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Pesticide Exposure and Risk for Parkinson's Disease

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Objective: Chronic, low-dose exposure to pesticides is suspected to increase the risk for Parkinson's disease (PD), but data are inconclusive.

Methods: We prospectively examined whether individuals exposed to pesticides have higher risk for PD than those not exposed. The study population comprised participants in the Cancer Prevention Study II Nutrition Cohort, a longitudinal investigation of US men and women initiated in 1992 by the American Cancer Society. Follow-up surveys were conducted in 1997, 1999, and 2001. The 143,325 individuals who returned the 2001 survey and did not have a diagnosis or symptoms of PD at baseline (1992) were included in the analyses.

Results: Exposure to pesticides was reported by 7,864 participants (5.7%), including 1,956 farmers, ranchers, or fishermen. Individuals exposed to pesticides had a 70% higher incidence of PD than those not exposed (adjusted relative risk, 1.7; 95% confidence interval, 1.2–2.3; $p = 0.002$). The relative risk for pesticide exposure was similar in farmers and nonfarmers. No relation was found between risk for PD and exposure to asbestos, chemical/acids/solvents, coal or stone dust, or eight other occupational exposures.

Interpretation: These data support the hypothesis that exposure to pesticides may increase risk for PD. Future studies should seek to identify the specific chemicals responsible for this association.

Ann Neurol 2006;60:197–203

The results of twin studies suggest that factors other than inherited genes have a prominent role in the cause of Parkinson's disease (PD).[1,2] The recognition that 1-methyl-4-phenyl-1,2,3,6-tetrahydropiridine (MPTP), a chemical with structural similarity to the herbicide paraquat, can cause degeneration of dopaminergic neurons and parkinsonism[3] raised the question of whether chronic low-dose exposure to pesticides could be a risk factor for PD. Accumulating data support this hypothesis.[4] Exposure to pesticides is associated with increased risk for PD in several ecological[5,6] and case–control studies,[7–9] and the selective dopaminergic toxicity of rotenone and other pesticides has been demonstrated in experimental studies.[10–12] Nevertheless, the overall evidence, albeit suggestive, remains inconclusive, in part because the results of case–control studies could be systematically affected by recall or selection bias. These limitations could be overcome by using a prospective study design. Two investigations have examined prospectively the relation between pesticide exposure and PD risk. In one investigation, the risk for PD was increased among individuals exposed to pesticides, but the association did not reach statistical significance[13]; in the other investigation, a small study among elderly subjects, pesticide exposure predicted the risk for PD among men, but not among women.[14] We therefore examined whether individuals exposed to pesticides have an increased risk for PD in a large cohort of US men and women, comprising more than 140,000 participants and 413 incident cases of PD.

Subjects and Methods

Study Population

The study was conducted among participants in the Cancer Prevention Study (CPS) II Nutrition Cohort, a prospective investigation of 184,190 individuals (86,404 men and 97,786 women) initiated in 1992 by the American Cancer Society to investigate the roles of diet and other lifestyle factors in cancer incidence.[15] At enrollment, participants completed a questionnaire on smoking, physical activity, and

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Received Jan 25, 2006, and in revised form Mar 27. Accepted for publication Apr 28, 2006.

Published online Jun 26, 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20904

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other aspects of lifestyle. Follow-up surveys were conducted in 1997, 1999, and 2001, the last including for the first time a question on lifetime occurrence of PD. The 143,325 individuals who returned the 2001 survey (89% of all living cohort members) and did not have a diagnosis or symptoms of PD at baseline (1992) were included in this study. Participants in the study were mostly white (97.5%), and mean age at enrollment was 63.7 years for men (median, 63; range, 41–90 years) and 62.0 years for women (median, 62; range, 40–85 years).

The study was approved by the Human Subject Committee at Harvard School of Public Health and the Institutional Review Board at Emory University.

Assessment of Exposure

Participants in the CPS II Nutrition Cohort were drawn from a cohort of 1.2 million US men and women recruited by American Cancer Society volunteers in 1982 for a study of cancer mortality (CPS II).[16] In 1982, as part of the original CPS II mortality study, participants completed a four-page survey that included questions on occupation and exposure to selected chemicals or dusts. Specifically, participants were asked whether they were currently exposed or had been regularly exposed in the past to asbestos, chemicals/acids/solvents, coal or stone dust, coal tar/pitch/asphalt, diesel engine exhaust, dyes, formaldehyde, gasoline exhaust, pesticides/herbicides, textile fibers/dust, wood dust, or x-ray/radioactive material. If exposed, participants were asked to report the duration of exposure in years. Information on chemical exposures was not updated in subsequent surveys. Participants were also asked to report their current job and the job kept for the longest period of time. The reported jobs were categorized according to the 1980 Bureau of the Census occupational titles.[17] Individuals were considered as farmers if any of the jobs reported were coded with the Census category "farmers, ranchers or fishermen"; farming was the longest held occupation in 76% of these individuals.

Case Ascertainment

The case ascertainment procedures were similar to those that we used in our previous studies of PD.[18] In brief, we wrote to all participants who reported a diagnosis of PD on the 2001 questionnaire and asked for permission to contact their treating neurologists and obtain copy of the medical records. We then asked their treating neurologists (or internists if the neurologists did not respond) to complete a diagnostic questionnaire or to send a copy of the medical record. The questionnaire included questions about cardinal signs of PD (rest tremor, rigidity, bradykinesia, and postural instability), response to L-dopa treatment, and the presence of signs and symptoms or other features that may corroborate a diagnosis of PD or suggest an alternative diagnosis. Confirmed cases were those for whom the PD diagnosis was considered definite or probable by the treating neurologist or internist, or if the medical record included either a final diagnosis of PD made by a neurologist or evidence at a neurological examination of at least two of the four cardinal signs (with one being rest tremor or bradykinesia), a progressive course, and the absence of unresponsiveness to L-dopa or other features suggesting an alternative diagnosis. The review of medical records was conducted by our movement disorder specialist (M.A.S.), who was blinded to the exposure status.

A total of 840 participants reported a diagnosis of PD at any time in the past, and 677 (81%) of these participants provided informed consent for contacting the treating neurologists. We obtained a completed diagnostic questionnaire or medical record for 648 (96%) of the 677 participants who provided consent, and then confirmed the diagnosis in 588 (91%). Of these, 175 had onset before 1992 and were excluded from the analyses. Among the 413 PD incident cases included in this report, 67.6% of the diagnoses were confirmed by the treating neurologists (55.0%) or movement disorder specialists (12.6%), 21.1% by review of the medical records, and 11.4% by the treating internists or family physicians. The proportion of cases confirmed by different methods did not vary significantly by occupation (farmers vs nonfarmers) or exposure to pesticides.

Statistical Analyses

Although data on pesticide exposure were collected in 1982, we used the date of return of the 1992 questionnaire as the date when follow-up began for PD. Follow-up ended on the date of the first symptoms of PD for PD cases or September 30, 2001, for participants without PD. For each environmental exposure, we defined participants as exposed if they responded "yes" to the question about regular current or past exposure or nonexposed if they responded "no." Participants who left blank the answer to one or more questions on chemical exposures were initially considered as a separate group, with missing exposure. Because the risk for PD (adjusted for age, sex, and smoking) among the latter group was virtually identical to that in the corresponding unexposed group, in final analyses, these individuals were considered as not exposed. Exposed individuals were further categorized according to self-reported duration of exposure.

Primary analyses were conducted using Cox proportional hazard models, adjusted for age, sex, and smoking (never, past, or current: 1–14 cigarettes/day, 15–24 cigarettes/day, 25+ cigarettes/day). Further analyses were adjusted for potential risk factors for PD, including coffee consumption (none, 3–6 cups/week, 1 cup/day, 2–3 cups/day, 4–5 cups/day, 6+ cups/day, missing); education (five categories, from some high school to college graduate or higher); use of ibuprofen, aspirin, or other NSAIDs ($<$2 tablets/week, 2–6.9 tablets/week, and $\geq$1 tablet/day)[19]; and physical activity (metabolic equivalents, in quintiles). These variables were derived from the 1992 survey, except for coffee consumption and education, which were derived from the 1982 questionnaire, because they were not included in the 1992 survey. Stratified analyses were conducted according to occupation (farmers and nonfarmers), attained age ($<$65 and $\geq$65 years), and smoking status (never smokers and ever smokers). Because there were no apparent differences in the association between pesticide exposure and PD risk by sex, we presented the results for men and women combined unless otherwise specified. Additional sensitivity analyses were conducted by censoring the follow-up at age 75 years for all participants, excluding PD cases not confirmed by a neurologist, and by including participants who confirmed the diagnosis of PD but did not provide consent for review of their medical records. All p values are two-tailed and consid-

ered significant if less than 0.05. Because the primary hypothesis of the study was that exposure to pesticides/herbicides, but not other chemical exposures, would be associated with increased risk for PD, we did not adjust the statistical tests for multiple comparisons.

Results

Overall, exposure to pesticides was reported by 5,203 men (8.2%) and 2,661 women (3.3%). Individuals who reported exposure to pesticides were 14 times more likely to report their occupation as "farmer, rancher or fisherman" and twice more likely to be blue collar workers than those not exposed (Table 1). Furthermore, the educational level was slightly lower among the exposed, whereas smoking behavior, coffee consumption, and other aspects of lifestyle were similar between the two groups (see Table 1).

The mean age at PD onset was 70 years and was unrelated to pesticide exposure (70.9 years in exposed and 70.3 years in nonexposed participants). Age-specific incidence rates of PD per 100,000 person-years were: in men: 31.8 (age range, 50–59 years), 53.2 (60–69 years), 86.8 (70–79 years), and 102 (80–89 years); and in women: 10.1 (50–59 years), 28.0 (60–69 years), 48.3 (70–79 years), and 29.1 (80–89 years), but the latter rate is based on small numbers and is thus unstable. In analyses adjusted for age, sex, and smoking, the risk for PD was 70% higher among individuals exposed to pesticides than among those not exposed (relative risk [RR], 1.7; 95% confidence interval [CI], 1.2–2.3; $p = 0.002$); this association was slightly stronger in analyses adjusted for other potential confounders (RR, 1.8; 95% CI, 1.3–2.5; $p = 0.0003$). In contrast, none of the other exposures was significantly associated with risk for PD (Fig). The association between pesticide exposure and PD did not differ appreciably in analyses restricted to cases confirmed by a neurologist or movement disorder specialist, or within strata defined by occupation (farmers or nonfarmers), sex, age, or smoking status (Table 2). Only 2,308 (28%) of the individuals who reported exposure to pesticides indicated the duration of exposure. The risk for PD was not significantly different among individuals who reported exposure for 10 or more years (multivariate RR, 2.3; 95% CI, 1.1–4.9) compared with those with shorter (RR, 2.1; 95% CI, 0.7–6.5) or missing (RR, 1.7; 95% CI, 1.2–2.5) duration.

Finally, we were concerned about the possibility of bias due to the exclusion of those subjects with PD who did not provide permission to review their medical records. Prevalence of exposure to pesticides among these cases was higher than among cases who consented to medical record review (6.9 vs 4.6%), and their exclusion may thus have attenuated the association between exposure and PD. Because the diagnosis of PD in this group is not confirmed and the date of onset of PD symptoms is unknown, these participants could not be included in the primary analyses. To examine the robustness of the results, we repeated the analyses assuming that all individuals who denied consent for

Table 1. Age- and Sex-Adjusted Population Characteristics in 1992 According to Exposure to Pesticides/Herbicides

Characteristics	Exposed to Pesticides in 1982	Not exposed to Pesticides in 1982
N	7,864	135,461
Mean age, yr	63.3	62.7
Male, %	66.2	42.9
Race, %		
White	97.4	97.5
Black	1.1	1.3
Other	1.5	1.2
Body mass index, kg/m^2	26.4	25.9
Current smokers, %	7.9	7.6
Past smokers, %	42.5	45.7
Coffee drinkers, %		
None	12.8	12.0
≤1 cup/day	15.8	14.9
2-3 cups/day	27.3	29.0
4+ cups/day	21.2	20.8
Missing	23.0	23.3
Ibuprofen use, %		
No use	72.4	74.7
<2 tablets/week	6.1	6.4
2-6.9 tablets/week	5.2	5.0
≥1 tablet/day	7.0	6.1
Missing	9.4	7.9
Alcohol intake, %		
None	43.7	38.3
<1 drink/day	37.2	39.4
1+ drinks/day	15.4	18.5
Missing	3.8	3.9
Supplement use (% current users)		
Vitamin E	22.6	19.7
Vitamin C	28.2	25.9
Multivitamin	37.3	37.5
Occupation, %		
White collar	43.8	55.9
Blue collar	24.0	12.4
Housewife	18.3	17.2
Unknown occupation	13.9	14.4
Physical activity in leisure time, %		
None or low	76.6	80.0
Moderate	19.3	15.9
High	2.6	2.6
Missing	1.6	1.4
Total mets/week	29.9	25.8
Education, %		
Some high school or less	7.8	5.4
High school graduate	27.2	25.1
Vocational	5.8	5.6
Some college	23.7	22.9
College graduate or higher	35.3	40.2
Farmer, rancher, or farm hand (% main occupation)	15.0	1.1

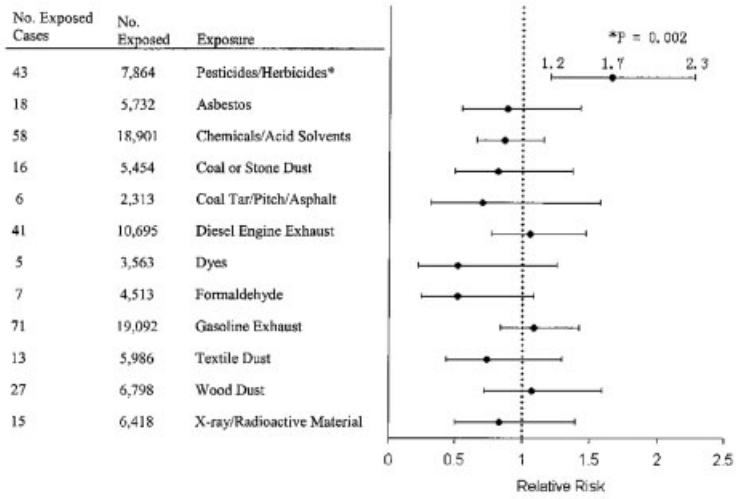

Fig. Relative risks and 95% confidence intervals of Parkinson's disease according to environmental exposures reported in 1982 in the Cancer Prevention Study II Nutrition Cohort (1992–2001).

Table 2. Relative Risk for Parkinson's Disease according to Exposure to Pesticides in Stratified Analyses

Characteristics	Cases, N	Person-Time	RR (95% CI)
All			
Not exposed to pesticide	370	1,167,603	Reference
Exposed to pesticide	43	67,901	1.8 (1.3–2.5)
Only cases confirmed by neurologist[a]			
Not exposed to pesticide	329	1,167,773	Reference
Exposed to pesticide	37	67,935	1.8 (1.3–2.5)
Occupation			
Not a farmer			
Not exposed to pesticide	362	1,148,865	Reference
Exposed to pesticide	30	50,938	1.7 (1.2–2.5)
Farmer			
Not exposed to pesticide	8	18,738	0.9 (0.5–1.9)
Exposed to pesticide	13	16,964	1.6 (0.9–2.7)
Sex			
Men			
Not exposed to pesticide	232	504,816	Reference
Exposed to pesticide	34	45,210	1.8 (1.2–2.6)
Women			
Not exposed to pesticide	138	662,787	Reference
Exposed to pesticide	9	22,691	1.9 (1.0–3.8)
Age			
<65 yr			
Not exposed to pesticide	171	719,681	Reference
Exposed to pesticide	19	40,072	1.7 (1.1–2.8)
≥65 yr			
Not exposed to pesticide	199	447,922	Reference
Exposed to pesticide	24	27,829	1.9 (1.2–2.9)
Smoking			
Never smoker			
Not exposed to pesticide	179	532,365	Reference
Exposed to pesticide	22	29,762	2.1 (1.3–3.3)
Ever smoker			
Not exposed to pesticide	183	620,815	Reference
Exposed to pesticide	21	37,229	1.7 (1.1–2.7)

[a]Cases were confirmed by treating neurologist, movement disorder specialist, or neurologist review of medical records.

RR = relative risk; CI = confidence interval.

review of the medical records were confirmed incident cases of PD (with date of onset arbitrarily set at middle of follow-up). In these analyses, as expected, we found a slightly stronger association between exposure to pesticides and risk for PD (RR, 1.8; 95% CI, 1.4–2.4; $p < 0.0001$).

Discussion

In this large cohort study of more than 140,000 people, those who reported exposure to pesticides/herbicides before 1982 had a 70% higher incidence of PD 10 to 20 years later than those not exposed to these chemicals. Exposure to many other environmental contaminants was not related to PD risk. Strengths of this study include the large sample size, the prospective collection of information on pesticide exposure, and the availability of information on several potential confounders. The main limitation of the investigation is the lack of detailed information about the duration, frequency, and intensity of the exposure and information on specific pesticides. Furthermore, information on exposure was not updated after 1982, and therefore we cannot exclude the possibility that more recent pesticide exposure contributed to the increased risk for PD. Information on pesticide use, however, was collected 10 or more years before the onset of PD, and the misclassification of exposure is thus most likely nondifferential with respect to PD risk. This misclassification would be expected to attenuate any true association between exposure to pesticides and PD risk, and it is unlikely to result in a spurious positive association. Additional potential sources of bias are differential diagnostic errors or underreporting of PD. Although some diagnostic errors are inevitable, bias from this source is probably modest, because in recent clinicopathological studies, the positive predictive value of clinical diagnoses of PD has been found to be high: 90% for diagnoses made by neurologists[20] and 98% for diagnoses made by movement disorder specialists.[21] Selective underrecognition or underreporting of PD among individuals not exposed to pesticides could also theoretically induce a spurious positive association between pesticide exposure and PD risk, but it would have to be rather extreme to account for the marked increase in risk observed in our study.

Several individual compounds commonly used as pesticides in US agriculture have been found to cause dopaminergic degeneration in the substantia nigra and motor abnormalities when administered at high doses to experimental animals. These include rotenone,[10,22] paraquat,[11] and the combination of paraquat with maneb or other dithiocarbamates.[12,23–25] A variety of mechanisms have been proposed for the deleterious effects of these pesticides, including oxidative stress, interference with dopamine transporters, mitochondrial dysfunction,[26] promotion of α-synuclein fibrillation,[27] and inflammation.[28] The relevance of these mechanisms in PD remains uncertain.

In postmortem studies, higher levels of organochlorine insecticides have been found in the substantia nigra or striatum of individuals with PD.[29–31] This finding reflects that organochlorine insecticides, unlike organophosphates and most other pesticides, persist in tissues for many years after cessation of exposure. These measurements indicate that organochlorine pesticides reach the affected tissue, but do not prove that they cause PD and also do not identify which pesticides may be responsible. Ecological and case–control studies support the association of PD with rural residence,[5,32–39] use of private wells,[33,34,37,38,40] farming,[5,35,41,42] as well as exposure to insecticide and herbicide products.[6,35,36,39,41,43–49] According to a meta-analysis of case–control studies, the risk for PD is about 90% higher among individuals who reported exposure to pesticides than among those not exposed.[7] Consistent results have been obtained in more recent investigations.[8,9,50] Finally, in a previous prospective study of more than 8,000 men of Japanese ancestry in Hawaii, a significant positive association was found between duration of work in a plantation and risk for PD.[13] The RR comparing men who worked in a plantation for 20 or more years with men who never worked in a plantation was 1.9 (95% CI, 1.0–3.5); a positive trend, albeit nonsignificant, was also found between exposure to pesticides and risk for PD.

Exposure to pesticides could be a marker of other unspecified aspects of rural living, rather than the actual cause of disease. A direct effect of pesticides, however, is supported by the finding in this study that farmers not exposed to pesticides were not at increased risk for PD. Consistent with an adverse effect of pesticides is also the finding of stronger associations between use of pesticides and PD among individuals who are poor debrisoquine metabolizers because of genetic variation in the CYP2D6 gene.[8] The CYP2D6 gene encodes the enzyme debrisoquine hydroxylase, which metabolizes several xenobiotics, including MPTP, the herbicide atrazine, and organophosphate pesticides.[51] Identification of the pesticides directly related to increased PD risk is critical, because generic attempts to reduce overall exposure may be insufficient and impractical. Further investigations should focus on populations exposed to specific chemicals and examine the role of variations in genes affecting xenobiotic metabolism and their functional consequences.

In summary, the findings of this large prospective investigation support the hypothesis that exposure to pesticides is a risk factor for PD. Future studies should seek to identify the specific compounds associated with risk.

This study was supported by the Michael J. Fox Foundation, (A.A.) Kinetic Foundation, (A.A.) and National Institute of Environmental Health Sciences (ES10804, M.A.S.).

We thank E. Jiménez and E. Thacker for technical support.

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Productie 18

Eur J Epidemiol (2011) 26:547–555
DOI 10.1007/s10654-011-9574-5

NEURO-EPIDEMIOLOGY

Parkinson's disease risk from ambient exposure to pesticides

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Received: 10 December 2010 / Accepted: 23 March 2011 / Published online: 20 April 2011

Abstract Due to the heavy and expanding agricultural use of neurotoxic pesticides suspected to affect dopaminergic neurons, it is imperative to closely examine the role of pesticides in the development of Parkinson's disease (PD). We focus our investigation on pesticide use in California's heavily agricultural central valley by utilizing a unique pesticide use reporting system. From 2001 to 2007, we enrolled 362 incident PD cases and 341 controls living in the Central Valley of California. Employing our geographic information system model, we estimated ambient exposures to the pesticides ziram, maneb, and paraquat at work places and residences from 1974 to 1999. At workplaces, combined exposure to ziram, maneb, and paraquat increased risk of PD three-fold (OR: 3.09; 95% CI: 1.69, 5.64) and combined exposure to ziram and paraquat, excluding maneb exposure, was associated with a 80% increase in risk (OR:1.82; 95% CI: 1.03, 3.21). Risk estimates for ambient workplace exposure were greater than for exposures at residences and were especially high for younger onset PD patients and when exposed in both locations. Our study is the first to implicate ziram in PD etiology. Combined ambient exposure to ziram and paraquat as well as combined ambient exposure to maneb and paraquat at both workplaces and residences increased PD risk substantially. Those exposed to ziram, maneb, and paraquat together experienced the greatest increase in PD risk. Our results suggest that pesticides affecting different mechanisms that contribute to dopaminergic neuron death may act together to increase the risk of PD considerably.

Keywords Case–control study · Geographic information systems (GIS) · Paraquat · Parkinson's disease · Pesticide · Ziram

Abbreviations

CA DPR	California department of pesticide regulation
CI	Confidence interval
GIS	Geographic information system
HIPAA	Health insurance portability and accountability act
OR	Odds ratio
PD	Parkinson's disease
PLSS	Public land survey system
PUR	Pesticide use report
UCLA	University of California Los Angeles
UPS	Ubiquitin proteasome system

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Introduction

Parkinson's disease (PD) is a common movement disorder associated with the degeneration of dopaminergic neurons of the substantia nigra. PD has an estimated annual incidence of approximately 17 per 100,000 and an increasing prevalence worldwide due to the growth of aging populations [1]. Recently, a number of animal studies have suggested biologic mechanisms for specific pesticides that may increase PD risk. Paraquat has been shown to damage dopaminergic neurons by promoting oxidative stress and cell death [2–5]. Exposure to manganese ethylene-bis-dithiocarbamate, the major active ingredient in the dithiocarbamate fungicide maneb, selectively produces dopaminergic neurodegeneration in mice by disrupting mitochondrial function, increasing oxidative stress, and inhibiting proteasomal function [6, 7]. Ziram, another dithiocarbamate, has been shown to cause dopaminergic neuron damage in cell culture by inhibiting the E1 ligase of the ubiquitin proteasome system (UPS) [8]. Recent animal studies reported that the dopaminergic toxicity of paraquat is enhanced when co-administered with maneb [9–11]. These studies suggest that different toxins may potentially act together and contribute to PD pathology via different pathways linked to dopaminergic neurodegeneration.

The impact of pesticide exposures on humans in agricultural communities is of special concern. Not only are pesticide applicators disproportionately exposed to pesticides due to infrequent use of personal protective equipment and improper pesticide mixing and application, but those living and working near farms are also exposed due to drifting pesticide spray [12–14]. Even though the association between PD, farm work, and pesticide exposures is supported by the literature [15–17], very few studies to date have reported findings for specific chemical agents [18–24]. Many studies in human populations employed a case–control design that lends itself to recall bias when pesticides are assessed retrospectively via self-report [23, 25]. Occupational cohort studies of PD to date have been limited by a paucity of PD cases handling specific pesticides or relying on participant recall to obtain data on specific pesticides [19].

We accessed data from the Pesticide Use Report (PUR) system maintained by California's Department of Pesticide Regulation (CA DPR) and used a geographic information system (GIS) to assess ambient exposures to specific pesticide [26]. For the first time, we assess ambient exposures to ziram, maneb, and paraquat derived from occupational in addition to residential addresses. We focus on ziram because it is structurally related to maneb and is a more potent inhibitor of the UPS [8].

Materials and methods

All procedures described have been approved by the UCLA-IRB for human participants and informed consent was obtained from all participants.

Participant recruitment

We recruited persons with PD and population controls from Fresno, Tulare, and Kern counties ("tri-county" area), largely agricultural areas in Central California, details are provided elsewhere [27]. Briefly, PD cases newly diagnosed between January 1998 and January 2007, residing in the tri-county area and living in California for at least 5 years prior to diagnosis were recruited into our study within 3 years of diagnosis. We collaborated with practicing neurologists, Kaiser Permanente, Kern and Visalia Medical Centers and the Veteran's Administration, Parkinson's disease support groups, local newspapers, and radio stations that broadcast public service announcements to recruit participants in the tri-county area.

Of the 1,167 PD cases we invited and who responded to participate in the study, 604 were not eligible: 397 had been diagnosed more than 3 years prior to contact, 51 denied a PD diagnosis, 134 lived outside the tri-county area, and 22 were too ill to participate. Of the 563 cases found eligible, 473 were examined by a UCLA movement disorder specialist at least once and confirmed as having clinically "probable" or "possible" PD; the remaining 90 potential cases could not be examined or interviewed (54% withdrew, 32% were too ill or died, and 14% moved away). Among those examined, we excluded 83 for whom we were unable to confirm a diagnosis of idiopathic PD, leaving us with 390 cases. We were able to re-examined 71% of the cases and excluded another 21 participants misdiagnosed with PD. Of the remaining 369 cases, 362 provided all information needed for analyses.

Initially controls older than 65 years of age were identified from Medicare enrollee lists in 2001 and were invited to participate in our study, but due to Medicare prohibiting the continued use of enrollees after HIPAA implementation, we changed our recruitment plan and recruited the remaining 70% of our controls from randomly selected residential units (parcels) from tri-county tax assessor records. We mailed letters of invitation to a random selection of parcels and also attempted to identify head-of-household names and telephone numbers for these parcels using marketing companies' services and Internet searches. We contacted 1,212 potential controls by mail and/or phone for eligibility screening to recruit one person per household. Eligibility criteria were: (1) not having PD, (2)

being at least 35 years of age, (3) currently residing primarily in one of the three counties, and (4) having lived in California for at least 5 years prior to the screening. Of the 457 ineligible controls, 409 were too young, 44 were terminally ill and 4 primarily resided outside the study area. Of the 755 eligible population controls, 409 declined participation, were too ill or moved out of the area before honoring an appointment and 346 were enrolled, and 341 provided all information needed for analyses.

For all study participants, we conducted telephone interviews to obtain demographic and exposure information.

GIS-based ambient pesticide exposures assessment

Employing our GIS-based system, we combined PUR data, land use maps, and geocoded address information [26, 28] to produce estimates of pesticide exposure within a 500-m radius buffer around participants' occupational and residential addresses as suggested in previous literature [29–31]. A technical discussion of our GIS-based approach is provided elsewhere, here we briefly summarize the data sources and exposure modeling process [26]. In a previous validation study, our GIS-derived measure for organochlorine exposures identified those with high serum dichlorodiphenyldichloroethylene levels with high specificity (87%) [32].

Pesticides use reporting

Since 1974, the CA DPR has recorded agricultural application of restricted-use pesticides (defined as "agents with harmful environmental or toxicological effects"), and for all agriculturally applied pesticides from 1990 onwards. The location of each PUR record is referenced to the Public Land Survey System (PLSS), a nationwide grid that parcels land into sections at varying resolutions. Each PUR record includes the name of the pesticide's active ingredient, the poundage applied, the crop and acreage of the field, the application method, and the date of application.

Land use maps

Because the PUR records only link an agricultural pesticide application to a whole PLSS grid section, we added information from land use maps to more precisely locate the pesticide application as described in detail elsewhere [28]. Briefly, the California Department of Water Resources periodically (every 7–10 years) performs countywide surveys of location and extent of land use and crop cover. We constructed historical electronic maps of land use and crop type from digital maps from recent surveys [33] (1996–1999) and manually digitized earliest available paper maps (1977–1995). Using the PLSS grid section and crop type reported on the PUR, we further refined pesticide applications using the more detailed land use geography.

Geocoding

We obtained historical occupational and residential addresses from all study participants. Addresses reported for the period of 1974–1999 in the tri-county area were automatically geocoded to TigerLine files (Navteq 2006), and then manually resolved in a multi-step process similar to that described by McElroy [30]. We considered geocoded addresses as having high accuracy if we were able to geocode to the actual address, a parcel/lot centroid, street centroid, or street intersection. Inaccurately geocoded addresses were considered to be those geocoded at the zipcode, city, county, state centroids, or did not have enough information to be geocoded.

Pesticide exposure estimates at occupational and residential addresses

First we combined the PUR data, land use maps, and geocoded address information and created 500 meter buffers around addresses in our GIS for each year in the 26-year period from 1974 to 1999. Then we calculated annual ambient exposures to the individual pesticides, maneb, ziram, and paraquat, for each participant by summing the pounds of pesticides applied in each buffer and weighting the total poundage by the proportion of the acreage treated. For each of the three pesticides examined in this study, we summed the annual pounds applied per acre to obtain 26 annual exposure values for each pesticide separately for occupational and residential addresses.

Average pesticide exposures were then calculated for the following exposure time windows: (1) 1974–1999, (2) 1974–1989, (3) 1990–1999 to address a possible extended induction period for PD and assess the influence of age at exposure. A participant was considered exposed to a particular pesticide when the pounds per acre measured was greater than zero during the time window. We created exposure measures for single and combined pesticides by creating categories of co-exposures to different pesticides. Participants who did not work or live in the tri-county area between 1974 and 1999 could not be assigned an exposure estimate and were considered unexposed.

In the same manner, we also created exposure estimates for organophosphates and organochlorines, two pesticide classes that also contribute to neurodegeneration [34, 35]. Participants were considered exposed if they had any exposure to at least one organophosphate or organochlorine pesticide.

Statistical analysis

We conducted analyses of occupational and residential exposures to maneb, ziram, and paraquat individually and in different combinations. We also conducted analyses stratified by exposure time window and by age. We adjusted for age at diagnosis (cases) or age at interview (controls), sex, ethnicity (White vs. non-White), education (<12 years, 12 years, >12 years), having a 1st degree family member with PD (yes, no), and smoking (current, former, never). We also adjusted for organophosphate and organochlorine exposure in some analyses.

We used SAS 9.1 (SAS Institute Inc., Cary, NC, USA) to perform unconditional logistic regression analyses.

Results

Study participants were predominantly White, over the age of 60, and the minority reported a family history of Parkinson's disease (Table 1). Cases were slightly older than controls, more often male, and had completed fewer years of education. They were also more likely to have never smoked cigarettes.

When assessing combinations of exposure to all three pesticides, combined exposure to all three pesticides at both workplaces (OR: 3.09; 95% CI: 1.69, 5.64) and residences (OR: 1.86; 95% CI: 1.09, 3.18) was most strongly associated with PD risk, followed by combined exposure to ziram and paraquat only at workplaces (OR: 1.82; 95% CI: 1.03, 3.21) (Table 2). Adjustment for exposure to organophosphate and organochlorine pesticides, shifted risk estimates slightly towards the null value and increased confidence interval sizes (results not shown), but combined exposure to maneb, ziram, and paraquat at workplaces remained strongly associated with PD risk (organophosphate and organochlorine adjusted OR: 2.61; 95% CI: 1.24, 5.48). The rarity of exposure to maneb alone and exposure to ziram and maneb without paraquat precludes estimation of effects for these combinations of pesticides. Exposure to paraquat alone was not associated with PD risk at residences but was associated with an increased risk at workplaces.

When considering the main effects of exposure to ziram, maneb, and paraquat, participants exposed to these three pesticides at both residences and work places experienced a greater increase in risk of PD than those exposed at residences or workplaces only (Table 3). Participants exposed to maneb experienced a similar increase in PD risk when exposed at either workplaces or residences only. However, those exposed to ziram at workplaces only experienced higher PD risk than those exposed at residences only. PD risk did not increase for participants exposed to paraquat at workplaces or residences only.

Table 1 Demographic characteristics of the study population

	Case		Control		OR	95% CI
	(N = 362)	%	(N = 341)	%		
Age (mean and range)	68.2 (34–88)		67.6 (34–92)			
≤60	77	21	87	26	1.00	Reference
>60	285	79	254	74	1.27	(0.89, 1.80)
Sex						
Female	156	43	165	48	1.00	Reference
Male	206	57	176	52	1.24	(0.92, 1.67)
1st deg. relative with PD						
No	307	85	303	89	1.00	Reference
Yes	55	15	37	11	1.47	(0.95, 2.30)
Race						
White	291	80	279	82	1.00	Reference
Non-white	71	20	62	18	1.10	(0.75, 1.60)
Education						
<12 years	68	19	38	11	1.19	(0.72,1.98)
12 years	96	27	64	19	1.00	Reference
>12 years	198	55	239	70	0.55	(0.38, 0.80)
Smoker status						
Never smoker	191	53	146	43	1.00	Reference
Ex smoker	151	42	161	47	0.72	(0.53, 0.98)
Current smoker	20	6	34	10	0.45	(0.25, 0.81)

Table 2 Effect estimates (ORs and 95% CIs) for ambient pesticide exposures to paraquat, maneb, and ziram in the Central California Valley study population for the 1974–1999 time window of exposure

	Occupational[b]				Residential[c]			
	Case (N = 362)	Control (N = 341)	Adjusted OR[a]	95% CI	Case (N = 362)	Control (N = 341)	Adjusted OR[a]	95% CI
Not exposed to paraquat, maneb, or ziram	164	191	1.00	Reference	122	136	1.00	Reference
Exposed to paraquat, not maneb or ziram	81	78	1.26	(0.86, 1.86)	109	125	0.91	(0.63, 1.31)
Exposed to maneb, not ziram or paraquat	1	3	–[d]	–[d]	2	1	–[d]	–[d]
Exposed to ziram, not maneb or paraquat	6	6	1.37	(0.42, 4.49)	4	3	1.48	(0.32, 6.85)
Exposed to ziram and maneb, not paraquat	1	0	–[d]	–[d]	1	0	–[d]	–[d]
Exposed to maneb and paraquat, not ziram	26	21	1.41	(0.75, 2.68)	34	21	1.59	(0.86, 2.95)
Exposed to ziram and paraquat, not maneb	37	24	1.82	(1.03, 3.21)	37	27	1.37	(0.78, 2.42)
Exposed to maneb, ziram, and paraquat	46	18	3.09	(1.69, 5.64)	53	28	1.86	(1.09, 3.18)

[a] Adjusted for age, sex, education, smoking, family history of PD, and race

[b] Pesticide exposure derived from self-reported occupational addresses

[c] Pesticide exposure derived from self-reported residential addresses

[d] Not calculated due to insufficient cell counts

Table 3 Effect estimates (ORs and 95% CIs) for ambient exposures to ziram, maneb, and paraquat at residences and workplaces for the 1974–1999 time window of exposure

	Case (N = 341)	Controls (N = 341)	Adjusted OR[a]	95% CI
Ziram				
Not exposed to ziram	229	253	1.00	Reference
Exposed at residences only	43	40	1.13	(0.70, 1.82)
Exposed at workplaces only	38	30	1.52	(0.90, 2.58)
Exposed at both residences and workplaces	52	18	3.01	(1.69, 5.38)
Maneb				
Not exposed to maneb	236	266	1.00	Reference
Exposed at residences only	52	33	1.71	(1.06, 2.77)
Exposed at workplaces only	36	25	1.77	(1.02, 3.09)
Exposed at both residences and workplaces	38	17	2.26	(1.22, 4.20)
Paraquat				
Not exposed to paraquat	101	110	1.00	Reference
Exposed at residences only	71	90	0.77	(0.50, 1.17)
Exposed at workplaces only	28	30	1.07	(0.59, 1.96)
Exposed at both residences and workplaces	162	111	1.50	(1.03, 2.18)

[a] Adjusted for age, sex, education, smoking, family history of PD, and race

Combined exposure to ziram and paraquat at workplaces was associated with a two-fold increase in PD risk in the overall 1974–1999 time window (Table 4). Furthermore, this combination exposure contributed to PD risk at workplaces in both early and late time windows, while only the early time window contributed to PD risk at residences. These patterns were also observed for combined exposure to maneb and paraquat.

Estimated PD risk increase was generally much larger for those diagnosed with PD at a younger age (age ≤60) (Table 5). Younger onset patients that were exposed to a combination of ziram and paraquat at workplaces (OR:

Table 4 Effect estimates (ORs and 95% CIs) for ambient exposures to maneb, ziram, and paraquat by time window of exposure

Time window of exposure	Occupational[b]				Residential[c]			
	Case (N = 362)	Control (N = 341)	OR[a]	95% CI	Case (N = 362)	Control (N = 341)	OR[a]	95% CI
Maneb and paraquat exposure								
1974–1999 overall time window								
Not exposed to maneb or paraquat	170	197	1.00	Reference	126	139	1.00	Reference
Exposed to parauqat, not maneb	118	102	1.37	(0.97, 1.94)	146	152	0.98	(0.70, 1.38)
Exposed to maneb, not paraquat	2	3	0.96	(0.16, 5.99)	3	1	3.21	(0.32, 32.68)
Exposed to maneb and paraquat	72	39	2.15	(1.36, 3.41)	87	49	1.73	(1.11, 2.68)
1974–1989 time window								
Not exposed to maneb or paraquat	180	212	1.00	Reference	144	165	1.00	Reference
Exposed to maneb or paraquat	124	96	1.43	(0.99, 2.07)	145	137	1.15	(0.81, 1.63)
Exposed to maneb and paraquat	58	33	1.82	(1.08, 3.07)	73	39	2.05	(1.23, 3.40)
1990–1999 time window								
Not exposed to maneb or paraquat	269	279	1.00	Reference	227	228	1.00	Reference
Exposed to maneb or paraquat	71	52	1.15	(0.74, 1.81)	110	95	0.88	(0.61, 1.28)
Exposed to maneb and paraquat	22	10	1.69	(0.74, 3.84)	25	18	0.91	(0.46, 1.82)
Ziram and paraquat exposure								
1974–1999 overall time window								
Not exposed to ziram or paraquat	165	194	1.00	Reference	124	137	1.00	Reference
Exposed to parauqat, not ziram	107	99	1.30	(0.91, 1.86)	143	146	0.99	(0.70, 1.41)
Exposed to ziram, not paraquat	7	6	1.65	(0.52, 5.17)	5	3	1.75	(0.40, 7.62)
Exposed to ziram and paraquat	83	42	2.37	(1.52, 3.68)	90	55	1.60	(1.05, 2.46)
1974–1989 time window								
Not exposed to ziram or paraquat	175	211	1.00	Reference	144	165	1.00	Reference
Exposed to ziram or paraquat	121	90	1.55	(1.06, 2.26)	154	139	1.13	(0.80, 1.61)
Exposed to ziram and paraquat	66	40	1.71	(1.05, 2.78)	64	37	1.79	(1.05, 3.05)
1990–1999 time window								
Not exposed to ziram or paraquat	267	277	1.00	Reference	218	227	1.00	Reference
Exposed to ziram or paraquat	62	53	1.04	(0.66, 1.64)	93	81	1.03	(0.70, 1.51)
Exposed to ziram and paraquat	33	11	2.16	(1.01, 4.63)	51	33	1.06	(0.61, 1.84)

[a] Adjusted for age, sex, education, smoking, family history of PD, and race; exposure time windows are mutually adjusted for each other

[b] Pesticide exposure derived from self-reported occupational addresses

[c] Pesticide exposure derived from self-reported residential addresses

5.98; 95% CI: 1.95, 18.32) experienced a greater risk of PD than when exposed at residences (OR: 2.78; 95% CI: 1.10, 7.07). Similarly, for younger onset patients, exposure to maneb and paraquat alone and in combination was associated with a much larger risk at workplaces than at residences.

Discussion

The population-based case–control study of PD we conducted in a heavily agricultural region of California shows that combined exposure to ziram and paraquat, apart from maneb exposure, conferred an increased risk for developing PD. Our results suggest that exposure to paraquat, maneb and ziram may act together to increase the risk of PD more strongly than exposure to each individual pesticide alone or exposure to any combination of two pesticides. Only the early time window was important for ambient residential exposures to either ziram and paraquat or maneb and paraquat. In contrast, ambient workplace exposure during the early or late time window to either ziram and paraquat or maneb and paraquat increased PD risk, suggesting that although there may be a long induction period for these combinations of pesticides, potentially more intense occupational exposures later in life may also contribute to risk of developing PD. Finally, younger participants consistently experienced the greatest risks when

Table 5 Effect estimates (ORs and 95% CIs) for ambient exposures to maneb, ziram, and paraquat by age at PD diagnosis for the 1974–1999 time window of exposure

	Occupational[b]				Residential[c]			
	Case (N = 362)	Control (N = 341)	OR[a]	95% CI	Case (N = 362)	Control (N = 341)	OR[a]	95% CI
Maneb and paraquat exposure								
60 years old or younger								
Not exposed to maneb or paraquat	30	56	1.00	Reference	20	38	1.00	Reference
Exposed to maneb or paraquat	29	28	1.78	(0.87, 3.64)	36	42	1.53	(0.73, 3.19)
Exposed to maneb and paraquat	18	3	8.75	(2.31, 33.19)	21	7	4.82	(1.69, 13.76)
Over 60 years old								
Not exposed to maneb or paraquat	140	141	1.00	Reference	106	101	1.00	Reference
Exposed to maneb or paraquat	91	77	1.22	(0.82, 1.83)	113	111	0.89	(0.60, 1.32)
Exposed to maneb and paraquat	54	36	1.48	(0.88, 2.50)	66	42	1.28	(0.78, 2.09)
Ziram and paraquat exposure								
60 years old or younger								
Not exposed to ziram or paraquat	28	53	1.00	Reference	21	38	1.00	Reference
Exposed to ziram or paraquat	30	29	1.90	(0.91, 3.93)	35	37	1.65	(0.79, 3.45)
Exposed to ziram and paraquat	19	5	5.98	(1.95, 18.32)	21	12	2.78	(1.10, 7.07)
Over 60 years old								
Not exposed to ziram or paraquat	137	141	1.00	Reference	103	99	1.00	Reference
Exposed to ziram or paraquat	84	76	1.17	(0.76, 1.72)	113	112	0.88	(0.59, 1.30)
Exposed to ziram and paraquat	64	37	1.93	(1.10, 3.03)	69	43	1.38	(0.85, 2.26)

[a] Age stratified models adjusted for age, sex, education, smoking, family history of PD, and race

[b] Pesticide exposure derived from self-reported occupational addresses

[c] Pesticide exposure derived from self-reported residential addresses

exposed to a combination of either maneb and paraquat or ziram and paraquat. We not only confirm our previous results for residential exposures to paraquat and maneb with our new occupational address based exposure measures [18], but also observe that risk estimates at workplaces were generally larger than at residences and that exposures at both work places and residences together further increase risks.

The vast majority of previous epidemiological studies relied on self-reported pesticide exposures and thus may suffer from biased exposure assessment as study participants may misreport their historical pesticide use [36–40]. The issue of recall bias is especially problematic when attempting to estimate exposures to specific pesticides via self-report. The Agricultural Health Study cohort [19] attempted to estimate effects for several specific pesticides but found no pesticide or functional group to be more than weakly associated with incident PD, possibly due to the small number of cases who reported exposure to specific pesticides. Furthermore, self-reported pesticide exposure cannot account for risk in those not actively applying pesticides who nevertheless are potentially chronically exposed to pesticides from drift and contact with contaminated dust in heavily agricultural areas [14].

A strength of our study is that our GIS-based pesticide exposure assessment allow us to derive pesticide exposure information for participants who work or live near agricultural pesticide applications and may be unknowingly exposed due to pesticide drift. Additionally, our GIS-based methods employing the PUR data is an improvement over pesticide exposure assessment methods based on recall only, since it identifies the exact type, amount, and location of a pesticide active ingredient applied historically, and eliminates differential recall of exposure according to case status. Another strength is that we were able to obtain exposure data at occupational in addition to residential addresses. Since agricultural pesticides are applied during working hours, exposure estimates at workplaces may more accurately reflect true pesticide exposure and risk estimates are expected to be of greater magnitude if participants are present when pesticides are applied to fields. Finally, our population-based study is the only study to date in which movement disorder specialists examined patients multiple times to confirm diagnoses, thus reducing disease misclassification.

Our GIS-based method, which uses a 26-year average pesticide estimate at participants' occupational and residential addresses, cannot be considered a quantitative

measure of exposure because the derived poundage of active ingredient per acre applied does not translate easily into a measure of human neurotoxicity across pesticides or pesticide classes. In addition, pesticides vary in toxicity so that fewer pounds of a highly toxic pesticide may have the same effect as a greater poundage of a less toxic pesticide. Thus, we considered participants exposed if they experienced any exposure and created mutually exclusive pesticide exposure categories to assess multiple pesticides.

Another limitation is that the accuracy of our GIS-based pesticide exposure estimation relies on the quality of self-reported addresses. Occupational addresses were generally geocoded less accurately than residential addresses and addresses with lower geocoding accuracy tended to be assigned less exposure than accurately geocoded addresses (results not shown). Exposure estimates could only be obtained for participants with an occupational address located in the tri-county area between 1974 and 1999. Of the 703 participants, 26% of cases and 26% of controls were missing occupational address information, while only 4% of cases and 4% of controls were missing residential address information. Different from our previously published work [18], we classified participants with missing data as unexposed to maintain statistical power when assessing the risk of pesticide exposures at occupational addresses. This approach would bias effect estimates towards the null as long as the resulting exposure misclassification is non-differential by case status, as suggested by the comparable percentages of cases and controls with missing address information.

Despite these limitations, we believe that our GIS model provides us with an accurate qualitative indicator of ambient pesticide exposure from applications and drift in close proximity to workplaces and residences. It is unlikely that our GIS-based results are affected by selection bias because participants were likely unaware of their historical ambient workplace or residential exposure to specific pesticides associated with PD risk, thus their enrollment would not be associated with pesticide exposure.

Our study confirms observations from cell culture studies conducted by our research group that implicate ziram in the pathology of PD [8] and is the first epidemiologic study that provides strong evidence in a human population that (1) the combination of maneb, ziram, and paraquat confers a greater risk of PD than exposure to these individual chemicals alone, suggesting the pesticides that affect different mechanisms leading to dopaminergic cell death may act together to increase the risk of PD; (2) exposure to ziram and paraquat increases the risk of PD independent of combined exposures to maneb and paraquat; and (3) ambient exposure derived from workplaces is associated with a greater risk for developing PD than ambient exposure at residences and those exposed at both workplaces and residences experience the greatest PD risk.

Acknowledgments This work was supported by National Institute of Environmental Health Science [grant numbers ES10544, U54ES12078, 5P30 ES07048], National Institute of Neurological Disorders and Stroke [grant number NS 038367], and Department of Defense Prostate Cancer Research Program [grant number 051037]; in addition, initial pilot funding was provided by the American Parkinson's Disease Association.

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Productie 19

Original Investigation

Elevated Serum Pesticide Levels and Risk for Alzheimer Disease

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Editorial page 273

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IMPORTANCE The causes of late-onset Alzheimer disease (AD) are not yet understood but likely include a combination of genetic, environmental, and lifestyle factors. Limited epidemiological studies suggest that occupational pesticide exposures are associated with AD. Previously, we reported that serum levels of dichlorodiphenyldichloroethylene (DDE), the metabolite of the pesticide dichlorodiphenyltrichloroethane (DDT), were elevated in a small number of patients with AD (n=20).

OBJECTIVE To evaluate the association between serum levels of DDE and AD and whether the apolipoprotein E (*APOE*) genotype modifies the association.

DESIGN, SETTING, AND PARTICIPANTS A case-control study consisting of existing samples from patients with AD and control participants from the Emory University Alzheimer's Disease Research Center and the University of Texas Southwestern Medical School's Alzheimer's Disease Center. Serum levels of DDE were measured in 79 control and 86 AD cases.

MAIN OUTCOMES AND MEASURES Serum DDE levels, AD diagnosis, severity of AD measured by the Mini-Mental State Examination score, and interaction with *APOE4* status.

RESULTS Levels of DDE were 3.8-fold higher in the serum of those with AD (mean [SEM], 2.64 [0.35] ng/mg cholesterol) when compared with control participants (mean [SEM], 0.69 [0.1] ng/mg cholesterol; $P < .001$). The highest tertile of DDE levels was associated with an odds ratio of 4.18 for increased risk for AD (95% CI, 2.54-5.82; $P < .001$) and lower Mini-Mental State Examination scores (−1.605; range, −3.095 to −0.114; $P < .0001$). The Mini-Mental State Examination scores in the highest tertile of DDE were −1.753 points lower in the subpopulation carrying an *APOE* ε4 allele compared with those carrying an *APOE* ε3 allele (P interaction = .04). Serum levels of DDE were highly correlated with brain levels of DDE ($\rho = 0.95$). Exposure of human neuroblastoma cells to DDT or DDE increased levels of amyloid precursor protein.

CONCLUSIONS AND RELEVANCE Elevated serum DDE levels are associated with an increased risk for AD and carriers of an *APOE4* ε4 allele may be more susceptible to the effects of DDE. Both DDT and DDE increase amyloid precursor protein levels, providing mechanistic plausibility for the association of DDE exposure with AD. Identifying people who have elevated levels of DDE and carry an *APOE* ε4 allele may lead to early identification of some cases of AD.

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JAMA Neurol. 2014;71(3):284-290. doi:10.1001/jamaneurol.2013.6030
Published online January 27, 2014.

Alzheimer disease (AD) is the most common neurodegenerative disease worldwide and cases are expected to increase 3-fold over the next 40 years.[1] The most common form of AD is the late-onset form, which typically develops after 60 years of age. The etiological factors of late-onset AD are not yet completely understood but include genetic, environmental, and lifestyle factors that influence a person's risk for developing the disease.[2] Although there is a growing list of AD susceptibility genes, only having an apolipoprotein E4 (*APOE4*) allele has a relatively strong effect (relative risk approximately 2-3), and, cumulatively, the more than 10 genes identified thus far account for only less than half of AD cases.[3] To our knowledge, few studies have explored the potential of environmental exposures to contribute to AD, but occupational exposure to metals, solvents, and pesticide is reported to be a potential environmental contributor.[4,5] Previously, we reported that serum levels of p,p'-dichlorodiphenyldichloroethylene (DDE), a metabolite of the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT), were significantly higher in a small cohort (n = 20) of patients with AD compared with control participants, and that there was a significant association between DDE levels and a diagnosis of AD.[6] In the present study, we evaluated the associations between serum DDE levels, AD, and Mini-Mental State Examination (MMSE) scores in a larger number of cases and control participants from 2 geographical sites, and we explored differential susceptibility by *APOE4* genotype status. We also examined the relationship between brain and serum levels of DDE and whether DDT or DDE alters the expression of the amyloid precursor protein (APP) in cultured neuronal cells.

Methods

Study Population

Existing serum samples were obtained from control participants and patients with AD who were evaluated in the Alzheimer's Disease Research Centers at the University of Texas Southwestern Medical Center (UTSW) and Emory University between 2002 and 2008. Participants who provided samples were diagnosed and assigned to AD or normal control groups based on consensus diagnosis. Normal control participants were determined to have normal neurological/clinical examinations and neuropsychological functioning findings on standardized testing. The inclusion criteria were as follows: (1) MMSE score of 28 to 30 for the control participants, (2) no structural brain abnormalities indicated by magnetic resonance imaging; and/or (3) normal general neurological examination; and (4) normal Consortium to Establish a Registry for Alzheimer's Disease battery results. Patients with AD were diagnosed by applying National Institute of Neurological and Communicative Disorders-Alzheimer's Disease and Related Disorders Association criteria for probable AD based on neurological and neuropsychological examination results, brain imaging, and laboratory assessments to rule out other causes of dementia at both UTSW and Emory University. Blood samples for serum preparation and genotyping were generally taken at enrollment along with MMSE. All participants had *APOE* genotype determined by standard TaqMan polymerase chain reaction.

Data from 43 control samples and 20 AD samples from UTSW reported in our first study were included in this analysis.[6] An additional 11 control and 41 AD samples were provided by UTSW, and 25 control and 25 AD samples were provided by Emory University. Serum samples were randomly selected from existing samples collected between 2002 and 2008, and an attempt was made to match these based on age, sex, and race/ethnicity.

Matched brain and serum samples of patients with AD were obtained from the Alzheimer's Disease Center at Washington University to determine whether serum levels of DDE correlated with brain levels. All samples (n = 11; average age = 85.7 years) were from patients diagnosed as having AD by National Institute of Neurological and Communicative Disorders-Alzheimer's Disease and Related Disorders Association criteria and verified histopathologically following death. Blood samples were taken an average of 193 days before death and brain samples were obtained after an average postmortem interval of 12 hours.

The institutional review boards of UTSW, Washington University, Emory University, and the Robert Wood Johnson Medical School approved all of the protocols and procedures. All participants reviewed and signed written approved informed consent documents.

Assessment of Pesticide Levels

Serum DDE levels were determined by gas chromatography/mass spectrometry, as described previously,[6,7] and expressed in terms of free cholesterol levels. The limit of detection for DDE was approximately 100 pg/mL. For brain pesticide determination, approximately 150 mg of temporal cortex was sonicated in a 1:1 mixture of acetone and hexane containing 5 µL of an internal standard (4-4'-DDT ^{13}C, 1 mg/mL). Following an overnight incubation, the sample was centrifuged for 10 minutes at 3500 rpm and supernatant removed. The extraction procedure was repeated 4 times and the extract reduced by evaporation under nitrogen. The dried residue was reconstituted in acetone:hexane and applied to a solid-phase extraction column containing 5 g of Florisil and 1.5 g of anhydrous sodium sulfate preconditioned with 8 mL of hexane. Analytes were eluted with methyl tert-butyl ether, evaporated to dryness, and reconstituted in 1.8 mL of hexane for GC/MS analysis, as described previously.[6,7]

In Vitro Exposure to DDT/DDE and Analysis of APP Levels

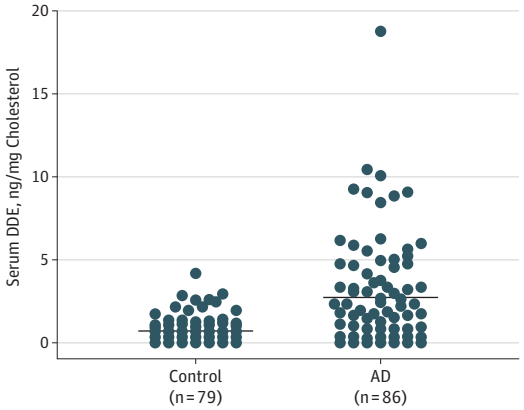
SH-SY5Y cells (ATCC) were differentiated by reducing serum concentration to 1% and the addition of 1 µM retinoic acid to culture media. Cells were then exposed to DDE or DDT for 48 hours, washed with phosphate-buffered saline, and fixed in 4% paraformaldehyde. Cells were incubated with anti-APP (Sigma Aldrich) and MAP2 (Millipore) primary antibodies, followed by species-appropriate fluorescently labeled secondary antibodies (Jackson Laboratories). Images were captured on a Zeiss Observer D1 microscope (Zeiss Inc) with an X-Cite series 120Q fluorescent illuminator and a Jenoptik camera with ProgRes CapturePro 2.8 software (Jenoptik). Optical density per intensity of fluorescence against APP stain was quanti-

Table 1. Description of the Study Population

Characteristic	Control (n = 79)	AD (n = 86)
Age, mean (SD), y	70.2 (8.8)	74.1 (8.4)
Sex, No. (%)		
Female	47 (59.5)	47 (54.7)
Male	32 (40.5)	39 (45.3)
Race/ethnicity, No. (%)		
White	69 (87.3)	79 (91.9)
African American	10 (12.7)	7 (8.1)
Family history, No. (%)	30 (38.0)	42 (48.8)
Education, mean (SD), y	15.6 (2.4)	14 (3.5)
MMSE score, mean (SD)	28.9 (1.7)	18.9 (8.1)
APOE, No. (%)		
ε4 positive	28 (35.4)	56 (65.1)
ε4 negative	51 (64.6)	30 (34.9)
Site, No. (%)		
UTSW	54 (68.4)	61 (70.9)
Emory	25 (31.6)	25 (29.1)
DDE nondetects	24 (30.4)	17 (19.8)

Abbreviations: AD, Alzheimer disease; APOE, apolipoprotein E; DDE, dichlorodiphenyldichloroethylene; MMSE, Mini-Mental State Examination; UTSW, University of Texas Southwestern Medical Center.

Figure 1. Serum Levels of Dichlorodiphenyldichloroethylene (DDE)



Serum levels of DDE are elevated in Alzheimer disease (AD). Data were pooled from University of Texas Southwestern Medical Center and Emory University. Levels of DDE are significantly higher in patients with AD (mean [SEM], 2.64 [0.35]) vs control participants (mean [SEM], 0.69 [0.10]; $P < .001$).

fied in individual cells using Image-Pro Plus 7.0 software (Media Cybernetics Inc). Data were calculated as mean (SEM) density/intensity from 3 individual experiments, each performed in triplicate, and data calculated as the percentage of control.

Statistical Analyses

All analyses were conducted with SAS software version 9 (SAS Institute Inc) or Stata version 12. We used nonparametric analysis of variance (Kruskal-Wallis) for bivariate analysis to explore the association between DDE, AD, and other covariates. Correlations between serum and brain levels of DDE were examined using the Pearson correlation coefficient.

Unconditional logistic regression, controlling for age, sex, and location, was used to estimate odds ratios (ORs) and their 95% CIs for the association between serum DDE levels and AD diagnosis in the UTSW and Emory study locations. Generalized estimating equations were used to determine the odds of having AD or decrease in MMSE score per tertile of DDE level in the full study population, controlling for age, sex, race/ethnicity, education, and *APOE* genotype and accounting for location. Confounders were selected on the basis of biological plausibility and 10% change in effect estimate. For samples with nondetectable levels of DDE (n=46), we imputed a value equal to half the limit of detection (0.075 ng/mL), as described by Lubin and colleagues[8] and corrected for cholesterol levels. Regression analysis including the nondetects as zero value did not significantly change the OR estimate. To explore whether the presence of an ε4 allele of *APOE* modified the association between DDE levels and MMSE scores, we either stratified the data by genotype or used an interaction model (DDE***APOE4*) with generalized estimating equations.

Results

Baseline characteristics of the cohort are shown in **Table 1**. There were a total of 165 samples representing 79 control and 86 AD cases. The cohort comprised 94 women and 71 men, with women comprising 60% of the control and 55% of the AD cases. The presence of at least 1 *APOE4* allele was found in 35% of control and 65% of AD cases.

Dichlorodiphenyldichloroethylene (DDE) was detected in 70% of control and 80% of AD cases (Table 1), with mean levels 3.8-fold higher in the serum of AD cases (mean [SEM], 2.64 [0.35] ng/mg cholesterol) compared with control participants (mean [SEM], 0.69 [0.1] ng/mg cholesterol; $P < .001$; **Figure 1**). No other organochlorine pesticide besides DDE was found to be elevated in AD samples compared with control participants (data not shown). The association between serum DDE levels and AD is presented in **Table 2**. Levels of DDE were divided into tertiles, with the nondetects designated at half the limit of detection, and the OR was estimated using generalized estimating equations and corrected for age, sex, race/ethnicity, and location. Compared with the first tertile, the OR for AD diagnosis in the third tertile of DDE level was significantly increased (OR, 4.18; 95% CI, 2.54-5.82; $P < .0001$). The presence of an *APOE* ε4 allele alone was associated with increased AD diagnosis (OR, 3.70; 95% CI, 2.97-4.60; $P < .0001$). However, adjustment for *APOE* genotype did not significantly alter the association between DDE levels and AD diagnosis (Table 2). Furthermore, DDE levels did not differ based on *APOE* genotype (data not shown). To explore the potential influence of nondetects of DDE on AD diagnosis, we performed a sensitivity analysis by comparing the highest tertile of DDE against the nondetects and comparing the highest tertile against the lowest tertile when nondetects were excluded. Similar ORs to our original analysis were observed (eTable 1 in Supplement). Likewise, similar ORs were ob-

Table 2. Odds of AD per Tertile of DDE Distribution

Variable	Serum DDE Level, ng/mg Cholesterol/Tertile of Distribution			*P* Value[a]
	0.09-0.26	0.27-1.64	1.66-18.75	
Odds (95% CI) of AD diagnosis (n = 160)				
Adjusted for age, sex, race/ethnicity, and location	1 [Reference]	0.70 (0.19-2.55)	4.18 (2.54-5.82)	<.001
Adjusted for age, sex, race/ethnicity, location, and covariates[b]	1 [Reference]	0.54 (0.13-2.18)	3.40 (1.70-6.82)	<.001

Abbreviations: AD, Alzheimer disease; DDE, dichlorodiphenyldichloroethylene.

[a] *P* value is for the third tertile compared with the first.

[b] Covariates include education and apolipoprotein E status.

Table 3. *APOE4* Polymorphism Modifies the Association Between DDE and MMSE Scores[a]

MMSE	β (95% CI)	*P* Value	*P* Value for Interaction
Independent effects in main effects model			
DDE (3rd tertile vs 1st tertile)	−0.84 (−1.60 to −0.08)	.03	
APOE4	−3.56 (−4.59 to −2.54)	<.0001	
Effect of DDE by *APOE* genotype-stratified model			
APOE4	−1.70 (−3.29 to −0.11)	.04	
APOE2/E3	−0.53 (−0.62 to −0.43)	<.0001	.04
Interaction model			
APOE4	−1.80 (−2.30 to −1.28)	<.0001	
APOE2/3	−1.75 (−3.40 to −0.11)	.04	

Abbreviations: APOE, apolipoprotein E; DDE, dichlorodiphenyldichloroethylene; MMSE, Mini-Mental State Examination.

[a] Controlling for age, sex, race/ethnicity, education, and location in the models.

served when the nondetects were assigned a value of zero (OR, 3.60; 95% CI, 1.23-10.57; $P < .001$).

Mini-Mental State Examination scores were significantly lower in the highest DDE tertile (−0.841; 95% CI, −1.604 to −0.079; $P = .03$) (**Table 3**). Sensitivity analysis demonstrated that excluding nondetects (−1.605; 95% CI, −3.095 to −0.114; $P = .04$) or designating nondetects as zero (−2.628; 95% CI, −5.363 to 0.107; $P = .06$) resulted in similar effect estimates (eTable 2 in Supplement). There was a significant interaction between *APOE* status and DDE levels, where the MMSE score in those with an ε4 allele and DDE levels in the third tertile (OR, −1.6995; 95% CI, −3.293 to −0.106; $P = .04$) was significantly lower (*P* interaction = .04) compared with those without an ε4 allele (OR, −0.5272; 95% CI, −0.623 to −0.432); $P < .0001$). Serum DDE levels did not differ by genotype, suggesting this is a functional interaction.

To determine the relationship between serum and brain levels of DDE, we measured DDE in 11 matched brain and serum samples from patients with AD collected from the Washington University Alzheimer's Disease Center. For these samples, the serum samples were taken within a year before death and collection of brain tissue. Mean (SEM) serum levels of DDE were 2.69 (0.75) ng/mg cholesterol and were not significantly different from and highly correlated with mean (SEM) brain levels (1.89 [0.46] ng/mg cholesterol; ρ = 0.95; eFigure in Supplement).

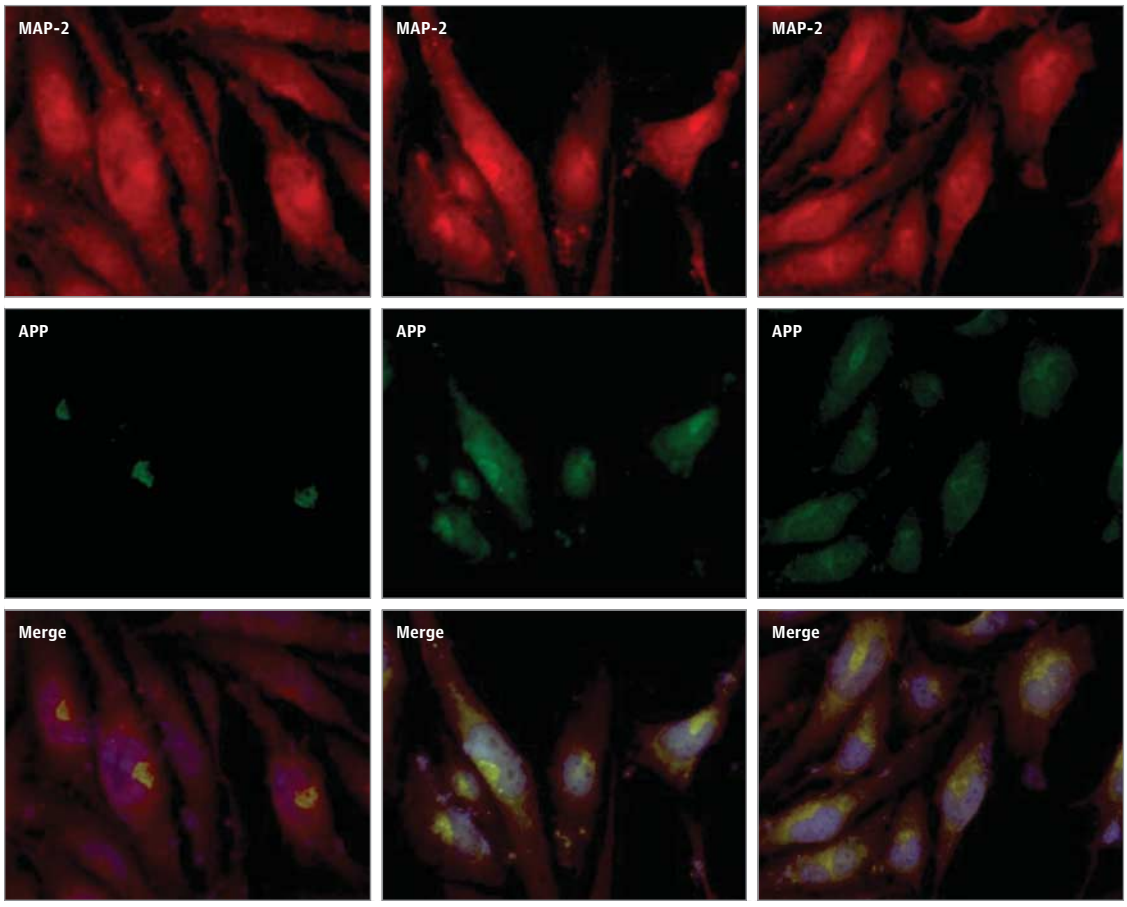
Finally, we sought to determine whether DDE, or its parent compound DDT, has a mechanistic association with AD. A recent study reported that altered network activity in a transgenic AD model, the APP overexpressing mouse, was associated with altered sodium channels.[9] Because sodium channels are the molecular target of DDT and APP overexpression is a causative factor in AD,[10] we hypothesized that DDT or DDE would increase APP levels. To determine this, we exposed cultured neuronal cells and measured the levels of APP, whose genetic overexpression is a risk factor for AD. Exposure of differentiated SY5Y cells to 1-μM DDE or DDT for 48 hours significantly increased APP levels by almost 50% (**Figure 2**).

Discussion

Few studies to date have explored the potential for environmental exposures to contribute to AD. Here, we demonstrated that serum levels of DDE, the metabolite of the organochlorine pesticide DDT, are associated with AD diagnosis and AD severity, as assessed by MMSE. Furthermore, serum DDE levels in the third tertile and the presence of an *APOE* ε4 allele resulted in even greater cognitive impairment. Finally, we demonstrated that concentrations of DDE and its parent compound DDT, similar to those observed in highly exposed individuals in the general population of the United States,[11,12] increased APP levels in cultured neuronal cells. Together, these data identify DDT/DDE exposure as an environmental risk factor for AD.

Dichlorodiphenyltrichloroethane (DDT) was extensively used from the 1940s through 1972 in the United States both in agriculture as a broad-spectrum insecticide and for control of vector-borne diseases including malaria. Although DDT undoubtedly led to major public health victories, the Environmental Protection Agency banned the use of DDT in the United States in 1972 because of concerns regarding its environmental persistence and potential effects on wildlife. At its peak in 1962, production of DDT in the United States was approximately 82 million kg.[13] Currently, several countries around the world continue to use DDT legally and illegally for agricultural purposes, and it is an ingredient in the pesticide Dicofol. Although controversial, the World Health

Figure 2. Dichlorodiphenyltrichloroethane (DDT) and Dichlorodiphenyldichloroethylene (DDE) Increase Levels of Amyloid Precursor Protein (APP) in Differentiated SH-SY5Y Cells



SY5Y cells were differentiated with retinoic acid and exposed to 1-μM DDT or DDE for 48 hours. Cells were fixed and stained with antimicrotubule-associated protein–2 (MAP-2) and anti-APP. Original magnification ×20.

Organization[14,15] supported reintroduction of DDT for malaria eradication in 2006. Thus, there is still significant exposure of human populations.

Levels of DDT and DDE have decreased significantly in the environment over the past 3 decades in the United States. However, DDE is still found in 75% to 80% of serum samples from the Centers for Disease Control and Prevention's cross-sectional National Health and Nutrition Examination Survey.[16] This is likely the result of the exceptionally long half-life of DDE (approximately 8-10 years) and continuing exposure from the import of food from countries where DDT is still used or from legacy contamination of soil and waterways in the United States.[17] The serum DDE concentrations reported here are consistent with those reported by the National Health and Nutrition Examination Survey, with the highest levels in the same range as observed in the 95th percentile.[16] Serum concentrations of DDE are much higher elsewhere in the world where DDT was phased out later or is still used such as Spain and India.[18,19] Importantly, we also found that serum levels are highly correlated with brain levels, which has not been reported before, but is consistent with the high correlation between serum and adipose tissue.[20] Thus, serum levels appear to be an accurate surrogate for DDE levels in the brain.

Although DDT exerts its insecticidal activity through disruption of the nervous system, neither DDT nor DDE are particularly toxic (rat oral LD50s = 113 and 880 mg/kg, respectively).[21] Indeed, administration of DDT or DDE to human individuals for up to 18 months did not cause overt toxicity.[22,23] However, chronic exposure to DDT and DDE has raised concerns about a variety of potential adverse health effects.[13,14,24] Unfortunately, to our knowledge, there are few human studies that have explored the potential neurotoxicity of DDT/DDE. Cueto and colleagues[23] exposed volunteers to 3.5 or 35 mg DDT per day for 12 to 18 months and observed no effects on neurological function. However, 2 other studies found that workers engaged in spraying DDT displayed cognitive dysfunction, although no measurements of DDT or DDE were available for either study.[25,26] Likewise, a large community-based study identified that occupational exposure to organochlorine pesticides was associated with dementia and AD.[5]

One small study reported that DDT was found more often in AD brains (n = 7) compared with control participants (n = 14).[27] Recently, we found an association of serum DDE levels with a diagnosis of AD in a small pilot study, and another study in India found higher serum levels of several organochlorine pesticides, including DDE, in patients with AD.[6,28] Taken together with these studies, our data provide strong support for a role of DDT/DDE in AD. However, we questioned whether this association was mechanistically plausible.

Treatment of SH-SY5Y cells with concentrations of DDT and DDE in the range of concentrations observed in the serum of humans administered 5 to 20 mg of DDT or DDE for 2 to 6 months,[22] in people from an Alabama community with high levels of DDT exposure from industrial dumping of DDT[11] and in people residing in a community near a Superfund site in Maryland,[12] resulted in increased levels of APP, suggesting a possible mechanism for our epidemiological finding.

Our study had a number of strengths including the largest sample size to date for this type of study and the use of well-characterized clinical populations from 2 different geographical locations. The sensitivity analysis ensures that the results were not driven by differences in sampling site or differences in nondetects between cases and control participants. The serum levels of DDE are consistent with those most recently reported by the National Health and Nutrition Examination Survey, suggesting that the cases and control participants were representative of the general population of the United States.[2] Additionally, we provided data demonstrating that serum levels of DDE are highly correlated with brain levels.

There were also limitations to our study. As with our previous study and one other that measured pesticides in the serum of patients with AD, we were limited to studying the persistent organochlorine pesticides.[6,28] Thus, the possibility that other nonpersistent pesticides, such as organophosphates,[5] may contribute to the development of AD in our cohorts cannot be ruled out. A recent study from India found that in addition to DDE, dieldrin and β-hexachlorocyclohexane were elevated in serum samples from patients with AD.[28] However, no detectable levels of dieldrin were found in this study or in more than 200 human serum samples we analyzed in previous studies,[6,7] and β-hexachlorocyclohexane levels significantly decreased in the United States and were not associated with AD in this study.[7] Some patients with AD in our cohort (17 of 86) had nondetectable levels of DDE and control participants were present in the top tertile of DDE levels. This suggests that exposure to DDE may contribute to AD only in a subset of cases, perhaps those with genetic polymorphisms that render them more susceptible to DDT/DDE exposure.

Conclusions

Our findings support epidemiological studies reporting an association of AD with occupational exposure to organochlorine pesticides[5,28,29] and extend them by identifying DDT/DDE as a specific organochlorine pesticide linked to AD in a clinical population from the United States. Indeed, the OR for the association of elevated serum DDE levels with AD is as high as that for *APOE* and the recently reported *TREM2*.[3,30,31] Because elevated DDE levels were associated with significantly worse MMSE performance and exacerbated by the presence of an *APOE* ε4 allele, measurement of serum DDE levels accompanied by *APOE* genotyping might be a useful clinical measure to identify individuals who may be at increased risk for AD. The finding that DDT and DDE increase APP levels in cells provides a mechanistic plausibility to the association between these exposures and AD. If elevation of APP by DDT and/or DDE is confirmed in animal studies and humans, it may provide an avenue for a targeted treatment of individuals with high levels of DDE, such as beta-site APP-cleaving enzyme inhibitors, to prevent cleavage of elevated APP to amyloid-β 42.

ARTICLE INFORMATION

Accepted for Publication: December 4, 2013.

Published Online: January 27, 2014. doi:10.1001/jamaneurol.2013.6030.

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Author Contributions: Dr Richardson had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Acquisition of data: Richardson, von Stein, Hossain, Buckley, Gearing, Levey, German.
Analysis and interpretation of data: Richardson, Roy, Shalat, Levey, German.
Drafting of the manuscript: Richardson, Roy, Buckley, German.
Critical revision of the manuscript for important intellectual content: Richardson, Roy, Shalat, von Stein, Hossain, Gearing, Levey, German.
Statistical analysis: Richardson, Roy, Shalat.
Obtained funding: Richardson, Levey, German.
Administrative, technical, and material support: von Stein, Hossain, Buckley, Gearing, Levey.
Study supervision: Richardson.

Conflict of Interest Disclosures: None reported.

Funding/Support: This research was supported in part by National Institutes of Health grants P30ES005022, T32ES07148, R21NS072097, R01ES015991, P30AG012300, P50AG05681, and P50AG025688.

Role of the Sponsor: The National Institutes of Health had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Interdiscip Toxicol. 2014; **Vol. 7**(3): 123–133.
doi: 10.2478/intox-2014-0017

interdisciplinary toxicology

ORIGINAL ARTICLE

Younger age at onset of sporadic Parkinson's disease among subjects occupationally exposed to metals and pesticides

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ITX070314A02 • Received: 07 May 2014 • Revised: 13 September 2014 • Accepted: 14 September 2014

ABSTRACT

An earlier age at onset of Parkinson's disease (PD) has been reported to be associated with occupational exposures to manganese and hydrocarbon solvents suggesting that exposure to neurotoxic chemicals may hasten the progression of idiopathic PD. In this study the role of occupational exposure to metals and pesticides in the progression of idiopathic PD was assessed by looking at age at disease onset. The effects of heritable genetic risk factors, which may also influence age at onset, was minimized by including only sporadic cases of PD with no family history of the disease (n=58). Independent samples Student *t*-test revealed that subjects with occupational exposure to metals and/or pesticides (n=36) were significantly (p=0.013) younger than unexposed controls (n=22). These subjects were then divided into three groups [high (n=18), low (n=18), and unexposed (n=22)] to ascertain if duration of exposure further influenced age at onset of PD. One-way ANOVA revealed that subjects in the high exposure group were significantly (p=0.0121) younger (mean age: 50.33 years) than unexposed subjects (mean age: 60.45 years). Subjects were also stratified by exposure type (metals *vs.* pesticides). These results suggest that chronic exposure to metals and pesticides is associated with a younger age at onset of PD among patients with no family history of the disease and that duration of exposure is a factor in the magnitude of this effect.

KEY WORDS: occupational exposure; pesticides; metals; Parkinson's disease; onset; age

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized clinically by tremor, bradykinesia, gait disturbances, cogwheel rigidity, postural instability, hypomimia, hypophonia, and micrographia. The clinical manifestations associated with PD result from the loss of pigmented dopaminergic neurons in the pars compacta of the substantia nigra (Klawans & Cohen, 1970). Although the etiology of PD has not been fully elucidated studies suggest that PD is a multi-factorial disorder, which involves genetic and environmental factors (Tanner & Goldman, 1996; Seidler *et al.*, 1996; Kamel *et al.*, 2014; Tanner *et al.*, 2014; Singh *et al.*, 2014). Although studies have revealed evidence for familial forms of PD a genetic factor has not been identified in the majority of cases. As a result, the possible role of environmental and occupational exposures to neurotoxicants in the development of idiopathic PD has received considerable attention from the medical and public health communities (Ballard *et al.*, 1985; Pezzoli *et al.*, 1995; Tanner & Goldman, 1996; Seidler *et al.*, 1996; Menegon *et al.*, 1998; Savolainen *et al.*, 1998; Smargiassi *et al.*, 1998; Saunders-Pullman *et al.* 1999; Feldman & Ratner, 1999; Priyadarshi *et al.*, 2000; Petrovitch *et al.*, 2002; Dawson & Dawson, 2003).

Environmental factors that have been associated with an increased risk of developing PD include pesticide exposure, rural living, well water consumption, and diet (Rajput *et al.*, 1987; Barbeau, 1987; Calne *et al.* 1987; Liou *et al.*, 1997; Smargiassi *et al.*, 1998; Kuopio *et al.*, 1999; Petrovitch *et al.*, 2002). Despite decades of research, exposure to a specific neurotoxic chemical has never been shown to increase the

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incidence or prevalence of idiopathic PD. Research has however begun to accumulate, suggesting that exposure to neurotoxic chemicals can influence age at onset of PD. Racette *et al.* (2001) found a significantly younger age at onset of PD among manganese-exposed welders. The mean age at onset among these welders was 46 years while the unexposed controls had a mean age at onset of 63 years ($p<0.0001$). A similar study by Pezzoli *et al.* (2000) which looked at hydrocarbon exposure also revealed a younger age at onset among exposed subjects with PD. The neurotoxic substances most frequently encountered by the exposed subjects were acetone, 2-di-methyl-ethyl-ketone, n-hexane and its isomers, cyclo-hexane and its isomers, hepthane and its isomers, ethyl-acetate, isobutylacetate, butyl-acetate, dichloropropane, trichloroethylene, trichloroethane, tetrachloroethylene, freon, toluene, and 1-methoxy-2-propanol. The exposed group had a mean age at onset of 55.2 years (±9.8 years) compared with 58.6 years (±10 years) for unexposed controls. The severity of symptoms was correlated with the duration and the intensity of exposure to hydrocarbons. In addition, the exposed subjects were less responsive to treatment and required a higher mean dosage of levodopa than did the unexposed controls. These findings were interpreted to suggest that hydrocarbon exposure might be involved in the pathogenesis of PD, which does not appear to have a major genetic component. These observations are important because these data provide evidence for a scientifically plausible interaction between a genetic predisposition and environmental risk factors. However, although Pezzoli *et al.* (2000) and Racette *et al.* (2001) have both provided evidence that age at onset of PD is influenced by occupational exposure to chemicals neither of these studies took specific measures to minimize the opportunity for interactions between exposure and known heritable genetic risk factors which may also influence age at onset and thus, it is difficult to interpret these findings in relation to sporadic PD. Although Racette *et al.* (2001) reported that family history of PD was similar among exposed subjects and unexposed controls, over 50% (n=8) of the 15 exposed subjects included this study had a positive family history of PD making the influence of genetics on age at onset in this relatively small sample population particularly difficult to interpret. Pezzoli *et al.* (2000) also reported that family history of PD was similar (approximately 25%) among exposed subjects and controls but again the influence of genetics on these data is difficult to interpret since subjects with a positive family history for PD were not excluded from the study or by stratification during data analysis.

To minimize the influence of genetic factors on age at onset of PD while further elucidating on the role of chemical exposure history we elected to look at age at onset among subjects with no family history of the disease (sporadic PD). It is hoped that this approach will not only reveal effects of exposure that could be masked by dominant genotypes but may also provide valuable insight that will be useful in the development of novel therapeutics that may be more beneficial for treating sporadic PD.

Table 1. Occupations associated with exposures to metal and pesticides. Subjects were asked if they had ever worked in any of the following industries with recognized risk for occupational exposure to metals of pesticides.

Metals	Pesticides
Chemical manufacturing	Farm ranch or orchard
Metal finishing industry	Landscaping
Lumber or wood manufacturing	Forestry, paper mill, or woodworking
Electroplating	Rodent control
Battery manufacturing	Weed control
Glass, stone, or clay manufacturing	Crop dusting
Paper or pulp manufacturing	Pesticide manufacturing
Foundry	Pesticide applicator
Autobody repair	
Smelter	

Methods

Human subjects

Human subjects and controls (n=58) participating in this study were drawn from the clinical population of patients with PD seen at the Movement Disorder Center at the Boston University Medical Center, Boston, Massachusetts. All subjects signed a Human Subjects Committee consent form. All subjects included in this study reported having no family history of PD among their first-degree relatives. These subjects were initially deemed eligible for the GenePD study (Maher *et al.*, 2002) but were later excluded because of a negative family history of PD among their first-degree relatives. No effort was made to include or exclude subjects based on gender or race. The diagnosis of PD was confirmed by a clinical evaluation by a Board Certified Neurologist specializing in movement disorders. Every subject included in this study met the Ward and Gibb diagnostic criteria for selection of subjects for research in PD (Ward & Gibb, 1990; Taylor *et al.*, 1999; Maher *et al.*, 2002) (Table 1).

A trained research assistant interviewed each subject about their occupational exposure to chemicals and recorded the information obtained during the interview on a structured questionnaire form as previously described (Maher *et al.*, 2002). The subject was questioned by the research assistant about whether he or she had worked for more than six months (cumulative exposure) in various industries with a recognized risk for occupational exposure to metals or pesticides. If the subject affirmed having worked in a specific industry, then he or she was asked about his or her history of exposure to specific neurotoxic compounds commonly encountered by workers employed in these industrial settings (Tables 1 and 2).

ISSN: 1337-6853 *(print version)* | 1337-9569 *(electronic version)*

Categorization of exposed cases and unexposed controls

Exposed subjects

Subjects with no family history of PD who reported to have experienced at least six months of occupational exposure to metals and/or pesticides were included as exposed subjects in this study (n=36). Subjects reported their occupational exposure histories to a trained interviewer who recorded specifics about the nature of the exposure circumstances in a narrative note and categorized the subject's exposure circumstances based on the following four objective grouping criteria obtained from the questionnaire form used for the GenePD study (Maher *et al.*, 2002).

1. Less than once a month for less than 10 years.
2. Less than once a month for 10 years or more.
3. Once a month or more for less than 10 years.
4. Once a month or more for 10 years or more.

A maximum number of possible exposure days were calculated for each exposure category using these definitions (Table 3).

To determine if exposure duration influenced age at onset of PD we constructed a two–tiered exposure duration index based on the exposure data obtained by the interviewer. For the purposes of this study a low-level exposure was defined as that which occurred less than once per month for at least six months but for less than 10 years (category 1 from questionnaire). For example, one of the subjects in this category reported "light exposure" to insecticides, herbicides, fungicides, and rodenticides while working with stored grain and agricultural products at age 10. This low level exposure category was deemed the most likely to select for those subject's who had worked briefly either as teenagers or during career changes in occupational settings where there was a risk for occasional exposure to metals and pesticide. Exposure categories 2 though 4 from the questionnaire were defined as high-level exposures. For example, one subject in this category reported working as an autobody repair mechanic for 3 years with exposures to lead more than once per month. This grouping pattern was deemed the best for identifying those subjects who had either worked in occupations where overt recognized exposures occurred relatively frequently (more than once per month) or had worked for more than 10 years in careers that involved the risk for recognized occasional exposures and the potential for unrecognized exposures. The interviewer's narrative notes were used to aid in categorizing the subject's exposure level and in determining the average duration of exposure for subjects in these two groups.

Unexposed controls

Subjects with no family history of PD and no history of occupational exposure to heavy metals and/or pesticides were assigned to the control group (n=22).

Data analysis

Data was analyzed using SPSS software installed on a Macintosh computer (Apple Computer, Cupertino, CA) at 95% confidence intervals (p=0.05). Independent samples Student t-test was used for analysis of the effects of gender and smoking history on age at onset of PD. One-way analysis of variance (ANOVA) was used to ascertain if duration of exposure significantly influenced mean age at onset among the three groups of PD subjects stratified by levels of exposure to neurotoxic chemicals. ANOVA was also used to determine if age at onset of PD among subjects and controls was influence by exposure to metals or pesticides. Post Hoc analysis of ANOVA data were performed using Tukey's HSD and the Least Significant Difference (LSD) tests The Pearson correlation coefficient was used to determine if age at onset of PD was correlated with duration of occupational exposure.

Table 2. Potential occupational chemical exposures in various industries. List of metals and pesticides exposures inquired about and recorded on questionnaire by interviewer. Specific examples of common insecticides, herbicides, fungicides, and rodenticides are provided.

Metals	Pesticides
Mercury	Insecticides
Copper	*Organochlorines (DDT)*
Zinc	*Carbamates (Sevin)*
Manganese	*OPCs (chlorpyrifos)*
Iron	*Pyrethroids*
Magnesium	Herbicides
Lead	*Paraquat*
Other metals	Fungicides
	Maneb
	Rodenticides
	Diphacinone

Table 3. Mathematical calculations showing differences in maximum possible days of occupational exposure for subjects in each category.

Exposure category	Maximum possible exposure *
Category 1	11 days × 9 years = max. exposure = 99 days
Category 2	11 days × 10 years = max. exposure >110 days
Category 3	13 days × 9 years = max. exposure >117 days
Category 4	13 days × 10 years = max. exposure >130 days

*Assumes that subjects exposed less than once per month were not exposed more than 11 times per year and that subjects exposed more than once per month were exposed at least 13 times per year by definition. Likewise, assumes that subjects exposed for less than 10 years were not exposed for more than 9 years and subjects exposed for 10 years or more exposed for a minimum of 10 years by definition.

Results

The mean age at onset of PD among the entire sample population (n=58) studied was 55.4 (range 34 to 80 years;

SD±10.95). Analysis of skewness and kurtosis indicated that the sample population had a normal distribution (data not shown).

The *a priori* hypothesis for this study was that occupational exposure to neurotoxic chemicals such as heavy metals and pesticides influenced age at onset of PD among subjects with no family history of the disease. To ascertain if occupational exposure history influenced age at onset of PD we compared exposed subjects with unexposed controls. The exposed subjects (n=36) were younger with a mean age at onset of PD of 53.09 years (SD±10.29). The unexposed controls (n=22) had a mean age at onset of PD of 60.45 years (SD±10.71). Importantly, independent samples *t*-test revealed that the difference in age at onset of PD in these two groups was significantly different (t=2.5634; df=56; p=0.013) (Figure 1).

To determine if age at onset of PD was further influenced by duration of exposure we stratified our subjects into three groups based on self-reported occupational exposure history. A two-tiered exposure duration index was used to stratify exposed subjects into high and low exposure groups. A low-level exposure was defined as that which occurred less than once per month for at least six months but for less than ten years. A high-level exposure was defined as that which occurred either: A) less than once per month for ten years or more; B) more than once per month for at least six months but for less than ten years; or C) more than once per month for ten years or more. The mean age at onset among subjects in the high-level exposure group which consisted of those subjects who were more likely to have held full-time professional positions working with metals or pesticides for several years or more was 50.33 years (SD±8.75). The mean age at onset among subjects in the low exposure group, which consisted primarily of subjects who briefly worked with metals or pesticides while young adults, was 56 years (SD±11.15). The mean age at onset among unexposed controls was 60.45 years (SD±10.71). The range for ages at onset among the subjects in the high-level exposure group was 34 to 66 years with a median of 50.33 years. This is in contrast to the range of 45 to 80 years with mean age at onset of 60.45 years among the unexposed controls. ANOVA of the age at onset of PD data from subjects stratified into three groups (high, low and unexposed) at 95% confidence intervals using Tukey's HSD for post hoc comparisons revealed a significantly (F=4.7913; p=0.0121) younger age at onset of PD among subjects from the high exposure group suggesting that duration and frequency of exposure influences age at onset of PD (Figure 2). Independent samples *t*-test indicated that age at onset of PD among subjects in the low exposure group did not significantly differ (p=0.207) from that of the unexposed subjects in this study.

There was a significant (r=−0.384; p=0.008) negative correlation between age at onset of PD and duration of occupational exposure (Figure 3). By squaring of the absolute value of the correlation coefficient we derived the coefficient of determination (R^2=0.147) for these data which indicates that only about 15% of the variance in age at onset of PD seen in this sample population can be explained by duration of exposure. This finding indicates that other factors such as magnitude or intensity of exposure to metals and pesticides may have also influenced the younger age at onset seen in this population.

To better understand the influence of pesticide and metal exposures on age at onset of PD we stratified subjects by their pesticide and metal exposure histories. Although few subjects reported exposure to only one chemical, were able to stratify subjects by their primary exposure. Forty-seven percent (17/36) of the occupationally exposed subjects participating in this study reported occupational exposure to pesticides the remaining were

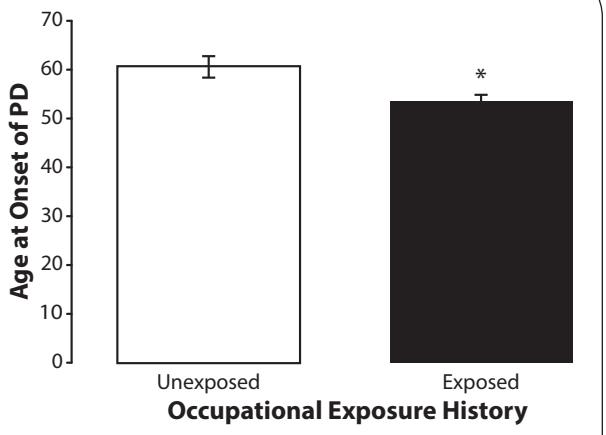

Figure 1. Means and standard errors of the means for age at onset of PD among occupationally exposed (n=36) and unexposed (n=22) subjects. The exposed subjects were significantly younger (t=2.5634; df=56; p=0.013) than the unexposed controls.

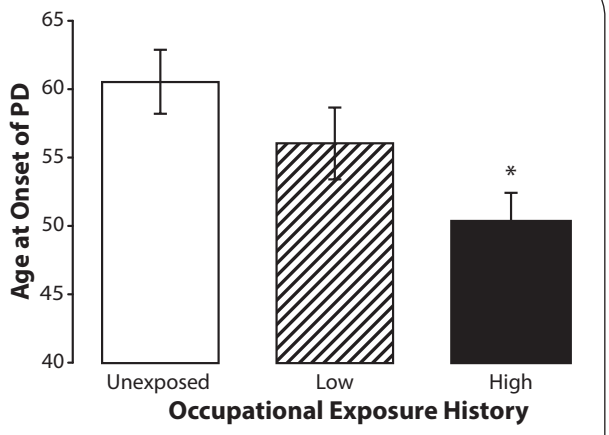

Figure 2. Means and standard errors of the means for age at onset of PD among subjects in high (n=18) and low (n=18) exposure groups and unexposed controls (22). Subjects in the high exposure group were significantly younger (F=4.7913; p=0.0121) than the unexposed controls.

Figure 3. Scatter plot and regression line with confidence bands demonstrating the negative correlation between exposure history and age at onset of PD (r=–0.384; p=0.003).

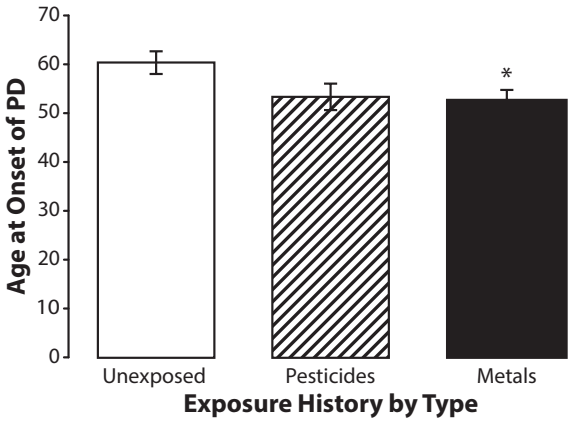

Figure 4. Means and standard errors of the means for age at onset of PD among subjects exposed to metals (n=19), subjects exposed to pesticides (n=17), and unexposed controls (n=22). Post hoc analysis using the Least Significant Different test revealed that subjects exposed to metals were significantly younger (F=3.2848; df=56; p=0.045) than unexposed controls. Subjects exposed to pesticides were younger than the unexposed controls but this strong trend difference did not reach statistical significance (p=0.057).

exposed to metals. ANOVA using the Least Significant Difference (LSD) test for post hoc analysis revealed that subjects who reported occupational exposure to metals had a significantly (p=0.045) younger age at onset of PD (mean age at onset 52.84 years; SD±9.66 years) than the unexposed controls (mean age at onset 60.45 years; SD±10.71 years) (Figure 4). Subjects exposed to primarily to pesticides were younger than unexposed controls with a mean age at onset of 53.53 years (SD 11.23 years) but his difference was not statistically significant (p=0.057). The mean age at onset of PD among subjects exposed to metals and pesticides was very similar and did not approach statistical significance (Figure 5).

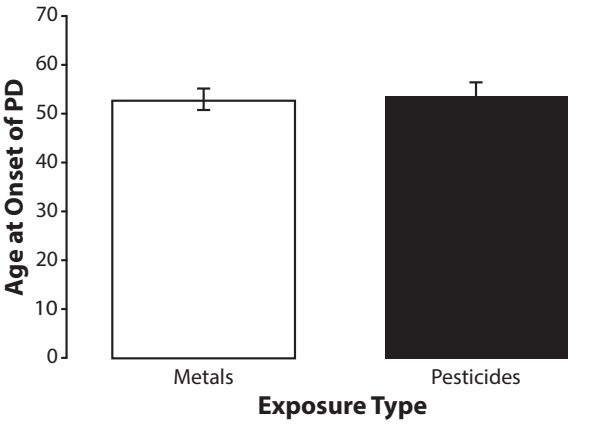

Figure 5. Means and standard errors of the means for age at onset of PD among subjects exposed to metals (n=19) and pesticides (n=17). The difference in mean age at onset of PD among the subjects in these two groups did not even approach statistical significance (t=0.1974; df=34; p=0.845).

To determine if smoking history, which has previously been associated with a decrease in the incidence of PD (Smargiassi *et al.*, 1998; Taylor *et al.*, 1999), had any influence on age at onset of sporadic PD among the subjects participating in this study we stratified subjects by smoking history. None of the subjects in this study currently smoked. Smoking history was similar for unexposed (40%) and exposed subjects (47%). The mean age at onset of PD among former unexposed smokers (n=26) was 59.08 (SD±11.31). The mean age at onset of PD among former exposed smokers (n=32) was 53.38 (SD±10.12). Independent samples t-test revealed that this difference was significant (p=0.048) (Figure 6). Occupationally exposed former smokers (n=17) were also older with mean age at onset of 55.82 years (SD±10.75) than the exposed non-smokers (n=19) who had a mean age at onset of 50.79 years (SD±9.50) but this difference was not statistically significant (p=0.145) (Figure 7). A similar effect was found among unexposed nonsmokers (n=13) who were also younger than unexposed former smokers (n=9) but again this difference did reach statistical significance (p=0.082) (Figure 8). We also found that the percentage of former smokers in the low exposure group was 61% (11/18) which was nearly double the percentage of former smokers in the high exposure group 33% (6/18) suggesting that some of the variance in age at onset of PD among subjects in the high and low exposure groups may have been due to smoking history.

Because the majority of subjects in this study were men we elected to determined if gender had any influence on age at onset PD. The sample population (n=58) included 13 women and 45 men. The mean age at onset of PD among the male subjects (n=45) was 55.49 years

(SD±11.03 years). The mean age at onset of PD among the female subjects (n=13) was 57.46 years (SD±10.98 years). Independent samples *t*-test indicated that gender did not significantly (p=0.572) influence age at onset among the subjects in this sample population (Figure 9).

Eleven of the subjects included in this study reported experiencing a loss of consciousness prior to the onset of their symptoms of PD. The age at onset among those subjects with a positive history for loss of consciousness was 59 years (SD±13.53). By contrast, the mean age at onset among the 45 subjects with a negative history for loss of consciousness was 55.2 years (SD±10.30). These findings indicate that loss of consciousness did not account for the younger age at onset found among the subjects with sporadic PD included in this study.

Discussion

Exposure history

The results of this study suggest that occupational exposure to metals and/or pesticides is associated with a significantly younger age at onset of idiopathic PD among subjects with no family history of the disease among their first-degree relatives. This observation is particularly important because in contrast to previous studies the data presented here indicate that the effect of occupational exposure to chemicals on age at onset of PD is significant even when the influence of heritable genetic factors is minimized by excluding subjects with a positive family history of the disease (Pezzoli *et al.*, 2000; Racette *et al.*, 2001).

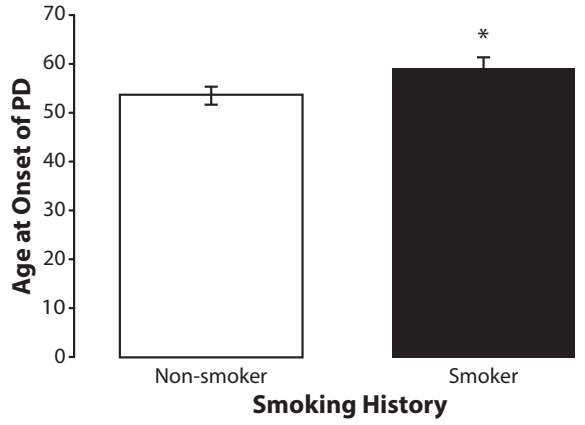

Figure 6. Effects of smoking on age at onset of PD. Means and standard errors of the means for age at onset of PD among former non-smokers and smokers. Former smokers (n=26) are significantly (t=2.0250; df=56; p=0.048) older than non-smokers (n=32).

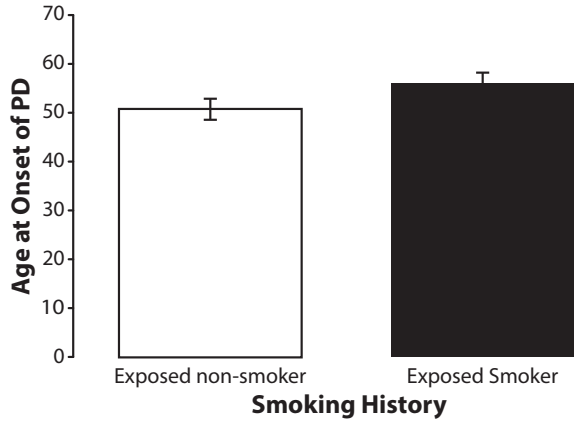

Figure 7. Means and standard errors of the means for age at onset of PD among former occupationally exposed non-smokers and smokers. Although former exposed smokers (n=17) were older at onset of PD than the nonsmokers (n=19), the difference in age at onset of PD was not statistically significant (t=1.4914; df=34; p=0.145).

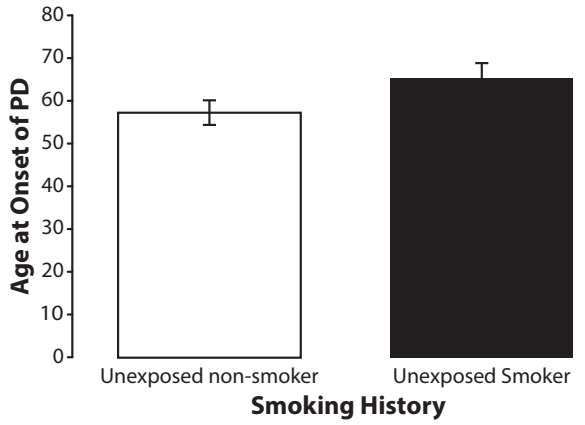

Figure 8. Means and standard errors of the means for age at onset of PD among unexposed non-smokers and smokers. Although the unexposed smokers (n=9) were slightly older at onset of PD than the unexposed nonsmokers (n=13), the difference in age at onset was not statistically significant (t=1.8329; df=20; p=0.082).

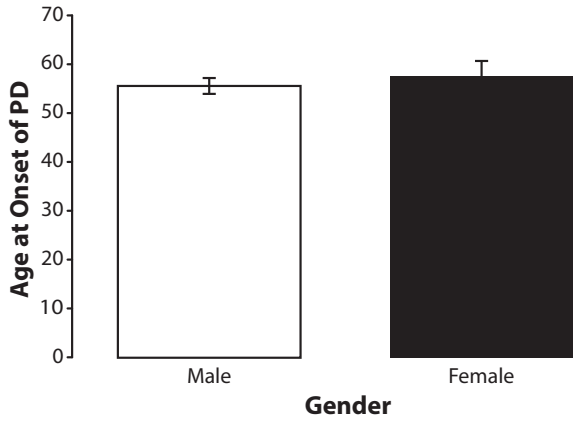

Figure 9. Effects of gender on age at onset of PD. Means and standard errors of means for age at onset of PD male (n=45) and female (n=13) subjects with PD. Although age at onset for the males was slightly younger than that of the females in this study the difference was not significant (t=0.5687; df=56; p=0.572).

To ascertain the effect of duration of exposure on age at onset of sporadic PD we stratified our subjects by duration of occupational exposure. Subjects in the high exposure group, which consisted of those subjects with longest duration of occupational exposure, were found to be significantly younger than the unexposed controls (p=0.0121). Although the subjects in the low exposure group were slightly younger than the unexposed controls, this difference was not statistically significant (p=0.207). Duration of occupational exposure to metals and/or pesticides negatively correlated with age at onset of PD in this study. However, the coefficient of determination indicated that only 15% of the variance in age at onset of PD was associated with exposure duration in this study. This observation indicates that other factors such as intensity or type of exposure and genetic factors that influence detoxification of neurotoxicants may also play an important role in age at onset in exposed subjects with sporadic PD. It is important to note that herbicide exposure has been shown to modify the relationship between glutathione-*S*-transferase pi gene polymorphisms and age at onset in familial PD (Wilk *et al.*, 2006).

Estimating the level of exposure over a lifetime is difficult using historical exposure data obtained via questionnaire because this method does not provide for access to the actual occupational exposure levels necessary to ascertain the influence of exposure intensity on the outcome measured (Fidler *et al.*, 1987). The use of duration of exposure, which inherently ignores intensity of exposure, has its limitations but this parameter has nevertheless been used in many other studies (Hane *et al.*, 1977; Harkonen *et al.*, 1978; Lindstrom & Martelin, 1980; Seppalainen *et al.*, 1980; Iregren, 1982; Olson, 1982). These limitations of duration as the sole parameter are most likely reflected in our data, which suggest that 85% of the variance in age at onset PD seen in this study can be attributed to variables other than duration of exposure. These findings indicate the need for additional research using subjects with well-documented exposure history that will permit stratification by intensity as well as duration of exposure.

Analysis of the influence of exposure type on age at onset of PD in this study was conducted by stratifying our subjects into two groups based on whether they had been exposed primarily to metals or pesticides. Subjects who reported occupational exposure to metals were significantly younger than unexposed controls (p=0.045). Subjects exposed to pesticides were also younger than controls and that although this difference did not reach statistical significance (p=0.057) – there was a very strong trend. Comparison of mean age at onset of PD among subjects exposed to metals with that of subjects exposed to pesticides also failed to reveal a significant difference (p=0.845).

Previous reports suggest that subjects exposed to manganese (mean: 46 years) have a younger mean age at onset than subjects exposed to hydrocarbons (mean: 55.2 years). However, comparing the data sets from these two studies in an attempt to arrive at any conclusions about the effects of a specific chemical on age at onset of PD is difficult since the exposure levels as well the specific type of chemical exposure may have varied considerably (Pezzoli *et al.*, 2000; Racette *et al.*, 2001). Although our findings also suggest that metal exposure may have a greater influence on age at onset than pesticide exposure additional research is nevertheless needed to ascertain how exposure to specific chemicals may differentially influence age at onset of sporadic PD.

Other factors possibly influencing age at onset of PD

Gender

Although men out numbered women in this study, gender did not significantly influence age at onset of sporadic PD among subjects occupationally exposed to metals and/or pesticides. These data in subjects with sporadic PD are also consistent with the findings of Maher *et al.* (2002) who reported no difference in mean age at onset of PD among male and female siblings with PD. However, it should be noted that Maher *et al.* (2002) did find that females were more likely to have a first-degree relative with PD, suggesting that male subjects are more likely to be afflicted with sporadic PD. Since our subjects were derived from the same population this pattern may account for the ratio of males to females in our entire sample population of subjects with sporadic PD. The ratio of exposed males to females seen in this study is also consistent with other studies, which too have reported that men are more likely to have a history of occupational exposure to hydrocarbons and manganese than women (Pezzoli *et al.*, 2000; Racette *et al.*, 2001). More work is needed to determine how gender interacts with exposure history and genetics to influence age at onset of PD among subjects with sporadic and familial PD.

Smoking behavior

The results of this study revealed a significantly (p=0.048) older age at onset of PD among former smokers suggesting that smoking may provide a protective effect against the preclinical progression of sporadic PD. The effect of smoking on age at onset PD was opposite that of occupational exposure to metals and pesticides. Although smoking history was associated with an older age at onset, it most likely did not account for the older age at onset seen among the unexposed subjects in this study because smoking history was similar for the unexposed and exposed subjects. In fact, exposed subjects were actually more likely to have smoked than were unexposed subjects (47% versus 40% respectively) and therefore more likely to have been afforded the protective benefits of smoking.

The reduced risk for PD among smokers is fairly well documented but the effect of smoking on age at onset of sporadic PD is less clear (Smargiassi *et al.*, 1998; Taylor *et al.*, 1999; Hernan *et al.*, 2001; Petrovich *et al.*, 2002; Maher *et al.*, 2002). No association between age at onset and smoking history (smoked versus never smoked) has been observed in the GenePD study suggesting that the protective effect of smoking may greater in sporadic PD than in familial PD (personal communication, Jemma Wilk).

Although inhibition of monoamine oxidase B (MAO B) is suspected to be involved in the reduced risk of PD, the mechanism by which smoking could slow the progression of PD has not been fully elucidated. MAO B polymorphisms such as the G to A transition in intron 13 have been shown to interact with catechol-O-methyltransferase (COMT) polymorphisms to influence relative risk for PD (Wu *et al.*, 2001). However, the MAO B intron 13 polymorphism does not appear to have a major role in the risk for PD, either by itself or by interacting with smoking (Hernan *et al.*, 2002). Additional research is needed to further elucidate on the role of these observations in age at onset of sporadic PD.

Prior head trauma and loss of consciousness

History of loss of consciousness prior to onset of PD was reported among eleven of the subjects included in this study. The age at onset among subjects in this study with a positive history for loss of consciousness was 59 years. By contrast, the mean age at onset among the 45 subjects in this study who reported a negative history for loss of consciousness was 55.2 years indicating that loss of consciousness could not possibly have accounted for the younger age at onset of PD we observed among the occupationally exposed subjects in this study.

The relationship between prior head trauma and/or loss of consciousness and risk for developing PD is unclear. A study by Factor and Weinstein (1991) evaluated the relationship between head trauma, loss of consciousness, and PD among 97 patients with PD. Sixty-four spouses served as controls. Thirty-one PD patients reported head trauma before onset of PD whereas only 11 controls reported head injury before completing the study survey. Twenty PD patients and five controls reported head injury associated with alteration or loss of consciousness. Injury occurred a mean of 37.7 years before onset of PD and 37.2 years before survey completion in the two groups, respectively. No significant differences were found between the two groups after controlling for sex. However, a trend toward significance was observed when examining head trauma with alteration of consciousness.

Other studies have found an association between a younger age at onset of PD among subjects who reported a history of head trauma and/or loss of consciousness prior to disease onset (Taylor *et al.*, 1999). Maher *et al.* (2002) found an association between head trauma and a younger age at onset of PD among a sample of 203 siblings pairs diagnosed with PD suggesting that factors such as head trauma which may contribute to a global loss of neurons throughout the central nervous system (CNS) as well as neuronal loss in the basal ganglia and substantia nigra may influence age at onset of PD.

Studies looking at young onset (<40 years old) PD patients found that head injury and exercise were the significant predictors of risk. Keeping all other variables constant, head injury was a risk factor and exercise appeared to be a protective factor. These findings suggest that head trauma may trigger and expedite the appearance of parkinsonian features, but such acceleration may be prevented through regular exercise (Tsai *et al.*, 2002). More research is needed to determine how occupational exposures to neurotoxic chemicals and prior head trauma or loss of consciousness may interact to influence age at onset of sporadic PD.

Genetics

This research has expanded on the earlier work of Pezzoli *et al.* (2000) and Racette *et al.* (2001). Although Pezzoli *et al.* (2000) and Racette *et al.* (2001) provided evidence that age at onset of PD is influenced by occupational exposure to chemicals both studies failed to specifically control for known heritable genetic risk factors that may influence age at onset of PD by excluding subjects with a family history of the disease.

The influence of genetic factors on age at onset of PD were minimized in this study access the influence of metals and pesticides on age at onset of PD by excluding subjects with a positive family history of the disease among their first-degree relatives. Because genetic factors can influence age at onset of PD as well as lifetime risk for the disease, family history of PD must be considered when interpreting data related to age at onset of the disease. The greater similarity for age at onset than for year at onset among siblings with PD together with an increased risk for the disease among subject's biological relatives compared with subject's spouses stresses the importance of controlling for heritable genetic components (Maher *et al.*, 2002). Although we could not control for all genetic variables in this retrospective study, the results presented here nevertheless suggest that age at onset of PD is influenced by occupational exposure history in the absence of recognized heritable genetic risk factors for the disease.

The genetic factors that were able to control for in this study were those associated with a positive family history of the disease including: the ubiquitin C-terminal hydrolase gene located on chromosome 4p14/PARK5; the α-synuclein gene on chromosome 4q21-23/PARK1; and, the parkin gene located on chromosome 6q25-27/PARK2. α-Synuclein aggregation may be involved in Lewy body formation and in the pathogenesis of autosomal dominant forms of familial PD (Polymeropoulos *et al.*, 1997). The ubiquitin C-terminal hydrolase gene located on chromosome 4p14/PARK5 has been associated with autosomal dominant PD (Leroy *et al.*, 1998).

Unfortunately, even by excluding subjects with a positive family history of PD we could not fully control for the influence the parkin gene (Kitada *et al.*, 1998; Periquet *et al.*, 2003). Parkin mutations have been associated with about 15% of early-onset cases (≤45 years-old) without a family history of PD; this proportion decreased significantly with increasing age at onset (Periquet *et al.*, 2003). Because it is conceivable that some of the subjects with no family history in our sample population might still have the parkin gene mutation, future studies to control for this recessive genetic variable are recommended.

This study relied on retrospective data provided by the subjects about the medical history of their first-degree relatives. The lack of actual medical record reviews to confirm

the diagnoses should be recognized as a potential source of error. However, because this same method for subject selection has been used extensively in the GenePD study we therefore deemed it reliable for the purposes of this study as well (Maher *et al.*, 2002; DeStefano *et al.*, 2002).

Bias

Because the subjects evaluated in this study were selected first on the basis of familial history of PD selection bias was minimized and not based on exposure history per se selection bias was minimized. Occupational exposure history data was ascertained by a blinded interviewer who was not involved in the subject selection process nor in data analysis thereby further minimizing potential for selection bias. Stratification of subjects was performed by the PI but was based on exposure history alone with no regard for age at onset. All data on age at onset was ascertained after subjects were stratified by exposure history.

Conclusions

The results of this study suggest that environmental factors such as occupational exposure to metals and pesticides can influence age at onset of sporadic PD. This study is the first to demonstrate an association between occupational exposures to chemicals while attempting to specifically control for the influences of heritable genetic factors by excluding subjects with a positive family history of PD among first-degree relatives.

The results of this study also suggest that exposure duration is negatively correlated with age at onset of PD. However, because 85% of the variance in age onset of PD could be accounted for by other factors such as intensity of exposure, additional studies are needed to better define this relationship.

The observation that smoking is associated with an older age at onset among subjects with sporadic PD regardless of exposure history is intriguing. Additional research is needed to determine how smoking history differentially influences age at onset among subjects with sporadic and familial forms of PD.

The data from this study along with the previous findings of Racette *et al.* (2001) and Pezzoli *et al.* (2000) suggest that a common mechanism of action may underlie the effects of occupational chemical exposure on age at onset of PD. Because oxidative stress has been associated with PD and many of the chemicals these subjects were potentially exposed to may increase oxidative stress it is conceivable that increased oxidative stress is the common mechanism which may have hastened the progression of PD in these subjects. Future studies could be developed to test this hypothesis by ascertaining how genetic polymorphisms that influence the activity of enzymes such as glutathione-*S*-transferase which are involved in the metabolism and detoxification of neurotoxicants interact with exposure history to influence age at onset of sporadic PD (Offen *et al.*, 1996; Seaton *et al.*, 1997; Shimoda-Matsubayashi *et al.*, 1997; Menegon *et al.*, 1998; Feldman & Ratner, 1999; Dawson & Dawson, 2003). Studies looking at age at onset of PD may help to provide information about the etiology as well as the progression of this disabling disease and may contribute to the development of novel preventative and therapeutic strategies.

Acknowledgements

This work is dedicated to the memory of Robert G. Feldman, M.D. for his unparalleled contributions to understanding, treating, and preventing Parkinson's disease. We would like to thank Genevieve Von Thesling for her assistance with subject intake. We would also like to thank Dr. Jemma Wilk for her assistance with the statistical analysis of the data for this project. This work was supported in part by a grant from the American Parkinson's Disease Association.

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Productie 21

Environment International 107 (2017) 266–273

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Occupational pesticide use and Parkinson's disease in the Parkinson Environment Gene (PEG) study

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ARTICLE INFO

Keywords:
Occupational pesticide use
Parkinson's disease
Case control study

ABSTRACT

Objective: To study the influence of occupational pesticide use on Parkinson's disease (PD) in a population with information on various occupational, residential, and household sources of pesticide exposure.

Methods: In a population-based case control study in Central California, we used structured interviews to collect occupational history details including pesticide use in jobs, duration of use, product names, and personal protective equipment use from 360 PD cases and 827 controls. We linked reported products to California's pesticide product label database and identified pesticide active ingredients and occupational use by chemical class including fungicides, insecticides, and herbicides. Employing unconditional logistic regression, we estimated odds ratios and 95% confidence intervals for PD and occupational pesticide use.

Results: Ever occupational use of carbamates increased risk of PD by 455%, while organophosphorus (OP) and organochlorine (OC) pesticide use doubled risk. PD risk increased 110–211% with ever occupational use of fungicides, herbicides, and insecticides. Using any pesticide occupationally for > 10 years doubled the risk of PD compared with no occupational pesticide use. Surprisingly, we estimated higher risks among those reporting use of personal protective equipment (PPE).

Conclusions: Our findings provide additional evidence that occupational pesticide exposures increase PD risk. This was the case even after controlling for other sources of pesticide exposure. Specifically, risk increased with occupational use of carbamates, OPs, and OCs, as well as of fungicides, herbicides, or insecticides. Interestingly, some types of PPE use may not provide adequate protection during pesticide applications.

1. Introduction[1]

Parkinson's disease (PD) is a chronic and progressive movement disorder. Many previous epidemiologic investigations identified occupational pesticide exposures as risk factors for PD (Brown et al., 2006). Studies reporting associations of PD with occupational exposures to pesticides, herbicides, insecticides, and fungicides, however, are of varying quality, size, and consistency in terms of the agents they examined. Also, some studies assessed exposures rather crudely (ever/never occupational exposure), or employed self-reports only (Brown et al., 2006), with little more than a handful of studies creating job exposure matrixes (JEMs) based on various types of information and levels of detail (Baldi et al., 2003a; Baldi et al., 2003b; Liew et al., 2014; Elbaz et al., 2009; Feldman et al., 2011; van der Mark et al., 2014), and the Agricultural Health Study (AHS) being the only cohort of licensed pesticide applicators and spouses with a prospective design and detailed assessment of pesticide use (Kamel et al., 2007).

In our California population based case control study of PD (Kang et al., 2005; Ritz et al., 2016), we conducted a detailed historical assessment of active occupational use of pesticides and personal protective equipment (PPE) use which we are reporting on for the very first time. Our previous reports relied on extensive information for other sources of pesticide exposure for this population, specifically, household pesticide use and ambient pesticide exposures from agricultural applications at workplaces and residences. Here, we present results for primarily farming-related occupational pesticide use self-reported by participants and complemented by information on chemicals from the California pesticide registration system. Different from

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[1] Abbreviations: PEG, Parkinson Environment Gene study; PD, Parkinson's disease; OP, organophosphorus; OC, organochlorine; PPE, personal protective equipment; HIPAA, Health Insurance Portability and Accountability Act; MMSE, Mini-Mental State Examination; CAPIT, Core Assessment Program for Intracerebral Transplantation; CDPR, California Department of Pesticide Regulation; PAN, Pesticide Action Network; DDT, Dichlorodiphenyltrichloroethane; TEPP, Tetraethyl pyrophosphate; GIS, geographic information system; DTC, dithiocarbamate; PQ, paraquat

http://dx.doi.org/10.1016/j.envint.2017.04.010
Received 7 October 2016; Received in revised form 9 April 2017; Accepted 10 April 2017
Available online 02 August 2017

previous studies in other populations, we are able to adjust for other pesticide exposures (gardening and household use and ambient bystander exposures) common in agricultural environments; also, we are only the second study to examine whether PPE use modifies risk from occupational pesticide use (Kamel et al., 2007; Furlong et al., 2015).

2. Materials and methods

2.1. Study subjects

The Parkinson Environment Gene (PEG) study is a population-based case-control study of Parkinson's disease, with participants recruited from the mostly rural California counties Kern, Fresno, and Tulare. Cases were enrolled within three years of PD diagnosis, from 2001 through 2007, and population controls were enrolled between 2001 and 2011. Descriptions of PD case diagnostic criteria (Kang et al., 2005) and subject recruitment (Wang et al., 2011) can be found in our prior publications.

Briefly, through local neurologists, medical groups, and public service announcements, we identified 1167 PD patients. We excluded 397 diagnosed > 3 years before contact, 134 not living in the target counties, 51 without a PD diagnosis, and 22 who were too ill to participate. Of 563 remaining eligible cases, 90 declined, moved, became too ill or died before we could examine them. We further excluded 107 who did not meet criteria for idiopathic PD at exam (Kang et al., 2005), and six withdrew prior to interview leaving us with 360 patients (78.9% acceptance rate for PD cases).

In the first year of our study, controls 65 years or older were randomly selected from Medicare enrollee lists for all three counties, but after the Health Insurance Portability and Accountability Act (HIPAA) was instated, controls were instead randomly selected from residential parcel listings on tax assessor records. We used two strategies to enroll controls. First, we mailed letters to selected residential units and enrolled through mail and phone only. Using a second strategy, we recruited controls from randomly selected clusters of five neighboring households from parcel listings, and trained field staff conducted home visits to determine eligibility and enrolled controls at the doorstep. Only one eligible person per household was allowed to enroll as a control in our study (Liew et al., 2014).

We have depicted control selection in a flowchart in the supplement to this article (Fig. S1, Supplemental material). Using the first sampling strategy, we contacted 1212 potential controls of whom 457 were ineligible (409 were < 35 years of age, 44 too ill to participate, and 4 lived outside target counties). We recruited 346 controls via phone and mail, since an additional 409 eligible controls declined, became too ill, or moved after screening and prior to interview. An additional 5 controls out of the 346 were missing data, leaving 341 controls with complete data. Through an early mailing, for which the number of eligible subjects who did not respond remains unknown, we recruited and interviewed 62 from among controls randomly selected from residential parcel listings. We screened 4753 individuals for eligibility at their door step and found 3512 to be ineligible (88% due to age criteria), leaving 1241 eligible controls, of whom 634 declined participation and 607 enrolled. Of the 607 controls enrolled through the second sampling strategy, 183 subjects agreed to participate in an abbreviated interview only and did not provide occupational information. Altogether, we have 827 controls available.

This study was approved by the University of California, Los Angeles (UCLA) Institutional Review Board, and we obtained written informed consent from all participants.

2.2. Data collection

Trained interviewers collected information by telephone on demographic characteristics, smoking, household pesticide use, lifetime residential addresses, lifetime occupations and addresses, and screened for jobs with exposures of interest, i.e., fertilizers, pesticides, metals, wood, paint strippers, and solvents. We conducted interviews in the preferred language of the participant and employed bilingual staff.

PD cases (290 out of 360) and controls (619 out of 827), who screened positive in this main telephone interview, i.e., reported (1) ever having worked regularly (i.e., once a week or more) with any one of the agents of interest, or who reported having ever (2) lived on a farm, or (3) worked on a farm, were invited for an additional telephone interview to collect more details on specific occupational exposures. We provide a flowchart in the supplement showing the participation in the detailed occupational interview (Fig. S2, Supplemental material).

Of those who screened positive for fertilizers or pesticide use, or ever working or living on a farm (N = 754), 78.7% (192/244) of cases and 80% (408/510) of controls agreed to participate in the detailed occupational interview. Of the 228 cases and 457 controls who participated in the detailed interview, there are 36 cases and 49 controls who screened positive for using chemicals other than pesticides, i.e., metals, wood, paint strippers and solvents and did not report ever working on a farm or living on a farm. Of these, 3/36 (8.3%) cases and 4/49 (8.2%) controls reported pesticide use in the supplemental occupational interview. Therefore, it is unlikely that those who screened positive for using other chemicals only (metals, wood, paint strippers, and solvents), who also reported not living on a farm and not working on a farm, and refused to participate in occupational interviews (10 cases and 60 controls) would have used pesticides occupationally.

All of our PD patients were seen at least once – many multiple times over a period of 10 years – by our UCLA movement specialists to confirm idiopathic PD according to United Kingdom Brain Bank, Core Assessment Program for Intracerebral Transplantation (CAPIT) rating scale, and Gelb criteria (Kang et al., 2005). We also conducted a Mini-Mental State Examination (MMSE) over the phone or in person, with phone scores converted into predicted in-person scores as recommended (Newkirk et al., 2004).

2.3. Active occupational pesticide use

In this paper, we utilize extensive information from the additional interview in which participants self-reported active occupational pesticide use of fungicides, herbicides, insecticides, and other pesticides (rodenticides, defoliants) including the name of pesticide products used, purpose or site of usage (e.g., crop, plant, animal, insect), at what ages they used pesticides, duration (in years) of use, location of use (Fresno, Kern, or Tulare counties; California; United States or abroad), whether subjects mixed or loaded pesticides, application methods (tractor with/out an enclosed cab, hand sprayer, backpack or aerial application, etc.), and PPE use (gloves, mask, coveralls, boots, goggles, respirator, etc.). In order to reduce subject burden and recall issues, we started our collection of all self-reported pesticide product and usage data (i.e., purpose, age of use, duration, location, job tasks, and PPE) by asking about pesticide groups (fungicides/herbicides/insecticides/other pesticides). We asked, “Have you ever handled fungicides at work?” If the participant responded, “Yes”, we followed with, “What type of fungicide was it?”, “For what purpose did you use a fungicide?”, etc., collecting self-reported fungicidal product names if the participant remembered them. We asked the same series of questions for herbicides, insecticides, and other pesticides.

We identified the main active ingredient of each self-reported pesticide product, relying on the California Department of Pesticide Regulation (CDPR) product label database (California Department of Pesticide Regulation, 2013), which lists the active ingredients of all pesticide products sold on the California market, with over 70% of products having registrations dated 1970 and later. We obtained the main active ingredient (in terms of product weight), by comparing the reported pesticide product name and purpose of use with CDPR database names, purposes (e.g., crop, plant, animal, insect), use types

(e.g., fungicides, herbicides, insecticides), and product registration dates during the years of reported use.

When information on product composition was not available through CDPR (i.e., use prior to 1970), the most probable main active ingredient was identified based on products with the same brand names (e.g., Lannate) and purposes/sites of usage (e.g., cotton, alfalfa). If the chemical composition of a product varied over time, we considered the user as exposed to all main active ingredients the product contained in the period of its use. To identify the chemical classes of the main active ingredients (e.g., dicarboximide, inorganic, amide, etc.), we used the Pesticide Action Network (PAN) pesticide database (Kegley et al., 2000-2016) and the Compendium of Pesticide Common Names (Wood, 1995-2017). When the reported information was inadequate to identify chemical class we still were able to identify pesticide use type (fungicide/insecticide/herbicide/other pesticides).

From the self-reported occupational pesticide use information we derived ever/never use for each main active ingredient. We additionally summarized over the categories of all pesticides, pesticide use types (fungicides, insecticides, herbicides, and other pesticides), and chemical classes (carbamates, organochlorines, organophosphorus). We considered 'ever users' those who used products containing any ingredient within the category prior to the index time (year of diagnosis for cases and year of interview for controls). Carbamates we identified in reported products include aldicarb, carbaryl, methomyl, benomyl, and propoxur. Organochlorines include DDT, chlordane, dicofol, lindane, toxaphene, aldrin, dieldrin, chlorothalonil, dicofol, and methoxychlor. Organophosphorus pesticides include chlorpyrifos, diazinon, dimethoate, malathion, methyl parathion, parathion, phorate, acephate, demeton, bensulide, TEPP, phosmet, mevinphos, tribufos, disulfoton, naled, methamidophos, and ethion.

Since our screening process for the additional occupational interview included other chemical exposures (i.e., metals, wood, paint strippers, and solvents), participants screening positive did not necessarily use pesticides occupationally. We considered subjects who screened negative (68 cases, 201 controls), who refused to participate in the occupational interview (60 cases, 159 controls), or who participated but did not provide responses to questions about pesticide use (15 cases, 15 controls) as never occupational pesticide users if during screening they reported no regular work (i.e., once a week or more) with fertilizers or pesticides. Participants lacking information on occupational pesticide use from either the screening question or additional interview (7 cases, 13 controls) were excluded from analyses of ever versus never occupational pesticide use (Fig. S2, Supplemental material).

Different from the ambient workplace pesticide exposure assessments described in Section 2.4, we did not utilize occupational addresses to infer active occupational pesticide use.

2.4. Ambient pesticide exposures

A geographic information system (GIS) was used to obtain estimates of ambient workplace and ambient residential pesticide exposures prior to the index date. The lifetime workplace and residential addresses for the period 1974–1999 were geocoded and combined with data on pesticide use records from CDPR and land use maps from the California Department of Water Resources (Wang et al., 2011). We estimated the pounds per acre per year of pesticides applied within a 500 meter radius surrounding each address. We then summed the exposures over the years in the 26-year period during which participants worked or lived in California and calculated 26-year average exposures. Participants who were missing a workplace or residential address were considered unexposed during that time. Median 26-year averages for exposures in controls were calculated separately for each individual pesticide at residences and workplaces. Those subjects with exposure at workplace or residential addresses greater than or equal to the median 26-year average exposure in exposed controls for four types of pesticides (organochlorines (OC), organophosphorus (OP), dithiocarbamates (DTC) and paraquat (PQ)) were assigned a value of 1 for workplace or residential exposure, respectively. For example, a participant assigned a 1 for ambient residential pesticide exposures, was residentially exposed at or above the median level (in exposed controls) to one or more of the pesticides among the four types we assessed. Those with exposures at workplace or residential addresses below the median 26-year average exposure in exposed controls for all four types of pesticides were considered unexposed and assigned a 0 for workplace or residential exposure, respectively.

2.5. Active household pesticide use

We previously created a measure of household pesticide use frequency (Narayan et al., 2013), identifying main active ingredients of reported home and garden use pesticide products from the CDPR product label database in the manner described for occupational products. We calculated the lifetime average frequency of any household pesticide use (personal application indoors or outdoors in yards, on lawns, or in gardens) prior to index age, considering use at or above the median value in exposed controls 'frequent use' and use below the median 'never/infrequent use'.

2.6. Statistical analyses

We calculated odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression for ever occupational use of any pesticide, pesticide use types (i.e., fungicides/insecticides/herbicides/other pesticides), and exposure to specific chemicals and classes. To allow for comparison to prior studies on occupational pesticide exposures, we used a reference group of never occupational pesticide users, which included participants with other sources of pesticide exposures (i.e., frequent household pesticide use and/or ambient pesticide exposures). We report on chemical classes with at least 5 exposed cases and 5 exposed controls for analyses and specifically examined carbamates, OPs, and OCs. We conducted analyses of self-reported duration of work with pesticides in years, examining those with 1) > 0 and ≤ 10 years and 2) > 10 years of work with pesticides, and calculating a p-trend based on the median of each category. We also analyzed household pesticide use frequency, ambient residential and workplace exposures to pesticides, use of protective measures during work with pesticides (i.e., use of any PPE/tractor with enclosed cab [yes/no], type of PPE used, frequency of PPE use, tractor with enclosed cab use [yes/no]), and job tasks of mixing/loading or applying pesticides at work.

We adjusted analyses for sex, smoking (ever/never), age at index date (continuous), education ($<$ 12 years, 12 years, and $>$ 12 years), and race (white/non-white). In separate sensitivity analyses, we additionally adjusted for PD family history (yes/no), MMSE scores, other types of exposures (includes regular, i.e., once a week or more, work with metals, wood, chemical solvents, or paint strippers), estimated associations for males only, excluded controls who were interviewed later than cases (i.e., between 2009 and 2011), excluded the 62 controls from an unknown base population, and excluded participants with low MMSE scores ($<$ 27).

We used two methods to address co-exposures of different types of pesticides from various exposure sources. First, when estimating the effect of occupational pesticide use we adjusted for other sources of pesticide exposure (frequent household use, ambient workplace, ambient residential) or mutually adjusted for occupational use (yes/no) of other types of pesticides (i.e., OPs, OCs, DTCs, paraquat, rotenone, carbamates, triflumizole, captan, and propargite, pesticides for which we have previously seen associations of ambient exposures or frequent household use with PD (Ritz et al., 2016; Wang et al., 2011; Narayan et al., 2013)). Second, we analyzed our data after creating alternative exposure categories combining the pesticide exposure measures from

Table 1
Characteristics of participants.

	Cases (360) n (%)	Controls (827) n (%)
Age		
Mean (SD)	68.3 (10.2)	66 (11.7)
≤ 60 years	76 (21.1)	264 (31.9)
> 60 years	284 (78.9)	563 (68.1)
Range	34–88	35–99
Sex		
Male	206 (57.2)	382 (46.2)
Female	154 (42.8)	445 (53.8)
Race[a]		
White	290 (80.6)	569 (68.8)
Black	3 (0.83)	28 (3.4)
Latino	47 (13.1)	160 (19.4)
Asian	4 (1.1)	25 (3)
Native American	16 (4.4)	43 (5.2)
Education		
< 12 years	67 (18.6)	123 (14.9)
12 years	96 (26.7)	172 (20.8)
> 12 years	197 (54.7)	532 (64.3)
Family history of PD		
Positive	53 (14.7)	65 (7.9)
Negative	307 (85.3)	762 (92.1)
Smoking status		
Never	188 (52.2)	400 (48.4)
Former	152 (42.2)	333 (40.3)
Current	20 (5.6)	94 (11.4)

[a] There were 2 controls for whom we were missing information on race.

different sources. Participants in the reference category for this analysis 1) did not use pesticides occupationally, 2) were unexposed to ambient residential and workplace OP, OC, DTC pesticides and paraquat (i.e., exposed below the median of exposed controls), and 3) were never/infrequent users of household pesticides. Of note, this reference group includes individuals with some pesticide exposures i.e., low ambient exposures to pesticides at workplaces or residences or low household pesticide exposures from infrequent use.

All analyses were conducted in SAS version 9.3.

3. Results

The majority of our participants were older than 60 years of age and of European ancestry. Cases were more often male, less educated than controls, and more likely to be never smokers than controls (Table 1). Participants using pesticides for occupational purposes were almost exclusively men (86.7%; versus 13.3% women).

We found frequent household pesticide use, ambient residential exposure to pesticides, and ambient workplace exposure to pesticides each to be associated with PD, increasing PD risk between 46 and 68% (Table 2).

Those with ever active occupational use of any pesticides, fungicides, insecticides, and herbicides had 29 to 89% increased risk for PD (Table 2). On average, cases used pesticides longer than controls, and most effect estimates were much larger for those having used pesticides for > 10 years. Adjusting for other sources of pesticide exposure (i.e., frequent household use, ambient residential and ambient workplace) attenuated our estimates. Concerning pesticide groups, we estimated a strong association for ever use of carbamates (OR = 3.45, 95% CI: 1.19, 10.02) but not for ever OP or OC occupational use (Table S1, Supplemental material).

Active occupational users who also reported using PPE were at increased risk, especially those using gloves, while our data suggested a smaller risk increase for ever pesticide users without PPE, and the highest OR for those always using PPE (Table 3). Among occupational pesticide users, use of gloves, masks, and coveralls were all moderately correlated based on Spearman's rank correlation coefficients (rho values of approximately 0.6, p-values all < 0.0001) (Table S2, Supplemental material). Of those wearing gloves, 72% also wore masks and 60% wore coveralls. Out of the 8 cases and 10 controls who reported using a tractor with an enclosed cab, 7 cases and 9 controls also used personal protective equipment. Use of an enclosed cab was only weakly correlated with use of gloves, masks, and coveralls (Table S2, Supplemental material).

We also saw a positive association for the job tasks of mixing and loading pesticides (OR = 1.62, 95% CI: 1.00, 2.60) (Table S3, Supplemental material).

When we conducted analyses combining different sources of pesticide exposure, we found ORs for PD to be elevated for all categories of occupational pesticide use compared with the reference including never pesticide users having likely low exposures from ambient and household use pesticides (Table 4).

In sensitivity analyses additionally adjusting for PD family history, MMSE scores, or other types of exposures (includes regular, i.e., once a week or more, work with metals, wood, chemical solvents, or paint strippers), results did not change. Associations were also similar when we excluded females, controls who were interviewed later than cases, and the 62 controls from an unknown base population.

4. Discussion and conclusions

Our findings for occupational pesticide use are in agreement with earlier studies showing an increase in PD risk and our own studies of increased PD risk with ambient workplace exposures to OP pesticides, dieldrin, and benomyl (Ritz et al., 2016). Our results are also consistent with expectations in terms of duration of exposure such that longer years of use were associated with higher risk, and the highest risks were estimated for job activities (mixing/loading) known to result in particularly high exposures (Rutz and Krieger, 1992). Interestingly, those who reported PPE use, especially always use of PPE and use of gloves, were at highest risk of PD, possibly because these farm workers felt compelled to use PPE when handling toxic pesticides; however, the types of PPE they used may have failed to protect them adequately. Different from previous studies, we also adjust our estimates for other sources of pesticide exposure in addition to all major confounding variables.

Toxicologic studies in animals, cells, and in vitro experiments with pesticides provided evidence of neurotoxicity in support of the hypothesis that pesticides are involved in PD pathogenesis (Ritz et al., 2016; Sherer et al., 2002; Uversky et al., 2002; Manning-Bog et al., 2002). Mechanisms by which pesticides may be related to PD pathogenesis include oxidative stress and inhibition of mitochondrial complex I (Sherer et al., 2002). Pesticides, including rotenone, DDT, 2,4-dichlorophenoxyacetic acid (2,4-D), dieldrin, diethyldithiocarbamate, paraquat, maneb, trifluralin, parathion, and imidazoldinethione, were found to accelerate the formation of α-synuclein fibrils in vitro (Uversky et al., 2002), and mice exposed to paraquat had increases in brain levels of α-synuclein and α-synuclein containing aggregates in the substantia nigra pars compacta (Manning-Bog et al., 2002). Lab and epidemiologic studies from our group show that benomyl inhibits aldehyde dehydrogenase, which detoxifies the dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL), in mesencephalic rat neurons and inhibits the ubiquitin-proteasome system in SK-N-MC^{u} neuroblastoma cells (Ritz et al., 2016).

Previously, ten cohort studies – six occupational – examined associations between PD and occupational pesticide exposures or work in occupations involving pesticide exposures (Baldi et al., 2003b; Feldman et al., 2011; Kamel et al., 2007; Wastensson et al., 2006; Ascherio et al., 2006; Petrovitch et al., 2002; Hofmann et al., 2006; Kenborg et al., 2012; Tomenson and Campbell, 2011; Tuchsen and Jensen, 2000), and reported relative risk estimates ranging from 0.66 to 5.6. However, since PD is a rare event in all but very large cohorts, these studies relied on as few as 1 and a maximum of 134 exposed

Table 2
OR (95% CI) for self-reported active household pesticide use, ambient residential pesticide exposure, ambient workplace pesticide exposure, self-reported active occupational pesticide use, years of active occupational use, and PD risk.

	Cases (360) n (%)	Controls (827) n (%)	Unadjusted OR	Adjusted[a] OR (95% CI)	Adjusted[b] OR (95% CI)
Household pesticide use[c]					
Never/infrequent users	196 (54.4)	502 (60.7)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Frequent users	161 (44.7)	302 (36.5)	1.37	1.46 (1.13, 1.91)	–
Ambient residential exposure to pesticides[d]					
Exposed below the median	102 (28.3)	312 (37.7)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Exposed at or above the median	258 (71.7)	508 (61.4)	1.55	1.56 (1.18, 2.05)	–
Ambient workplace exposure to pesticides[d]					
Exposed below the median	130 (36.1)	394 (47.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Exposed at or above the median	230 (63.9)	426 (51.5)	1.64	1.68 (1.29, 2.19)	–
Analyses of self-reported active occupational pesticide use and years of use					
No occupational pesticide use[e]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)	1.00 (Ref)
Occupational pesticide users[f]					
Any pesticides					
Ever use	74 (20.6)	114 (13.8)	1.63	1.50 (1.05, 2.14)	1.36 (0.95, 1.95)
Duration of use in years					
Mean (SD)	18.2 (15.4)	13.0 (13.2)	–	–	–
> 0 and ≤ 10	29 (8.1)	55 (6.7)	1.32	1.27 (0.77, 2.09)	1.22 (0.74, 2.02)
> 10	35 (9.7)	40 (4.8)	2.20	1.98 (1.20, 3.28)	1.69 (1.01, 2.83)
p-trend[g]			*0.0009*	*0.0073*	*0.0426*
Pesticide product types[h]					
Fungicides					
Ever use	31 (8.6)	39 (4.7)	2.00	1.89 (1.12, 3.19)	1.62 (0.95, 2.76)
Duration of use in years					
> 0 and ≤ 10	14 (3.9)	18 (2.2)	1.95	1.97 (0.93, 4.17)	1.86 (0.87, 3.95)
> 10	13 (3.6)	16 (1.9)	2.04	1.82 (0.83, 3.97)	1.46 (0.66, 3.23)
p-trend[g]			*0.0363*	*0.0969*	*0.2776*
Insecticides					
Ever use	51 (14.2)	87 (10.5)	1.47	1.29 (0.87, 1.94)	1.15 (0.76, 1.74)
Duration of use in years					
> 0 and ≤ 10	20 (5.6)	41 (5.0)	1.22	1.12 (0.62, 1.99)	1.05 (0.58, 1.90)
> 10	23 (6.4)	29 (3.5)	1.99	1.71 (0.94, 3.10)	1.45 (0.79, 2.65)
p-trend[g]			*0.0146*	*0.0771*	*0.2315*
Herbicides					
Ever use	41 (11.4)	60 (7.3)	1.72	1.51 (0.96, 2.36)	1.34 (0.84, 2.12)
Duration of use in years					
> 0 and ≤ 10	8 (2.2)	31 (3.8)	0.65	0.65 (0.29, 1. 46)	0.59 (0.26, 1.35)
> 10	26 (7.2)	22 (2.7)	2.97	2.41 (1.31, 4.44)	2.07 (1.12, 3.85)
p-trend[g]			*0.0005*	*0.0070*	*0.0290*
Other pesticides (rodenticides, defoliants, etc.)					
Ever use	20 (5.6)	37 (4.5)	1.36	1.37 (0.76, 2.47)	1.27 (0.70, 2.33)
Duration of use in years					
> 0 and ≤ 10	6 (1.7)	18 (2.2)	0.84	0.98 (0.37, 2.59)	1.01 (0.38, 2.69)
> 10	9 (2.5)	8 (1.0)	2.82	2.60 (0.95, 7.12)	2.05 (0.74, 5.69)
p-trend[g]			*0.0536*	*0.0764*	*0.1829*

[a] Adjusted for sex, smoking (ever/never), age (continuous), education (< 12 years, 12 years, and > 12 years), race (white/non-white).
[b] Adjusted for sex, smoking (ever/never), age (continuous), education (< 12 years, 12 years, and > 12 years), race (white/non-white), household pesticide use frequency (frequent vs never/infrequent), and ambient residential and work address pesticide exposures.
[c] In this particular analysis only, the household pesticide use variable is lagged 10 years, i.e., it is defined based on the median value of the lifetime average frequency of any household pesticide use (personal application indoors or outdoors in yards, on lawns, or in gardens) prior to 10 years before the index age, considering use at or above the median value in exposed controls 'frequent use' and use below the median 'never/infrequent use'. We excluded 3 cases and 23 controls missing information on household pesticide use from analyses.
[d] Reference category includes those unexposed to any OP pesticides, organochlorines, dithiocarbamates, and paraquat at or above the median value in exposed controls over the 26 year period. Those considered exposed, had exposure at or above the median value in exposed controls. We excluded 7 controls missing information on ambient residential and workplace exposures to pesticides from analyses.
[e] Reference group for all comparisons in analyses of self-reported active occupational pesticide use. Reference group is composed of self-reported never users of pesticides occupationally. These participants may have other pesticide exposures (such as frequent household pesticide use, ambient residential, and/or ambient workplace pesticide exposures).
[f] We excluded 7 cases and 13 controls missing information on occupational pesticide use from analyses.
[g] Based on median of each category.

incident PD cases. Exposure assessment in these studies was based on self-report, broad occupational categories listed in national databases, and few used employee records (Wastensson et al., 2006; Tomenson and Campbell, 2011) or job-exposure matrices (Baldi et al., 2003b; Feldman et al., 2011). The Agricultural Health Study (Kamel et al., 2007) and a French (PAQUID) study (Baldi et al., 2003b) performed the most detailed exposure assessments, but still only had 68 and 8 exposed PD cases available for analysis, respectively. Some studies collected exposure information only once at baseline, possibly ignoring long periods of exposure during follow-up and prior to diagnosis that might be relevant (Feldman et al., 2011; Ascherio et al., 2006; Petrovitch et al., 2002; Tuchsen and Jensen, 2000). Case control studies enrolling larger numbers of PD cases might have higher diagnostic accuracy if patients are examined by experts, but many were small (< 200 cases) and included prevalent cases with long (> 5 years) or unspecified disease duration (Baldi et al., 2003a; Elbaz et al., 2009; van der Mark et al., 2014; Koller et al., 1990; Semchuk et al., 1992; Hertzman et al., 1994; Chan et al., 1998; McCann et al., 1998; Fall et al., 1999; Baldereschi et al., 2003; Fong et al., 2007; Petersen et al., 2008; Gorell et al., 1998; Kuopio et al., 1999). Few included incident cases

Table 3
OR (95% CI) for self-reported active occupational pesticide use with or without personal protective equipment (PPE) use, tractor with enclosed cab use, and PD risk.

	Cases (360) n (%)	Controls (827) n (%)	Unadjusted OR	Adjusted[a] OR (95%CI)
No occupational pesticide use[b]	279 (77.5)	700 (84.6)	1.00 (Ref)	1.00 (Ref)
Occupational pesticide users				
Used any PPE/enclosed cab[c]				
No	28 (7.8)	49 (5.9)	1.43	1.33 (0.80, 2.20)
Yes	46 (12.8)	65 (7.9)	1.78	1.64 (1.06, 2.53)
Used specific types of PPE				
Gloves				
No	34 (9.4)	61 (7.4)	1.40	1.25 (0.78, 1.99)
Yes	40 (11.1)	53 (6.4)	1.89	1.82 (1.14, 2.90)
Mask				
No	43 (11.9)	68 (8.2)	1.59	1.54 (1.00, 2.37)
Yes	31 (8.6)	46 (5.6)	1.69	1.45 (0.87, 2.41)
Coveralls				
No	50 (13.9)	77 (9.3)	1.63	1.51 (1.01, 2.27)
Yes	24 (6.7)	37 (4.5)	1.63	1.48 (0.84, 2.62)
Used engineering control method: tractor with enclosed cab				
No	66 (18.3)	104 (12.6)	1.59	1.51 (1.05, 2.18)
Yes	8 (2.2)	10 (1.2)	2.01	1.42 (0.53, 3.77)
PPE use frequency[d]				
Never	28 (7.8)	49 (5.9)	1.43	1.33 (0.80, 2.21)
Sometimes	23 (6.4)	39 (4.7)	1.48	1.40 (0.79, 2.45)
Always	20 (5.6)	21 (2.5)	2.39	2.21 (1.14, 4.30)

[a] Adjusted for sex, smoking (ever/never), age (continuous), education (< 12 years, 12 years, and > 12 years), race (white/non-white).
[b] Reference group for all comparisons. Reference group is composed of self-reported never users of pesticides occupationally. These participants may have other pesticide exposures (such as frequent household pesticide use, ambient residential, and/or ambient workplace pesticide exposures).
[c] Here, we include gloves, masks, coveralls, applying pesticides in a tractor with an enclosed cab, and other sorts of protection, such as boots, goggles, etc. For 14 participants who used pesticides, information on PPE use was not available, and we assigned them to the no PPE category; another 9 participants with partially missing information on PPE use were also assigned to the no PPE category, as they reported no use. Of the 8 cases and 10 controls reporting use of a tractor with an enclosed cab, only 1 case and 1 control used a tractor with enclosed cab and no PPE.
[d] The PPE use frequency does not incorporate information on use of a tractor with an enclosed cab. We did not collect frequency of tractor use. Also, 2 cases and 4 controls reported using PPE, but did not provide a frequency of PPE use.

Table 4
OR (95% CI) for self-reported active occupational pesticide use and PD risk. Analyses combine multiple sources[a] of pesticide exposure.

	Cases (360) n (%)	Controls (827) n (%)	Unadjusted OR	Adjusted[b] OR (95%CI)
Low exposure to pesticides[c]	34 (9.4)	134 (16.2)	1.00 (Ref)	1.00 (Ref)
Exposure to other pesticide sources[d]	250 (69.4)	563 (68.1)	1.75	1.89 (1.25, 2.87)
Occupational pesticide users				
Any pesticide use	74 (20.6)	114 (13.8)	2.56	2.50 (1.50, 4.15)
Pesticide product types[e]				
Fungicide use	31 (8.6)	39 (4.7)	3.13	3.11 (1.65, 5.88)
Insecticide use	51 (14.2)	87 (10.5)	2.31	2.10 (1.22, 3.60)
Herbicide use	41 (11.4)	60 (7.3)	2.69	2.45 (1.37, 4.36)
Other pesticide use (rodenticides, defoliants, etc.)	20 (5.6)	37 (4.47)	2.13	2.22 (1.11, 4.44)
Chemical class of main active ingredients				
Carbamate use	10 (2.8)	6 (0.7)	6.57	5.55 (1.81, 17.04)
Organochlorine use	10 (2.8)	17 (2.1)	2.32	1.97 (0.81, 4.82)
Organophosphorus use	16 (4.4)	31 (3.8)	2.03	1.92 (0.92, 4.04)

[a] The multiple sources include self-reported active occupational pesticide use, self-reported active household pesticide use, ambient residential pesticide exposure, and ambient workplace pesticide exposures.
[b] Adjusted for sex, smoking (ever/never), age (continuous), education (< 12 years, 12 years, and > 12 years), race (white/non-white).
[c] Reference group for all comparisons. Reference category participants have low exposure to ambient residential and ambient workplace pesticides (OPs, OCs, DTCs, & paraquat; i.e., exposed below the median of exposed controls), are never/infrequent users of household pesticides, and did not use pesticides occupationally. We excluded 2 cases and 16 controls from analyses who could not be assigned to an exposure category due to missing information on occupational pesticide use, household pesticide use, exposure to ambient residential pesticides, and/or exposure to ambient workplace pesticides.
[d] These participants did not self-report active occupational pesticide use but were exposed to pesticides based on other measures of pesticide exposure (frequent household pesticide use, ambient residential, and/or ambient workplace pesticide exposures).
[e] Note that participants may be counted in multiple sub-categories (e.g., fungicides, insecticides, herbicides, other pesticides, carbamates, organochlorines, organophosphorus pesticides) of pesticide usage.

(Elbaz et al., 2009; Firestone et al., 2010), raising concerns about survivor bias, differential recall due to cognitive impairment in prevalent cases, and temporal ambiguity.

Strengths of our California case control study are that it is to date among the largest in terms of the prevalence of occupational pesticide use among PD cases (21%) and that we enrolled incident PD cases diagnosed by UCLA movement disorder specialists and re-evaluated most patients at multiple follow-up occasions, limiting misclassification of disease status. Our study is one of few that evaluated risk of PD from exposure to specific pesticides and also duration and intensity/type of exposure, only the second study of occupational pesticide use which assessed use of personal protective equipment, and the first that controlled for other sources of pesticide exposures in residents of largely agricultural counties in which few can be considered completely

unexposed. We had to rely on recall for our exposure assessment, which allows for non-differential misclassification bias as well as differential recall bias. Restricting analyses to subjects with high cognitive scores (i.e., MMSE) indicated that our results were not greatly affected by impaired cognition. Our occupational pesticide use variable accounts for active pesticide use only. Other agricultural workers, such as fieldworkers and packers, would only be considered 'ever occupational users of pesticides' if they reported to also have actively used pesticides at work, but not otherwise. Since participants often do not know or remember what active ingredients the product they used contained, we compared reported pesticide brand names, purposes, and dates of use with information in the CDPR database to identify the main active pesticide ingredients in reported pesticide products. We did not account for other active ingredients, which may change often and are therefore difficult to identify, nor did we have information on inert ingredients in pesticide products. Another possible limitation is that our results may be impacted by selection bias if occupational pesticide use were related to participation in our study, since a larger proportion of eligible cases compared with controls participated in our study.

We collected detailed information about PPE use during occupational work with pesticides. Findings from the Agricultural Health Study (AHS) (Kamel et al., 2007) and the Farming and Movement Evaluation (FAME) case-control study nested within the AHS cohort (Furlong et al., 2015) suggested that PPE use in pesticide applicators may reduce PD risk and that protective glove use (chemically resistant rubber gloves, plastic gloves, and rubber gloves) > 50% of the time while mixing and applying pesticides reduced PD risk from use of paraquat and permethrin. A family-based case-control study found PPE use to not alter associations between pesticide use at home and work and PD (Hancock et al., 2008). Our results suggest that PPE use, especially glove use, may not protect against risk but rather may even be a surrogate marker for the use of more toxic pesticides or, alternatively, the PPE participants used may not protect from exposure to the agents handled. Indeed, most of our study participants did not report using highly protective PPE (e.g., respirators, chemically resistant rubber gloves). We may have seen elevated PD risks in glove users, since many did not report using chemically resistant gloves and may have used gloves inadequate for protection from exposure. Other characteristics of gloves, such as their cleanliness and whether they were in good condition, could have affected our PPE results. Perhaps workers using PPE or tractors with enclosed cabs may not have felt the need to change their clothes, wash their hands, or take a bath or shower right after completing their pesticide work. Other job-related behaviors of the pesticide mixers, loaders, and applicators may also be important to consider. Some literature on PPE use in farming is consistent with the idea that glove users may be more exposed to pesticides in general than non-glove users. For example, a large randomized educational study conducted in pesticide applying farmers resulted in an increased use of gloves 6 months post intervention but unfortunately did not succeed in reducing dermal exposures to pesticides during applications (Perry and Layde, 2003). Interestingly, the same research group also compared self-reported pesticide applications and PPE use of 86 farmers with urinary biomarkers for a commonly used herbicide 8 hours after pesticide application; they found that self-reported chemical-resistant glove use was, contrary to what they had hypothesized, positively associated with urinary biomarker levels (Perry et al., 2006). Limitations of the self-reported PPE and tractor with enclosed cab use data in our study include missing information and the possibility that cases may recall PPE use and glove use differently from controls. A survey of almost 2000 California farmers found that those concerned about specific medical problems were more likely to use protective equipment, i.e., risk perception influenced behavior (Schenker et al., 2002). This might suggest reverse causation bias in our study if farmers who later developed PD and were affected by non-motor symptoms that are characteristic of PD during its long (up to 20 years) prodromal phase changed their risk perception and chose to use PPE more often than possibly healthier controls. Additional research targeting PPE use when assessing health risks from chronic pesticide exposures is needed.

Our subjects reported occupational use of 149 different pesticides, with 42% and 40% of exposed cases and controls, respectively, reporting use of more than one pesticide up to a maximum of 29 different pesticides, limiting our ability to estimate effects for single pesticide exposures of interest for PD based on animal, cell, or previous human data. Of cases and controls who reported occupational pesticide use, 35% of cases and 25% of controls did not recall the specific products used. Chemicals that our participants commonly used include DDT, 2,4-D, malathion, and glyphosate, but these have not been previously linked to PD. Our difficulty in interpreting results as pesticide specific is due to co-exposure to multiple pesticides applied simultaneously or sequentially by study participants. When we mutually adjusted for occupational use of other pesticides we previously identified as relevant for PD, estimates for occupational carbamate use remained elevated, but confidence intervals widened (OR = 4.46, 95% CI: 0.66, 30.25). Importantly, in our reference group of never occupational pesticide users, a majority were exposed to other sources of pesticides including household and gardening pesticides or ambient exposures at residences or workplaces from agricultural applications in these counties. Of note, in additional analyses we created an alternate reference group accounting for multiple exposure sources and found even more strongly increased risks with occupational use of carbamates, organochlorines, and organophosphorus pesticides (Table 4). While gene-pesticide interactions are important to explore in relation to PD etiology and to understand whether or not results can be replicated, as we have done previously for ambient pesticide exposure (Ritz et al., 2016), unfortunately, for occupational use of specific pesticides we are lacking statistical power to examine gene-pesticide interactions.

In this population based study of incident PD, we found evidence of increased PD risk with occupational pesticide use, increasing years of pesticide use, and job tasks resulting in the highest exposures to pesticides such as mixing and loading pesticides. We also found some evidence of an association for the specific pesticide groups of carbamates, OPs, and OCs. Finally, personal protective equipment use did not result in reduced PD risk from pesticide exposures at the workplace, and our findings suggest that the equipment, especially gloves, may not protect the applicators sufficiently. However, public health implications for PPE use cannot be determined from this study alone; for this, additional studies with more comprehensive data on PPE use and job-related behaviors are necessary.

Funding

This work was supported by the National Institute of Environmental Health Sciences [ES10544, P01ES016732, U54ES12078, 5P30 ES07048] and pilot funding received from the American Parkinson Disease Association. The funding organizations had no role in the study design, data collection, data analysis, interpretation, writing of the report, or the decision to submit the paper for publication.

Competing interests

Dr. Narayan, Dr. Liew, and Dr. Ritz declare no competing interests. Dr. Bronstein reports grants from the National Institute of Environmental Health Sciences during the conduct of the study.

Ethics approval

This research was reviewed and approved by the UCLA Institutional Review Board. All enrolled participants provided written informed consent.

Acknowledgments

We gratefully acknowledge our study participants and Dr. Myles Cockburn at the University of Southern California for assisting us with our GIS pesticide exposure assessments. This manuscript is based on work published as part of SN's dissertation, referenced directly below, which is available in ProQuest.

Narayan, Shilpa. "An Investigation into Household and Occupational Pesticide Exposures with Genetic Variants as Risk Factors for Parkinson's Disease." Order No. 3680243 University of California, Los Angeles, 2015. Ann Arbor: ProQuest. Web.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2017.04.010.

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Productie 22

Environmental Research 191 (2020) 110186

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Pesticide use and incident Parkinson's disease in a cohort of farmers and their spouses

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ARTICLE INFO

Keywords:
Pesticides
Parkinson's disease
Agricultural health study
Farmers

ABSTRACT

Background: Extensive literature suggests an association between general pesticide use and Parkinson's disease (PD). However, with few exceptions, little is known about associations between specific pesticides and PD.
Objective: We evaluated use of pesticides and incident PD in 38,274 pesticide applicators and 27,836 of their spouses in the Agricultural Health Study cohort followed over 20 years.
Methods: We used self-reported information on ever-use of 50 specific pesticides as of enrollment for both applicators and spouses, and considered intensity-weighted lifetime days (IWLD) reported at enrollment and through the first 5-year follow-up among applicators. We estimated covariate-adjusted hazard ratios (HR) and 95% confidence intervals (CI) using Cox regression. We also examined heterogeneity in associations by history of head injury and chemical resistant glove use.
Results: A total of 373 applicators and 118 spouses self-reported incident doctor-diagnosed PD. Ever-use of the insecticide terbufos (HR:1.31, 95%CI:1.02–1.68) and the herbicides trifluralin (HR:1.29, 95%CI: 0.99–1.70) and 2,4,5-T (HR:1.57, 95%CI:1.21–2.04) was associated with elevated PD risk. On the other hand, diazinon (HR:0.73, 95%CI: 0.58–0.94) and 2,4,5-TP (HR:0.39, 95%CI:0.25–0.62) were associated with reduced risk. We observed heterogeneity in ever-use associations by head injury and chemical-resistant glove use for some pesticides, with higher risk among those who reported a history of head injury, or who did not use gloves. PD risk was also elevated for applicators in the highest category of IWLD for dichlorvos, permethrin (animal use), and benomyl.
Conclusions: We found evidence of increased PD risk for some pesticides. Our results also suggest higher susceptibility for pesticide-associated PD among individuals with head injury as well as protection with use of chemical resistant gloves, although further research is needed to understand the impact of head injury. Research on current and newer pesticides, including mechanisms relevant to PD, is important given widespread pesticide use.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting around 1–2% of adults over the age of 65 years (Hirtz et al., 2007). PD is associated with substantial economic burden (Kowal et al., 2013) which is likely to increase with the aging of the population (US Administration on Aging, 2012). Many pesticides are neurotoxic, and some epidemiologic studies have linked general pesticide use with PD (Goldman et al., 2017; Pezzoli and Cereda, 2013; van der Mark et al., 2012). Although most of these studies evaluated functional (i.e., fungicides, insecticides, and herbicides) or chemical (i.e., organochlorine or organophosphate insecticides) classes, rather than

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https://doi.org/10.1016/j.envres.2020.110186
Received 5 June 2020; Received in revised form 27 August 2020; Accepted 28 August 2020
Available online 10 September 2020
0013-9351/Published by Elsevier Inc.

individual pesticides, some evidence from human and toxicological studies points to associations of PD with the insecticides dieldrin and rotenone and with the herbicides 2,4-dichlorophenoxyacetic acid (2, 4-D) and paraquat (Goldman et al., 2017; Kanthasamy et al., 2005; Tanner et al. 2009, 2011; Weisskopf et al., 2010). Given that some of these and other pesticides continue to be widely used, with annual usage of all pesticides totaling over one billion pounds in the United States (US) alone (Atwood and Paisley-Jones, 2017), identifying links between specific pesticides and PD can have important implications.

The Agricultural Health Study (AHS) is a prospective cohort study of farming populations from North Carolina and Iowa (Alavanja et al., 1996), with follow-up ongoing for over 20 years. Two previous investigations on pesticides and PD were conducted in the AHS. The first included data from the full cohort and examined pesticide exposure data collected at enrollment in relation to self-reported PD through the first study follow-up, approximately 5 years later (Kamel et al., 2007). The second effort, the Farming and Movement Evaluation (FAME) study, was a case-control study nested within the cohort, which assessed PD cases through the first follow-up, but with self-reported PD confirmed by in-person assessment by movement disorder specialists and with collection of additional exposure data for specific pesticides (identified *a priori*) including some not well covered in the original AHS surveys (Tanner et al., 2011). Since then, self-reported incident PD was ascertained in two additional follow-up surveys. A recent update of mortality in the AHS found that pesticide applicators experience higher than expected mortality from PD than the general populations of Iowa and North Carolina, indirectly implicating farming exposures including pesticides (Shrestha et al., 2019a). Therefore, with additional PD cases identified from extended follow-up as well as updated exposure data, we examined associations between individual pesticides and incident PD that occurred over 20 years of follow-up among private pesticide applicators and their spouses.

2. Material and methods

2.1. Study population

The AHS is described in detail elsewhere (Alavanja et al., 1996). In 1993–1997 (Phase 1), 52,394 private pesticide applicators (97.4% male, mainly farmers) completed an enrollment questionnaire at pesticide licensing locations (see Supplemental Fig. 1 for study timeline). A take-home questionnaire requesting additional pesticide use information, was completed by 22,916 (44% of those who enrolled). Applicators were also given a questionnaire to be filled out by their spouses; 32,345 spouses (75% of married spouses, 99.3% female) enrolled in the study. Enrollment questionnaires were self-administered. Computer-assisted follow-up telephone interviews were conducted in 1999–2003 (Phase 2) and 2005–2010 (Phase 3). Participants completed either self-administered mailed questionnaires or computer-assisted telephone interviews in 2013–2016 (Phase 4). Questionnaires can be found at https://aghealth.nih.gov/collaboration/questionnaires.html. The Phase 2 survey was completed by 33,456 applicators and 23,796 spouses, Phase 3 by 24,170 applicators and 19,959 spouses, and Phase 4 by 24, 145 applicators and 18,186 spouses. The institutional review boards of the National Institute of Environmental Health Sciences and the National Cancer Institute approved the study.

2.2. Pesticide use

The applicator enrollment questionnaire asked about ever-use of 50 pesticides, and duration and frequency of use for 22 specific pesticides. The applicator take-home questionnaire asked participants to provide duration and frequency of use for the remaining 28 pesticides, and to complete a checklist of ever-use of additional specific pesticides ("other pesticides used") that were not covered in the enrollment questionnaire. Our current analysis focuses on the 50 pesticides for which detailed information on duration and frequency of use were collected either in the enrollment or the take-home questionnaire (although other pesticides were considered in some analyses as noted). These questionnaires also sought detailed information on pesticide use practices including application methods, mixing processes, personal protective equipment use, and other workplace hygiene factors. The enrollment questionnaire asked applicators what type of personal protective equipment they generally wore when they personally handled pesticides, including respirator/gas mask, fabric/leather gloves, and chemical-resistant gloves. The enrollment spouse questionnaire only asked about ever-use of the 50 specific pesticides. All participants were asked about their overall use of any pesticides, including years and days personally mixed or applied pesticides.

We also used pesticide information collected at Phase 2 (conducted 2–10 years after enrollment, 5 years on average). At this interview, applicators and spouses were asked to provide the names and number of days of use of specific pesticides in the year prior to the interview (or most recent year used) and information on pesticide use practices. Although the Phase 2 interview asked only about pesticide use in the most recent year, when estimating cumulative exposure, we assumed that year represented pesticide use during the period since the Phase 1 exposure assessment.

We used several approaches to characterize pesticide exposures. First, we examined ever-use of the 50 specific pesticides. Exposure intensity weights were previously derived using an algorithm that incorporates information on mixing practices, application methods, repair status, and personal protective equipment use (Coble et al., 2011). We then used intensity-weighted lifetime days (IWLD) of pesticide use (i.e., the product of years of use and days used per year weighted by exposure intensity) as a measure of cumulative exposure for applicators. IWLD days were categorized using cut-points based on the exposure distribution of the full sample and number of PD cases (i.e., at least five cases) in each exposure category. Specifically, we created a four-category exposure variable (never use and three categories among users with cut-points at tertiles of IWLD). When sample size was limited, we created a three-category variable by cutting at the median of IWLD. As only applicators were asked about duration and frequency of use of specific pesticides in Phase 1, the IWLD analyses were limited to the applicators. We further restricted these analyses to male applicators due to the small number of female applicators.

In addition to examining individual pesticides, we created two ever-use pesticide groups based on potential mechanisms implicated in PD pathogenesis. The first group included use of any pesticides linked to mitochondrial complex I inhibition (namely, benomyl, permethrin, rotenone, dichlorvos, and thiabendazole) (Binukumar et al., 2010; Tanner et al., 2011); the second group included pesticides linked to aldehyde dehydrogenase inhibition (namely, benomyl, captan, folpet, aldrin, dieldrin, mancozeb/maneb, ferbam, thiram and ziram) (Fitzmaurice et al. 2013, 2014). Some pesticides of interest, including rotenone, thiabendazole, folpet, ferbam, and thiram, were not among the 50 main pesticides queried at enrollment and were only asked of applicators (not spouses) on the checklist of "other pesticides used" in the Phase 1 take home questionnaire. Although both applicators and spouses could have reported their use in the Phase 2 open-ended survey, we considered only Phase 1 exposures for these analyses to maximize the analytical sample with complete information on these pesticides and for analytical simplicity. To accommodate the fact that not all participants provided data and only a portion completed the take-home questionnaire, we conducted analyses (that focused on Phase 1 exposures only) in two different analytical subsets. We first considered only those participants with complete data on all individual pesticides in a group (so, the analysis was limited to the male applicators who returned the take-home questionnaire). In a secondary analysis in the overall sample, we considered participants as exposed if they indicated they used *at least one* of the pesticides in the group, regardless of missing information on other pesticides in that group.

2.3. Parkinson's disease

Potential PD cases were identified by self-report in all AHS surveys (i.e., positive response to "has a doctor ever told you that you had been diagnosed with Parkinson's disease?), as well as via linkage to the National Death Index and state death registries (with PD recorded as an underlying or contributing cause of death). Self-reported PD cases identified through Phase 2 were previously confirmed by movement disorder specialists as a part of the FAME study, via structured clinical examinations and medical records; self-reported PD was confirmed in 84% (Tanner et al., 2011). Between 2012 and 2017 (around and following the Phase 4 survey), we attempted to validate all potential PD cases (prevalent as well as incident), including those considered PD cases in FAME (n = 810). Briefly, each participant with potential PD, or their proxy (if deceased or too ill), was asked to complete a detailed screening questionnaire on PD diagnosis, symptoms, characteristics, and treatment. We also requested consent to obtain medical records from their treating or diagnosing physician. Screeners were obtained for 510 prevalent and incident cases. The PD screeners were evaluated by a movement disorder specialist to adjudicate PD status using criteria analogous to clinical diagnostic criteria proposed by Gelb et al. (1999). This evaluation classified 75% as probable or possible PD, 11% as questionable or other neurological disorders, and 14% as not having PD. Among those for whom medical records were obtained (n = 65), 91% were confirmed as PD by medical records and 9% were considered questionable (because of conflicting information from multiple physicians and/or physician's reporting of inadequate evidence to distinguish from other neurological disorders).

After excluding self-reported prevalent cases (age at diagnosis ≤ age at enrollment) and those with no information on age at diagnosis, we had 598 eligible incident potential cases (440 with and 158 without screener data; Supplemental Fig. 2). We excluded cases without supporting PD symptoms or medications (99 of 440 participants screened) and those who did not provide consistent responses across surveys (8 of the 158 without screener information), leaving 491 cases for analysis. Overall, 80.6% of the 491 cases had some confirmatory information from a validation screener, medical record, FAME evaluation, or death certificate. We used the age at diagnosis provided at the earliest survey in which age at diagnosis was reported.

2.4. Study sample

Participants eligible for our analysis included a total of 38,798 applicators and 28,238 spouses who completed at least one follow-up survey or the PD validation screening questionnaire (Fig. 1). After excluding prevalent cases, those with inconsistent PD information across surveys, or those lacking other supporting information, we had 38,274 applicators and 27,836 spouses for ever-use of pesticides analyses (n = 66,110; 491 with PD). For IWLD analyses of the 22 pesticides for which frequency and duration of use were asked in the enrollment questionnaire, the final sample size included 37,284 male applicators (372 PD cases) and for the 28 pesticides for which frequency and duration of use were asked in the take-home questionnaire, the final sample size included 19,068 male applicators (237 PD cases).

2.5. Statistical analysis

2.5.1. Pesticide use at enrollment

We first examined bivariate relations of incident PD with baseline covariates that included applicator status, sex, state of residence, cigarette smoking, alcohol consumption, and education. We used Cox proportional hazards regression to estimate hazard ratios (HRs) and 95% confidence intervals (95% CI) for associations between pesticide use reported at enrollment and incident PD. We used attained age as the time scale with left truncation at enrollment and always adjusted for sex, state of residence, smoking status, and education. Models for individual pesticides were additionally adjusted for the top four pesticides among those whose Spearman correlation with the pesticide of interest was 0.40 or greater. Whenever the proportional hazards assumption failed for a pesticide (p-value for interaction between age and pesticide ≤ 0.10), we allowed hazards to vary by the median age (63 years). Ever-use analyses were conducted in a combined sample of applicators and spouses, and separately for male applicators (n = 37,284) and female spouses (n = 27,673) (female applicators and male spouses, respectively, were excluded from these analyses due to small numbers). In the IWLD analyses among male applicators, we conducted a test for trend using the median value for each exposure category as an ordinal variable in regression models.

Information on smoking (n = 691) and education (n = 2474) was missing for some participants, and further, some participants reported 'something else' for education (n = 2625). We treated 'something else'

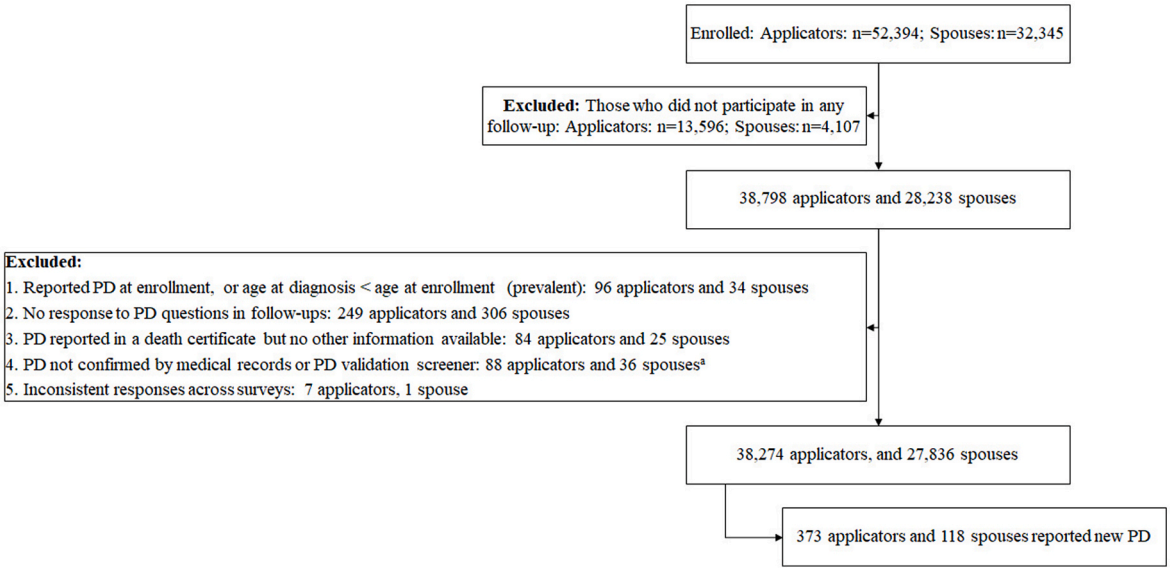

Fig. 1. Sample selection for pesticide and Parkinson's disease (PD) analysis in the Agricultural Health Study. [a]includes n = 2 spouses selected for validation based on FAME screening who did not report PD.

as a missing covariate and used multiple imputation to impute missing covariates (i.e., education and smoking). We created five imputed datasets, performed regression analysis in each dataset, and combined those results to estimate parameters and their standard errors using SAS PROC MIANALYZE (SAS Institute Inc, 2015).

Wearing chemical-resistant gloves was previously shown to modify PD associations with some pesticides (Furlong et al., 2015). Further, individuals with head injury may be more susceptible to pesticide-associated PD risk – the underlying hypothesis being combinations of risk factors acting in concert increase disease vulnerability (the "multiple-hit hypothesis") (Lee et al., 2012). We examined potential heterogeneity in the associations of PD with ever-use of pesticides by these characteristics (by testing for the interaction between pesticides and these characteristics), when each cross-classified category of exposure and factor contained at least five cases. Applicators were asked about a history of head injury requiring medical attention only in the take-home questionnaire, whereas all spouses were asked about head injury, and thus heterogeneity by head injury was evaluated in a smaller subset (19,222 applicators and 26,666 spouses resulting in a total of 45,888 participants). Only applicators (in the enrollment questionnaire) but not spouses were asked about chemical resistant glove use and thus heterogeneity by chemical resistant glove use was evaluated in male applicators only (n = 32,816). We also stratified the analysis by follow-up time (≤10 years and >10 years) for ever-use analysis. Potential heterogeneity was not examined for IWLD due to limited sample size.

To examine the potential impact of loss-to-follow up, we performed a sensitivity analysis using inverse probability of censoring weights (Howe et al., 2016). Briefly, we used weighted Cox models to estimate HRs and 95% CIs, adjusting for covariates and using stabilized inverse probability weights. For stabilized weight estimation, first we transformed our data from a single record per person into person-year data (i.e., with multiple records per person). Then, we used logistic regression analyses to calculate the denominator of the weights, or probability of overall participation in Phase 4 conditional on exposure, year and baseline covariates (age, sex, education, smoking, alcohol use, state of residence; missing values imputed for covariates whenever applicable), as well as to calculate the numerator of the weights, or probability of overall participation in Phase 4 conditional only on year. We estimated stabilized weights as the ratio of cumulative conditional probabilities.

Lastly, we used logistic regression to analyze two other groups of cases (i) all "confirmed" prevalent and incident PD cases (n = 66,216 with 597 PD cases), and (ii) all "potential" prevalent and incident PD cases (any self-reported cases or reported on death certificates) (n = 84,739, with 860 PD cases). Statistical significance was determined using two-sided tests with α of 0.05. We performed statistical analyses using SAS version 9.4 (SAS Institute, Inc, Cary, NC).

2.5.2. Pesticide use through Phase 2

We also examined associations between cumulative pesticide use through Phase 2 and incident PD. However, given the lower exposure and outcome prevalence in spouses, we performed this analysis only in male applicators. About 14% of the applicators included in our analysis were missing Phase 2 exposure data due to Phase 2 non-response. To account for the missing exposure data due to non-response, we used a multiple imputation approach developed specifically for AHS applicators (Heltshe et al., 2012). This approach used information on several factors including demographics, farm characteristics, prior pesticide use, and medical conditions that predicted missingness to impute use of specific pesticides for the Phase 2 non-responders. We created five imputed datasets which were then converted to person-year datasets allowing pesticide exposure information (ever-use and IWLDs) through Phase 2 to vary until their time at risk. We applied a Cox model applied to each imputed dataset and combined those results to obtain an HR and 95% CI using SAS PROC MIANALYZE (SAS Institute Inc, 2015). This analysis was limited to the previously described 50 specific pesticides. Information on smoking and education was missing for only 1% and 4% of the sample, and we used a missing indicator category for this analysis.

3. Results

Characteristics of participants at enrollment differed by PD status (Table 1). Older participants, applicators, males, and those from North Carolina were more likely to develop PD, while current smokers and alcohol drinkers were less likely to develop PD. Chemical resistant glove use and a history of head injury requiring medical attention were similar between the two groups, although when adjusted for age, sex, state, education, and smoking status, we found an inverse association between having a head injury and incident PD (HR: 0.71, 95% CI: 0.46, 1.09).

3.1. Phase 1 pesticides

In the analysis examining lifetime days of *any* pesticide use in relation to incident PD in the overall sample, we generally observed positive HRs for higher lifetime days compared to never use, although we did not see a monotonic increasing trend (for example, HRs for the third and the fourth quartiles compared to never use were 1.27 (95% CI: 0.82, 1.98) and 1.07 (95% CI: 0.69, 1.67), respectively, Supplemental Table 1). In the female spouses only analysis, we observed increased risk (HR: 1.58,

Table 1
Characteristics of Agricultural Health Study participants at enrollment (n = 66,110).

Characteristics	No PD (n (%))[a] (n = 65,619)	Incident PD (n (%))[a] (n = 491)
Age (years)		
≤45	31,843 (48)	53 (11)
46–55	16,479 (25)	109 (22)
56–65	12,382 (19)	206 (42)
>65	4915 (7)	123 (25)
Participant		
Spouse	27,718 (42)	118 (24)
Applicator	37,901 (58)	373 (76)
Sex		
Female	28,546 (44)	117 (24)
Male	37,073 (56)	374 (76)
State of residence		
Iowa	43,319 (66)	299 (61)
North Carolina	22,300 (34)	192 (39)
Education[b]		
≤ High school graduate	31,301 (50)	300 (64)
1–3 years beyond high school	16,507 (26)	94 (20)
College graduate or more	12,732 (20)	77 (16)
Something else	2624 (4)	1 (0)
Smoking status[c]		
Never smoker	40,305 (62)	296 (61)
Former smoker	16,573 (26)	159 (33)
Current smoker	8056 (12)	30 (6)
Alcohol consumption (past 12 months)[d]		
No	23,979 (38)	221 (49)
Yes	38,420 (62)	230 (51)
Chemical resistant glove use[e]		
No	6193 (19)	65 (20)
Yes	26,299 (81)	259 (80)
Head injury requiring medical attention[f]		
No	41,911 (92)	316 (93)
Yes	3638 (8)	23 (7)

[a] % may not add to 100% due to rounding.
[b] Education missing for n = 2474.
[c] Smoking status missing for n = 691.
[d] Alcohol consumption missing for n = 3260.
[e] Chemical resistant glove use information was not sought from spouses and missing for n = 5458 applicators.
[f] Applicators provided information on head injury only in the take-home questionnaire.

95% CI: 1.00, 2.50) in those exposed to more than the median days as compared to never use. In the male applicators only analysis, associations for higher quartiles of lifetime days compared to the lowest quartile were slightly inverse. In a combined analysis of applicators and spouses (Table 2), we found positive associations for the organophosphate insecticide terbufos (HR:1.30, 95% CI: 1.02, 1.68) and the herbicides trifluralin (HR:1.29, 95% CI: 0.99, 1.70) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) (HR:1.57, 95% CI: 1.21, 2.04), and inverse associations for ever-use of the organophosphate insecticide diazinon (HR: 0.73, 95% CI: 0.58, 0.94), the fumigant ethylene dibromide (HR: 0.35, 95% CI: 0.14, 0.84), and the herbicide 2,4,5-TP [2,4,5-T,P, 2-(2,4,5-trichlorophenoxy) propionic acid] (HR: 0.39, 95% CI: 0.25, 0.62). These associations remained when analyses were performed separately for male applicators (Supplemental Table 2). Separate analyses for female spouses (Supplemental Table 2) were limited to only a few pesticides due to fewer PD cases; elevated (HR > 1.40), yet imprecise, risk was observed for the herbicides glyphosate, trifluralin, and cyanazine.

We found heterogeneity in associations for ever-use of some pesticides and PD risk by head injury (Table 3). We found higher PD risk for the three organochlorine insecticides chlordane, dichlorodiphenyltrichloroethane (DDT), and toxaphene, the two organophosphate insecticides diazinon and phorate, the insecticide permethrin (animal and crop use combined), the fumigant methyl bromide, and the herbicides paraquat and pendimethalin among those who reported a history of head injury as compared to reduced or null associations among those did not report a history of head injury (p for heterogeneity ≤0.10). For example, the HR for paraquat among those with a history of head injury was 3.20 (95%CI: 1.38, 7.45) versus 1.00 (95%CI: 0.71, 1.41) for those without a history (p for heterogeneity = 0.01).

Similarly, we found that five herbicides (dicamba, imazethapyr, metolachlor, trifluralin, and metribuzin) were associated with elevated PD risk among those who did not use chemical-resistant gloves as compared to reduced or null associations among glove users, although directions were reverse for metalaxyl (Table 3). In the analyses stratified by follow-up time (≤10 years and >10 years), we found that HRs for some herbicides including alachlor, butylate, chlorimuron ethyl, trifluralin, 2,4-D, and atrazine were elevated for the first 10 years of follow-up, but not for later years (Supplemental Table 3).

In the analyses examining IWLD through Phase 1 in male applicators (Table 4), we saw no clear monotonic exposure-response for pesticides associated with elevated PD risk. There were a few suggestive patterns. Specifically, we saw elevated HRs for individuals in the highest category of IWLD of the insecticides dichlorvos [HR:1.46 (95% CI: 0.98, 2.19), p-trend:0.06] and permethrin (animal use)[HR:1.44 (95% CI: 0.85, 2.44), p-trend: 0.21], and the fungicides benomyl (HR: 1.34 (95% CI: 0.64, 2.80), p-trend:0.31], captan [(HR: 1.27 (95% CI: 0.74, 2.20), p-trend:0.36], and chlorothalonil [HR: 1.29 (95% CI: 0.66, 2.56), p-trend:0.41] as compared to those who never used those pesticides, although risk estimates were very imprecise as reflected by the wide confidence intervals. For the herbicides terbufos and trifluralin (for which we observed significant positive association in the ever-use analysis), HRs were generally elevated for all tertiles as compared to never use. For heptachlor, HRs were higher for the two lower tertiles than for the upper. HRs in the higher tertiles of the insecticides aldrin, toxaphene, carbaryl, diazinon, and malathion were lower than in the never use category. The results (odds ratio estimates) were similar when we included "confirmed" prevalent cases (Supplemental Tables 4 and 5), or any "potential" PD cases (data not shown). The HR estimates using inverse probability weights were also similar (Supplemental Table 6).

In the male applicators returning take-home questionnaires, none of the pesticide groups – mitochondrial complex I inhibitors [HR: 0.96 (95%CI: 0.71, 1.29)] or aldehyde dehydrogenase inhibitors [(HR: 0.84 (95%CI: 0.65, 1.11)] – were associated with increased PD risk, although we observed heterogeneity by head injury for ever-use of mitochondrial complex I inhibitors with higher HR among those who experienced head injury [HR: 2.42 (95%CI: 0.91, 6.47)] vs reduced HR among those

Table 2
Ever-use of pesticide reported at enrollment and Parkinson's disease (PD) risk in all participants (n = 66,110).

Pesticide	No PD, n (%)[a]	PD, n (%)[b]	HR (95% CI)[c]
Organochlorine insecticide			
Aldrin	6507 (11.1)	98 (23.7)	0.91 (0.68, 1.23)
Chlordane	9758 (16.5)	125 (29.8)	1.05 (0.82, 1.34)
Dieldrin	2440 (4.1)	38 (9.2)	0.88 (0.60, 1.30)
DDT	8954 (15.4)	143 (34.8)	0.86 (0.67, 1.12)
Heptachlor	5442 (9.4)	87 (21.3)	1.01 (0.74, 1.38)
Toxaphene	5160 (8.7)	59 (14.1)	0.80 (0.60, 1.08)
Lindane	7250 (12.1)	74 (17.7)	0.92 (0.71, 1.19)
Carbamate insecticide			
Aldicarb	3809 (6.5)	28 (6.9)	1.05 (0.68, 1.62)
Carbaryl	27,180 (45.5)	231 (55.4)	1.09 (0.87, 1.37)
Carbofuran	10,017 (16.7)	110 (26.6)	0.95 (0.74, 1.21)
Organophosphate insecticide			
Chlorpyrifos	16,700 (26.8)	143 (30.7)	0.92 (0.74, 1.13)
Coumaphos	3423 (5.7)	35 (8.4)	1.04 (0.73, 1.47)
Diazinon	13,979 (23.3)	105 (25.1)	0.73 (0.58, 0.94)
Dichlorvos	4425 (7.3)	48 (11.5)	1.12 (0.83, 1.53)
Fonofos	8219 (13.6)	75 (17.7)	0.91 (0.70, 1.19)
Malathion	28,496 (48.7)	253 (62.6)	1.01 (0.78, 1.30)
Parathion	5661 (9.5)	62 (14.8)	0.98 (0.74, 1.30)
Phorate (≤63 y)[d]	5618 (18)	39 (36.1)	1.33 (0.85, 2.08)
>63 y	5786 (22.1)	73 (26.1)	0.71 (0.52, 0.97)
Terbufos	13,718 (23.8)	138 (35.4)	1.30 (1.02, 1.68)
Permethrin insecticide			
Permethrin (Crops)	5263 (8.8)	36 (8.8)	0.99 (0.70, 1.40)
Permethrin (Animals)	5696 (9.4)	41 (9.8)	1.07 (0.77, 1.48)
Fumigant			
Carbon disulfide/Carbon tetrachloride	2099 (3.5)	31 (7.3)	1.03 (0.71, 1.50)
Aluminum phosphide	1707 (2.8)	16 (3.8)	1.08 (0.65, 1.78)
Ethylene dibromide	1294 (2.2)	5 (1.2)	0.35 (0.14, 0.84)
Methyl bromide	5707 (9.5)	46 (10.8)	0.86 (0.59, 1.25)
Fungicide			
Benomyl[e]	*3492 (6)*	*26 (6.4)*	*0.80 (0.48, 1.31)*
Benomyl (≤ 63y)[d, e]	*1664 (5.3)*	*4 (3.6)*	*0.35(0.11, 1.10)*
> 63y	*1828 (6.8)*	*22 (7.5)*	*0.99 (0.58, 1.68)*
Captan	4617 (7.7)	33 (8)	0.84 (0.59, 1.20)
Chlorothalonil	2899 (4.8)	21 (5)	0.97 (0.59, 1.60)
Maneb (≤63 y)[d]	1685 (5.2)	8 (7)	1.43 (0.63, 3.22)
>63 y	2030 (7.3)	21 (7)	0.75 (0.44, 1.25)
Metalaxyl	7968 (13.6)	58 (14.3)	0.85 (0.61, 1.18)
Herbicide			
Alachlor	19,057 (32.1)	187 (45.6)	1.13 (0.88, 1.45)
Butylate (≤63 y)[d]	5750 (18.3)	38 (34.9)	1.31 (0.86, 2.01)
>63 y	5245 (19.7)	65 (23.4)	0.87 (0.64, 1.20)
Chlorimuron ethyl	12,693 (21.8)	101 (25.6)	1.04 (0.80, 1.36)
Dicamba	17,945 (31)	161 (41.2)	0.94 (0.72, 1.22)
EPTC	7049 (12.2)	54 (14.1)	0.84 (0.61, 1.15)
Glyphosate	35,406 (58.6)	291 (67.4)	1.10 (0.87, 1.39)
Imazethapyr	15,124 (26.3)	126 (32.6)	1.04 (0.79, 1.37)
Metolachlor	16,114 (27.9)	127 (32.6)	0.80 (0.62, 1.03)
Paraquat	8526 (14.2)	87 (20.4)	1.09 (0.84, 1.41)
Pendimethalin	15,250 (26.1)	127 (31.9)	1.07 (0.83, 1.37)
Petroleum distillate	16,756 (28.9)	146 (37)	0.93 (0.73, 1.18)
Trifluralin	18,665 (32.2)	182 (46.8)	1.29 (0.99, 1.70)
2,4-D	28,871 (49.8)	262 (66.7)	1.06 (0.79, 1.43)
2,4,5-T	7264 (12.5)	116 (28.3)	1.57 (1.21, 2.04)
2,4,5-TP	3287 (5.5)	23 (5.5)	0.39 (0.25, 0.62)
Atrazine	25,297 (42.8)	237 (58.2)	1.03 (0.77, 1.38)
Cyanazine	14,641 (25.2)	133 (33.6)	0.90 (0.69, 1.18)
Metribuzin	15,500 (26.8)	137 (35.7)	0.86 (0.65, 1.14)

Abbreviation: 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; 2,4,5-T,P, 2-(2,4,5-trichlorophenoxy) propionic acid; CI, Confidence Intervals; DDT, Dichlorodiphenyltrichloroethane; EPTC, S-Ethyl dipropylthiocarbamate; HR, Hazard Ratio; PD, Parkinson's disease.

[a] Exposed individuals who did not develop PD.

[b] Exposed individuals who developed PD.

[c] HR adjusted for sex, state of residence, smoking status, education, and ever-use of correlated pesticides (other pesticides whose ever-use variable had Spearman correlation ≥ 0.40 with the ever-use variable of the target pesticide).

[d] Hazard ratio allowed to vary by the median age (i.e., 63 years) for pesticides that did not meet proportional hazards assumption (p ≤ 0.10).

[e] Proportional hazards assumption did not meet for those in italics, but there was not adequate sample size meeting the criteria of at least five exposed cases in cross-classified categories.

without head injury [HR: 0.83 (95%CI: 0.61, 1.12), p for heterogeneity: 0.04]. The results were similar (i.e., no independent associations for the pesticide groups but heterogeneity by head injury for the mitochondrial complex I inhibitors), when we also considered participants as exposed if they indicated they used *at least one* individual pesticide in the group in the overall sample.

3.2. Pesticide exposure through Phase 2

Among male applicators, associations between ever-use of individual pesticides through Phase 2 were similar to the results using information reported at enrollment; specifically, PD risk was reduced among those who ever-used diazinon, ethylene dibromide, and 2,4,5-TP, and elevated among those who ever-used terbufos, 2,4,5-T, and trifluralin (Supplemental Table 7). Results that used IWLD through Phase 2 were also similar (Table 4).

4. Discussion

In this study, we found that ever-use of the insecticide terbufos and the herbicides trifluralin and 2,4,5-T was associated with elevated PD risk. Positive associations of PD with ever-use of the herbicides trifluralin and 2,4,5-T are consistent with the prior AHS-wide analysis based on 78 self-reported incident PD cases identified through Phase 2 (Kamel et al., 2007). We also found lower PD risk for ever-use of some pesticides including diazinon and 2,4,5-TP. In IWLD analyses, however, we did not see evident monotonic exposure response gradients for these pesticides, although HRs for higher exposure categories reflected findings of ever-use analyses. We observed heterogeneity in the pesticide-PD associations by head injury and chemical-resistant gloves use, indicating higher PD risk for use of certain organochlorine insecticides (chlordane, DDT, and toxaphene), organophosphate insecticides (diazinon and phorate), insecticide permethrin, and herbicides (paraquat and pendimethalin) among those who reported head injury and for use of certain herbicides (dicamba, imazethapyr, and trifluralin) among those who did not use chemical-resistant gloves.

To the best of our knowledge, no studies have linked the insecticide terbufos with PD, although a few prior studies have linked other individual organophosphate insecticides that also act by inhibiting the enzyme acetylcholinesterase with PD (Gatto et al., 2009; Wang et al., 2014). We also found elevated PD risk in AHS applicators who were exposed to higher IWLD of the organophosphate insecticide dichlorvos. Chronic dichlorvos exposure in rats has been shown to induce degeneration of nigrostriatal dopaminergic neurons and alpha-synuclein aggregation, the hallmarks of PD pathogenesis, as well as to inhibit mitochondrial complexes and alter mitochondrial structures (Binukumar et al., 2010). We are aware of only one study on dichlorvos and PD, and in that study, individuals in the lower, although not the highest, exposure-day category of dichlorvos had elevated PD risk as compared to the individuals who were never exposed (van der Mark et al., 2014). On the other hand, we saw an inverse association between the organophosphate insecticide diazinon and PD risk in the overall sample and among those without head injury but saw elevated yet not statistically significant risk among those with head injury. A few prior studies, although not all, have linked diazinon with increased PD risk (Firestone et al., 2010; Gatto et al., 2009; Narayan et al., 2013). We observed

Table 3
Ever-use of pesticides reported at enrollment and Parkinson's disease (PD) risk by head injury status and chemical resistant glove use.

Pesticide	Head injury	Exposed/Unexposed PD cases	HR (95% CI)[a]	P[b]
Chlordane	No	77/206	1.10 (0.81, 1.51)	0.01
	Yes	16/6	4.08 (1.58, 10.55)	
Diazinon	No	57/223	0.64 (0.47, 0.88)	0.07
	Yes	10/11	1.48 (0.62, 3.51)	
DDT	No	87/189	0.86 (0.62, 1.19)	0.06
	Yes	15/7	2.12 (0.85, 5.31)	
Methyl bromide	No	22/265	0.67 (0.40, 1.11)	0.01
	Yes	6/15	2.85 (1.06, 7.65)	
Paraquat	No	45/239	1.00 (0.71, 1.41)	0.01
	Yes	10/12	3.20 (1.38, 7.45)	
Pendimethalin	No	65/207	0.90 (0.65, 1.25)	0.03
	Yes	10/6	2.85 (1.02, 7.91)	
Permethrin (animal and crop use combined)	No	33/260	0.79 (0.54, 1.14)	0.08
	Yes	6/12	2.04 (0.76, 5.44)	
Phorate	No	60/205	0.74 (0.53, 1.04)	0.03
	Yes	10/6	2.47 (0.89, 6.89)	
Toxaphene	No	31/248	0.69 (0.46, 1.03)	0.08
	Yes	7/15	1.64 (0.66, 4.04)	
	Chemical resistant glove[c]			
Dicamba	No	21/20	2.10 (1.11, 3.98)	0.008
	Yes	127/100	0.85 (0.63, 1.15)	
Imazethapyr	No	18/23	3.34 (1.75, 6.39)	0.0002
	Yes	100/127	0.92 (0.69, 1.24)	
Metalaxyl	No	8/39	0.44 (0.20, 0.96)	0.05
	Yes	48/191	1.00 (0.70, 1.43)	
Metolachlor	No	18/26	1.60 (0.88, 2.94)	0.01
	Yes	98/132	0.70 (0.54, 0.91)	
Metribuzin	No	17/25	1.48 (0.78, 2.81)	0.06
	Yes	110/109	0.78 (0.58, 1.06)	
Trifluralin	No	25/18	2.64 (1.42, 4.92)	0.03
	Yes	144/80	1.24 (0.91, 1.68)	

Abbreviation: CI, Confidence Intervals; DDT, Dichlorodiphenyltrichloroethane; HR, Hazard Ratio; PD, Parkinson's disease.

[a] HR adjusted for state of residence, smoking status, education, and ever-use of correlated pesticides (other pesticides whose ever-use variable had Spearman correlation ≥ 0.40 with the ever-use variable of the target pesticide); HR for head injury also adjusted for sex.

[b] P-value for test for heterogeneity.

[c] Male applicators only.

similar heterogeneity by head injury for the organophosphate phorate. One prior study has reported an association between phorate exposure and elevated PD risk (Wang et al., 2014). Apart from a common pathway for pesticidal action, i.e., inhibition of acetylcholinesterase, individual organophosphate insecticides may exert neurotoxicity through a wide range of mechanisms including oxidative stress and neuroinflammation (Terry, 2012) resulting in varying degrees of toxicity. We are uncertain, however, about the reasons underlying the observed inverse association for some pesticides in the overall sample or among those without head injury.

Besides the prior AHS reports (Furlong et al., 2015; Kamel et al., 2007), we are not aware of other epidemiologic evidence linking the herbicides trifluralin and 2,4,5-T and PD, although an *in vitro* study has shown that trifluralin accelerates the formation of alpha-synuclein fibrils, a finding relevant to PD pathogenesis (Uversky et al., 2002). In another analysis, AHS applicators who experienced high pesticide exposure events involving trifluralin were also more likely to report olfactory impairment, one of the important prodromal symptoms of PD (Shrestha et al., 2019b). To our knowledge, the only other study (based on only four and seven exposed cases and controls respectively) that examined 2,4,5-T in relation to PD did not find any association (Dhillon et al., 2008). We found that the herbicide dicamba was associated with increased PD risk among those who did not use chemical-resistant gloves during handling of pesticides. Dicamba, structurally similar to the phenoxy herbicide 2,4,5-T (Bradberry et al., 2004), was associated with increased, although statistically non-significant, PD risk in the prior AHS investigation in the overall sample (Kamel et al., 2007). We observed an unexpected inverse association with the herbicide 2,4,5-TP, another phenoxy pesticide structurally similar to 2,4,5-T. Use of both 2,4,5-T and 2,4,5-TP was suspended in the US in 1979 due to potential contamination by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and associated health concerns (Gintautas et al., 1992; Lilienfeld and Gallo, 1989; Ware, 1988).

We found that ever-use of certain individual pesticides and the pesticide group mitochondrial complex I inhibitors was associated with increased PD risk among those who reported a history of head injury requiring medical attention, although head injury itself was not independently associated with elevated PD risk. While sequelae of traumatic brain injury, including microglial activation, alpha-synuclein aggregation, mitochondrial dysfunction, and other chronic inflammatory responses have been suggested as potential mechanisms for PD predisposition (Acosta et al., 2015; Hutson et al., 2011; Lifshitz et al., 2004; Loane et al., 2014), findings of prior epidemiologic studies on head injury and PD risk have been conflicting (Gardner et al., 2015; Kenborg et al., 2015; Taylor et al., 2016). With the notion that traumatic brain injury potentially requires synergistic factors to lead to PD, a case-control study examined PD risk in relation to joint exposure to head injury and paraquat (assessed using geographical information system-based land use and historic pesticide use reporting data); it found that paraquat-associated PD risk was greater among individuals with head injury and that the joint exposure was associated with higher PD risk as compared to exposure to paraquat or head injury alone (Lee et al., 2012). An experimental study in rats also demonstrated that acute traumatic brain injury induced progressive degeneration of nigrostriatal dopaminergic neurons, microglial activation, and alpha-synuclein accumulation were exacerbated when the animals were exposed to concentrations of paraquat that alone would not induce nigrostriatal death (Hutson et al., 2011). We are not aware of reports that examined interaction between other pesticides and head injury, but potential interaction is plausible as some of these pesticides have been implicated in PD pathogenesis (Furlong et al., 2015; Wang et al., 2014). We note several limitations in this particular analysis – our questionnaire did not capture head injury not requiring medical attention, and limited information was available on age at injury which precluded analysis on the timing of injury occurrence.

Although our subgroup analysis did hint at higher PD risk for paraquat as well as for the pesticide group mitochondrial complex I inhibitors among individuals with head injury, we found limited evidence for independent associations of incident PD with these pesticides, whereas both were independently associated with PD in FAME (Tanner et al., 2011). Among other specific pesticides previously examined in FAME, we saw some suggestions of elevated PD risk for those with higher IWLD of the fungicide benomyl and the insecticide permethrin (animal use), though HR estimates were imprecise.

Limited reproducibility of FAME findings in the current study could be due to differences in study design, exposure data, and criteria for inclusion in analyses. FAME, although conducted within the AHS framework, collected more granular exposure data on some pesticides suspected to be etiologically relevant to PD, some of which were infrequently-used and therefore covered superficially at AHS enrollment (ever-use of "other pesticides"). The AHS questionnaires at enrollment focused, in part, on frequently-used pesticides. Further, AHS questionnaires differed for applicators and spouses, leading to lack of information in the AHS on certain pesticides of interest in FAME. For example, information on rotenone (included in the group mitochondrial complex I inhibitor) was not asked of spouses and was collected only from applicators who completed the take-home questionnaire. Likewise, although all participants were asked about ever-use of paraquat, information on duration and frequency of paraquat use was not asked of spouses and was collected only from the applicators returning the take-home questionnaire. Differences in study design and outcome ascertainment also could have contributed to differences in findings. Our analysis included all cohort members with at least some follow-up information and involved a longer follow-up period, whereas FAME involved a small subset of the cohort with fewer PD cases and shorter follow-up. Our current analysis utilized pesticide data obtained before PD diagnosis; whereas the exposure data in FAME were collected retrospectively after PD diagnosis (from participants or their proxies if participants were deceased), thereby opening the possibility of bias associated with differential recall of pesticide use (for example, if cases were more likely to recall such exposures). On the other hand, FAME benefitted from more detailed exposure information on relevant pesticides. Further, in FAME, both cases and controls underwent in-person assessment, while in the current study, we mainly relied on self-reports and those who self-reported to be PD-free did not undergo additional evaluation. Since we also included the FAME cases, however, a portion of our cases had an earlier in-person exam. Disease misclassification is possible and could have led to diminish estimates of relative risk in our analyses. In fact, while pesticide-use agreement was good overall, we did see some evidence of differential reporting by cases and controls in FAME when comparing data reported in both FAME and in the main AHS enrollment questionnaire (Supplemental Table 8 presents some comparisons, although we note that exposure timeframes are different as FAME asked exposures before PD diagnosis for cases or a reference date for controls). Lastly, FAME and our current cohort-wide effort are capturing different time windows of exposure relative to disease onset. The insidious onset of PD that is difficult to capture in non-clinical settings together with limited knowledge of induction and latent periods makes determination of exposure-relevant time windows difficult.

Specifically, for the herbicide paraquat, animal and earlier human studies offer persuasive evidence for a potential link with PD, despite continuing debate (Goldman et al., 2017; Jones et al., 2014). Some subgroups, including those with specific genetic makeup, head injury, and certain dietary intake have been found particularly vulnerable to PD following paraquat exposure (Goldman et al., 2012; Kamel et al., 2014; Lee et al., 2012; Ritz et al., 2009). We cannot rule out the possibility that limited evidence of independent associations between PD and ever-use of some pesticides (including paraquat) in the current study resulted from non-differential bias attenuating HR estimates; for example, the HR for ever-use of paraquat was elevated [HR: 1.09 (95% CI: 0.84, 1.41)], but not statistically significant. Nevertheless, we were still able to observe associations among those potentially more susceptible due to head injury.

Table 4
Intensity-weighted lifetime days of pesticide use at enrollment and incident PD in male applicators.

		Pesticide exposure through enrollment				Pesticide exposure through Phase 2				
Pesticide	Lifetime days[a]	No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]	Lifetime days[a]	No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]
Organochlorine insecticide										
Aldrin [d]	Never use	13,427 (82.7)	145 (75.5)	Ref	0.08	–	–	–	–	
	>0–≤315	994 (6.1)	18 (9.4)	0.84 (0.50, 1.42)						
	>315–≤980	911 (5.6)	17 (8.9)	0.82 (0.47, 1.41)						
	>980	905 (5.6)	12 (6.3)	0.56 (0.30, 1.06)						
Chlordane [d]	Never use	13,516 (80.7)	144 (72.7)	Ref	0.69	–	–	–	–	
	>0–≤236	1090 (6.5)	18 (9.1)	1.04 (0.63, 1.71)						
	>236–≤735	1111 (6.6)	17 (8.6)	0.92 (0.55, 1.53)						
	>735	1039 (6.2)	19 (9.6)	1.02 (0.62, 1.68)						
Dieldrin [d]	Never use	15,739 (96.2)	178 (93.2)	Ref	0.61	–	–	–	–	
	>0–≤338	307 (1.9)	8 (4.2)	1.24 (0.60, 2.59)						
	>338	308 (1.9)	5 (2.6)	0.77 (0.31, 1.93)						
DDT [d]	Never use	12,823 (78.3)	117 (60.6)	Ref	0.61	–	–	–	–	
	>0–≤341	1221 (7.5)	21 (10.9)	0.84 (0.52, 1.37)						
	>341–≤1675	1175 (7.2)	35 (18.1)	1.39 (0.92, 2.08)						
	>1675	1150 (7)	20 (10.4)	0.87 (0.53, 1.43)						
Heptachlor [d]	Never use	14,332 (87.4)	155 (78.7)	Ref	0.93	–	–	–	–	
	>0–≤280	673 (4.1)	14 (7.1)	1.41 (0.79, 2.51)						
	>280–≤882	729 (4.4)	17 (8.6)	1.44 (0.85, 2.46)						
	>882	660 (4)	11 (5.6)	1.02 (0.54, 1.94)						
Toxaphene [d]	Never use	16,128 (88.6)	200 (89.7)	Ref	0.12	–	–	–	–	
	>0–≤315	714 (3.9)	7 (3.1)	0.54 (0.26, 1.16)						
	>315–≤1181	670 (3.7)	8 (3.6)	0.66 (0.32, 1.33)						
	>1181	681 (3.7)	8 (3.6)	0.59 (0.29, 1.21)						
Lindane	Never use	15,591 (86.3)	186 (85.3)	Ref	0.56	Never use	15,424 (84.4)	183 (83.6)	Ref	0.62
	>0–≤315	823 (4.6)	7 (3.2)	0.56 (0.26, 1.2)		>0–≤341	944 (5.2)	8 (3.7)	0.59 (0.29, 1.21)	
	>315–≤1232	839 (4.6)	16 (7.3)	1.23 (0.73, 2.06)		>341–≤1232	961 (5.3)	18 (8.2)	1.26 (0.76, 2.07)	
	>1232	815 (4.5)	9 (4.1)	0.77 (0.40, 1.51)		>1232	940 (5.1)	10 (4.6)	0.80 (0.42, 1.51)	
Carbamate insecticide										
Carbaryl	Never use	9547 (57.8)	111 (56.3)	Ref	0.12	Never use	9194 (52)	106 (53)	Ref	0.11
	>0–≤387	2432 (14.7)	32 (16.2)	0.96 (0.65, 1.44)		>0–≤441	2904 (16.4)	37 (18.5)	0.90 (0.60, 1.36)	
	>387–≤2460	2403 (14.6)	30 (15.2)	0.81 (0.53, 1.26)		>441–≤2320	2918 (16.5)	30 (15)	0.78 (0.50, 1.22)	
	>2460	2123 (12.9)	24 (12.2)	0.64 (0.38, 1.08)		>2320	2675 (15.1)	27 (13.5)	0.63 (0.37, 1.05)	
Carbofuran [e]	-	–	–	–		Never use	23,500 (71.3)	198 (64.5)	Ref	0.41
						>0–≤368	3133 (9.5)	41 (13.4)	0.90 (0.60, 1.36)	
						>368–≤1370	3200 (9.7)	41 (13.4)	0.78 (0.50, 1.22)	
						>1370	3127 (9.5)	27 (8.8)	0.63 (0.37, 1.05)	
≤ 63y	Never use	13,827 (76.4)	52 (61.9)	Ref	0.28	–	–	–	–	
	>0–≤784	2156 (11.9)	24 (28.6)	1.88 (1.15, 3.05)						
	>784	2117 (11.7)	8 (9.5)	0.66 (0.31, 1.4)						
>63y	Never use	10,581 (67.5)	148 (66.4)	Ref	0.93					
	>0–≤784	2534 (16.2)	38 (17)	0.99 (0.69, 1.42)						
	>784	2551 (16.3)	37 (16.6)	1.02 (0.71, 1.46)						
Organophosphate insecticide										
Chlorpyrifos	Never use	18,564 (55)	191 (58.2)	Ref	0.60	Never use	19,755 (55.4)	220 (61.1)	Ref	0.78
	>0–≤455	5003 (14.8)	54 (16.5)	1.14 (0.84, 1.55)		>0–≤490	5251 (14.7)	55 (15.3)	1.04 (0.77, 1.41)	
	>455–≤1848	5165 (15.3)	33 (10.1)	0.68 (0.47, 0.99)		>490–≤1903	5406 (15.2)	38 (10.6)	0.71 (0.5, 1.01)	
	>1848	4994 (14.8)	50 (15.2)	1.12 (0.82, 1.54)		>1903	5243 (14.7)	47 (13.1)	0.99 (0.72, 1.35)	
Coumaphos	Never use	29,725 (91.2)	271 (90)	Ref	0.99	Never use	29,678 (91)	271 (90)	Ref	0.93
	>0–≤380	955 (2.9)	9 (3)	0.87 (0.45, 1.7)		>0–≤385	975 (3)	9 (3)	0.85 (0.44, 1.65)	
	>380–≤1418	979 (3)	12 (4)	1.07 (0.6, 1.91)		>385–≤1428	986 (3)	12 (4)	1.07 (0.6, 1.91)	
	>1418	938 (2.9)	9 (3)	0.99 (0.51, 1.92)		>1428	966 (3)	9 (3)	0.96 (0.49, 1.87)	
Diazinon[f]	*Never use*	*13,412 (79.2)*	*162 (81)*	Ref	0.23	Never use	13,202 (75.4)	162 (79.8)	Ref	0.11
	>0–≤328	*1194 (7.1)*	*13 (6.5)*	*0.79 (0.45, 1.40)*		>0–≤350	1443 (8.2)	13 (6.4)	0.68 (0.38, 1.21)	

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Table 4 (*continued*)

Pesticide	Lifetime days[a]	Pesticide exposure through enrollment				Pesticide exposure through Phase 2				
		No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]	Lifetime days[a]	No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]
	>328–≤1274	*1213 (7.2)*	*14 (7)*	*0.78 (0.45, 1.35)*		>350–≤1270	1476 (8.4)	16 (7.9)	0.81 (0.48, 1.36)	
	>1274	*1116 (6.6)*	*11 (5.5)*	*0.69 (0.37, 1.29)*		>1270	1391 (7.9)	12 (5.9)	0.6 (0.32, 1.12)	
Dichlorvos	Never use	29,516 (89.2)	264 (86.3)	Ref	0.06	Never use	29,409 (88.9)	264 (86.3)	Ref	0.06
	>0–≤1344	1783 (5.4)	15 (4.9)	0.79 (0.46, 1.33)		>0–≤1360	1844 (5.6)	15 (4.9)	0.79 (0.46, 1.33)	
	>1344	1773 (5.4)	27 (8.8)	1.46 (0.98, 2.19)		>1360	1832 (5.5)	27 (8.8)	1.46 (0.98, 2.19)	
Fonofos	Never use	25,838 (77.6)	240 (77.4)	Ref	0.32	Never use	25,820 (77.5)	240 (77.4)	Ref	0.32
	>0–≤455	2467 (7.4)	26 (8.4)	1.06 (0.7, 1.61)		>0–≤455	2468 (7.4)	26 (8.4)	1.06 (0.7, 1.61)	
	>455–≤1680	2526 (7.6)	24 (7.7)	0.92 (0.60, 1.42)		>455–≤1696	2538 (7.6)	24 (7.7)	0.92 (0.6, 1.41)	
	>1680	2463 (7.4)	20 (6.5)	0.80 (0.50, 1.27)		>1696	2470 (7.4)	20 (6.5)	0.8 (0.5, 1.27)	
Malathion	Never use	6436 (35.7)	76 (35)	Ref	0.08	Never use	6107 (30.4)	69 (29.2)	Ref	0.12
	>0–≤368	3832 (21.3)	53 (24.4)	1.13 (0.79, 1.61)		>0–≤384	4797 (23.9)	68 (28.8)	1.26 (0.89, 1.79)	
	>368 ≤ 1440	3948 (21.9)	46 (21.2)	0.89 (0.62, 1.29)		>384–≤1344	4603 (22.9)	47 (19.9)	0.93 (0.64, 1.35)	
	>1440	3795 (21.1)	42 (19.4)	0.75 (0.51, 1.10)		>1344	4584 (22.8)	52 (22)	0.83 (0.57, 1.2)	
Parathion	Never use	16,605 (92.1)	201 (91)	Ref	0.97	Never use	16,580 (91.9)	201 (90.5)	Ref	0.76
	>0–≤882	718 (4)	10 (4.5)	0.94 (0.49, 1.78)		>0–≤880	728 (4)	10 (4.5)	0.86 (0.44, 1.69)	
	>882	697 (3.9)	10 (4.5)	0.99 (0.52, 1.89)		>880	726 (4)	11 (5)	1.05 (0.56, 1.94)	
Phorate	Never use	11,467 (68.4)	121 (61.4)	Ref	0.55	Never use	11,523 (67.8)	122 (61.3)	Ref	0.47
	>0–≤315	1771 (10.6)	25 (12.7)	1.18 (0.75, 1.86)		>0–≤320	1818 (10.7)	25 (12.6)	1.14 (0.72, 1.79)	
	>315–≤1176	1809 (10.8)	34 (17.3)	1.61 (1.07, 2.41)		>320–≤1176	1874 (11)	35 (17.6)	1.62 (1.09, 2.41)	
	>1176	1715 (10.2)	17 (8.6)	0.84 (0.50, 1.40)		>1176	1781 (10.5)	17 (8.5)	0.8 (0.48, 1.34)	
Terbufos	Never use	19,869 (59.8)	168 (54.4)	Ref	0.50	Never use	19,649 (59.1)	168 (54)	Ref	0.53
	>0–≤646	4397 (13.2)	46 (14.9)	1.34 (0.96, 1.88)		>0–≤660	4497 (13.5)	48 (15.4)	1.35 (0.97, 1.89)	
	>646–≤2400	4536 (13.7)	54 (17.5)	1.46 (1.06, 2.00)		>660–≤2436	4623 (13.9)	53 (17)	1.39 (1.01, 1.91)	
	>2400	4411 (13.3)	41 (13.3)	1.16 (0.82, 1.65)		>2436	4480 (13.5)	42 (13.5)	1.16 (0.82, 1.64)	
Permethrin insecticide										
Permethrin (crops)	Never use	28,383 (86.5)	269 (89.4)	Ref	0.21	Never use	27,839 (84.8)	265 (88.3)	Ref	0.27
	>0–≤273	1470 (4.5)	15 (5)	1.30 (0.77, 2.2)		>0–≤288	1639 (5)	17 (5.7)	1.19 (0.7, 2.01)	
	>273–≤1080	1492 (4.5)	11 (3.7)	1.01 (0.55, 1.84)		>288–≤1117	1695 (5.2)	11 (3.7)	0.94 (0.51, 1.72)	
	>1080	1466 (4.5)	6 (2)	0.59 (0.26, 1.33)		>1117	1643 (5)	7 (2.3)	0.66 (0.31, 1.4)	
Permethrin (animals)	Never use	28,783 (86.3)	272 (88.6)	Ref	0.21	Never use	28,163 (84.3)	270 (87.9)	Ref	0.16
	>0–≤368	1574 (4.7)	11 (3.6)	0.93 (0.50, 1.70)		>0–≤392	1737 (5.2)	11 (3.6)	0.84 (0.46, 1.53)	
	>368–≤1418	1505 (4.5)	9 (2.9)	0.77 (0.40, 1.51)		>392–≤1512	1781 (5.3)	9 (2.9)	0.68 (0.34, 1.35)	
	>1418	1493 (4.5)	15 (4.9)	1.44 (0.85, 2.44)		>1512	1721 (5.2)	17 (5.5)	1.49 (0.9, 2.46)	
Fumigant										
Carbon disulfide/carbon tetrachloride [d]	Never use	17,467 (95.8)	209 (94.6)	Ref	0.74	–	–	–	–	
	>0–≤172	398 (2.2)	6 (2.7)	0.82 (0.36, 1.86)						
	>172	364 (2)	6 (2.7)	0.88 (0.39, 1.98)						
Methyl Bromide	Never use	28,072 (84.9)	274 (85.9)	Ref	0.58	Never use	28,084 (84.8)	276 (85.7)	Ref	0.52
	>0–≤320	1613 (4.9)	13 (4.1)	0.82 (0.46, 1.47)		>0–≤326	1669 (5)	14 (4.3)	0.78 (0.44, 1.41)	
	>320–≤1372	1670 (5.1)	17 (5.3)	1.03 (0.60, 1.78)		>326–≤1395	1673 (5.1)	17 (5.3)	0.98 (0.57, 1.7)	
	>1372	1706 (5.2)	15 (4.7)	0.82 (0.46, 1.48)		>1395	1696 (5.1)	15 (4.7)	0.8 (0.45, 1.42)	
Fungicide										
Benomyl	Never use	14,990 (92.8)	174 (91.6)	Ref	0.31	Never use	14,977 (92.4)	174 (90.6)	Ref	0.35
	>0–≤868	591 (3.7)	5 (2.6)	0.62 (0.24, 1.61)		>0–≤868	623 (3.8)	6 (3.1)	0.73 (0.31, 1.75)	
	>868	574 (3.6)	11 (5.8)	1.34 (0.64, 2.80)		>868	613 (3.8)	12 (6.3)	1.34 (0.64, 2.79)	
Captan	Never use	29,167 (89.8)	274 (90.7)	Ref	0.36	Never use	28,708 (88.2)	270 (88.8)	Ref	0.26
	>0–≤9	1224 (3.8)	8 (2.6)	0.83 (0.41, 1.68)		>0–≤10	1278 (3.9)	8 (2.6)	0.78 (0.39, 1.59)	
	>9–≤212	1046 (3.2)	6 (2)	0.65 (0.29, 1.46)		>10–≤540	1311 (4)	10 (3.3)	0.83 (0.44, 1.57)	
	>212	1060 (3.3)	14 (4.6)	1.27 (0.74, 2.20)		>540	1264 (3.9)	16 (5.3)	1.33 (0.80, 2.21)	
Chlorothalonil	Never use	30,547 (92.8)	293 (94.2)	Ref	0.41	Never use	30,371 (92.2)	291 (93.6)	Ref	0.47
	>0–≤1535	1132 (3.4)	7 (2.3)	0.74 (0.34, 1.61)		>0–≤1613	1245 (3.8)	7 (2.3)	0.71 (0.32, 1.55)	
	>1535	1221 (3.7)	11 (3.5)	1.29 (0.66, 2.56)		>1613	1312 (4)	13 (4.2)	1.41 (0.75, 2.66)	
Maneb[g]	-	–	–	–		Never use	15,464 (92.3)	186 (93)	Ref	0.34
						>0–≤1268	660 (3.9)	8 (4)	0.69 (0.31, 1.53)	
						>1268	636 (3.8)	6 (3)	0.59 (0.25, 1.4)	

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Table 4 (*continued*)

Pesticide	Lifetime days[a]	Pesticide exposure through enrollment				Pesticide exposure through Phase 2				
		No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]	Lifetime days[a]	No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]
Metalaxyl	Never use	14,301 (81.6)	177 (82.3)	Ref	0.14	Never use	14,239 (79.1)	175 (80.6)	Ref	0.47
	>0–≤312	1060 (6.1)	17 (7.9)	1.27 (0.77, 2.11)		>0–≤312	1241 (6.9)	18 (8.3)	1.26 (0.76, 2.08)	
	>312–≤1568	1094 (6.2)	15 (7)	1.18 (0.66, 2.11)		>312–≤1488	1286 (7.1)	15 (6.9)	1.17 (0.64, 2.13)	
	>1568	1061 (6.1)	6 (2.8)	0.53 (0.22, 1.27)		>1488	1240 (6.9)	9 (4.1)	0.79 (0.38, 1.65)	
Herbicide										
Alachlor	Never use	15,100 (45.7)	127 (41.9)	Ref	0.80	Never use	14,974 (45.3)	124 (40.9)	Ref	0.73
	>0–≤809	5925 (18)	63 (20.8)	1.11 (0.82, 1.52)		>0–≤809	6011 (18.2)	65 (21.5)	1.15 (0.85, 1.57)	
	>809–≤3132	6056 (18.3)	55 (18.2)	0.95 (0.69, 1.30)		>809–≤3145	6121 (18.5)	56 (18.5)	0.97 (0.7, 1.33)	
	>3132	5927 (18)	58 (19.1)	1.07 (0.78, 1.46)		>3145	5977 (18.1)	58 (19.1)	1.09 (0.8, 1.5)	
Butylate	Never use	11,964 (71.9)	144 (72.7)	Ref	0.22	Never use	13,263 (73)	164 (73.9)	Ref	0.24
	>0–≤473	1564 (9.4)	25 (12.6)	1.26 (0.81, 1.95)		>0–≤473	1626 (9)	26 (11.7)	1.26 (0.81, 1.95)	
	>473–≤1519	1583 (9.5)	16 (8.1)	0.85 (0.50, 1.45)		>473–≤1512	1659 (9.1)	18 (8.1)	0.91 (0.54, 1.53)	
	>1519	1531 (9.2)	13 (6.6)	0.73 (0.41, 1.30)		>1512	1619 (8.9)	14 (6.3)	0.73 (0.41, 1.3)	
Chlorimuron Ethyl	Never use	12,384 (68)	163 (73.4)	Ref	0.44	Never use	12,187 (65.4)	162 (72.6)	Ref	0.22
	>0–≤245	1930 (10.6)	25 (11.3)	1.22 (0.80, 1.87)		>0–≤263	2140 (11.5)	30 (13.5)	1.28 (0.85, 1.9)	
	>245–≤784	1977 (10.9)	17 (7.7)	0.80 (0.49, 1.33)		>263–≤817	2169 (11.6)	15 (6.7)	0.69 (0.4, 1.18)	
	>784	1910 (10.5)	17 (7.7)	0.85 (0.52, 1.41)		>817	2127 (11.4)	16 (7.2)	0.77 (0.46, 1.28)	
Dicamba	Never use	15,344 (47.7)	141 (48)	Ref	0.33	Never use	14,269 (44.3)	131 (44.4)	Ref	0.13
	>0–≤564	5548 (17.2)	50 (17)	0.90 (0.63, 1.28)		>0–≤694	5897 (18.3)	58 (19.7)	0.99 (0.7, 1.41)	
	>564–≤2184	5761 (17.9)	46 (15.6)	0.81 (0.56, 1.17)		>694–≤2380	6126 (19)	43 (14.6)	0.83 (0.56, 1.22)	
	>2184	5524 (17.2)	57 (19.4)	1.11 (0.79, 1.56)		>2380	5950 (18.5)	63 (21.4)	1.25 (0.88, 1.77)	
EPTC	Never use	26,190 (79.7)	249 (83)	Ref	0.74	Never use	26,155 (79.6)	249 (83)	Ref	0.73
	>0–≤315	2215 (6.7)	19 (6.3)	0.93 (0.58, 1.49)		>0–≤315	2222 (6.8)	19 (6.3)	0.93 (0.58, 1.49)	
	>315–≤1181	2245 (6.8)	14 (4.7)	0.67 (0.39, 1.15)		>315–≤1190	2261 (6.9)	14 (4.7)	0.66 (0.39, 1.14)	
	>1181	2192 (6.7)	18 (6)	0.97 (0.60, 1.57)		>1190	2205 (6.7)	18 (6)	0.97 (0.6, 1.57)	
Glyphosate	Never use	8307 (23.3)	86 (24.2)	Ref	0.09	Never use	5247 (14.8)	62 (17.5)	Ref	0.10
	>0–≤677	8996 (25.2)	106 (29.8)	1.17 (0.88, 1.55)		>0–≤970	9965 (28)	132 (37.2)	1.21 (0.88, 1.65)	
	>677–≤2604	9313 (26.1)	91 (25.6)	0.99 (0.73, 1.33)		>970–≤3352	10,318 (29)	84 (23.7)	0.92 (0.64, 1.34)	
	>2604	9015 (25.3)	73 (20.5)	0.85 (0.62, 1.17)		>3352	10,018 (28.2)	77 (21.7)	0.88 (0.62, 1.25)	
Imazethapyr	Never use	17,941 (55.5)	173 (58.6)	Ref	0.38	Never use	17,152 (53.1)	169 (56.9)	Ref	0.64
	>0–≤341	4874 (15.1)	42 (14.2)	1.00 (0.70, 1.45)		>0–≤403	5007 (15.5)	47 (15.8)	1.03 (0.72, 1.47)	
	>341–≤1008	4752 (14.7)	41 (13.9)	1.05 (0.73, 1.52)		>403–≤1176	5205 (16.1)	42 (14.1)	0.92 (0.63, 1.35)	
	>1008	4733 (14.7)	39 (13.2)	1.18 (0.81, 1.72)		>1176	4964 (15.4)	39 (13.1)	1.11 (0.76, 1.62)	
Metolachlor	Never use	17,519 (52.8)	182 (60.1)	Ref	0.79	Never use	16,273 (49)	174 (57.4)	Ref	0.71
	>0–≤720	5255 (15.8)	35 (11.6)	0.67 (0.46, 0.96)		>0–≤760	5600 (16.9)	41 (13.5)	0.72 (0.51, 1.03)	
	>720–≤2688	5322 (16)	44 (14.5)	0.84 (0.6, 1.17)		>760–≤2700	5776 (17.4)	44 (14.5)	0.78 (0.55, 1.1)	
	>2688	5079 (15.3)	42 (13.9)	0.90 (0.64, 1.26)		>2700	5585 (16.8)	44 (14.5)	0.89 (0.63, 1.25)	
Paraquat	Never use	15,305 (84.1)	188 (82.5)	Ref	0.45	Never use	15,216 (81.9)	188 (81.7)	Ref	0.36
	>0–≤289	961 (5.3)	13 (5.7)	1.03 (0.58, 1.81)		>0–≤308	1111 (6)	13 (5.7)	0.92 (0.51, 1.63)	
	>289–≤1232	975 (5.4)	18 (7.9)	1.42 (0.86, 2.33)		>308–≤1308	1135 (6.1)	20 (8.7)	1.49 (0.92, 2.41)	
	>1232	960 (5.3)	9 (3.9)	0.74 (0.37, 1.49)		>1308	1113 (6)	9 (3.9)	0.69 (0.34, 1.38)	
Pendimethalin	Never use	11,440 (62.9)	154 (68.1)	Ref	0.25	Never use	10,685 (53.9)	145 (60.9)	Ref	0.57
	>0–≤341	2262 (12.4)	32 (14.2)	1.13 (0.77, 1.66)		>0–≤378	3003 (15.2)	38 (16)	1.1 (0.77, 1.57)	
	>341–≤1320	2263 (12.4)	23 (10.2)	0.90 (0.58, 1.40)		>378 ≤ 1232	3114 (15.7)	32 (13.4)	0.97 (0.65, 1.44)	
	>1320	2227 (12.2)	17 (7.5)	0.76 (0.46, 1.26)		>1232	3005 (15.2)	23 (9.7)	0.89 (0.57, 1.39)	
Petroleum	Never use	14,257 (78.9)	184 (83.6)	Ref	0.72	Never use	14,169 (78.1)	183 (82.8)	Ref	0.59
	>0–≤515	1266 (7)	9 (4.1)	0.57 (0.29, 1.11)		>0–≤495	1317 (7.3)	11 (5)	0.67 (0.36, 1.23)	
	>515–≤2500	1286 (7.1)	13 (5.9)	0.91 (0.52, 1.61)		>495–≤2408	1355 (7.5)	13 (5.9)	0.88 (0.5, 1.55)	
	>2500	1261 (7)	14 (6.4)	0.89 (0.52, 1.53)		>2408	1312 (7.2)	14 (6.3)	0.85 (0.49, 1.47)	
Trifluralin	Never use	14,464 (45.7)	116 (40.4)	Ref	0.07	Never use	14,106 (44.5)	113 (39.4)	Ref	0.10
	>0–≤1008	5653 (17.9)	61 (21.3)	1.40 (1.01, 1.95)		>0–≤1046	5779 (18.2)	64 (22.3)	1.42 (1.02, 1.97)	
	>1008–≤3828	5877 (18.6)	47 (16.4)	1.05 (0.73, 1.52)		>1046–≤3906	6144 (19.4)	49 (17.1)	1.05 (0.73, 1.52)	
	>3828	5669 (17.9)	63 (22)	1.50 (1.06, 2.11)		>3906	5672 (17.9)	61 (21.3)	1.48 (1.04, 2.1)	
2,4-D	Never use	8108 (22.9)	72 (20.5)	Ref	0.52	Never use	6928 (19.5)	67 (18.9)	Ref	0.52

(*continued on next page*)

Table 4 (*continued*)

Pesticide	Lifetime days[a]	Pesticide exposure through enrollment				Pesticide exposure through Phase 2				
		No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]	Lifetime days[a]	No PD, n (%)	PD, n (%)	HR (95% CI)[b]	p[c]
	>0–≤1269	8944 (25.3)	84 (23.9)	1.07 (0.78, 1.47)		>0–≤1440	9486 (26.6)	97 (27.3)	1.08 (0.79, 1.49)	
	>1269–≤5104	9303 (26.3)	97 (27.6)	1.04 (0.76, 1.43)		>1440–≤5394	9767 (27.4)	86 (24.2)	0.87 (0.62, 1.22)	
	>5104	9035 (25.5)	99 (28.1)	0.96 (0.7, 1.31)		>5394	9432 (26.5)	105 (29.6)	0.93 (0.67, 1.29)	
2,4,5-T[d]	Never use	13,328 (80.9)	143 (71.9)	Ref	0.71	–	–	–	–	
	>0–≤289	1068 (6.5)	20 (10.1)	1.21 (0.75, 1.95)						
	>289–≤1006	1069 (6.5)	20 (10.1)	1.27 (0.78, 2.05)						
	>1006	1007 (6.1)	16 (8)	1.11 (0.65, 1.89)						
Atrazine	Never use	9709 (27.3)	95 (27)	Ref	0.64	Never use	8473 (23.8)	87 (24.7)	Ref	0.53
	>0–≤1050	8525 (23.9)	87 (24.7)	1.14 (0.84, 1.54)		>0–≤1221	8960 (25.2)	97 (27.6)	1.17 (0.86, 1.59)	
	>1050–≤4456	8826 (24.8)	85 (24.1)	0.99 (0.73, 1.34)		>1221–≤4666	9250 (26)	83 (23.6)	0.95 (0.69, 1.3)	
	>4456	8556 (24)	85 (24.1)	0.99 (0.73, 1.34)		>4666	8943 (25.1)	85 (24.1)	0.98 (0.72, 1.34)	
Cyanazine	Never use	19,018 (57.2)	174 (57.4)	Ref	0.79	Never use	18,910 (56.9)	173 (57.1)	Ref	0.66
	>0–≤560	4706 (14.2)	40 (13.2)	0.84 (0.59, 1.20)		>0–≤588	4808 (14.5)	39 (12.9)	0.81 (0.57, 1.17)	
	>560–≤2268	4850 (14.6)	45 (14.9)	0.91 (0.64, 1.28)		>588–≤2279	4792 (14.4)	46 (15.2)	0.94 (0.66, 1.32)	
	>2268	4665 (14)	44 (14.5)	1.00 (0.71, 1.42)		>2279	4716 (14.2)	45 (14.9)	1.03 (0.73, 1.45)	
Metribuzin	Never use	9599 (59.7)	115 (61.5)	Ref	0.33	Never use	9513 (58)	115 (60.8)	Ref	0.37
	>0–≤319	2148 (13.4)	30 (16)	1.10 (0.71, 1.69)		>0–≤341	2358 (14.4)	31 (16.4)	1.01 (0.65, 1.56)	
	>319–≤1024	2193 (13.6)	21 (11.2)	0.77 (0.47, 1.27)		>341–≤1054	2259 (13.8)	21 (11.1)	0.75 (0.46, 1.23)	
	>1024	2128 (13.2)	21 (11.2)	0.81 (0.49, 1.33)		>1054	2260 (13.8)	22 (11.6)	0.81 (0.50, 1.34)	

Abbreviation: 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; 2,4,5-T,P, 2-(2,4,5-trichlorophenoxy) propionic acid; CI, Confidence Intervals; DDT, Dichlorodiphenyltrichloroethane; EPTC, S-Ethyl dipropylthiocarbamate; HR, Hazard Ratio.

[a] Tertile cut-off and n (%) may differ between Phase 1 and Phase 2 exposure because of difference in exposure information and missingness.

[b] HR adjusted for sex, state of residence, smoking status, education, and ever-use of correlated pesticides (other pesticides whose ever-use variable had Spearman correlation ≥ 0.40 with the ever-use variable of the target pesticide).

[c] P-value for test for trend.

[d] HR not presented if, at Phase 2, pesticide exposure since enrollment was not reported.

[e] HR allowed to vary by the median age (i.e., 63 years) for pesticides that did not meet proportional hazards assumption ($p \leq 0.10$).

[f] Proportional hazards assumption not met for those in italics, but there was no adequate sample size to provide stratified estimates by the median age.

[g] HR not presented as there were less than 5 exposed cases.

Our study also suggests that use of chemical resistant gloves may have conferred some protection against PD in pesticide applicators using certain herbicides. Chemical resistant gloves, but not other types of gloves, have been shown in the AHS to offer protection from pesticide exposure (Hines et al., 2011; Thomas et al., 2010). In FAME, associations of several pesticides including permethrin and paraquat with PD risks were greater in those who used chemical-resistant gloves less than 50% of the time compared to those who used >50% (Furlong et al., 2015).

4.1. Limitations and strengths

Our study has several limitations. First, pesticide-use data were self-reported; thus, some exposure misclassification is likely. However, AHS farmers have been shown to report both reliable and valid pesticide usage (Blair et al., 2002; Hoppin et al., 2002); for lifetime exposures, we used exposure intensity, which correlates better with urinary biomarkers of pesticides than uncorrected days of use (Coble et al., 2011; Hines et al., 2011). Due to our prospective design, exposure misclassification was likely non-differential for PD. Non-differential misclassification might have biased effect estimates towards the null for binary pesticide-use variables; but, for polytomous categories, directionality of bias is uncertain (Rothman et al., 2008).

Second, in the current analyses, although we incorporated pesticide usage through the first follow-up for applicators, we could not do so for spouses and we could not account for more proximal exposures for applicators because data were not available for all participants due to cohort attrition. The time duration from Phase 2 (when exposures were updated for this analysis) to Phase 4 (i.e., end of follow-up) was 13 years on average. Failure to account for exposure occurring during this window could have heightened exposure misclassification, for those pesticides that are still on the market.

Third, our effort to validate all potential PD cases using medical records suffered from low response from both participants (or their proxies) and their physicians, so we relied on PD self-report. We attempted to minimize potential PD misclassification by restricting analysis to individuals providing consistent responses on PD across surveys and for those with relevant questionnaire data, restricting to cases with supporting data on neurological symptoms and medication use. We did find that those medical records we obtained were in high agreement with self-reported PD. Further, agreement between PD self-report and clinical diagnostic evaluation was found to be high in FAME (84%) (Tanner et al., 2011) as well in other studies (Jain et al., 2015), indicating PD self-reports are, in general, reasonably reliable. Furthermore, we observed reduced PD risk in smokers [age and sex adjusted HR: 0.75 (95%CI: 0.61, 0.91) for former smoking and 0.55 (95%CI: 0.38, 0.81) for current smoking] in the current study, which is consistent with prior literature (Hernan et al., 2002) and thus indirectly supports the validity of PD self-reports in the AHS.

Fourth, we were unable to account for possible PD in participants who were lost to follow-up, although we were able to identify participants who had PD recorded on their death certificates (but did not report PD in surveys). We included such cases in our analysis if their proxy provided adequate information in the validation screener to support a PD diagnosis. We had similar results in analyses using inverse probability weighting to make inference on all enrolled participants. Nevertheless, we cannot completely rule out selection bias due to loss-to-follow up or bias due to selective mortality before enrollment resulting from higher pesticide exposures. Fifth, we found inverse associations for some pesticides which may be due to reverse causality – for instance, if individuals with symptomatic but undiagnosed PD accumulate less exposure due to reduced farming activities compared to those individuals that are "healthy" and continue farming. We know of no reason why this reverse causality would apply only to certain pesticides.

Sixth, we also did not adjust for multiple comparisons given the exploratory nature of our study and therefore some of the observed associations may be false positives and thus our findings should be interpreted with caution. Seventh, participants were exposed to multiple pesticides. Although we adjusted for several correlated pesticides, we cannot rule out lack of complete control of confounding due to other pesticides. Lastly, our current analytical approach focusing on a single exposure fails to account for the overall PD risk associated with multiple pesticide exposures. Pesticide use in the AHS is not easily addressed using current methods for the analysis of chemical mixtures. Applicators report a lifetime of use, with one or two possibly different pesticides being used in any given year. Chemicals used may have changed over time in relation to specific crops planted, environmental conditions, changes in availability of banned or restricted chemicals, pesticide costs and economic constraints, and much more. The development and application of new methods to address this complex and unique mixture situation is warranted.

Countering these limitations, the strengths for the current investigation include large sample size, prospective design, long-term follow-up, comprehensive information on lifetime use of pesticides, and detailed information on PD risk factors. Although we found evidence of increased PD risk for a few pesticides, most pesticides were not associated with PD nor, for the most part, were pesticides/groups that were previously implicated for PD. Continued research on pesticide-PD risk that can focus on specific chemicals is important because of continued widespread use of pesticides worldwide.

Credit author statement

Srishti Shrestha and Dale P. Sandler conceptualized the investigation, led the analysis and prepared the first draft of the manuscript. Srishti Shrestha and Marie Richards-Barber conducted data analysis. Dale P. Sandler provided supervision and funding acquisition. Dale P. Sandler, Christine G. Parks, and Laura E. Beane Freeman were involved in project administration. All the authors were involved in data interpretation and in reviewing, critiquing, and editing the manuscript, and provided final manuscript approval.

Funding

This work was supported by the Intramural Research Program of the National Institute of Health, National Institute of Environmental Health Sciences (Z01-ES-049030) and National Cancer Institute (Z01-CP-010119).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank Drs. Freya Kamel and Caroline Tanner for their assistance with Parkinson's disease case validation and Drs. Katie O'Brien, Caroline Tanner, and Emily Werder for thoughtful review of the draft of the manuscript. We used AHS data releases AHSREL20170600, P1REL20170100, P2REL20170100, P3REL201808_00, and P4REL20180904 for the analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2020.110186.

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Scand J Work Environ Health 2012;38(1):65-69
Published online: 17 Jun 2011, Issue date: Jan 2012

doi:10.5271/sjweh.3176

Parkinson's disease among gardeners exposed to pesticides – a Danish cohort study

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Refers to the following texts of the Journal: 2000;26(4):359-362 2007;33(6):465-469 1986;12(2):97-107 2011;37(5):427-436

The following article refers to this text: 2017;43(3):197-209

Key terms: cohort study; Denmark; epidemiology; gardener; gardening; occupational health; occupational health; Parkinson's disease; pesticide

This article in PubMed: www.ncbi.nlm.nih.gov/pubmed/21687921

Print ISSN: 0355-3140 Electronic ISSN: 1795-990X

Original article

Scand J Work Environ Health 2012;38(1):65–69. doi:10.5271/sjweh.3176

Parkinson's disease among gardeners exposed to pesticides – a Danish cohort study

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Kenborg L, Lassen CF, Lander F, Olsen JH. Parkinson's disease among gardeners exposed to pesticides – a Danish cohort study. *Scand J Work Environ Health.* 2012;38(1):65–69. doi:10.5271/sjweh.3176

Objective Several studies have found positive associations between exposure to pesticides and Parkinson's disease. As Danish gardeners have had frequent, intensive exposure to pesticides, the aim of this study was to investigate their risk for Parkinson's disease.

Methods The cohort was comprised of 3124 male members of the Danish Union of Gardeners on 1 April 1975. Hospital register data were used to follow them for a primary diagnosis of Parkinson's disease during 1977–2008 and to calculate standardized hospitalization rate ratios (SHR) for this disease among gardeners and the general Danish population for comparison. Data from the Danish Cancer Registry were used to calculate standardized incidence rate ratios (SIR) for smoking-related cancers among gardeners and the general population.

Results The SHR for Parkinson's disease among gardeners was close to that of the general population [1.14, 95% confidence interval (95% CI) 0.76–1.65]. In a birth cohort analysis, a downward trend was observed, with the highest risk among gardeners born before 1915 (SHR 1.55, 95% CI 0.77–2.77). The SIR for smoking-related cancers did not differ from that of the general population.

Conclusion The results indicate a weak but dose-related association between exposure to pesticides and risk for Parkinson's disease; however, the results were based on 28 cases and the possibility of no association cannot be ruled out.

Key terms Denmark; epidemiology; gardening; occupational health.

Parkinson's disease is a common neurodegenerative disorder engendered by loss of dopaminergic neurons in the substantia nigra pars compacta of the brain (1). The etiology of the disease is probably a complex interplay of aging, genetics, and the environment (2), except for a small proportion of cases that can be attributed to gene mutations (3). It has been suggested that environmental factors might cause a direct effect, such as a toxic agent causing cell death, or an indirect effect through DNA expression or repair processes (4).

Gardeners are exposed to a wide range of environmental factors, including pesticides, some of which have shown to be neurotoxic to humans (5). Although several studies have addressed an association between working in occupations with exposure to pesticides and the risk for Parkinson's disease, they mainly focused on farmers. No study has focused solely on professional gardeners. The aim of this study was to examine the risk for Parkinson's disease in a cohort of Danish gardeners exposed to pesticides.

Methods

Study population

The cohort comprised 3124 professional male gardeners identified from a cross-sectional file covering all members of the Danish Union of Gardeners. The gardeners worked primarily in greenhouses, nursery gardens, or public parks, gardens and cemeteries, and the majority were regularly exposed to a mixture of pesticides during their active working life (6). All the members of

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the cohort had a valid personal identification number, were working on 1 May 1975, and alive and living in Denmark on 1 January 1977 (baseline).

Parkinson's disease and cancer

We linked the cohort members with the files of the Danish National Hospital Register to identify those who had been diagnosed with Parkinson's disease (ICD-8 code 342 and ICD-10 code G20) when they were aged ≥35 years. The Hospital Register contains data on all hospital admissions for somatic diseases in Denmark since 1977; information on outpatients and emergency ward contacts was added to the Register in 1995 (7). Only in- and outpatients with a primary diagnosis of Parkinson's disease were identified. For patients discharged more than once with this diagnosis, only the first record was retained and used as the date of diagnosis.

We also linked the study population to the Danish Cancer Registry to identify incident cases of the major smoking-related cancers [ie, of the lung (ICD-7 162; ICD-10 C33–C34 and C39), larynx (ICD-7 161; ICD-10 C32) and urinary bladder (ICD-7 181; ICD-10 C67)] (8). As previous studies have repeatedly found a protective effect of smoking on the risk for Parkinson's disease (9, 10), we included these cancers as proxies for smoking among gardeners.

Statistical analysis

Gardeners were followed for a primary diagnosis of Parkinson's disease from 1 January 1977 or when they reached 35 years until the earliest diagnosis of this disease, death, emigration, loss to follow-up, or 31 December 2008. We compared the observed numbers of first hospital contacts for a primary diagnosis of Parkinson's disease among gardeners with those expected from age-, gender- and calendar-period-specific rates for the general population in 1977–2008. The rates were calculated by multiplying the numbers of person–years for cohort members by the in- and outpatient rates for a primary diagnosis of Parkinson's disease of Danish men in 5-year age groups and calendar periods.

Similarly, we calculated the incidences of cancers of the lung, larynx and urinary bladder among the gardeners and compared them with those expected from the age- and calendar-period-specific incidence rates in the general male population. The observed and expected numbers were derived from the Danish Cancer Registry for the period 1977–2008.

Standardized hospitalization rate ratios (SHR) for Parkinson's disease were computed, as were standardized incidence rate ratios (SIR) for cancers of the lung, larynx, and urinary bladder. For each risk estimate, the associated 95% confidence interval (95% CI) was calculated on the assumption of a Poisson distribution of the observed numbers.

As the working environment of gardeners and their exposure to pesticides has changed during the twentieth century, we also conducted a birth cohort analysis (gardeners born in 1900–1914, 1915–1934, or 1935–1958) to evaluate the presence of a time trend for risk of Parkinson's disease. Gardeners in the early birth cohort were ≥30 years old in the period after World War II, when pesticide use became widespread. As heavy use began to decline in the 1960s, gardeners in the late birth cohort were not exposed to the same extent as their counterparts in the two earlier birth cohorts (11).

Results

The cohort consisted of 3124 gardeners born between 1900–1958, who accrued 68 323 person–years of follow-up (range, 4 days to 32 years), with an average of 24.7 years (table 1).

During follow-up, 28 of the gardeners were hospitalized with a primary diagnosis of Parkinson's disease, while 24.5 were expected from the rates for the general population, yielding an overall slightly increased SHR of 1.14 (95% CI 0.76–1.65) (table 1). When we stratified the analysis on the three birth cohorts, the risk estimate was 1.55 (95% CI 0.77–2.77) for the early birth cohort (table 2).

In the analysis of smoking-related cancers among the gardeners, the risk estimate for lung cancer was close to that of the Danish population (SIR 1.03, 95% CI 0.86–1.20) (table 3).

Discussion

In this follow-up study of 3124 professional gardeners, the overall risk for a first hospitalization for Parkinson's disease was close to that of the general Danish population. In the birth cohort most intensively exposed to pesticides, we observed 11 cases of Parkinson's disease when 7.5 were expected, a difference that did not reach statistical significance.

This is the first study, to our knowledge, to investigate the risk for Parkinson's disease among gardeners. Several studies have found positive associations between farming and Parkinson's disease (12–16), including a Danish study, in which men working in agriculture and horticulture had a significantly higher risk for a first hospital admission for Parkinson's disease (SHR 132, 95% CI 111–156) than the general Danish population (17). It has been suggested that the increased risks of farmers

Table 1. Selected characteristics of 3124 Danish professional gardeners

Characteristics	Number	%
Period of birth		
1900–1914	501	16.0
1915–1934	1185	37.9
1935–1958	1438	46.0
Censoring of follow-up due to		
Parkinson's disease	28	0.9
Emigration / disappeared	18	0.6
Dead	1529	48.9
End of follow-up, 31 December 2008	1549	49.6
Year of Parkinson's disease diagnosis		
1977–1979	1	3.6
1980–1989	12	42.9
1990–1999	12	42.9
2000–2006	3	10.7
Diagnosis of Parkinson's disease obtained from		
Outpatient clinic	4	14.3
Inpatient clinic	24	85.7

Table 2. Standardized hospitalization rate ratios (SHR) and 95% confidence intervals (95% CI) for Parkinson's disease among 3124 Danish professional gardeners, by birth cohort

Birth cohort	Observed cases	SHR	95% CI
All	28	1.14	0.76–1.65
1900–1914	11	1.55	0.77–2.77
1915–1934	16	1.15	0.66–1.87
1935–1958	1	0.28	0.00–1.58

Table 3. Standardized incidence rate ratios (SIR) and 95% confidence intervals (95% CI) for cancer of the lung, urinary bladder, and larynx among 3124 Danish professional gardeners

Site of cancer	Observed cases	SIR	95% CI
Lung	139	1.02	0.86–1.20
Larynx	9	0.72	0.33–1.37
Urinary bladder	59	0.82	0.62–1.05

and agricultural workers are related to exposure to pesticide (17, 18), and this hypothesis has been examined in several recent epidemiological studies, most of which found positive associations (18–27), while few were unable to find such effects (28–30). Four of the studies were cohort studies (18, 25–27), while the remaining had a case–control design. However, the results of the case–control studies might have been affected by differential recall by case status as suggested in a recent study of the potential for recall bias in case–control studies of pesticides and Parkinson's disease (31).

The exposure of Danish gardeners to pesticides was described by the Danish National Environmental Board (32) and in studies of their cancer risk (6, 11), showing that, in Denmark, gardeners have had more frequent and intense exposure to pesticides than any other occupational group (11), including farmers who usually spray crops only a few times a year. Furthermore, as most Danish male gardeners stay in the trade from a young age until retirement, they are considered to constitute a very stable job group. When the cohort of gardeners was established for the studies of cancer risks, it was estimated that one third had been directly exposed to pesticides, while the remainder has been indirectly exposed by skin contact with newly sprayed plants or by inhalation when working in newly sprayed areas (6). Several of the pesticides used by Danish gardeners have shown to be neurotoxic to humans, including organochlorines [eg, dieldrin, dichlorodiphenyltrichloroethane (DDT) and lindane], paraquat, organophosphates (eg, malathion) and dithiocarbamates (eg, maneb) (5, 11, 30, 33). Gardeners in the early birth cohort (born before 1915) were potentially exposed to the highly toxic pesticides introduced after World War II, which might have resulted in the observed elevated risks for certain neoplasms, such as soft tissue sarcoma and leukemia, found in an earlier study (11).

The strengths of our study include the long follow-up time (up to 32 years), complete follow-up of the cohort members, and ascertainment of diagnoses of Parkinson's disease and smoking-related cancers from national registers, thus minimizing the possibility of bias in selection of study subjects or reporting disease outcomes. The potential limitations of the study include the finding that, although Parkinson's disease is a common disorder, only 28 of the gardeners had a primary diagnosis of this disease during follow-up. This limited the statistical power of the study, especially for assessing trends in risks across birth cohorts. Furthermore, we did not have individual information on exposure to pesticides or smoking habits. Cigarette smoking has repeatedly been associated with a decreased risk for Parkinson's disease. In the analysis of smoking-related cancers, however, the risk for lung cancer did not differ from that of the general population, although the risk for urinary bladder and larynx cancer was slightly decreased, indicating neutral or slightly reduced smoking frequencies among gardeners.

The identification of cases with Parkinson's disease from a hospital register might have introduced some degree of disease misclassification, because a smaller unknown percentage of patients with secondary parkinsonism or symptomatic related diseases are discharged with a Parkinson's disease diagnosis. To minimize the inclusion of misdiagnosed gardeners, we only included those who had a primary diagnosis of Parkinson's disease during follow-up as this diagnosis is considered to be more accurate and reliable than a supplementary diagnosis. A previous study of patients with Parkinson's disease in Denmark showed that patients with such a diagnosis more often are diagnosed in a neurological department compared to patients with a supplementary diagnosis (34). We also lacked information on date of

first symptoms. Most patients with Parkinson's disease begin drug treatment months to years before they are hospitalized with a primary diagnosis of Parkinson's disease (34), indicating a delay in diagnosis when solely identifying cases in a hospital register. Furthermore, when patients in the early phase of the disease are not in contact with either in- or outpatient clinics, less severe cases of Parkinson's disease were potentially not detected during follow-up. However, this also applies to the general Danish population, thus leading to non-differential disease misclassification.

The gardeners were followed for Parkinson's disease from 1977 (the same year the Danish National Hospital Register was instituted), so some prevalent cases might have been registered in the first years. However, when we lagged the analysis by excluding the first three years of follow-up (1977–1979), the overall risk estimate changed only slightly (SHR 1.17, 95% CI 0.77–1.70).

Since all the gardeners in the cohort were working at the time of inclusion, they constitute a healthier group than the average population, which includes both the unemployed and invalids. Parkinson's disease mainly affects older people, thus it is unlikely that the disease has influenced employment earlier in life, but might have caused a selection out of the workforce due to the physical limitations of the movement disorder. This healthy worker survivor effect tends to underestimate an exposure–disease relationship, thus potentially biasing the risk estimate toward the null. However, the strength of the healthy worker effect tends to diminish with length of time since entry (35), so the long follow-up time in this study might have weakened the effect, if there was any.

In conclusion, the study indicates a weak but dose–related association between exposure to pesticides and Parkinson's disease. Further, larger studies are warranted to clarify whether gardeners exposed to pesticides have a higher risk for Parkinson's disease.

Acknowledgments

We thank Andrea Meersohn, Institute of Cancer Epidemiology, Danish Society, Copenhagen, Denmark for conducting the statistical analyses.

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Received for publication: 11 March 2011

Productie 24

Toxicology Letters 230 (2014) 85–103

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases—A mechanistic approach

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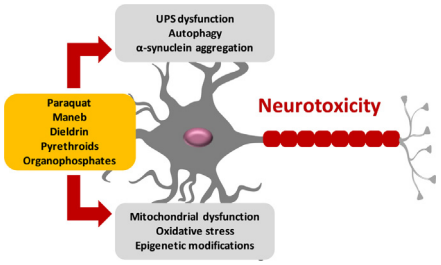
HIGHLIGHTS

- The review provides new information regarding the neurotoxicity mechanisms of herbicides and pesticides.
- New perspectives concerning chronic pesticide exposure with amyotrophic lateral sclerosis.
- Novel information regarding paraquat exposure and Parkinson's disease.
- New insights regarding chronic pesticide exposure and Alzheimer's disease.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Available online 3 February 2014

Keywords:
Pesticides
Paraquat
Parkinson's disease
Alzheimer's disease
Amyotrophic lateral sclerosis
Neurodegenerative diseases

ABSTRACT

The etiology of most neurodegenerative disorders is multifactorial and consists of an interaction between environmental factors and genetic predisposition. The role of pesticide exposure in neurodegenerative disease has long been suspected, but the specific causative agents and the mechanisms underlying are not fully understood. For the main neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis there are evidences linking their etiology with long-term/low-dose exposure to pesticides such as paraquat, maneb, dieldrin, pyrethroids and organophosphates. Most of these pesticides share common features, namely the ability to induce oxidative stress, mitochondrial dysfunction, α-synuclein fibrillization and neuronal cell loss. This review aims to clarify the role of pesticides as environmental risk factors in genesis of idiopathic PD and other neurological syndromes. For this purpose, the most relevant epidemiological and experimental data is highlighted in order to discuss the molecular mechanisms involved in neurodegeneration.

1. Introduction

The World Health Organization currently estimates that around a billion people worldwide are affected by a neurodegenerative disease (WHO, 2006). As aging corresponds to the greatest risk factor for neurodegeneration, the prevalence of neurological disorders is expected to increase dramatically in next few years due to

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http://dx.doi.org/10.1016/j.toxlet.2014.01.039

the higher life expectancy worldwide (Brown et al., 2005). Another risk factor for neurodegeneration, alongside with the aging process, is long-term/low-dose pesticide exposure. The role of pesticide exposure in the genesis of neurodegenerative diseases has been especially scrutinized for Parkinson's disease (PD) (Franco et al., 2010).

PD is a progressive movement disorder characterized by progressive bradykinesia (slowness of voluntary movement), rigidity, rest tremor, and postural disturbances. Nonetheless, it is also increasingly recognized that non-motor symptoms, including autonomic and cognitive impairment, sleep disturbances, olfactory dysfunction, and depression occur in PD patients, and these features are probably due to the spread of the pathology beyond the basal ganglia (Shulman et al., 2011). The PD's motor manifestations are attributed to the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc) resulting in secondary dysfunction of the basal ganglia, which are involved in the initiation and execution of movements (Shulman et al., 2011). The subcellular hallmarks of PD are the intraneuronal inclusions of various structures consisting mostly of fibrillar α-synuclein (Lewy bodies and Lewy neurites) (Halliday et al., 2011).

The etiology of PD is currently unknown but it is assumed that there is a significant non-genetic contribution. It seems that the disease results from combination and accumulation of environmental exposures, and complex gene–environment interactions sustained by the slow and progressive development during aging (Cannon and Greenamyre, 2011; Dinis-Oliveira et al., 2006). Most of the forms of PD are idiopathic, but approximately 10–30% of the cases have a familial history, and in a minority of them the disease follows a Mendelian inheritance pattern. The disease is characterized by an early-onset (typically under 40 years) and so far 15 PD loci (PARK1-15) and 11 genes for PARK loci, specially *a-synuclein, leucine-rich repeat kinase 2*, *parkin*, *PTEN-induced putative kinase 1*, *DJ-1*, and *ATP13A2* have been described to cause typical forms of inherited PD or parkinsonian syndromes (Coppede, 2012). Parkinsonism is often observed as one of the symptoms in other monogenic diseases, when mutations in non-PARK loci (*MAPT*, *SCA1*, *SCA2*, *spatacsin*, *POLG1*) occur. In sporadic PD, genetic polymorphisms in four loci (*SNCA*, *MAPT*, *GBA* and *LRRK2*) are considered strong risk factors (Coppede, 2012). It is consensual that both etiologies share the same pathological pathways. SNpc is highly sensitive to diverse genetic, cellular and environmental factors that independently or concomitantly cause cell death over time. For instance, evidence suggests that mitochondrial dysfunction, accumulation of misfolded and aggregated proteins (ubiquitin-proteasome system and autophagy pathway impairment) and oxidative and nitrosative stress play an essential role in the pathogenesis of both idiopathic and familial forms of PD (Kanthasamy et al., 2012).

Since the discovery of the ability of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to reproduce some of the features of PD, research has been focused on finding other environmental risk factors implicated in the etiology of PD, which revealed that occupational exposures to paraquat (PQ), maneb (MB) and rotenone have been associated with higher incidence of PD.

Besides PD, several studies have also suggested that Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), dementia and deficits in cognitive function are linked to occupational pesticide exposure (Cannon and Greenamyre, 2011; Chang and Wu, 2009; Kamel and Hoppin, 2004; Kamel et al., 2012; Migliore and Coppede, 2009a; Sutedja et al., 2009). AD is a chronic disease characterized by progressive loss of memory and cognitive capacity, ultimately leading to dysfunction in daily life or work abilities. The neurodegeneration seen in AD involves two main protein aggregates, senile/amyloid plaques and neurofibrillary tangles (Palop and Mucke, 2010). Senile plaques are deposits of fibrils of the β-amyloid peptide, a fragment derived from the proteolytic processing of the amyloid precursor protein whereas neurofibrillary tangles are a compact filamentous network of helical filaments from hyperphosphorylated tau protein (Maccioni et al., 2001). Initially, the entorhinal cortex and hippocampus are particularly affected, as shown by the impaired synaptic transmission, especially reduction in the glutamatergic synaptic transmission strength and plasticity, and cholinergic dysfunction (Danysz and Parsons, 2012; Nyakas et al., 2011). The cause for development of AD as other neurodegenerative diseases is not fully understood, however roughly 0.1% of the cases arise from mutation in three genes (*APP*, *PSEN 1* and *PSEN 2*) that result in a familial early-onset (<65 years) autosomal dominant forms (Migliore and Coppede, 2009b). Metals, pesticides, solvents, electromagnetic fields, brain injuries, inflammation, educational levels, lifestyles and dietary factors have been proposed as environmental AD risk factors (Cannon and Greenamyre, 2011; Dosunmu et al., 2007). Carbamates, organophosphates (OPs) and organochlorines are the pesticides more frequently associated with occupational exposure and AD (Hayden et al., 2010; Parron et al., 2011; Tyas et al., 2001; Zaganas et al., 2013).

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease evidenced by progressive loss of motor neurons at the anterior horn of the spinal cord and brain, resulting in progressive weakness, muscle atrophy, and respiratory failure within 3–5 years after diagnosis (Al-Chalabi et al., 2012). Around 5% of the cases are familial forms of ALS that arise from mutations in several genes including *SOD1*, *TARDBP*, *FUS*, *UBQLN2*, *C9orf72*, and *TAF15* (Al-Chalabi et al., 2012; Lill et al., 2011; Maccioni et al., 2001). Studies in mutations in *SOD1* have provided new insight into the pathogenesis of ALS, namely the generation of reactive oxygen (ROS) and nitrogen species (RNS), dramatic gliosis characterized by abnormalities of astrocytes, widespread astrocytosis, increased expression of inducible nitric oxide synthase (NOS) and activated microglial cells (Almer et al., 1999; Cha et al., 2000; Nagy et al., 1994). Interestingly, the neuronal cytoplasmic inclusions of ALS are constituted by aggregates of proteins encoded by the mutated genes described above (Ince et al., 2011). ALS and sporadic frontotemporal lobar degeneration share the same pathologic lesion, the 'ubiquitin-only inclusion' body, within lower motor neurons and cerebral neurons (hippocampal and frontotemporal neocortex neurons), and both are considered proteinopathies (Ince et al., 2011). The remaining 95% of cases do not have an obvious family history of ALS and appear to occur sporadically throughout the community (Schymick et al., 2007). Despite this fact, the etiology of the majority of sporadic ALS cases is presumably due to several interactions between genetic and environmental factors. The genes that have been identified as being causative of ALS are related to DNA repair (*APEX1* and *hOGG1*), angiogenesis (ANG and VEGF), paraoxonases (*PON1*, *PON2* and *PON3*), iron metabolism (HFE), neurofilaments (*NEFH*), and survival motor neuron (*SMN1* and *SMN2*) (Maccioni et al., 2001; Migliore and Coppede, 2009b; Schymick et al., 2007). Several studies suggest that occupational exposure to pesticides is a significant risk factor of ALS (Bonvicini et al., 2010; Govoni et al., 2005; Kamel et al., 2005; Kanavouras et al., 2011; Qureshi et al., 2006). For instance, Horner and colleagues reported a two-fold increase in the risk of ALS among veterans of the 1991 Gulf War over the subsequent 10 years (Coffman et al., 2005; Horner et al., 2003; Miranda et al., 2008). Although the information about the chemicals to which soldiers were exposed is scarce and biased, the possibilities include OPs, other pesticides, nerve gases, pyridostigmine, petrochemicals and depleted uranium (Spencer et al., 1998).

Recently, two different research groups conducted a retrospective meta-analysis of studies relating ALS and pesticides as a group (Malek et al., 2012), and one of them investigated the association of ALS with specific pesticides, using data from the Agricultural Health Study, a cohort including 84739 private pesticide applicators and spouses (Kamel et al., 2012). From the eight case–control

studies (Bonvicini et al., 2010; Chancellor et al., 1993; Deapen and Henderson, 1986; Granieri et al., 1988; Gunnarsson, 1994; McGuire et al., 1997; Morahan and Pamphlett, 2006; Savettieri et al., 1991) and one cohort study (Weisskopf et al., 2009), the major finding was the strong association observed between agricultural activities and ALS, although the chemical or class of agrochemical was not specified by the majority of studies (Kamel et al., 2012; Malek et al., 2012; Sutedja et al., 2009). On the other hand, in the Agricultural Health Study, ALS was not associated with pesticides as a group, but was associated with use of organochlorine insecticides, herbicides, pyrethroids, and fumigants (Kamel et al., 2012).

There is mounting evidence that long-term/low dose pesticide exposure is potentially neurotoxic and increases risk of PD and with lesser extent other neurological diseases, such as AD and ALS. PQ and MB are the most studied pesticides, though pesticides such as dieldrin, pyrethroids and OPs are also described as neurotoxics. The pesticides addressed in this review represent the five important classes of pesticides that have been more extensively studied in this matter. The neonicotinoids, acetamiprid and imidacloprid belong to a new class of insecticides and the latter is considered the most widely used within this class in the world. Nonetheless, so far neonicotinoids are considered safer than other compounds due to their higher selectivity for the nicotinic receptors of insects than mammalian receptors (Casida and Durkin, 2013). There are few studies reporting nervous system depression following acute poisoning with imidacloprid or acetamiprid, but currently there is no evidence or human data reporting health effects on humans after prolonged exposure to neonicotinoids (Imamura et al., 2010; Karatas, 2009; Phua et al., 2009; Proenca et al., 2005).

This review aims to clarify some of the mechanisms involved in the genesis of idiopathic PD and other neurological syndromes and the role of pesticides as environmental risk factors.

2. Paraquat

2.1. Oxidative stress and inflammation

The herbicide PQ has increasingly been reported in epidemiological studies to enhance the risk of developing PD. PQ belongs to the chemical class of bipyridyl (also called bipyridylium) quaternary ammonium herbicides characterized by two covalently linked methylpyridine rings (Calderbank, 1968). The toxicity of PQ has been extensively described concerning the effects to the main target organ, the lungs, and also the kidneys, liver and heart (Dinis-Oliveira et al., 2008). However, only in the past decade the research has been focusing on the effect of PQ in the brain after several reports of brain damage in individuals exposed to lethal doses of PQ (Baltazar et al., 2013; Dinis-Oliveira et al., 2006; Grant et al., 1980; Hughes, 1988; Soontornniyomkij and Bunyaratvej, 1992). The mechanisms of PQ-induced neurotoxicity are not fully comprehended yet, but several pathways have been proposed: induction of oxidative stress, mitochondrial dysfunction, apoptosis and autophagy, inhibition of the ubiquitin-proteasome system, induction of synucleinopathy and tauopathy (Dinis-Oliveira et al., 2009; Franco et al., 2010) (Fig. 1).

A reliable amount of evidence has demonstrated that oxidative stress is considered to have a key role in the pathogenesis of PD (Drechsel and Patel, 2008). Dopaminergic neurons are markedly exposed to oxidative stress injury, mainly due to the oxidative metabolism of dopamine (DA), which contributes to sustain a higher production of ROS in the SNpc when compared to other regions of the brain (Fahn and Cohen, 1992). The other factors contributing to the selective vulnerability of the nigrostriatal system to oxidative stress include the following:

(a) The capability of DA auto-oxidation products, quinones and semiquinones to adduct proteins containing a thiol group such as glutathione and DNA (Graham, 1978; Hastings, 1995; Levay and Bodell, 1993).
(b) High aerobic respiration activity (Bueler, 2009).
(c) Increased iron concentration in the *substantia nigra* that leads to the production of hydroxyl radical ($HO^{\bullet}$) through Fenton reaction (Andersen, 2004; Kaur and Andersen, 2004).

PQ is widely known as a redox cycling agent, capable of accepting one electron from several cellular diaphorases (enzymes that transfer one electron from NAD(P)H), mainly NADPH-cytochrome P450 reductase, to form the monocation radical ($PQ^{\bullet+}$). Other enzymes are able to reduce PQ^{2+} to $PQ^{\bullet+}$, which comprise mitochondrial NADH:ubiquinone oxidoreductase (Gray et al., 2007), xanthine oxidase, NOS (Day et al., 1999), NADPH-oxidases from NOx family (Cristovao et al., 2009), and thioredoxin reductase (Gray et al., 2007). The $PQ^{\bullet+}$ is then rapidly reoxidized, regenerating its parent compound, in the presence of O_2, originating superoxide ($O_2^{\bullet-}$). The $O_2^{\bullet-}$ produced can react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$) or be dismutated by superoxide dismutase (SOD) to hydrogen peroxide (H_2O_2). In the presence of iron (II), H_2O_2 is reduced to $HO^{\bullet}$, a far more damaging ROS that rapidly oxidizes DNA, proteins and lipids (Klein and Ackerman, 2003) (Fig. 1).

The role of oxidative stress in PQ-induced neurodegeneration has been demonstrated in several cellular models (Castello et al., 2007; Cocheme and Murphy, 2008; Shimizu et al., 2003b; Wu et al., 2005) and *in vivo* studies (Czerniczyniec et al., 2011; McCormack et al., 2002, 2005; Thiruchelvam et al., 2000b; Wills et al., 2012). PQ exposure was shown to cause depletion of GSH and increase GSSG levels in the *substantia nigra* of mice (Kang et al., 2009), augment malondialdehyde and protein carbonyls concentration, as well as DNA fragmentation (Yang and Tiffany-Castiglioni, 2005). PQ-induced increase in NADPH oxidase expression might result from initial ROS produced by the PQ redox cycle or mitochondria (Cristovao et al., 2009) (Fig. 1). However, continued production of ROS might be sustained by constant generation of NADPH oxidase-mediated $O_2^{\bullet-}$. When cells were pretreated with apocynin, a putative NADPH oxidase inhibitor, PQ-induced ROS generation and dopaminergic cell death were significantly reduced. PQ has been shown to activate other signaling pathways such as protein kinase delta (PKC δ) or ERK1/2, which were reported as NADPH oxidase transcriptional activators (Miller et al., 2007). Exposure of microglial cells to PQ resulted in a rapid phosphorylation of ERK1/2 and phosphorylation of the cytosolic subunits of NADPH oxidase by PKC δ, resulting in increased ROS production and cell death (Miller et al., 2007).

Increasing evidences support that microglia, the resident macrophages in the brain, are a chronic source of inflammation and ROS responsible for progressive neuron damage (Surace and Block, 2012). Purisai and colleagues demonstrated that microglial activation is a priming event leading to PQ-induced dopaminergic cell degeneration (Purisai et al., 2007) (Fig. 1). Furthermore, a single-PQ exposure triggered an increase in the number of cells with immunohistochemical, morphological and biochemical characteristics of activated microglia, including induction of NADPH-oxidase, but failed to cause oxidative stress and neurodegeneration (Purisai et al., 2007). However, when PQ was repeatedly administered or challenged with other pro-inflammatory stimuli, such as lipopolysaccharide (LPS), the susceptibility of dopaminergic neurons to toxic injury was dramatically exacerbated (Mangano and Hayley, 2009; Purisai et al., 2007). The current knowledge supports the hypothesis that inflammatory insults may influence dopaminergic neuronal sensitivity to subsequent environmental xenobiotics by modulating the state of glial and immune factors, and these findings may be important for multifactorial

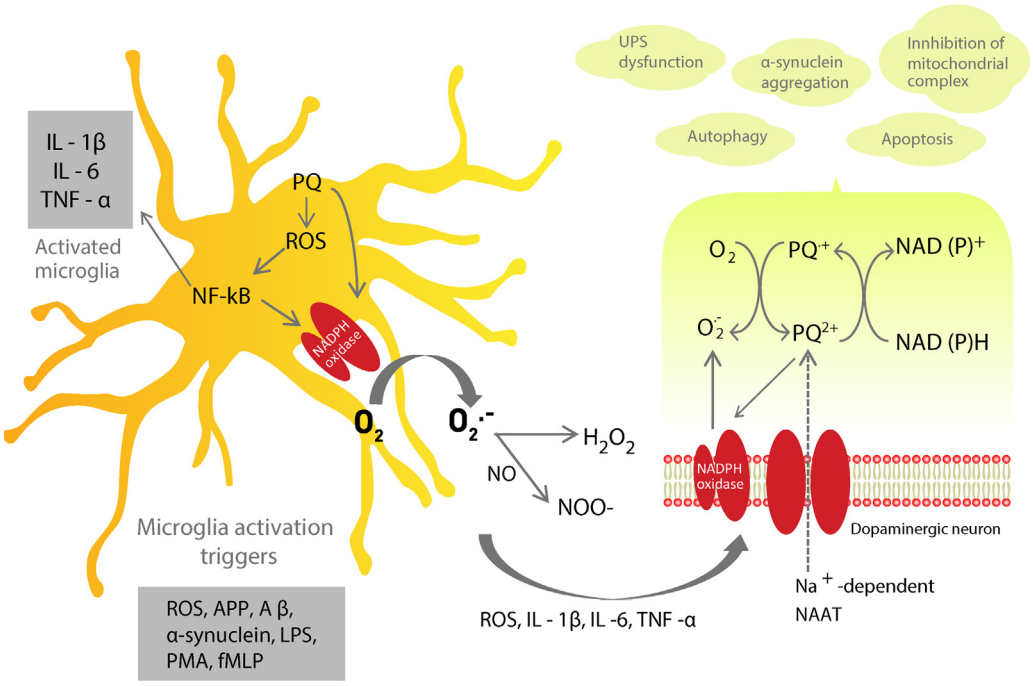

Fig. 1. PQ increases NADPH oxidase expression possibly due to the ROS produced by the PQ redox cycle or mitochondria or the activation of NF-κB. Activated microglia releases inflammatory cytokines and superoxide radicals, which damage the adjacent neurons. Brain and striatal neurons uptake of PQ is supposed to be mediated by the neutral amino acid transport system (NAAT) in a Na^+-dependent manner. Within the cells, PQ^{2+} is reduced to $PQ^{\bullet +}$ by several oxidoreductases. The $PQ^{\bullet +}$ is then rapidly reoxidized, regenerating PQ^{2+}, in the presence of O_2, originating superoxide ($O_2^{\bullet -}$). The redox cycle leads to the generation of ROS and cellular damage. The other neurotoxicity mechanisms of PQ include mitochondrial dysfunction, apoptosis and autophagy, inhibition of the ubiquitin-proteasome system, induction of synucleinopathy.

neurodegenerative conditions, such as PD (Mangano and Hayley, 2009; Miller et al., 2007; Surace and Block, 2012).

Subchronic systemic injection of PQ (10 mg/kg) to C57BL/6 mice induced a specific dose and age-dependent neurodegeneration of the dopaminergic neurons in the SNpc populations, whereas GABA-ergic cells in the *substantia nigra pars reticulata* and cholinergic neurons in the hippocampus were not affected (McCormack et al., 2002). Noteworthy, the authors claimed that the discrepancy observed between neurodegeneration and lack of significant DA loss represents another important feature of PQ model and is probably a reflection of compensatory mechanisms by which neurons that survive damage are capable of restoring neurotransmitter tissue levels (Ossowska et al., 2006). Other authors also corroborated the same findings (Kuter et al., 2007; Ossowska et al., 2005), nonetheless the combined exposure to PQ and MB resulted in nigral DA loss associated with decreased tyrosine hydroxylase (TH) protein levels and nigral dopaminergic neurodegeneration (Li et al., 2005; Thiruchelvam et al., 2002).

McCormack and colleagues reported that ferritin transgenic mice are resistant to PQ-induced neuronal loss and lipid peroxidation, possibly by avoiding the generation of HO• *via* Fenton reaction (McCormack et al., 2005). In the same study, wild type C57BL/6 mice showed simultaneous dose-dependent loss of nigrostriatal dopaminergic neurons and an increase in the counts of neurons immunoreactive for 4-hydroxinonenal, and nitrotyrosine, biomarkers of lipid peroxidation and nitrosative damage, respectively. Similarly to the *in vivo* studies (McCormack et al., 2002, 2005), midbrain cells exposed to a single treatment with 10 mM PQ did not show a decrease in the number of dopaminergic neurons, although sequential treatments with 10 mM PQ for 2 days considerably killed dopaminergic neurons (Shimizu et al., 2003b). These results strongly support that the constant exposure to low levels of PQ would lead to the vulnerability of dopaminergic neurons in the nigrostriatal system. Moreover, an indirect excitotoxic pathway involving the NMDA receptors has been proposed by Shimizu and colleagues (Shimizu et al., 2003a). Glutamate and ROS including NO have been hypothesized to play a pivotal role in neuronal cell loss (Sawada et al., 1996). The toxic mechanism of PQ involves the stimulation of glutamate efflux from non-NMDA receptors, resulting in activation of NMDA receptor-channels. The high intracellular influx of Ca^{2+} stimulates NOS. Released NO can diffuse to the dopaminergic terminals and further induce mitochondrial dysfunction and interaction with other ROS, with subsequent formation of peroxynitrite, resulting in continuous and long-lasting dopamine overflow (Shimizu et al., 2003a).

2.2. Mitochondrial-derived ROS

Impairment of mitochondrial dynamics and function has emerged as one of the key mechanisms underlying the pathogenesis of both sporadic and familial PD (Bueler, 2009). Schapira and colleagues showed, for the first time, that the activity of the mitochondrial respiratory complex I (NADH-quinone oxidoreductase) is reduced in the *substantia nigra* of PD patients (Schapira et al., 1989). The susceptibility of nigral dopaminergic neurons to impairments of complex I activity is due to the oxidative metabolism of dopamine and iron content and, possibly from the low mass content of mitochondria in this region, compared to other neurons in the midbrain. The potential role of mitochondria in PQ-induced ROS production is controversial but ongoing research has revealed that mitochondria can be a major source of PQ-induced ROS production (Castello et al., 2007; Cocheme and Murphy, 2008). Mice deficient in two mitochondrial antioxidant enzymes, MnSOD and Gpx are more sensitive to PQ than wild type (Van Remmen et al., 2004). Mitochondrial expression of antioxidant enzymes, such as catalase or peroxiredoxin 5, protects against PQ toxicity more effectively than cytosolic expression (Mockett et al., 2003; Nguyen-nhu and Knoops, 2003). Castello and colleagues (Castello et al., 2007) claimed that mitochondrial respiratory complexes I and III both serve as targets for PQ-mediated ROS generation, with complex III

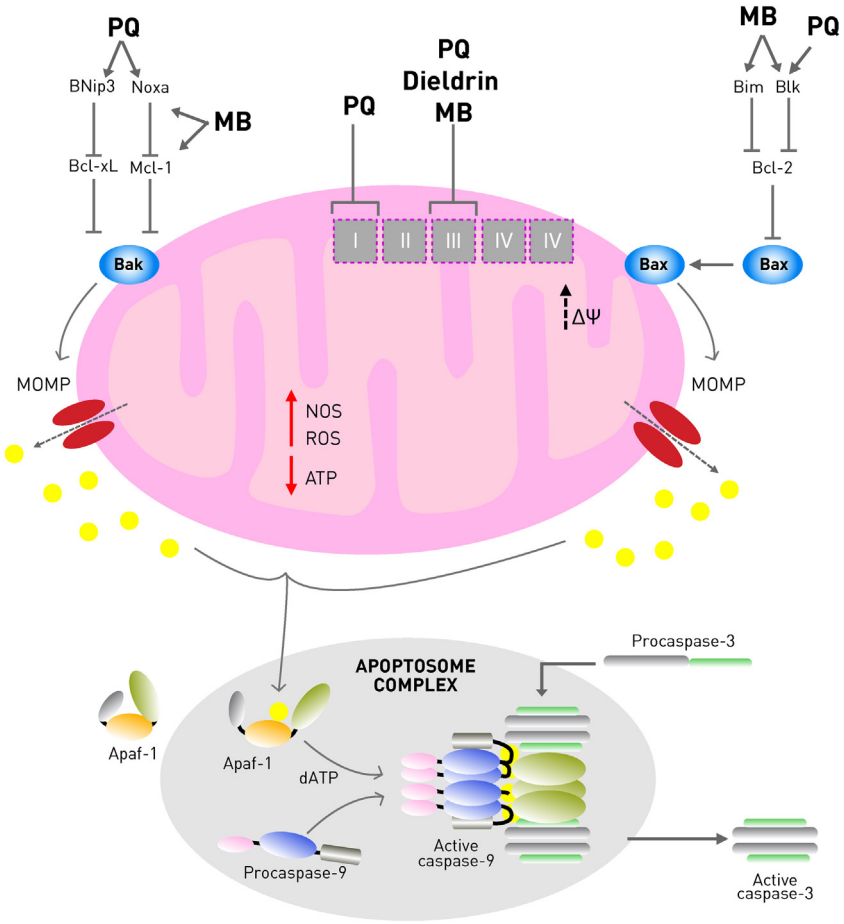

Fig. 2. Mitochondrial dysfunction is a defect occurring early in the pathogenesis of both sporadic and familial Parkinson's disease (PD). Pesticide-induced dopamine neurons death has been also associated with their ability to alter the mitochondrial function. Paraquat (PQ) inhibits mainly the complex I and III, and maneb (MB) and dieldrin inhibit only the complex III of the mitochondrial respiratory chain, generating reactive oxygen (ROS) and nitrogen species (RNS), leading to decreased ATP synthesis, oxidation of matrix proteins and mitochondrial DNA damage. PQ induces the BH3-only members Noxa and BNip3, and MB induces Noxa and Mcl-1. Noxa specifically binds to Mcl-1 and BNip3 binds to Bcl-xL, two major inhibitors of Bak. Binding of Noxa to Mcl-1 and BNip3 to Bcl-xL causes disinhibition of Bak. PQ and MB also disinhibit Bim, Blk, leading to disinhibition of Bax. The availability of Bak and Bax causes transient membrane disruptions, referred to as mitochondrial outer membrane permeabilization (MOMP), leading to the release of cytochrome c. Cytoplasmic cytochrome c complexes with Apaf-1 and procaspase-9 to form an apoptosome that activate executioner caspases, such as caspase-3, leading to apoptosis.

showing a higher sensitivity, while other authors argued that complex I is the most likely site for damage (Cocheme and Murphy, 2008) (Fig. 2).

Mitochondrial aconitase (m-aconitase) is highly sensitive to $O_2^{\bullet -}$, which causes oxidation of the $[4Fe–4S]^{2+}$, promoting the removal of a labile iron that *via* Haber-Weiss reaction forms H_2O_2 (Cantu et al., 2009). In accordance, exposure to PQ was shown to induce m-aconitase-dependent increase in H_2O_2, Fe^{2+} and cell death as seen by the attenuation of H_2O_2 production when m-aconitase expression was reduced by RNA interference (Cantu et al., 2011).

2.3. Inhibition of proteossomal pathways and synucleinopathies/tauopathies

Progressive loss of DA neurons of the nigrostriatal system and deposition of filamentous α-synuclein aggregates are the main characteristics of PD (Puschmann et al., 2012). Indeed, besides the fact that α-synuclein is a natively unfolded protein that plays a central role in the control of synaptic membrane processes and biogenesis, when it becomes misfolded, it aggregates, and accumulates in neuronal inclusion bodies, the Lewy bodies (Bellucci et al., 2012). Remarkably, several studies indicate that multiplications or mutations of the *SNCA* gene are causative of autosomal dominant PD, and specific polymorphisms in the promoter region of the *SNCA* gene (REP1) increase the risk to develop PD. Other alterations that promote α-synuclein aggregation include nitration, hyperphosphorylation at Ser129, and the presence of DA adducts (Valente et al., 2012).

PQ markedly induced the *in vitro* conformational changes in α-synuclein, and accelerated the rate of aggregation of α-synuclein (Uversky et al., 2001, 2002). *In vivo* experiments have corroborated these results, showing that brain, levels and aggregation of α-synuclein were significantly increased in PQ-treated mice, 2 days after each of three weekly PQ injections and with protein levels returning to control values by day 7 post-treatment (Manning-Bog et al., 2002). α-Synuclein overexpression induces the formation of membrane pore-like structures that increase membrane conductance (Feng et al., 2010). The same authors concluded that leak channel conductance occurred prior to substantial cell death suggesting that pore formation may contribute to the overall cell vulnerability (Feng et al., 2010). More recently, Feng and colleagues demonstrated that co-treatment with PQ and DA in dopaminergic cells enhances α-synuclein-induced leak channel conductivity leading to a disruption of ionic imbalance, and eventually cell death (Feng and Maguire-Zeiss, 2011). PQ-treated mice striata showed significant accumulation of α-synuclein and hyperphosphorylation of Tau through activation of p-GSK-3β, a major Tau kinase (Wills

et al., 2012). Notably, the specific sites of phosphorylation of Tau serine residues in PQ treated mice striata are the same sites found in PD *post mortem* striata (Wills et al., 2010). Besides, high levels of hyperphosphorylated (p-Tau) is strictly dependent on the presence of α-synuclein, as indicated by lack of any p-Tau formation in MPTP-treated α-synuclein −/− mice or in neuronal cells lacking α-synuclein (Duka et al., 2006, 2009). PQ, MPP^+, and rotenone, but not MB, are known to induce synucleinopathy and tauopathy (Duka et al., 2006, 2009; Hoglinger et al., 2005; Mitra et al., 2011; Wills et al., 2012).

Ubiquitin-proteasome system and autophagy are the two major pathways of degradation of misfolded, oxidized, and aggregated proteins. In PD, ubiquitin-proteasome system is impaired possibly due to depletion of ATP levels caused by mitochondrial dysfunction, oxidative stress or in cases of familial PD, mutations in *parkin* and *UCHL1* genes (Valente et al., 2012). Ubiquitin-proteasome system dysfunction leads to α-synuclein aggregation and the protein itself is capable of reducing even more the proteasomal activity (Branco et al., 2010). DJ-1 deficient mice treated with PQ showed impaired proteasome activity and increased ubiquitinated protein levels. Nevertheless, the authors claimed that PQ exposure or deficiency in *DJ-1* gene alone did not stimulate a decrease in proteasome activity. The same study also showed that 19S ATPase Rpt6 and 20S β5 subunits and a transcription factor Nrf2 were decreased in *DJ-1*-deficient mice treated with PQ. On opposition, more recent studies have shown that administration of PQ (10 mg/kg), twice weekly for six weeks, significantly reduced the 26S proteolytic activity without loss of either 19S or 20S components or changes in the assembly of the 26S proteasome (Wills et al., 2012). The authors hypothesized that PQ seems to interact directly with the 20S component of the proteasome. In view of the fact that PQ induces overexpression α-synuclein and p-tau it is expected that proteasomal activity is reduced, due in part to the direct inhibitory effects of α-synuclein and p-Tau (Wills et al., 2012).

2.4. Cell death

PQ induces selective neurodegeneration in dopaminergic neurons in the SNpc triggering different mechanisms of cell death. Apoptosis induced by PQ has been shown to involve mainly the intrinsic mitochondrial pathway (Fei et al., 2008), and more recently, some evidences suggest the contribution of endoplasmatic reticulum (ER) stress and (Niso-Santano et al., 2006) an autophagic process in neuronal cell death (Gonzalez-Polo et al., 2007b).

PQ triggers apoptosis *via* the intrinsic pathway by releasing cytochrome c and activation of caspase-9 due to the induction of Bcl-2 family members such as Bak, Bid, BNip3, and NOXA (Fei et al., 2008) (Fig. 2). Authors suggested that PQ neurotoxicity is mediated by a Bak-dependent mechanism by induction of Nox and BNip3 binding to Mcl-1 and Bcl-xL, respectively. This binding de-represses Bak, making it available to create mitochondrial outer membrane permeabilization with further release of cytochrome *c*, which will interact with Apaf-1 and procaspase-9 to create the apoptosome. The fully active apoptosome processes and activates executioner caspase-3 triggering apoptosis (Fei et al., 2008) (Fig. 2).

The ER is highly sensitive to oxidative stress, Ca^{2+} disturbances, and hypoxia (Boyce and Yuan, 2006). These disturbances cause accumulation of unfolded proteins in ER, triggering stress responses (Xu et al., 2005). Activation of inositol-requiring enzyme 1 (IRE1), apoptosis signal regulating kinase (ASK1), C/EBP homologous protein, and stressed-activated kinases lead to the activation/induction of pro-apoptotic Bcl-2 family members, which promotes the crosstalk between ER and the mitochondria-triggered apoptotic pathway, including release of cytochrome c from mitochondria and activation of caspase-3 (Paschen and Mengesdorf, 2005) (Fig. 3). An increasing body of evidence indicates that the stress-activated kinases, including c-Jun N-terminal kinase (JNK) and p38 kinase, play a critical role in the PQ-induced degeneration process (Choi et al., 2010; Klintworth et al., 2007). Sequential phosphorylation of JNK and the activation of caspase-3, and p53 transcription factors (Yang and Tiffany-Castiglioni, 2008) have been reported in animal and *in vitro* models of PD using PQ (Peng et al., 2004) (Fig. 3). These first studies demonstrated the involvement of p38/JNK signaling, however, how oxidative stress activates these pathways has not been established in culture or in animal models of PD. The current knowledge suggests that ASK1 acts upstream of JNK and p38 kinases throughout the phosphorylation of MKK3/6 and MKK4/7 (Yang et al., 2009) (Fig. 3). Moreover, Niso-Santano and colleagues investigated the role of the transcription factor Nrf2, a master regulator of cytoprotective genes, and its target thioredoxin (Trx), which binds and inhibits ASK1. PQ induced a dose-dependent decrease in Trx levels correlated with a major increase in phosphorylated ASK1, suggesting that Nrf2/Trx is crucial in PQ-induced apoptosis (Niso-Santano et al., 2010) (Fig. 3).

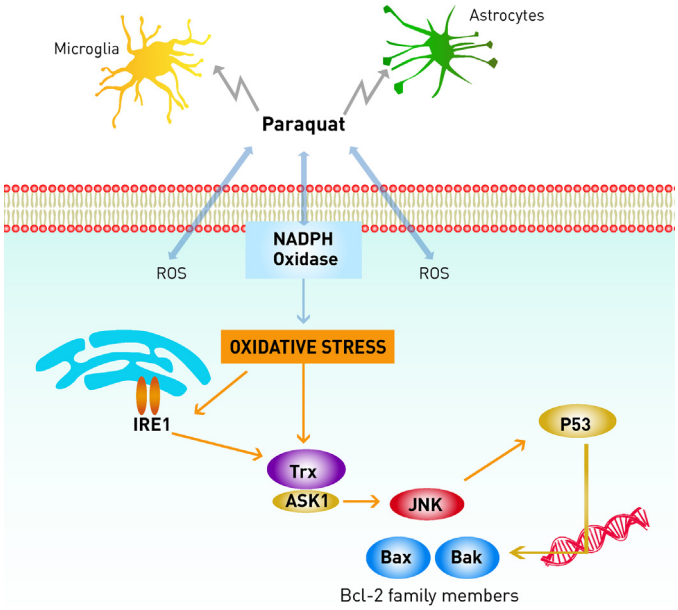

Fig. 3. PQ production of ROS through the redox cycling, activation of microglia and astrocytes, inhibition of mitochondrial electron transport chain, and/or induction/activation of ROS generating enzymes such as NADPH oxidases, mediates the activation of cell death signaling cascades. Oxidative stress triggers the induction of endoplasmatic reticulum stress and activation of inositol-requiring enzyme 1 (IRE1), then activating the ASK1/JNK signaling cascade. PQ induces a dose-dependent decrease in Trx levels leading to an increase in phosphorylated ASK1, suggesting that Nrf2/Trx is crucial in PQ-induced apoptosis. Phosphorylation of JNK induces p53 transcription factor that lead to activation/induction of pro-apoptotic Bcl-2 family members, culminating in apoptosis.
Adapted from Franco et al. (2010).

Autophagy is a mechanism involved in the degradation of oxidatively damaged proteins and in organelle turnover. This phenomenon has been observed in neurons from patients with various neurodegenerative diseases such as Huntington's disease, AD and PD (Anglade et al., 1997; Kegel et al., 2000; Nixon et al., 2000). However, the implication of autophagy in PD and whether environmental xenobiotics upregulate or downregulate autophagy is still controversial (Janda et al., 2012). The accumulation of α-synuclein-rich protein inclusions induced by some pesticides (rotenone and PQ) suggests that the autophagy pathways are rather inhibited than promoted. The first *in vitro* studies reported that PQ triggers autophagy, shown by the significant increase in LC3II levels, weak inhibition of mTOR phosphorylation, and increase in

LC3-GFP autophagic vesicles (Gonzalez-Polo et al., 2007a). The same authors claimed that PQ elicits autophagy as a defense mechanism to degrade the oxidized proteins by ROS since the inhibition of autophagy, using 3-MA, accelerated the apoptotic death process (Gonzalez-Polo et al., 2007a). From the six genes linked with hereditary PD, *α-synuclein*, *Parkin*, PTEN-induced kinase 1 (*PINK-1*), and *DJ-1* (Krebiehl et al., 2010) are the genes most strongly implicated in autophagy impairment. Gonzalez-Polo and colleagues defended that autophagy induced by PQ was dependent on *DJ-1*, as its knockdown reversed the autophagic response to PQ (Gonzalez-Polo et al., 2009). Unlike the *in vitro* experiments, the only *in vivo* study suggests an impairment of macroautophagy and proteasome function upon exposure to PQ (Wills et al., 2012). Both MB and PQ increased the levels of mTOR, an inhibitor of autophagy, and reduced LC3 II to LC3 I ratio, despite increases in autophagic proteins, such as beclin 1 and Agt12. In parallel, increased mTOR was also observed in post-mortem human PD striata, and a reduction in the LC3 II to LC3 I ratio as well (Wills et al., 2012). The controversial data raises doubts about the role of PQ in the autophagy pathway even though the *in vivo* studies suggest an impairment of macroautophagy. Further work will be necessary to elucidate the mechanisms underlying PQ-related modulation of autophagy.

2.5. Crosstalk between experimental and human data

Recently, case–control studies, cohort studies and cross-sectional studies were combined in two meta-analyses (van der Mark et al., 2012; Van Maele-Fabry et al., 2012). Despite not being completely consensual, the main conclusions reinforce the idea that there is evidence of an increased risk of PD associated with PQ exposure. The results suggested that heterogeneity was rather due to differences in the exposure assessment than with study design, source of control population, adjustment of results for potential confounders, or geographical area (van der Mark et al., 2012).

Kamel et al. (2007) analyzed the Agricultural Health Study (Alavanja et al., 1996) data from licensed private pesticide applicators and spouses to evaluate the relation of self-reported PD to pesticide exposure. There was a weak negatively association of prevalent PD with ever use of a pesticide and with personally mixing or applying pesticides and a positively association with incident PD. Incident PD was associated with the highest category of cumulative days of pesticide use at enrollment with personally applying pesticides more than half the time but not with prevalent PD. Considering only chemicals for which there were four or more exposed cases, OR's for prevalent PD were elevated (>1.4). Actually, frequent use of PQ, the OR's were 1.8 (95% CI, 1.0–3.4) for prevalent PD cases and 1.0 (95% CI, 0.5–1.9) for incident PD cases (Kamel et al., 2007).

Tanner and colleagues also analyzed the Agricultural Health Study data and conducted a case–control study focused to assess whether pesticides linked to mitochondrial dysfunction or oxidative stress, in a population with well characterized pesticide exposure, are associated with PD or clinical features of parkinsonism in humans (Tanner et al., 2011). From the eight pesticides classified as oxidative stressors, and from the seven classified as mitochondrial complex I inhibitors, only PQ and rotenone, respectively, were associated with PD. In 110 PD cases and 358 controls, use of PQ (OR = 2.5; 95% CI, 1.4–4.7 for men and women, and 2.7; 95% CI, 1.4–5.1 for men only), and rotenone (OR = 2.5; 95% CI, 1.3–4.7) were associated with PD, but for cumulative lifetime days of use, only PQ was positively correlated with duration of use (OR = 3.6; 95% CI, 1.6–8.1 for greater than the median). Despite the size of the study, wide variability of exposure, quality of diagnosis with movement disorders experts, and reliability of pesticide exposure information, the authors did not distinguish prevalent and incident cases, potential bias might have occurred during selection, and the subgroup analyses were not justified (Mandel et al., 2012). Also, the study could not rule out the possibility that the results were attributable to combined exposures or other agents other than those analyzed.

Even though long-term occupational exposure to pesticides might be linked with PD, most of the studies have not found a significant association with specific pesticides, namely, PQ. In a cohort study performed among men with high prevalence of parkinsonism and daily exposed to pesticides, Engel et al. found that the association of PD with PQ was negative (prevalence ratio = 0.8; 95% CI, 0.5–1.3). There was no correlation with duration of exposure as for the highest tertile of years of exposure and for highest acre-years of exposure the prevalence ratios were <1 (Engel et al., 2001). A study conducted in Taiwan with 120 patients and 240 controls, where the herbicide PQ is commonly sprayed over rice fields, reported an OR of 3.22 (95% CI, 2.41–4.31) for PD in PQ users compared with nonusers, and 6.44 (95% CI, 2.41–17.2 for the highest duration of use. However, subjects were highly exposed to other pesticides which difficult the comprehension of PQ involvement. Hertzman et al. (1990) compared personal histories of 57 cases and 122 age-matched controls in British Columbia to identify possible environmental determinants of PD and reported an increased risk of PD for working in orchards (OR = 3.69; 95% CI, 1.34–10.27) and a marginally significant increased risk associated with working in planer mills (OR = 4.11; 95% CI, 0.91–18.50). Based on Fisher's exact test of the association between PD development and PQ was statistically significant ($p = 0.01$). In a population-based case–control study of incident PD in western Washington State, the only increased risk estimate was for men exposed to parathion, whereas for PQ the association was negative (OR = 0.9; 95% CI, 0.15–5.43) (Firestone et al., 2010). These findings corroborate the previous study carried out by the same authors where there was no significantly increased odds ratio from exposure to PQ (OR = 1.67; 95% CI, 0.22–12.76) (Firestone et al., 2005). Three studies provided information about exposure to pesticides and increased risk of incident PD in agricultural areas of California (Costello et al., 2009; Gatto et al., 2009; Wang et al., 2011). In all studies, ambient exposure to pesticides was estimated from applications to agricultural crops employing a validated geographic information system. Gatto et al. (2009), concluded that, for PQ, the risk from well water consumption and ambient exposure were generally small and uninformative, which might be explained by the observations that exposure to PQ may require concomitant MB exposure to increase PD risk, as reported by Costello et al. (see below). The latest study from the same group, reported that for combined exposure to ziram, MB and PQ, and for combined exposure to ziram and PQ there was a significantly increased odds ratio but exposure to PQ alone (OR = 1.26; 95% CI, 0.86–1.86) adjusted for age, sex, education, smoking, family history of PD and race was no significantly increase was observed (Wang et al., 2011).

Concerning the evidences for the role of PQ exposure (*e.g.* during application of this herbicide, production or following acute poisoning) in the etiology of PD, many aspects have to be addressed. Firstly, PQ is poorly absorbed through intact human skin (0.03 μg/cm^2 over 24 h), with only 0.3% of the applied dose being absorbed within 24 h (Wester et al., 1984). Furthermore, the few occupational studies performed have shown that even after a dermal exposure of PQ during application, urine levels were either undetectable (Chester et al., 1993; Van Wendel de Joode et al., 1996) or very low, with 83.3, 47.1 and 63.9% of the samples being below the LOQ before-, during- and after-paraquat spray days, respectively (Lee et al., 2009). PQ has low volatility and the fraction of respirable particles (<5 μm) produced by standard spray nozzles is low, limiting the absorption by inhalation. Assessment of PQ exposure during handling of this herbicide reveals that dermal exposure is relatively high and that the degree of exposure *via* inhalation is below the permissible exposure limits set by United States National Institute of

Occupational Safety and Health (Baharuddin et al., 2011). Moreover, other occupational studies do not report the quantification of PQ in biological samples and therefore understanding the extension of the PQ absorbed through skin and air is neglected (Dalvie et al., 1999; Machado-Neto et al., 1998).

Secondly, the question of how PQ, a charged hydrophilic compound, enters the brain remains to be clarified. In rodents, transport into brain has been proposed to occur *via* a specific neutral amino acid transporter, although it reaches a brain concentration ten times lower than in peripheral tissues (McCormack and Di Monte, 2003; Shimizu et al., 2001). Rappold and colleagues demonstrated that, when PQ^{2+} is reduced to the monovalent cation $PQ^{\bullet+}$ (in the presence of either a reducing agent or NADPH oxidase on microglia), it is efficiently taken up by cells through DAT and organic cation transporter 3 (Rappold et al., 2011). Other studies, in the rhesus monkey and C57BL/6J mice reported that PQ uptake and the pattern of PQ distribution in the brain is similar across species, with higher concentrations being found in areas of the brain not fully protected by the blood–brain barrier such as the olfactory bulb, pineal gland and lateral ventricles (Bartlett et al., 2009; Breckenridge et al., 2013). It was also reported that PQ is slowly eliminated from brain, but whether PQ is bound to tissues or organelles within the brain, or whether specific neurons or other cells selectively retain PQ is unknown (Breckenridge et al., 2013). On the other hand, *in vivo* evaluation of the toxicokinetics of PQ shows that it accumulates in a linearly way, with a half-life of approximately one month in adult C57BL/6J mice after a single dose (10 mg/kg) resulting in accumulation of similar levels of PQ in the different regions of brain (striatum, frontal cortex, hippocampus, and cerebellum) (Prasad et al., 2007, 2009). Even when a low concentration of PQ (0.3 mg/ml) was given orally in drinking water, the brain concentration was ~0.12 ng/mg after 8 weeks of exposure (Prasad et al., 2009). However, neither of the studies quantified the urine and plasma levels, which hinder a possible correlation between the experimental data, the human exposure levels and epidemiological data.

Despite the literature evidences of the ability of PQ to reproduce some of the features of PD in mice models, the results should be interpreted carefully. Breckenridge and colleagues (Breckenridge et al., 2013) conducted a thorough experiment to evaluate the potential effects of PQ in the SNpc and striatum of male C57BL/6J mice. The scheme of treatment was similar to previous reported experiments (McCormack et al., 2002, 2005, 2006; McCormack et al., 2006; Richardson et al., 2005) whereas PQ dose was 10 mg/kg i.p. once per week for three consecutive weeks. Unlike others, their main finding is that under their conditions, there was minimal evidence of PQ-related neuronal degeneration without alteration of the concentration of dopamine (DA), homovanillic acid (HVA) or 3,4-dihydroxyphenylacetic acid (DOPAC), or increase DA turnover in the striatum. However, it should be noted that among the published animal studies on PQ neurotoxicity there is a lack of consistency in dose, species or strain, age of animals and timing of treatment. The doses fluctuate between 5 and 25 mg/kg, the frequency of dose ranges from 1 to 2 times per week, the length of the study from 1 to 4 weeks. The majority of the studies with PQ and PD use as model the C57BL/6 or C57BL/6J mice but other studies have also use the swiss albino mice (Mitra et al., 2011) and the rats strain Sprague–Dawley or Wistar (Czerniczyniec et al., 2011; Songin et al., 2011). Breckenridge and colleagues (Breckenridge et al., 2013) tested one type of inbred mouse strain that is considered the most susceptible to PQ neurotoxicity, the C57BL/6J mice, at 2 months of age. The difference between studies might also be linked with the age of the animals. Peng and colleagues suggested that age contributes to the greater susceptibility to PQ due to the age-related iron accumulation in the *substantia nigra* (Peng et al., 2007, 2009, 2010. Other recent studies also report significant PQ-induced TH staining loss in SNpc in 4–6 month old C57BL/6J mice (Jiao et al., 2012; Yin et al., 2011).

3. MB and PQ + MB

In rodent models, MB was shown to alter behavioral function, reduce locomotor activity and increase aggressiveness (Morato et al., 1989). Direct injection of MB to the rat lateral ventricles resulted in selective dopaminergic neurodegeneration, induced extensive striatal DA efflux, and preferentially inhibited mitochondrial complex III (Zhang et al., 2003). Barlow and colleagues also reported that MB and other dithiocarbamates were able to increase synaptosomal DA accumulation *in vitro* at concentrations as low as 500 nM, possibly by delaying DA efflux (Barlow et al., 2003). Furthermore, MB was shown to increase the tissue content of $^{[14C]}$PQ *in vivo* by a mechanism that appeared to be distinct from the DA transporter (DAT), suggesting that dithiocarbamates might augment other xenobiotics neurotoxicity by modulating their toxicokinetics (Barlow et al., 2003).

MB has been shown to exacerbate the pro-oxidant condition through its ability to disrupt the glutathione antioxidant system in dopaminergic neurons (Barlow et al., 2005), to catalyze the auto-oxidation of DA (Fitsanakis et al., 2002), and to disturb the mitochondrial function, as an inhibitory uncoupler of the electron transport chain (Domico et al., 2006). Additionally, exposure of dopaminergic cells to 6 μM Mn-EBDC for 7 days produced not only significant neurotoxicity but also decreased proteasomal function, and led to α-synuclein aggregation with formation of cytoplasmic inclusions that were immunoreactive for α-synuclein (Zhou et al., 2004). Despite *in vitro* inhibition of proteasomal activity and induction of α-synuclein aggregation, MB effects *in vivo* are somewhat different. MB was ineffective in increasing α-synuclein or p-Tau levels. When PQ and MB were concomitantly administered, the effects were similar to when PQ was administered alone (increased α-synuclein aggregation and p-Tau levels), which suggests that MB does not enhance the effects of PQ (Wills et al., 2012). Moreover, unlike PQ, MB did not directly inhibit soluble proteasomal activity, nor did it intensify the direct inhibitory effect of PQ on this activity. In the same study, MB increased levels of the autophagy inhibitor mammalian target of rapamycin, mTOR, suggesting impaired axonal autophagy, despite increases in certain autophagic proteins, such as beclin 1 and Agt12 (Wills et al., 2012).

Due to the fact that MB and PQ are used in geographically overlapping areas, and rural workers exposed to both pesticides have an increased risk of developing PD by 75% (Costello et al., 2009), several authors used PQ + MB as a PD model (Thiruchelvam et al., 2000a, 2000b). C57BL/6 mice exposed to PQ (10 mg/kg) and MB (30 mg/kg), i.p., once a week for 4 weeks, showed reduced locomotor activity, significant DA fiber loss, and altered levels of DA and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (Thiruchelvam et al., 2000a). These effects were preferentially expressed in the nigrostriatal dopaminergic system (Thiruchelvam et al., 2000b). Fei and Ehtell, found that MB potentiates PQ neurotoxicity by triggering Bax-dependent cell death through activation of three strong Bak inhibitors, Bfl-1, Bcl-xL and Mcl-1, and also induced Bax activators that included Bik and Bim (Fei and Ethell, 2008).

Idiopathic PD is typically considered as an aging-related neurodegenerative disorder, but its onset is still unclear, whether it could arise from events that occur during premature development, during adult life or from lifetime cumulative effects. Mice exposed developmentally to PQ + MB showed reduced levels of dopaminergic neurons (38% loss) in adulthood, while re-exposure of mice to the mixture of pesticides lead to a 70% loss of dopaminergic neurons in the *substantia nigra* and a concomitant decrease in locomotor

activity (Thiruchelvam et al., 2002). Developmental exposure to PQ or MB alone produced minimal changes. Conversely, the response to a second stimulus in adult life was exaggerated, suggesting that there is a period of silent neurotoxicity that predisposes adult animals to the toxicity of a re-exposure. Moreover, subsequent studies have shown that aging enhances sensitivity of nigrostriatal pathway to the combined exposure of PQ + MB (Thiruchelvam et al., 2003). Reduced levels of locomotor activity 24 h and even 3 months after treatment were age-related, since 5 and 18 months old mice did not recover, whereas 6 week old mice exhibited total recovery. Also, levels of striatal DA and dopaminergic neurons in the *substantia nigra*, particularly for PQ + MB treatment in both 5 and 18 months old mice were decreased and unchanged 3 months after the final exposure (Thiruchelvam et al., 2003).

4. Dieldrin

Dieldrin, an organochlorine, is one of the most environmentally persistent insecticides. Despite having been banned in the 1970s in most of the developed countries, its low volatility, and high lipophilic properties lead to an extensively bioaccumulation and biomagnification in non-target species and soil. Nowadays, humans continue to be chronically exposed to dieldrin through contaminated food, polluted ground water, and environmental residues (Jorgenson, 2001).

Post mortem studies indicate that exposure to dieldrin is closely associated with PD, since dieldrin levels in the caudate nucleus from PD patients were significantly higher than those in control brains (Corrigan et al., 1996, 1998, 2000). Also, previous studies revealed that dieldrin was detected in 6 of 20 PD brains, and in none of 14 control samples with a highly significant positive association between the insecticide and the diagnosis of PD (Fleming et al., 1994).

In vitro, dieldrin appears to be a relatively selective dopaminergic neurotoxin in mesencephalic cultures, indicated by the low neurotoxicity to GABA-ergic neurons compared to dopaminergic neurons (Sanchez-Ramos et al., 1998). Moreover, in rat and mice dopaminergic cell lines, dieldrin yields a depletion of intracellular DA levels, a decrease of DA metabolites, including 3,4-dihydroxyphenylacetic acid and homovanillic acid (Hatcher et al., 2007), a depolarization of mitochondrial membrane potential, generation of ROS (Chun et al., 2001), and apoptosis (Kitazawa et al., 2001) (Fig. 4). Dieldrin also activates brain microglia, inducing NADPH-dependent ROS production (Mao et al., 2007).

A mechanism underlying dieldrin-induced apoptosis has been recently proposed. Kitazawa and co-workers reported that PC12 cells exposed to dieldrin release cytochrome c, which is followed by the activation of the caspases cascade and caspase-3-dependent proteolytic activation of PKC δ (Kanthasamy et al., 2008; Kitazawa et al., 2003) (Fig. 4). In accordance, dieldrin-induced Poly (ADP-ribose) polymerase cleavage, chromatin condensation and DNA fragmentation, and caspase-3 activation were completely blocked in Bcl-2-overexpressed PC12 cells as compared to control cells. These findings suggest that dieldrin primarily alters mitochondrial function to initiate apoptotic cell death, since overexpression of the anti-apoptotic protein Bcl-2 prevents these events (Kitazawa et al., 2004). In mesencephalic dopaminergic neurons, dieldrin can rapidly induce the hyperacetylation of histones, specifically histones H3 and H4. The histone hyperacetylation in the striatum and *substantia nigra* was also observed in mice exposed to dieldrin (5.0 mg/kg) for 30 days (Fig. 4). The authors also found that the protein level of CBP, a well-known histone acetyltransferase, was increased in a time-dependent manner. This fact might be due to dieldrin-induced proteasomal dysfunction, resulting in accumulation of pivotal histone acetyltransferase. The inhibition of CBP

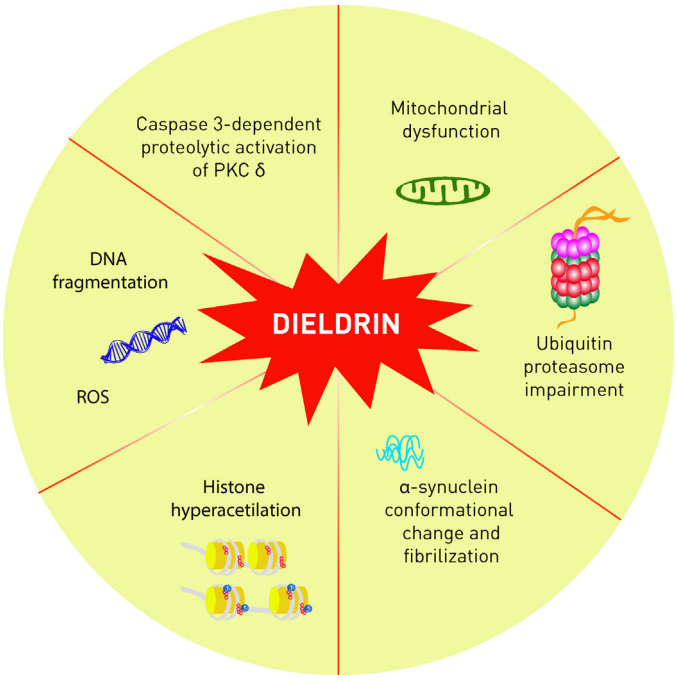

Fig. 4. Dieldrin and other cyclodienes are lipophilic compounds and are therefore capable of readily cross the blood brain barrier. Several *in vitro* and *in vivo* studies have shown that dieldrin reproduces many features of Parkinson's disease (PD). Dieldrin-induced release cytochrome c is followed by the activation of the caspases cascade and caspase-3-dependent proteolytic activation of protein kinase delta (PKC δ). Dieldrin has the ability to induce mitochondrial dysfunction, oxidative stress, oxidation of DNA, RNA, lipids and proteins. The dysfunction of the ubiquitin proteasome system (UPS) and reduced protein degradation is also responsible for dieldrin's induction of hyperacetylation of histone, probably due to the accumulation of pivotal histone acetyltransferases. Dieldrin induces α-synuclein aggregation. Together, UPS dysfunction and α-synuclein accumulation enhances the susceptibility of dopaminergic neurons to apoptotic cell death.

attenuated dieldrin-induced histone acetylation, caspase-3 activation, and PKC δ proteolytic activation, and DNA fragmentation in dopaminergic neurons (Song et al., 2010).

Dieldrin has been shown to induce a conformational change in α-synuclein and promote fibrillization of α-synuclein (Uversky et al., 2001) (Fig. 4). The overexpression of α-synuclein has been reported to inhibit proteasomal function (Snyder et al., 2003). For that reason, Sun and colleagues exposed α-synuclein overexpressing dopaminergic neurons to dieldrin (Sun et al., 2005). Their results showed that dieldrin impairs ubiquitin proteasome function additively with α-synuclein, and enhances the susceptibility of dopaminergic neurons to apoptotic cell death. Together, the results suggest that combination of α-synuclein overexpression due to genetic mutations or exposure to environmental pesticides that also increase α-synuclein levels, are likely to contribute to the overall vulnerability of dopaminergic neurons (Sun et al., 2005).

As mentioned above, exposure to pesticides during the perinatal period or early age may result in either permanent damage, progressive lesions of the nigrostriatal dopaminergic system or enhanced adult vulnerability to a future neurotoxicant challenges. Perinatal exposure of mice to low levels of dieldrin (0.3, 1, or 3 mg/kg every 3 days) resulted in a long-term enhancement of protein and mRNA levels of the DAT and vesicular monoamine transporter 2 (VMAT2) (Richardson et al., 2006). The increase DAT:VMAT ratio appears to be correlated with higher susceptibility of dopamine neurons to degeneration in PD (Miller et al., 1999). Indeed, when dieldrin-exposed mice were challenged with MPTP (2 × 10 mg/kg s.c.) at 12 week of age, the neurotoxicity was exacerbated as shown by the increase of α-synuclein levels and

Table 1
Summary of the structural and biological differences between class I and II of pyrethroids.

Pyrethroids		
Type I	Type II	
Devoided of a cyano moiety at the α-position, produce aggressive behavior, fine tremor progressing to whole-body tremor and prostration (*i.e.* permethrin, allethrin, cimethrin, bifenthrin, bioallethrin)	Possess a α-cyano moiety, produce hypersensitivity, coarse tremor, clonic seizure and profuse salivation (*i.e.* deltamethrin, cypermethrin, fenvalerate, cyfluthrin)	
Mechanisms of neurotoxicity		
Voltage-gated sodium channels	Voltage-gated chloride channels	GABA-gated chloride channels
Slower activation or opening of the channels, shifting the voltage dependence of the gates to more hyperpolarized potentials. Type I and Type II	Decreased opening probability of the channels which amplify the sodium channel-mediated signs of intoxication. Only Type II pyrethroids	Inhibition of GABA-gated chloride channels. Only Type II pyrethroids

a greater reduction of striatal DA, which was associated with a greater DAT:VMAT2 ratio.

Although dieldrin shows many features of PD including the ability to induce mitochondrial dysfunction, oxidative stress and apoptosis, induction of α-synuclein aggregation, and DA depletion, it fails to provoke motor deficits and dopaminergic neuron loss. Other issues are the lack of more extensive studies that associate dieldrin exposure with PD, the high concentrations used in *in vitro* and *in vivo* studies and whether induction of oxidative stress is a primary or secondary event in this pesticide-induced neurotoxicity.

5. Pyrethroids

Pyrethroids are a class of synthetic insecticides derived from the naturally occurring pyrethrins isolated from the *Chrysanthemum* genus of plants (Ray and Fry, 2006). Pyrethroids are divided into two classes of compounds based on their toxic signs and structure:

(a) Type I or T (tremor) syndrome (*i.e.* permethrin, allethrin, cimethrin, bifenthrin, bioallethrin) – are devoid of a cyano moiety at the α-position (α-cyano), produce aggressive behavior, fine tremor progressing to whole-body tremor and prostration;
(b) Type II or choreoathetosis syndrome (CS) (*i.e.* deltamethrin, cypermethrin, fenvalerate, cyfluthrin) – possess a α-cyano moiety, produce hypersensitivity, coarse tremor, clonic seizure and profuse salivation (Nasuti et al., 2003) (Table 1).

The main target of pyrethroids-induced neurotoxicity is voltage-gated sodium channels. These insecticides slow the activation or opening of the channels, shifting the voltage dependence of the gates to more hyperpolarized potentials (Clark and Symington, 2012). Therefore, the channel is held open for longer periods, allowing more sodium ions to cross, maintaining a sustained membrane depolarization. Pyrethroids also decrease the opening probability of voltage-gated chloride channels which amplify the sodium channel-mediated signs of intoxication. At relatively high concentrations, deltamethrin and cypermethrin inhibit GABA-gated chloride channels and, as with voltage-gated chloride channels, these effects are specific of type II pyrethroids (Ray and Fry, 2006). These mechanisms are responsible for the observed hyperexcitability of peripheral sites (type I) or central nervous system (type II) in acute poisonings (Table 1).

Currently, the major concerns of exposure to pyrethroids are developmental neurotoxicity and nigrostriatal dopaminergic neurodegeneration (Shafer et al., 2005; Singh et al., 2012a) (Table 2). Rat pups exposed to deltamethrin 0.7 mg/kg/day over postnatal days 9–13, resulted in a delayed appearance of radial glial fibers, which guide the migration of granule cells of cerebellum (Patro and Patro, 2005). The same group, years later, showed that deltamethrin at the same dose regimen and postnatal period, induced the up-regulation of S100β, a biomarker of brain damage, reduced dendritic arbor with short primary dendrites of purkinje neurons and much reduced stumpy and hypertrophied dendritic branches (Patro et al., 2009). However, the effect of these insecticides in muscarinic receptors, disruption of voltage-dependent sodium channels and other cellular targets, are poorly correlated with the adverse outcomes in adulthood (Ray and Fry, 2006; Shafer et al., 2005).

Low dose of permethrin (0.8–1.5 mg/kg) given to C57 B1/6 mice caused a 33% increase in DA uptake (Karen et al., 2001), similarly with another studies (Bloomquist et al., 2002; Pittman et al., 2003), and a significant increase of DAT protein levels 28 days post treatment (Gillette and Bloomquist, 2003). Unlike DAT, the up-regulation of α-synuclein protein was maximal one day post-treatment and returned to normal levels by the 14- and 28-day (Gillette and Bloomquist, 2003). Kou and colleagues showed that 3-month exposure to permethrin (1.5 mg/kg, once a week) had no effect on the expression of TH and DAT protein in striatal dopaminergic terminals, while exposure for longer period (6 months) to either 0.8 mg/kg or 1.5 mg/kg up-regulated TH expression, but did not alter the expression of DAT (Kou and Bloomquist, 2007). Concomitant treatment of permethrin (0.8 mg/kg or 1.5 mg/kg) with MPTP (20 mg/kg) for 3 or 6 months did not augment the neurotoxicity of MPTP on the striatal dopaminergic system (Kou and Bloomquist, 2007). Despite some changes were observed across the studies, there is lack of neurodegeneration of dopaminergic neurons after long-term, low-dose exposure to permethrin alone.

Deltamethrin is a type II pyrethroid insecticide, for which the main target is the central nervous system as the compound has little or no peripheral effects common to other pyrethroids. Deltamethrin induces apoptotic cell death in cultured cerebral cortical neurons (Wu et al., 2003), affects different neuronal subtypes in hippocampus, and interferes with cholinergic and dopaminergic neurotransmission mechanisms in different models (Wu and Liu, 2000). Lazarini and colleagues reported that deltamethrin increases DOPAC levels without changes in DA levels in the adult striatum after prenatal exposure of dams to a non-toxic deltamethrin dose. During their adult life, male rats showed a decreased immobility latency to float and in general activity after the swimming test (Lazarini et al., 2001). Dermal exposure to deltamethrin (30 mg/kg/day, 4 weeks) using an administration schedule mimicking a possible long-lasting occupational skin contamination is accompanied by cerebrocortical injury and loss of hippocampal and striatal DA and DA transporter (Tayebati et al., 2009).

Cypermethrin has been the pyrethroid that has raised more concerns regarding the increase of risk of developing PD. Singh and colleagues conducted several studies that highlighted the nigrostriatal dopaminergic neurotoxicity of this pesticide (Singh

Table 2
Referenced studies in developmental neurotoxicity of pyrethroids.

Model	Compound/dose	Major Findings	References
Rat pups PND 0–7 and 9–13	Deltamethrin 0.7 mg/kg/day, i.p.	Up-regulation of S100β. Reduced dendritic arbor with short primary dendrites of purkinje neurons.	Patro et al. (2009)
Rat pups PND 5–19	Cypermethrin 1.5 mg/kg, 2× week (2 weeks); rechallenged in adulthood 15 mg/kg (2× week, 4, 8, 12 weeks) i.p.	Neurodegeneration only after 12 weeks in adult rats. Postnatal preexposure enhances the susceptibility, when rechallenged during adulthood	Singh et al. (2012a,b)
Rat pups PND 5–19	Cypermethrin 1.5 mg/kg, 2× week (2 weeks); rechallenged in adulthood 15 mg/kg (2× week, 4, 8, 12 weeks) i.p.	Reduction of DA levels, motor dysfunction and loss of TH^+ cells-microglial activation dependent	Singh et al. (2011a,b)
Rat pups PND 5–19	Cypermethrin 1.5 mg/kg, 2× week (2 weeks); rechallenged in adulthood 15 mg/kg (2× week, 4, 8, 12 weeks) i.p.	ROS generation, lipid peroxidation and modulation of VMAT 2, CYP2E1, GSTA4-4 expressions	Tiwari et al. (2010)
Pregnant rats GD 6–15	Deltamethrin 0.08 mg/kg, p.o., once daily	PND 21: no differences in locomotion frequency and immobility duration of male and female; increased male rearing frequency. PND60 males: decreased immobility latency to float; increased DOPAC, DOPAC/DA	Lazarini et al. (2001)

CYP2E1: cytochrome P450 isoform 2E1; DA: dopamine; DOPAC: 3,4-dihydroxyphenylacetic acid; GSTA4-4: glutathione-S-transferase; PND: postnatal days; ROS: reactive oxygen species; TH^+: tyrosine hydroxylase positive; VMAT 2: vesicular monoamine transporter 2.

et al., 2011a, 2011b, 2012a, 2012b). Their main findings show that cypermethrin induces neurodegeneration only after long-term exposure (12 weeks) in adult rats and that postnatal pre-exposure enhances the susceptibility, when rechallenged during adulthood (Singh et al., 2012b). Cypermethrin induced-reduction of DA levels, impairment in motor activities and loss of TH^+ cells are microglial activation-dependent (Singh et al., 2011a). Besides microglial activation, other mechanisms of cypermethrin-mediated neurotoxicity have been proposed such as generation of ROS, modulation of antioxidant enzymes and CYP2E1 (Giray et al., 2001; Tiwari et al., 2010) (Table 2).

6. Organophosphates and carbamates

OP are a group of acetylcholinesterase (AChE) inhibitors and represent the largest group of insecticides sold worldwide. Acute OP poisonings leads to the development of three main syndromes: (i) acute cholinergic crisis; (ii) intermediate syndrome (IMS), and (iii) OP-induced delayed polyneuropathy (OPIDP) (Moretto, 1998). Both IMS and OPIDP result of an acute exposure to OP, usually after a suicide attempt or accidental ingestion. The acute cholinergic crisis results from the inhibition of AChE leading to overstimulation of nicotinic and muscarinic receptors in the central and peripheral nervous systems and the consequent signs and symptoms (Lotti, 2001).

IMS is considered a spectrum disorder of the neuromuscular junction that occurs 24–96 h after ingestion of an OP in conscious patients who received treatment for the acute cholinergic syndrome. Respiratory failure associated with IMS is a major contributor to the high morbidity, mortality, and cost of OP poisoning (Abdollahi and Karami-Mohajeri, 2012). The pathophysiology of IMS remains poorly understood although several possible causes such as delayed AChE inhibition, muscle necrosis, down regulation or desensitization of postsynaptic acetylcholine receptors, failure of postsynaptic acetylcholine release, and oxidative stress-related myopathy have been considered to be involved in IMS (Jayawardane et al., 2008, 2009; Yang and Deng, 2007). The toxicokinetics and chemical properties of certain OP critically contribute to the higher probability to develop IMS. For instance, more lipophilic OP are well distributed into fat, leading to a delayed and prolonged AChE inhibition. Other factors reflect the detoxification of OP such as polymorphisms in *cytochrome P450-paraoxonase 1* (*PON1*), *glutathione S-transferases*, and *cytochromes P450* (Androutsopoulos et al., 2011; Furlong, 2000; La Du et al., 2001; Xiao et al., 2003).

OPIDP is a relatively rare sensory-motor distal axonopathy in humans characterized by degeneration of long axons in the central and peripheral nervous system that appears about 2–3 weeks after exposure or later [reviewed by Jokanovic et al., 2011]. The irreversible inhibition of neuropathy target esterase (NTE) is thought to be the main mechanism involved in the pathogenesis of OPIDP (Lotti and Moretto, 2005). At least 70% of peripheral nerves NTE must be inhibited and subsequently aged to cause the instigation of OPIDP (Johnson, 1990). The irreversible phosphorylation of NTE induces a toxic gain of function by leading to calcium entry, elevation of axonal calpain activity and Wallerian-type degeneration (Glynn, 2000).

Carbamates also act by carbamylating the same site on AChE and NTE, which reversibly inhibits these enzymes activity. Carbamates include insecticides and drugs, such as pyridostigmine and disulfiram. Several clinical cases of polyneuropathy associated with exposure to high levels of carbamates have been reported (Lotti and Moretto, 2006). In three cases, intoxication with methylcarbamates resulted in a clinical and electrophysiological evaluation consistent with peripheral polyneuropathy with distal axonopathy similar to that of OPIDP (Dickoff et al., 1987; Umehara et al., 1991; Yang et al., 2000). More recently, Hu and colleagues reported a case of self-poisoning with a mixture of methomyl–alphamethrin that resulted in cortical blindness and delayed neuropathy (Hu et al., 2010).

Despite the toxic cholinergic effects of OP, there is now substantial evidence that non-cholinergic mechanisms might be associated with the adverse consequences from repeated exposures to low levels of certain OP, such as impairments in attention, memory, and other domains of cognition, as well as chronic illnesses where these symptoms are manifested (*e.g.*, Gulf War Illness, AD) (see reviews, Androutsopoulos et al., 2013; Terry, 2012). Chronic low-level exposure to the OP dichlorvos, in adult rats, triggered neuronal apoptosis, elicited an oxidative stress and inflammatory response with impaired mitochondrial complexes I, III and IV activities (Kaur et al., 2007). Binukumar and colleagues also showed that dichlorvos caused nigrostriatal dopaminergic degeneration, reduction in striatal DA and tyrosine hydroxylase levels and positive inclusions for α-synuclein and ubiquitin, resembling the PD features (Binukumar et al., 2010). Others suggested that OP, at doses that were not associated with acute signs of toxicity, can lead to deficits in axonal transport, mitochondrial dynamics (Middlemore-Risher

et al., 2011; Terry et al., 2003, 2007) similar to what has been proposed to be involved in the pathogenesis of ALS and AD (Reddy et al., 2012; Shi et al., 2010; Stokin and Goldstein, 2006). Cdk5-dependent hyper-phosphorylation of tau has been considered a biomarker for AD pathology (Maccioni et al., 2001). Chlorpyriphos induced the dysregulation of the D1 receptor/cAMP/PKA signaling pathway, potentiation of corticostriatal glutamatergic transmission, hyperphosphorylation of tau, and induction of aberrant activity of the neuronal protein kinase Cdk5 (Torres-Altoro et al., 2011). As mentioned above, the toxicokinetics of OP significantly contribute to their toxicity. PON1 is an A-esterase which detoxifies several organophosphate-oxons that result from phase-I metabolism of OP such as diazinon, parathion and chlorpyriphos (Costa et al., 2003). There are two main polymorphisms in PON1, one that affects the catalytic site of the enzyme, PON1 192Q/R polymorphism and other, PON1 55L/M polymorphism that is associated with low serum concentration of the enzyme. Importantly, PON1 genotypes might be associated with PD, AD and ALS (Androutsopoulos et al., 2011; Dardiotis et al., 2013). Carriers of PON155L/M allele, variant MM genotype and homozygotes for 192R allele possess an increased risk in developing PD (Akhmedova et al., 2001; Manthripragada et al., 2010). However, the data is controversial and several studies have not found an association between PON1 genotypes and PD (Akhmedova et al., 1999; Wingo et al., 2012). The studies focused in the study of the role of PON1 genotypes in AD prevalence have also found positive and negative associations (Androutsopoulos et al., 2011). In a large population of 730 Caucasian and 467 African American AD cases, the authors found a significant association with PON1 S161 C/T polymorphism (Erlich et al., 2006). More recently, the groups of Leduc and Erlich have corroborated these results, showing that low levels of PON1 protein, lesser catalytic activity toward paraoxon, and presence of the methionine allele of the 55L/M polymorphism are risk factors for AD (Erlich et al., 2012; Leduc et al., 2009). Morahan and colleagues, found that PON1 promoter polymorphisms were strongly associated with ALS by reducing PON1 expression and possibly modulating the susceptibility of motor neurons to OP (Morahan et al., 2007). As for the other neurodegenerative diseases, despite the epidemiological results demonstrating the association of PON1 and ALS, it is still controversial whether paraoxonases are implicated in this disease pathogenesis (Androutsopoulos et al., 2011).

The main concern regarding the neurotoxicity of OP is related to neurobehavioral changes after long-term exposure to low doses of OP. Recently, Starks and colleagues conducted a study to assess neurobehavioral function in licensed pesticide applicators enrolled in the Agricultural Health Study in Iowa and North Carolina (Starks et al., 2012). The study included 701 male participants and quantitative measures of nine neurobehavioral tests to assess memory, motor speed and coordination, sustained attention, verbal learning and visual scanning and processing. Frequent use of at least one OP pesticide was negatively associated with performance on three of nine neurobehavioral tests and with significantly better performance on six of nine tests. Only malathion was significantly associated with reduced performance on a test of visual scanning and processing (Starks et al., 2012). The inconsistency of association between long-term low or moderate exposure to OP and impaired neurobehavioral function or other neurological effects is seen across several other epidemiological studies (Colosio et al., 2009; Farahat et al., 2003; Kamel and Hoppin, 2004). The main limitations of the available data are the reduced number of neurobehavioral function tests used per study, and the fact that several studies include cases of a previous acute poisoning to high levels of OP (Roldan-Tapia et al., 2006). Therefore, the observed neurological alterations could be due to an unspecific brain injury, resulting from ischemia/hypoxia, or post-traumatic stress disorder.

Colosio and colleagues reviewed 24 papers published on human neurobehavioral effects of OP and/or carbamates, and found that only 6 papers considered the whole spectrum of functions, the studies yielding positive or uncertain results being 13 for cognitive function, 11 for psychomotor function, 11 for sensory-motor function, and 11 for psychological function impairment. In 46% of the positive studies a previous severe acute poisoning was reported (Colosio et al., 2009). Another limitation of the studies is the absence of qualitative and quantitative measurement of OP, correlation to AChE activity and neurobehavioral function. Few studies have evaluated these aspects and found positive correlation (Rasoul et al., 2008; Rothlein et al., 2006). In other studies, confounding factors such as exposure to different OP and other pesticides, laboratory methodology, and size of the population, might have led to the poor validity of the cause–effect relationship of OP exposure to the neurobehavioral effects, despite the consistency in the neurobehavioral findings (Rohlman et al., 2011). Even experimental studies in animal models fail to reproduce low dose, long-term exposures to OP. In general, the doses used are sufficiently high to exert signs of acute toxicity and exposure is no longer than 1–3 months. Additionally, neurobehavioral adverse effects only appear when AChE is inhibited (Moser, 2007).

Over the past decade, a growing body of epidemiological and experimental evidence suggests that the oxon metabolites of phosphorothionates insecticides, especially chlorpyriphos (CP) and diazinon, are responsible for the neurodevelopmental toxicity of OP (Bouchard et al., 2011; Flaskos et al., 2007; Flaskos and Sachana, 2010; Rohlman and McCauley, 2010) (Table 3). In different type of primary neuronal or culture cells, CP affected the expression of activated of Ca^{2+}/cAMP response element binding protein (CREB), a transcription factor involved in brain development, and impaired neurite outgrowth, an *in vitro* index of neuronal differentiation (Flaskos et al., 2011; Schuh et al., 2002). CP also decreased the activity of choline acetyltransferase, glutamate decarboxylase, glutamine synthase and cyclic nucleotide phosphohydrolase, biomarkers of neuronal cells, astrocytes and oligodendrocytes, respectively (Monnet-Tschudi et al., 2000). Flaskos and colleagues reviewed several studies revealing that CP and diazinon oxons's deleterious effects on neuritogenesis are not etiologically related to the inhibition of the enzymatic activity of AChE (Flaskos, 2012). In fact, besides the AChE classical role in synaptic transmission, it also has other 'non-classical' effects such as cell adhesion, shown by the detection of a new class of proteins, the cholinesterase-like adhesion molecules (Paraoanu and Layer, 2008). These adhesion properties are intimately involved in AChE promotion of neurite outgrowth and neural network formation. The suggested mechanism by which AChE might regulate neuritogenesis is associated with its non-catalytic morphogenic activity, protein–protein interactions that may act as a neurite-attractive, as well as network-stabilizing protein during neural development, and neurodegenerative diseases. Alterations in glial cell development result in a higher vulnerability to myelination, synaptic plasticity, and architectural modeling, which is extended until adolescence (Garcia et al., 2002). In glial cells, CP was shown to inhibit cell replication and disrupt cell differentiation. Additionally, CP altered the integrity of the microtubule network and decreased the levels of the microtubule-associated protein MAP 1B and, particularly, tubulin, as well as a reduction in the levels of the cytoskeletal glial fibrillary acidic protein (GFAP) (Garcia et al., 2005). In summary, the above *in vitro* data reveals that organophosphate-oxons are capable of disrupting separately most phases of nervous system development namely, neuronal cell proliferation, differentiation and apoptosis and glial cells proliferation and differentiation.

Bouchard and colleagues, conducted a birth cohort study to assess the association between prenatal and postnatal exposure to OP pesticides, indicated by urinary dialkyl phosphate (DAP)

Table 3
Referenced *in vitro* studies in long-term exposure to low doses of organophosphates and neurodevelopment.

Model	Concentration/dose	Major findings	References
Fetal rat (DIV 5–15 and DIV 25–35) aggregating cell culture of telencephalon	CP (10^{-8} to 10^{-4} M), CP oxon (10^{-10} to 10^{-5} M), parathion (10^{-10} to 10^{-4} M), paraoxon (10^{-10} to 10^{-5} M), during 10 days	Decreased choline acetyltransferase, glutamate decarboxylase, glutamine synthase and cyclic nucleotide phosphohydrolase activities	Monnet-Tschudi et al. (2000)
Rat pups hippocampal and cortical neurons	CP and CP oxon (0.001–10 μM)	AChE-independent increase of pCREB	Schuh et al. (2002)
N2a neuroblastoma cells	CP oxon (1–10 μM)	Inhibition of axon outgrowth; reduced levels of protein-43 and NFH	Flaskos et al. (2011)
PC12 pheochromocytoma and C6 glioma cells	CP and CP oxon (30 μM)	AChE-independent inhibition of DNA synthesis	Qiao (2001, #577)
N2a neuroblastoma cells	DZ (10 μM)	Inhibition of neurite outgrowth	Flaskos et al. (2007)
C6 glioma cells	CP (5 μg/ml)	Impairment of G-protein signaling, impairment of cell differentiation, reduced expression of the transcription factor Sp1, ↑ROS; the effects were greater in undifferentiated C6 cells but were still detectable in differentiating cells	Garcia (2001, #578)
C6 glioma cells	DZ oxon (1, 5 and 10 μM)	Decreased GFAP expression, reduced levels of tubulin and MAP1B. Reduced outgrowth of extensions from C6 cells under differentiation-promoting conditions	Sidiropoulou (2009, #579)
C6 glioma cells	CP and CP oxon (1–10 μM)	Inhibition of the outgrowth of differentiating cells, reduced levels of tubulin and MAP1B	Sachana (2008′ #580)

AChE: acetylcholinesterase; CP: chlorpyriphos; DZ: diazinon; GFAP: glial fibrillary acidic protein; MAP1B: microtubule associated protein 1B; NFH: neurofilament heavy chain; pCREB: phosphorylated Ca2+/cAMP response element binding protein; ROS: reactive oxygen species.

metabolite concentrations, in urine collected during pregnancy and from children at 6 months and 1, 2, 3, 3.5, 5 years of age and cognitive abilities of 7-year-olds (Bouchard et al., 2011). Prenatal, but not postnatal, urinary DAP concentrations were associated with poorer intellectual development in 7-year-old children (Bouchard et al., 2011). Rauh and colleagues reported that prenatal exposure to high levels of CP was associated with higher cognitive deficits evaluated by two different indices, Working Memory Index and Full-Scale IQ in children at 7 years of age (Rauh et al., 2011). Accordingly, Horton and colleagues found that males exposed to CP during the prenatal period were more susceptible to experience a decrement in working memory than females (Horton et al., 2012).

7. Concluding remarks

In humans, pesticides can be responsible for diverse acute and long-term health effects. Even though not consistent, there is a growing body of epidemiologic evidence linking long-term/low-dose pesticide exposure to cancer, reproductive health issues, neurodegenerative diseases such as AD, PD, and neurodevelopment impairments in children. Experiments concerning the environmental etiology of PD are more frequent than for other diseases, and several different animal models have been proposed (Cicchetti et al., 2009; Drechsel and Patel, 2008; Moretto and Colosio, 2011). However, a crucial issue is translation for real human exposure to pesticides, tissue concentration reached, and dose regimen used in animal experiments. In these models, a relatively high dose or few consecutive doses of a single compound is usually administered during a short period of time (days or weeks) and the behavioral and/or biochemical analyses are performed within few weeks as well. In contrast, increased health risks are associated with exposure to low levels for several years to decades to a combination of different environmental toxicants. Therefore, there is an urgent need to standardize the doses, age, species or strain, duration of treatment and the methodology to assess neurodegeneration.

Particularly, PQ and MB exposure has been largely associated with PD. Other pesticides such as rotenone, dieldrin and diquat have also been shown to reproduce some features of PD in animal models. However, no single compound, including the non-pesticide MPTP, is able to reproduce all the hallmarks of human PD (Blesa et al., 2012; Cicchetti et al., 2009). Combined exposure to PQ + MB, or MPTP + PQ/MB yields potentiated damage to dopaminergic system, producing cell damage and loss, even when the doses of each compound are non-toxic. Most likely, PD might result from a prolonged contact to sub-toxic multi-hits at different targets within the dopaminergic system.

Despite the numerous studies, association between neurobehavioral adverse effects and OP is only consistent regarding subjects acutely poisoned, instead of the uncertainty in data concerning subjects chronically exposed to low doses of OP. Because of the complexity of the effects of environmental exposures on human health, the current available data do not support a good correlation between actual pesticide exposure and development of PD or other neurodegenerative diseases. Further research should focus on the improvement of the characterization of exposure in epidemiological studies (pesticide identification and quantification), particularly the categorization of previously acute poisoned subjects and prevalent/incident cases. Future investigations should also concentrate in designing animal studies that better simulates human exposure and measures the same aspect of neurological function and outcomes.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

This work received financial support of the Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), CRL and of the Faculty of Medicine of Porto University. Ricardo Dinis-Oliveira and Maria Teresa Baltazar, acknowledges FCT for their investigator (IF/01147/2013) and PhD grants (SFRH/BD/65387/2009), respectively. The Research Centre on Physical Activity Health and Leisure (CIAFEL) is supported by Pest-OE/SAU/UI0617/2011. We also acknowledge the designer Joana Macedo for the artwork performed for the article.

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ORIGINAL ARTICLE

Occupational exposure to pesticides and endotoxin and Parkinson disease in the Netherlands

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▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/oemed-2014-102170).

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Received 16 February 2014
Revised 11 July 2014
Accepted 23 July 2014
Published Online First 7 August 2014

ABSTRACT

Objectives Previous research has indicated that occupational exposure to pesticides and possibly airborne endotoxin may increase the risk of developing Parkinson disease (PD). We studied the associations of PD with occupational exposure to pesticides, specifically to the functional subclasses insecticides, herbicides and fungicides, and to airborne endotoxin. In addition we evaluated specific pesticides (active ingredients) previously associated with PD.

Methods We used data from a hospital-based case–control study, including 444 patients with PD and 876 age and sex matched controls. Exposures to pesticides from application and re-entry work were estimated with the ALOHA+job-exposure matrix and with an exposure algorithm based on self-reported information on pesticide use. To assess exposure to specific active ingredients a crop-exposure matrix was developed. Endotoxin exposure was estimated with the DOM job-exposure matrix.

Results The results showed almost no significant associations. However, ORs were elevated in the higher exposure categories for pesticides in general, insecticides, herbicides and fungicides, and below unity for endotoxin exposure. The analyses on specific active ingredients showed a significant association of PD risk with the fungicide benomyl.

Conclusions This study did not provide evidence for a relation between pesticide exposure and PD. However, the consistently elevated ORs in the higher exposure categories suggest that a positive association may exist. The possible association with the active ingredient benomyl requires follow-up in other studies. This study did not provide support for a possible association between endotoxin exposure and PD.

What this paper adds

- Occupational pesticide exposure has been associated with an increased risk of developing Parkinson disease (PD), but it is unclear which specific active ingredients are responsible.
- It has been postulated that occupational exposure to endotoxin may increase PD risk as well.
- In a multicentre case–control study on PD in the Netherlands, no significant associations with insecticides, herbicides or fungicides were observed, but active ingredient-specific analyses revealed a possible association with benomyl, a benzimidazole fungicide.
- No association between endotoxin exposure and PD was apparent.

INTRODUCTION

It has been frequently reported in epidemiological and toxicological studies that exposure to pesticides may increase the risk of developing Parkinson disease (PD).[1–3] Pesticides are widely used in agriculture and therefore farm work is an important source of exposure. Exposure occurs during the actual application of pesticides, but also during contact with crops treated with pesticides when carrying out so-called re-entry activities such as weeding or thinning. Exposure from re-entry work could be substantial depending on the crop.[4 5]

Since a wide range of pesticides (active ingredients) have been used in the past, it is unclear which active ingredients are responsible for the reported increase in PD risk in epidemiological studies. The accuracy of self-reported information on performed applications in the past is limited for specific active ingredients.[6] Moreover, farm workers who have performed re-entry work but were not involved in the application or purchase of pesticides might not be able to accurately report on the use of pesticides. The use of occupational histories to assign exposure is less affected by recall problems.[7] However, only a few of the previous epidemiological studies on PD risk used job titles[8–11] or a combination of job titles with self-reported information[12 13] to assess exposure to pesticides or functional subclasses of pesticides, for example, insecticides, herbicides and fungicides.

Recently, it has been postulated that not only pesticides may increase PD risk, but also endotoxin exposure, by inducing inflammation-mediated neurodegeneration.[11 14] Endotoxins are the lipopolysaccharide components of Gram-negative bacterial cell walls, and exposure is common during agricultural work.

We present the results of analyses on the possible associations between occupational exposure to pesticides and endotoxin and PD risk within a recently conducted hospital-based case–control study in the Netherlands. We used the existing general

To cite: van der Mark M, Vermeulen R, Nijssen PCG, *et al*. *Occup Environ Med* 2014;**71**:757–764.

population ALOHA+job-exposure matrix (JEM)[15] and self-reported exposure information to estimate exposure to insecticides, herbicides and fungicides through mixing and application work and through re-entry work in treated crops. Furthermore, we constructed a time-dependent crop-exposure matrix to estimate exposure to specific active ingredients based on self-reported cultivation of crops. For exposure to endotoxin we used the recently developed DOM-JEM.[16]

METHODS

Cases and controls

Details about the study methodology were described previously.[17] Briefly, cases and controls were recruited between April 2010 and June 2012 from five hospitals in four different areas in the Netherlands. We set out to include all patients who had an initial PD diagnosis in one of the participating hospitals between January 2006 and December 2011. In the Netherlands, with a universal healthcare system, all patients with PD are seen in a hospital. Patients with PD included can therefore be regarded to be representative for all patients with PD in the service areas of the participating hospitals. In each hospital, one neurologist reviewed the medical files of all potential participants. For each confirmed patient with PD, two matched controls were selected from persons who were seen at the department of neurology between January 2006 and December 2011 for non-neurodegenerative symptoms (median nerve neuropathy; International Classification of Diseases, 10th revision (ICD-10) G56.0 and G56.1, ulnar nerve neuropathy; ICD-10 G56.2, thoracic and lumbar disc disease; ICD-10 G55.1, G54.3 and G54.4, and sciatica; ICD-10 M54.3 and M54.4). The controls were matched to the cases on hospital, visiting date (within 3 years of the case diagnosis year), sex and age. Cases and controls were initially contacted via an invitation letter from the hospitals' neurology departments together with a reply form for giving informed consent or to decline study participation. The study information explained that the study objective was to assess risk factors for neurological disorders, without further specification. Non-responders were sent a reminder after 1 month, and one phone call attempt was performed after another month.

At recruitment, 1001 (93% of total) eligible patients with PD were still alive and of 993 of those we had a valid current address. There were 448 persons who participated (45%), 406 who declined participation and 139 who did not reply. The participation rate for controls was 35%. About 50% of the non-participants provided a reason for their decline. Health-related reasons were reported most frequently, but compared with cases, more controls reported to be not interested. For 12 cases only 1 suitable control was found and for 4 cases no controls were found, leaving 444 cases and 876 controls who were included in the analyses.

Data collection

Participants were interviewed in a standardised computer-assisted telephone interview by one of three trained interviewers. The questionnaire contained an occupational history in which all jobs performed for at least 6 months were included. Study participants reported on years and hours per week worked, job title, type of industry, company name and main tasks. Supplemental questions about occupational application of insecticides, herbicides and fungicides were asked to those who reported to have worked on a farm or as a gardener. The annual number of days on which applications were performed at the job (<1/year, 1–5/year, 6–20/year, 21–50/year or >50/year) was asked. Questions about application method and use of protective equipment were asked to participants who had personally applied pesticides. Furthermore, participants who worked on a farm were asked to name the main crop types cultivated at the farm with a maximum of three. Participants reporting having worked at their parents' farm during childhood were not always asked additional questions on working hours and pesticide applications (n=40). Based on what was reported by other participants with similar jobs, we assumed that those participants helped 8 h per week at the farm from age 12 to 18 and in case no farm type was provided we assumed it was a mixed farm.

All jobs were coded according to the International Standard Classification of Occupations 1968 and 1988 (ISCO68 and ISCO88).

Assessment of pesticide exposure

Exposures were estimated from 1955 until the calendar year before diagnosis, as after this year synthetic pesticides became commonly used in the Netherlands. Occupational exposure to pesticides was estimated using three different methods.

The first method estimated pesticide exposure by linking all reported jobs to the ALOHA+JEM.[15] This JEM assigns exposure to pesticides, and to functional subclasses (ie, insecticides, herbicides and fungicides) using arbitrary weights of 0, 1 and 4 for no, low and high exposure. For farm and gardener jobs the JEM score was set to 0 if the participant reported that no insecticides (n=40), herbicides (n=41) or fungicides (n=77) had been applied. For jobs coded ISCO88 code 9333 (freight handlers), exposure to insecticides was only assigned to those jobs coded ISCO68 code 97120 (dockers). Cumulative exposures were estimated by multiplying the JEM scores with years worked in a job, summed across all jobs of a participant's occupational history.

The second method estimated more specifically the exposure for participants who held a farm or gardener job. For these jobs, we developed an exposure model (algorithm) for insecticides, herbicides and fungicides, based on the algorithm developed for the US Agricultural Health Study (AHS) for estimating applicator exposure.[18] We extended the algorithm by including estimates for exposure due to re-entry work.

Applicator exposure was calculated for participants who reported to have applied insecticides, herbicides or fungicides personally. Exposure intensity was estimated based on the application method and use of personal protective equipment (PPE) in accordance to the AHS algorithm. However, as we had no information on performing maintenance or repair of application equipment, and because essentially all applicators in our study mixed the pesticides before use, these factors included in the AHS algorithm were left out in our study.

For the application method, the AHS model uses relative exposure values of one for distribution of tablets/granules, three for a boom sprayer on a tractor, eight for a backpack sprayer and nine for a hand sprayer. Since the European Predictive Operator Exposure Model (EUROPOEM) shows roughly a factor two difference in exposure between manual and tractor spraying, we adjusted the AHS values slightly to one for distribution of tablets/granules, four for tractor application and eight for manual application by backpack or hand spray.[19 20]

The values for the use of PPE were based on the AHS algorithm and were 1 for individuals not using PPE, 0.8 for individuals using gloves, rubber boots or goggles, and 0.5 for individuals who also used impermeable clothing or facemasks. If a participant reported that the use of PPE had changed over the

years of working in a particular job, average values of categories were assigned.

Yearly applicator exposure was calculated by multiplying the intensity level with the number of applications per year using the midpoint of the answer categories and the percentage of applications performed by the participant at the farm (mostly/always: 0.9, sometimes: 0.5 and rarely: 0.1).

Yearly applicator exposure=application method×PPE×applications/year×percentage self-application by participant.

Yearly exposure from re-entry work was estimated by multiplying the intensity level for a day of re-entry work with the number of days of re-entry work. Based on the EUROPOEM applicator and re-entry worker models, we estimated that a day of re-entry work would result in 10% of the exposure of a typical day of application work.[20 21]

Since most applications were conducted by tractor or manually by backpack or hand spray, which in the applicator part of the model have on average an intensity level of 6, we used an intensity level of 0.6 for a day of re-entry work. The yearly number of days with re-entry work was calculated from the reported number of applications per year at the farm, the number of days a pesticide was assumed to be present on the crop after application and an estimated number of days that a participant performed re-entry work after each application. The number of applications per year at a farm was imputed for participants who did not know if pesticides were applied at a job or at what frequency, based on the most frequently reported answer by other participants for similar jobs. Although the number of days a pesticide is present on the crops after application depends on pesticide type, crop type and weather conditions, we assumed a period of 14 days based on data in the literature.[5 22] Furthermore, we estimated that workers who worked 40 h/week at farms with horticultural or fruit crops performed 5 days/week re-entry work (ISCO68 job codes: 61230, 62320, 61270, 62720, 62730) and workers at farms with field crops 1 day/week (ISCO68 job codes: 61110, 62105, 61220, 62210). These estimations were adjusted for the number of hours per week a participant had worked.

Cumulative total exposure was calculated by multiplying the yearly exposure from pesticide application plus re-entry work with the number of years in a job, summed across all jobs of a participant's occupational history.

The third method assigned exposure to specific active ingredients by linking reported crops cultivated at the participant's farm to a crop-exposure matrix. In this crop-exposure matrix, per-decade estimations are given for the percentage of farms that applied a specific active ingredient on a type of crop and the yearly frequency of application. Active ingredients included in the crop-exposure matrix were based on previous studies linking specific pesticides to PD risk and for which we had sufficient data to estimate historical application. The included active ingredients were the insecticides: dichlorvos, lindane, parathion and permethrin; the herbicides: 2,4-D, atrazine, dinoseb and paraquat; and the fungicides: benomyl and maneb.

Expert judgment on the probabilities and frequencies of application were provided by former extension workers, two per crop type. These experts estimated probability and frequency of use of active ingredients allowed for use on potatoes, cereals, beets, maize, tulip bulbs and fruit, back to the year 1960. More details about the expert estimations can be found elsewhere (Brouwer *et al*, submitted). Estimates for other field crops, and vegetables and flowers in green houses, which were not covered by the experts, were derived from data of Statistics Netherlands that gathered statistics on use of specific active ingredients since 1995. For earlier decades, probability and frequency of application for those crops were extrapolated from time trends for crops for which expert estimations were available. Statistics Netherlands also gathered data since 1976 on active ingredient use in public places, which we used to estimate exposure for gardeners.[23]

Cumulative exposures to these active ingredients were calculated by summing the yearly probability of application of a specific active ingredient at the farm multiplied with the yearly frequency across all years worked at farms. For farms where exposure to an active ingredient was assigned to more than one crop, the probability and frequency of use for the crop with the highest probability of use were taken for calculating cumulative exposure. As in the approach with the JEM analyses described earlier, no exposure was assigned to a farm job when on that farm, according to the participant, no insecticides, herbicides or fungicides had been applied.

Assessment of endotoxin exposure

High, low or no exposure (weights of 4, 1, 0, respectively, were assigned to reflect the multiplicative nature of occupational exposure distributions) to endotoxin was assigned by linking the DOM-JEM with the reported jobs.[16] Cumulative exposure was estimated by multiplying the JEM scores with years worked in a job, summed across all jobs of a participant's occupational history.

Statistical analysis

ORs and 95% CIs were calculated using conditional logistic regression. The exposed participants were categorised in two or three groups based on either median or tertiles of the distribution of the different exposures among controls. In Model 1, all analyses were adjusted for pack-years of smoking (5 levels), total coffee consumption (4 levels) and categories for occupational skill and status (high-skilled white-collar worker, low-skilled white-collar worker, high-skilled blue-collar worker and low-skilled blue-collar worker). In Model 2, the pesticide analyses were additionally adjusted for cumulative endotoxin exposure (4 levels).

RESULTS

Of the patients with PD, 63.3% were men with a median age at diagnosis of 67 years (see table 1). Cases more often had high-skilled white-collar jobs than controls, smoked less and consumed less coffee. Prevalence of pesticide exposure was 19.3% for cases and 19.1% for controls as assessed by the JEM approach (see table 2). The prevalence of exposure to the functional subclasses insecticides, herbicides and fungicides was slightly higher for cases compared with controls for the JEM approach and the exposure algorithm. More controls (41.7%) than cases (38.1%) ever had a job with low or high endotoxin exposure. Most participants who held a job with high endotoxin exposure were individuals who worked at a farm with livestock. The most reported jobs with low endotoxin exposure were other farm jobs and cleaning jobs. Correlations between exposure to subclasses of pesticides as shown in table 2 were high (Spearman correlation coefficients: 0.66–0.87). More moderate correlations (Spearman correlation coefficients: 047–0.60) were observed between endotoxin exposure and exposure to (subclasses of) pesticides.

Pesticide exposure as assessed by the JEM approach

In table 3, the analyses of cumulative pesticide exposure based on the ALOHA+JEM augmented with self-reported information on

Table 1 General characteristics of cases and controls

	PD cases (n=444)	Controls (n=876)
Men, N (%)	281 (63.3)	557 (63.6)
Age at interview, median (range)	68 (34–91)	68 (34–90)
Age at diagnosis, median (range)	67 (34–90)	–
Cigarette smoking*		
Never smoked, N (%)	207 (46.6)	243 (27.7)
>0–7.8 pack-years, N (%)	86 (19.4)	161 (18.4)
>7.8–17.5 pack-years, N (%)	67 (15.1)	155 (17.7)
>17.5–29.4 pack-years, N (%)	45 (10.1)	160 (18.3)
>29.4–103 pack-years, N (%)	39 (8.8)	157 (17.9)
Coffee consumption†		
0–97 consumption-years, N (%)	128 (28.8)	220 (25.1)
>97–156 consumption-years, N (%)	146 (32.9)	221 (25.3)
>156–214 consumption-years, N (%)	90 (20.3)	216 (24.7)
>214–720 consumption-years, N (%)	80 (18.0)	218 (24.9)
Occupational skill and status‡		
High-skilled white-collar worker, N (%)	198 (44)	335 (38)
Low-skilled white-collar worker, N (%)	87 (20)	187 (21)
High-skilled blue-collar worker, N (%)	101 (23)	202 (23)
Low-skilled blue-collar worker, N (%)	58 (13)	152 (17)

*Pack-years of cigarette smoking was calculated by dividing average number of cigarettes per day by 20 multiplied with the number of years of smoking. Pack-years were divided based on the quartiles of the exposure distribution among the controls.
†Consumption-years were calculated by multiplying the average amount of coffee consumptions per day (cups per day) with the estimated number of years of coffee consumption. Consumption-years were divided based on the quartiles of the exposure distribution among the controls. Coffee consumption information was missing for one control.
‡The categories of occupational skill and status were made according to major International Standard Classification of Occupations 1988 (ISCO88) groups (first digit of the job codes). 1–3: high-skilled white-collar jobs, 4–5: low-skilled white-collar jobs, 6–7: high-skilled blue-collar jobs, and 8–9: low-skilled blue-collar jobs. The participants were categorised according to the group in which they had worked most years during their career.
PD, Parkinson disease.

actual use of pesticides are presented. The analyses revealed no statistically significant results, but overall, relatively more cases than controls were in the higher exposure tertiles for all groups of pesticides. Increased ORs were most pronounced for exposure to insecticides. Analyses without using augmentation on self-reported actual use of insecticides, herbicides or fungicides within farm or gardener jobs, resulted in ORs closer to 1 (see online supplementary material table S1).

Pesticide exposure as assessed by the exposure algorithm

A total of 94 cases (21%) and 183 controls (21%) stated to have ever worked on a farm or as a gardener and consequently were asked supplemental questions on pesticide applications. The results of the analyses using the adjusted AHS exposure algorithm are shown in table 4. Relatively more cases than controls were in the third tertile of cumulative exposure for insecticides, herbicides and fungicides, although the elevated ORs did not reach statistical significance. Sixty-five per cent of the insecticide-exposed, 63% of the herbicide-exposed and 55% of the fungicide-exposed had not personally applied pesticides, thus for those participants only re-entry work contributed to the exposure estimates. This was especially the case for women: of the 60 women exposed to insecticides, herbicides and/or fungicides, there were only two women who had actually applied pesticides. We also performed analyses on application work only (see online supplementary material table S2). Ever having performed applications showed higher non-significant elevated ORs for insecticides and herbicides than for fungicides.

Exposure to specific active ingredients as assessed by the crop-exposure matrix

Table 5 shows the results for specific active ingredients as assessed based on self-reported crops, self-reported actual use of pesticides and applying the active ingredient-specific crop-exposure matrix. For the active ingredient benomyl (a benzimidazole fungicide), a positive association with PD was observed for the highest exposed individuals (OR=2.46; 95% CI 1.16 to 5.22), which remained statistically significant after adjustment for potential confounders. Analyses without reclassifying to non-exposed if persons reported that insecticides, herbicides or fungicides had not been applied generally resulted in lower ORs (see online supplementary material table S3).

Table 2 Prevalence of pesticide, herbicide, insecticide, fungicide, and endotoxin exposure and their correlations

	Exposed cases n (%)	Exposed controls n (%)	Correlation* insecticides	Correlation* herbicides	Correlation* fungicides	Correlation* endotoxin
JEM approach†						
Cumulative exposure						
All pesticides	86 (19.3)	167 (19.1)	0.86	0.82	0.85	0.60
Insecticides	67 (15.1)	123 (14.0)	–	0.83	0.76	0.53
Herbicides	60 (13.5)	109 (12.4)	0.83	–	0.73	0.49
Fungicides	68 (15.3)	130 (14.8)	0.76	0.73	–	0.47
Endotoxin	169 (38.1)	365 (41.7)	0.53	0.49	0.47	–
Exposure algorithm‡						
Cumulative exposure						
Insecticides	58 (13.1)	108 (12.3)	–	0.87	0.70	–
Herbicides	58 (13.1)	106 (12.1)	0.87	–	0.66	–
Fungicides	32 (7.2)	57 (6.5)	0.70	0.66	–	–

*Spearman correlation coefficient.
†Cumulative exposures were estimated by multiplying the job-exposure matrix (JEM) assigned exposures (0 for no, 1 for low and 4 for high exposure) with years worked in a job, summed across all jobs of a participant's occupational history from 1955. The JEM score was set to 0 for exposure to pesticides, insecticides, herbicides or fungicides if the participant reported that those were not applied.
‡Cumulative exposures were estimated by multiplying the exposure algorithm assigned exposure from pesticide application and re-entry work with all years worked in a job, summed across all jobs of a participant's occupational history from 1955.

Table 3 Cumulative exposure to pesticides, specific subclasses and endotoxin and Parkinson disease risk: job-exposure matrix (JEM) approach

	Cases n (%)	Controls n (%)	Crude OR (95% CI)	Model 1* OR (95% CI)	Model 2† OR (95% CI)
Pesticides‡					
Never	358 (80.6)	709 (80.9)	1	1	1
1–19	26 (5.9)	56 (6.4)	0.92 (0.57 to 1.48)	0.91 (0.55 to 1.52)	0.90 (0.52 to 1.56)
20–32	22 (5.0)	56 (6.4)	0.77 (0.46 to 1.30)	0.74 (0.43 to 1.27)	0.75 (0.42 to 1.36)
33–216	38 (8.6)	55 (6.3)	1.36 (0.89 to 2.09)	1.28 (0.79 to 2.10)	1.56 (0.86 to 2.83)
Insecticides‡					
Never	377 (84.9)	753 (86.0)	1	1	1
1–20	22 (5.0)	46 (5.3)	0.95 (0.56 to 1.61)	1.05 (0.60 to 1.84)	1.10 (0.60 to 2.03)
21–36	16 (3.6)	38 (4.3)	0.85 (0.47 to 1.55)	0.77 (0.40 to 1.47)	0.83 (0.42 to 1.66)
37–216	29 (6.5)	39 (4.5)	1.48 (0.90 to 2.41)	1.46 (0.84 to 2.53)	1.79 (0.95 to 3.37)
Herbicides‡					
Never	384 (86.5)	767 (87.6)	1	1	1
1–6	16 (3.6)	37 (4.2)	0.85 (0.46 to 1.58)	0.97 (0.51 to 1.85)	1.00 (0.51 to 1.97)
7–23	23 (5.2)	39 (4.5)	1.19 (0.70 to 2.03)	1.16 (0.66 to 2.04)	1.30 (0.70 to 2.39)
24–168	21 (4.7)	33 (3.8)	1.26 (0.71 to 2.24)	1.13 (0.59 to 2.15)	1.25 (0.62 to 2.53)
Fungicides‡					
Never	376 (84.7)	746 (85.2)	1	1	1
1–7	19 (4.3)	54 (6.2)	0.70 (0.41 to 1.19)	0.67 (0.38 to 1.18)	0.64 (0.35 to 1.17)
8–25	22 (5.0)	34 (3.9)	1.33 (0.75 to 3.37)	1.35 (0.73 to 2.50)	1.41 (0.74 to 2.71)
26–168	27 (6.1)	42 (4.8)	1.26 (0.76 to 2.07)	1.12 (0.65 to 1.93)	1.24 (0.69 to 2.23)
Endotoxin‡					
Never	275 (61.9)	511 (58.3)	1	1	
1–7	63 (14.2)	128 (14.6)	0.90 (0.64 to 1.27)	1.09 (0.76 to 1.57)	1.18 (0.78 to 1.79)
8–21	46 (10.4)	117 (13.4)	0.74 (0.51 to 1.07)	0.79 (0.52 to 1.18)	0.76 (0.48 to 1.18)
22–244	60 (13.5)	120 (13.7)	0.93 (0.67 to 1.30)	0.95 (0.64 to 1.40)	0.83 (0.51 to 1.34)

*The first adjusted model includes cigarette smoking (5 categories), coffee consumption (4 categories) and occupational skill and status (4 categories). Since information on coffee consumption was missing for one control, this participant was excluded from adjusted analyses.
†Additionally mutually adjusted: pesticide exposures to endotoxin and vice versa.
‡Conditional logistic regression analyses of pesticide and endotoxin exposure as assessed by the JEM approach. Cumulative exposures were estimated by multiplying the JEM assigned exposures (0 for no, 1 for low and 4 for high exposure) with years worked in a job, summed across all jobs of a participant's occupational history from 1955. The JEM score was set to 0 for exposure to pesticides, insecticides, herbicides or fungicides if the participant reported that those were not applied. The exposed were divided based on the tertiles of the exposure distribution among the controls.

Table 4 Cumulative exposure to specific subclasses of pesticides and Parkinson disease risk: exposure algorithm

	Cases n (%)	Controls n (%)	Crude OR (95% CI)	Model 1* OR (95% CI)	Model 2† OR (95% CI)
Insecticides‡					
Never	386 (86.9)	768 (87.7)	1	1	1
>0–20	18 (4.1)	36 (4.1)	0.99 (0.55 to 1.78)	0.94 (0.51 to 1.75)	0.99 (0.51 to 1.92)
>21–194	17 (3.8)	37 (4.2)	0.92 (0.51 to 1.68)	1.03 (0.54 to 1.96)	1.12 (0.57 to 2.22)
>195–9702	23 (5.2)	35 (4.0)	1.31 (0.76 to 2.26)	1.29 (0.71 to 2.34)	1.46 (0.76 to 2.81)
Herbicides‡					
Never	386 (86.9)	770 (87.9)	1	1	1
>0–11	17 (3.8)	35 (4.0)	0.96 (0.53 to 1.74)	0.92 (0.50 to 1.71)	0.98 (0.51 to 1.92)
>11–147	18 (4.1)	37 (4.2)	0.98 (0.55 to 1.76)	1.12 (0.60 to 2.09)	1.21 (0.62 to 2.33)
>147–9702	23 (5.2)	34 (3.9)	1.36 (0.78 to 2.37)	1.33 (0.73 to 2.43)	1.52 (0.78 to 2.97)
Fungicides‡					
Never	412 (92.8)	819 (93.5)	1	1	1
>0–73	9 (2.0)	19 (2.2)	0.96 (0.43 to 2.15)	1.08 (0.47 to 2.51)	1.17 (0.49 to 2.81)
>73–314	9 (2.0)	19 (2.2)	0.97 (0.44 to 2.14)	1.52 (0.64 to 3.56)	1.66 (0.69 to 4.00)
>314–6684	14 (3.2)	19 (2.2)	1.44 (0.72 to 2.88)	1.23 (0.59 to 2.60)	1.38 (0.64 to 2.99)

*The first adjusted model includes cigarette smoking (5 categories), coffee consumption (4 categories) and occupational skill and status (4 categories).
†The second adjusted model additionally includes endotoxin exposure (4 categories). Since information on coffee consumption was missing for one control, this participant was excluded from adjusted analyses.
‡Conditional logistic regression analyses of pesticide exposure as assessed by the exposure algorithm. Cumulative exposures were estimated by multiplying the exposure algorithm, assigned exposure from pesticide application and re-entry work with all years worked in a job, summed across all jobs of a participant's occupational history from 1955. The exposed were divided based on the tertiles of the exposure distribution among the controls.

Table 5 Exposure to specific active ingredients and Parkinson disease risk: crop-exposure matrix

	Cases n (%)	Controls n (%)	Crude OR (95% CI)	Model 1* OR (95% CI)	Model 2† OR (95% CI)
Paraquat‡					
Never	411 (92.6)	818 (93.4)	1	1	
>0–3.80	18 (4.1)	29 (3.3)	1.27 (0.68 to 2.35)	1.33 (0.68 to 2.60)	1.42 (0.71 to 2.85)
>3.80	15 (3.4)	29 (3.3)	1.03 (0.54 to 1.95)	0.91 (0.45 to 1.84)	1.01 (0.48 to 2.12)
Maneb‡					
Never	419 (94.4)	835 (95.3)	1	1	1
>0–1.38	12 (2.7)	21 (2.4)	1.16 (0.56 to 2.42)	1.33 (0.60 to 2.93)	1.42 (0.63 to 3.21)
>1.38	13 (2.9)	20 (2.3)	1.28 (0.64 to 2.57)	1.16 (0.55 to 2.45)	1.31 (0.60 to 2.85)
Atrazine‡					
Never	423 (95.3)	845 (96.5)	1	1	1
>0–0.35	9 (2.0)	14 (1.6)	1.30 (0.56 to 3.00)	1.03 (0.42 to 2.52)	1.05 (0.42 to 2.61)
>0.35	12 (2.7)	17 (1.9)	1.40 (0.66 to 2.98)	1.08 (0.48 to 2.43)	1.15 (0.50 to 2.65)
Benomyl‡					
Never	420 (94.6)	841 (96.0)	1	1	1
>0–0.27	7 (1.6)	20 (2.3)	0.69 (0.29 to 1.66)	0.80 (0.31 to 2.05)	0.88 (0.34 to 2.27)
>0.27	17 (3.8)	15 (1.7)	2.46 (1.16 to 5.22)	2.23 (1.01 to 4.82)	2.47 (1.05 to 5.78)
Dinoseb‡					
Never	422	844 (96.3)	1	1	1
>0–3.46	11 (2.5)	16 (1.8)	1.38 (0.64 to 2.96)	1.34 (0.60 to 3.03)	1.41 (0.61 to 3.26)
>3.46	11 (2.5)	16 (1.8)	1.36 (0.62 to 2.97)	0.91 (0.38 to 2.18)	0.98 (0.39 to 2.46)
Dichlorvos‡					
Never	430 (96.8)	850 (97.0)	1	1	1
>0–9.6	7 (1.6)	12 (1.4)	1.18 (0.45 to 3.07)	2.04 (0.73 to 5.72)	2.06 (0.72 to 5.90)
>9.6	7 (1.6)	14 (1.6)	0.97 (0.38 to 2.46)	0.87 (0.32 to 2.37)	0.96 (0.34 to 2.66)
Lindane‡					
Never	407 (91.7)	810 (92.5)	1	1	1
>0–0.35	17 (3.8)	35 (4.0)	0.95 (0.52 to 1.74)	0.85 (0.45 to 1.60)	0.89 (0.46 to 1.73)
>0.35	20 (4.5)	31 (3.5)	1.29 (0.72 to 2.31)	1.26 (0.67 to 2.38)	1.39 (0.70 to 2.75)
Parathion‡					
Never	404 (91.0)	796 (90.9)	1	1	1
>0–1.53	17 (3.8)	41 (4.7)	0.81 (0.45 to 1.44)	0.85 (0.46 to 1.57)	0.85 (0.45 to 1.63)
>1.53	23 (5.2)	39 (4.5)	1.16 (0.68 to 1.99)	1.09 (0.60 to 1.98)	1.22 (0.64 to 2.31)
Permethrin‡					
Never	427 (96.2)	852 (97.3)	1	1	1
>0–1.03	7 (1.6)	12 (1.4)	1.18 (0.45 to 3.07)	1.32 (0.47 to 3.74)	1.37 (0.47 to 3.99)
>1.03	10 (2.3)	12 (1.4)	1.62 (0.70 to 3.75)	1.44 (0.57 to 3.67)	1.60 (0.60 to 4.30)
2,4-D‡					
Never	415 (93.5)	832 (95.0)	1	1	1
>0–0.25	11 (2.5)	22 (2.5)	1.02 (0.48 to 2.18)	1.05 (0.46 to 2.38)	1.13 (0.49 to 2.64)
>0.25	18 (4.1)	22 (2.5)	1.68 (0.87 to 3.24)	1.51 (0.74 to 3.08)	1.68 (0.81 to 3.49)

*The first adjusted model includes cigarette smoking (5 categories), coffee consumption (4 categories) and occupational skill and status (4 categories).
†The second adjusted model additionally includes endotoxin exposure (4 categories). Since information on coffee consumption was missing for one control, this participant was excluded from adjusted analyses.
‡Conditional logistic regression analyses of exposure to specific active ingredients as assessed based on self-reported crops and applying the crop-exposure matrix. Exposure was calculated by summing the estimated chance of use at the farm times the frequency of use, summed for all years working on farms. Exposed were divided based on the median of the exposure distribution among the controls. No exposure was assigned to a farm job when on that farm according to the participant no insecticides, herbicides, or fungicides were applied.

Exposure to endotoxin

In table 3, the results for cumulative exposure to endotoxin and PD risk based on the DOM-JEM are reported. ORs below unity were observed for the highest tertiles, but no trend with cumulative exposure was observed. Previous pesticide and endotoxin exposure in one model resulted in lower ORs for endotoxin and higher ORs for pesticides (see adjusted model two in tables 3–5).

DISCUSSION

We performed a case–control study on PD and used complementary methods to assign pesticide exposure. We used (1) a JEM that accounts for pesticide exposures in all jobs and industries, (2) an exposure algorithm that accounts for exposure during application and re-entry work at farms and (3) a crop-exposure matrix enabling estimation of exposure to specific active ingredients. The comprehensive evaluation revealed no evidence for an association with pesticides and the functional subclasses: insecticides, herbicides and fungicides. However, elevated ORs, which were observed in most analyses for the highest exposure categories, suggest that an overall effect may exist but that the overall limited number of high-exposed cases precluded any statistical significance. Our analyses on specific

active ingredients suggest an association with benomyl, a fungicide. In addition, we found no indication for an increased PD risk following occupational exposure to endotoxin.

A strength of our study was the use of a JEM and a crop-exposure matrix to estimate exposure. The results of our recent meta-analysis showed that studies estimating pesticide exposure based on job titles found higher ORs than studies using self-reported data only.[1] An explanation for this might be that study participants are not able to accurately remember past exposures leading to non-differential exposure misclassification and bias towards the null when solely relying on self-reported data.[24] We only downgraded the estimated exposures from the JEM and crop-exposure matrix for farm workers and gardeners who informed us that insecticides, herbicides or fungicides were not used on the farm where they had worked. We believe that this approach increased the specificity of the exposure assessment, and this is indirectly supported by the analyses that showed regression to the null if this information was not used. However, we cannot exclude that some recall bias was introduced if cases were less likely than controls to report that pesticides had not been applied at the job.

In addition, we analysed exposure to pesticides for individuals with farm or gardener jobs using an exposure algorithm to estimate exposure in more detail by relying more on self-reported data on exposure determinants. The exposure algorithm was adapted from an existing algorithm for applicator exposure.[18] We added re-entry work to the model, because exposures during re-entry work can be substantial,[4 5] and the majority (57%) of the participants who had worked at farms where pesticides were applied did not personally perform applications but did potentially perform tasks that included re-entry work. We found ORs in the same range as with the JEM approach, showing the robustness of our results. In addition, we also analysed mixing and application work only so as to keep the analyses comparable to previous work. These analyses showed non-significant increases in ORs for participants who applied pesticides, which seemed to be stronger for exposure to insecticides and herbicides than fungicides. This is in line with our recent meta-analysis that showed increased summary estimates for ever applying insecticides and herbicides but not for fungicides.[1] Interestingly, our analyses using a crop-exposure matrix to estimate exposure to specific pesticides found an increased OR for benomyl, which is a fungicide. Exposure to benomyl was only assigned to 1/3 or 1/2 of the participants who were assigned an exposure to fungicides based on the JEM and exposure algorithm, respectively. This points to the necessity of assigning exposure to specific active ingredients as noteworthy observations might be missed when grouped together.

A limitation of the study was the relatively low participation rate, especially among the oldest participants. The participation rate among cases and controls age 70 years or younger was 66% and 39%, respectively. Sensitivity analyses limited to younger participants resulted in higher ORs than in the overall analyses (data not shown). The finding of associations in a subgroup with a higher participation rate strengthens the evidence for a relation between pesticides and PD. However, this finding might also reflect a better recall of exposures by younger participants compared with older participants, or it could relate to differences in active ingredients used between earlier and later decades.

A limitation of using hospital controls is that the conditions included in the control group might relate to pesticide or endotoxin exposure or may suffer from referral bias and therefore may have influenced results. However, repeating the analyses leaving out one of the four categories of neurological conditions from the control group at a time resulted in almost identical results, suggesting that the results are not unduly driven by one specific control group.

No formal validation of the JEMs has been carried out, but the JEMs were based on expertise from experts with a long experience with agricultural-related exposures such as pesticides and endotoxin. A potential source of exposure misclassification in the JEM analyses is that the same exposure is assigned to all participants with the same job code. For the analyses on pesticides, this was partly solved by adjusting for self-reported non-application of pesticides at the farm, but some non-differential misclassification may still exist because differences in performed tasks within similar jobs were not taken into account. Also, the exposure algorithm used to assess pesticide exposure has some limitations. Owing to no specific questions on job tasks being asked, days with re-entry work had to be estimated from farm type only. In addition, weighting factors in the algorithm were based on the AHS and EUROPOEMs, and it is uncertain how well they correspond to the actual exposures in our study. These uncertainties most likely resulted in some non-differential exposure misclassification and likely attenuation of results.

The crop-exposure matrix analyses were limited in that exposures to active ingredients were assigned based on estimated probability and frequency of use. Especially when probability of use on a crop was low this could have resulted in incorrect assignment of exposure. For this reason, we categorised those exposed into two exposure groups, and based our conclusions on the highest exposure category for which exposure was most certain. Another limitation is that crops in greenhouses and field crops other than potatoes, beets, cereals or maize were not covered by the experts and probabilities of exposure were based on information from Statistics Netherlands. Exposure for those crops had to be extrapolated for time periods before 1995, resulting in higher chance of incorrect estimations and non-differential exposure misclassification. Also, for some potentially interesting active ingredients that were withdrawn from the market before 1995 and that were mainly used on crops not covered by the experts (eg, some organochlorines such as dieldrin and DDT), we could not assign an exposure based on the crop-matrix. Therefore our study is not informative for these active ingredients.

Our crop-specific analyses add evidence for a possible relation between benomyl and PD. Benomyl is a fungicide from the benzimidazole family that has been used for three decades on a wide range of crops. In 2001, it was banned in large parts of the world including the Netherlands. Benomyl has been investigated in two previous epidemiological studies on PD. A non-significant elevated OR of 1.9 for self-reported use of benomyl was found within a nested case–control study in the AHS.[25] A case–control study using registration data of pesticide applications showed a trend for increased risk for ambient exposure to benomyl at occupational addresses but, however, not at residential addresses.[26] Besides these epidemiological studies there is also toxicological evidence supporting a possible association between benomyl and PD risk, through a mechanism where benomyl inhibits microtubule assembly thereby stimulating aggregation of α-synuclein, or by inhibition of aldehyde dehydrogenase activity resulting in accumulation of a toxic dopamine metabolite.[26 27]

Although only active ingredients previously linked to PD were analysed, the observed association for benomyl might be caused by other factors such as other pesticides related to the crops associated with benomyl exposure. Therefore, we conducted

separate analyses on those crop groups. In our study, most individuals in the highest exposure category for benomyl had worked for a large part of their career on farms with field crops, mainly potatoes, cereals and/or beets. Analyses on working 10 or more years after 1955 on a farm with those field crops resulted in non-significant elevated ORs for beets (OR=1.84 (95% CI 0.82 to 4.12)) and cereals (OR=1.71 (95% CI 0.80 to 3.66)) but not for potatoes (OR=1.09 (95% CI 0.47 to 2.56)). In addition, among those participants with the highest benomyl exposure a number of participants had worked on farms cultivating strawberries. Ever working on a farm with strawberries showed a non-significant association with PD (OR=2.87 (95% CI 0.87 to 9.44)). Given that we observe increased risks for most of these crops suggests that the observed effect might be benomyl specific or attributable to a pesticide used in combination with benomyl.

No increase in PD risk was observed after exposure to endotoxin. The fact that more controls than cases had endotoxin exposure and adjusting the pesticide results for endotoxin exposure led to higher ORs also points to a possible protective effect of exposure to endotoxin. As no exposure response relation for endotoxin exposure was observed, this result should be interpreted with caution and the association between endotoxin exposure and PD should be investigated in more detail, for example, using quantitative data on endotoxin exposure.

CONCLUSIONS

In summary, we studied the relation between exposure to insecticides, herbicides, fungicides and endotoxin and PD in a multicentre case–control study in the Netherlands. The results did not provide evidence for the postulated increase in risk after endotoxin exposure. Also, no evidence for an association with exposure to pesticides was found. However, statistically non-significant elevated ORs observed in the higher exposure categories for pesticides, insecticides, herbicides and fungicides are in line with earlier evidence that exposure to pesticides might increase PD risk. Active ingredient-specific analyses revealed a possible association with benomyl, a benzimidazole fungicide that has previously been associated with PD risk.

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Contributors PCGN and HK initiated the study. All authors were involved in the design of the study. MvdM, RV, AH and HK were involved in the statistical analyses and interpretation of the data. The manuscript was drafted by MvdM and was revised with contributions from all authors.

Funding This work was supported by Stichting Internationaal Parkinson Fonds (The Netherlands); and The Netherlands Organization for Health Research (ZonMW) within the programme Electromagnetic Fields and Health Research under grant number 85800001.

Competing interests None.

Patient consent Obtained.

Ethics approval Medical Ethics Committee of St Elisabeth Hospital, Tilburg, The Netherlands.

Provenance and peer review Not commissioned; externally peer reviewed.

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Occupational exposure to pesticides and endotoxin and Parkinson disease in the Netherlands

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Occup Environ Med 2014 71: 757-764 originally published online August 7, 2014
doi: 10.1136/oemed-2014-102170

Productie 26

American Journal of Epidemiology
Vol. 157, No. 5

Printed in U.S.A.
DOI: 10.1093/aje/kwf216

ORIGINAL CONTRIBUTIONS

Neurodegenerative Diseases and Exposure to Pesticides in the Elderly

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Received for publication January 31, 2002; accepted for publication September 11, 2002.

The authors investigated the hypothesis that exposure to pesticides could be related to central nervous system disorders in a prospective cohort study of 1,507 French elderly (1992–1998). Lower cognitive performance was observed in subjects who had been occupationally exposed to pesticides. In men, the relative risks of developing Parkinson's disease and Alzheimer's disease for occupational exposure assessed by a job exposure matrix were 5.63 (95% confidence interval: 1.47, 21.58) and 2.39 (95% confidence interval: 1.02, 5.63), respectively, after confounding factors were taken into account. No association was found with having a primary job in agriculture or with environmental pesticide exposure, nor was an association found in women. These results suggest the presence of neurologic impairments in elderly persons who were exposed occupationally to pesticides.

aged; Alzheimer disease; nervous system diseases; occupational exposure; Parkinson disease; pesticides

Abbreviations: CI, confidence interval; MMSE, Mini-Mental State Examination.

Mortality due to neurodegenerative diseases is expected to increase 119–231 percent in the United States between 1990 and 2040, depending on the population model used (1–3). A recent meta-analysis on the relation between pesticide exposure and Parkinson's disease revealed a positive association (4), even if no dose-response relation has been established and no specific type of pesticide identified. Other studies have found delayed cognitive impairments in relation to pesticide exposure (5–8), which could be predictive of dementia before the clinical diagnosis of disease (9). However, few studies have dealt with the relation between Alzheimer's disease and pesticide exposure (10–12). The aim of the present study was to search for an association between lifelong cumulative exposure to pesticides and neurodegenerative diseases in an elderly cohort.

MATERIALS AND METHODS

Study population

The study population was that of the PAQUID Study, a cohort study involving elderly people aged 65 years or older at inclusion in 1987 who were living at home or in an institution in Gironde, southwestern France. The methodology of the PAQUID Study has been described elsewhere (13). Figure 1 presents the dates of each step, the numbers of subjects under follow-up, and the times of collection of the data used in our analysis.

Assessment of pesticide exposure

Occupational exposure. Detailed occupational histories were generated for all subjects from specific questionnaires that had been filled in during a face-to-face interview at the 5-year follow-up. All jobs were coded using the classification of the Institut National de la Statistique et des Etudes Economiques (14). The likelihood of and level of exposure to pesticides (including insecticides, herbicides, and fungicides) with reference to the job codes were assessed by a panel of six experts blinded to neurologic status, and the median of the experts' judgments was used. The assessment finally provided a job exposure matrix (table 1). Experts

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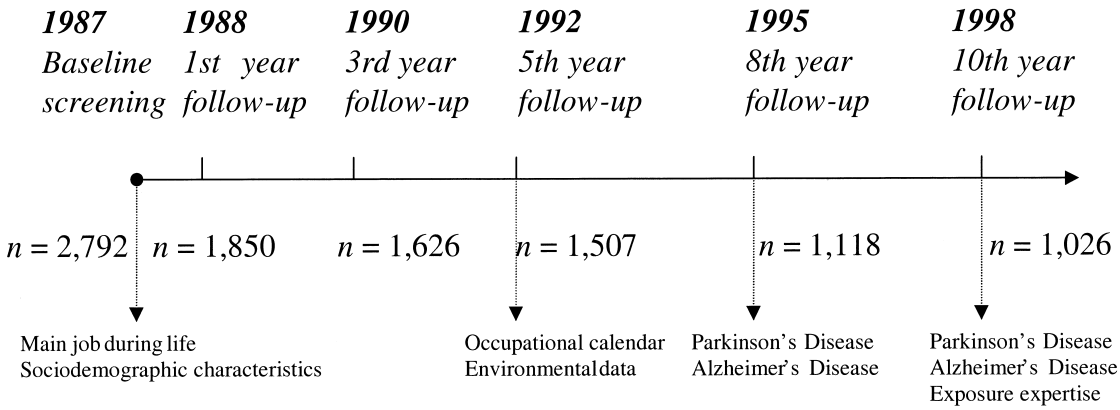

FIGURE 1. Design of the PAQUID Study, Gironde, France, 1987–1998.

considered that workers on and owners of small farms had experienced higher exposure, since they had mixed and sprayed pesticides themselves using older and less efficient equipment. Cumulative occupational exposure to pesticides was calculated as the sum of exposures incurred in all calendar periods, exposure itself being the product of the period duration and the level indicated by the job exposure matrix. Quartiles of the distribution of the cumulative index values were considered.

Environmental exposure. Since the median duration of residence in the same district was 40 years (interquartile range, 20–63 years), we hypothesized that place of residence at baseline was an indicator for environmental exposure to pesticides, and we established two variables: rural residency and residency in a district with vineyards.

Rural residency. The classification of the Institut National de la Statistique et des Etudes Economiques was used to code the place of residence at baseline. According to this classification, the definition of an "urban area" relied on the continuity of the housing (buildings less than 200 m apart) and the gathering of 2,000 inhabitants or more.

Residency in a district with vineyards. The proportion of surface area planted with vineyards was calculated for each district of residency at baseline. We used 5 percent coverage as the threshold for considering that a district was planted with vineyards.

TABLE 1. Titles of jobs involving a nonnull level of exposure to pesticides, PAQUID Study, Gironde, France, 1992–1998

Job title	Exposure level
Wine grape grower or fruit grower on <7 hectares	3
Worker in wine grape growing or fruit growing	3
Farmer on <20 hectares	2
Truck farmer or horticulturist on <1.5 hectares	2
Wine grape grower or fruit grower on 7–20 hectares	2
Wine grape grower or fruit grower on 20–40 hectares	2
Worker in truck farming or horticulture	2
Poultry or mixed animal breeder on <10 hectares	1
Farmer on 20–40 hectares	1
Gardener	1
Carpenter	1
Farm worker	1
Herbivore breeder on <10 hectares	0.5
Farmer on >40 hectares	0.5
Furniture craftsperson	0.5
Veterinarian	0.5
Technician in agriculture	0.5
Fire fighter	0.5
Farm or forest machine driver	0.5
Breeding worker	0.5

Neurologic assessment

Cognitive impairment. Neuropsychological tests on standardized questionnaires were administered at each subject's home by psychologists who were supervised by a researcher in neuropsychology and were not aware of the study hypothesis. We considered here results from the Mini-Mental State Examination (MMSE), which is the sum of subscores that measure orientation to time and place, the recording of three words, language, and visual construction (15). Depression, which influences neuropsychological performance, was evaluated with the French version of the Center for Epidemiologic Studies Depression Scale (16).

Parkinson's and Alzheimer's diseases. A simple algorithm based on a standardized questionnaire classified the subjects as either suspected or not suspected of having dementia. Thereafter, subjects who met the *Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised*, criteria for dementia were evaluated by a neurologist, who filled in information on the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's and Related Disorders Association criteria and the Hachinski score (17, 18). Cases were definitively classified by considering the results of jointly available comple-

TABLE 2. Sociodemographic characteristics of the study population at the 5-year follow-up according to pesticide exposure (assessed by a job exposure matrix), PAQUID Study, Gironde, France, 1992–1998

	Not exposed		Exposed		Total	
	No.	%	No.	%	No.	%
Sex						
Men	454	38.2	131	40.9	585	38.8
Women	733	61.8	189	59.1	922	61.2
Mean age (years)	78.4 (6.3)*		79.2 (6.4)		78.6 (6.3)	
Main job						
Housewife	124	10.5	20	6.3	144	9.6
Blue-collar worker	219	18.5	101	31.7	320	21.3
Office employee	477	40.4	79	24.8	556	37.0
Farmer†	14	1.2	87	27.3	101	6.7
Craftsperson or shopkeeper	179	15.2	20	6.3	199	13.3
Executive	168	14.2	12	3.8	180	12.1
Educational level						
Certificat d'Études‡	254	21.4	159	49.7	413	27.4
Higher level	933	78.6	161	50.3	1,094	72.6

* Numbers in parentheses, standard deviation.
† Farm workers ($n = 72$) constituted 4.8% of the total population.
‡ Degree formerly obtained at the end of primary school education in France.

mentary examinations. At baseline screening and 5-year follow-up, diagnosis of Parkinson's disease was ascertained by a two-phase design (19). In the first stage, the following two validated questions were used to screen for Parkinson's disease: "Do your arms or legs shake at rest?"; "Do you experience slowness or stiffness in your movements?". All subjects who gave positive answers to both questions and/or were taking anti-Parkinson's disease drugs (including anticholinergic medications, dopamine agonists, and monoamine oxidase inhibitors) were visited at home by a trained neurologist for confirmation or exclusion of a diagnosis of Parkinson's disease. At 8 and 10 years of follow-up, the possibility of Parkinson's disease was explored solely by the question, "Do you have Parkinson's disease?".

Other variables. The questionnaire also collected information on history of smoking and sociodemographic factors: age, sex, and educational level considered in two classes (whether or not a subject had received the "Certificat d'Études," a primary school diploma, which was previously found in PAQUID to be the best educational threshold for predicting cognitive impairment (20)).

Analysis

Analyses were performed separately in men and women for the effect of each occupational or environmental pesticide exposure. First, because information on occupational exposure was collected at the 5-year follow-up, MMSE results obtained at that time were compared according to past pesticide exposure to explore the cross-sectional association with general cognitive functioning. Since there was no normative reference value in our population, the MMSE threshold used was the 25th percentile of the distribution of the scores. Potentially confounding factors associated with MMSE performance in univariate analysis with p values less than 0.25 were retained in the multivariate models.

We also explored the association between each pesticide exposure variable and incident cases of diagnosed Parkinson's disease or Alzheimer's disease between 5-year follow-up and 10-year follow-up, based on the Cox proportional hazard models in which the time scale was the age of the subjects. Thus, age was not entered as an explanatory variable in the model. Indeed, several authors have advocated that in the study of age-associated diseases, the appropriate time scale for survival models is age rather than time since the baseline survey (21, 22). We used a Cox model with delayed entry to take into account the left-truncation process (subjects entered the sample only if they had not developed a neurodegenerative disease prior to their age at entry). Dates of Alzheimer's and Parkinson's disease occurrence were estimated to lie at the center of the interval between the follow-up period in which the disease was observed and the follow-up period in which the subject was considered Alzheimer's and Parkinson's disease-free.

RESULTS

Study population

The sample was initially composed of 1,122 men (40.2 percent) and 1,670 women. At the 5-year follow-up point, 623 subjects had died, 614 refused to be visited, and 48 could not be contacted, while 1,507 were still alive and could be visited. They were predominantly women (61.2 percent) (table 2). The mean age was 78.6 years (standard deviation,

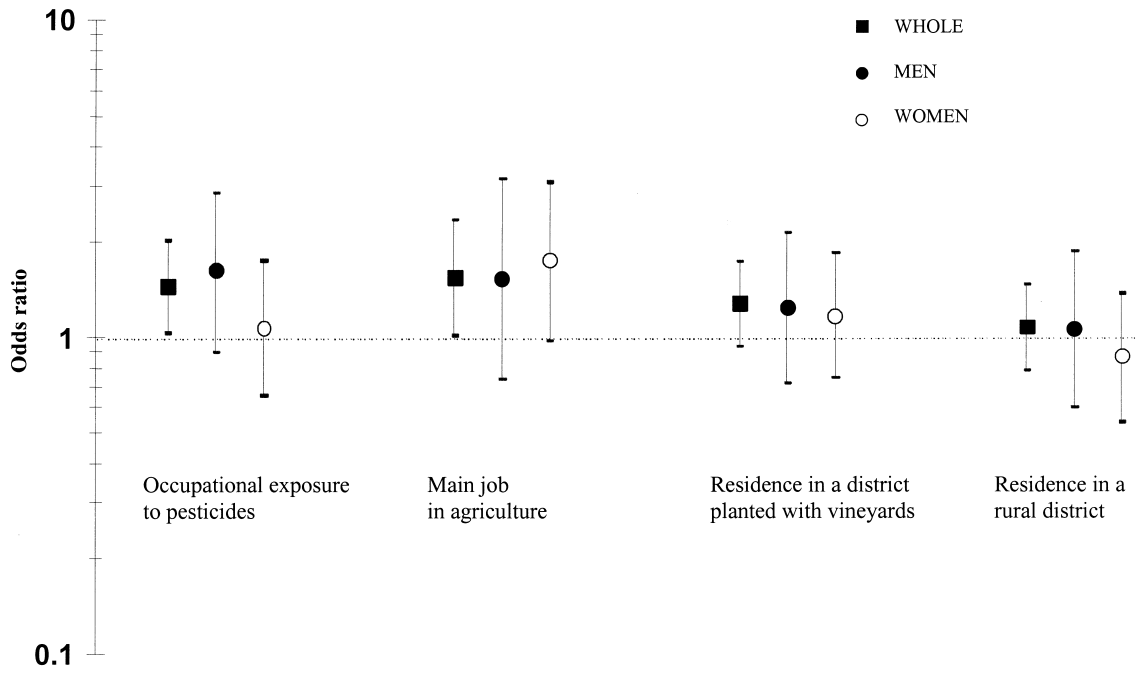

FIGURE 2. Risk of scoring low on the Mini-Mental State Examination (threshold: 25th percentile) in the whole study population and separately in men and women, according to occupational exposure to pesticides (job exposure matrix), having a main job in agriculture, residency in a district planted with vineyards, and rural residency, PAQUID Study, 1992–1998. Results are expressed as odds ratios adjusted for age, education, and depressive symptoms.

6.3). Most of the subjects were office employees (37.0 percent) or blue-collar workers (21.3 percent). A high level of education was more represented than a low level (72.6 percent).

Pesticide exposure assessment

Nineteen job titles were estimated to involve nonnull pesticide exposure (table 1), corresponding to 320 subjects (21.2 percent). A cumulative exposure index could be calculated for 228 subjects (71.3 percent). In others, dates of the ending or beginning of jobs were missing. The median exposure duration was 28 years, and the median delay since the end of exposure was 20 years. Having a primary job in agriculture was reported by 173 of the subjects (11.5 percent). One subject out of four was living in a rural district (26.5 percent), and 414 were living in a district planted with vineyards. The median duration of residence at baseline was 54.5 years for subjects with Alzheimer's disease and 47 years for subjects with Parkinson's disease.

Cross-sectional analysis of MMSE performance at 5-year follow-up

Performances on the MMSE were significantly associated with educational level, age, and depressive symptoms in both men and women. Each additional year of age increased the risk of scoring low by 13 percent. A lower educational level was a risk factor for scoring low, by a factor of 5.9 in women and a factor of 10.6 in men. Depressive symptoms also had a negative impact on performance, with risks of 2.4 in women and 3.9 in men.

Taking these factors into account, the risk of scoring low on the MMSE remained higher among subjects with pesticide exposure (figure 2). For occupational exposure determined by the job exposure matrix, the increase was significant, with an odds ratio of 1.45 (95 percent confidence interval (CI): 1.04, 2.02); it was almost significant for men separately (odds ratio = 1.61, 95 percent CI: 0.90, 2.86) but was not significant for women (odds ratio = 1.07, 95 percent CI: 0.66, 1.75). Risk of scoring low on the MMSE was also higher among subjects who reported working primarily in agriculture. Living in a district planted with vineyards tended to be associated with an increase in risk of scoring low, but not significantly.

Incidence of neurodegenerative diseases between the 5- and 10-year follow-ups

Parkinson's disease. Twenty-four cases of Parkinson's disease (10 in men and 14 in women) arose between the 5-year and 10-year follow-ups, corresponding to an incidence of 5 per 1,000 person-years.

Eight cases occurred in occupationally exposed subjects (8.9 cases per 1,000 person-years) and 16 in nonexposed subjects (4.1 cases per 1,000 person-years) ($p = 0.07$).

In men, univariate analysis showed a significant association between Parkinson's disease and occupational exposure

TABLE 3. Incidence of Parkinson's disease and Alzheimer's disease between the 5- and 10- year follow-ups, PAQUID Study, Gironde, France, 1992–1998*

	Parkinson's disease (n = 24 incident cases)			Alzheimer's disease (n = 96 incident cases)		
	Crude RR†	Adjusted‡ RR	95% CI†	Crude RR	Adjusted‡ RR	95% CI
Occupational exposure						
Men	6.05	5.63	1.47, 21.58	2.86	2.39	1.02, 5.63
Women	0.81	1.02	0.22, 4.82	1.23	0.89	0.49, 1.62
Main job in agriculture						
Men	2.35	1.62	0.31, 8.63	1.75	1.32	0.43, 4.10
Women	0.67	0.81	0.10, 6.40	1.15	0.85	0.40, 1.86
Rural residency						
Men	1.76	1.45	0.38, 5.49	0.96	0.74	0.30, 1.86
Women	1.20	1.31	0.40, 4.30	0.93	0.76	0.44, 1.31
Residency in a district planted with vineyards						
Men	0.59	0.46	0.09, 2.29	1.00	0.84	0.35, 2.01
Women	0.78	0.87	0.24, 3.19	1.03	0.88	0.52, 1.50

* Cox models with left-truncation.
† RR, relative risk; CI, confidence interval.
‡ Relative risks were adjusted for smoking and education.

as determined by the job exposure matrix, with a relative risk of 6.0 that remained significant at 5.6 (95 percent CI: 1.5, 21.6) after adjustment for smoking and educational level (table 3). The adjusted relative risk in men increased from 5.3 (95 percent CI: 0.6, 49.3) in the first quartile to 5.7 (95 percent CI: 0.5, 60.3) in the second quartile and 10.9 (95 percent CI: 1.7, 70.3) in the third quartile. In the fourth quartile, no case was exposed. Results were not significant for other pesticide exposures.

In women, there was no significant association between Parkinson's disease and any pesticide exposure variable.

Alzheimer's disease. We analyzed 96 incident cases of Alzheimer's disease between the 5- and 10-year follow-ups (71 in women and 25 in men). The incidence of Alzheimer's disease in our study was 21 per 1,000 person-years. Twenty-six cases occurred in exposed subjects (30.7 cases per 1,000 person-years) and 70 in nonexposed subjects (18.9 cases per 1,000 person-years) ($p = 0.02$).

In men, univariate analysis showed a significant association between Alzheimer's disease and occupational exposure, with a relative risk of 2.9 that remained significant after adjustment for education and smoking (relative risk = 2.4, 95 percent CI: 1.0, 5.6) (table 3).

The adjusted relative risk in men increased from 1.2 (95 percent CI: 0.2, 9.5) in the first quartile to 3.8 (95 percent CI: 0.8, 17.1) in the second quartile and to 3.9 (95 percent CI: 0.8, 17.6) in the third quartile. The relative risk was 2.6 (95 percent CI: 0.6, 12.2) in the fourth quartile. Results were not significant for other pesticide exposure variables or in women.

DISCUSSION

In this cohort of French elderly, we found an association between past occupational exposure to pesticides and low cognitive performance, together with an increase in the risk of developing Alzheimer's disease or Parkinson's disease. The fact that only occupational exposure was related to neurologic outcomes (MMSE score, Alzheimer's disease, and Parkinson's disease) and that the relation appeared exclusively in men is consistent with our knowledge of pesticide use in vineyards, since pesticide treatment tasks (i.e., pesticide mixing and application) are performed almost exclusively by males.

Unfortunately, because of industrial and trade interests, information on the use of specific pesticides in defined geographic areas like Gironde is not available. We characterized the pattern of pesticide use in Gironde by analyzing 60 treatment calendars collected from farm owners in the region in 1992 and 1993. The number of different pesticides used on any given farm each year ranged from three to 23. Dithiocarbamates accounted for 37 percent of the organic substances applied (7.2 kg/hectare/year) and folpet for 26 percent (5 kg/hectare/year). Organophosphates and carbamates corresponded to 1 percent and 2 percent, respectively, and were sprayed at mean doses of 230 g and 390 g per hectare per year.

From the present study, we cannot provide definitive conclusions about the specific pesticides responsible for the effects observed. However, these findings allow hypotheses to be drawn about the pesticide exposures currently incurred in vineyards, since one particularity of this agricultural setting is the predominance of fungicide use. Our assessment of Parkinson's disease at 8 and 10 years of follow-up relied

solely on answers to questionnaires, which could have induced a differential bias. However, we do not think such misclassification could be related to pesticide exposure, because the hypothesis of this association was revealed neither to the subjects nor to the interviewers. In any case, the incidence of Parkinson's disease observed in our study is consistent with that seen in other studies (23).

For Alzheimer's disease, the diagnosis was based on neurologic examination at each follow-up of the cohort. The incidence rates in the PAQUID Study did not differ from those observed in other European cohorts (24).

The difference in risk between men and women is noteworthy. In case-control studies of Parkinson's disease, a risk of approximately 2 is usually found, which corresponds to the risk we would find when pooling men and women.

Our results are coherent with those of other studies of Parkinson's disease but raise new questions regarding differences between the sexes in pesticide-related Parkinson's disease. They indicate that conclusions might depend largely on exposure assessment. They throw new light on the possible relation with Alzheimer's disease, as previous studies failed to demonstrate an association. They suggest that cognitive disturbances persist in occupationally exposed subjects, even long after work cessation. Therefore, attention should be paid to the possibility of long-term neurologic effects of pesticide use in agricultural settings where fungicides account for much of the occupational exposure.

ACKNOWLEDGMENTS

This study was supported by grants from the following organizations: Fondation de France, Novartis Pharma, Axa Insurance Group, Caisse Nationale d'Assurance Maladie, Caisse Primaire d'Assurance Maladie de Dordogne, Conseil Général de la Dordogne, Conseil Général de la Gironde, Conseil Régional d'Aquitaine, Danone, Ministère de la Recherche et de la Technologie, Mutualité Sociale Agricole de Gironde et de Dordogne, Mutuelle Générale de l'Education Nationale, Société Péchiney, 2010 Média, and Caisse de Retraite Interentreprise, Capimmec, Institut du Cerveau, Direction Régionale des Affaires Sanitaires et Sociales.

The authors thank members of the PAQUID study team involved in cohort set-up and data collection, especially C. Helmer for her helpful advice. They also thank Professor F. Tison for his role in Parkinson's disease diagnosis.

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Occupational exposure to pesticides increases the risk of incident AD

The Cache County Study

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ABSTRACT

Background: Commonly used organophosphate and organochlorine pesticides inhibit acetylcholinesterase at synapses in the somatic, autonomic, and central nervous systems and may therefore have lasting effects on the nervous system. Few studies have examined the relationship of pesticide exposure and risk of dementia or Alzheimer disease (AD). We sought to examine the association of occupational pesticide exposure and the risk of incident dementia and AD in later life.

Methods: Residents of the agricultural community of Cache County, UT, who were aged 65 years and older as of January 1995, were invited to participate in the study. At baseline, participants completed detailed occupational history questionnaires that included information about exposures to various types of pesticides. Cognitive status was assessed at baseline and after 3, 7, and 10 years. Standardized methods were used for detection and diagnosis of dementia and AD. Cox proportional hazards survival analyses were used to evaluate the risk of incident dementia and AD associated with pesticide exposure.

Results: Among 3,084 enrollees without dementia, more men than women reported pesticide exposure ($p < 0.0001$). Exposed individuals (n = 572) had more years of education ($p < 0.01$) but did not differ from others in age. Some 500 individuals developed incident dementia, 344 with AD. After adjustment for baseline age, sex, education, *APOE* ϵ4 status, and baseline Modified Mini-Mental State Examination scores, Cox proportional hazards models showed increased risks among pesticide-exposed individuals for all-cause dementia, with hazard ratio (HR) 1.38 and 95% confidence interval (CI) 1.09–1.76, and for AD (HR 1.42, 95% CI 1.06–1.91). The risk of AD associated with organophosphate exposure (HR 1.53, 95% CI 1.05–2.23) was slightly higher than the risk associated with organochlorines (HR 1.49, 95% CI 0.99–2.24), which was nearly significant.

Conclusions: Pesticide exposure may increase the risk of dementia and Alzheimer disease in late life. ***Neurology*® 2010;74:1524–1530**

GLOSSARY

3MS = Modified Mini-Mental State Examination; **AD** = Alzheimer disease; **CI** = confidence interval; **DQ** = Dementia Questionnaire; ***DSM-III-R*** = *Diagnostic and Statistical Manual of Mental Disorders*, 3rd edition, revised; **HR** = hazard ratio.

Few researchers have evaluated the potential association between pesticide exposure and later risk of Alzheimer disease (AD). Studies of this potential association are of particular interest for several important reasons. First, some pesticides are acetylcholinesterase inhibitors or have similar effects.[1] There is evidence that long-term pesticide exposure may have lasting toxic effects on the CNS.[2,3] Second, the use of pesticides has increased dramatically in the last 50 years.[4] As noted previously,[5] the Environmental Protection Agency reports that "In the U.S., more than 18,000 products are licensed for use . . . each year >2 billion pounds of pesticides are applied to crops, homes, schools, parks, and forests . . . ."[6] These numbers highlight a potentially important public health issue. Finally, older adults are more likely to have been exposed to persistent pesticides such as DDT, which

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Study funding: Supported by NIH/NIA R01-AG1380 and P30-AG 028377 [*APOE* genotyping].

Disclosure: Author disclosures are provided at the end of the article.

was in use from the 1940s until its ban in 1972. The peak year for DDT use was 1959, when 80 million pounds were applied across the United States.[7]

There have only been a few case-control studies and 2 relatively small cohort studies on the association between pesticide exposure and AD.[8] Larger sample sizes and better methods of exposure classification are needed in order to get a clear picture of the potential risk associated with occupational exposure to pesticides. Our objective was to evaluate the effects of occupational pesticide exposure on AD risk in a well-characterized cohort of elderly individuals who reside in the agricultural community of Cache County, UT.

METHODS

Cache County is known as one of Utah's primary agricultural regions. Cache leads the state in barley production; other products include winter wheat, spring wheat, dry beans, corn for silage, apples, and alfalfa hay.[9] Roughly 12% of the population under study reported farming as a primary occupation.

Standard protocol approvals, registrations, and patient consents. Procedures and protocols of the Cache County Memory Study, a prospective cohort study, were approved by the Institutional Review Boards of Utah State University, Duke University, and The Johns Hopkins University. Informed consent was obtained from all study participants at each stage of the study. Spouses or next of kin gave consent when participants were unable to provide it.

Ascertainment of dementia status. The Cache County Memory Study methods have been described in detail previously.[10,11] At the start of the study, all residents of the county aged 65 and older in 1995 were invited to participate.[10] Baseline cognitive screening and risk factor questionnaires were completed by a total of 5,092 participants (90% of the population aged 65 or older). A version of the Modified Mini-Mental State Examination (3MS)[12] adapted for epidemiologic studies[13] in combination with the Dementia Questionnaire (DQ)[14] were used as a 2-stage screening tool at the baseline evaluation and 3 years later at the first follow-up. At baseline, individuals falling below a predefined cutpoint in screening and all participants aged 90+ (at baseline) or aged 85+ (at subsequent evaluations) were assessed with a full clinical evaluation for dementia and for milder cognitive syndromes. Final diagnoses were assigned at consensus conferences using standard criteria. *DSM-III-R*[15] and the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria[16] were used for AD diagnoses and the National Institute of Neurological Disorders and Stroke and the Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria,[17] as operationalized by Tatemichi et al.,[18] was used to diagnose vascular dementia. Dementia onset age was defined as the year in which a participant unambiguously met *DSM-III-R* criteria for dementia.

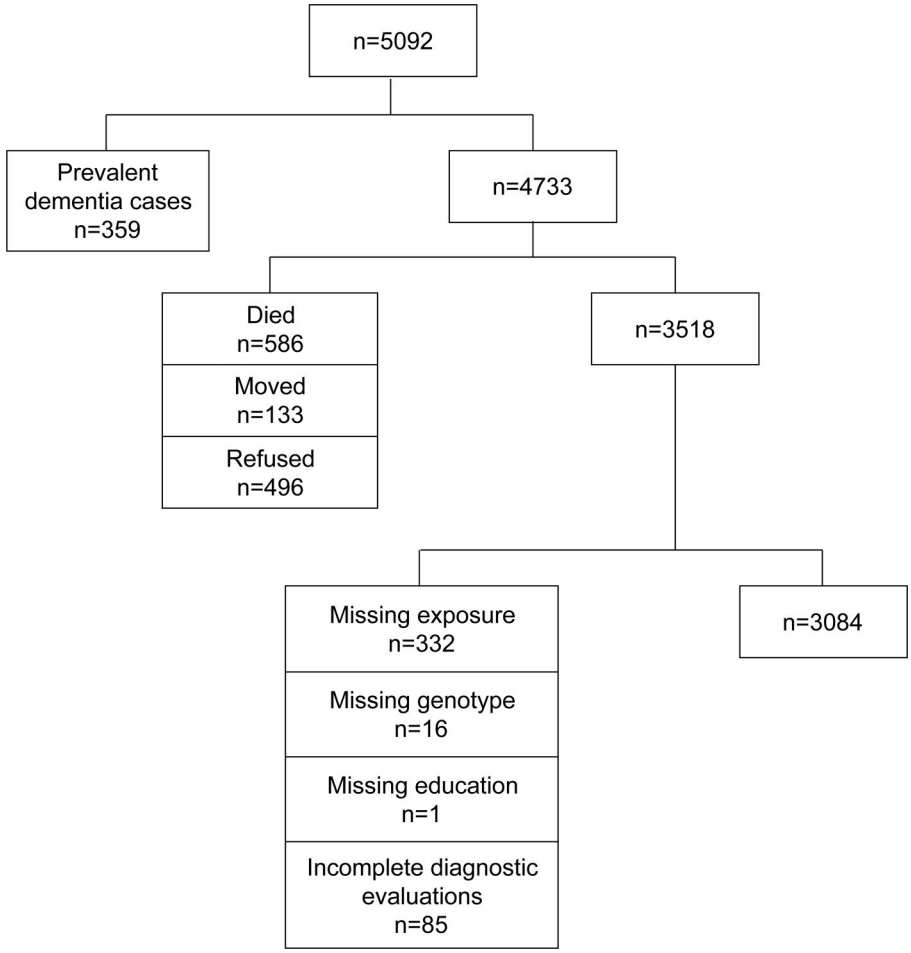
Exposure assessment. At baseline, participants completed detailed occupational questionnaires, providing information about their work histories and associated exposures to various substances. This structured, detailed questionnaire was administered in person and was used to classify occupations and assess occupational exposures including cues for exposures to specific agents. Initial questions determined if participants held a job to support their family and the kind of work they performed for the majority of their lives. If a person had multiple jobs, they were asked if they worked at each type of job for 5 years or more. If the 5-year threshold was met, the participant was asked about industry, employer, and primary duties. A second series of detailed questions were asked about exposures on the job. If pesticide use was endorsed, further probe questions were used to determine the type of pesticide, the frequency of use, and the duration of use in years. There were no questions about the use of safety equipment or the intensity of exposure. Questions addressed pesticides in general and 4 specific types of pesticides including organophosphates, carbamates, organochlorines (DDT), and methyl bromides. Responses to the occupational questionnaire were coded by an occupational health nurse according to the Dictionary of Occupational Titles.[19] Participants were classified as exposed if they endorsed any pesticide exposure.

Analytic approach. Demographic characteristics of the exposed and unexposed members of the sample were compared using χ^2 tests for categorical variables and t tests were used for continuous variables. Cox proportional hazard analysis was used to assess the relationship between prior pesticide exposure and the risk of incident dementia and AD. Unexposed participants were used as the reference group; age on study was used as the time axis. Participants were censored at dementia onset or last observation if dementia was not diagnosed. Additional models were constructed to address the risk associated with organophosphates and organochlorines specifically. Models were adjusted for factors known to independently influence the risk of dementia and AD including age at baseline (centered at age 65), sex, education in years, and *APOE* ε4 status. We also included a term for baseline 3MS scores to control for baseline cognitive status. In addition, we tested whether the estimated hazard of the association between pesticide exposure and dementia was proportional over the analytic time scale by including an interaction term between the variable for pesticide exposure and age on study. Analyses were performed using SAS statistical software.[20]

RESULTS

At baseline, a total of 5,092 participants were screened for dementia and completed detailed risk factor questionnaires. Each participant was classified by pesticide exposure. We excluded from the current analysis 359 individuals with prevalent dementia. A total of 586 individuals died prior to the first follow-up evaluation, 133 moved out of the area, and 496 refused to participate, leaving a potential sample of 3,518 individuals (figure). Another 434 individuals were excluded from the analysis due to missing data (exposure = 332, genotype = 16, education = 1, and diagnosis = 85). The final analytic sample comprised a total of 3,084 individuals without dementia at baseline who provided self-report information about pesticide exposures; 572 reported some pesticide exposure. Over 40% of those exposed reported farming as the primary occupation for most of their working lives. Follow-up evaluations were conducted 3, 7, and 10 years after the initial evaluation;

Figure Sample selection flowchart



cognitive status was reevaluated at each time point. The average duration of follow-up was 7.2 (3.5) years. Compared to those included in the study sample, those who were excluded tended to be older ($p < 0.0001$), were more likely to be female ($p < 0.0001$), and had fewer years of education ($p < 0.0001$). There were no differences between groups in *APOE* status (1 or more *APOE* ϵ4 alleles vs no ϵ4 alleles) ($p = 0.49$) or pesticide exposure status ($p = 0.86$) among those providing such information (data not shown).

Among those retained in the analytic sample, more men reported exposures than women ($p < 0.0001$); there were no differences in baseline age between the exposed and unexposed groups (overall mean age 74.4, SD 6.4) (table 1). Those who reported exposure had more education ($p < 0.01$). This difference is due to the fact that men had higher mean education levels than women (14.1 vs 13.0 years, $p < 0.0001$) and there were few women in the exposed group. There was a small but significant difference between groups' baseline 3MS scores. Of 572 individuals reporting pesticide exposure, 316 were exposed to organophosphates, 256 were exposed to organochlorines, 25 were exposed to carbamates, and 28 reported exposure to methyl bromides. A number of individuals reported more than one type of exposure (n = 186) and 164 individuals' exposures were not classified because participants' responses either indicated an agent that was not among the 4 major classes or their responses were nonspecific. A total of 500 individuals were identified with incident dementia; 344 had a primary or secondary diagnosis of AD. A total of 108 individuals who reported exposure to pesticides were later diagnosed with dementia.

Table 1 Participant characteristics (n = 3,084)

Characteristics	Unexposed (n = 2,512)	Exposed (n = 572)	Total (n = 3,084)	*p* Value
Baseline age, y, mean (SD)	74.5 (6.4)	74.1 (6.4)	74.4 (6.4)	
Male, n (%)	906 (36.1)	502 (87.8)	1,408 (45.7)	<0.0001
APOE ϵ4+, n (%)	783 (31.2)	168 (29.4)	951 (30.8)	
Years of education, mean (SD)	13.4 (2.8)	13.8 (3.4)	13.5 (2.9)	<0.01
Baseline 3MS score	91.9 (5.8)	91.3 (6.2)	91.8 (5.8)	<0.05

Table 2 Cox proportional hazards ratios for dementia incidence by pesticide exposure

	Unadjusted models				Adjusted models[a]	
Model	No dementia	Incident dementia	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Any pesticide exposure	2,582	500	1.25 (1.01–1.55)	0.04	1.38 (1.09–1.76)	0.008
Organophosphates	2,380	446	1.17 (0.88–1.55)	0.29	1.31 (0.96–1.78)	0.09
Organochlorines	2,327	439	1.23 (0.91–1.67)	0.18	1.33 (0.96–1.85)	0.09

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a] Models are adjusted for baseline age centered at age 65, sex, education level in years, baseline Modified Mini-Mental State Examination score, and *APOE* ϵ4 status (1 or more *APOE* ϵ4 alleles vs none).

Initial univariable Cox proportional hazard models for incident dementia are presented in table 2. Additional models were created adjusting for baseline age (centered at age 65), education, and *APOE* ϵ4 status because these factors are all known to significantly influence the risk of dementia and AD. We included a gender term because prior work in our study has shown a difference in risk for AD between men and women[11]; in the current sample, most of the exposed individuals are men. A term for baseline 3MS score was used to control for global cognitive status at baseline; age on study was used as the time axis. Results showed there was an increased risk of dementia in those exposed to any pesticide (hazard ratio [HR] 1.38, 95% confidence interval [CI] 1.09–1.76). When the outcome was restricted to AD only (including cases with AD as the primary or secondary diagnoses) the risk was similar (HR 1.42, 95% CI 1.06–1.91) (table 3). A test of the proportionality assumption indicated that the hazards associated with each group were proportional ($p = 0.17$).

Separate models were created to test the association between risk of incident dementia or AD and specific pesticides. In these models, we set aside individuals who reported exposure to pesticides other than the group under study in order to clearly distinguish between the exposed and unexposed groups. A total of 256 individuals reporting exposures other than organophosphates were set aside in the analysis of organophosphates and 316 individuals were set aside in the analysis of organochlorines. The risk of dementia associated with exposure to organophosphates after adjustment for baseline age, sex, education, baseline 3MS score, and *APOE* ϵ4 status was increased among the exposed (HR 1.31, 95% CI 0.96–1.78) although marginally nonsignificant ($p = 0.09$). The risk of AD was 53% higher (HR 1.53, 95% CI 1.05–2.23). In the evaluation of organochlorines, we found similarly marginal increased risks for dementia after adjustment for baseline age, sex, education, baseline 3MS score, and *APOE* ϵ4 status (HR 1.33, 95% CI 0.96–1.85) and for AD (HR 1.49, 95% CI 0.99–2.24). Because 177 participants reported exposures to both organophosphates and organochlorines, we performed an additional analysis to determine if the risk for dementia and AD was increased in this group, but none of our findings were significant (data not shown).

DISCUSSION Thus far, there have been few reports on the association between pesticides and AD.[2,21-25] Findings have been equivocal and the samples under study have been relatively small. Methods of exposure determination in these studies vary, ranging from proxy reports[22-24] to self-administered mail-in questionnaires[25] to structured in-person interviews.[2,24] Determination of the level of occupational pesticide exposure is particularly difficult because it depends on the duration of use, the method of application, and whether safety equipment was used, or used properly.[5] An individual who lives proximal to treated fields may also be exposed. Exposure classifi-

Table 3 Cox proportional hazards ratios for Alzheimer dementia incidence by pesticide exposure

	Unadjusted models				Adjusted models[a]	
Model	No dementia	Incident AD	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Any pesticide exposure	2,738	344	1.15 (0.88–1.49)	0.32	1.42 (1.06–1.91)	0.02
Organophosphates	2,513	313	1.19 (0.85–1.67)	0.32	1.53 (1.05–2.23)	0.03
Organochlorines	2,460	306	1.18 (0.81–1.71)	0.39	1.49 (0.99–2.24)	0.06

Abbreviations: AD = Alzheimer dementia; CI = confidence interval; HR = hazard ratio.
[a] Models are adjusted for baseline age centered at age 65, sex, education level in years, baseline Modified Mini-Mental State Examination score, and *APOE* ϵ4 status (1 or more *APOE* ϵ4 alleles vs none).

cation can therefore be a problem in studies of the effects of pesticides on the risk of dementia. Case-control studies in dementia research, which necessarily rely on informant reports, are likely to produce somewhat conservative estimates as proxy informants have been shown to underreport pesticide exposures.[26,27] Evaluations of data from cohort studies have also shown that informant reports tend to underestimate exposures while reports from farmers themselves tend to be reliable.[27,28]

Using self-reported exposure data, we showed that occupational pesticide exposure (including all forms endorsed) was associated with an increased risk of dementia. When the outcome was restricted to AD, the association remained. Two classes of pesticides, organophosphates and organochlorines, were associated with an increased risk of dementia and more specifically AD, although the associations were only marginal. We found a stronger risk for dementia with "any pesticide" exposure than for specific types likely because the "any pesticide" group included a number of exposures that could not be accurately classified. As a result, there were more individuals in the exposed group as well as a greater number of outcome events under study in the all-cause dementia group. In the analysis of specific classes of pesticides, we set aside individuals who claimed pesticide exposures other than the exposure of interest in order to reduce noise in the analysis. As a result, we found stronger risks for AD among those exposed to organophosphates and organochlorines than for all-cause dementia. We should note that 177 individuals reported exposure to both and were included in both analyses. A post hoc evaluation showed that there was no additional increased risk in individuals exposed to both pesticides. The fact that the associations we found were slightly stronger when the outcome was restricted to AD suggests that effects of pesticide exposure may be specific to AD and not other forms of dementia.

Our findings are generally consistent with another study that found an elevated risk of AD associated with occupational pesticide exposure.[2] Others have found elevated risks but probably lacked the power to find significant associations.[21,24,25] In a carefully conducted study from the PAQUID cohort,[2] pesticide exposures were evaluated in great detail. In-person questionnaires were used in combination with job coding classifications; a panel of experts assessed the likelihood of exposure associated with each job classification and a job exposure matrix was produced. Cumulative exposures were estimated by taking the product of the duration of exposure and the levels of exposure per the exposure matrix. Place of residence in proximity to vineyards was also taken into consideration. A significant association between pesticide exposure and later onset of AD in men was found (adjusted relative risk 2.36, 95% CI 1.02–5.63). These findings highlight the importance of exposure ascertainment as exposure to pesticides can be particularly difficult to determine.[5]

In the current study, our exposure ascertainment is derived from in-person structured interviews with cues for types of pesticides. Results of investigations into the reliability of data collection methods used in the Agricultural Health Study[27] suggest that our methods may be more accurate than those applied in studies that use mail back questionnaires[25] and reports from proxy informants which were necessarily used in case-control studies.[22-24] The Agricultural Health Study analysis showed that structured questionnaires with cues yield better information than open-ended questioning.[27] Farmers' self-reported pesticide use has shown good reliability[27,29] regardless of the age or education level of participants.[29] It is possible that farmers' recollection of pesticide use is strengthened because of the certification processes required for pesticide applicators. At the same time, the reliability of these reports decreases when more specific details are requested.[29]

There are several strengths and limitations of note in this study. First, this is one of the largest samples used to investigate this question to date, and the follow-up period is relatively long (mean 7.2 years). We have used well-established methods for identification and classification of dementia status. The sample is relatively stable, with low in and out migration, and high response rates (90% participation at baseline) dramatically reducing nonresponder bias.[30] They are also relatively homogenous with regard to education levels and access to health care. Over 90% of the sample are members of the Church of Jesus Christ of Latter-Day Saints, a factor which influences the amount of alcohol and tobacco use in the population. For these reasons, the sample may tend to be healthier, with lower rates of chronic disease. While it is unlikely that these factors influence the effects of pesticide exposures, it is possible that our findings may not be generalizable to other populations. Some nonspecific pesticide exposures were recorded and some individuals reported multiple exposures (177 were exposed to both organophosphates and organochlorines). While we recorded exposures to other pesticides (carbamates and methyl bromides), not enough were reported to analyze them separately. In general, although many forms of pesticides are available, most farmers choose from about 5.[27] The "any pesticide" category additionally captured exposures that either did not fit into one of the 4 major classifications that we targeted or the subject's report of the

particular agent was nonspecific such that it could not be classified. In order to reduce measurement error in our analyses of organophosphates and organochlorines, we set aside individuals who reported exposure to other pesticides or exposures that were not classified. It is possible that some reports among the unclassified exposures were not related to occupational exposure and could be considered measurement error.

Although participants were queried about the duration and extent of exposures, these questions were completed with less frequency. We choose to classify participants as ever/never users in order to retain the largest possible sample for analysis. This decision is bolstered by prior reports which indicate that ever/never use of pesticides is reported more reliably than responses to more detailed questions.[29,31] Regardless of our efforts, there is always the possibility of misclassification. In the current study, it is likely that the misclassification is nondifferential with regard to disease status. Given that exposures are classified dichotomously and the outcome is determined exhaustively, it is likely that the resulting effect sizes are conservative.[29,32,33]

These findings add to a small but growing literature suggesting that exposure to pesticides may have adverse long-term effects on the nervous system, thereby increasing the risk of AD in late life. Future epidemiologic work should focus on specific types of pesticides and the intensity of exposure to fully characterize the potential association with AD. Toxicologic studies may help elucidate the biologic mechanism for the association.

ACKNOWLEDGMENT

The authors thank the study participants; the neurogenetics laboratory of the Bryan AD Research Center at Duke University for *APOE* genotyping; and study coordinators Nancy Sassano (Utah State University) and Michelle McCart (Duke University) and Cara Brewer, Tony Calvert, Tiffany Newman, Roxane Pfister, Sarah Schwartz, and Joslin Werstak of Utah State University for technical assistance in data collection and entry.

DISCLOSURE

Dr. Hayden serves as Senior Editor of *Alzheimer's & Dementia* and receives research support from the NIH (R01-AG1380 [investigator], K01-AG029336 [PI], and P30-AG028377 [administrative support]). Dr. Norton receives research support from the NIH (AG-11380 [Project manager], AG-11380 [Subcontract PI], AG-18712 [Co-I], AG-211386 [Co-I], and AG-31272 [PI]). Dr. Darcey receives research support from Cobalt Development Institute and the CDC/NIOSH (educational training grant). Dr. Østbye has received travel expenses and honoraria for educational activities not funded by commercial entities; has received/receives research support from the NIH (NIDDK R01-DK-07549 [PI], NCI R01-CA-114392-01A1 [Co-I], and NIDDK R01-DK-64986 [PI]); and has served as a legal consultant for AstraZeneca. Dr. Zandi receives research support from the NIH; work on this project was supported by AG-11380 [Subcontract PI]. Dr. Breitner serves on scientific advisory boards for the N. Bud Grossman Center for Research in Memory and Care, the Florida ADRC (NIA and State of FL), and for the Chicago Health and Aging Project; serves on the editorial boards of *Alzheimer's Disease and Associated Disorders*, *PLOS-One, Academics*, *Alzheimer's and Dementia*, and the *American Journal of Alzheimer's Disease*; and receives research support from the NIH (NIA U01-AG-15477 [PI]) and the Minnesota Medical Foundation. Dr. Welsh-Bohmer has served on a scientific advisory board for Medivation, Inc.; has received funding for travel and speaker honoraria from Medivation, Inc. and Elan Corporation/Wyeth; has served/serves as an Associate Editor of *Neuropsychology Review*, on the editorial boards of *Alzheimer's & Dementia*, the *Journal International Neuropsychological Society*, and the *Journal of Experimental & Clinical Neuropsychology*, and as a consulting editor for *Aging, Neuropsychology, and Cognition* and *Neuropsychology*; holds US Patent 6867236 (issued 2005): use patent for nonsteroidal anti-inflammatory compounds for the treatment of Alzheimer's disease; receives royalties from the publication of *Geriatric Neuropsychology: Assessment and Intervention* (Guildford Publications, 2006); and receives research support from the NIH (NIA AG11380 [PI] and NIA AG028377 [PI]).

Received October 23, 2009. Accepted in final form February 5, 2010.

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Toxicology Letters 326 (2020) 31–51

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Pesticides, cognitive functions and dementia: A review

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ARTICLE INFO

Keywords:
Dementia
Cognitive function
Pesticides
Alzheimer's disease
Toxicity
Pesticide exposure
Real-life risk simulation

ABSTRACT

Pesticides are widely-used chemicals commonly applied in agriculture for the protection of crops from pests. Depending on the class of pesticides, the specific substances may have a specific set of adverse effects on humans, especially in cases of acute poisoning. In past years, evidence regarding sequelae of chronic, low-level exposure has been accumulating. Cognitive impairment and dementia heavily affect a person's quality of life and scientific data has been hinting towards an association between them and antecedent chronic pesticide exposure. Here, we reviewed animal and human studies exploring the association between pesticide exposure, cognition and dementia. Additionally, we present potential mechanisms through which pesticides may act neurotoxically and lead to neurodegeneration. Study designs rarely presented homogeneity and the estimation of the exposure to pesticides has been most frequently performed without measuring the synergic effects and the possible interactions between the toxicants within mixtures, and also overlooking low exposures to environmental toxicants. It is possible that a Real-Life Risk Simulation approach would represent a robust alternative for future studies, so that the safe exposure limits and the net risk that pesticides confer to impaired cognitive function can be examined. Previous studies that evaluated the effect of low dose chronic exposure to mixtures of pesticides and other chemicals intending to simulate real life exposure scenarios showed that hormetic neurobehavioral effects can appear after mixture exposure at doses considered safe for individual compounds and these effects can be exacerbated by a coexistence with specific conditions such as vitamin deficiency. However, there is an overall indication, derived from both epidemiologic and laboratory evidence, supporting an association between exposure to neurotoxic pesticides and cognitive dysfunction, dementia and Alzheimer's disease.

1. Introduction

Pesticides are a broad category of various, widely-used chemicals, mainly applied in agriculture to protect the crops from pests; the nature of pests ranges from insects and birds, to fungi and microorganisms (Zaganas et al., 2013). Depending on the desired target and mechanism of action, there are several classes of pesticides, the most representative being organophosphates (OPs), organochlorines (OCs), carbamates, pyrethroids and neonicotinoids (Costa et al., 2008). Though these compounds do not exert a specific toxicity, they may result in human

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https://doi.org/10.1016/j.toxlet.2020.03.005
Received 24 December 2019; Received in revised form 2 March 2020; Accepted 3 March 2020
Available online 04 March 2020
0378-4274/

poisoning.

More specifically, OPs and carbamates both inhibit the enzyme acetylcholinesterase (AChE), leading to the accumulation of acetylcholine (ACh) in the synapses. Although their acute toxicity symptoms are similar, poisoning caused by carbamates is usually quickly reversible, as opposed to OPs. Furthermore, OPs have been linked to more chronic adverse effects compared to carbamates, such as organophosphate-induced delayed polyneuropathy (OPIDP), neuropsychological sequelae and developmental neurotoxicity (Dardiotis et al., 2019b; Roldan-Tapia et al., 2005; Ruszkiewicz et al., 2019). Pyrethroids slow down the activation and inactivation of the sodium channels, leading to hyperexcitability, and only a few human poisonings have been reported with these substances (Ray and Fry, 2006). OCs, nowadays banned in most countries worldwide, have diverse sites of action, such as the sodium channels of the axonal membrane or the chloride channels, antagonizing γ-aminobuturic acid (GABA) inhibition. Pyrethroids και OCs have been reported to have acute and chronic side-effects, the latter mostly affecting the function of the liver and the organs of the reproductive system (Costa et al., 2008). Neonicotinoids, on the other hand, exert their action and their toxicity via the activation of nicotinic receptors; these newer compounds are relatively safe, since they are specialized for insect rather than mammalian receptors (Costa et al., 2008). Additionally, pesticides, besides these adverse effects, have been reported to be associated with carcinogenesis (Falzone et al., 2016; Rapisarda et al., 2017), with numerous studies tying them to various forms of cancers (Brody et al., 2004; Brown et al., 1990; Hardell and Eriksson, 1999).

Highly water-soluble pesticides, such as 2,4-D, a known herbicide, are less persistent in the environment and most likely to biodegrade quickly and not end up bioconcentrated in organisms. Lipid-soluble pesticides such as DDT and DDE, a member of the OC group, are especially prone to bioaccumulation (Katagi, 2010). Although very little is known about pesticide detoxification in the central nervous system (CNS) and detoxifying systems are highly polymorph, leading to great inter-individual variability in susceptibility to the neurotoxic effects of insecticides, chronic pesticide effects during the period of cerebral development are also an issue of particular interest (Cassereau et al., 2017).

Pesticide molecules, small and lipophilic in nature, can enter from blood to brain and then in neurons, glial cells and brain microvessels (Pulgar, 2018). Pesticides target and disrupt blood- brain barrier receptors in the CNS (Moura et al., 2019), enhancing pesticidal chronic toxicity and affecting the physiological process of receptor-mediated transcytosis, as well as blocking pharmaceutical transport and delivery through the brain endothelial cells towards the parenchyma, thus leading to drug ineffectiveness and other adverse effects (Moura et al., 2019).

Accumulating epidemiologic evidence over the past decades suggests that repeated exposure to low levels of pesticides negatively affects the CNS in various ways (Stephens et al., 1995), while agrichemical exposure has been identified as a risk factor for a plethora of adverse CNS effects (London et al., 1997). Repeated or even single acute episodes have been associated with long-term pesticide exposure, but also low-level chronic exposure has been reported to be more strongly implicated in acute episodes, though relevant studies have not been consistent (Baldi et al., 2011; Jamal et al., 2002; Kamel et al., 2003). Additionally, pesticides have been linked to a series of neurological diseases, such as amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD), with the latter presenting the strongest association (Dardiotis et al., 2013; Freire and Koifman, 2012; Kamel et al., 2012). Pesticide exposure has been eventually associated with cognitive impairment and dementia, including that due to Alzheimer's disease (AD) (Dardiotis et al., 2019c).

Taking into account the burden associated with such disease, particularly but not only in the Western countries and their aging populations, and the associated costs in terms of both economic loss and low quality of life, identifying the factors that pertain to these disorders and that could halt their progression if modified, is crucial; regarding the matter in hand, highlighting pesticide safety issues for individuals. Pesticides, due to their wide-spread use, affect the majority of people and if they are really implicated in neurodegenerative processes such as dementia, their restriction could substantially contribute in reducing the incidence of these diseases. Several studies on animals and human subjects have been conducted in order to pinpoint possible links between pesticides and cognition/dementia and AD, yet, to our knowledge, no consensus in the scientific community on the matter exists, and recent reviews on this issue are lacking. Therefore, in this article, we have attempted to identify the mechanisms underpinning the toxicity relevant to cognitive impairment, AD and dementia separately for each substance and for pesticides in general. Moreover, we aimed to present the results of studies on pesticides and dementia/cognitive impairment, to provide further insight in the way they interact.

2. Possible mechanisms of pesticide-associated cognitive impairment

The multifactorial nature of pesticide-related cognitive impairment is consistent with experimental results showing distinct roles for pesticide exposure, years of exposure and age of exposed individuals. The extent to which each of the aforementioned factors contributes to cognitive deficits remains under-explored. Several theories have been suggested and the most dominant one hybridizes the various approaches and offers a combined interpretation under the suggested theoretical models. The main culprit, incriminated by the overwhelming majority of relevant studies, could be the oxidative stress induced by the substances; neuronal cells are more susceptible to oxidative stress due to their high polyunsaturated fat content in the myelin sheaths, low anti-oxidative capabilities, enzymatic systems with transient metals that aid in the production of free radicals, and high oxygen demand and glucose metabolism rate (Chen et al., 2015; Mostafalou et al., 2012). Studies have also investigated the effect pesticides have on the pathological hallmarks of Alzheimer's disease (AD)- the archetype of cognitive impairment in humans- such as the Aβ amyloid plaques and the Tau protein dysfunction (Chen et al., 2015; Palop and Mucke, 2010), with some studies also describing the implication of epigenetic modifications (Chen et al., 2015). As it is already well-known, pathologically, amyloid plaques and neurofibrillary tangles are the main forms of aggregated proteins involved in AD (Nousia et al., 2019, 2018). Amyloid plaques are visible protein aggregates derived from the dimers and oligomers of brain amyloid-β protein, while neurofibrillary tangles are composed of a compact filamentous network of helical filaments of hyperphosphorylated tau protein. Together these neuropathological changes are thought to result in the loss of synapses and neuronal cell death, leading in cognitive dysfunction (https://pathways.nice.org.uk/pathways/dementia).

Paraquat (PQ), one of the most commonly used herbicides, has been the focus of numerous studies. Its main mechanism of action is the induction of oxidative stress and mitochondrial damage (Baltazar et al., 2014; Drechsel and Patel, 2008), as it can enter the organelles and use electrons from the electron transport chain to generate reactive oxygen species (ROS) (Cocheme and Murphy, 2008) and enhance H2O2 production (Castello et al., 2007). Chen et al. (2015) revealed that PQ-exposed mice, regardless of their Amyloid Precursor Protein (APP) gene status, had lower complex I and IV activities, and that genetically modified rats with triple peroxiredoxin 3 (PRDX3, anti-oxidative mitochondrial enzyme, central to H202 removal) activity were protected against oxidative stress (Chen et al., 2015; Chen, 2012). In another study conducted by the same researchers, PQ-exposed mice performed more poorly than controls in cognitive tests, while the markers of mitochondrial damage correlated directly with the impairment in associative learning and memory skills; again, PRDX3 overexpression attenuated differences in performance (Chen et al., 2015; Chen, 2012). PQ

also led to an increase in Aβ amyloid aggregation in APP carrier mice, a phenomenon linked to oxidative stress, since PRDX3-overexpressing APP subjects showed suppressed Aβ amyloidogenesis (Chen, 2012). Additionally, the involvement of the NLPR3 inflammasome, a key component in inflammation commencement in response to ROS in neuronal damage (Kepp et al., 2011), was evinced by the elevation of IL-1b and caspase-1 in PQ-exposed mice regardless of their APP status (Chen et al., 2015). Similarly, PRDX3-overexpressing mice showed decreased levels of these markers, further validating the role of H2O2 in CNS inflammation (Chen et al., 2015).

PQ has also been reported to affect the glutaminergic system in PD. It has been shown to enhance glutamate release from non-NMDA receptors, leading to a raised influx of calcium and the stimulation of nitrogen oxide species, which can in turn induce mitochondrial damage alongside other ROS (Shimizu et al., 2003). Autophagy also seems to play a part, since it degrades oxidated-by-ROS proteins and its inhibition has been shown to accelerate cell death after exposure to PQ (Gonzalez-Polo et al., 2007); it was also noted in neurons of AD patients (Nixon et al., 2000). Finally, PQ exposure has been linked to increased p-Tau via the activation of a phosphorylating kinase, and tubulin alterations, hinting towards cytoskeleton remodeling (Wills et al., 2012).

OPs constitute a class of pesticides encountered worldwide, with widespread use, and therefore, exposure. Though their main mechanism of action involves the inhibition of AChE and the consequent toxic accumulation of Ach in the synapses (Dardiotis et al., 2019b), current evidence suggests that non-cholinergic systems are implicated in the neuropsychological and chronic manifestations of OP exposure, such as the Gulf War Illness (Androutsopoulos et al., 2013; Jokanovic, 2009; Vucinic et al., 2017). Kaur et al. (2007) described deficits in mitochondrial complex I, II and IV activities after chronically exposing rats to dichlorvos; the researchers pinpointed oxidative stress as the culprit, after finding lower glutathione (GSH) and lower superoxide dismutase (SOD) activity levels (Kaur et al., 2007). Abou-Donia (2003), describing the entity of OP-ester induced chronic neurotoxicity (OPICN), which involves many neurobehavioral symptoms such as attention and memory deficits, included oxidative stress in its main mechanisms, via the critical depletion of mitochondrial energy, the activation of proteolytic enzymes, and DNA fragmentation, all giving way to apoptosis (Abou-Donia, 2003). Consequently, OPs considerably disrupt mitochondrial function and lead to oxidative stress in the CNS (Middlemore-Risher et al., 2011).

Flaskos et al. (2011) have extensively studied the effects of OPs, chlorpyrifos (CP) in particular, in typical neuronal development, and their studies have shown that AChE inhibition is not involved in the noted disruptions; other pathways involve the deregulation of transcription factors central to neurite growth and of enzymatic biomarkers of CNS-cell differentiation (Flaskos, 2012; Flaskos et al., 2011). CP also seems to affect typical development via alterations in structural proteins and glial cell proliferation (Garcia et al., 2002, 2005). Concerning Aβ amyloidogenesis, the in vivo studies that have focused on CP have so far yielded contradictory results. In an animal model, the amyloid levels were raised in the hippocampus and the cortex of exposed mice, while this change has been accompanied by memory deficits (Salazar et al., 2011); nevertheless, Peris-Sampedro's (2014) similar two-phase study failed to replicate the same findings (Peris-Sampedro et al., 2014).

Other mechanisms, such as the OP-triggered induction of a xanthine oxidase, may also play a role (Gultekin et al., 2000) in cognitive impairment, and OPs' versatile activity does not seem to stop at enzymes, but rather extends to the genetic level as well. OPs affect DNA methylation in several hundred genes, and this has been demonstrated to occur in vivo in both human and animal models alike (Hodjat et al., 2017; Xing et al., 2014; Zhang et al., 2012a, b). Abou-Donia (2003) in their OPICN review, reported that OPs also seem to induce the AChE gene expression, enhancing apoptosis, and implicated the glutamatergic system as well, with the activation of NMDA glutamate receptors and the subsequent calcium influx in the post-synaptic neurons, that leads to degeneration (Abou-Donia, 2003). As Terry (2012) concluded, the OP delayed toxicity may also involve axonal disruption and neurotrophins (Terry, 2012) (see (Reddy et al., 2012; Slotkin, 2006) for similar findings in AD).

Finally, the aforementioned findings cannot undermine the importance of OPs' main mode of action; AChE inhibition. It has been widely acknowledged that acetylcholine excitoxicity, through repeated stimulation, quickly consumes the available cellular ATP since it requires more energy (Dettbarn et al., 2006). This action may also constitute another route through which OP exposure results in mitochondrial dysfunction, since prolonged depletion of ATP has been shown to reduce mitochondrial membrane potentials, which in turn leads to electron transfer enzymatic complexes' dysfunction and the formation of ROS (Carlson and Ehrich, 1999; Milatovic et al., 2006; Mostafalou et al., 2012).

Additional pesticide classes seem to lead to cognitive impairment through the same mechanisms activated by PQ and OPs. Maneb has been shown to obstruct the glutathione system (Baltazar et al., 2014). OCs, such as DDE and DDT, aggravated Aβ amyloidogenesis in in-vivo models (Li et al., 2015; Richardson et al., 2014) and their lipophilicity has motivated research tying obesity and dementia to OCs (Lee et al., 2016). Amyloidogenesis has also been implicated in triazine- and pyrazole-associated cognitive impairment, as it has been shown to induce the production of Aβ42 over Aβ40, another hallmark of AD, via the potentiation of amyloid secretases (Cam et al., 2018; Portelius et al., 2016). Lastly, animal models involving carbamates and pyrethroids have suggested Tau protein malfunction as a plausible mechanism underlying cognitive impairment (Chen et al., 2015).

Moving on, various pesticides bind to growth factors (IGF1, TGF-α, EGF, EDGF and VEGF), chemokines, hormones, transmitters [e.g. G protein-coupled receptors (GPCRs), involving fast-responding GABA receptors (Tanaka, 2019), muscarinic or nicotinic acetylcholine receptors, glycine and 5-HT3], and integrin, and thus play a key role in the activation of a secondary downstream intracellular messenger system. The activation of this secondary signaling through PLC, IP3/DAG, PKC (Yamasaki et al., 2009), adenylatecyclase, cAMP and phosphatidylinositol signal pathway, deregulate gene expressions related, among others, to cellular immune responses (Chaffey, 2003),vascular permeability (Fan et al., 2018), β-amyloid precursor protein expression, neuronal death, synaptic dysfunction, and memory and learning, the deficits of which are pathological findings in AD (Del Prete et al., 2014).

Synthetic pesticides, such as carbamates, are inducers of nuclear factor-kappa B (NF-κB) –a transcription factor that induces a plethora of genes, some of which involved in the process of inflammation- activity through ROS (Bowie and O'Neill, 2000; Schieven et al., 1993), lipoxygenases, TNFα and IL-1β (Westbrook et al., 2012). Pesticide-induced NF-kB activation (Gutierrez et al., 2005) alongside growth factors such the brain-derived neurotrophic factor (BDNF) (Zaheer et al., 2001), the Wnt/β-catenin signaling (Kobayashi et al., 2015), IL-1β (Chung et al., 2017), TNFα (Reid et al., 2009) and TNFR (Qiu et al., 2004) cytokines, PKAc (Kaltschmidt et al., 2006) or alteration of testosterone (Hofbauer et al., 2002), estrogen (Michael et al., 2005) and aromatase levels (Hofbauer et al., 2002), transcriptionally (Martín Millán, 2015) and post-transcriptionally (Jia et al., 2017) affect gene expressions (Santoro, 2016), but are also implicated in processes of synaptic plasticity (Boersma et al., 2011) and memory (Meffert et al., 2003).

Fungicides that act as AChR agonists, such as fludioxonil, fenhexamid, mepanipyrim, cyprodinil, pyrimethanil, and chlorpyrifos-methyl (OP and pyrethroid combination), directly and indirectly, via ER, AR and multiple other receptor pathways, transcriptionally remodel gene expression, resulting in the deregulation of neural differentiation, observed and in birth defects (Medjakovic et al., 2014). Furthermore, pesticide ligands of NRs and AChR disrupt AHR mRNA/HES1 oscillations and the MASH-1/ Notch signaling pathway (Akahoshi et al., 2006). Organotin, for example, activates RXR-PPAR heterodimers that exert their mutagenic and carcinogenic capabilities (Prival et al., 1977),

and deregulate the behavioral and circadian rhythms (Ema et al., 1991), resulting in circadian clock disruption, mood and CNS disorders (Logan and McClung, 2019), and global prevalence of obesity by both HFD-induced disruption of metabolic rhythms (Eckel-Mahan et al., 2012) and ER/FFAs/PPARγ signaling activation (Liu et al., 2013), which suppresses the Per1 gene expression (Killilea et al., 2017; Kawai et al., 2010; Kawai et al., 2010; Killilea et al., 2017).

Preactivation of the suprachiasmatic nucleus (SCN) by AChR agonist-pesticides, such as CP, carbendazim and thiabendazole, blocks the glutamate-induced phase resetting of the SCN electrical activity rhythm (Mukai and Tischkau, 2007), suggesting that chronic pesticidal activation directly within the SCN impacts the ability of the clock to respond to phase-resetting stimuli e.g. the daily rhythm of thyroid hormone secretion that is governed by SCN-signalling via the rhythmic TSH secretion (Fahrenkrug et al., 2017). Similarly, AChR-binding pesticides change the behavioral and electrical activity of circadian clock genes Per1 and Bmal1 in the liver and the SCN (Amakura et al., 2016), attenuating light-induced induction of Per1 in the SCN (Takeuchi et al., 2008), providing an essential mechanism for pesticidal AChR activation on light-induced changes by daily photic cues transmitted from the retina (Barakat et al., 2005) via the retinohypothalamic tract in behavioral rhythmicity (Popovska-Gorevski et al., 2017).

At this point, we would like to also bring attention to another factor of pesticide toxicity; their absorption. A recent study showed that a high percentage of the pesticides studied (81.4 %) exhibited high intestinal absorption and a 38.5 % displayed brain permeation (Chedik et al., 2017). Since many neurodegenerative disorders seem to be induced by insecticides and herbicides, most notably OPs, OCs, phenoxyacetic acids and triazine compounds (Mostafalou and Abdollahi, 2017), the knowledge of their gut absorption and brain permeation kinetics and dynamics is necessary for predicting chronic and direct toxicity in the neurovascular unit. Infants and young children are particularly sensitive to these contaminants, as their brains and organ systems are not fully developed and the field of neurodevelopmental pesticide toxicity is gaining more ground in the scientific community. For this reason, it is important for future researchers to be aware of the mechanisms of chronic pesticide neurotoxicity, which we have summarized in Fig. 1, and the various other biological mechanisms which exceed the purposes of this manuscript, but have been presented in Fig. 2.

In conclusion, the pathways through which pesticides cause cognitive impairment exhibit great heterogeneity and no single mechanism has been identified. Oxidative stress and mitochondrial dysfunction seem to play the dominant role, with several other modes of actions, such as epigenetic alterations and amyloid production elevation, contributing considerably to cognitive impairment as well.

3. Animal studies

Ever since suspicions on the effects pesticides seem to have on cognition were raised, several research groups have attempted to conduct experiments in order to investigate this association and elucidate the mechanisms that underlie it. Experiments on rats remain the most popular, with a wide variety being conducted mainly via the administration of pesticides on subjects and the subsequent assessment of their performance on tasks designed to test specific aspects of the subjects' cognitive functioning, (e.g. attention span or learning ability), such as mazes and hidden target platforms (Abdullah et al., 2011; Levin et al., 2010; Phillips and Deshpande, 2016; Terry et al., 2003). Here, we have included 15 animal studies, that we believe have provided important results, and connect them with findings from other studies and reviews as well.

More specifically, Levin, Slotkin et al. have presented a series of studies on rats following pesticide exposure, mainly OPs. The studies have exhibited cognitive impairment to be closely linked to deficient Ach function in the synapses (Levin et al., 2001, 2010; Slotkin, 2004, 2006), even at concentration levels that do not lead to diffuse cholinesterase inhibition (Slotkin, 2004, 2006). Visuospatial memory performance has also been shown to be highly dependent on ACh function; also, in their experiments, they have measured several indices tied to ACh and serotonin function, known to be affected by developmental exposure to OPs, such as 5HT1A/2R, activity of choline acetyltransferase (ChAT), and the concentration of α4β2 nicotinic ACh receptors (nAChRs) (Levin et al., 2010; Yatham et al., 1999, 2000). The same researchers have located deficits in ACh presynaptic activity, accompanied by a rise in compensatory mechanism indices, in regions where ACh mediates several important behaviors such as reward, similar to what has been found in AD (Bissette et al., 1996; Levin et al., 2010; Siokas et al., 2020; Slotkin et al., 1994; Stamati et al., 2019; The Canadian Study of Health and Aging, 1994). Testing a non-toxic prenatal exposure to parathion, they described a long-lasting effect on

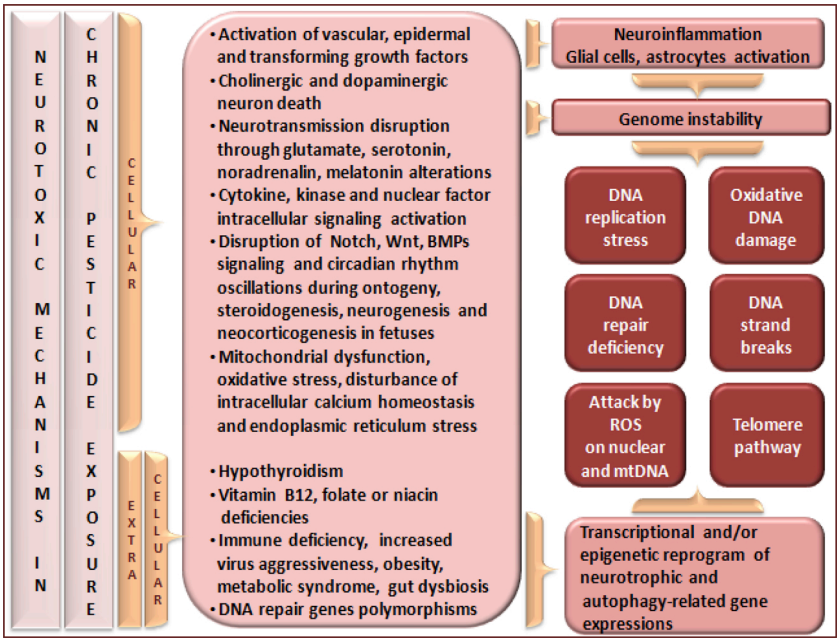

Fig. 1. Cellular mechanisms of dementia and cognition decline due to long - term pesticide exposures.

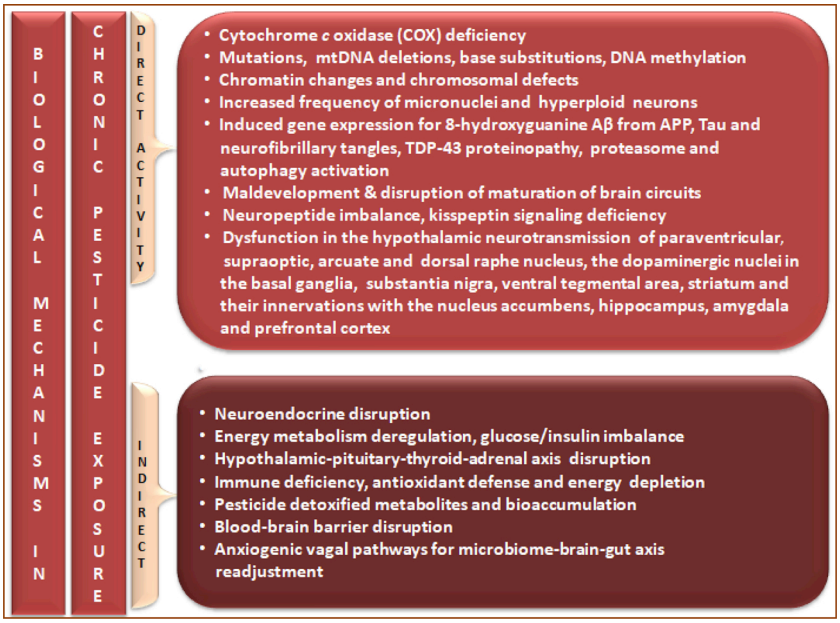

Fig. 2. Direct and indirect long-term pesticide activities during dementia.

several brain regions of relatively young rats, with the pathological findings being akin to those found in subjects of deep senescence. Crucially, parathion effects were not found to be subject to sex differences; exposed male rats did not present better performance scores than female rats in visuospatial memory tasks, as expected based on their sex (Levin et al., 2001, 2010). A possible explanation could be the faster recovery and plasticity that female brains present when exposed to these substances (Levin et al., 2010). In an another relevant study, visuospatial impairment was also noted in malathion-exposed test subjects, at concentrations that did not inhibit AChE, thus implying that additional mechanisms are implicated as well (dos Santos et al., 2016).

Some studies have also highlighted the role of oxidative stress in the reported cognitive deficits (Gubandru et al., 2013; Sayapina et al., 2017). Jain et al. (2013) found that triazophos-exposed rats presented learning and memory deficits, and had much lower levels of BDNF and GSH in their hippocampi (Jain et al., 2013). Deficits in mitochondrial complex activity were further noted in chlorpyrifos (CP)-exposed rats' hippocampus neurons, whose activity was positively correlated to performance in object location tasks, suggesting that damage in the respiratory chain of these neurons was the primary cause of the cognitive impairment (dos Santos et al., 2016). In Akande et al.'s (2014) study, taurine, a known anti-oxidant, was found to counteract the oxidative processes caused by the exposure to CP and lead acetate in exposed rats (Akande et al., 2014); CP has been shown to compromise both learning and memory skills in exposed tested subjects (Ambali and Aliyu, 2012). It has also been shown that BDNF is involved in memory and learning skills located in the hippocampus (Alonso et al., 2005; Linnarsson et al., 1997; Numakawa et al., 2011; Siokas et al., 2019), therefore its depletion could reflect cognitive deficits, alongside oxidative stress. Chen et al. studied PQ exposure and found poorer performance in associative learning and memory tests in exposed mice, in direct correlation to mitochondrial damage, while mice over-expressing an anti-oxidating enzyme were found to be relatively protected (Chen et al., 2015; Chen, 2012).

Finally, in a similar vein, several animal models have attempted to replicate the Gulf War Illness, which is now considered to be caused by exposure to OP nerve gas Sarin, with substances such as diisopropylfluorophosphate and permethrin, and further explore its effects (Abdullah et al., 2011; Hattiangady et al., 2014; Phillips and Deshpande, 2016). These studies showed that the exposed mice presented disturbances in circadian rhythms, psychomotor skills, endocrine and immune system proteins, as well as anxiety-like or depression-like behaviors, all commonly reported by Gulf War veterans (Abdel-Rahman et al., 2004; Abdullah et al., 2011; Hattiangady et al., 2014; Phillips and Deshpande, 2016). Abdullah et al. (2011) reported a delayed cognitive impairment in exposed mice, in learning and memory skills, and attributed it to compensatory mechanisms that lower synaptic ACh levels in response to the chronic elevation of the substance after exposure to chemicals such as pyridostigmine bromide (Abdullah et al., 2011). The researchers also reported astrogliosis simultaneous to the cognitive impairment manifestation, which aligns with the findings of other studies (Abdel-Rahman et al., 2004; dos Santos et al., 2016), thus providing converging evidence on an underlying inflammatory/immune-mediated etiology of the cognitive impairment (Abdullah et al., 2011). Phillips and Deshpande (2016) and Hattiangady et al. (2014) reported depression and deficits in motivation and spatial memory, and attributed them to hippocampal lesions revealed by histological analyses, a finding that was also captured in studies involving Gulf War veterans (Hattiangady et al., 2014; Phillips and Deshpande, 2016). In line with previous studies' hypotheses, the activation of the glutaminergic system, as a response to AChE inhibition, could explain the damage to the hippocampus neurons via an elevation in calcium levels and the subsequent excitoxicity (Deshpande et al., 2010).

All in all, there is a wide variety of experiments with pesticides based on animal models, mainly involving rodents and organophosphates, yet their results are equally versatile; it seems that several mechanisms are involved in the pathogenesis of cognitive impairment following exposure to these substances, which were extensively described in the preceding sections.

4. Human studies

4.1. Pesticides and cognitive and neuropsychological function

Besides the notion that pesticide exposure may enhance the risk of dementia, it has long been suggested in the scientific community that such an exposure, if chronic, may affect specific cognitive functions even without reaching a diagnosis of dementia. Several studies have examined this potential association, with some paying particular attention to differentiating between chronically-exposed subjects and those suffering from episodes of acute pesticide poisoning, an event that could influence the final outcome (Ames et al., 1995; Kamel et al.,

2003; Mackenzie Ross et al., 2010; Steenland et al., 2000; Stephens and Sreenivasan, 2004). A wide array of tests has been administered by scientific groups to evaluate participants' cognitive functioning. Some studies have applied gold-standard, widely acknowledged tests such as the Mini Mental State Examination (MMSE), while others have used neurobehavioral batteries, such as the World Health Organization's Neurobehavioral Core Test Battery, which includes numerous tasks and questions. Since the parameters and variables examined were numerous and heavily differentiated from task to task and from study to study, we deemed it better not to include the full extent of numerical values and statistics that each study presented. Instead, we concisely describe their results and encourage the readers to turn to the original articles for further details and numerical values. In this section, we have included 30 relevant studies, spanning from 1992 to 2019, with the last search performed in August 2019.

Early studies failed to show a link between pesticides and cognitive impairment. Daniell et al. (1992) compared 49 OP applicators to 40 controls with self-reporting questionnaires and measurements of cholinesterase serum levels, and found no clinically important effects in the neuropsychological performance of the subjects before and after the spraying season (Daniell et al., 1992). In a similar vein, Ames et al. (1995) compared 45 chronically-exposed-to-anticholinesterase-agents subjects to 90 controls, and found no evidence of increased neurological sequelae in the test parameters (Ames et al., 1995). London et al. (1997) performed a cross-sectional study using exposure questionnaires, measurements of cholinesterase levels and a wide array of neurobehavioral tests in 163 OP applicators and 84 non-exposed workers. According to the results, the study revealed weak effects of occupational exposure on the participants' cognitive and behavioral scores. A number of factors have been implicated for the inconsistencies found, or the lack of effects, such as misclassification of exposure, cross-cultural differences and confounders, namely alcohol consumption and history of head trauma (London et al., 1997). After assessing 40 OP-exposed farmers and 40 age-, sex- and education-matched controls, Bayrami et al. (2012) found increased incidence of clinical symptoms such as eczema and nausea, alongside elevated biochemical markers of oxidative stress, but no evidence of impaired cognitive functions in any of the tests performed (Bayrami et al., 2012). Finally, in a prospective longitudinal study, Berent et al. (2014) evaluated 53workers exposed to CP, an important member of the OP group, and 60 referent workers, yet found no evidence of cognitive impairment; surprisingly, Berent et al.'s (2014) study reported higher cognitive function scores for the exposed subjects in specific domains one year after the initial evaluation (Berent et al., 2014). The authors mention higher marriage and education rates in CP workers, (Berent et al., 2014), a factor which could have influenced the results, since, to our understanding and knowledge, a higher education level and a married life which could be in accordance with lower stress levels, both contribute to higher cognition scores. The overall findings hint towards cognitive impairment in exposed individuals as a consequence of the interaction among several factors, including exposure itself.

Additional studies found evidence in favor of neuropsychological effects stemming from pesticide exposure, though such effects were not deemed to be major. Steenland et al. (2000) assessed 191 current and former applicators exposed to CP, along with 189 controls, yet found no big group differences either in clinical performance scores, or in comparisons conducted between formerly and currently exposed workers; worse cognitive performance in specific domains was only reported for 8 subjects that had a history of acute CP poisoning. Exposed subjects in general, did exhibit higher reporting frequency of fatigue, memory deficits, and tension (Steenland et al., 2000). In an observational study by Salvi et al. (2003), 37 farmers exposed to OPs for 3 months were monitored, and no differences in MMSE and word span scores between them and the general populous were noticed; almost half of them, however, had a diagnosis of either generalized anxiety disorder or major depression. Twenty-five of these subjects were re-evaluated after a 3-month period free of OP exposure, and the occurrence of concurrent psychiatric diagnoses had considerably dropped (Salvi et al., 2003).

As can be so far understood, psychological effects stemming from pesticide exposure constitute a frequently reported finding of studies focusing on possible links between cognitive functioning and pesticide exposure. Stephens et al. (1995) compared 146 OP-exposed farmers and 143 non-exposed subjects via the use of occupational questionnaires and a wide array of neuropsychological tests. Besides the exposed group's poorer mental health, the study revealed significant impairment in the group's information processing speed and sustained attention abilities; no effects were reported for short-term memory and learning skills (Stephens et al., 1995). Jamal et al. (2002) performed a case-control study of 79 OP-exposed farmers nested within a larger cross-sectional study of 685 subjects. Participants were administered detailed questionnaires and neuropsychological battery tests. Though there were no signs of memory deficits, the exposed group exhibited slower processing speed compared to the control group. This specific study has primarily focused on the peripheral neuropathology of the subjects, reporting that this subgroup of individuals was also characterized by depression and anxiety with evidence of peripheral nerve damage (Jamal et al., 2002). Slow processing speed for exposed individuals has also been reported by Stephens and Sreenivasan (2004), who applied the sensitive Syntactic Reasoning test, among others, on 37 sprayers chronically exposed to CP, other OPs and carbamates, in the biochemically-proven absence of recent exposure and with no history of acute poisoning, versus 57 non-exposed workers (Stephens and Sreenivasan, 2004). Cole et al. (1997) conducted a cross-sectional study comparing 144 farm-involved rural citizens and 72 age- and education-matched non-farm-involved individuals. The study reported general cognitive deficits for the farm cohort, which were mainly evident in spatial, psychomotor and motor functions; the subgroup of OP and carbamate applicators were also found to perform worse than controls in attention tests (Cole et al., 1997). Lastly, addressing the class of fumigants, Calvert et al.'s(1998) study with 123 fumigation workers and 120 controls showed a connection between high-level exposure to sulfuryl fluoride and lower scores on cognitive and visual memory tests; yet, this specific study failed to show an over-generalized drop of performance in the fumigation worker's cognitive function abilities (Calvert et al., 1998).

Some studies have reported more global cognitive impairments following chronic exposure to pesticides. In a cross-sectional study by van Wendel de Joode et al. (2001), 36 chronically DDT-exposed workers, alongside 31 controls, performed consistently worse in the neurobehavioral tests administered, with a mean performance decrease of about 20 %, which considerably deteriorated as exposure years increased; drop in cognitive performance in the same study was also found to associate positively with the workers'exposure levels. Verbal attention, and visuomotor speed and sequencing were the two domains most heavily influenced, while exposed subjects also reported psychiatric symptoms at a higher frequency (van Wendel de Joode et al., 2001). In another cross-sectional study (Kamel et al., 2003) with 288 farm workers(exposed to unspecified pesticides) and 51 controls, workers showed consistent deficits in cognitive, psychomotor and balance functions, with the deficits being especially stronger for the experimental subjects who had performed farm work one year before testing. Crucially, poorer performance persisted even after the subjects that had suffered from acute poisoning were removed from the sample, which suggests that chronic exposure does indeed affect the nervous system (Kamel et al., 2003). Deficits in numerous neurobehavioral tests were also reported in Farahat et al.'s study (2003) with 52 OP-exposed workers and 50 age- and education-matched controls. Participants were administered questionnaires, neurobehavioral battery tests and serum measurements. According to the findings, there was impairment in visuomotor and verbal speed, visual and auditory memory, and verbal abstraction; subjects also exhibited higher neuroticism scores. Impairment levels were found to positively correlate with the duration of

occupational exposure, while no subject showed biochemical evidence of acute OP poisoning at the time of testing (Farahat et al., 2003). Further evidence in favor of chronic cholinesterase-inhibitor exposure leading to neuropsychological impairment has been provided by Roldan-Tapia et al. (2005), who assessed 40 farm workers and 26 controls with methods similar to those of Farahat et al. (2003). Impairments were reported for perceptive and visuospatial processing, as well as integration time only for those individuals who wereOP exposed for more than 10 years. Though Roldan-Tapia et al. (2005) used no syntactic reasoning tasks,there seemed to be no great group effect in reasoning skills; picture completion and similarities test scores, with educational level as a covariate, showed relative risks of 1.35 (0.042–2.66) and 0.94 (0.26–1.62) respectively (Roldan-Tapia et al., 2005).

Following the same line of reasoning, Rothlein et al. (2006) compared the performance of 96 agricultural, OP-exposed workers to 45 controls. The former group exhibited poorer scores in the majority of the neurobehavioral battery tests, namely, in sustained attention, information processing and motor speed, as well as coordination. Though urinary OP metabolites were associated with lower scores in some of the tests, this specific association was rather deemed to most likely reflect an approximation of the exposure level due to the metabolites' short life and the nature of the tests, rather than a sensitive predictor of cognitive performance (Rothlein et al., 2006). Baldi et al.'s (2011) follow-up *PHYTONER* study with 614 vineyard workers (unspecified pesticides) showed that the workers exhibited low performance in the neurobehavioral tasks testing processing speed, attention and strategic thinking, with ORs spanning from 1.35 to 5.60; cognitive performance was associated with pesticide exposure, with direct exposure only elevating the risk by some decimals compared to the indirect exposure condition (Baldi et al., 2011). Effects of direct vs. indirect exposure (mostly to cholinesterase inhibitors) were also investigated by Corral et al.'s (2017) study with 32 directly-exposed, 32 indirectly-exposed workers, and 38 controls. The directly-exposed group performed worse in executive functions, verbal fluency, visual and auditory memory tests than the indirectly-exposed workers, who in turn did worse than the control group. Both exposed groups showed higher decline rates than controls, a finding which suggests that low-level chronic exposure, either direct or indirect, does influence cognitive functioning (Corral et al., 2017). Finally, Jamal et al. (2016) compared 187 OP-exposed sprayers to 187 controls, and found that the former group exhibited considerably worse performance inpsychomotor speed, selective attention, divided attention, verbal, non-verbal, and prospective memory, spatial functioning, and initiative, alongside higher rates of anxiety and depression. The study also addressed the fact that the sprayers were negligent to protection measures, such as face masks, gloves and showers, although these were available (Jamal et al., 2016).

The nature of occupation has also been found to play an important role in exposure and, par consequence, to the selection of experimental subjects in relevant research. A particular group often tested in pesticide-cognition studies comprises of sheep dippers and farmers, whose profession entails chronic exposure to low levels of OPs. Stephens et al. (1995), as mentioned before, found information-processing speed and sustained attention deficits, besides poorer overall mental health, in 146 dippers (vs. controls) (Stephens et al., 1995). Similarly, Jamal et al. (2002) reported slower processing speed for 79 sheep farmers (Jamal et al., 2002). Mackenzie Ross et al.'s (2010) study was the first to include subjects that retired on the grounds of ill-health, also assessing the PON1 genotype, since mutations are thought to confer susceptibility to OP poisoning (Androutsopoulos et al., 2011; Dardiotis et al., 2019b, 2018; Mackenzie Ross et al., 2010), and excluding farmers with a history of acute poisoning or other conditions that might have accounted for their reported poor health. The cohort of 128 sheep farmers in Mackenzie Ross et al.'s (2010) study exhibited greater impairments in memory, response speed, fine motor control, mental flexibility and strategy making, alongside higher rates of anxiety and depression, compared to the 78 unexposed controls, though verbal and visuospatial abilities remained largely intact. Some parameters were also found to correlate positively with the duration of exposure (Mackenzie Ross et al., 2010).

The observation of an exponential increase in dementia and cognitive decline prevalence with age has motivated the inclusion of older people in studies focusing on links between exposure and cognitive impairment. Within the context of the *Maastricht Aging Study*, Bosma et al. (2000) have examined 830 non-demented 50-to-80-year-old individuals and reported that exposed (to unspecified pesticides) individuals had a five-fold risk of mild cognitive dysfunction as compared to the non-exposed group (Bosma et al., 2000). Steenland et al. (2013) assessed 400 elderly residents of government-run clinics. Those who had been occupationally exposed to unspecified pesticides in the past performed worse on dementia screening tests, while and a trend of performance decrease was observed for individuals reporting more years of exposure (Steenland et al., 2013). Kim et al. (2015) studied 644 elderly participants of the *National Health and Nutrition Examination Survey* of the U.S., also looking for possible associations between cognitive decline and OC pesticides. All OC compounds were inversely correlated with cognition scores. OPs or pyrethroids did not give rise to any correlation, which, according to the authors, may be attributed to the high lipophilicity of OC compounds, a trait that allows them to stay within the adipose tissue of an organism and be released in small quantities over time (Kim et al., 2015). In a similar vein, Lee et al. (2016) studied 989 people of 70 years or older enrolled in the *Prospective Investigation of the Vasculature in Uppsala Seniors*. According to the results, the measure of 3 OC pesticides after adjusting for weight change was a good predictor of cognitive impairment (Lee et al., 2016). In a large study conducted by Tang et al. (2016), 3,471 urban and 4,429 rural residents over the age of 50 were examined for risk factors pertaining to cognitive impairment, including exposure to pesticides, with an OR of 4.68 95 % CI: 1.27–17.21) (Tang et al., 2016). Similarly, Paul et al.'s (2018) study with a subset of 430 individuals over the age of 60 from the *Sacramento Area Latino Study on Aging* found that OP-exposed subjects exhibited faster cognitive decline rates in 3MSE and SENAS (Spanish English Neuropsychological Assessment Scales) tests when compared to the non-exposed group (Paul et al., 2018). Rohlman et al. (2007) studied 56 adolescents and 119 adults spraying OPs occupationally; adolescents scored higher than adults, while the profession duration (in years) was found to be positively correlated with deficits in cognitive performance tests(Rohlman et al., 2007). The overall data prompts us to think about the underlying dimensionality of the various factors, including age, years of exposure and pesticides, which may all act synergistically towards cognitive impairment.

Experimental evidence in favor of the multifaceted nature of cognitive impairment in an exposure context also derived from Sullivan' et al. (2018) study with 159 Gulf War veterans, who were exposed to pesticides or pyridostigmine bromide. High levels of exposure in both groups were associated with slower information-processing speed, visual memory and attention deficits, as well as mood deterioration. Crucially, the high-pesticide exposure group was found to perform worse than the pyridostigmine bromide exposure group on several cognitive tasks, with the worst level of performance being reported for the visual memory recall test (Sullivan et al., 2018). Finally, data from a population-based cohort study of older adults (HEllenic Longitudinal Investigation of Aging and Diet-HELIAD) in Greece, suggested that non-demented individuals who reported that they had been living in areas near sprayed fields, presented an overall poorer neuropsychological performance, particularly evident in language, executive and visual-spatial functioning, and attention (Dardiotis et al., 2019c).

Table 1 presents an overview of the selected and included studies that have so far investigated the relationship between pesticide exposure and various cognitive functions in humans.

Table 1
Selected studies examining the relationship between of pesticide exposure and cognitive functions in humans.

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
Daniell et al./ 1992/USA	Organophosphate insecticides	Motor speed Motor coordinationProcessing speed Memory	Finger tapping Hand-Eye Coordination Continuous Performance Symbol Digit Pattern Memory	Prospective longitudinal	49 apple orchard pesticide applicators and 40 controls – Initial evaluation and follow-up evaluation 1month after the end of spraying season	Questionnaire – occupational exposure + cholinesterase serum levels	No evidence of clinically significant adverse neuropsychological effects
Ames et al./1995/ USA	Organophosphate insecticides	Motor speed Attention Motor coordination Processing speed Memory	Finger Tapping Sustained Attention Hand-Eye Coordination Simple Reaction Time Symbol Digit Pattern Memory Serial Digit Learning	Cross-sectional	45 male subjects with history of moderate cholinesterase inhibition and 90 controls	Cholinesterase serum levels from previous reports	No evidence of chronic or long term neurologic sequelae
Stephens et al./ 1995/United Kingdom	Organophosphate insecticides	Short term memory Sustained attention Processing speed Long term memory	Digit Span Visual Spatial Memory Simple Reaction Time Symbol-Digit Substitution Synaptic Reasoning Category Search Serial Word Learning	Cross-sectional	146 sheep farmers exposed to organophosphates and 143 controls	Questionnaire – occupational exposure	Worse performance of sheep farmers on tests of sustained attention and processing speed – No group differences on short term memory and learning
Cole et al./1997/ Equador	Organophosphate and carbamate insecticides	Attention Visuospatial function Psychomotor function Motor function	Digit Span Benton Visual Retention Digit Symbol Simple Reaction Time Santa Anna Pursuit Aiming Test Digit Vigilance Trails A & B Block Design	Cross-sectional	144 farmers (23 consumers, 28 exposed, 123 applicators) and 72 controls	Questionnaire – occupational exposure + cholinesterase serum levels	Worse performance in the farmers' group
London et al./ 1997/South Africa	Organophosphate insecticides	Dexterity Clerical speed Visuospatial function Attention Motor Speed Apprehension Encoding Short-term memory Semantic access	Santa Anna Pursuit Aiming Digit Symbol Substitution Benton Visual Retention Digit Span Simple Reaction Time Inspection Time Reaction Time Continuous Number Checking Short-term Memory Scanning Manipulating Numbers I, II and III Animal Postures I and II Speaking Arrows Stimulus Resistance Pointing Arrows Echopraxis	Cross-sectional	163 fruit farmers and 84 controls	Questionnaire – occupational and non-occupational exposure + cholinesterase serum levels	Weak evidence of neurobehavioral effects
Calvert et al./ 1998/USA	Fumigants: methyl bromide and sulfuryl fluoride	Motor Coordination Processing speed	Hand-eye Coordination Simple Reaction Time Continuous	Cross-sectional	123 structural fumigation workers and 120 controls	Questionnaire – occupational exposure	Worse performance on specific cognitive functions (Pattern Memory Test and Santa Anna Dexterity Test)

(*continued on next page*)

Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
		Memory Psychomotor function	Performance Symbol Digit Pattern Memory Serial Digit Learning Santa Anna Dexterity				
Steenlandet al./ 2000/USA	Organophosphate insecticides: chlorpyriphos	Attention Processing Speed Memory Motor Coordination	Digit Span Continuous Performance Simple Reaction Time Symbol Digit Pattern Memory Hand-eye coordination	Cross-sectional	191 termiticide applicators and 189 controls	List of professional applicators by the State of North Carolina – occupational exposure + TCP levels in the urine	Non significant differences
Van Wendel et al./ 2001/Costa Rica	Dichlorodiphenyltrichloroethane (DDT)	Attention Processing Speed Visuomotor function Motor function	Digit Span Digit Vigilance Digit Symbol Trails Pursuit Aiming Simple Reaction Time Finger Tapping Santa Anna Dexterity Purdue Pegboard	Cross-sectional	36 retired malaria-control workers and 31 controls	Questionnaire – occupational exposure	Worse performance for DDT exposed workers
Jamal et al./ 2002/United Kingdom	Organophosphate insecticides	Psychomotor performance Learning Memory	Motor Screening Reaction Time Matching to sample visual search Pattern Recognition Spatial Recognition Paired Associate Learning Spatial Span Rey Auditory Verbal Learning	Case-control	79 sheep farmers	Questionnaire – occupational exposure	slowing down of processing speed – No evidence of memory impairment
Kamel et al./ 2003/USA	Pesticides	Processing speed Psychomotor function	Digit Span Symbol Digit Latency Tapping Santa Anna	Cross-sectional	288 farm workers and 51 controls	Questionnaire – occupational exposure	Worse performance on specific tests (Digit Span, Tapping, Santa Anna) and weak effect on others tests (Symbol Digit)
Salvi et al./2003/ Brazil	Organophosphate (OP) pesticides	Orientation Immediate and short term memory Naming Sequencing	Mini mental Word Span	Longitudinal	37 workers in agriculture of tobacco exposed to OP for 3 months – 25 of these workers after 3 months off OP exposure	Questionnaire – occupational exposure + Serum acetylcholinesterase	No influence of exposure to OP
Farahat et al./ 2003/Egypt	Organophosphorus (OP) pesticides	Verbal abstraction Visuomotorspeed Problem solving Attention Memory	Similarities Digit Symbol Trailmaking part A & B Block Design Paced Auditory Serial Addition Test (PASAT) Letter Cancellation Digit Span Benton Visual Retention Story Recall parts A & B	Cross-sectional	52 male workers in cotton crops and 50 controls	Questionnaire – occupational exposure + Serum acetylcholinesterase	Worse performance on specific tests (Similarities, Digit Symbol, Trailmaking part A & B, Letter Cancelation, Digit Span, Benton Visual Retention)
Stephens/2004/ United Kingdom	Organophosphates (OP)	Processing Speed Memory Verbal abilities	Simple Reaction Time Digit Span Symbol-Digit	Cross-sectional	37 orchard sprayers exposed to OP and 26 pig farm workers and 31	Questionnaire – occupational exposure	Slower performance on Synaptic Reasoning Test

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
			Substitution Synaptic Reasoning Location Recognition Category Search Serial Word Learning		construction workers not exposed to OP	+ DEP and DETP in the urine	
Roldan–Tapia et al./2005/ Spain	Organophosphates (OPs) and carbamates	Attention Reasoning Memory Perception Visuomotorskills Expressive language Motor performance	Stroop "A" Cancellation Letter Trail Making Digit Vigilance Picture Completion Similarities Digit Span Rey-Auditory Verbal Learning Rey-Osterreich Complex Figure Benton Visual Retention Logical Memory Benton Visual Form Discrimination Poppelreuter Block Design Boston Naming Luria	Cross-sectional	40 farm workers and 26 controls	Questionnaire – occupational exposure + Serum cholinesterase level	Worse performance on specific tests
Rothleinet al./ 2006/USA	Organophosphates	Psychomotor functions Processing speed Attention Memory	Finger Tapping Simple Reaction Time Progressive Ratio Symbol Digit Digit Span Selective Attention Serial Digit Learning Continuous Performance	Longitudinal	96 agricultural workers and 45 controls	DAP metabolites in the urine	Worse performance on the majority of tests
Rohlman et al./ 2007/USA	Pesticides	Response speed Coordination Coding Complex functioning Attention Memory Motivation Learning	Finger Tapping Symbol Digit Simple Reaction Time Digit Span Progressive Ratio Selective Attention Serial Digit Learning Continuous Performance Match-to-Sample Reversal Learning	Cross-sectional	119 adults and adolescents currently working in agriculture and 56 adults and adolescents not currently working in agriculture	Questionnaire – occupational exposure	Worse performance on specific tests (Digit Span, Match-to-Sample, Continuous Performance)
Ross et al./2010/ United Kingdom	Organophosphate pesticides (OPs)	Memory Response speed Mental flexibility Language Fine motor control Strategy making Reaction time Visuospatial skills	Wechsler Adult Intelligence Scale-III Wechsler Memory Scale-III Trail Making A& B Stroop Graded Naming Verbal fluency California	Cross-sectional	127 sheep farmers and 78 controls	Questionnaire – occupational exposure	Worse performance on specific tests (response speed, memory, mental flexibility, fine motor control, strategy making)

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
			Computerised Assessment Package				
Bayrami et al./ 2011/Iran	Organophosphate pesticides (OPs)	Orientation Registration Attention Calculation Recall Language Visual Construction	Mini Mental State Examination	Cross-sectional	40 horticulture farmers and 40 controls	Questionnaire – occupational exposure + Serum cholinesterase level	No significant group difference in cognitive function
Baldi et al./2011/ France	Pesticides	Orientation Registration Attention Calculation Recall Language Visual Construction Memory Processing speed	Mini Mental State Examination Wechsler Paired-Associates Benton Visual Retention Five words Trail Making Part A Isaacs Set Finger Tapping Stroop Wechsler Similarities	Prospective case control	614 workers classified as directly exposed, indirectly exposed or non-exposed – 4 year follow-up	Questionnaire – occupational exposure	Worse performance on tests
Steenland et al./ 2013/Costa Rica	Pesticides	Orientation Registration Attention Calculation Recall Language Visual Construction	Mini Mental State Examination		400 elderly subjects	Questionnaire – occupational exposure	Worse performance on tests
Berent et al./ 2014/USA	Chlorpyrifos (CPF)	General ability Attention/information processing Memory-visual Memory-verbal Problem solving Psychomotor	Wide Range Achievement Test-Third Edition Reaction Time Rapid Visual Information Processing Signal Detection Matching to Sample Visual Search Change Score in milliseconds Delayed Matching to Sample CogniSyst Story Recall Stockings of Cambridge Moves Motor Performance Series	Prospective longitudinal	53 CPF workers and 60 controls – Baseline and 1 year later evaluation	TCPy in the urine, plasma butyrylcholinesterase (BuChE) activity and red blood cell acetylcholinesterase activity (AChE)	No evidence for impaired neurobehavioral domains
Kim et al./2015/ USA	Organochlorine pesticides	Visuospatial and motor speed of processing	Digit-Symbol Substitution Test	Cross-sectional	644 elders	Serum organochlorine pesticides	p,p′-dichlorodiphenyltrichloroethane (DDT), p,p′-dichlorodiphenyldichloroethylene (DDE), trans-nonachlor, oxychlordane, heptachlor epoxide, and β-hexachlorocyclohexane showed statistically significant or marginally significant inverse associations with cognitive score after adjusting for covariates
Jamal et al./ 2016/India	Organophosphates		Subjective Neuro-cognition Inventory	Cross-sectional		Questionnaire – occupational exposure	neurocognitive impairment

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
		Attention Memory Psychomotor retardation			187 pesticide sprayers of mango plantation and 187 controls	+ serum acetylcholinesterase	
Lee at al/2016/ Sweden	Organochlorine (OC) pesticides	Overall cognitive function	Diagnostic criteria for AD/MCI	Prospective	989 men and women aged 70 years – Baseline examination and 10 year follow-up	Plasma concentrations of 3 OC pesticides	Increased risk of cognitive impairment (MCI and dementia)
Tang et al./2016/ China	Pesticides	Orientation Registration Attention Calculation Recall Language Visual Construction	Mini Mental State Examination	Cross-sectional	7900 individuals aged ≥50years (4429 from rural area and 3471 from urban area)	Questionnaire – occupational exposure	Increased risk of cognitive impairment (OR = 4.68 95 %CI 1.27–17.21)
Corral et al./ 2017/Chile	Organophosphate pesticides	Memory Executive functions Attention Language Visuoconstruction	Mini Mental State Examination Digit Span Rey-Osterrieth Complex Figure Stroop d2 Test of Attention Frontal Assessment Battery Semantin Verbal Fluency	Cross-sectional	32 agricultural workers directly exposed to pesticides, 32 individuals environmentally exposed to pesticides and 38 controls	Questionnaire – occupational exposure + Place of residence - environmental exposure	Higher cognitive deficits in both exposed groups
Paul et al./2018/ USA	Organophosphorus	Overall cognitive performance	Modified Mini Mental State Exam	Prospective cohort	430 persons aged ≥ 60 years – Baseline examination and every 12–15 months for up to seven study visits	Place of residence - environmental exposure	Faster cognitive decline for persons with high OP exposure
Sullivan et al./ 2018/Gulf War	Organophosphates Carbamates Organochlorines Pyrethroids DEET	Language Attention/executive domain Psychomotor domain Visuospatial domain Memory	Boston Naming Trail-making Computerized Continuous Performance Wisconsin Card Sorting Finger Tapping Grooved Pegboard Hooper Visual Organization Rey OsterriethComplex Figure Stanford-Binet Copying California Verbal Learning		159 Gulf War military pesticide applicators	Environmental exposure report by the Department of Defense and questionnaire - environmental exposure	Worse performance of persons with high pesticide exposure
Bosma et al./ 2000/ Netherlands	Pesticides	Main cognitive domains	Stroop Colour Word Verbal Learning Letter Digit Coding Word FLuency	Prospective cohort	830 non-demented people aged 50–80 years – Baseline examination and follow-up after 3 years	Questionnaire – occupational exposure	Increased risk of Mild Cognitive Dysfunction

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Table 1 (*continued*)

Authors/Year/ Country	Pesticides	Cognitive and Psychomotor Functions	Tests	Study Design	Sample Description	Exposure Assessment	Findings
Dardiotis et al./ 2019/Greece	Pesticides	Main cognitive domains and overall performance	composite, memory, executive, visual-spatial, language and attention z-scores	Cross sectional analysis	1,397 non-demented (1,218 cognitively healthy individuals and 179 individuals with MCI)	Self-reported-pesticides exposure	Non-demented individuals who reported that they had been living in areas near sprayed fields, had poorer neuropsychological performance. The poorer performance was evident in language, executive and visual-spatial functioning, and attention. These associations remained after excluding subjects with MCI.

4.2. Pesticides and dementia

Dementia refers to an acquired objective cognitive or behavioral impairment that represents a decline from a previous level of performance that interferes with daily function (McKhann et al., 2011). Dementia is an umbrella term that covers many types of severe cognitive impairment, including AD, vascular dementia (VaD), frontotemporal dementia (FTD) and Lewy body dementia (LBD). The symptoms of the different dementias partly overlap and partly differentiate one another; however, the diagnosis of dementia is not easy, as many concomitant diseases and atypical symptoms may exist, such as psychosis (Dardiotis et al., 2019a; Nussbaum et al., 2017; Rayner et al., 2006; Siokas et al., 2018; Tsatsakis et al., 2019a). Unfortunately, many of the epidemiological studies that have investigated the association between pesticide exposure and cognitive impairment have focused on a general diagnosis of dementia without making a distinction between its different phenotypes, or the different pesticides involved. Here, we have included 18 studies, spanning from 1984 to 2015.

Occupational exposure to unspecified pesticideshas been associated with increased risk of all-cause dementia [Hazard Ratio (HR) = 1.38 and 95 % Confidence Interval (95 %CI) = 1.09–1.76)] in the Cache County Study (Hayden et al., 2010). This prospective cohort study enrolled 3,084 residents of the agricultural community of Cache County, UT, aged 65 years and above without dementia, and assessed their cognitive status at baseline and after 3, 7 and 10 years. The participants completed detailed questionnaires and after adjustments for various factors, an increased risk of dementia was shown for the exposed group. The study also evaluated the risk of a specific type of dementia, namely AD, in late life after occupational pesticide exposure and found a similar association (HR = 1.42 and 95 %CI 1.06–1.91) (Hayden et al., 2010). In another study, Povey et al. (2014) examined the effects of pesticides, otherwise unspecified, on the incidence of neuropsychiatric disorders in British sheep farmers (Povey et al., 2014). The results showed no association between dementia and low dose chronic exposure -defined as ever handling a pesticide concentrate. However, the outcome was different for the participants who sought advice for pesticide poisoning. In the latter, acute exposure showed a considerable association with dementia [Odds Ratio (OR) = 6.94 and 95 %CI = 3.44–14.00] (Povey et al., 2014). The Prospective Netherlands Cohort Study by Koeman et al. (2015) investigated the effects of various occupational factors, pesticides included, on non-vascular dementia (Koeman et al., 2015). Unexpectedly, the authors found an inverse association between pesticides and non-vascular dementia among men. A possible explanation for this oddity was the healthier living habits of farmers, who were however exposed to higher levels of pesticides (Koeman et al., 2015). Finally, a population-based case-control study in Taiwan compared patients with a history of acute OP or carbamate poisoning with age- and sex-matched controls, and found a hazard risk of 1.98 (95 % CI = 1.59–2.47) for all-cause dementia for the exposed subjects (Lin et al., 2015). However, in a follow up analysis of the data stemming from the Canadian Study of Health and Aging, Medehouenou et al. (2014) measured polychlorinated biphenyls (PCBs) and OCs plasma concentrations in 2023 participants and after adjusting for several factors such as ApoE4 allele, age, and sex, found no, or an inverse association between the measured concentrations and dementia; higher concentrations of some PBC and OC metabolites were tied to a reduced prevalence of dementia [e.g.: PCB105: OR = 0.87, 95 % CI = 0.77–0.99; PCB 118:OR = 0.86, 95 % CI = 0.74–0.99, HCB (OC) partially adjusted OR = 0.85, 95 % CI = 0.75–0.97; fully adjusted OR = 0.87, 95 % CI = 0.77–0.99, p,p′-DDT (OC) partially adjusted OR = 0.82, 95 % CI = 0.72–0.93; fully adjusted OR = 0.84, 95 % CI = 0.74–0.96], and the majority showed no higher or lower risk (Medehouenou et al., 2014).

The most common form of dementia is AD. AD patients typically exhibit gradually progressive cognitive and behavioral impairment with functional decline. AD has been extensively reported to affect many,

and common symptoms include deficits in short term memory, language processing, and executive functions (Apostolova, 2016). Several epidemiological studies have examined the relationship between pesticide exposure and risk of AD with conflicting results. There are four case-control studies, three prospective cohort studies and one ecological study that have so far focused on the aforementioned association, from 1985 until 2011. Three of the case-control studies showed no association between pesticide exposure and AD, while the fourth found increased risk of AD. More specifically, French et al. (1985) obtained information from 78 male patients with AD and 76 controls in order to assess various possible risk factors for AD, and observed a much commoner occurrence of head trauma among patients (OR = 4.5, Confidence Limits = 1.44–15.69), but no difference in occupational exposure of any kind, including unspecified pesticides (OR = 0.8, Confidence Limits = 0.29–2.19) (French et al., 1985). These findings have been replicated by Gun et al. (1997) in Australia, as well as by Gauthier et al. (2001) in Canada (Gauthier et al., 2001; Gun et al., 1997), whereby no relation was reported between generic pesticide exposure and risk of AD. On the contrary, the Canadian Study of Health and Aging, (1994) designed to identify risk factors for AD (among other purposes) found an increased risk of AD tied to occupational exposure to unspecified pesticides (OR = 2.17 and 95 %CI = 1.18–3.99) after examining 258 patients with probable AD of recent onset and 535 controls. The risk also seemed to be higher for less educated subjects (The Canadian Study of Health and Aging, 1994).

Considerably different results were yielded by three prospective cohort studies, spanning from 2001 to 2010. Besides the Cache County Study by Hayden et al. (2010) mentioned above, a population-based longitudinal study by Tyas et al. (2001) in Canada analyzed the data of 694 subjects who were cognitively intact on baseline examination and for whom cognitive status assessment was obtained after 5 years (Hayden et al., 2010; Tyas et al., 2001). On this follow-up examination, 36 subjects were diagnosed with AD. The study found an increased risk of AD associated with occupational exposure to fumigants/defoliants [Risk Ratio (RR) = 4.35 and 95 %CI 1.05–17.90]. However, the increased risk was found lower, though still somewhat elevated for pesticides/fertilizers as a whole (RR = 1.45 and 95 %CI = 0.57–3.68) (Tyas et al., 2001). The PAQUID Study (2003) in France, on the other hand, was a large prospective cohort study comprised of 1507 elderly participants aged ≥65 years, who were followed-up for 5 and 10 years (Helmer et al., 2006; Ramaroson et al., 2003). Questionnaires with detailed occupational background questions were used to collect information on participants 'occupational exposure to pesticides, while the place of residence was manipulated as an index of environmental exposure. The authors analyzed 96 cases with AD during the follow-up session and found a strong association between occupational exposure and AD in men (RR = 2.39 and 95 %CI = 1.02–5.63). No association was revealed for women, which was partially explained by the fact that pesticides are mixed and mainly applied by men; no major association was found for environmental exposure either (Baldi et al., 2003). Finally, Parron et al. (2011) conducted an ecological study in Spain in order to examine whether environmental pesticide exposure was associated with various neuropsychiatric conditions (Parron et al., 2011). The study population included 17,429 patients who were recruited from hospital records. Environmental exposure in Parron et al.'s (2011) study was deemed high or low based on selected geographical areas. The analyses showed an increased risk of AD in populations living in districts with high generic pesticide use (OR = 2.10 and 95 %CI = 1.96–2.25) (Parron et al., 2011). In general, when examining etiological relationships between risk factors and AD or more generally any chronic disease, cohort studies are preferable to case-control studies, due to the absence of recall bias (Yan et al., 2016). However, the well conducted case-control studies, with excellent exposure assessment, and particularly cohort-nested case-control studies can still be more reliable, particularly when compared to the limited reliability of the evidence yielded by the ecologic studies.

Possible associations of pesticide exposure with the other, less common forms of dementia have been less explored. Vascular dementia (VaD), the second commonest form of the disease (Lin et al., 2015), is diagnosed on the basis of cognitive impairment with vascular contribution manifested by history, physical examination, cognitive tests and neuroimaging data (Smith, 2016). Various risk factors for VaD were studied in a follow up publication from Lindsay et al., (1997) mentioned before, which recruited 129 VaD patients of recent onset and 535 controls. The diagnosis of VaD was made on the basis of the ICD-10 criteria as well as on clinical grounds without routine neuroimaging. According to the study's findings, occupational exposure to unspecified pesticides and fertilizers was elevated for the VaD patients only (OR = 2.60, 95 %CI = 1.30–5.23) (Lindsay et al., 1997). Results were similar in Hebert et al.'s (2000) study, based on the second phase of the Canadian Study of Health and Aging. The DSM-IV and NINDS-AIREN criteria were used to identify VaD patients and all subjects underwent a CT scan. Analysis of the data of 105 VaD patients and 802 controls revealed that exposure to pesticides or fertilizers was an important risk factor for VaD (OR = 2.05, 95 %CI = 1.03–3.85) (Hebert et al., 2000).

Frontotemporal dementia (FTD) is a progressive neurodegenerative disorder mainly characterized by behavioral changes and/or language deficits, usually affecting adults in their fifties to sixties. There are several clinical subtypes of FTD, including behavioral variant FTD, semantic, non-fluent/agrammatic and logopenic-variant primary progressive aphasia, as well as FTD associated with motor neuron disease (MND) (Finger, 2016). A study on the medical and environmental risk factors for FTD including pesticides in general was carried out in Netherlands by Rosso et al. (2003). This case-control study included 80 FTD patients and 124 controls matched on age, sex and surrogate informant relations. The study found no association between exposure to pesticides and sporadic FTD. The only major risk factor identified was head trauma. It follows that more studies, including prospective cohort studies, are needed to shed more light on potential relationships between exposure to pesticides, either occupational or environmental, and FTD. It would be particularly interesting to examine possible associations between FTD and MND, given the fact that numerous epidemiological studies have found that the risk of MND, of ALS in particular, is associated with pesticide use (Kamel et al., 2012; Vinceti et al., 2012, 2017).

Lewy Body Dementia (LBD) includes both Parkinson Disease Dementia (PDD) and Dementia with Lewy Bodies (DLB). There is a clinicopathologic overlap between these two entities, alongside several differences. PDD occurs after an established diagnosis of Parkinson's disease, while in DLB, parkinsonian signs develop with or after the presence of dementia (Armstrong, 2019). A case-control study by Hubble et al. (1998) in Kansas provided evidence in favor of a genetoxin interaction as a risk factor for PDD (Hubble et al., 1998). The analysis of the data of 43 PDD patients and 51 PD patients without dementia showed that subjects exposed to unspecified pesticides and carrying a susceptibility-for-PDD allele had a predicted probability of PDD reaching 83 % (Hubble et al., 1998). Review of relevant literature indicates a relationship between pesticides and PD (Brown et al., 2006; Freire and Koifman, 2012), that may be influenced by genetic factors, implying an increased risk of developing PD after exposure to pesticides, for genetically susceptible people (Dardiotis et al., 2013). This association suggests that potential links may exist between pesticides and LBD as well, since PD and LBD share similar pathophysiological and clinical characteristics (Zaganas et al., 2013).

All the aforementioned epidemiological studies assessed exposure to pesticides either by using questionnaires and/or the place of residence in order to clarify occupational and/or environmental exposure, respectively. Few case-control studies were based on biomarkers of exposure, i.e. have examined serum pesticide levels in AD patients and controls. The study by Singh et al. (2013) in India, for example, measured serum levels of various OCs in 70 patients with AD and 75 controls, and found significantly increased levels of β-

Table 2
Selected studies examining the relationship between of pesticide exposure and risk of dementia in humans.

Authors/Year/Country	Clinical Entity	Study Design	Sample Description	Exposure Assessment	Adjustments - Matching	Findings
Hayden et al./ 2010/ USA	Dementia and AD	Prospective cohort	3084 non-demented people ≥65 years – Baseline examination and follow up evaluations after 3, 7, 10 years	Questionnaire – occupational exposure	Adjusted for age, sex, education, baseline MMSE score, APOE ε4 status	Increased risk of all-cause dementia (HR:1.38 95 %CI 1.09–1.76) Increased risk of AD (HR:1.42 95 %CI 1.06–1.91)
Povey et al./ 2014/ Great Britain	Dementia	Cross-sectional	1350 British sheep farmers who were ≥13 years in 1970	Questionnaire – occupational exposure [Acute exposure was defined as ever seeking advice for pesticide poisoning – Low dose chronic exposure was defined as ever handling the pesticide concentrate]	Adjusted for age, sex, region, ever smoker, lambing, slaughtered livestock, somatic score	No association between dementia and ever handling the pesticide concentrate (OR = 1.18 95 %CI 0.61–2.29) Increased risk of dementia in those ever seeking advice for pesticide poisoning (OR = 4.27 95 %CI 1.85–9.83)
Koeman et al./ 2015/ Netherlands	Dementia (non-vascular)	Case cohort	682 males and 870 females who had died with non-VaD out of 120.852 subjects who had enrolled in the Netherlands Cohort Study in 1986	Baseline questionnaire in 1986 - occupational exposure	Adjusted for age, smoking, physical activity, BMI	Inverse association of non-vascular dementia among men (HR = 0.61 95 %CI 0.40 – 0.93)
French et al./1984/ USA	AD	Case control	78 male AD patients and 76 controls	Questionnaire – occupational exposure	Matching by age, sex, race	non-statistically significant differences between AD patients and controls (OR = 0.8 95 %CI 0.29–2.19)
Canadian Study of Health and Aging (CSHA)/ 1994/Canada	AD	Case control	258 AD patients and 535 controls	Questionnaire – occupational exposure	Adjusted for age, sex, education, residence	Increased risk of AD (OR = 2.17 95 %CI 1.18–3.99) – the increased risk was greater in participants with low education level
Gun et al./1997/ Australia	AD	Case control	170 AD patients and 170 controls	Questionnaire – occupational exposure	Matching by age, sex	non-statistically significant differences between AD patients and controls (OR = 2.54 95 %CI 0.41–27.06)
Gauthier et al./2001/ Canada	AD	Case control	67 AD patients and 67 controls	Residential history - environmental exposure + Questionnaire – occupational exposure	Matching by age, sex - Adjusted for education level, family cases, APOE ε4 allele	Non-statistically significant differences between AD patients and controls (OR = 0.97 95 %CI 0.38–2.41)
Tyas et al./2001/Canada	AD	Prospective cohort	694 cognitively intact subjects – Baseline examination and follow up evaluation after 5 years	Questionnaire – occupational exposure	Adjusted for age, education, sex	Increased risk of AD after exposure to fumigants/defoliants (RR = 4.35 95 %CI 1.05–17.90) – non- statistically significant association with AD after exposure to pesticides (RR = 1.45 95%CI 0.57–3.68)
Baldiet al./2003/France (PAQUID Study)	AD	Prospective cohort	1507 French elderly ≥65 years – Baseline examination and follow-up evalutaion after 5, 10 years	Questionnaire – occupational exposure + Place of residence - environmental exposure	Adjusted for age, smoking, education	Increased risk of AD in men (RR = 2.39 95 %CI 1.02–5.63)
Parron et al./2011/Spain	AD	Ecological	17.429 patients diagnosed with various neuropsychiatric disorders, including AD	Different selected areas -environmental exposure	Adusted for age, gender	Increased risk of AD (OR = 2.10 95 %CI 1.96–2.25)
Lindsay et al./1997/Canada (Canadian Study of Health and Aging, CSHA)	VaD	Case control	129 VaD patients and 535 controls	Questionnaire – occupational exposure	Adjusted for age, sex, education, residence in community or institution	Increased risk of VaD (OR = 2.60 95 %CI 1.30–5.23)
Hebert et al./2000/Canada (Canadian Study of Health and Aging, CSHA)	VaD	Prospective case control	105 VaD patients and 802 controls	Questionnaire – occupational exposure	Adjusted for age, sex, education, region	Increased risk of VaD (OR = 2.05 95 %CI 1.03–3.85)
Rosso et al./2003/ Netherlands	FTD	Case control	80 FTD patients and 124 controls	Questionnaire – occupational exposure	Adjusted for age, sex, surrogate informant relationship	No association with FTD
Hubble et al./1998/USA	PDD	Case control	43 PD patients with dementia and 51 PD patients without dementia	Questionnaire – occupational exposure		Increased risk of PDD in combination with a genetic trait (CYP 2D6 29B + allele) (OR = 3.17 95 %CI 1.11–9.05)
Singh et al./2012/India	AD	Case control	70 AD patients and 75 controls	Serum levels		Increased levels of β-HCH, dieldrin and pp'-DDE in AD patients

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Table 2 (*continued*)

Authors/Year/Country	Clinical Entity	Study Design	Sample Description	Exposure Assessment	Adjustments - Matching	Findings
Richardson et al./2014/USA	AD	Case control	86 AD patients and 79 controls	Serum levels	Adjusted for age, sex, race/ethnicity, education, APOE genotype, location	Increased levels of DDE in patients with AD
Medehouenou et al./2014/ Canada (Canadian Study of Health and Aging, CSHA)	Dementia and AD	Cross-sectional	2,023 participants, 574 with dementia, 399 with AD in particular	Polychlorinated biphenyls and OCs plasma concentrations	Adjusted for blood collection period, total plasma lipids, age, sex, education, apolipoprotein E e4 allele, tobacco and alcohol use, rural/urban residence, and comorbidities	Elevated plasma PCB and OC pesticide concentrations were not associated with higher prevalenceof all-cause dementia and AD
Lin et al./2015/Taiwan	Dementia	Case control	9,616 patients hospitalized for acute OP and CM poisoning and 38,510 controls	Hospital records	Adjusted for age, sex and year of hospitalization	1.98-fold increased risk of dementia compared with the control cohort (95 % CI = 1.59–2.47)

hexachlorocyclohexane (β-HCH), dieldrin and pp'-dichlorodiphenyldichloroethylene (pp'-DDE) in the patients (Singh et al., 2013). Richardson et al. (2014) also found elevated levels of dichlorodiphenyldichloroethylene (DDE) in the serum of patients with AD, thus supporting the reliability of epidemiological studies that show increased risk of AD after pesticide exposure (Richardson et al., 2014). Table 2 presents an overview of the included studies that have so far investigated the relationship between pesticide exposure and risk of dementia in humans.

5. Discussion

The research on the intricate mechanisms and the relations between pesticides and cognition is far from being complete. Considering that many studies have indeed reported an association between exposure and cognitive impairment, it would probably be naïve to assume that they were all mere coincidences and that chronic exposure to deeply harmful substances such as pesticides leaves the brain unscathed.

However, there are many reasons which may explain why researchers could not reach a consensus on this issue so far. First of all, a sadly big number of studies did not assess exposure to specific substances, pesticide categories, or compounds. In many occasions, exposure to and application of pesticides are assessed via questionnaires and personal interviews, where the subjects cannot name the exact compounds, or are heterogeneous, and no specific pesticide class can be pinpointed. It has also been challenging to reliably quantify exposure levels, as few biomarkers exist, and many of them do not accurately depict chronic exposure; they may have short half-lives, or different pathways may be activated in acute and chronic exposure instances. Animal studies have shown that prolonged exposure to low-doses of OPs that do not produce AChE inhibition or clinical symptoms of OPs intoxication leads to inflammatory responses that can in turn lead to neuronal damage (Chapman et al., 2006; Collombet et al., 2005; Henderson et al., 2002; Lim et al., 2011). It is speculated that OP-induced inflammation has a big impact on the pathogenesis of OP neurotoxicity so new biomarkers of inflammation used in epidemiological and biomonitoring studies could help for a better evaluation of OPs impact on cognitive function and risk of dementia.T he methods of assessment tend to also be inherently fairly unreliable and subject to bias, for example recall bias in the case of questionnaires (Tsatsakis et al., 2016, 2018), and a relatively small number of studies included biochemical measurements, such pesticide levels or their respective biomarkers, for example the bio-scavenger butyrylcholinesterase (BChE) in the case of OPs. Additionally, the populations under study exhibited broad heterogeneity in terms of exposure, extending from workers directly and indirectly exposed, to unexposed subjects; population heterogeneity has also been further broadened by differences in ethnicity. There should be a consensus between the studies analyzed regarding what counts as chronic exposure and if the period under study is relevant or sufficient for developing the neurological toxic effects. Sometimes the delayed neuropathies can manifest outside of the timeline of the study. For example, the study of Daniel et al. (Daniell et al., 1992), that could not show any evidence of clinically significant adverse neuropsychological effects, evaluated the pesticide applicators before the spraying season and at 1 month after the end of the spraying system, while in the studies that showed a significant correlation between worse neurological performance and pesticide exposure, the follow-up period was usually more than 1 year (Baldi et al., 2011, 2003; Berent et al., 2014; Bosma et al., 2000; Helmer et al., 2006; Lee et al., 2016; Tyas et al., 2001). In fact, the studies that reported a positive association, when compared to those that did not, seemed to include larger populations and larger periods of time, ranging from months to even 10 years. Conclusively, besides determining what chronic exposure means, specific cut-off points in the timeframes of the studies should be established, for proper comparisons to be feasible in the future; is one month after the pesticide applying period enough? Should

subjects be studied at least 3 months or even years after acute/occupational exposure? How many years of inhabiting a high-exposure area are deemed enough for a subject to be named “chronically exposed to low doses”? All these questions should be addressed if the scientific community wishes to reach dependable conclusions.

Confounding factors also play a crucial role in assessing risk and associations. Firstly, polymorphisms in the genes involved in pesticide metabolism can also lead to different response after exposure to the same level of pesticides. For instance, regarding OPs, mutations in the genes of BCHE, cytochrome P450 enzymes (CYP2D6, CYP2C19, CYP3A), paraoxonase-1 (PON1) and glutathione S-transferase (GSTM1, GSTT1) have been shown to be involved in increased risk after exposure to OP pesticides (Howard et al., 2010). Consequently, further studies that analyze the correlation between genetic variability in genes associated with enzymes implicated in pesticide metabolism, can better simulate the real life cases. It would also be beneficial for studies to incorporate adjustments for risk factors proven to associate with cognitive deficits, such as head trauma. As can be seen, in most cases, controls and cases were age- and sex- matched, while few further adjustments were made. It is also important to address whether subjects had a history of acute poisoning when handling the subject of pesticides, since some studies did show a difference between those who had and those who did not (Lin et al., 2015; Povey et al., 2014). One study also addressed the matter of adequate self-protection (Jamal et al., 2016); this would have been particularly interesting if more studies had included such a section. It is possible that the extent of the protective measures’ application may heavily vary from country to country and from field of work to field of work, and so discrepancies in the results may result from this factor as well.

Study designs rarely presented homogeneity either, so drawing clear conclusions from the available studies still remains difficult. Moreover, the estimation of the exposure to pesticides has been most frequently performed without measuring the synergic effects and the possible interactions between the toxicants within mixtures, and also overlooking low exposures to environmental toxicants (Docea et al., 2018; Tsatsakis et al., 2019b, 2016; Tsatsakis et al., 2017). Exposure to such substances known to affect cognitive function could also occur alongside pesticide exposure, and influence the results. One such example is lead, with studies suggesting that it aggravates AD (Wu et al., 2020; Zhou et al., 2018), so adjustments for potential exposure confounders are crucial in drawing accurate results. It is possible that a Real-Life Risk Simulation approach may represent a robust alternative in order for the safe exposure limits and the net risk that pesticides confer to impaired cognitive function to be examined (Hernandez and Tsatsakis, 2017; Kostoff et al., 2018; Tsatsakis et al., 2017). Previous studies that evaluated the effect of low dose chronic exposure to mixtures of pesticides and other chemicals with the intention of simulating real life exposure scenarios showed that hermetic neurobehavioral effects can appear after mixture exposure at doses considered safe for individual compounds and these effects can be exacerbated by the coexistence with conditions such as vitamin deficiency (Tsatsakis et al., 2019c, d). Finally, some study limitations, such as inadequate size with consequent lack of statistical precision and selection bias particularly in case-control studies, markedly affected the epidemiologic studies, thus hindering the possibility to adequately assess the effect of these compounds on cognitive function in humans.

Another point that arises when one wants to answer the question of whether pesticides can cause cognitive deficits, is the question of what happens with the general population. Most studies have examined groups of people that have been exposed, such as pesticide applicators. However, a few studies did involve people indirectly exposed via their occupation and did show a degree of cognitive impairment, while the study of Parron et al. (2011), for instance, did not even assess occupational exposure; they only used geographical areas with higher pesticide use and found a higher risk for AD (Parron et al., 2011). It is no news that the general population becomes exposed to pesticides via water, nutrition and other factors (Murphy et al., 1983), and that pesticides reach groups not traditionally linked to exposure in a variety of ways; for example, the levels of OC pesticides were found higher than the European Union highest allowed level in Nigerian personal care products (Adekunle et al., 2018). Population-based studies assessing pesticide exposure in general are few and far between, such as the aforementioned HELIAD study, where individuals with some degree of environmental exposure to pesticides showed poorer cognitive performance (Dardiotis et al., 2019c). It seems that if we are to accept that pesticides do in fact affect cognition in directly exposed individuals, the general population is not “safe” either, since exposure is so widespread and low doses seem to suffice in producing chronic sequelae without ever needing to surpass the levels of acute poisoning.

Nonetheless, there seems to be enough scientific evidence to support the existence of adverse effects of pesticides on individuals’ mental condition. Although pesticide exposure may not be the sole factor involved in dementia and cognitive impairmentin general, it may be enough to trigger the process, often in combination with other factors such as genetic polymorphisms and other toxicants, as it promotes phenomena such as oxidative stress. Therefore, we think it is safe to assume that modifying pesticide use may protect humans against dementia. Such perspective, of course, needs to be further explored and validated by future studies. In the next step, further studies with larger cohorts of participants, comparable study-designs and a Real-Life Risk Simulation approach are needed to better elucidate the relationship between pesticides and cognitive impairment. Special attention should be paid to identifying the specific pesticide categories most detrimental to cognition, as well as the toxicological mechanisms through which they act.

6. Conclusions

Given the complicity of the matter, the relationship between pesticides and cognitive impairment seems particularly tricky to decipher. There is a plethora of studies available, either on animals or involving humans, which have attempted to shed light on this subject, but their results are considerably heterogeneous. It is not to wonder, however, since the study designs and the parameters in hand are completely different from study to study and many aspects remain underexplored. The evidence seems to hint towards the existence of an association between pesticides and cognitive impairment but in order for this association to be confirmed, future research groups should try to follow similar designs, addressing the points analyzed above. Absolute proof of this association could have a tremendous effect in preventing dementia, at least in part, via adequate self-protection measures or specific compound restriction.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Ann Ist Super Sanità 2010 | Vol. 46, No. 3: 284-287
DOI: 10.4415/ANN_10_03_10

Exposure to pesticides and risk of amyotrophic lateral sclerosis: a population-based case-control study

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Summary. A few epidemiologic studies have suggested an association of agricultural work and pesticides exposure with a severe degenerative disease of the motor neurons, amyotrophic lateral sclerosis (ALS), though conflicting results have also been provided. We investigated through a population-based case-control study the possible relation between overall occupational exposure to pesticides and ALS risk in the northern Italy municipality of Reggio Emilia. By administering a questionnaire, we investigated occupational history and leisure-time habits of the 41 ALS patients diagnosed in the 1995-2006 period, and of 82 age- and sex-matched randomly sampled population controls. More cases than controls were found to have been exposed to pesticides for at least six months (31.7% *vs* 13.4%, respectively), in all cases within the occupational environment. In a conditional logistic regression model, we found an excess ALS risk associated with exposure to pesticides, with a relative risk of 3.6 (95% confidence interval 1.2-10.5). Such association persisted after inclusion in the statistical analysis of potential confounders. Despite the limited statistical stability of the risk estimates, these results appear to indicate that occupational exposure to pesticides is a risk factor for ALS, suggesting the need to further investigate this issue.

Key words: amyotrophic lateral sclerosis, pesticides, epidemiology, case-control studies.

Riassunto *(Esposizione a pesticidi e rischio di sclerosi laterale amiotrofica: uno studio caso-controllo di popolazione)*. Da studi epidemiologici recenti è emersa, sebbene con risultati talvolta contrastanti, una possibile correlazione tra l'esposizione complessiva a pesticidi in ambito occupazionale e il rischio di sclerosi laterale amiotrofica (SLA). Abbiamo studiato questa ipotesi attraverso uno studio caso-controllo nella popolazione del Comune di Reggio Emilia. Abbiamo identificato i 41 nuovi casi di SLA diagnosticati nel periodo 1995-2006 e selezionato nella popolazione generale 82 controlli, appaiati per sesso e età ai pazienti. I soggetti inclusi nell'indagine (o uno stretto familiare quando non altrimenti possibile) hanno compilato un questionario sull'attività professionale e su alcuni fattori dello stile di vita. I pazienti sono risultati caratterizzati da una maggiore esposizione a pesticidi in ambito professionale rispetto ai controlli (rispettivamente 31,7% e 13,4%). Il rischio relativo di SLA associato all'esposizione a pesticidi è risultato pari a 3,6 (intervallo di confidenza al 95% 1,2-10,5), permanendo dopo aggiustamento per alcuni possibili fattori confondenti. Questi risultati suggeriscono nel complesso, nonostante la limitata stabilità statistica delle stime di rischio, una correlazione tra SLA ed esposizione professionale a pesticidi.

Parole chiave: sclerosi laterale amiotrofica, pesticidi, epidemiologia, studi caso-controllo.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare and severe neurodegenerative disease, occurring in sporadic and familial forms, whose etiology is still unknown [1].

Many genetic variants have been investigated as possible risk factors for the familial form, but results are still conflicting [2], while sporadic ALS is considered to be a multifactorial disease with different

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environmental and genetic risk factors suspected to contribute to motor neuron degeneration [3-5].

The association between ALS and exposure to neurotoxic chemicals, such as solvents, some pesticides categories and a few metals and metalloids, has been investigated in several epidemiologic studies with inconsistent results [6-8]; the difficulty in attaining a high level of evidence has been attributed to methodological factors such as inadequate exposure assessment [6]. However, several studies have suggested a relation between exposure to pesticides and ALS risk, and biological plausibility of such association is provided by the well-known neurotoxicity of several of these compounds [8-15].

We investigated through a population-based case-control study the possible relation between occupational exposure to pesticides and ALS risk in an Italian population.

METHODS

We aimed at identifying all residents in the northern Italy municipality of Reggio Emilia (around 150 000 inhabitants) who received a first-time diagnosis of ALS during 1995-2006 period, and provided that they had been resident in the municipality for at least six months, using a methodological approach that has already been described in detail elsewhere [16]. Briefly, we reviewed the hospital discharge register of the Emilia Romagna Region for both inpatients and outpatients of public and private hospitals from 1995 to 2006, as well as the death certificates from 1996 to 2007, and the prescriptions of a drug specific for ALS, riluzole. After having identified the potential cases of the disease, we ascertained the exact diagnosis by reviewing the hospital clinical records when available, or we contacted the general practitioners in case of uncertain diagnosis or missing data. Only the 41 patients fulfilling the El Escorial diagnostic criteria for probable or definite ALS [17], and residing in the Reggio Emilia municipality at the time of diagnosis, were included.

We extracted a group of matched controls from the general population of Reggio Emilia, identifiable through annual directories of residents made available by the General Registry Office of the Region. Using the calendar-year specific file of municipal residents corresponding to the year of diagnosis for each case, we randomly selected two controls matched to the case for year of birth and sex. We administered a questionnaire to cases and controls or, for the deceased cases (39) and controls (13), to the closest available relative, generally the marital/cohabiting partner or a son/daughter. All cases/relatives but one agreed to participate, whilst 11 of the initially sampled 82 controls could not be enrolled, and were replaced by other controls. We collected information about occupational history, and we considered the subject as exposed to pesticides when he/she had been involved in agricultural work and other pesticide-related professional activities for at least six months. We also ascertained occupational exposures to industrial chemicals and magnetic fields, antecedent sources of drinking water, dietary habits, smoking, coffee consumption, physical activity, and history of trauma. Moreover, we collected information about family history of ALS in first-degree relatives, residential history, and educational attainment level, and we checked residential history information reported by the subject against the files of the Municipal Registry Office, also to eventually assess residential exposure to magnetic fields from high-voltage power lines [18].

We estimated the relative risk (RR) of ALS associated with pesticides exposure from odds ratios estimated from conditional logistic regression bivariate and multivariate models, using the statistical package STATA version 10.1 (Stata Corp., TX, 2009).

RESULTS

Forty-one ALS patients and eighty-two age- and sex-matched randomly sampled population controls were eventually enrolled in the study. No occurrence of ALS was reported among the first degree relatives of the cases.

More cases (13/41) than controls (11/82) were found to have been occupationally exposed to pesticides for at least six months in their life (31.7% versus 13.4%, respectively), in all cases due to agricultural work activities. The number of exposed subjects was 10 among cases (33.3%) and 8 among controls (13.3%) in males, while in females 3 cases (27.3%) and 3 controls (13.6%) had been exposed.

In a conditional logistic regression model, we found an excess ALS risk associated with pesticides exposure, with an odds ratio of 3.6 and a 95% confidence interval of 1.2-10.5. Such association persisted after inclusion in the statistical analysis of potential confounders, such as educational attainment level, exposure to chemicals, and residential and occupational exposure to magnetic fields *(Table 1)*.

In sex-specific analysis, the association between pesticide exposure and ALS risk was present both

Table 1 | *Relative risk (RR) with 95% confidence interval (CI) of amyotrophic lateral sclerosis according to occupational exposure to pesticides in conditional logistic regression models, Reggio Emilia municipality, Northern Italy, 1995-2006*

Model	RR	95% CI
Crude	3.6	1.2-10.5
Multivariate – 1[a]	5.3	1.6-17.1
Multivariate – 2[b]	3.3	1.1-9.6
Multivariate – 3[c]	3.7	1.3-11.1
Multivariate – 4[d]	4.7	1.4-15.5

[a] Adjusting for educational attainment level. [b] Adjusting for occupational and residential exposure to magnetic fields. [c] Adjusting for exposure to chemicals. [d] Adjusting for all potential confounders (educational attainment level, exposure to chemicals and magnetic fields).

Table 2 | *Relative risk (RR) with 95% confidence interval (CI) of amyotrophic lateral sclerosis related to occupational pesticides exposure according to sex and age, Reggio Emilia municipality, Northern Italy, 1995-2006*

Model	Sex				Age			
	Males		Females		< 68 yrs		> 68 yrs	
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Crude	4.4	1.2-16.7	2.4	0.4-15.0	2.4	0.4-15.0	4.4	1.2-16.7
Multivariate – 1[a]	6.8	1.5-30.1	3.1	0.4-22.4	4.8	0.6-39.1	5.5	1.3-22.9
Multivariate – 2[b]	3.7	1.0-14.4	2.2	0.3-15.4	6.0	0.6-58.2	3.9	1.0-15.1
Multivariate – 3[c]	4.7	1.2-18.8	2.4	0.4-15.0	2.1	0.3-13.0	6.7	1.3-35.8
Multivariate – 4[d]	5.3	1.2-23.9	3.0	0.4-25.7	2.4	0.4-15.5	6.2	1.2-32.7

[a] Adjusting for educational attainment level. [b] Adjusting for occupational and residential exposure to magnetic fields. [c] Adjusting for exposure to chemicals. [d] Adjusting for all potential confounders (educational attainment level, exposure to chemicals and magnetic fields).

in males and in females, though evidence for an excess risk was stronger in males, both in the crude and in the adjusted analysis. After stratifying for age using 68 years (median age at disease onset) as cutoff, the excess ALS risk associated with pesticides exposure was considerably higher in the oldest group *(Table 2)*.

DISCUSSION

In our population-based study, we found a strong association between occupational exposure to pesticides overall considered and ALS risk. In males such association resulted to be stronger than in females, suggesting the influence of sex-related factors, such as metabolic patterns, or different features of the occupational exposures to pesticides occurring in males, though the small number of subjects limits the interpretation of this finding. Moreover, ALS relative risk resulted to be higher in the oldest group, indicating the possibility of an interaction between pesticides and age in favouring ALS onset, possibly due to a long induction period after exposure required to trigger disease onset.

Exposure to pesticides have already been reported to be associated with ALS risk in some investigations, sometimes with evidence of dose-response effects, but inconsistent results have also been reported [8-13, 15]. The hypothesis of an association between exposure to pesticides used in football grounds and ALS risk has also been proposed to explain the increased disease incidence found in soccer players [19-21]. Alternatively, intense physical activity has been suggested as a possible explanation of this observation, but the lack of increased ALS risk in professional road cyclists and basketball players indicates that the disease is not related to physical activity per se [21].

ALS has been previously found to be associated, in few but not all investigations, with other environmental factors such leisure-time habits, education, smoking, electromagnetic fields and chemical agents exposure, physical activities and history of trauma [1]. In our study, inclusion in the statistical analysis of three potential confounders, education and exposure to chemicals and magnetic fields, did not reduce the association between pesticides and ALS risk, but it even enhanced it. When we further adjusted the analysis for additional factors such as consumption of dietary supplements, coffee and alcohol consumption, smoking habits, physical activity, and history of trauma, the result did not change substantially (data not shown).

We found that all ALS cases were of the sporadic form of the disease, which is generally considered to be a multifactorial disease with several genetic and environmental potential risk factors [2-5]. Recent investigations have shown a possible association between sporadic ALS and gene polymorphisms of paraoxonase (PON), an enzyme detoxifying the organophospate pesticides [22, 23]. Exposure to organophosphates, in particular when associated with a genetic tendency to a less efficient detoxification, has been proposed as a possible explanation for ALS increased risk in Gulf War veterans [24]. Nevertheless, in contrast to these studies, a more recent large meta-analysis showed no significant association of ALS risk with the PON locus [25], suggesting the need to further investigate this issue.

Major strengths of our study were the use of multiple sources of data in identifying new cases, including pharmacological prescriptions, the accuracy in confirming diagnosis of ALS and the methodology to identify the controls, matched for age, sex and calendar-year to the cases. On the converse, some limitations of the present investigation must be acknowledged. First, the small size of our sample, as reflected by the low statistical stability of the risk estimates, which is particularly evident in the multivariate analysis. Moreover, we adopted a "crude" measure of exposure to pesticides, *i.e.* a dichotomous indicator of overall occupational history related to use of these compounds, without taking into consideration dose and duration of exposure as well as the specific nerotoxic pesticides to which the subjects may have been exposed [14]. This is a crucial issue, since several but not all the compounds belonging

to the pesticides classes (insecticides, herbicides, fungicides and rodenticides) are recognized neurotoxicants, and even in these cases their neurotoxic effects are due to different and still not entirely understood mechanisms [14, 26]. Therefore, results of the present study should be interpreted as an indication that overall occupational exposure to pesticides is a risk factor for sporadic ALS in this Italian population, but they also suggest the need of further in-depth investigation of this relation in larger studies, focusing on careful assessment of exposure to single pesticides classes or compounds.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

Acknowledgements

The authors acknowledge the cooperation of all subjects and their families who agreed to participate in the study. Financial support to this study was provided by Unità Sanitaria Locale (the Local Health Unit) of Reggio Emilia and by the Reggio Emilia Section of Associazione Italiana Sclerosi Laterale Amiotrofica -AISL.

Submitted on invitation.
Accepted on 22 April 2010.

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Environmental Research 117 (2012) 112–119

Contents lists available at SciVerse ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: A meta-analysis of epidemiological studies ☆
Pesticide exposure as a risk factor for ALS

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ARTICLE INFO

Article history:
Received 20 June 2011
Received in revised form
25 May 2012
Accepted 15 June 2012
Available online 20 July 2012

Keywords:
Amyotrophic lateral sclerosis
Pesticides
Occupational exposures
Epidemiology
Meta-analysis

ABSTRACT

Background: Exposure to pesticides and agricultural chemicals has been linked to amyotrophic lateral sclerosis (ALS) although findings have been inconsistent. A meta-analysis of studies published through May, 2011 was conducted to investigate the association of pesticide exposure and risk of ALS.
Methods: Six peer-reviewed studies that met criteria were included in a meta-analysis of men involving 1,517 ALS deaths from one retrospective cohort study and 589 ALS or motor neuron disease cases from five case-control studies. A random effects model was used to calculate sex-specific pooled odds ratios (ORs).
Results: Evidence was found for an association of exposure to pesticides and risk of ALS in male cases compared to controls ($OR=1.88$, 95% CI: 1.36–2.61), although the chemical or class of pesticide was not specified by the majority of studies.
Conclusion: This meta-analysis supports the relationship of exposure to pesticides and development of ALS among male cases compared to controls. The weight of evidence links pesticide exposure to ALS; however, additional prospective studies with a target exposure group are necessary to better elucidate the relationship. Future research should focus on more accurate exposure assessment and the use of job exposure matrices.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease in adults with an incidence of approximately 1–3 per 100,000 persons worldwide each year (Kiernan et al., 2011; Migliore and Coppede, 2009). Ninety to ninety-five percent of ALS is sporadic with no known cause, while the remaining 5%–10% is familial or hereditary. Men have a 50% greater risk of developing ALS compared to women although the inequality seems to balance out after menopause (Kamel et al., 2005; Migliore and Coppede, 2009). ALS risk increases with age with an average age of onset of 58–63 years for sporadic ALS (Kamel et al., 2005; Kiernan et al., 2011). After 75 years of age, the incidence of ALS decreases (Migliore and Coppede, 2009). ALS is characterized by progressive degeneration of both the upper and lower motor neurons resulting in muscle weakness, atrophy, impaired respiration, and ultimately death (Borasio and Miller, 2001). The median survival after onset of ALS is about 2–4 years (Borasio and Miller, 2001).

There are very few known risk factors for ALS identified from previous epidemiologic investigations, and those identified are very general and include male sex and age (Morahan and Pamphlett, 2006; Nelson, 1995). The 3.0–2.0 male to female ratio argues for a possible environmental or occupational exposure not experienced in a widespread manner in women. Genetic susceptibility to various environmental exposures is also suspected to be related to ALS.

Since 1950, pesticide use has risen over 50% and pesticide toxicity has increased ten-fold (Tweedy, 1981). The main varieties of toxic pesticides include: (1) organophosphates, (2) carbamates, (3) organochlorines, (4) fungicides, and (5) fumigants. Three million cases of acute severe pesticide poisoning and over 200,000 deaths are reported annually and include both occupational and general exposures (World Health Organization, 1990; Ferrer and Cabral, 1995).

☆This research was funded by a pilot grant from the University of Pittsburgh, Center for ALS Research.

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http://dx.doi.org/10.1016/j.envres.2012.06.007

Pesticides, many of which are related to widespread agricultural application, are considered to be potentially neurotoxic.

It is well known that acute high level exposure to organophosphates, carbamates, organochlorines, fungicides, and fumigants affects the nervous system (Kamel and Hoppin, 2004). Paraoxonase 1 (PON1) is an enzyme that hydrolyzes organophosphates. Therefore, those with higher levels of PON1 have less toxicity as they are able to metabolize even higher doses of organophosphates. Single nucleotide polymorphisms in the PON1 gene have been found to be related to sporadic ALS through the mechanism of alteration of PON1 function (Cronin et al., 2007; Landers et al., 2008; Morahan et al., 2007; Saeed et al., 2006; Slowik et al., 2006; Valdmanis et al., 2008).

In addition, previous studies have reported an association between pesticide exposure and risk of Parkinson's disease and Alzheimer's disease (Elbaz et al., 2007; Migliore and Coppede, 2009; Stozicka et al., 2007). Exposure to pesticides with characteristics similar to rotenone, paraquat, MPTP, as well as others, has been linked to Parkinson's disease and parkinsonism (Kamel and Hoppin, 2004; Migliore and Coppede, 2009). In a few cases, herbicides have been associated with changes in neurobehavioral performance (Dobbs 2009). Neurotoxicity of pesticide exposure at moderate levels is debatable.

To date, only one meta-analysis has been carried out to investigate the association of pesticide exposure and risk of ALS (Kamel et al., 2012). Two systematic reviews were conducted in 2009 but did not include a meta-analysis due to heterogeneity of the studies (Sutedja et al., 2009a; Sutedja et al., 2009b). This meta-analysis will focus on the broad category of occupational exposure to pesticides in order to evaluate the overall risk estimates presented in the peer-reviewed literature to date.

2. Materials and methods

2.1. Study identification

A systematic review of published articles in Pubmed was conducted to identify epidemiological studies of the association between exposure to pesticides and risk of ALS or motor neuron disease through May, 2011. Motor neuron disease was included as ALS accounts for the majority of cases. The database was searched for potential studies of all languages to be included in the meta-analysis using the following medical subject headings (MeSH) and search terms: amyotrophic lateral sclerosis or ALS or motor neuron disease or MND in combination with agrochemicals or pesticides. Studies containing gardening-related exposures were excluded to eliminate the potential confounding effect of hobby-related exposures.

The search method and exclusion criteria for studies included in the meta-analysis are shown by the QUOROM diagram (Fig. 1). The literature review identified 141 studies of which 69 were relevant to neurological disease. In addition, a manual review of references from the primary and review articles identified thirteen additional studies. Of the 82 relevant studies identified (69 from the Pubmed search and 13 from the manual review), 10 met the inclusion

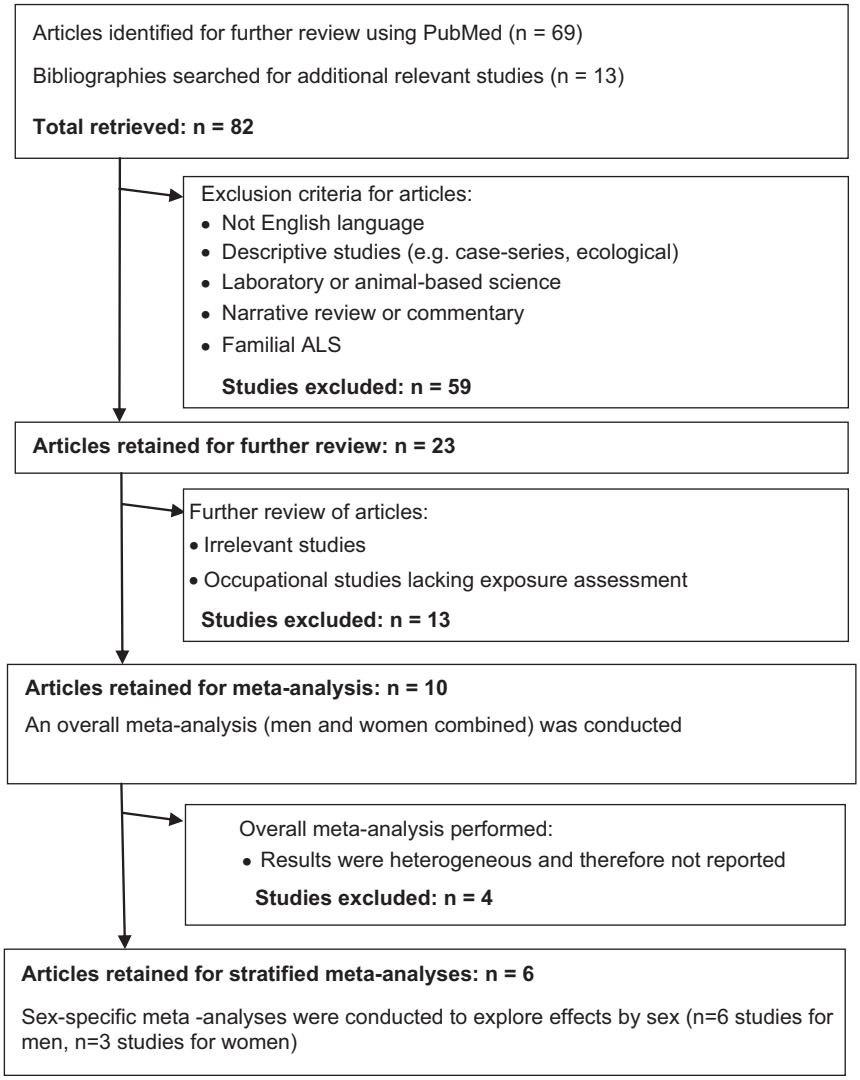

Fig. 1. QUORUM summary diagram of studies included in meta-analyses.

Table 1
Characteristics of Studies Included in the Meta-Analysis.

Author, location of study	Year	Study design	Source of controls	ALS/MND diagnostic criteria	Pesticide exposure characterization and source	No. of cases/ controls (Total N)	Matching factors	Adjusting factors
Bonvicini, Italy [37]	2010	Case-control	Population controls	ALS (El Escorial criteria)	Self-reported occupational pesticide exposure $>=6$ months (men and women, women only, and men only)	41, 82 ($N=123$)	Age (year of birth), sex	Education
Burns, U.S. [27]	2001	Cohort	Deaths identified from cohort of Dow Chemical Company employees	ALS (ICD-8: 348.0)	Job exposure matrix used to rank time weighted average of occupational exposure to the herbicide, 2,4-dichlorophenoxy-acetic acid, (men only) by: very low (only 1983-94 cohort: less than 50% time in low exposure area), low (<0.1 mg/m^3), moderate (0.1–1.0 mg/m^3), or high (>1.0 mg/m^3) categories for various durations of time (1.3, 1.8, or 12.5 years) and years (1947–9, 1950-1, and 1968-86). Cumulative exposure calculated as: very low (<0.05), low (0.05–0.49), moderate (0.5–4.9), or high ($>=5.0$).	1517, 40,600 ($N=42,117$)	Sex	Age, year
Chancellor, Scotland [34]	1993	Case-control	Patient controls	MND standard diagnostic criteria (SALS: multiple spinal level upper and lower motor neuron signs)	Self-reported occupational pesticide exposure (men and women, women only, and men only) $>=12$ months	103,103 ($N=206$)	Age ($\pm$ date of birth nearest to patient), sex	None reported
Deapen, U.S. [40]	1986	Case-control	Co-workers, neighbors, acquaintance controls	ALS (patient registries from ALS Society)	Self-reported occupational pesticide exposure (men and women): long-term exposure	518 ($N=1,036$)	Age (± 5 years), sex	None reported
Granieri, Italy [35]	1988	Case-control	Neurology and non-neurology hospital controls	MND (based upon clinical findings of PMA, PBP and ALS)	Agricultural and forestry occupations as identified by self-report; Agricultural chemical substances (men and women): continuous occupational exposure	70,210 ($N=280$)	Age (± 5 years), sex, same period of hospital admission (± 40 day), residency in study area	None reported
Gunnarsson, Sweden [36]	1992	Case-control	Population controls	MND (pure motor symptoms, progressive course, no signs of polyneuropathia) ALS (LMN symptoms in at least 2 regions and 2 UMN symptoms within 3 years after onset)	Self-reported occupational exposure to pesticides and insecticides (women only and men only): duration not reported	92,372 ($N=464$)	Age (same age range,45–79 years)	None reported
McGuire, U.S. [39]	1997	Case-control	Population controls	ALS [progressive MND affecting both UMN and LMN (ALS), and progressive muscular atrophy and progressive bulbar palsy (variants of ALS)]	Self-report and blinded panel assessment of occupational exposures by four industrial hygienists overall and by sex. An exposure index estimate, monthly frequency of exposure, annual index, and a lifetime cumulative index of exposure were calculated. The panel assessed occupational exposures occurring between age 15 and 10 years prior to ALS diagnosis/reference date. Agricultural chemical exposure ever (men and women, women only, and men only) and low/high (men only); <3 years and >3 years exposure to agricultural chemicals (men only); Fertilizers and the classes of pesticides: fungicides, insecticides, herbicides, and other pesticides exposure ever (men and women and men only) and low/high (men only); Agricultural exposure due to accident/spill (excess exposure by self-report) (men and women)	174,348 ($N=522$)	Age (± 5 years), sex, and respondent type (self or proxy)	Age, education

Morahan, Australia [13]	2006	Case-control	Community, spouse, acquaintance controls	ALS (probable or definite modified El Escorial criteria)	Self-reported: herbicide/pesticide exposure ever, occasional, and regular; Farming herbicide/pesticide exposure ever, occasional, and regular; Industrial herbicides/pesticides exposure ever, occasional, and regular (men and women, women only, and men only)	179,179 ($N=358$)	Age (not specified), sex, ethnicity	None reported
Savettieri, Italy [33]	1991	Case-control	Acquaintance controls	ALS (did not report diagnostic criteria)	Self-reported exposure to agricultural chemicals (men and women): continual	46, 92 ($N=138$)	Age (± 5 years), sex, residence (urban/rural), SES	None reported
Weisskopf, U.S. [28]	2009	Cohort	Deaths identified from CPS-II cohort of ACD	ALS (ICD-9: 335.2, or ICD-10: G12.2 revision)	Self-reported occupational exposure to pesticides/herbicides (men and women): duration not reported for full cohort analysis and currently or regularly exposed for restricted cohort analysis	1,156 986,030 ($N=987,186$)	None reported	Age, sex, smoking, military, education, alcohol use, occupation, vitamin E use, and other chemical classes (full cohort)

Abbreviations: ALS, Amyotrophic Lateral Sclerosis; ICD, International Classification of Diseases; SALS, Sporadic Amyotrophic Lateral Sclerosis; MND, Motor Neuron Disease; PMA, Progressive Muscular Atrophy; PBP, Progressive Bulbar Palsy; No., Number; LMN, Lower Motor Neurons; UMN, Upper Motor Neurons; CPS-II, Cancer Prevention Study II; ACS, American Cancer Society; and SES, Socioeconomic Status.

criteria of: (1) peer-reviewed, (2) case-control or cohort design, (3) published in the English language, (4) provided measures of odds ratios (ORs) or relative risks (RRs) (e.g., unadjusted or adjusted OR) for ALS, or provided the number of individuals (either cases and controls, or cases and person-years), and (5) sporadic ALS or motor neuron disease as the outcome. Review articles, case-series, commentaries, laboratory science studies, and any non-relevant studies were excluded from analysis. Due to the presence of heterogeneity, an overall meta-analysis of the 10 studies was not possible. However, 6 of the 10 studies were included in the sex-specific meta-analyses ($n=6$ studies for men and $n=3$ studies for women).

Standardized data extraction forms were used to extract the following data from each study: location, year, study design, source of cases/controls, diagnostic criteria, pesticide exposure source, number of cases/controls, total number of subjects, matching factors, adjusting factors, measures of effect, and confidence intervals. We attempted to include other studies; however, we were unsuccessful in contacting the corresponding authors to obtain the relevant information required for meta-analysis. Table 1 displays characteristics of studies included in the meta-analysis. One author performed the data extraction and again verified the data to check for inconsistencies.

2.2. Statistical analysis

The reviewed studies measured exposure to pesticides by duration, frequency, concentration, or class of pesticide, depending on the study. All case-control studies included age and sex-matched controls. Some case-control studies additionally matched upon respondent type in terms of self or proxy (McGuire et al., 1997), the same period of hospital admission (± 40 days) and residency in the study area (Granieri et al., 1988), and SES and place of residence (urban/rural) (Savettieri et al., 1991). Cohort members of the retrospective cohort study were matched by sex (Burns et al., 2001).

Results were reported as ORs with 95% confidence intervals (CIs) by the majority of studies (Bonvicini et al., 2010; Chancellor et al., 1993; Deapen and Henderson, 1986; Granieri et al., 1988; Gunnarsson et al., 1992; McGuire et al., 1997; Morahan and Pamphlett, 2006; Savettieri et al., 1991). Four studies presented measures of effect adjusted for confounders (Bonvicini et al., 2010; Burns et al., 2001; McGuire et al., 1997; Weisskopf et al., 2009). One prospective study and one case-control study provided relative risks (Burns et al., 2001; Weisskopf et al., 2009). The retrospective cohort study reported standardized mortality ratios (SMRs) (Granieri et al., 1988). The statistical software package, Comprehensive Meta-Analysis, required calculation of an OR by all included studies in order to calculate the OR summary effect estimate.

ALS is a rare disease, occurring among approximately 1–3 per 100,000 persons annually (Borenstein et al., 2005). When the incidence of disease is rare ($<10\%$), an odds ratio is a good approximation to the relative risk. Calculation of the OR for the pooled analysis required the number of events (deaths) that occurred among exposed and unexposed ALS cases and controls. To perform the meta-analysis, the statistical software transformed all values to log values then displayed the results converted back to ratio values. Thus, odds ratios were calculated for the two cohort studies based upon data obtained from the corresponding authors if these data were not available through the manuscripts. The weighted average estimate of the effect of pesticide exposure on ALS across studies served as our summary effect estimate.

Heterogeneity of studies was assessed by calculation of both Q and I^2 statistics. The Q-statistic is a standardized measure yielding the weighted sum of squares, although it does not provide any information regarding the degree of heterogeneity (Thompson and Sharp, 1999). Heterogeneity was considered statistically significant by a Q-statistic p-value of <0.1 in our meta-analysis (Higgins and Thompson, 2002). The I^2 statistic is used to determine the extent of true variability. An I^2 statistic of 25, 50, or 75 indicates low, medium, or high heterogeneity, respectively (Higgins and Thompson, 2002). Meta-analyses are assumed to contain some degree of heterogeneity as they combine a number of studies conducted by various investigators in different places. Moreover, heterogeneity in our analysis may be due to: chance, differing diagnostic criteria, design characteristics, or patient level covariates (which cannot be explored further without individual-level data). The heterogeneity may also be unexplainable.

Peer-reviewed studies that met the required inclusion criteria were included in our meta-analysis. Sex was evaluated separately as a potential source of heterogeneity between studies. A random effects model was used in the presence of heterogeneity with the understanding that the pooled standard error (SE) would be inflated over a fixed effects model. This is because it accounts for the extra variability of differing effects estimated by studies. In addition, the random effects model can lead to incorrect inferences as it does not account for or explain heterogeneity.

Funnel plots were visually assessed to evaluate potential publication bias among studies (Sterne and Egger, 2001). The x-axis contains the log of the ORs while the y-axis contains the standard error (SE) of the log of ORs. The presence of publication bias was determined by an asymmetrical plot. Comprehensive Meta-Analysis V2 software was used to conduct all analyses (Borenstein et al., 2005).

3. Results

3.1. Description of studies

Review of the literature identified two cohort studies (one prospective and one retrospective) and eight retrospective case-control studies that met criteria for inclusion in the meta-analysis. However, due to heterogeneity, an overall meta-analysis was not possible and thus sex-specific meta-analyses were carried out. The meta-analysis of men involved one retrospective cohort study and five retrospective case-control studies, and the meta-analysis of women included three case-control studies. Table 1 summarizes characteristics of studies included in the meta-analysis. The studies varied by control selection, exposure characterization and source, measure of effect, and geographic location. Studies were conducted in the U.S. ($n=4$), Italy ($n=3$), Scotland ($n=1$), Australia ($n=1$), and Sweden ($n=1$) (Bonvicini et al., 2010; Chancellor et al., 1993; Gunnarsson et al., 1992; Morahan and Pamphlett, 2006; Savettieri et al., 1991). The U.S. studies included a national study as well as studies conducted in Michigan, California, and Washington state (Burns et al., 2001; Deapen and Henderson, 1986; Granieri et al., 1988; McGuire et al., 1997; Weisskopf et al., 2009).

Depending on the study, ALS was diagnosed according to El Escorial Criteria, standard diagnostic criteria as described in detail, or was identified by death certificates (ICD-8 348.0, ICD-9 code 335.2, or ICD-10 code G12.2) (Bonvicini et al., 2010; Burns et al., 2001; Gunnarsson et al., 1992; McGuire et al., 1997; Morahan and Pamphlett, 2006; Weisskopf et al., 2009). Deapen et al. utilized patient registries available from the ALS Society to identify cases (Deapen and Henderson, 1986). One study did not specify the ALS diagnostic criteria used (Savettieri et al., 1991). Motor neuron disease was diagnosed according to standard diagnostic criteria by two studies and by the presence of pure motor symptoms, a progressive course, and no signs of polyneuropathia by one study (Chancellor et al., 1993; Granieri et al., 1988; Gunnarsson et al., 1992). Studies using standard diagnostic criteria were conducted prior to publication of El Escorial criteria in 1994.

A total of 1,029,303 participants from two cohort studies (2,673 ALS deaths and 1,026,630 controls) and 3,127 participants from eight case-control studies (1,223 cases and 1,904 controls) were considered for meta-analysis. All of the case-control studies included age and sex-matched controls. Three studies involved population controls (Bonvicini et al., 2010; Gunnarsson et al., 1992; McGuire et al., 1997); however, some used patient (Chancellor et al., 1993), hospital (neurology and non-neurology department) (Granieri et al., 1988), or acquaintance controls (Deapen and Henderson, 1986; Morahan and Pamphlett, 2006; Savettieri et al., 1991). The national population register of Sweden was consulted to randomly select 500 population controls for one study (Gunnarsson et al., 1992). Annual resident directories of the General Registry's Office were consulted to identify controls for each region of a study in the Italian municipality of Reggio Emilia (Bonvicini et al., 2010).

Morahan and Pamphlett, 2006 study involved a combination of age, sex, and ethnicity-matched spouse, community volunteer, and patient acquaintance controls. Age and sex-matched co-worker, neighbor, and acquaintance controls participated in Deapen et al's study (Deapen and Henderson, 1986). Healthy friends or neighbors of cases served as controls for one study, provided they were not co-workers (Savettieri et al., 1991). Patient controls, referred to as community controls by Chancellor et al., were defined as age and sex-matched individuals identified through the General Practitioner's register of cases (Chancellor et al., 1993).

Death certificates were consulted for verification of ALS mortality by the cohort studies using the following International Classification of Diseases (ICD) codes for ALS: ICD-8: 348.0, ICD-9: 335.2, or ICD-10: G12.2 revision (Burns et al., 2001; Weisskopf et al., 2009). Results were adjusted for by age, education, and year, among other potential confounders, by four studies (Bonvicini et al., 2010; Burns et al., 2001; McGuire et al., 1997; Weisskopf et al., 2009). The remaining studies did not report adjusting for confounders.

3.2. Pesticide exposure

The majority of studies obtained information related to pesticide exposure solely by self-report through a questionnaire or interview (Bonvicini et al., 2010; Chancellor et al., 1993; Deapen and Henderson, 1986; Granieri et al., 1988; Gunnarsson et al., 1992; McGuire et al., 1997; Morahan and Pamphlett, 2006; Savettieri et al., 1991; Weisskopf et al., 2009). Duration of exposure to pesticides was reported by some studies and frequency of exposure by fewer studies. One study assessed self-reported occupational exposure to pesticides for $\geq$6 months (Bonvicini et al., 2010), while another assessed exposure for $\geq$12 months (Chancellor et al., 1993). Self-reported long-term occupational exposure to pesticides was also evaluated by one study (Deapen and Henderson, 1986). Frequency of self-reported exposure to herbicides/pesticides, farming herbicides/pesticides, and industrial herbicides was evaluated as ever, occasional, or regular by one study (Morahan and Pamphlett, 2006). Other studies obtained self-reported continuous occupational exposure to agricultural chemical substances (Granieri et al., 1988) and continual exposure to agricultural chemicals (Savettieri et al., 1991). In addition, the prospective study assessed self-reported occupational exposure to pesticides/herbicides as currently or regularly exposed in a restricted cohort analysis, and as duration not reported in a full cohort analysis (Weisskopf et al., 2009).

A case-control study carried out by McGuire et al. evaluated self-reported occupational exposure as well as involved a panel assessment of four industrial hygienists blinded to self-reported exposure and disease status (McGuire et al., 1997). The panel examined job history as coded by U.S. occupational and industry coding (McGuire et al., 1997; Office of Federal Statistical Policy and Standards, 1980; Office of Management and Budget, 1987). Results from the panel assessment are included in our meta-analysis. The panel assessment used a previously developed scaling system that considered exposure intensity, duration of employment, job tasks, frequency of contact, and the use of protective equipment (Gerin et al., 1985; McGuire et al., 1997; Siemiatycki, 1991). An exposure index estimate, monthly frequency of exposure, annual index, and a lifetime cumulative index of exposure were calculated. The panel assessed ever and low/high occupational exposure to agricultural chemicals among both men and women as well as for <3 years and >3 years exposure to agricultural chemicals among men only (McGuire et al., 1997). Occupational exposure to fertilizers and the following classes of pesticides: fungicides, insecticides, herbicides, and other pesticides was examined as ever exposed among men and women, and among men only (McGuire et al., 1997). Low/high exposure was examined among men only (McGuire et al., 1997). Men and women also self-reported excess agricultural exposure resulting from an accident or spill (McGuire et al., 1997).

Occupational exposure to a specific chemical or pesticide was investigated by only one study, which assessed potential exposure to the herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), among employees of The Dow Chemical Company (Burns et al., 2001). The Dow Chemical Company consisted of four plants in Midland, Michigan that manufactured, formulated, esterified, aminated, and packaged the product (Burns et al., 2001). Information related to potential exposure to 2,4-dichlorophenoxyacetic

acid was obtained from complete work histories available in the employee registry of all full-time employees who worked more than 3 days per week from 1945–1994 (Burns et al., 2001). A job exposure matrix was used to rank time weighted average occupational exposure to 2,4-dichlorophenoxyacetic acid as: very low (only 1983-94 cohort: less than 50% time in low exposure area), low (<0.1 mg/m^3), moderate (0.1–1.0 mg/m^3), or high (>1.0 mg/m^3) for various durations (1.3, 1.8, or 12.5 years) and years (1947–9, 1950–1, and 1968–86). Cumulative exposure was calculated as: very low (<0.05 mg/m^3), low (0.05–0.49 mg/m^3), moderate (0.5–4.9 mg/m^3), or high (≥ 5.0 mg/m3).

3.3. Results of the meta-analysis

The meta-analysis of men included a total of 1,517 ALS deaths from the retrospective cohort study and 589 ALS or motor neuron disease cases from five case-control studies that met the inclusion criteria. The meta-analysis of women involved a total of 144 ALS cases from three case-control studies. Studies with a small number of women as well as studies in which only cases reported exposure to pesticides (and not controls) were unable to be included in the meta-analysis (Chancellor et al., 1993). Table 2 summarizes results of the forest plots for the sex-specific meta-analyses. Evidence was found for exposure to pesticides and the risk of ALS among male cases ($OR=1.88$, 95% CI: 1.36–2.61) compared to controls through a random effects model (Table 2). No relationship was found for exposure to pesticides and risk of ALS among female cases compared to controls ($OR=1.31$, 95% CI: 0.69–2.47) by a random effects model (Table 2).

Results were reported as ORs with 95% CIs, as previously mentioned (Table 3). As expected, results of the fixed effect and random effects meta-analyses were equivalent. The study specific ORs were considered heterogeneous at $p<0.1$. Results of the Q-test were not heterogeneous for men ($Q=2.86$, $df=5$, $p=0.721$) or women ($Q=0.67$, $df=2$, $p=0.716$) demonstrating that the studies shared a common effect size. Due to estimation of different effects by the studies, a random effects model was used to capture any additional variability. The I^2 statistic was 0.00 for the sex-specific analyses of men and women indicating no heterogeneity.

3.4. Publication bias

No evidence of publication bias was suggested by the funnel plots for any of the analyses as the studies were all symmetrical around the mean (Figs. 2 through 3).

4. Discussion

The relation of exposure to pesticides and risk of ALS, as observed in our meta-analysis, is an important finding. Overall,

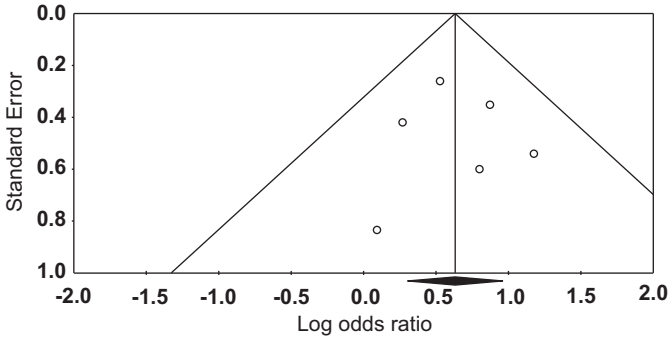

Fig. 2. Funnel plot of studies involving men.

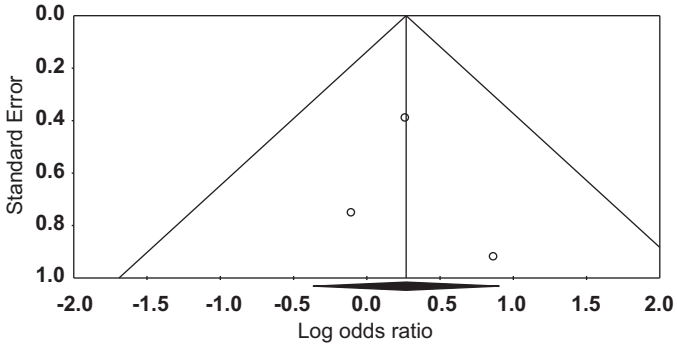

Fig. 3. Funnel plot of studies involving women.

Table 2
Results of Forest Plot for Random Effects Meta-Analyses of Studies by Sex.

Population	Model	Study name	Odds ratio	Lower limit	Upper limit	Relative weight
Men	Random	Bonvicini	3.25	1.12	9.41	9.50
		Burns Chancellor	2.23 1.31	0.69 0.58	7.26 3.00	7.73 15.75
		Gunnarsson	1.10	0.21	5.66	4.00
		McGuire	2.40	1.20	4.80	22.35
		Morahan	1.70	1.02	2.84	40.68
		Effect estimate	1.88	1.36	2.61	
Women	Random	Bonvicini	2.38	0.39	14.38	12.41
		McGuire	0.90	0.21	3.92	18.57
		Morahan	1.30	0.61	2.79	69.01
		Effect estimate	1.31	0.69	2.47	

Table 3
Results for Random-Effects Meta-Analyses of Studies by Sex.

Population	Model	Effect size and 95% confidence interval (CI)			Heterogeneity		
		Number of Studies	Odds Ratio	95% CI	*p*	Q-value	I^2
Men	Random	6	1.88	1.36–2.61	0.721	2.86	0
Women	Random	3	1.31	0.69–2.47	0.716	0.67	0

evidence was found for the association of exposure to pesticides and ALS among male cases compared to controls.

Studies published through May, 2011 were included in this quantitative meta-analysis investigating the association of pesticide exposure and risk of ALS. Pesticide class (i.e. herbicide, fungicide, and insecticide) was examined by only two studies, one of which specified the name of the herbicide. Therefore, we were unable to carry out meta-analyses by class of pesticide due to the small number of studies. Some studies provided duration of pesticide exposure; however, there were not enough studies with similar exposures to be combined in a stratified meta-analysis. Thus, a gap identified in this field of research is quantification of the class of pesticide and active ingredient, frequency and duration of exposure, and feasible monitoring of blood or urine analysis for better dose estimation.

The systematic review identified a large prospective study of nearly 1 million participants that met inclusion criteria for an overall meta-analysis but due to the presence of heterogeneity, results are not reported. Heterogeneity may be due to differences between studies such as the methodology of pesticide exposure, study design, study population, patient-level covariates, or even low power. The sex-specific analyses of men and women produced an I^2 of 0.00 indicating no heterogeneity between studies. One possible explanation may be the weighting of studies as reflected by the inverse of the study's variance. The sex-specific analyses also resulted in a T^2 (between-studies variance) of 0.00. The Q-statistic ($p<0.1$) is not a reliable estimate of heterogeneity when a small number of studies are included in the meta-analysis. Thus, results of the random effects model are reported for the sex-specific meta-analyses. The meta-analysis of men was not heterogeneous due possibly to exclusion of a large prospective study, differences in methodology, study population, or power. It is possible that with the addition of more (and larger) studies a stronger association may be detected.

We failed to find an association between pesticide exposure and risk of ALS in female cases compared to controls. This may be due to the small number of studies ($n=3$) and women in our analysis; therefore, resulting in a lack of power to detect an association. In addition, studies in which only female cases were exposed were excluded from analysis. Our results may indicate that men are more likely to be occupational exposed to pesticides and for longer periods of time than women.

4.1. Comparison with previous research

Only one meta-analysis of pesticide exposure and ALS has been conducted to date (Kamel et al., 2012). However, systematic reviews have been carried out without meta-analyses due to heterogeneity (Sutedja et al., 2009a; Sutedja et al., 2009b). Reviews and epidemiological studies investigating the relationship of pesticide exposure and ALS have produced conflicting results. Some authors have reported an association (Bonvicini et al., 2010; Govoni et al., 2005; McGuire et al., 1997; Morahan and Pamphlett, 2006), others have found non-significant increases (Deapen and Henderson, 1986; Savettieri et al., 1991), and still others have failed to replicate findings of the association of pesticide exposure and ALS (Chancellor et al., 1993; Granieri et al., 1988). Presently, only a small number of epidemiological studies have been carried out to investigate the relationship between exposure to pesticides and risk of ALS development. The study designs have varied and have included case-series, case-control studies, and only a few prospective studies. Controls selected for case-control studies have not always been population-based, which limits the representativeness of the results.

In addition, epidemiological studies conducted thus far have failed to report the names of specific pesticides under review. No epidemiological studies have attempted to obtain adequate exposure assessments through the use of blood samples or biomarkers, such as blood cholinesterase activity and urinary metabolites, which can only assess recent exposure. It may be possible, however, to draw a correlation between results of farming or toxicology studies measuring pesticide concentrations and those of epidemiological studies. For example, an exposure study carried out to evaluate exposure to glyphosate, a common herbicide used in farming, among farm families in South Carolina and Minnesota found an average urine concentration among farmers on application day of 3.2 parts-per-billion (ppb) (Fishel, 2009). Following pesticide application, the concentration decreased. This is considerably lower than the lowest no-effect level as determined by the Environmental Protection Agency (EPA) (175 ppm) (Fishel, 2009). This study, as well as other exposure studies, provides valuable information regarding the level of pesticides to which farmers are potentially exposed.

To date, exposure has primarily been obtained through self-report; however, McGuire et al."s study also incorporated a panel assessment of four blinded industrial hygienists to serve as a comparison. Differences in exposure levels were identified for both cases and controls. The type and magnitude of pesticide exposure is not usually obtained or reported. This is likely because self-report is not always an accurate measure of exposure. However, years worked with or around the pesticide, the number of hours exposed, and the specific pesticide or chemical exposed to could be asked of participants. Therefore, the gold standard for future epidemiological studies investigating the association of pesticide exposure and risk of ALS would be to obtain a thorough exposure assessment from multiple sources.

4.2. Strengths and limitations

Our analysis of men was fortunate to have a large sample size which allowed for sufficient power to detect an effect of exposure to pesticides and risk of ALS. Few studies examined pesticide exposure by class, duration, or intensity. Therefore, a meta-analysis of these subgroups was not possible due to the small number of studies (Burns et al., 2001; McGuire et al., 1997; Morahan and Pamphlett, 2006). Excluding gardening in our analysis helped to eliminate the potential confounding effect of hobby-related exposures. Furthermore, most studies included age and sex-matched controls to alleviate potential confounding effects.

A limitation of our study is the possibility of publication bias due to the literature search limits, accessing only one database, and the inclusion of studies in the English language, although Pubmed was searched for articles of all languages. However, the funnel plots were symmetric and publication bias does not appear to have significantly affected the positive association found between pesticide exposure and ALS among men. As only studies in the English language were included, the results may not accurately reflect the breadth of available literature.

We must also take into account the limitations of the primary study designs included in the meta-analysis. In general, those who participate in research studies may be different than those who do not participate. A number of biases may be present within the case-control and cohort study designs such as bias involved with self-reported exposure which may overestimate risk estimates. This is particularly important in retrospective studies as exposure assessment is conducted in an indirect manner. In addition, recall bias may play a role in that cases may more accurately remember exposures or information as compared to healthy controls.

The potential relationship between pesticide exposure and ALS has been difficult to establish as most studies have failed to obtain

details regarding pesticide class (insecticide, herbicide, fungicide, etc.), chemical name, or duration of exposure. In our analysis, the majority of studies reported occupational exposure to agricultural chemicals but did not specify the chemicals or jobs involved. Categorizing subjects by level or duration of exposure (i.e. low, high, long-term, etc.) is helpful, although a meaningful conclusion cannot be made if the number of subjects in each group was too small as is the case among women with occupational exposure to pesticides. Grouping all pesticide classes together may dilute the effect of one class and result in a lack of an association. The chemical composition of pesticides may not be known, but commonly used brand names or uses of specific pesticides could be provided in the questionnaire or interview to better identify exposures. In addition, study questionnaires can discriminate by class of pesticide although this may be problematic for some agricultural workers who are exposed to multiple classes of pesticides at through different routes of exposure, for different durations, and different times of the year.

Misclassification is also a concern when occupational groups, such as farming, combine various job titles regardless of exposure. The group "farmers" includes a number of different types of farmers such as soybean, livestock, corn, etc. Awareness of job exposures is necessary before grouping into occupational categories. Job exposure matrices are also very valuable in identifying and quantifying occupational exposures, and should be incorporated in future studies. Multi-site studies or collaborations between different institutions, states, or countries would be an excellent way to improve sample size and power. These implications serve as only a starting point from which to expand future research. Studies included in our meta-analysis provided these details in some, but not all, instances.

5. Conclusions

After examining all related articles through May, 2011, the meta-analysis found a relationship between exposure to pesticides and risk of ALS among male cases compared to controls. Future research should focus on more accurate exposure measurement and the use of job exposure matrices. In addition, protective equipment should be worn on the job as well as during household use of pesticides to help circumvent any potential exposures and to prevent "take-home" exposures to others.

In conclusion, more research must be conducted to determine whether an association truly does exist between suspected pesticide exposure and risk of ALS. ALS is a debilitating and devastating disease, and one which is certainly deserving of additional research.

Acknowledgements

The authors would like to thank Dr. David Lacomis and Judith Rager for their review of the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2012.06.007.

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Productie 31

Toxicology and Applied Pharmacology 268 (2013) 157–177

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Invited Review Article

Pesticides and human chronic diseases: Evidences, mechanisms, and perspectives

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ARTICLE INFO

Article history:
Received 4 October 2012
Revised 30 January 2013
Accepted 31 January 2013
Available online 9 February 2013

Keywords:
Pesticides
Systematic review
Chronic diseases
Cancer
Birth defects
Reproductive toxicity
Parkinson
Alzheimer
Amyotrophic lateral sclerosis
Diabetes
Asthma
Cardiovascular disease
Nephropathy
Genetic damage
Epigenetics
Endocrine disruption
Mitochondrial dysfunction
Oxidative stress
Endoplasmic reticulum stress
Proteotoxicity

ABSTRACT

Along with the wide use of pesticides in the world, the concerns over their health impacts are rapidly growing. There is a huge body of evidence on the relation between exposure to pesticides and elevated rate of chronic diseases such as different types of cancers, diabetes, neurodegenerative disorders like Parkinson, Alzheimer, and amyotrophic lateral sclerosis (ALS), birth defects, and reproductive disorders. There is also circumstantial evidence on the association of exposure to pesticides with some other chronic diseases like respiratory problems, particularly asthma and chronic obstructive pulmonary disease (COPD), cardiovascular disease such as atherosclerosis and coronary artery disease, chronic nephropathies, autoimmune diseases like systemic lupus erythematous and rheumatoid arthritis, chronic fatigue syndrome, and aging. The common feature of chronic disorders is a disturbance in cellular homeostasis, which can be induced via pesticides' primary action like perturbation of ion channels, enzymes, receptors, etc., or can as well be mediated via pathways other than the main mechanism. In this review, we present the highlighted evidence on the association of pesticide's exposure with the incidence of chronic diseases and introduce genetic damages, epigenetic modifications, endocrine disruption, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress and unfolded protein response (UPR), impairment of ubiquitin proteasome system, and defective autophagy as the effective mechanisms of action.

Contents

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0041-008X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2013.01.025

Introduction

Pesticides are considered as one of the main factors involved in environmental contamination of today's world. These chemicals are on purpose designed to be toxic to pest and vectors of diseases. These compounds are among more than 1000 active ingredients that are marketed as insecticide, herbicide, and fungicide. Nevertheless, formulation of new and potent pesticides is increasingly on the order of researchers and manufacturers because of pest resistance, hygienic controls, and major human need for more food as the world population grows. Although pesticides have largely benefited the human life through enhancement of agricultural products and controlling infectious diseases, their extensive use, in turn, has offended human health from side to side of occupational or environmental exposures. Long-term contact to pesticides can harm human life and can disturb the function of different organs in the body, including nervous, endocrine, immune, reproductive, renal, cardiovascular, and respiratory systems. In this regard, there is mounting evidence on the link of pesticide's exposure with the incidence of human chronic diseases, including cancer, Parkinson, Alzheimer, multiple sclerosis, diabetes, aging, cardiovascular and chronic kidney disease (Abdollahi et al., 2004c; De Souza et al., 2011; Mostafalou and Abdollahi, 2012a). In this overview, we discuss the association of pesticide's exposure with the incidence of different types of human chronic diseases as well as general mechanisms of disease's process, which can be involved in pesticide-induced toxicities.

Evidences for the link between pesticide exposure and incidence of chronic diseases

Chronic diseases are characterized by their generally slow progression and long term duration, which are considered as the leading cause of mortality in the new world, representing over 60% of all deaths. According to the WHO report, 36 million people died from chronic disease in 2008, of which nine million were under 60 and 90% of these premature deaths occurred in low- and middle-income countries (http://www.who.int/topics/chronic_diseases/en/).

Cancer

The first reports on the association of pesticides with cancer were presented around 50 years ago regarding higher prevalence of lung and skin cancer in the farmers using insecticides in grape fields (Jungmann, 1966; Roth, 1958; Thiers et al., 1967). During the past half century, a wide spectrum of population-based studies has been carried out in this respect leading to a significant progress in understanding the relationship of pesticides to the incidence of different types of malignancies (Penel and Vansteene, 2007). The International Agency for Research on Cancer (IARC) has conducted several cohort studies on the incidence of cancers in people exposed to pesticides somehow during their lives (Baldi and Lebailly, 2007). Based on rising evidence given by epidemiological and agricultural health studies associated with exposure to pesticides, different types of neoplasm have been reported such as breast cancer, prostate cancer, lung cancer, brain cancer, colorectal cancer, testicular cancer, pancreatic cancer, esophageal cancer, stomach cancer, skin cancer and non-Hodgkin lymphoma (Alavanja and Bonner, 2012; Jaga and Dharmani, 2005; Weichenthal et al., 2010). Van Maele-Fabry et al. (2006, 2007, 2008) pointed out exposure to pesticides as a possible risk factor for prostate cancer and leukemia by a meta-analysis of risk estimates in pesticide manufacturing workers. In a series of agricultural health studies, Lee et al. (2004a,b, 2007) found an association between exposure to pesticides and cancer incidence, particularly lymphohematopoietic cancers for alachlor, lung cancer for chlorpyrifos, and colorectal cancer for aldicarb. Nowadays, chronic low-dose exposure to pesticides is considered as one of the important risk factors for cancer expansion. Therefore, carcinogenicity tests are now applied to detect carcinogenic potential of pesticides before allowing them to be marketed. Carcinogenicity testing is a long-term (around two years) rodent bioassay using two species of both sexes. According to a new list of Chemicals Evaluated for Carcinogenic Potential by EPA's Pesticide Program published in 2010, more than 70 pesticides have been classified as a probable or possible carcinogen. This classification has been accomplished based on the information extracted from animal studies, metabolism studies, structural relationship with other carcinogens, and if available, epidemiologic findings in human (http://www.epa.gov/pesticides/carlist/).

Carcinogenic properties of pesticides can be influenced by a series of complex factors including age, sex, individual susceptibility, amount and duration of exposure, and simultaneous contacts with other cancer causing chemicals. However, carcinogenic mechanisms of pesticides can be explored in their potential to affect genetic material directly via induction of structural or functional damage to chromosomes, DNA, and Histone proteins, or indirectly disrupting the profile of gene expression through impairment of cellular organelles like mitochondria and endoplasmic reticulum, nuclear receptors, endocrine network, and the other factors involved in maintenance of cell homeostasis (George and Shukla, 2011; Rakitsky et al., 2000). Table 1 is indicating data extracted from epidemiological studies implicating on the relation between exposure to specific pesticides and increased risk of some kind of cancers.

Birth defects and developmental toxicity

Birth defects or congenital disorders are defined as structural or functional abnormalities existing at birth or before birth that causes physical or mental disabilities. Ranging from mild to fatal, diverse types of birth defects have been recognized and deliberated as the principal cause of death for infants during the first years of life. Any material which can induce birth defects is called teratogen (Rogers and Kavlock, 2008). The history of sensibility on the topic of developmental toxicity of pesticide returns to an incidence of congenital disorders induced by DDT and other organochlorines in the wildlife in Laurentian Great Lakes (Hamlin and Guillette, 2010). That concern was more intensified when reports associating with elevated rate of birth defects in defoliant sprayed areas of Vietnam appeared after

Table 1
Pesticides associated with elevated incidence of cancer in epidemiological studies.

Type of cancer	Pesticide	Reference
Leukemia	Chlordane/heptachlor	Purdue et al. (2007)
	Chlorpyrifos	Lee et al. (2004b)
	Diazinon	Beane Freeman et al. (2005)
	EPTC	van Bemmel et al. (2008)
	Fonofos	Mahajan et al. (2006)
Non-Hodgkin's lymphoma	Lindane	Purdue et al. (2007)
	Oxychlordane/chlordane	Spinelli et al. (2007)
Multiple myeloma	Permethrin	Rusiecki et al. (2009)
Brain cancer	Chlorpyrifos	Lee et al. (2004b)
Prostate cancer	Fonofos	Mahajan et al. (2006)
	Methylbromide	Alavanja et al. (2003)
	Butylate	Lynch et al. (2009)
	Clordecone	Multigner et al. (2010)
	DDT, lindane, simazine	Band et al. (2011)
Colon cancer	Aldicarb	Lee et al. (2007)
	Dicamba	Samanic et al. (2006)
	EPTC	van Bemmel et al. (2008)
	Imazethapyr	Koutros et al. (2009)
	Trifluralin	Kang et al. (2008)
Rectum cancer	Chlordane	Purdue et al. (2007)
	Chlorpyrifos	Lee et al. (2004b)
		Lee et al. (2007)
	Pendimethalin	Hou et al. (2006)
Pancreatic cancer	EPTC, pendimethalin	Andreotti et al. (2009)
	DDT	Garabrant et al. (1992)
Lung cancer	Chlorpyrifos	Lee et al. (2004b)
	Diazinon	Beane Freeman et al. (2005)
	Dicamba	Alavanja et al. (2004)
	Dieldrin	Purdue et al. (2007)
	Metolachlor	Alavanja et al. (2004)
	Pendimethalin	Hou et al. (2006)
Bladder cancer	Imazethapyr	Koutros et al. (2009)
Melanoma	Carbaryl	Mahajan et al. (2007)
	Toxaphene	Purdue et al. (2007)
	Carbaryl, parathion, maneb/mancozeb	Dennis et al. (2010)

war in late 1960. Defoliant or the famous Agent Orange is composed of phenoxy herbicides, which included small amounts of highly toxic dioxin (TCDD) as a byproduct (Ngo et al., 2006). Currently, there is much epidemiological evidence linking pre- and post-natal exposures to pesticides with congenital disorders (Weselak et al., 2007). A meta-analysis of literature published from 1966 to 2008 by Rocheleau et al. (2009) indicated that higher incidence of hypospadias resulted from parental exposure to pesticides. Parental exposure to Agent Orange has also been associated with increased risk of birth defects given by a meta-analytical review of epidemiological studies (Ngo et al., 2006). Furthermore, experimental data have indicated adverse developmental outcomes of some pesticides in laboratory animals as evidenced by intrauterine death, in utero growth retardation, visceral and skeletal malformations or dysfunctions (Cavieres, 2004).

In addition to the rate of placental transfer and systemic absorption as a determinant factor for chemicals to be teratogen, their potential in induction of genetic damage, neuronal cell defects, endocrine disruption, and oxidative stress has been proposed as the main mechanism of developmental toxicity (van Gelder et al., 2010).

Reproductive disorders

Reproductive disorders are defined as conditions prejudicing the capacity of the reproductive system to reproduce. Vast body of literature has detailed adverse effects of environmental exposures, particularly pesticides on both male and female reproductive systems (Kumar, 2004; Shojaei Saadi and Abdollahi, 2012). Decreased fertility in both sex, demasculinization (antiandrogenic effects), elevated rate of miscarriage, altered sex ratio, and change in the pattern of maturity are among the most reported reproductive dysfunctions induced by chronic exposure to pesticides (Frazier, 2007). These effects of pesticides deemed more important when their link to endocrinal disruption was explained. A number of pesticides, mostly the old organochlorine types like aldrin, chlordane, DDT, dieldrin, and endosulfan, the herbicide atrazine, and the fungicide vinclozolin have been identified as commonly believed endocrine disrupting chemicals (PAN, 2009). Interfering with functions of the endocrine system has been implicated in most pesticides that caused reproductive toxicities (Cocco, 2002; Figa-Talamanca et al., 2001; Tiemann, 2008).

Parkinson

Parkinson disease is a motor progressive disorder of CNS characterized by degeneration of dopaminergic neurons in the substantia nigra. The cause of this degeneration is not well-known but post-mortem studies have indicated that oxidative stress and mitochondrial dysfunction play the main role in development of this late-onset disorder. There are large numbers of population studies that prove higher incidence of Parkinson disease in the people exposed to pesticides (Bonetta, 2002; Freire and Koifman, 2012; Van Maele-Fabry et al., 2012). A new meta-analysis published by van der Mark et al. (2012) reviewed updated literature, including 39 case–control studies, four cohort studies, and three cross-sectional studies and found that exposure to insecticides, and herbicides can lead to augmented risk of Parkinson disease. Furthermore, elevated levels of some pesticides in the serum of patients with Parkinson disease have been reported (Richardson et al., 2009). These results were followed up by other researchers who designed developmental models to analyze the link between Parkinson disease and pesticide exposure in several environmental health studies (Cory-Slechta et al., 2005). It can be said that Parkinson and other neurodegenerative disorders have been most studied in case of exposure to neurotoxic pesticides such as organophosphates, carbamates, organochlorines, pyrethroids and some other insecticides since they interfere with neurotransmission and function of ion channels in the nervous system (Costa et al., 2008).

Alzheimer

Evidence implicating on the role of pesticide in developing Alzheimer's disease is lesser than that of Parkinson. Most of the studies carried out in this respect are relatively small and vague until a longitudinal population-based cohort study was published in 2010 (Jones, 2010). Elderly people living in an agricultural area who contributed in the survey for 10 years showed a higher rate of cognitive performance and risk of Alzheimer's disease. When researchers specifically tested CNS affecting pesticides, they found a direct and significant association between occupational exposure to organophosphates, acetylcholinesterase inhibitor compounds, and developing Alzheimer's disease later in life (Hayden et al., 2010). Furthermore, in an ecologic study, Parron et al. (2011) showed that people living in areas with high level of pesticides usage had an elevated risk of Alzheimer's disease.

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is the nearly all common form of the motor neuron diseases characterized by degeneration of both upper and lower motor neurons. The symptoms include rapidly progressive weakness, muscle atrophy and fasciculations, muscle spasticity, dysarthria (difficulty speaking), dysphagia (difficulty swallowing), and a decline in breathing ability. Irrespective of familial ALS which can be easily ruled out, there is no known cause for this disease but many evidence-based potential risk factors have been proposed for its development where chemical exposures have been bolded (Morahan and Pamphlett, 2006; Sutedja et al., 2009). A population-based case–control study conducted by McGuire and colleagues in 1997 was almost the starting point of pesticide-focused investigations in association with ALS. In that study, occupational exposure to three

Table 2
The list of studies whose results implicate on the association of exposure to pesticides with incidence of chronic diseases.

Disease	Types	Reports			
		Case control	Cohort	Ecological	Others
Cancer	Childhood leukemia	Alderton et al. (2006) Alexander et al. (2001) Buckley et al. (1989) Buckley et al. (1994) Infante-Rivard et al. (1999) Lafiura et al. (2007) Laval and Tuyns (1988) Leiss and Savitz (1995) Lowengart et al. (1987) Ma et al. (2002) Magnani et al. (1990) Meinert et al. (1996) Menegaux et al. (2006) Monge et al. (2007) Mulder et al. (1994) Rau et al. (2012) Reynolds et al. (2005) Rudant et al. (2007) Rull et al. (2009) Shu et al. (1988) Soldin et al. (2009)		Carozza et al. (2008)	Turner et al. (2010)
	Adult leukemia	Alavanja et al. (1990) Brown et al. (1990) Ciccone et al. (1993) Clavel et al. (1996) Miligi et al. (2006)	Beane Freeman et al. (2005) Beard et al. (2003) Blair et al. (1983) Bonner et al. (2010) Cantor and Silberman (1999)	Chrisman Jde et al. (2009) Delzell and Grufferman (1985) Mills (1998)	Cuneo et al. (1992) Merhi et al. (2007) Van Maele-Fabry et al. (2008)
	Hodgkin's lymphoma	Orsi et al. (2007) Persson et al. (1993) Rudant et al. (2007) van Balen et al. (2006)	Flower et al. (2004)	Carozza et al. (2008) Cerhan et al. (1998)	
	Non-Hodgkin's lymphoma	Alavanja et al. (1990) Buckley et al. (2000) Cantor (1982) Cantor et al. (1992) Chiu et al. (2006) De Roos et al. (2003) Eriksson et al. (2008) Hardell and Eriksson (1999) Hardell et al. (2002) Hoar et al. (1986) McDuffie et al. (2001) Meinert et al. (2000) Miligi et al. (2006) Nordstrom et al. (1998) Pearce et al. (1985) Rudant et al. (2007) Schroeder et al. (2001) t Mannetje et al. (2008) Vajdic et al. (2007) Woods et al. (1987) Zahm et al. (1990) Zahm et al. (1993)	Bonner et al. (2010) Kristensen et al. (1996b) Kross et al. (1996) Morrison et al. (1994) Purdue et al. (2007) Ritter et al. (1990) Zhong and Rafnsson (1996)		Khuder et al. (1998) Merhi et al. (2007)
	Multiple myeloma	Burmeister et al. (1983) Pearce et al. (1985)	Kristensen et al. (1996b) Landgren et al. (2009) Lope et al. (2008)	Cerhan et al. (1998)	Merhi et al. (2007)
	Neuroblastoma	Daniels et al. (2001) Walker et al. (2007)	Feychting et al. (2001) Giordano et al. (2006) Kristensen et al. (1996b) Littorin et al. (1993)	Carozza et al. (2008)	
	Soft tissue sarcoma	Kogevinas et al. (1995) Leiss and Savitz (1995) Magnani et al. (1989)		Carozza et al. (2008) Chrisman Jde et al. (2009)	
	Childhood brain cancer	Bunin et al. (1994) Cordier et al. (1994) Davis et al. (1993) Efird et al. (2003) Gold et al. (1979) Holly et al. (1998) Pogoda and Preston-Martin (1997) Rosso et al. (2008) Ruder et al. (2006) Searles Nielsen et al. (2010)	Kristensen et al. (1996a)		

Table 2 (*continued*)

Disease	Types	Reports			
		Case control	Cohort	Ecological	Others
Cancer	Childhood brain cancer	van Wijngaarden et al. (2003) Wilkins and Koutras (1988) Wilkins and Sinks (1990)			
	Adult brain cancer	Lee et al. (2005) Musicco et al. (1988) Provost et al. (2007) Rodvall et al. (1996) Samanic et al. (2008) Zheng et al. (2001)	Blair et al. (1983) Figa-Talamanca et al. (1993) Kross et al. (1996) Viel et al. (1998)	Delzell and Grufferman (1985) Mills (1998) Wesseling et al. (1999)	Smith-Rooker et al. (1992)
	Bone cancer	Merletti et al. (2006) Moore et al. (2005)	Holly et al. (1992)	Carozza et al. (2008) Wesseling et al. (1999)	
	Prostate cancer	Cerhan et al. (1998) Dosemeci et al. (1994) Forastiere et al. (1993) Meyer et al. (2007) Mills and Yang (2003) Settimi et al. (2003)	Alavanja et al. (2003) Chamie et al. (2008) Dich and Wiklund (1998) Fleming et al. (1999) Kross et al. (1996) MacLennan et al. (2002) Morrison et al. (1993)	Chrisman Jde et al. (2009) Delzell and Grufferman (1985) Mills (1998)	Keller-Byrne et al. (1997) Sharma-Wagner et al. (2000)
	Breast cancer	Band et al. (2000) Brophy et al. (2002) Duell et al. (2000) Mills and Yang (2005) Teitelbaum et al. (2007)	Dolapsakis et al. (2001)		Ortega Jacome et al. (2010)
	Colorectal cancer	Cerhan et al. (1998) Forastiere et al. (1993) Lo et al. (2010)	Kang et al. (2008) Koutros et al. (2009) Kross et al. (1996) Lee et al. (2007) Samanic et al. (2006) van Bemmel et al. (2008) Zhong and Rafnsson (1996)	Wesseling et al. (1999)	
	Pancreatic cancer	Alguacil et al. (2000) Forastiere et al. (1993) Ji et al. (2001) Kauppinen et al. (1995) Lo et al. (2007) Partanen et al. (1994)	Andreotti et al. (2009) Cantor and Silberman (1999)	Chrisman Jde et al. (2009)	
	Kidney cancer	Buzio et al. (2002) Fear et al. (1998) Forastiere et al. (1993) Hu et al. (2002) Karami et al. (2008) Mellemgaard et al. (1994) Olshan et al. (1993) Sharpe et al. (1995) Tsai et al. (2006)	Kristensen et al. (1996b)	Carozza et al. (2008)	
	Lung cancer	Brownson et al. (1993) Bumroongkit et al. (2008) Pesatori et al. (1994)	Alavanja et al. (2004) Barthel (1981) Beane Freeman et al. (2005) Blair et al. (1983) Lee et al. (2004b) Rusiecki et al. (2006) Samanic et al. (2006)	Wesseling et al. (1999)	
	Stomach cancer	Forastiere et al. (1993) Mills and Yang (2007)		Van Leeuwen et al. (1999)	
	Esophageal cancer	Jansson et al. (2006)		Chrisman Jde et al. (2009) Wesseling et al. (1999)	
	Liver cancer		Giordano et al. (2006)	Carozza et al. (2008) Wesseling et al. (1999)	
	Testicular cancer	Mills et al. (1984)	Fleming et al. (1999)	Mills (1998)	
	Bladder cancer	Forastiere et al. (1993)	Koutros et al. (2009)	Wesseling et al. (1999)	
	Gallbladder cancer		Giordano et al. (2006)	Wesseling et al. (1999)	
	Thyroid cancer		Ward et al. (2010)	Carozza et al. (2008)	
	Melanoma	Fortes et al. (2007)	Dennis et al. (2010) Mahajan et al. (2007)	Carozza et al. (2008) Wesseling et al. (1999)	
	Eye cancer	Carozza et al. (2008)	Kristensen et al. (1996b)		
	Lip cancer		Wiklund (1983)	Cerhan et al. (1998) Chrisman Jde et al. (2009)	
	Mouth cancer		Tarvainen et al. (2008)		
	Larynx cancer			Wesseling et al. (1999)	
	Sinonasal cancer				Tisch et al. (2002)
	Ovarian cancer	Donna et al. (1989)		Wesseling et al. (1999)	
	Uterine cancer			Wesseling et al. (1999)	
	Cervical cancer		Fleming et al. (1999)		

(*continued on next page*)

Table 2 (*continued*)

Disease	Types	Reports			
		Case control	Cohort	Ecological	Others
Birth defects		Brender et al. (2010) Brucker-Davis et al. (2008) Dugas et al. (2010) Nassar et al. (2010) Ren et al. (2011)	Chevrier et al. (2011) Perera et al. (2003) Petit et al. (2010)	de Siqueira et al. (2010) Garry et al. (1996) Schreinemachers (2003) Winchester et al. (2009)	Benachour and Seralini (2009) Enoch et al. (2007) Greenlee et al. (2004) Qiao et al. (2001) Rauch et al. (2012) Richard et al. (2005) Rocheleau et al. (2009) Sherman (1996)
Reproductive disorders		Greenlee et al. (2003) Swan et al. (2003b)	Saiyed et al. (2003) Snijder et al. (2011) Tiido et al. (2005) Tiido et al. (2006)	Swan et al. (2003a)	Anway et al. (2005) Cavieres et al. (2002) Fei et al. (2005) Gray et al. (1999) Joshi et al. (2011) Meeker et al. (2006) Oliva et al. (2001) Orton et al. (2011) Stanko et al. (2010) Wang et al. (2011b)
Neuro degenerative diseases	Parkinson	Baldi et al. (2003a) Butterfield et al. (1993) Chan et al. (1998) Costello et al. (2009) Dick et al. (2007) Dutheil et al. (2010) Elbaz et al. (2009) Fall et al. (1999) Firestone et al. (2005) Fong et al. (2007) Frigerio et al. (2006) Gatto et al. (2009) Gorell et al. (1998) Hancock et al. (2008) Hertzman et al. (1994) Hubble et al. (1993) Hubble et al. (1998) Koller et al. (1990) Manthripragada et al. (2010) Menegon et al. (1998) Ritz et al. (2009) Seidler et al. (1996) Semchuk et al. (1992) Stephenson (2000) Tanner et al. (2009) Tanner et al. (2011) Wang et al. (2011a) Zorzon et al. (2002)	Ascherio et al. (2006) Baldi et al. (2003b) Kamel et al. (2007) Petrovitch et al. (2002) Tuchsen and Jensen (2000)	Barbeau et al. (1987) Ritz and Yu (2000) Schulte et al. (1996)	Barlow et al. (2004) Barlow et al. (2005) Caudle et al. (2005) Chou et al. (2008) Jia and Misra (2007) Priyadarshi et al. (2000) Priyadarshi et al. (2001) Purisai et al. (2007) Richardson et al. (2006)
	Alzheimer		Baldi et al. (2003b) Hayden et al. (2010) Tyas et al. (2001)	Parron et al. (2011)	
	ALS	Bonvicini et al. (2010) Das et al. (2012) McGuire et al. (1997) Morahan and Pamphlett (2006) Pamphlett (2012) Qureshi et al. (2006)	Burns et al. (2001)		Choy and Kim (2011) Doi et al. (2006) Kanavouras et al. (2011)
Cardio-vascular diseases	Hypertension		Morton et al. (1975)		
	Atherosclerosis				Fokina and Bezuglyi (1978) Antov and Aianova (1980)
	Coronary artery disease				Zamzila et al. (2011)
Respiratory diseases	Asthma	Salam et al. (2004)	Beard et al. (2003) Hoppin et al. (2002) Hoppin et al. (2008) Slager et al. (2009)		Draper et al. (2003) Kolmodin-Hedman et al. (1982) Lessenger (1992) Moretto (1991) Salameh et al. (2003) Vandenplas et al. (2000) Wagner (2000) Weiner and Worth (1969)
	COPD	Arifkhanova et al. (2007) Ubaidullaeva (2006)	Chakraborty et al. (2009) Hoppin et al. (2007) LeVan et al. (2006) Valcin et al. (2007)		Barczyk et al. (2006) Faria et al. (2005)
Diabetes	Type 1, 2 and gestational	Lee et al. (2010)	Montgomery et al. (2008) Saldana et al. (2007)	Kouznetsova et al. (2007)	Everett and Matheson (2010) Patel et al. (2010)

Table 2 (*continued*)

Disease	Types	Reports			
		Case control	Cohort	Ecological	Others
Chronic renal diseases	Chronic renal failure				Peiris-John et al. (2006) Wanigasuriya et al. (2007)
	Chronic kidney disease				Siddharth et al. (2012)
Autoimmune diseases	Rheumatoid arthritis		Parks et al. (2011)		Gold et al. (2007)
	Systemic lupus erythematous	Cooper et al. (2004)	Parks et al. (2011)		Gold et al. (2007)

groups of chemicals, including solvents, metals, and pesticides in relation to the incidence of ALS was evaluated and the results showed the role of agrochemicals in most of the cases (McGuire et al., 1997). During the past decade, several reports indicated the association of ALS development with exposure to pesticides (Bonvicini et al., 2010; Doi et al., 2006; Freedman, 2001). Pesticides have reserved the most prominent role in the most of the surveys focusing on the association of environmental and occupational exposures with ALS, which have been carried out up to now, and it would not be unlikely to consider them as a risk factor for developing this neurological disorder (Johnson and Atchison, 2009; Kamel et al., 2012; Vinceti et al., 2012).

Diabetes

Diabetes can be said that has become epidemic since 347 million people worldwide are appraised to be diabetic and based on WHO belief, diabetes deaths are expected to double between 2005 and 2030 (http://www.who.int/diabetes/en/index.html). Unlike diseases mentioned above, diabetes, particularly type 2 has some identified risk factors, including rich diet, obesity and sedentary manner of living but the extent of reports implicating on the relation of exposure to environmental pollutants, particularly pesticides and development of diabetes is rapidly growing (Mostafalou and Abdollahi, 2012b; Rahimi and Abdollahi, 2007). The possibility of studying diabetes in experimental models allowed researchers to investigate effects of exposure to pesticides on glucose homeostasis in laboratory animals. In this regard, there were lots of reports on disrupting effects of pesticides particularly organophosphates and organochlorines on glucose metabolism in association with imbalanced insulin secretion and response in animals (Abdollahi et al., 2004a; Karami-Mohajeri and Abdollahi, 2011; Pournourmohammadi et al., 2007). A couple of epidemiological studies whose results published during the past few years indicated that exposure to pesticides can be a potential risk factor for developing diabetes (Everett and Matheson, 2010; Montgomery et al., 2008; Saldana et al., 2007). It has also been suggested that exposure to some pesticides can be a promoter for other risk factors of diabetes like obesity by distressing neural circuits that regulate feeding behavior or altering differentiation of adipocytes (Thayer et al., 2012).

Cardiovascular diseases

About the relationship between pesticide's exposure and cardiovascular diseases, there are just a few random reports carried out in varied forms. In addition to a report concerning hypertension in Oregon pesticide formulating workers (Morton et al., 1975), there have been a few evidences on the link between exposure to pesticides and atherosclerosis (Antov and Aianova, 1980; Fokina and Bezuglyi, 1978). Recently, it was reported that chronic exposure to organophosphate pesticides can potentiate the risk of coronary artery disease presumably through diminished paraoxonase activity (Zamzila et al., 2011).

Chronic nephropathies

Higher incidence of the late-onset nephropathies like chronic kidney disease and chronic renal failure has been reported in middle-aged people (40–60 years) living in the agricultural areas with more prevalence in men. The results of a survey in North Central Province of Sri Lanka have presented a significant relationship between chronic renal failure and environmental factors in farming areas (Wanigasuriya et al., 2007). Exposure to acetylcholinesterase inhibiting pesticides was associated with chronic renal failure (Peiris-John et al., 2006). Furthermore, higher level of organochlorine pesticides was detected in chronic kidney disease patients along with a reduced glomerular filtration and increased oxidative stress (Siddharth et al., 2012).

Chronic respiratory disease

Asthma is considered as the most common disorder among chronic respiratory dysfunctions affecting both children and adults. Its close relationship with work-related exposures has been known from 18 centuries so that occupational asthma is characterized as a disease in medicine. There have been several reports on increased rate of asthma in people occupationally exposed to pesticides (Hernandez et al., 2011). Moreover, the result of an agricultural health study indicated that exposure to some pesticides may increase the risk of chronic obstructive pulmonary disease (COPD) in farmers (Hoppin et al., 2007).

Other chronic diseases

However, there are sporadic reports on the association of exposure to pesticides with different types of human chronic diseases, including chronic fatigue syndrome (Behan and Haniffah, 1994), autoimmune diseases like systemic lupus erythematous and rheumatoid arthritis (Cooper et al., 2004; Gold et al., 2007; Parks et al., 2011) which need further investigations for more proof (Table 2).

Molecular mechanisms linking pesticide exposure to chronic diseases

Genetic damages

Genetic damages are caused by direct interaction with genetic material resulting in DNA damage or chromosomal aberrations and considered as a primary mechanism for chronic diseases within the context of carcinogenesis and teratogenesis. They are studied in the field of genetic toxicology and can be detected by distinctive kinds of genotoxicity tests. Growing body of data concerning genetic toxicity of pesticides have been collected from epidemiological and experimental studies using different types of examinations, including chromosomal aberrations, micronucleus, sister chromatid exchanges and comet assay (Bolognesi, 2003; Bull et al., 2006).

Indeed, genetic damages are classified into three groups as follows: 1. Premutagenic damages like DNA strand breaks, DNA adducts or unscheduled DNA synthesis; 2. Gene's mutation which means insertion or deletion of a couple of base pairs; 3. Chromosomal aberrations, including loss or gain of whole chromosome (aneuploidy), deletion or breaks (clastogenicity), and chromosomal segments or rearrangements. Premutagenic damages may be repaired prior to cell division while the damages in the second and third groups are permanent and have the

Table 3
Genotoxicity biomarkers determined in populations occupationally exposed to different types of pesticides.

Genetic damage	Pesticides	Reference
DNA strand breaks	Acephate, chlorpyrifos, dimethoate, monocrotophos, phorate, cypermethrin, fenvalerate, carbendazim	Grover et al. (2003)
	Dimethoate, ethephon, omethoate, oxydemeton-methyl, thiometon, befenthrin, B-cyfluthrin, deltamethrin, mancozeb, carbendazim, endosulfan, chlorothalanil, iprodione, diflufenicanil, L-cyhalothrin, pyrimethanil, fluroxypyr, cyproconazole, epoxyconazole, flutriafol, tebucanazole, atrazine	Lebailly et al. (1998)
DNA adducts	Glyphosate, methamidophos, monocrotophos, parathion methyl, methomyl, metam-sodium, dazomet, zineb, benomyl, carbendazim, paraquat, captan, folpet, endosulfan	Peluso et al. (1996)
Chromosomal aberration	Acephate, chlorpyrifos, dimethoate, fenitrothion, fenthion, fosetyl, isofenphos, methamidophos, naled, pyrazophos, cypermethrin, deltamethrin, fenpropathrin, fenvalerate, methiocarb, methomyl, oxamyl, mancozeb, propineb, zineb, benomyl, diquat, paraquat, captan, folpet, procymidone, endosulfan, abamectin, kasugamycin, iprodione, oxadixyl, buripimate, metribuzin, linuron, methabenzthiazuron, triforine, vinclozolin, bitertanol, fenbutatin oxide, amitraz, propargite, dithiocarbamate	Carbonell et al. (1993)
	Diazinon, dichlorvos, dimethoate, malathion, ethylazinophos, monocrotophos, parathion, parathion methyl, phorate, prothoate, terbufos, trichlorofon, cypermethrin, fenpropathrin, permethrin, maneb, thiram, dazomet, mancozeb, zineb, ziram, thiabendazole, paraquat, captan, folpet, endosulfan, dodemorph, chlorothalonil, iprodione, acetic metaldehyde, barium polysulfide, copper oxychloride, copper sulfate, sulfur, white oil, dinocap, DNOC, alachlor, simazine, MCPA, linuron, vinclozolin, phenmedifam, methalaxyl, ethofumesate, 2,4-D, dicofol	De Ferrari et al. (1991)
	Dimethoate, mevinphos, monocrotofos, parathion, parathion methyl, aldicarb, maneb, dazomet, propineb, zineb, captan, endosulfan, aldrin, aramite, chlordimeform, heptachlor, tetradifon	Dulout et al. (1985)
	2,4-D	Garry et al. (2001)
	Chlorpyriphos, cypermethrin, deltamethrin, fenpropathrin, methomyl, thiram, pirimicarb, benomyl, carbendazim, endosulfan, chlorothalonil, iprodione, buprofezin, atrazine, triforine, vinclozolin, cyhexatin, fetin acetate, carboxin, 2,4-D, chloridazon, defenamide, oxadiazon, propargyl	Lander et al. (2000)
Micronucleus formation	Metham sodium, dodemorph, zineb, antracol, captan, dazomet, dichloropropane, dichloropropene	Bolognesi et al. (1993)
	Diazinon, dichlorvos, fosetyl-aluminum, malathion, ethamidophos, parathion methyl, cypermethin, carbaryl, methomyl, mancozeb, pirimicarb, benomyl, captan, endosulfan, lindane, diuron, 2,4-D, aldrin, ametrina, BHC, DDT, dacomil, dieldrin, di-syxtox, endrin, furadan, gusathion, javelin, metalaxyl, nuvacron, oxidemeton methyl, talstar, tordon	Gomez-Arroyo et al. (2000)
	Deltamethrin, carbaryl, mancozeb, propineb, benomyl	Pasquini et al. (1996)
Sister chromatid exchange	Azynphos methyl, dimethoate, malathion, methyl parathion, 2,4,5-T, 2,4-D	Laurent et al. (1996)
	Mancozeb-contained fungicide	Jablonicka et al. (1989)
	A complex mixture of pesticides (atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion)	Zeljezic and Garaj-Vrhovac (2002)
	DDT, BHC, endosulfan, malathion, methyl parathion, phosphamidon, dimethoate, monocrotophos, quinalphos fenvelrate, and cypermethrin	Rupa et al. (1991)
	DDT, BHC malathion, parathion, dimethoate, fenitrothion, urea and gromor	Rupa et al. (1988)

ability of transmission to daughter cells after cell division (Guy, 2005) (Fig. 1).

Between chromosomal assessments, micronucleus has been recognized as the most reliable and successful test as verified by the Organisation for Economic Co-operation and Development (OECD). A micronucleus is referred to the third nucleus formed during the metaphase/anaphase transition of mitosis. The group of these cytoplasmic bodies is called micronuclei having a portion of acentric chromosome or whole chromosome, which does not integrate in the opposite poles during the anaphase. This results in the formation of daughter cells without a part or all of a chromosome. Regarding sensitivity, reliability, and cost-effectiveness of this test, it has been proposed as a biomarker for genotoxicity calculations, and has been used in different studies on pesticide-exposed populations. Most of these surveys implied on the increased level of micronucleus formation in people dealing with pesticides for a long time (Costa et al., 2011; Ergene et al., 2007; Garaj-Vrhovac and Zeljezic, 2002).

Sister chromatid exchange (SCE) or exchange of genetic material between sister chromatids is another testing for chemicals suspected to be mutagenic. Elevated level of SCE has been observed in some diseases, including Bloom syndrome and Behçet's syndrome and maybe tumor formation. There are some reports on increased frequency of SCE in pesticide applicators who worked in agricultural fields (Carbonell et al., 1990; Rupa et al., 1991; Zeljezic and Garaj-Vrhovac, 2002).

Single-cell gel electrophoresis (SCGE) or Comet assay is a simple and sensitive testing for evaluation of DNA strand breaks in eukaryotic cells (Dhawan et al., 2009). This technique has been frequently used for biomonitoring genotoxic effect of pesticides in a large number of studies most of which implicate on induction of DNA damage by these chemicals (Grover et al., 2003; Mostafalou and Abdollahi, 2012c; Shadnia et al., 2005; Zeljezic and Garaj-Vrhovac, 2001).

Although, genotoxicity assays are among necessary tests applying for pesticides prior to introducing to the market, collected data from post-market monitoring studies have been evident for potential of allowed pesticides in induction of genetic damages. Considering genetic damages as one of the main events for cancer induction or development, further studies focusing on genotoxicity of pesticides, of course in appropriate models like exposure to their mixtures along with some other promoting factors, are required to understand the carcinogenic and tumorigenic mechanisms of pesticides (Table 3).

Epigenetic modifications

Epigenetic is referred to the heritable changes in gene expression or cellular phenotype without any alterations in the DNA sequence, and its mechanisms include DNA methylation, histone modifications and expression of non-coding RNAs. A growing body of evidence has implicated on the role of environmental exposures, particularly in early development, in the induction of epigenetic changes that may be transmitted to subsequent generations or may serve as a basis of diseases developed later in life. Furthermore, it has become so likely that epigenetics contribute to the causes or transmission of chronic disorders from one generation to another (Weinhold, 2006) (Fig. 2).

Several evidence collected from animal studies during the past decade suggested that exposure to pesticides can induce epigenetic changes. Heritable alterations of DNA methylation in male germline along with testis and ovarian dysfunction have been reported after exposure to some pesticides like vinclozolin and methoxychlor (Anway and Skinner, 2006; Anway et al., 2005; Guerrero-Bosagna et al., 2010; Zama and Uzumcu, 2009). Exposure to dichloroacetic acid and trichloroacetic acid has been associated with decreased methylation in promoter regions of *c-jun* and *c-myc* in liver of mice (Tao et al., 2000a,b). Global DNA hypomethylation has also been reported in

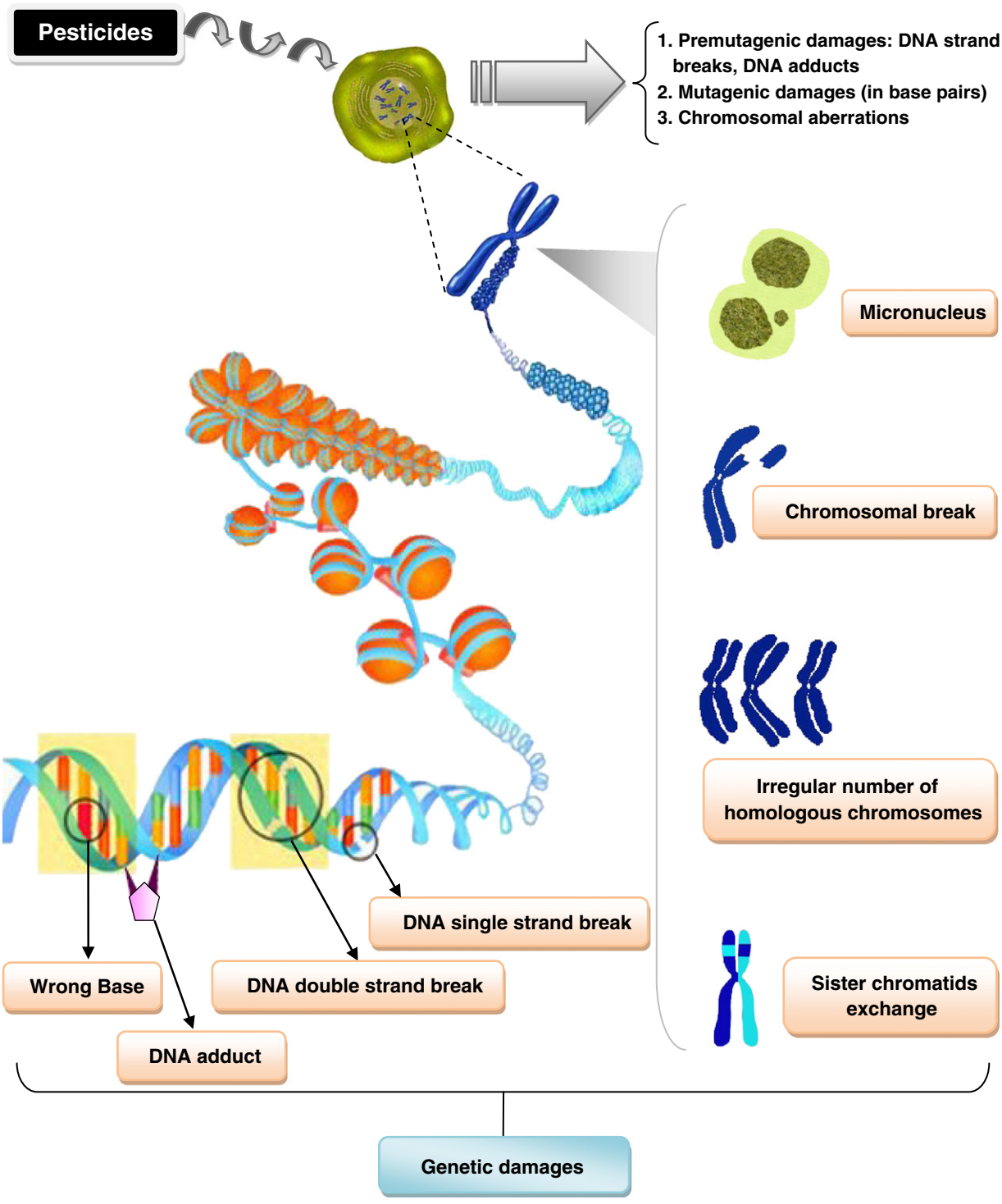

Fig. 1. A schematic model for induction of genetic damages including premutagenic, mutagenic and chromosomal effects by exposure to pesticides.

people who had an elevated blood level of pesticides and persistent organic pollutants in two surveys (Kim et al., 2010; Rusiecki et al., 2008). Furthermore, increased acetylation of core histones H3 and H4 has been reported by dieldrin, an organochlorine pesticide, in mouse models (Song et al., 2010).

On the other hand, growing progress has been made in the recognition of epigenetic modifications in human chronic diseases, particularly cancer. Cancer is now considered as an epigenetic disease the same as a genetic disease. There is tremendous evidence on the contribution of epigenetic events in the initiation, promotion and progression of different types of cancers, mainly through silencing of tumor suppressor genes and/or activation of proto-oncogenes. These modifications have allocated such a fundamental role in cancer development that epigenetic therapy of cancer is rapidly growing in medical sciences (Jones and Baylin, 2002). In addition, epigenetic changes currently have been a powerful tool for studying the carcinogenesis mechanisms of occupational and environmental exposures (Ziech et al., 2010). The first note on pesticide-induced carcinogenesis through epigenetic mechanisms was from a study carried out by Maslansky and colleagues in 1981. They reported hepatocarcinogenesis of organochlorine pesticides with no genotoxic effects in hepatocytes and suspected to epigenetic modifications disrupting intracellular

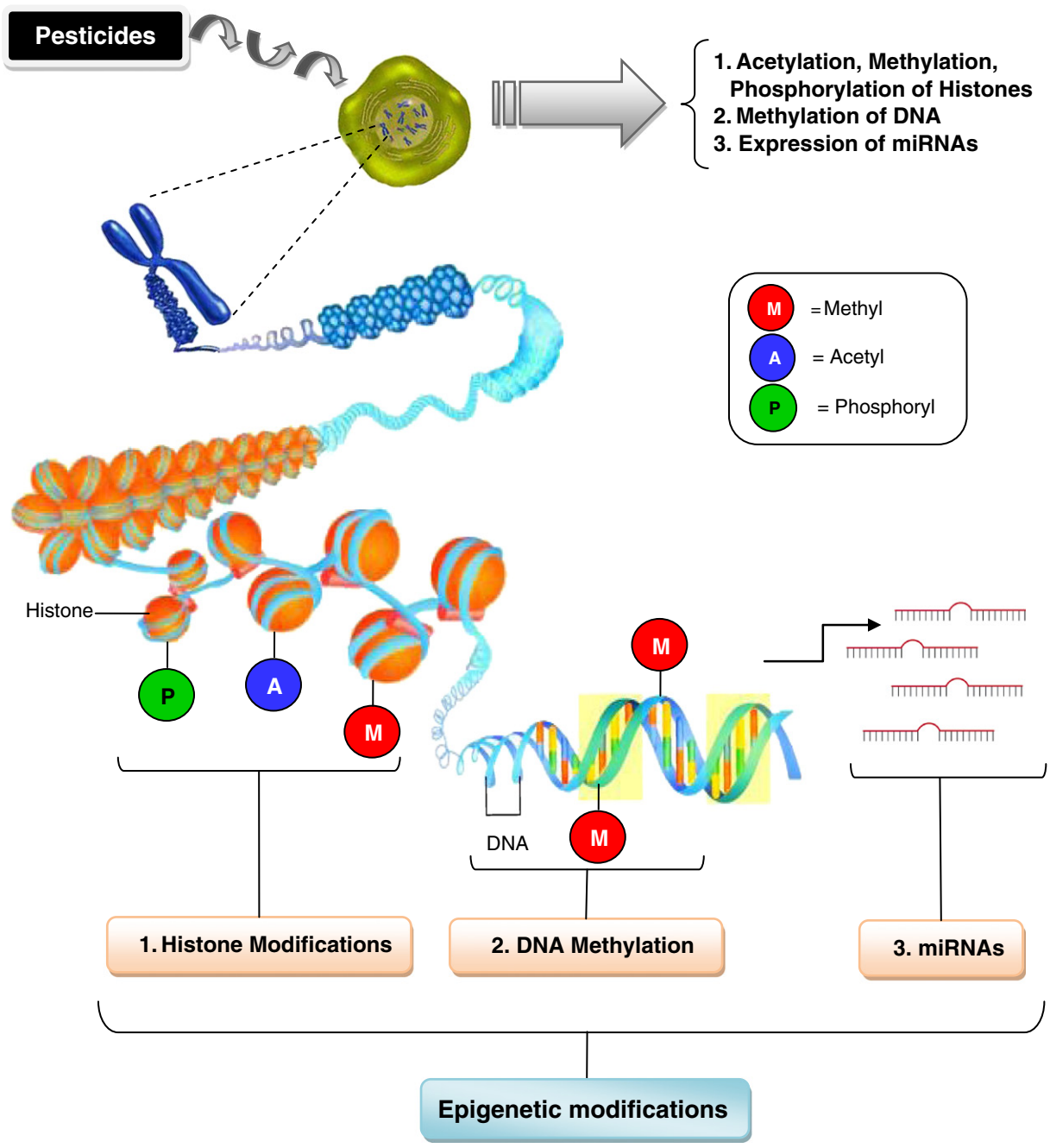

Fig. 2. A schematic model for induction of epigenetic modifications including DNA methylation, histone modification, and expression of miRNAs by pesticides.

communications (Maslansky and Williams, 1981). Later, reports presented about epigenetic actions of vinclozolin, a fungicide known to be an environmental endocrine disruptor, in association with adult-onset diseases, particularly tumor development (Skinner and Anway, 2007). Pesticides were introduced as carcinogens acting through epigenetic or nongenotoxic mechanisms (Rakitsky et al., 2000).

Other than cancer, epigenetic alterations have increasingly been detected and investigated in neurodegenerative diseases, including Parkinson (Habibi et al., 2011), Alzheimer (Kwok, 2010), ALS (Oates and Pamphlett, 2007), and multiple sclerosis (Burrell et al., 2011). On the role of epigenetic changes in pesticide-induced neurodegenerative disorder, recently neurotoxic insecticides were found to promote apoptosis in dopaminergic neurons through hyper-acetylation of core histones H3 and H4 (Song et al., 2010).

Epigenetic alterations have also been reported to be involved in some other late-onset diseases like diabetes (Simmons, 2007), aging (Gravina and Vijg, 2010), chronic kidney disease (Dwivedi et al., 2011), and atherosclerosis (Lund and Zaina, 2011). Nevertheless, presenting epigenetic modifications as a mechanism by which pesticides develop these chronic diseases depends on the future studies.

However, epigenetics has opened a new field for studying the influence of environmental exposures on transcriptional regulation of genes in association with human diseases. There are a lot of findings about changing the pattern of gene expressions in exposure to pesticides, which can be used as a tool in studying the process of human diseases (Pournourmohammadi and Abdollahi, 2011), but further studies are still required to determine the role of epigenetic mechanisms in these variations.

Other mechanisms involved in pesticide-induced chronic diseases

Endocrine disruption

At a cellular level, endocrine disruption refers to a mechanism of toxicity that interferes the ability of the cells to communicate hormonally and results in a wide variety of adverse health effects including birth defects, reproductive, developmental, metabolic, immune, and neurobehavioral disorders as well as hormone dependent cancers. The term "endocrine disruptor" (ED) was first introduced in 1991 referring to the substances that interfere with synthesis, secretion, transport, binding, action, metabolism or elimination of hormones in the body (Crisp et al., 1998). Up to now, a huge body of evidence has brought up on endocrine disrupting properties of pesticides so that currently a total of 101 pesticides have been listed as proven or possible EDs by the Pesticide Action Network UK (PAN, 2009). Most endocrine disrupting pesticides mimic estrogen function by acting as a ligand for receptor, converting other steroids to active estrogen or increasing the expression of estrogen responsive genes as shown by some organochlorines, organophosphates, carbamates, and pyrethroids. Antiandrogenic effects have also been reported for organochlorine and carbamate insecticides, as well as triazines, a group of herbicides through inhibition of binding natural ligand to receptors and androgen binding receptors. Competitive inhibition of thyroid hormone receptors by organophosphates and inhibition of progesterone action by pyrethroids are other findings regarding endocrine disruption by pesticides (McKinlay et al., 2008). However, the results of various transactivation assays using mammalian and yeast cells indicated agonistic or antagonistic activity of pesticides toward aryl hydrocarbon receptors and some members of the nuclear receptor superfamily including retinoic acid receptors, pregnane X receptors, and peroxisome proliferator-activated receptors (Kojima et al., 2010; Lemaire et al., 2005).

Mitochondrial dysfunction

As dynamic multifunctional organelles, mitochondria are the main source of ATP and reactive oxygen species (ROS) in the cell and have important roles in calcium homeostasis, synthesis of steroids and heme, metabolic cell signaling, and apoptosis. Abnormal function of the mitochondrial respiratory chain is the primary cause of imbalanced cellular energy homeostasis and has been widely studied in different types of human diseases most of all diabetes (Abdul-Ghani and DeFronzo, 2008; Kim et al., 2008; Lowell and Shulman, 2005; Ma et al., 2012) and neurodegenerative disorders (Johri and Beal, 2012). Perturbation of this organelle has been accepted as one of the crucial mechanisms of neurodegeneration since there is broad literature supporting mitochondrial involvement of proteins like α-Synuclein, Parkin, DJ-1, PINK1, APP, PS1 & 2, and SOD1 that have some known roles in major neurodegenerative disorders, including Parkinson, Alzheimer, and ALS (Martin, 2012). Some evidence even proposed the involvement of mitochondrial DNA and its alterations in development of these diseases (Lin and Beal, 2006). Parkinson was almost the first disease in which the role of mitochondrial dysfunction was uncovered when the classical inhibitor of complex I electron transport chain, metabolite of MPTP, was reported to cause Parkinsonism in drug abusers (Langston, 1996). In 2000, developing the symptoms of Parkinson was also reported for a broad-spectrum pesticide, rotenone, whose mechanism of action is selective inhibition of complex I mitochondrial respiratory chain so that it has been widely used to create Parkinson model in laboratory animals (Caboni et al., 2004). In this regard, interfering with mitochondrial respiratory chain functions has made a pattern in development of different types of pesticides, and many agrochemicals are known to inhibit electron transport chain activity as their primary or secondary mechanism of action. Most of the pesticides interfering with mitochondrial respiratory chain activities are mainly inhibitors of complex I electron transport chain and some others partially inhibit complexes II, III, and V (Gomez et al., 2007). Moreover, a wide variety of pesticides has been known as uncouplers of mitochondrial oxidative phosphorylation (Ilivicky and Casida, 1969). Nevertheless, impairment of oxidative phosphorylation has been reported in exposure to a large number of pesticides particularly neurotoxic agents through inhibition of a biosynthetic pathway essential for mitochondrial function or extramitochondrial generation of ROS (Ranjbar et al., 2010). Likewise, there is enough evidence on the role of mitochondrial dysfunction in pathophysiological features of diabetes, including insulin deficiency and insulin resistance. Pancreatic beta cell failure has been reported to be associated with mitochondrial dysfunction and can be caused by exposure to pesticides (Jamshidi et al., 2009; Pournourmohammadi et al., 2007). On the other hand, exposure to pesticides inhibiting complex I and III mitochondrial respiratory chain can lead to a diminished oxygen consumption and cellular energy supply which in turn can result in reduced insulin signaling cascade. In this way, organochlorines, atrazine, and some dioxin-like pesticides have been shown to decrease mitochondrial capacity in beta oxidation of fatty acids resulting in accumulation of intracellular fat, a situation considered to develop obesity and insulin resistance (Lee, 2011; Lim et al., 2009).

Oxidative stress

Increased production of ROS and/or decreased capacity of antioxidant defense can disrupt oxidative balance and result in damaging all components of the cell, including lipids, proteins, and DNA. Further, oxidative stress can disrupt various parts of cellular signaling because ROS are considered as one of the main messengers in redox signaling. However, the role of oxidative stress has been uncovered in induction and development of different kinds of human diseases, including cancer, diabetes, neurodegeneration, atherosclerosis, schizophrenia, chronic fatigue syndrome, and renal and respiratory disorders (Ahmad et al., 2010; Ciobica et al., 2011; Fendri et al., 2006; Lushchak and Gospodaryov, 2012; Nathan et al., 2011). On the other hand, there is a huge body of literature on induction of oxidative stress by pesticides, and it has been implicated in development of health problems mediated by exposure to pesticides (Grosicka-Maciag, 2011; Olgun and Misra, 2006; Slaninova et al., 2009; Soltaninejad and Abdollahi, 2009). It has been revealed that pesticides can disturb oxidative homeostasis through direct or indirect pathways, including mitochondrial or extramitochondrial production of free radicals, thiol oxidation, and depletion of cellular antioxidant reservoirs (Abdollahi et al., 2004b,c; Braconi et al., 2010; Mostafalou et al., 2012a). Considering the oxidative stress as a powerful promoter of other cellular pathways involved in disease process and as a unique attendant in inflammatory response, it has been put in the spotlight of the most mechanistic studies regarding the association of pesticide's exposure with chronic disorders. Oxidative stress has been implicated in the onset and progression of pesticide induced Parkinson disease (Singh et al., 2007). In this regard, organochlorine pesticides have been reported to cause degeneration of dopaminergic neurons by an oxidative dependent pathway in Parkinson model (Kanthasamy et al., 2002; Sharma et al., 2010). Additionally, disrupting effects of organophosphates on glucose homeostasis have been reportedly linked to oxidative damages and inflammatory cytokines and thought to be compensatory responses accompanied with reduced insulin signaling in insulin sensitive organs such as liver, muscle, and adipose tissue (Mostafalou et al., 2012b; Teimouri et al., 2006). As such further disruption of glucose homeostasis in diabetic models of laboratory animals exposed to organophosphate insecticides has been associated with enhanced lipid peroxidation and decreased activity of antioxidant enzymes (Begum and Rajini, 2011). Oxidative stress has also been reported to be involved in nephrotoxicity of some pesticides,

including diazinon, acephate, and paraquat (Poovala et al., 1998; Shah and Iqbal, 2010; Tomita et al., 2006).

Endoplasmic reticulum stress and unfolded protein response

As the first compartment of secretary pathway, endoplasmic reticulum (ER) is specialized for synthesis, folding, and delivery of proteins in addition to its fundamental role in the storage of calcium. Any disturbance in calcium homeostasis, redox regulation, and energy supply can cause perturbation of ER normal function resulting in accumulation of unfolded or misfolded proteins in this organelle, a situation which is called ER stress. Unfolded proteins occupy ER resident chaperones leading to release of transmembrane ER protein kinases which activate a series of phosphorylation cascades resulting in increased expression of genes, which act as molecular chaperones to reestablish ER folding capacity or promote ER associated degradation (ERAD) to remove misfolded proteins. This process is called unfolded protein response (UPR) aiming to adjust to the changing environment. In case if adaptation fails, ER stress results in expression of genes involved in programmed cell death pathways (Xu et al., 2005). Recent discoveries indicate that prolonged ER stress and UPR play an important role in the development of several human diseases particularly chronic ones, including insulin resistance, diabetes (Back et al., 2012; Kim et al., 2012; Scheuner and Kaufman, 2008), Parkinson, Alzheimer, ALS (Doyle et al., 2011; Lindholm et al., 2006; Nassif et al., 2010), tumor formation and progression (Koumenis, 2006; Lee and Hendershot, 2006), atherosclerosis, cardiomyopathy, chronic kidney diseases and renal failure (Dickhout et al., 2011; Tabas, 2010).

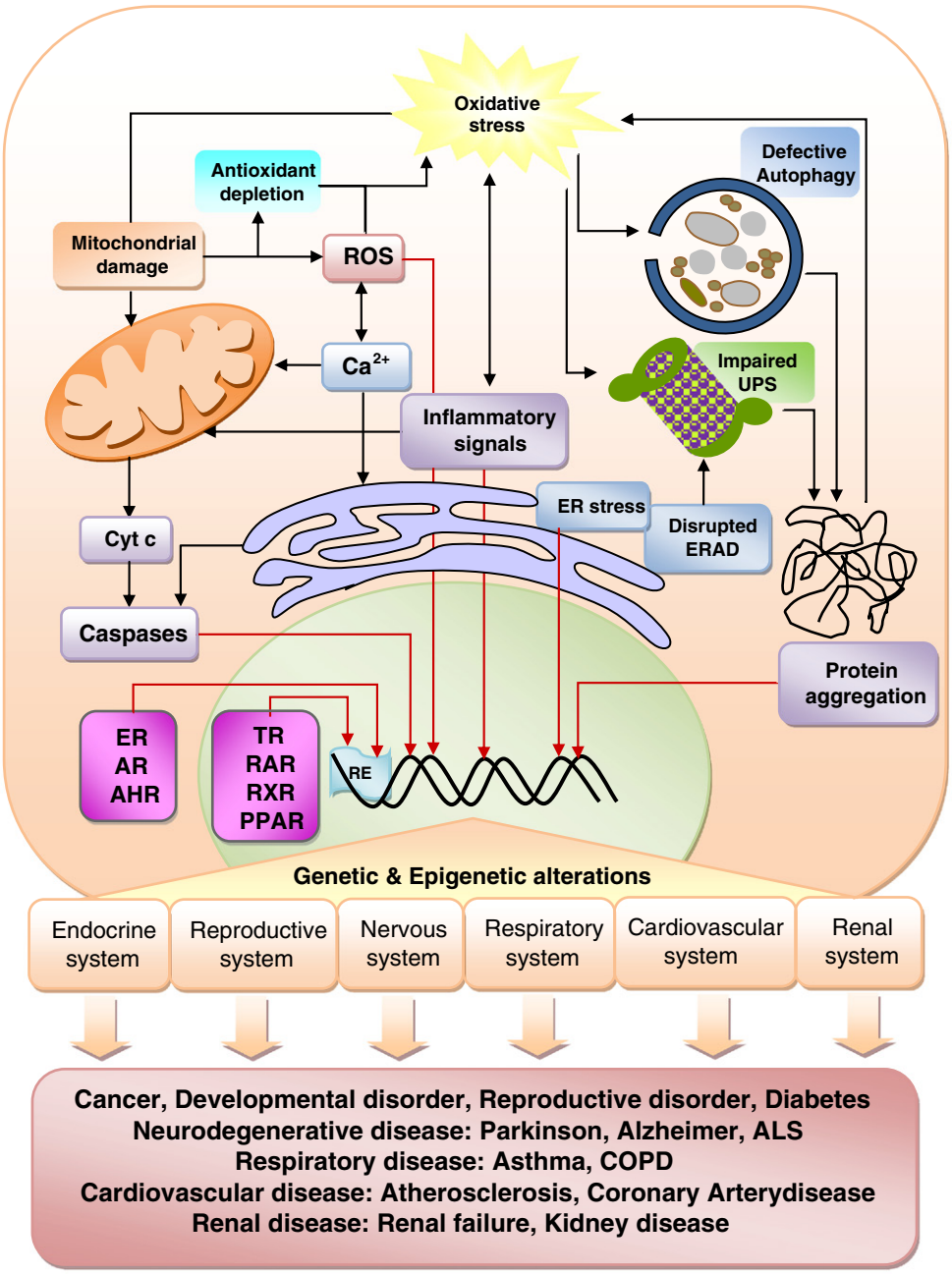

Fig. 3. A simplified model for mechanisms by which pesticides induce and develop chronic disease, ROS: reactive oxygen species, Cyt c: cytochrome c, UPS: ubiquitin proteasome system, ER stress: endoplasmic reticulum stress, ERAD: endoplasmic reticulum associated degradation, ER: estrogen receptor, AR: androgen receptor, AHR: aryl hydrocarbon receptor, TR: thyroid receptor, RAR: retinoic acid receptor, RXR: retinoid X receptor, PPAR: peroxisome proliferator-activated receptor, RE: response element, ALS: amyotrophic lateral sclerosis, COPD: chronic obstructive pulmonary disease.

On the other hand, ER stress and related pathways have been reported to be involved in cytotoxicity of some pesticides. Paraquat, a bipyridyl herbicide, which is suspected to increase the risk of Parkinson disease following chronic exposures, has been reported to induce ER stress and trigger dopaminergic cell death by enhanced cleavage of a small ER co-chaperone protein, p23, and inhibition of ERAD (Chinta et al., 2008). Elevated level of ER stress biomarkers like glucose-regulated protein 78 (GRP78), ER degradation-enhancing-α-mannosidase-like protein (EDEM), and C/EBP homologous protein (CHOP) has also been implicated in paraquat-induced toxicity in human neuroblastoma cells. Further, paraquat activated calpain and caspase 3 along with ER-induced cascade inositol-requiring protein 1 (IRE1)/apoptosis signal-regulating kinase 1 (ASK1)/C-Jun N-terminal kinase (JNK) (Yang et al., 2009). In another study carried out on neuroblastoma cells, rotenone-induced ER stress has become evident by increased phosphorylation of protein kinase RNA-like endoplasmic reticulum kinase (PERK), protein kinase RNA-activated (PKR), and eukaryotic initiation factor 2-a (eIF2a) as well as the expression of GRP78. Moreover, rotenone activates glycogen synthase kinase 3β (GSK3β), an ER related multifunctional serine/threonine kinase implicated in the pathogenesis of neurodegeneration (Chen et al., 2008). Deltamethrin, a pyrethroid pesticide, has been reported to induce apoptosis through ER stress pathway involving eIF2α, calpain and caspase 12 (Hossain and Richardson, 2011). Induction of apoptosis by pyrrolidine dithiocarbamate (PDTC)/Cu complex, a widely used pesticide, has also been linked to the ER stress-associated signaling molecules, including GRP78, GRP94, caspase-12, activating transcription factor 4 (ATF4), and CHOP in lung epithelial cells (Chen et al., 2010). Chloropicrin an aliphatic nitrate pesticide has been indicated to increase ER stress-related proteins, including GRP78, IRE1α, and CHOP/GADD 153 in human retinal pigment epithelial cells (Pesonen et al., 2012). Some other pesticides belonging to the organochlorines (endosulfan), carbamates (formetanate, methomyl, pyrimicarb), and pyrethroids (bifenthrin) have been evaluated for their effects on stress proteins among which upregulation of the ER chaperone GRP78 and downregulation of the cytosolic chaperone HSP72/73 were significant. These effects can occur when ER is under stress and the UPR result in increased expression of ER chaperones and decreased protein synthesis in the cytosol (Skandrani et al., 2006a,b).

Protein aggregation

Degradation of misfolded, damaged or unneeded proteins is a fundamental biological process which has a crucial role in maintenance and regulation of cellular function. There are two major cellular mechanisms for protein degradation; ubiquitin proteasome system (UPS) that mainly targets short-lived proteins by proteases, and autophagy that mostly clears long-lived and poorly soluble proteins through the lysosomal machinery (Gies et al., 2010). UPS is composed of ubiquitin for tagging and proteasomes for proteolysis of proteins, which are to be degraded. Deregulation of this system has been implicated in the pathogenesis of several chronic diseases, mostly neurodegeneration and cancers evidenced by decreased and increased proteasome activity, respectively (Paul, 2008). Environmental exposure to certain pesticides has been linked to proteasomal dysfunction in development of neurodegenerative diseases. The organochlorine pesticide dieldrin has been reported to decrease proteasome activity along with enhanced sensitivity to occurrence of apoptosis in dopaminergic neuronal cells (Sun et al., 2005). Proteasome inhibition has also been shown in neuroblastoma cells exposed to rotenone, ziram, diethyldithiocarbamate, endosulfan, benomyl, and dieldrin (Chou et al., 2008; Wang et al., 2006). Paraquat has also been noted to impair UPS given by decreased proteasome activity and increased ubiquitinated proteins in DJ-1 deficient mice and dopaminergic neurons (Yang and Tiffany-Castiglioni, 2007; Yang et al., 2007). Increased degradation of proteasome components has been presented as the mechanism of proteasome inhibition by rotenone, an inducer of Parkinson (Chou et al., 2010).

The lysosomal degradation pathway of autophagy is known as a self-digestion process by which cells not only get rid of misfolded proteins, damaged organelles and infectious microorganisms but also provide nutrients during fasting. Defect of this process has found an emerging role in many human diseases such as cancer, neurodegeneration, diabetes, aging, and disorders of the liver, muscle, and heart (Gonzalez et al., 2011; Levine and Kroemer, 2008; Shintani and Klionsky, 2004). There are a few reports on the involvement of defective autophagy in toxic effects of pesticides. A relationship between autophagy and paraquat-induced apoptosis in neuroblastoma cells was shown by Gonzalez-Polo and colleagues in 2007 (Gonzalez-Polo et al., 2007). This effect was confirmed in another study in which paraquat-induced autophagy was attributed to the occurrence of ER stress (Niso-Santano et al., 2011). Lindan, a broad-spectrum organochlorine pesticide, has been reported to promote its toxicity through disruption of an autophagic process in primary rat hepatocytes (Zucchini-Pascal et al., 2009) (Fig. 3).

Conclusion

Taken together, chronic diseases discussed above are considered as the major disorders affecting public health in the 21st century. The relationship between these diseases and environmental exposures, particularly pesticides increasingly continues to strengthen. Near to all studies carried out in the area of pesticides, and chronic diseases are categorized in the field of epidemiologic evidence or experimental investigation with mechanistic insight into the disease process. Some epidemiologic studies have been debated on their uncertainty in elicitation of a definite conclusion because of some restrictions. However, existence of more than a few dozen reports on the association of one case like brain cancer with exposure to pesticide is enough to create concern even without finding a direct link. Abundance of evidence in this regard has promoted scientist to evaluate the mechanisms by which pesticides develop chronic diseases. Although there remains a lot to do in this way, several mechanisms and pathways have been clarified for pesticide-induced chronic diseases. It should not be forgotten that these mechanisms work alongside or sequentially rather than singly in most cases, or they even can potentiate genetically susceptible individuals. However, the body of studies in this respect has become massive enough to consider pesticide exposure as a potential risk factor for developing chronic diseases. Considering chronic diseases as the most important global health problems it is time to find a preventive approach in association with agrochemicals by logical reducing pesticide use or pesticide dependency and find efficient alternatives for hazardous ones.

Conflict of interest statement

There is no competing interest.

Acknowledgments

Authors wish to thank assistances of INSF and TUMS.

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Arch Toxicol (2017) 91:549–599
DOI 10.1007/s00204-016-1849-x

REVIEW ARTICLE

Pesticides: an update of human exposure and toxicity

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Received: 11 August 2016 / Accepted: 8 September 2016 / Published online: 8 October 2016

Abstract Pesticides are a family of compounds which have brought many benefits to mankind in the agricultural, industrial, and health areas, but their toxicities in both humans and animals have always been a concern. Regardless of acute poisonings which are common for some classes of pesticides like organophosphoruses, the association of chronic and sub-lethal exposure to pesticides with a prevalence of some persistent diseases is going to be a phenomenon to which global attention has been attracted. In this review, incidence of various malignant, neurodegenerative, respiratory, reproductive, developmental, and metabolic diseases in relation to different routes of human exposure to pesticides such as occupational, environmental, residential, parental, maternal, and paternal has been systematically criticized in different categories of pesticide toxicities like carcinogenicity, neurotoxicity, pulmonotoxicity, reproductive toxicity, developmental toxicity, and metabolic toxicity. A huge body of evidence exists on the possible role of pesticide exposures in the elevated incidence of human diseases such as cancers, Alzheimer, Parkinson, amyotrophic lateral sclerosis, asthma, bronchitis, infertility, birth defects, attention deficit hyperactivity disorder, autism, diabetes, and obesity. Most of the disorders are induced by insecticides and herbicides most notably organophosphorus, organochlorines, phenoxyacetic acids, and triazine compounds.

Keywords Pesticide · Toxicity · Chronic disease · Review

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Introduction

Pesticides are a large and heterogeneous group of chemicals which have long been used to control and repel pests in different fields. Controlling pests have always been a concern for human life. The literature shows that natural and inorganic chemicals were sporadically used for this purpose, but development of new and potent organic chemical targets has brought pesticides into widespread use during the past century. Human has benefited pesticides in different fields like producing and keeping more and safe agricultural products, repelling home pests, and controlling infectious diseases among which malaria eradication program was a remarkable feature of insecticides' use. Human exposure to pesticides can occur through different routes, including occupations dealing with production, transport, delivery and application of pesticides, residing in the places high in pesticide residue, and circulation and accumulation of pesticides in the food chain. Since pesticides were born as chemicals to be toxic for living organisms, their toxicity for human and the other animal species is inevitable. This issue becomes further apparent in the huge and growing body of epidemiological and experimental evidence on the link between exposure to pesticides and the incidence of

various health disorders in human beings. Incidence of different human diseases like malignant, neurodegenerative, reproductive, developmental, respiratory, and metabolic diseases in association with exposure to pesticides has frequently become the research topic of numerous studies (Mostafalou and Abdollahi 2012a).

In the previous study, the relation between exposure to pesticides and incidence of different types of human chronic diseases was studied via a systematic review of epidemiological evidence and exploring the involved mechanisms. The results revealed that the largest share was accounted for incidence of cancers and then neurodegenerative, reproductive, and developmental disorders in association with exposure to pesticides (Mostafalou and Abdollahi 2013).

In the current work, diverse toxicities of pesticides within the context of known and prevalent human chronic diseases are updated via a systematic review.

Methodology

Article search

We performed a PubMed search of the literature on the association between pesticide exposure and human diseases. We restricted our search to articles published since 1980. The search used a combination of the following words: pesticides, cancer, bladder cancer, bone tumors, brain tumors, breast cancer, cervical cancer, colorectal cancer, eye cancer, gallbladder cancer, kidney cancer, laryngeal cancer, leukemia, lip cancer, liver cancer, lung cancer, lymphoma, melanoma, mouth cancer, multiple myeloma, neuroblastoma, esophageal cancer, ovarian cancer, pancreatic cancer, soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, uterine cancer, Alzheimer, Parkinson, amyotrophic lateral sclerosis (ALS), asthma, chronic bronchitis, birth defects, infertility, attention deficit hyperactivity disorder (ADHD), autism, developmental delay, diabetes, obesity, and humans. Details of the search are given in Fig. 1.

Article criteria

In order to identify eligible articles, the titles and abstracts and, if needed, the full text of the papers were reviewed. The following characteristics were considered as inclusion criteria for recording articles:

1. Written and published in English
2. Type of study as cross-sectional, case–control, cohort, ecological, and/or meta-analyses
3. Exposure assessment tool as interviews, questionnaires, geographic information system (GIS), job expo-

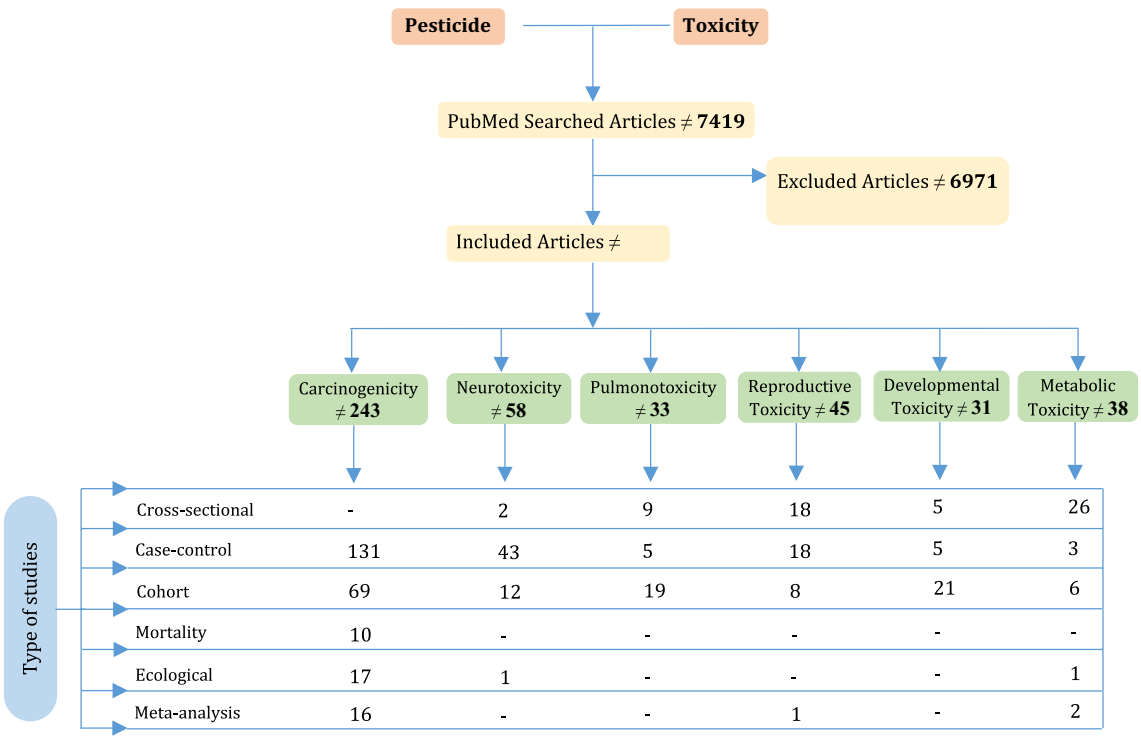

Fig. 1 Flow chart and category of the articles included and excluded in the systematic review

sure matrix (JEM), and/or residue detection in biological samples
4. Reported association of chronic diseases with pesticide exposures

Data extraction

The following information was extracted from eligible papers and presented in the classified tables:

1. Authors
2. Publication date
3. Type of study
4. Number of samples in each study
5. Exposure assessment tool
6. Type of exposure
7. Type of specific pesticide if reported
8. Type of specific disease in each category of toxicity
9. Quantitative risk estimate as the odd ratio (OR), hazard ratio (HR), relative risk or risk ratio (RR), proportional mortality ratio (PMR), standardized mortality ratio (SMR), mortality rate ratio (MRR), and standardized incidence ratio (SIR)
10. Confidence interval for reported risk estimates
11. Significance of the risk as *p* value if reported

Results

The PubMed searches yielded a total of 7419 unique articles. The number of records for each category of toxicity in combination with pesticides was as follows; carcinogenicity 3410, neurotoxicity 1342, pulmonotoxicity 512, reproductive toxicity 833, developmental toxicity 124, metabolic toxicity 1198. After screening the titles, abstracts, and full text of the papers, the irrelevant ones were excluded and the number of remaining articles for reviewing in each category became as follows: carcinogenicity 246, neurotoxicity 58, pulmonotoxicity 33, reproductive toxicity 46, developmental toxicity 31, and metabolic toxicity 38 (Fig. 1).

Disease-based evidence on carcinogenicity of pesticides

The World Health Organization (WHO) defines cancer as a generic term for a large group of neoplastic diseases affecting each part of the body. Cancer is the leading cause of mortality worldwide with almost 8.2 million cancer-related deaths in 2012. In the same year, new cases of cancer were estimated 14 million, which is expected to increase by 70 % over the next two decades. The most common cancer-related deaths are due to lung, liver, stomach, colorectal, breast, and esophageal cancer. Cancer is the result of genetics–environmental interactions, which can be relatively induced under the effect of biological, physical, and chemical exposures (WHO 2015). The association of exposure to different classes of pesticides, including insecticides, herbicides, and fungicides with incidence of cancers has been highlighted during the past half century. Different types of surveys have targeted the link of pesticides with cancers and reported various risk estimates. The number of reports evidencing a positive association between exposure to pesticides and cancer incidence is considerable, and the relevant ones resulted from population-based human studies have been reviewed and classified according to the site of cancer (Table 1).

Tumors of the nervous system

Brain tumors In general, studies concerning the environmental risk factors of brain tumors are separately conducted in children and adults. The link of childhood brain tumors (CBT) with pesticides is mostly studied in the form of parental, maternal, or paternal exposures. Results of a prospective cohort study of cancer in the offspring of agricultural censuses in Norway showed that parental exposure to pesticides is associated with three times higher incidence of CBT especially in children aged under 14 years (Kristensen et al. 1996). The other case–control studies assessing exposure to different classes of pesticides via organized questionnaire-based interviews indicated that incidence of CBT was increased up to 1.3–2 times in children parentally exposed to pesticides (Efird et al. 2003; Greenop et al. 2013; Pogoda and Preston-Martin 1997; Rosso et al. 2008; Shim et al. 2009; van Wijngaarden et al. 2003). Searles Nielsen and colleagues' studies on the role of genetic polymorphisms of *PON1* and *FMO1* in the link between pesticides and CBT implicated that prenatal and postnatal exposures to organophosphorus compounds and perhaps carbamates in people with reduced ability to detoxification were associated with a higher incidence of CBT (Searles Nielsen et al. 2005, 2010). However, there are other studies whose results have been meta-analyzed in some systematic reviews. A meta-analysis of 40 studies found an incidence risk of about times for CBT in children paternally exposed to pesticides (Vinson et al. 2011). In this regard, there is another study, which meta-analyzed the results of 15 studies on the association of CBT with paternal, maternal, and childhood exposures to pesticides and the highest risk estimate of CBT was reported for children whose fathers were exposed to pesticides before conception (Kunkle et al. 2014).

The link of adult brain tumors (ABT) with pesticides has been mostly studied in the populations occupationally dealing with pesticides. Since the disease has a high intrinsic severity, some researchers have reported high mortality ratio as of 200 and 270 due to brain tumors in licensed pesticide users (Blair et al. 1983; Figa-Talamanca et al.

Table 1 Carcinogenicity of pesticides evidenced by diseases

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Childhood brain tumors							
Greenop (2013)	CC	374/1467	Questionnaire	Par.	Home pest control	1.90 (1.08–3.36)	
					Pat. occupation	1.36 (0.66, 2.80)	
Searles Nielsen (2010)	CC	201/285	Interview	Res.	OPs + *PON1* polymorph OPs + *FMO1* polymorph	1.8 (1.1–3.0) 2.7 (1.2–5.9)	
Shim (2009)	CC	526/526	Interview	Par.	Herbicides	1.8 (1.1–3.1)	
Rosso (2008)	CC	318/318	Interview	Par.	During pregnancy After birth	1.6 (1.0, 2.5) 1.8 (1.2, 2.8)	
Searles Nielsen (2005)	CC	66/236	Interview	Par.	+*PON1* polymorph	2.6 (1.2–5.5)	
van Wijngaarden (2003)	CC	322/321	Interview	Pat.	Insecticides Herbicides Fungicides	1.5 (0.9, 2.4) 1.6 (1.0, 2.7) 1.6 (1.0, 2.6)	
Efird (2003)	CC	1218/2223	Interview	Mat.	Pesticide	2	
Pogoda and Preston-Martin (1997)	CC	224/218	Interview	Mat.	Flea/tick pesticides	1.7 (1.1–2.6)	
Kristensen (1996)	Co	323292	Census	Par.	–	3.37 (1.63–6.94)	
Vinson (2011)	MA	40 studies	Before birth	Pat.	–	1.49 (1.23–1.79)	
			After birth	Pat.	–	1.66 (1.11–2.49)	
Kunkle (2014)	MA 15 studies	3 5	Preconception	Pat.	–	2.29 (1.39–3.78)	
			In pregnancy	Pat.	–	1.63 (1.16–2.31)	
		5 7	Agricultural	Mat.	–	1.48 (1.18–1.84)	
			Non-agricultural	Mat.	–	1.36 (1.10–1.68)	
		4 5	Agricultural	Childhood	–	1.35 (1.08–1.70)	
			Non-agricultural	Childhood	–	1.32 (1.04–1.67)	
Adult brain tumors							
Samanic (2008)	CC	657/765	Interview	Occup.	Herbicides (in women)	2.4 (1.4, 4.3)	0.01
Provost (2007)	CC	221/442	Interview-JEM	Occup.	–	2.16 (1.10–4.23)	
Lee (2005)	CC	251/498	Interview	Occup.	Metribuzin Paraquat Bufencarb Chlorpyrifos Coumaphos	3.4 (1.2–9.7) 11.1 (1.2–101) 18.9 (1.9–187) 22.6 (2.7–191) 5.9 (1.1–32)	
Viel (1998)	Ec	89 units	GIS	Occup.	Vineyard pesticides	1.10 (1.03–1.18)	
Rodvall (1996)	CC	192/192	Questionnaire	Occup.	In men	1.8 (0.6–5.1)	
Figa-Talamanca (1993)	Mr	2310	Licensed users	Occup.	In men	SMR: 270 (108.6–556.9)	
Musicco (1988)	CC	240/742	Interview	Occup.	Insecticides, fungicides	2.0	0.006
Blair (1983)	Mr	3827	Licensed users	Occup.	In men	SMR: 200	
Neuroblastoma							
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	1.8 (1.5–2.1)	
Giordano (2006)	Co	168	Applicators	Occup.	–	SMR; 529.2 (144–1368)	
Daniels (2001)	CC	538/538	Interview	Res.	Home used Garden used	1.6 (1.0–2.3) 1.7 (0.9–2.1)	
Feychting (2001)	Co	235635	Census	Pat.	–	2.36 (1.27–4.39)	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Littorin (1993)	Co	2370	Applicators	Occup.	Insecticides, fungicides	SMR; 2.9 (1.1, 6.2)	
Kristensen (1996)	Co	323292	Census	Par.	–	2.38 (1.03–6.13)	
Esophageal cancer							
Meyer (2011)	CC	5782/5782	Workers	Occup.	–	1.38 (1.26–1.51)	
de Rezende Chrisman (2009)	Ec	11 states	Pesticide sales	Res., Occup.	–	MRR; 2.40 (2.34–2.45)	0.046
Jansson (2006)	CC	356/820	Airborne level	Occup.	–	2.3 (0.9 to 5.7)	
Stomach cancer							
Barry (2012)	Co	53588	Questionnaire	Occup.	Methyl bromide	3.13 (1.25–7.80)	0.02
Mills and Yang (2007)	Nested CC	100/210	Questionnaire	Occup.	2,4-D Chlordane Trifluralin herbicide	1.85 (1.05–3.25) 2.96 (1.48–5.94) 1.69 (0.99–2.89)	
Van Leeuwen (1999)	Ec	40 states	Drinking water	Env.	Atrazine	1.45 (1.20–1.70)	<0.05
Forastiere (1993)	CC	1674/480	Questionnaire	Occup.	–	1.77 (0.75–4.25)	
Colorectal cancer							
Lerro (2015b)	Co	33,484	Interview	Occup.	Acetochlor	1.75 (1.08–2.83)	
Salerno (2014)	Ec	Vercelli	GIS	Gen.	–	2.38 (1,76–2,87)	
Lo (2010)	CC	421/439	Interview	Occup.	Pesticides Insecticides Herbicides	2.6 (1.1–5.9) 3.2 (1.5–6.5) 5.5 (2.4–12.3)	
				Dietary	–	4.6 (1.5–14.6)	
Koutros (2009)	Co	57311	Questionnaire	Occup.	Imazethapyr	2.73 (1.42–5.25)	0.001
Kang (2008)	Co	50127	Questionnaire	Occup.	Trifluralin	1.76 (1.05–2.95)	
van Bemmel (2008)	Co	48378	Questionnaire	Occup.	EPTC	2.09 (1.26–3.47)	<0.01
Lee (2007b)	Co	56813	Questionnaire	Occup.	Chlorpyrifos Aldicarb	2.7 (1.2–6.4) 4.1 (1.3–12.8)	0.008 0.001
Samanic (2006)	Co	41969	Questionnaire	Occup.	Dicamba	3.29 (1.40–7.73)	0.02
Zhong and Rafnsson (1996)	Co	2449	Questionnaire	Occup.	–	2.94 (1.07–6.40)	
Forastiere (1993)	CC	1674/480	Questionnaire	Occup.	–	2.82 (0.75–9.32)	
Liver cancer							
VoPham (2015)	CC	3034/14991	GIS	Gen.	OCs	2.76 (1.58–4.82)	0.0004
de Rezende Chrisman (2009)	Ec	11 states	Pesticide sales	Res., Occup.	–	MRR; 1.49 (1.44–1.54)	
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	3.3 (2.1–5.0)	
Giordano (2006)	Co	168	Applicators	Occup.	–	SMR; 596.3 (204–1365)	
Gallbladder cancer							
Shukla (2001)	CC	30/30	Biliary level	–	HCB DDT	↑ level in cases ↑ level in cases	<0.04 <0.03
Giordano (2006)	Co	168	Applicators	Occup.	–	SMR; 723.8 (129–2279)	
Pancreatic cancer							
Lerro (2015b)	Co	33484	Interview	Occup.	Acetochlor	2.36 (0.98–5.65)	
Antwi (2015)	CC	2092/2353	Questionnaire	Gen.	–	1.21 (1.02–1.44)	
Andreotti (2009)	CC	93/82503	Questionnaire	Occup.	Pendimethalin EPTC herbicide	3.0 (1.3–7.2) 2.56 (1.1–5.4)	0.01 0.01
de Rezende Chrisman (2009)	Ec	11 states	Pesticide sales	Res., Occup.	–	MRR; 2.32 (2.23–2.40)	0.040
Lo (2007)	CC	194/194	Interview	Gen.		2.6 (0.97–7.2)	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Ji (2001)	CC	484/2095	JEM	Occup.	Pesticides Fungicides Herbicides	1.4 (1.0–2.0) 1.5 (0.3–7.6) 1.6 (0.7–3.4)	0.01
Alguacil (2000)	CC	164/238	JEM	Occup.	Arsenical pesticides Other pesticides	3.4 (0.9–12.0) 3.17 (1.1–9.2)	
Cantor and Silberman (1999)	Mr	9961		Occup.	–	2.71	
Forastiere (1993)	CC	1674/480	Questionnaire	Occup.	–	5.18 (1.55–16.7)	
Alavanja (1990)	Nested CC	22938	Death certificate	Occup.	–	SMR; 133	
Childhood leukemia							
Zhang et al.(2015)	CC	248/111	Urine level	Res.	OPs	1.9 (1.2–3.1)	<0.05
Maryam et al. (2015)	CC	94/94	Interview	Par.	–	4.2 (2.2–7.8)	<0.001
Metayer et al. (2013)	CC	269/333	Dust sample	Res.	Chlorthal	1.57 (0.90–2.73)	0.05
Ding G et al. (2012)	CC	176/180	Urine sample	–	Pyrethroids	2.75 (1.43–5.29)	
Bailey et al. (2011)	CC	388/870	Preconception In pregnancy After birth	Occup.	–	1.19 (0.83–1.69) 1.30 (0.86–1.97) 1.24 (0.93–1.65)	
Soldin et al. (2009)	CC	41/77	Questionnaire	Mat.	Insecticides	↑ risk	0.02
Rull et al. (2009)	CC	213/268	Questionnaire	Res.	Insecticides Herbicides Fungicides	1.5 (0.9–2.4) 1.2 (0.8–1.9) 1.2 (0.7–2.4)	
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	1.2 (1.1–1.3)	
Rudant (2007)	CC	764/1681	Questionnaire	Mat. Pat.	Household use Household use	2.1 (1.7–2.5) 1.5 (1.2–1.8)	
Monge P et al. (2007)	CC	334/579	Interview	Mat.	–	2.2 (1.0–4.8)	
Menegaux et al. (2006)	CC	280/280	Interview	Mat.	–	1.8 (1.2 to 2.8)	
Reynolds et al. (2005)	CC	2189/4335	Questionnaire	Mat.	Metam sodium Dicofol	2.05 (1.01–4.17) 1.83 (1.05–3.22)	
Ma et al. (2002)	CC	162/162	Interview	Mat.	Household use Insecticides	2.8 (1.4–5.7) 2.1 (1.3–3.5)	
Alexander FE et al (2001)	CC	136/266	Questionnaire	Mat.	Baygon/mosquito-cidal	5.14 (1.27–20.85)	0.02
Infante-Rivard et al. (1999)	CC	491/491	Questionnaire	Mat.	Insecticides Herbicides	2.47 (1.43–4.28) 1.84 (1.32–2.57)	
Meinert et al. (1996)	CC	173/175	Questionnaire	Par.	Garden used	2.52 (1.0–6.1)	
Leiss and Savitz (1995)	CC	252/222	Interview	Res.	Household use	1.7 (1.2–2.4)	
Mulder et al. (1994)	CC	14/52	Questionnaire	Res. Pat.	– –	6.0 (0.6–49.3) 3.2 (1.0–10.1)	
Buckley et al (1989)	CC	204/		Pat. job	–	2.7 (1.0–7.0)	0.06
Shu et al. (1988)	CC	309/618		Mat. job	–	3.5 (1.1–11.2)	
Chen (2015)	MA	16 studies		Res.	Indoor pesticides Herbicides	1.47 (1.26–1.72) 1.26 (1.10–1.44)	
Bailey 2014)	MA (13)	8236/14850		Mat.	–	1.01 (0.78–1.30) for ALL 1.94 (1.19–3.18) for AML	
		8169/14201		Pat.	–	1.20 (1.06–1.38) for ALL 0.91 (0.66–1.24) for AML	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Vinson (2011)	MA	40 CC		Mat.	–	1.48 (1.26–1.75)	
Van Maele-Fabry (2011)	MA	13 CC (1966–2009)		Res., Mat.	–	1.74 (1.37–2.21)	
Turner (2011)	MA	17 CC (1950–2009)		Res.	Pesticides Insecticides Herbicides	1.54 (1.13–2.11) 2.05 (1.80–2.32) 1.61 (1.20–2.16)	
Wigle (2009)	MA	31 CC (1950–2009)		Mat.	Pesticides Insecticides Herbicides	2.09 (1.51–2.88) 2.72 (1.47–5.04) 3.62 (1.28–10.3)	
Adult leukemia							
Baumann Kreuziger (2014)	Co	195	Interview	Occup.	Agent Orange	1.8 (0.7–4.5)	0.24
Bonner (2010)	Co	57310	Questionnaire	Occup.	Terbufos	2.38 (1.35–4.21)	
Miligi (2006)	CC	1925/1232	Questionnaire	Occup.			
Beane Freeman (2005)	Co	23106	Questionnaire	Occup.	Diazinon	3.36 (1.08–10.49)	0.026
Cantor and Silberman (1999)	Mr	9961		Occup.	–	SMR: 3.35	
Ciccone et al. (1993)	CC	67/246	Interview	Occup.	–	4.4 (1.7–11.5)	
Brown (1990)	CC	578/1245	Interview	Occup.	Crotoxyphos Dichlorvos Famphur Pyrethroids Methoxychlor	11.1 (2.2–55.0) 2.0 (1.2–3.5) 2.2 (1.0–5.0) 3.7 (1.3–10.6) 2.2 (1.0–5.0)	
Van Maele-Fabry (2008)	MA	14 studies (1984–2004)		Occup.	–	1.43 (1.05–1.94)	
Van Maele-Fabry (2007)	MA	17 Co (1979–2005)		Occup.	–	1.21 (0.99–1.48)	
Merhi (2007)	MA	13 CC (1990–2005)		–	–	1.35 (0.9–2)	
Hodgkin lymphoma							
Navaranjan (2013)	CC	316/1506	Interview		Insecticides OPs Carcinogen pesticides	1.88 (0.92–3.87) 3.16 (1.02–9.29) 2.47 (1.06–5.75)	
Karunanayake (2012)	CC	316/1506	Interview		Chlorpyrifos	1.19 (1.03–1.37)	
Pahwa (2009)	CC	316/1506	Interview		Dichlorprop	6.35 (1.56–25.92)	
Rudant (2007)	CC	130/1681	questionnaire	Mat.	Household use	4.1 (1.4–11.8)	
Orsi et al. (2007)	CC	824/752	Interview	Occup.	–	2.2 (1.0–4.7)	
van Balen et al. (2006)	CC	591/631	Interview	Occup.	Non-arsenicals	1.8 (1.1 to 2)	
Flower (2004)	Co	17357	Questionnaire	Par.	–	2.56 (1.06–6.14)	
Cerhan (1998)	Mr	88090	Death certificate	Occup.	–	PMR; 1.62 (1.04–2.54)	
Persson (1993)	CC	31/93	Questionnaire	Occup.	Phenoxy herbicides Other pesticides	2.6 (1.4–40) 2.0 (0.05–3.2)	
Non-hodgkin lymphoma							
Nordstrom (1998)	CC	121/484	Interview	Occup.	Insecticides Herbicides Fungicides	2.0 (1.1–3.5) 2.9 (1.4–5.9) 3.8 (1.4–9.9)	
Schinasi (2015)	Co	76493	Questionnaire	Occup.	Insecticides	1.12 (0.95–1.32)	
Coggon (2015)	Co	8036	Questionnaire	Occup.	Phenoxy herbicides	SMR; 1.85 (1.12–2.89)	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Schinasi and Leon (2014)	MA	44 studies			OPs Carbamates Phenoxy herbicides Lindane	1.6 (1.4–1.9) 1.7 (1.3–2.3) 1.4 (1.2–1.6) 1.6 (1.2–2.2)	
Alavanja (2014)	Co	54,306	Questionnaire	Occup.	Lindane DDT	2.5 (1.4–4.4) 1.7 (1.1–2.6)	0.004 0.02
Balasubramaniam (2013)	CC	390/1383	Interview	Occup.	–	3.1 (1.5–6.2)	<0.01
Karunanayake (2013)	CC	75/321	Questionnaire	Occup.	–	3.08 (1.26–7.53)♂	
Boccolini Pde (2013)	Ec	552 micro-region	GIS	Gen. –	– –	MRR; 2.92 (2.74–3.11)♂ MRR; 3.20 (2.98–3.43)♀	
Bräuner (2012)	Co	57053	Adipose tissue level		DDT cis-nonachlor Oxychlordane	1.35 (1.10–1.66) 1.13 (0.94–1.36) 1.11 (0.89–1.38)	
Pahwa (2012a)	CC	513/1506	Interview	Occup.	Phenoxy herbicide	2.67 (0.90–7.93)	
Viel (2011)	CC	34/34	Serum level	Res.	β-HCH DDT	1.05 (1.00–1.12) 1.20 (1.01–1.45)	
Bonner (2010)	Co	57310	Questionnaire	Occup.	Terbufos	1.94 (1.16–3.22)	
Ruder and Yiin (2011)	Co	2122	Plant workers	Occup.	Pentachlorophenol	SMR; 1.77 (1.03–2.84)	
Eriksson (2008)	CC	910–1016	Questionnaire		Herbicides Phenoxy herbicides Glyphosate Insecticides	1.72 (1.18–2.51) 2.81 (1.27–6.22) 2.26 (1.16–4.40) 1.28 (0.96–1.72)	
Vajdic (2007)	CC	694/694	Questionnaire	Occup.	–	4.23 (1.76–10.16)	
Rudant (2007)	CC	166/1681	Questionnaire	Mat. Pat.	Household use Household use	1.8 (1.3–2.6) 1.7 (1.2–2.6)	
Purdue (2007)	Co	57311	Questionnaire	Occup.	Lindane	2.6 (1.1–6.4)	0.04
Merhi (2007)	MA	13 CC (1990–2005)			–	1.35 (1.2–1.5)	
Chiu (2006)	CC	385/1432	Interview	Gen.	Animal insecticides Crop insecticides Herbicides Fumigants	2.6 (1.0–6.9) 3.0 (1.1–8.2) 2.9 (1.1–7.9) 5.0 (1.7–14.5)	
Miligi (2006)	CC	1925/1232	Questionnaire	Occup.	2,4-D	4.4 (1.1–29.1)	
De Roos (2003)	CC	870/2569	Interview	Gen.	Coumaphos Diazinon Fonofos Chlordane Dieldrin Atrazine Glyphosate	2.4 (1.0–5.8) 1.9 (1.1–3.6) 1.8 (0.9–3.5) 1.5 (0.8–2.6) 1.8 (0.8–3.9) 1.6 (1.1–2.5) 2.1 (1.1–4.0)	
Hardell (2002)	CC	515/1141	Questionnaire		Herbicides Insecticides Fungicides	1.75 (1.26–2.42) 1.43 (1.08–1.87) 3.11 (1.56–6.27)	
Schroeder (2001)	CC	182/	Questionnaire	Occup.	Dieldrin Toxaphene Lindane Atrazine Fungicides	3.7 (1.9–7.0) 3.0 (1.5–6.1) 2.3 (1.3–3.9) 1.7 (1.0–2.8) 1.8 (0.9–3.6)	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
McDuffie (2001)	CC	517/1506	Interview		Phenoxyherbicides Dicamba Carbamate OPs	1.38 (1.06–1.81) 1.88 (1.32–2.68) 1.92 (1.22–3.04) 1.73 (1.27–2.36)	
Meinert (2000)	CC	234/2588	Interview	Par.	Insecticides	2.6 (1.2–5.7)	0.02
Buckley (2000)	CC	268/268	Interview	Res.	Household use	7.3	0.05
Hardell and Eriksson (1999)	CC	442/884	Interview	Gen.	Herbicides Fungicides	1.6 (1.0–2.5) 3.7 (1.1–13.0)	
Kristensen (1996)	Co	323292	Census	Par.	–	2.47 (1.02–6.15)	
Clavel (1996)	CC	226/425	Interview	Occup.	–	1.7 (1.0–2.6)	
Cantor (1992)	CC	622/1245	Interview	Occup.	Carbaryl Chlordane DDT Diazinon Lindane Malathion	1.7 (0.9–3.1) 1.7 (1.0–2.9) 1.7 (1.2–2.6) 1.5 (0.9–2.5) 2.0 (1.0–3.7) 1.5 (0.8–2.7)	
Zahm (1990)	CC	201/725	Interview	Occup.	2,4-D	1.5 (0.9–2.5)	
Multiple myeloma							
Perrotta (2013)	CC	1959/6192	JEM	Occup.	Garden/nursery use	1.50 (0.9–2.3)	
Kachuri (2013)	CC	342/1357	Questionnaire		Fungicides Probably carcinogenic	1.73 (1.00–3.00) 1.57 (0.96–2.56)	0.04 0.03
Pahwa (2012b)	CC	342/1506	Questionnaire		Carbamate insecticide Captan fungicide Carbaryl	1.90 (1.11–3.27) 2.35 (1.03–5.35) 1.89 (0.98–3.67)	
Perrotta (2012)	CC	277/281	Questionnaire	Occup.	–	1.62 (1.01–2.58)	
Landgren (2009)	Co	57310	Questionnaire	Occup.	Age >50 years	6.8 (5.0–9.3)	
Lope (2008)	Co	2992166	Questionnaire	Occup.	In women	1.29 (0.83–2.00)	
Merhi (2007)	MA	13 CC (1990–2005)	–		–	1.16 (0.99–1.36)	
Cerhan (1998)	Mr	88090	Death certificate	Occup.	–	PMR; 1.17 (0.98–1.40))	
Kristensen (1996)	Co	323292	Census	Par.	–	2.03 (0.51–8.14)	
Bone cancer							
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	2.3 (1.8–2.9)	
Merletti (2006)	CC	96/2632	Interview	Occup.	–	2.33 (1.31–4.13)	
Moore (2005)	CC	196/196	Interview	Par.	–	3.0 (1.1–8.1)	
Holly (1992)	CC	43/193	Interview	Par.	–	6.1 (1.7–21.9)	0.002
Thorpe and Shirmohammadi (2005)	Ec	Maryland	Groundwater	Res.	Metolachlor	2.26 (0.97–5.24)	
Soft tissue sarcoma							
de Rezende Chrisman (2009)	Ec	11 states	Pesticide sales	Res., Occup.	–	MRR; 1.93(1.75–2.12)	0.015
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	1.7 (1.4–2.0)	
Kogevinas (1995)	Nested CC	11/55	Interview	Occup.	Phenoxy herbicides	10.3 (1.2–91)	
Leiss and Savitz (1995)	CC	252/222	Interview	Indoor	Yard treatment	4.1 (1.0–16.0)	
Kidney/renal cancer							
Karami (2008)	CC	1097/1476	Interview	Occup.	–	1.60 (1.00–2.55)	
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	3.3 (1.3–8.3)	
Tsai (2006)	CC	303/575	Interview	Mat.	–	1.41 (0.91–2.20)	Wilms

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Buzio et al. 2003	CC	100/200	Questionnaire	Occup.	+ *GSTM1* polymorph	3.46 (1.12–10.74)	
Buzio (2002)	CC	100/200	Questionnaire	Occup.	–	2.0 (0.8–4.7)	
Hu et al. (2002)	CC	1279/5370	Questionnaire	Occup.	Pesticides Herbicides	4.6 (1.7–12.5) 1.6 (1.3–2.0)	
Fear (1998)	Mr	167703		Pat.	–	PMR; 1.59 (1.18–2.15)	
Kristensen (1996)	Co	323292	Census	Par.	–	8.87 (2.67–29.5)	Wilms
Sharpe (1995)	CC	109/218	Interview	Pat. Mat.	–	3.24 (1.2–9.0) 128.6 (6.4–2,569)	Wilms Wilms
Mellemgaard (1994)	CC	365/396	Interview	Occup.	Insecticides/herbicides	2.2 (0.8–6.3)♂ 5.7 (0.6–58)♀	
Olshan (1993)	CC	200/233	Interview	Mat.	Household pesticide	2.16 (1.24–3.75)	
Forastiere (1993)	CC	1674/480	Questionnaire	Occup.	Olive crop used	3.16 (1.0–12.1)	<0.1
Bladder cancer							
Koutros (2015)	Co	57 310	Questionnaire	Occup.	Imazaquin herbicide Imazethapyr herbicide	1.54 (1.05–2.26) 3.03 (1.46–6.29)	0.005
Amr (2015)	CC	953/881		Occup.	–	1.68 (1.23–2.29)	
Matic (2014)	CC	143/114		Occup.	+ *GSTT1* polymorphism	4.5 (0.9–22.5)	
Sharma (2013)	CC	50/50	Blood level		Total-HCH, DDT	↑ risk	<0.05
Koutros (2009)	Co	57311	Questionnaire	Occup.	Imazethapyr herbicide	2.37 (1.20–4.68)	0.01
Prostate cancer							
Koutros (2013a, b)	CC	776/1444	Interview	Occup.	Malathion + *EHBP1*-SNP Aldrin +*TET2*-SNP	3.43 (1.44–8.15) 3.67 (1.43, 9.41)	0.003 0.006
Karami (2013)	CC	776/1444	Interview	Occup.	Parathion + Vit D gene	3.09 (1.10–8.68)	
Koutros (2013a, b)	Co	54412	Census	Occup.	Fonofos Malathion Terbufos Aldrin	1.63 (1.22–2.17) 1.43 (1.08–1.88) 1.29 (1.02–1.64) 1.49 (1.03–2.18)	0.001 0.04 0.03 0.02
Budnik et al. (2012)	MA	3 studies		Occup.	Methyl bromide	1.21 (0,98–1.49)	0.076
Barry (2011)	CC	776/1444	Interview	Occup.	Fonofos + *CT/TT*-SNP	3.25 (1.78–5.92)	
Cockburn (2011)	CC	173/162	GIS	Res.	Methyl bromide Organochlorines	1.62 (1.02–2.59) 1.64 (1.02–2.63)	
Band (2011)	CC	1516/4994	JEM	Occup.	Dichlone Maneb Ziram Simazine Azinphos-methyl Carbaryl DDT Diazinon Lindane Malathion	1.88 (1.01–3.52) 1.9 (1.09–3.30) 1.83 (1.08–3.10) 1.89 (1.08–3.33) 1.88 (1.06–3.32) 1.73 (1.09–2.74) 1.68 (1.04–2.70) 1.93 (1.21–3.08) 2.02 (1.15–3.55) 1.49 (1.02–2.18)	0.02 0.02 0.03 0.01 0.01 0.01 0.03 0.02 0.03 0.03
Koutros (2011)	CC	776/1444	Interview	Occup.	Terbufos + *MPO*-SNP	3.0 (1.5–6.0)	0.002

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Koutros (2010a, b)	Co	52394	Census	Occup.	Private use Commercial use	1.19 (1.14–1.25) 1.28 (1.00–1.61)	0.002
Multigner (2010)	CC	623/671	Plasma level	Gen.	Chlordecone	1.77 (1.21–2.58)	
Christensen (2010)	Co	47822	Questionnaire	Occup.	Coumaphos	1.65 (1.13–2.38)	0.004
Bonner (2010)	Co	57310	Questionnaire	Occup.	Terbufos	1.21 (0.99–1.47)	
Koutros (2010a, b)	CC	776/1444	Interview	Occup.	Fonofos + 8q24 variants	4.46 (2.17–9.17)	0.002
Parent (2009)	CC	49/183	Interview	Occup.	–	2.3 (1.1–5.1)	
de Rezende Chrisman (2009)	Ec	11 states	Pesticide sales	Res., Occup.	–	MRR; 1.66 (1.63–1.69)	0.019
Chamie (2008)	Co	13144	Veterans	Occup.	Agent Orange	2.19 (1.75–2.75)	
Meyer (2007)	CC	405/392	Interview	Occup.	–	1.6 (1.2–2.2)	
Settimi (2003)	CC	124/659	Interview	Occup.	Organochlorines	2.5 (1.4–4.2)	
Alavanja (2003)	Co	55332	Questionnaire	Occup.	Methyl bromide	3.47 (1.37–8.76)	0.004
Mills and Yang (2003)	Nested CC	222/1110		Occup.	Methyl bromide	1.59 (0.77–3.30)	0.25
MacLennan (2002)	Co	2045	Workers	Occup.	Triazine herbicides	SIR; 394 (128–920)	
Fleming (1999)	Co	33658	Applicators	Occup.	–	SIR; 1.91 (1.72–2.13)	
Cerhan (1998)	Mr	88090	Death certificate	Occup.	–	PMR; 1.26 (1.19–1.33)	
Dich and Wiklund (1998)	Co	20025	Applicators	Occup.	–	SIR; 1.13 (1.02–1.24)	
Forastiere (1993)	CC	1674/480	Questionnaire	Occup.	–	2.13 (0.64–6.49)	
Morrison (1993)	Ret. Co	1148	Acres sprayed	Occup.	Herbicides	2.23 (1.30–3.48)	<0.01
Testicular cancer							
Giannandrea (2011)	CC	50/48	Serum level	Res.	DDE, HCB	3.15 (1.00–9.91)	
Fleming (1999)	Co	33658	Questionnaire	Occup.	–	SIR; 2.48 (1.57–3.72)	
Breast cancer							
Parada (2016)	Mr	633	Blood level	–	DDT	2.72 (1.04–7.13)	
Niehoff (2016)	Co	50884	Interview	Gen.	DDT	1.3 (0.92–1.7)	
Lerro (2015a, b)	Co	30003	Questionnaire	Occup.	OPs	1.20 (1.01–1.43)	
Arrebola (2015)	CC	69/56	Serum level		β-HCH DDE	3.44 (1.30–9.72) 9.65 (1.81–63.33)	<0.1 <0.05
Yang (2015)	CC	75/79	Blood level		β-HCH, PCTA	↑ OCs level	<0.05
			Adipose tissue		β-HCH, DDE, PCTA	↑ OCs level	<0.05
Tang et al (2014a, b)	CC	78/72	Serum level	Diet	DDT	1.95 (0.95–4.00)	
El-Zaemey (2013)	CC	1743/1169	Self-report	Occup.	–	1.43 (1.15, 1.78)	
Boada (2012)	CC	121/103	Serum level		DDD	1.008 (1.001–1.015)	0.024
Ortega Jacome (2010)	CC	110/110	Questionnaire	Res.	–	2.15 (1.22–3.77)	
Teitelbaum (2007)	CC	1508/1556	Interview	Res.	–	1.39 (1.15, 1.68)	
Engel (2005)	Co	30454	Questionnaire	Occup.	2,4,5-TP Captan	2.0 (1.2–3.2) 2.7 (1.7–4.3)	
Charlier (2003)	CC	159/250	Blood level		DDT HCB	5.36 (1.89–15.19) 8.68 (2.83–26.62)	
Mills and Yang (2005)	CC	128/640	Questionnaire	Occup.	–	1.41 (0.66–3.02)	
Duell (2000)	CC	862/790	Interview	Occup.	–	1.8 (1.1–2.8)	
Ovarian cancer							
Lerro (2015a, b)	Co	30003	Questionnaire	Occup.	Diazinon	1.87 (1.02–3.43)	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Koutros (2010a, b)	Co	52394	Census	Occup.	Private use	2.45 (1.12–4.65)	
Donna (1989)	CC		Interview	Occup.	Triazine herbicides	2.7 (1.0–6.9)	
Cervical cancer							
Fleming (1999)	Co	33658	Questionnaire	Occup.	–	SIR; 3.69 (1.84–6.61)	
Eye cancer							
Abdolahi (2013)	CC	198/245	Interview	Pat.	10 years preconception 1 year preconception	1.64 (1.08–2.50) 2.12 (1.25–3.61)	
Carozza (2008)	Ec	1078 counties	GIS	Res.	–	2.6 (1.9–3.5)	
Kristensen (1996)	Co	323292	Census	Par.	–	3.17 (0.93–10.9)	
Laryngeal cancer							
Bravo (1990)	CC	85/170	Interview	Occup.	Insecticides	↑risk	
Lip cancer							
de Rezende Chrisman (2009)	Ec	11 states	Pesticide sales	Res. Occup.	–	MRR; 5.61 (4.88–6.35)	0.01
Rafnsson (2006)	Co	8311	Questionnaire	Occup.	Lindane	1.50 (1.08–2.04)♂ 9.09 (1.02–32.82)♀	
Cerhan (1998)	Mr	88090	Death certificate	Occup.	–	PMR; 1.58 (0.59–4.21)	
Wiklund (1983)	Ret. Co	354228	Questionnaire	Occup.	–	1.83 (1.62–2.05)	
Mouth cancer							
Tarvainen L	Co		JEM	Occup.	–	1.77 (0.85–3.26)	
Lung cancer							
Lerro (2015b)	Co	33484	Interview	Occup.	Acetochlor	1.74 (1.07–2.84)	
Zendehdel et al. (2014)	MA	5 Co		Occup.	Chlorophenols, phenoxyacetic acids	SMR; 1.18 (1.03–1.35)	0.014
Luqman (2014)	CC	400/800	Questionnaire	Occup.	–	5.1 (3.1–8.3)	
Bonner (2010)	Co	57310	Questionnaire	Occup.	Terbufos	1.45 (0.95–2.22)	
Samanic (2006)	Co	41969	Questionnaire	Occup.	Dicamba	2.16 (0.97–4.82)	0.02
Rusiecki (2006)	Co	50193	Questionnaire	Occup.	Metolachlor	2.37 (0.97–5.82)	0.03
Beane Freeman (2005)	Co	23106	Questionnaire	Occup.	Diazinon	2.41 (1.31–4.43)	0.005
Moore (2005)	CC	196/196	Questionnaire	Par.	Household pesticides	3.0 (1.1–8.1)♂	
Lee (2004a, b)	Co	54383	Questionnaire	Occup.	Chlorpyrifos	2.18 (1.31–3.64)	0.002
Alavanja (2004)	Co	57284	Questionnaire	Occup.	Metolachlor Pendimethalin Chlorpyrifos Diazinon	5.0 (1.7–14.9) 4.4 (1.2–15.4) 1.9 (0.9–4.0) 3.2 (1.1–8.9)	0.0002 0.003 0.03 0.04
Pesatori (1994)	Nested CC	65/294	Interview	Occup.	–	2.4 (1.0–5.9)	
Brownson (1993)	CC	429/294	Interview	Occup.	–	2.4 (1.1–5.6)	
Thyroid cancer							
Lerro (2015a, b)	Co	30,003	Questionnaire	Occup.	Malathion	2.04 (1.14–3.63)	
Freeman (2011)	Co	57,310	Questionnaire	Occup.	Atrazine	4.84 (1.31–17.93)	0.08
Lee (2004a, b)	Co	49,980	Questionnaire	Occup.	Alachlor	1.63 (0.42–6.37)	
Pukkala (2009)	Co	15,000,000	Farmers	Occup.	–	SIR; 1.18 (1.07–1.30)	
Skin cancer							
Lerro (2015b)	Co	33,484	Interview	Occup.	Acetochlor	1.61 (0.98–2.66)	

Table 1 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Segatto (2015)	CC	95/96	Interview	Occup.	–	2.03 (1.03–6.89)	
Dennis (2010)	Co	52394	Questionnaire	Occup.	Maneb/mancozeb	2.4 (1.2–4.9)	0.006
					Parathion	2.4 (1.3–4.4)	0.003
					Carbaryl	1.7 (1.1–2.5)	0.013
Mahajan (2007)	Co	21416	Questionnaire	Occup.	Carbaryl (>175 days)	4.11 (1.33–12.75)	0.07
Fortes (2007)	CC	287/299	Interview	Indoor	–	2.18 (1.07–4.43)	0.027

♂: risk found in male, ♀: risk found in female, *MA* meta-analysis, *CC* case–control, *CS* cross-sectional, *Co* cohort, *Ec* ecological, *Mr* mortality, *Ret.* retrospective, *Pros.* prospective, *Occup.* occupational, *Env.* environmental, *Mat.* maternal, *Pat.* paternal, *Par.* parental, *Res.* residential, *Gen.* general, *GIS* geographic information system, *JEM* job exposure matrix, *OR* odd ratio, *RR* relative risk, *HR* hazard ratio, *PMR* proportional mortality ratio, *SMR* standard mortality ratio, *MRR* mortality rate ratio, *SIR* standard incidence ratio, *ALL* acute lymphocytic leukemia, *AML* acute myeloblastic leukemia, *ChE* cholinesterase, *OPs* organophosphoruses, *OCs* organochlorines, *2,4-D* 2,4-dichlorophenoxyacetic acid, *2,4,5-T* 2,4,5-trichlorophenoxyacetic acid, *EPTC* S-ethyl-N,N-dipropylthiocarbamate, *HCB* hexachlorobenzene, *β-HCH* beta-hexachlorocyclohexane, *PCTA* pentachlorothioanisole, *DDT* dichlorodiphenyltrichloroethane, *DDE* dichlorodiphenyldichloroethylene, *DDD* dichlorodiphenyldichloroethane, *GST* glutathione-S-transferase

1993). Furthermore, Viel et al. (1998) reported higher mortality ratio of brain cancer in an ecological model assessment of vineyard pesticide-exposed farmers. Some other case–control studies found an approximately doubled risk of being occupationally exposed to pesticides in cases of brain tumors compared with control. Among these studies, some reported the association of ABT with specified class of pesticides such as herbicides albeit in women (Samanic et al. 2008), and insecticides/fungicides (Musicco et al. 1988), while other evidence of risk of ABT was referred to any class of pesticides (Provost et al. 2007; Rodvall et al. 1996). In this regard, Lee and colleagues' analysis of cases of ABT in comparison with controls revealed elevated odd ratios as 22.6, 18.9, 11.1, 5.9, and 3.4 for participants occupationally exposed to chlorpyrifos, bufencarb, paraquat, coumaphos, and metribuzin, respectively (Lee et al. 2005).

Neuroblastoma Because of the high prevalence of neuroblastoma in infancy and childhood, its association with parental exposure to pesticide has been well studied and the relative risk of about 2.3 reported by two surveys (Feychting et al. 2001; Kristensen et al. 1996). Carozza and colleagues studied the association of childhood cancers in an ecologic analysis of geographic information system (GIS) and reported an OR of 1.8 for the association of residential exposure to pesticides and neuroblastoma in children living in agricultural areas (Carozza et al. 2008). Residential exposure has also been studied in a case–control study of neuroblastoma, and the ORs were resulted as 1.6 and 1.7 for home-used and garden-used pesticides, respectively (Daniels et al. 2001). Furthermore, elevated SMRs due to neuroblastoma were reported by two cohort studies on pesticide applicators (Giordano et al. 2006; Littorin et al. 1993).

Tumors of the digestive system

Esophageal cancer Chrisman and colleagues' ecological study on 11 states in Brazil regarding residential or occupational exposure to pesticides revealed an elevated MRR of 2.4 for esophageal cancer (de Rezende Chrisman et al. 2009). There are also two case–control studies on the association of esophageal cancer with occupational exposure to pesticides among which one assessing the airborne level of pesticides has given an OR of 2.3 (Jansson et al. 2006) and the other death certificate-based study reported an OR of 1.38 (Meyer et al. 2011).

Stomach cancer A study on the link of stomach cancer with occupational exposure to pesticides in a questionnaire-based case–control analysis gave an OR of 1.77 without determining specified pesticides (Forastiere et al. 1993). But other studies in this regard have presented risk estimates of stomach cancer in association with exposure to specified pesticides. An ecological study of 40 ecodistricts in Ontario (Canada) assessed the relation between incidence of stomach cancer and environmental exposure to atrazine measured in drinking water and gave an OR of 1.45 implicated on a significant association (p value <0.05) (Van Leeuwen et al. 1999). Mills and colleagues carried out a nested case–control study on the link of stomach cancer with occupational exposure to different classes of pesticides and found elevated risks regarding 2,4-dichlorophenoxyacetic acid (2,4-D), chlordane, and trifluralin given by ORs of 1.85, 2.96, and 1.69, respectively (Mills and Yang 2007). A significant association of stomach cancer with occupational exposure to methyl bromide was also resulted by a cohort study (Barry et al. 2012).

Colorectal cancer In two separated questionnaire-based case–control studies determining occupational exposure to generally pesticides in cases and controls, the ORs of colorectal cancer were estimated as 2.6 and 2.8. One of these studies calculated the risk estimates of colorectal cancer in association with insecticides and herbicides as well and reported ORs of 3.2 and 5.5, respectively. Further estimate was carried out regarding dietary exposure to pesticides and risk of colorectal cancer represented by an OR of 4.6 (Forastiere et al. 1993; Lo et al. 2010). Among cohort studies analyzing the risk of colorectal cancer in people occupationally exposed to pesticides, apart from one study reporting a tripled incidence ratio (Zhong and Rafnsson 1996), the others estimated the risk regarding specified species of pesticides. Aldicarb, dicamba, imazethapyrc, chlorpyrifos, S-ethyl-N,N-dipropyl thiocarbamate (eptam or EPTC), trifluralin, and acetochlor were the pesticides, arranged in order, for which elevated risk ratios of colorectal cancer reported in occupationally exposed people (Kang et al. 2008; Koutros et al. 2009; Lee et al. 2007b; Lerro et al. 2015b; Samanic et al. 2006; van Bemmel et al. 2008). Recently, Salerno et al. carried out a GIS-based ecological study focusing on the cancer risk among farmers in a province of Italy and reported a double risk of colorectal cancer which may be representative of exposure to pesticides (Salerno et al. 2014).

Liver cancer Ecological analyses on the link of pesticides and incidence of liver cancer were carried out in two separated studies whose results implicated on the elevated OR as 3.3 for residential exposure assessed by GIS on 1078 counties (Carozza et al. 2008) and increased MRR as 1.49 for residential or occupational exposure estimated on the basis of pesticides sale in 11 states (de Rezende Chrisman et al. 2009). Moreover, a high SMR 596.3 due to liver cancer was found in a cohort study conducted on pesticide applicators (Giordano et al. 2006). VoPham et al. compared the cases of liver cancer with matched controls via a GIS-based exposure assessment tool and reported a significant association between exposure to organochlorine pesticides and the incidence of liver cancer given by the OR of 2.76 and a p value of 0.0004 (VoPham et al. 2015).

Gallbladder cancer In a cohort study conducted by Giordano and colleagues on pesticide applicators, a high mortality ratio due to gall bladder cancer was noted with an SMR of 723.8 (Giordano et al. 2006). Further, biliary level of some organochlorine pesticides was measured in a case–control study, and an increased level of hexachlorobenzene (HCB) (p value <0.04) and dichlorodiphenyltrichloroethane (DDT) (p value <0.03) was found in the gall bladder cancer cases in comparison with controls (Shukla et al. 2001).

Pancreatic cancer In 1990, Alavanja and colleagues published the results of cohort mortality and a nested case–control analyses of more than 22000 males. Subjects were enrolled in the life insurance program, and an estimated SMR of 133 due to pancreatic cancer among flour mill workers which were frequently exposed to pesticides (Alavanja et al. 1990). In the same decade, another study found a significantly elevated risk ratio of mortality due to pancreatic cancer among aerial pesticide applicators (Cantor and Silberman 1999). Chrisman and colleagues also reported a high mortality rate ratio of pancreatic cancer in association with per capita sales of pesticides in an ecological study conducted in 11 states of Brazil (de Rezende Chrisman et al. 2009). There are different types of case–control studies on the link between exposure to pesticides and incidence of pancreatic cancer. Regarding occupational exposure to pesticides, an OR of 5.18 was reported for pancreatic cancer by a case–control analysis (Forastiere et al. 1993). ORs including 1.2 and 2.6 were estimated for pancreatic cancer in association with regular exposure to pesticides in two separated case–control studies (Antwi et al. 2015; Lo et al. 2007). Alguacil et al. conducted a case–control study assessing exposure to pesticides via a job exposure matrix (JEM) and estimated a tripled risk of pancreatic cancer in people occupationally exposed to pesticides. In that study, arsenical pesticides were shown to be associated with higher incidence of pancreatic cancer given by OR of 3.4 (Alguacil et al. 2000). Another JEM-based case–control study found significantly elevated ORs including 1.4, 1.5, and 1.6 for pancreatic cancer due to occupational exposure to pesticides, fungicides, and herbicides, respectively (Ji et al. 2001). Occupational exposure to herbicides (EPTC and pendimethalin) was shown to be (p value <0.01) associated with double and triple risks of pancreatic cancer, respectively (Andreotti et al. 2009). Furthermore, an increased relative risk of pancreatic cancer (2.36) in association with occupational exposure to acetochlor herbicide was the finding of the Agricultural Health Study (Lerro et al. 2015b).

Tumors of the hematopoietic system

Leukemia Similar to the brain tumors, studies concerning the link of leukemia with exposure to pesticides are separately designed and conducted with respect to the age of the target population. Regarding childhood leukemia, lots of case–control and other types of epidemiological studies have targeted the association with different routes of exposure, including residential exposure due to indoor or outdoor use of pesticides and parental exposure due to maternal or paternal activities. The results of these studies have been frequently meta-analyzed in various models, and risk of childhood leukemia regarding exposure to pesticides has been estimated. A meta-analysis of 31 case–control studies

published between 1950 and 2009 reported a higher risk of leukemia in children whose mothers dealt with pesticides, insecticides, and herbicides given by ORs of 2.09, 2.72, and 3.62, respectively (Wigle et al. 2009). Another meta-analysis done by Vinson and colleagues on 40 case–control studies assessing maternal exposure to pesticides indicated an elevated OR of 1.48 for leukemia in children (Vinson et al. 2011). Regarding residential exposures, a meta-analysis of 17 case–control studies published between 1950 and 2009 found an elevated ORs including 1.54, 2.05, and 1.61 for incidence of leukemia in children exposed to pesticides, insecticides, and herbicides, respectively (Turner et al. 2011). Further, the results of 16 case–control studies assessing residential exposure in children were meta-analyzed, and 1.4 and 1.2 times elevated risks of childhood leukemia were found in respect to indoor-used pesticides and herbicides (Chen et al. 2015). A meta-analysis of 13 case–control studies published between 1966 and 2009 found an elevated risk estimate of about 1.74 for the link of childhood leukemia with maternal or residential exposure to pesticides (Van Maele-Fabry et al. 2011). Another type of meta-analysis done by Baily and colleagues pooled the results of 13 case–control studies assessing exposure to pesticides in the offspring of parents occupied in the pesticide-related jobs and found associations between childhood AML and maternal exposure, and between childhood ALL and paternal exposure, represented by elevated ORs as 1.9 and 1.2, respectively (Bailey et al. 2014).

Regarding the link of adult leukemia with pesticide exposures, several epidemiological studies have become evident so that their results have been meta-analyzed in different formats. A meta-analysis of 13 case–control studies published between 1990 and 2005 showed that exposure to pesticides and incidence of adult leukemia were associated with an OR of 1.35 (Merhi et al. 2007). Van Maele-Fabry and colleagues meta-analyzed the results of 17 cohort studies published between 1979 and 2005 and found an increased risk estimate (1.2) of leukemia in adults occupationally exposed to pesticides (Van Maele-Fabry et al. 2007). Their another meta-analysis of 14 studies published between 1984 and 2004 revealed 1.4 times higher risk of adult leukemia in association with occupational exposure to pesticides (Van Maele-Fabry et al. 2008). Occupational exposure to crotoxyphos, dichlorvos, famphur, pyrethroids, and methoxychlor was shown to be associated with higher incidence of leukemia in a case–control study (Brown et al. 1990). A high incidence of leukemia was also reported by three separated cohort studies conducted on people occupationally exposed to Agent Orange, terbufos, and diazinon with estimated risks of 1.8, 2.3, and 3.3, respectively (Baumann Kreuziger et al. 2014; Beane Freeman et al. 2005; Bonner et al. 2010). Moreover, Cantor et al. showed that the mortality ratio of leukemia was tripled in people occupationally exposed to pesticides (Cantor and Silberman 1999).

Lymphoma Regarding the tumors of the lymphoid tissues, pesticide exposures have been mostly studied for two main categories of lymphomas, including Hodgkin lymphomas (HL) and non-Hodgkin lymphomas (NHL). Other than one cohort and one mortality studies, 7 case–control analyses have been obtained through this systematic review for HL. The cohort study calculated nearly a triple risk of HL due to parental exposure to pesticides, while the mortality study found a PMR of 1.6 for HL in people occupationally exposed to pesticides (Cerhan et al. 1998; Flower et al. 2004). Among case–control studies, those without determining a specific class of pesticides estimated ORs ranging from 1.8 to 2.2 for HL in association with occupational exposure to pesticides, while the others have reported specified ORs including 1.88, 3.16, 2.47, 4.1, and 2.6 for the link of HL with, respectively, insecticides, organophosphorus compounds, carcinogenic pesticides, household-used pesticides, and phenoxy herbicides, and more specifically, 1.19 and 6.35 for chlorpyrifos and dichlorprop (Karunanayake et al. 2012; Navaranjan et al. 2013; Pahwa et al. 2009; Persson et al. 1993; Rudant et al. 2007).

However, the number of studies targeting the link of NHL and pesticide' exposures is much more than that of HL which may be due to higher prevalence (90 %) of NHL among lymphomas. In this systematic review, a total of 29 studies, including 20 case–control, 8 cohort, and 1 ecological analyses on the link of pesticide exposures with NHL have been collected from which 21 calculated the risk estimate regarding specified classes of pesticides.

Of seven studies not determining the type of pesticides in association with NHL, one cohort study reported RR of 2.47 due to parental exposure and one GIS-based ecological study reported MRRs of 2.9 and 3.2 due to NHL in men and women, respectively (Boccolini Pde et al. 2013; Kristensen et al. 1996). Remaining 5 studies are case–control whose results implicate on the higher incidence of NHL in people exposed to pesticides as given by ORs ranging from 1.7 to 7.3 (Balasubramaniam et al. 2013; Buckley et al. 2000; Clavel et al. 1996; Karunanayake et al. 2013; Rudant et al. 2007; Vajdic et al. 2007).

All of the studies in this systematic analysis, which have estimated the risk of NHL in association with the specified class or type of pesticides, have been designed and conducted in case–control or cohort format composed of different sample numbers. Elevated risk estimates ranging from 1.1 to 3, 1.6 to 2.9, and 1.8 to 3.8 have been reported for NHL in association with exposure to insecticides, herbicides, and fungicides, respectively. Further, five times higher incidence of NHL in people exposed to fumigants has been indicated by Chiu and colleagues (Chiu et al.

2006; Eriksson et al. 2008; Hardell and Eriksson 1999; Hardell et al. 2002; Meinert et al. 2000; Nordstrom et al. 1998; Schinasi et al. 2015; Schroeder et al. 2001).

Exposure to organophosphorus and carbamate compounds have been shown to be associated with, respectively, 1.7 and 1.9 times higher incidence of NHL by a case–control analysis, as such results have been calculated by a meta-analysis of 44 studies (McDuffie et al. 2001; Schinasi and Leon 2014). In these classes of insecticides, higher risk estimates of NHL were reported for people exposed to malathion (1.5), diazinon (1.5, 1.9), terbufos (1.9), coumaphos (2.4), fonofos (1.8), and carbaryl (1.7) (Bonner et al. 2010; Cantor et al. 1992; De Roos et al. 2003). Among organochlorine insecticides, elevated risk estimates ranging from 1.6 to 2.6, 1.2 to 1.7, 1.8 to 3.7, and 1.5 to 1.7 have been reported for lindane, DDT, dieldrin, and chlordane, respectively. The risk of NHL has also been calculated in association with exposure to the other organochlorine insecticides, including toxaphene (3.0), oxychlordane (1.1), cis-nonachlor (1.1), and beta-hexachlorocyclohexane (β-HCH) (1.05) (Alavanja et al. 2014; Brauner et al. 2012; Cantor et al. 1992; De Roos et al. 2003; Purdue et al. 2007; Schinasi and Leon 2014; Schroeder et al. 2001; Viel et al. 2011). Ruder and Yiin (2011) have shown higher mortality ratio of NHL in a cohort of plant workers who were occupationally exposed to pentachlorophenol.

The specific link of NHL and herbicides has been mostly evaluated for phenoxy class of herbicides for which elevated ORs ranging from 1.4 to 2.8 have been estimated by three separated case–control studies (Eriksson et al. 2008; McDuffie et al. 2001; Pahwa et al. 2012a). Coggon and colleagues conducted a cohort study on 8036 participants occupied in phenoxy herbicides-manufacturing plants, and the results revealed a high mortality ratio of NHL among workers (Coggon et al. 2015). The ORs as high as 1.5 and 4.4 have also been reported for the risk of NHL in people occupationally exposed to 2,4-dichlorophenoxy acetic acid (2,4-D) by two case–control studies (Miligi et al. 2006; Zahm et al. 1990). The link of NHL with exposure to other specific herbicides including atrazine and glyphosate has also been studied in three case–control studies, and increased ORs ranging from 1.6 to 1.7 and 2.1 to 2.3 have been estimated, respectively (De Roos et al. 2003; Eriksson et al. 2008; Schroeder et al. 2001).

Multiple myeloma Similar to the other malignancies of the hematopoietic system, multiple myeloma has also been the target of epidemiological health studies linking with exposure to pesticides. This review found totally 8 relevant including 4 case–control, 3 cohorts and one mortality studies on the link between incidences of multiple myeloma and exposure to pesticides. Cerhan et al. (1998) reported a PMR of about 1.2 due to multiple myeloma among farmers who had been occupationally exposed to pesticides. The results of two separate cohort studies showed that occupational exposure to pesticides is associated with higher incidences of multiple myeloma as given by risk estimates of 1.3 and 6.8 in women and people aged more than 50, respectively (Landgren et al. 2009; Lope et al. 2008). A cohort of agricultural workers occupationally exposed to pesticides showed a doubled RR of multiple myeloma among their offspring (Kristensen et al. 1996). Two case–control studies conducted by Perrotta and colleagues without determining a specific class of pesticides have given ORs of about 1.5 and 1.6 for the incidences of multiple myeloma in people occupationally dealing with pesticides (Perrotta et al. 2012, 2013). The comparison of cases of multiple myeloma with matching controls regarding prevalence of exposure to specific types of pesticides was made by two separate studies. Their results implicate elevated ORs for fungicides (1.7), probably carcinogenic pesticides (1.6), carbamates (1.9), captan (2.3), and carbaryl (1.9) (Kachuri et al. 2013; Pahwa et al. 2012b). A meta-analysis of 13 case–control studies published between 1990 and 2005 showed an OR of about 1.2 for the risk of multiple myeloma in association with exposure to pesticides (Merhi et al. 2007).

Tumors of the bone and soft tissues

Bone tumors There are two ecological and three case–control studies giving evidence on the link of bone cancer with exposure to pesticides. One of the ecological studies reported the link between residential exposure to pesticides and higher incidence of childhood bone tumors, while another GIS-based ecological study showed an OR of about 2.3 for bone cancers in relation to higher level of metolachlor in groundwater (Carozza et al. 2008; Thorpe and Shirmohammadi 2005). Two separate case–control studies evaluated the Ewing's sarcoma in children and showed its positive association with parental exposure to pesticides with ORs including 3.0 and 6.1 (Holly et al. 1992; Moore et al. 2005). Comparing adult cases of bone sarcoma in adults with matching controls, 2.3 times higher incidence of occupational exposure to pesticides has been found in cases (Merletti et al. 2006).

Soft tissue sarcoma Two ecological and three case–control studies are the results of a systematic review for the link of soft tissue sarcoma with exposure to pesticides. An ecological study assessing the rate of pesticide sales in 11 states of Brazil reported a higher MRR of soft tissue sarcoma in people of states with greater exposure to pesticides (de Rezende Chrisman et al. 2009). The GIS-based ecological study of Carozza and colleagues also indicated that residential exposure to pesticides is associated with 1.7 time higher inci-

dence of soft tissue sarcoma (Carozza et al. 2008). Indoor exposure to pesticides, especially those used for yard treatments, was shown to be 4.1 times higher in people diagnosed with soft tissue sarcoma by a case–control study (Leiss and Savitz 1995). It should be taken into consideration the finding of a nested case–control study estimating an OR of about 10 for the risk of soft tissue sarcoma in association with occupational exposure to phenoxy herbicides (Kogevinas et al. 1995).

Tumors of the urinary system

Kidney cancer A total of 12 including one ecological, one cohort, one mortality and 9 case–control studies have been collected by this systematic analysis regarding the relation between exposure to pesticides and the incidence of renal cancers. An ecological study conducted on health data of children residing in agriculturally intense areas in the USA revealed nearly tripled incidence risk of childhood renal carcinoma in association with residential exposure to pesticides (Carozza et al. 2008). Examining the records of childhood death revealed a PMR of about 1.6 due to kidney cancer in the offspring of fathers who had been occupationally exposed to pesticides (Fear et al. 1998). Furthermore, 8.9 times increment in the RR of Wilm's tumors was noted in a cohort of children whose parents had been exposed to pesticides due to engagement in agricultural activities (Kristensen et al. 1996). Those case–control studies focusing on adults estimated ORs ranging from 1.6 to 5.7 for the risk of kidney cancers in relation to occupational exposure to pesticides, while the others reported ORs between 1.4 and 128.6 for the risk of childhood renal carcinoma including Wilm's tumors in association with parental, paternal, or maternal exposure to pesticides (Buzio et al. 2002; Forastiere et al. 1993; Karami et al. 2008; Mellemgaard et al. 1994; Olshan et al. 1993; Sharpe et al. 1995; Tsai et al. 2006).

Bladder cancer In regard to the association of bladder cancer with pesticide exposures, there have been two cohorts and three case–control studies. Two cohort studies published by Koutros and colleagues in 2009 and 2015 have shown that people occupationally exposed to imazethapyr herbicides were 2.4 and 3 times more prone to be diagnosed with bladder cancer as given by respective p values of 0.01 and 0.005 (Koutros et al. 2009, 2015). An OR of about 1.7 was estimated by a case–control study for the risk of bladder cancer in people who had been occupationally exposed to pesticides (Amr et al. 2015), even though another report has further highlighted such a risk in cases carrying the *GSTT1* polymorphism (Matic et al. 2014). The remaining case–control study has implicated on a significantly higher blood concentration of HCH and DDT in bladder cancer cases when compared to matching controls (Sharma et al. 2013).

Tumors of the male reproductive system

Prostate cancer Since the prevalence of prostate cancer in men is higher than other malignancies, the surveys on its association with exposure to pesticides are sufficiently high. In this review, there have been 25 epidemiological studies on the link of pesticide exposures with incidence of prostate cancer, of which 13 are case–control, 10 are cohort, one is mortality, and one is ecological. The results of ecological and mortality studies implicated on the high mortality ratio of prostate cancer as calculated MRR of 1.7 and PMR of 1.3, respectively (Cerhan et al. 1998; de Rezende Chrisman et al. 2009). There were three cohort studies which linked occupational exposure to pesticides with elevated SIRs for prostate cancer ranging from 1.2 to 1.9 (Dich and Wiklund 1998; Fleming et al. 1999; Koutros et al. 2010a), while the other seven cohort studies estimated the risk in association with specified classes of pesticides. Morrison and colleagues surveyed the prostate cancer mortality in a cohort of pesticide applicators retrospectively and found an increased RR (2.2) in relation to acres sprayed with herbicides (Morrison et al. 1993). Another cohort including workers of a triazine herbicides-manufacturing plant found the prostate cancer incidence and increased SIR of about 390 (MacLennan et al. 2002). Furthermore, a total of 13144 Vietnam War veterans were examined by a cohort study in regard to Agent Orange exposure, and the results showed that twice as many exposed men were identified with prostate cancer (Chamie et al. 2008). Aldrin, malathion, fonofos, terbufos, coumaphos, and methyl bromide are the other pesticides whose association with prostate cancer was studied in different cohorts and increased risk estimated including 1.5, 1.4, 1.6, 1.2, 1.6, and 3.5, respectively (Alavanja et al. 2003; Bonner et al. 2010; Christensen et al. 2010; Koutros et al. 2013b). Similarly, case–control studies estimated the risk of prostate cancer in association with or without specified classes of pesticides, and, respectively, 10 and 3 case–control studies were collected in this review. Elevated ORs including 1.6, 2.1, and 2.3 were reported in three separate case–control studies conducted in cases of prostate cancer and their matched controls in regard to occupational exposure to ever used pesticides (Forastiere et al. 1993; Meyer et al. 2007; Parent et al. 2009). Three separate case–control studies compared the cases of prostate cancer with matching controls and estimated ORs of 1.6 and 2.5 for the risk in association with exposure to organochlorines, and an OR of 1.6 twice for the risk in association with exposure to methyl bromide (Cockburn et al. 2011; Mills and Yang 2003; Settimi et al. 2003). Exposure to malathion, DDT, carbaryl, chlordecone, ziram, dichlone, azinphos, simazine, maneb, diazinon, and lindane was shown to be associated with higher incidence of prostate cancer as evidenced by ORs ranging from 1.5 to 2 by two case–control studies (Band et al. 2011; Multi-

gner et al. 2010). The five remaining case–control studies evaluated the prostate cancer susceptibility against pesticide exposure in people carrying polymorphism of some variants. Elevated ORs including 3, 3.1, 3.2, 3.4, 3.7, and 4.5 have been estimated for the risk of prostate cancer in association with occupational exposure to terbufos in carriers of *MPO*-single nucleotide polymorphism (SNP), parathion in carriers of SNP in genes of vitamin D metabolism, fonofos in carriers of *CT/TT*-SNP, malathion in carriers of *EHBP1*-SNP, aldrin in carriers of *TET2*-SNP, and again fonofos in carriers of 8q24 variants, respectively (Barry et al. 2011; Karami et al. 2013; Koutros et al. 2010b, 2011, 2013a).

Testicular cancer A cohort of licensed pesticide applicators in Florida was evaluated, and a 2.5 times higher SIR was reported for the risk of testicular cancer in relation to occupational exposure to pesticides (Fleming et al. 1999). Giannandrea and colleagues have also measured and compared the serum level of dichlorodiphenyldichloroethylene (DDE) and HCB in cases of testicular cancer with matching controls and observed a significant tripled risk of testicular cancer in association with higher serum levels of mentioned pesticides (Giannandrea et al. 2011).

Tumors of the female reproductive system

Breast cancer Breast cancer is the most prevalent malignancy in female and has been frequently the topic of environmental health studies examining its association with pesticide exposures. In this systematic search, a total of 14 studies including 4 cohort and 10 case–control analyses have been reviewed. The Agricultural Health Study examined a cohort of 30454 farmers' wives prospectively, and breast cancer SIR of 2.0 and 2.7 was calculated for those who had been exposed to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and captan, respectively (Engel et al. 2005). A cohort of 30003 spouses of pesticide applicators have also been the participants of another Agricultural Health Study whose results implicated on breast cancer RR of 1.2 among women who had personal use of organophosphorus insecticides (Lerro et al. 2015a). Niehoff and colleagues conducted a prospective Sister Study cohort and reported a breast cancer HR of 1.3 in women who had been aged 0–18 years before the ban of DDT in the USA (Niehoff et al. 2016). Furthermore, blood level of DDT was evaluated in relation to women's survival following breast cancer in a prospective cohort study, and an HR of 2.7 was estimated for breast cancer-specific mortality in the highest tertile of blood DDT concentration (Parada et al. 2016). Five separate case–control studies published during 2000 and 2013 showed breast cancer ORs ranging from 1.4 to 2.1 in association with occupational or residential exposure to pesticides (Duell et al. 2000; El-Zaemey et al. 2013; Mills and Yang 2005; Ortega Jacome et al. 2010; Teitelbaum et al. 2007). The other case–control studies measured the blood concentration of organochlorine insecticides in breast cancer cases and their matched controls, and higher concentrations of DDT, DDE, dichlorodiphenyldichloroethane (DDD), β-HCH, and HCB in the blood were shown to be associated with breast cancer ORs of 5.3, 9.6, 1, 3.4, and 8.7, respectively (Arrebola et al. 2015; Boada et al. 2012; Charlier et al. 2003; Tang et al. 2014b). In addition to blood levels, Yang and colleagues reported that there is a positive association between adipose tissue levels of organochlorine insecticides, including DDE, β-HCH, and pentachlorothioanisole (PCTA) with incidence of breast cancer in women (Yang et al. 2015).

Ovarian cancer Updated data on cancer incidence in the Agricultural Health Study revealed an ovarian cancer SIR of 2.4 in association with occupational exposure to pesticides in private sectors (Koutros et al. 2010a). Recently, the results of the Agricultural Health Study with a focus on organophosphorus insecticides have revealed that diazinon use was associated with 1.9 times higher RR of ovarian cancer among spouses of pesticides applicators (Lerro et al. 2015a). In this regard, there is also a case–control study reporting an increased ovarian cancer OR (2.7) in association with occupational exposure to triazine herbicides (Donna et al. 1989).

Cervical cancer There is a cohort study conducted on licensed pesticide applicators in Florida which estimated a cervical cancer SIR of 3.7 in association with exposure to pesticides (Fleming et al. 1999).

Tumors of the head and neck

Eye cancer A GIS-based ecological study conducted by Carozza and colleagues estimated an eye cancer OR of 2.6 in people residing in counties with high density of pesticides (Carozza et al. 2008). In a follow-up of cancer incidence among offspring of parents who had been involved in agricultural activities, a tripled eye cancer RR has been found in association with parental exposure to pesticides (Kristensen et al. 1996). Furthermore, a case–control study focusing on the childhood sporadic bilateral retinoblastoma has found 2.1 and 1.6 times higher risk in children whose fathers had been exposed to pesticides, respectively, 1 year and 10 years before conception (Abdolahi et al. 2013).

Laryngeal cancer Bravo and colleagues have conducted a case–control study and found an increased occurrence of insecticide exposure in cases of laryngeal cancer compared with their matched controls (Bravo et al. 1990).

Lip cancer Association of lip cancer with pesticide exposures has been investigated in an ecological, a mortality, and two cohort studies. Chrisman and colleagues evaluated ecologically the rate of pesticide sales in 11 states and estimated a lip cancer mortality ratio of 5.6 in males who had been residentially or occupationally exposed to pesticides (de Rezende Chrisman et al. 2009). Extracting the causes of death from death certificates of Lowa farmers revealed a PMR of 1.6 due to lip cancer in association with occupational exposure to pesticides (Cerhan et al. 1998). A retrospective cohort study of Swedish agricultural workers presented a decreased risk of most cancers among the study group except lip cancer, which was shown to be greater than the national average by a factor of almost 1.8 (Wiklund 1983). Furthermore, another cohort study carried out on a population engaged in sheep dipping indicated that occupational exposure to lindane increased the risk of lip cancer by 1.5 time in men and by 9 times in women (Rafnsson 2006).

Mouth cancer A study converting the Census occupation to chemical exposures with a JEM-based approach revealed that occupational exposure to pesticides was associated with a mouth cancer SIR of about 1.8 in a cohort of Finns born between 1906 and 1945 (Tarvainen et al. 2008).

Miscellaneous

Lung cancer The link of pesticide exposures with lung cancer incidences has been studied by 11 epidemiological studies of which 7 are cohort and 4 are case–control analyses collected in this review. Occupational exposure to pesticides has been linked to higher incidence of lung cancer by three separated case–control studies estimating ORs including 2.4 and 5.1 (Brownson et al. 1993; Luqman et al. 2014; Pesatori et al. 1994), while the results of another case–control study has implicated on a tripled risk of lung cancer in the sons whose parents had been exposed to household pesticides (Moore et al. 2005). All of cohort studies in this review have determined the incidence of lung cancer in association with specified types of pesticides, as such an elevated risk estimate of 1.7 has been found for occupational use of acetochlor, 1.4 for terbufos, 2.1 for dicamba, 2.4 and 5 for metolachlor, 2.4 and 3.2 for diazinon, 1.9 and 2.2 for chlorpyrifos, and 4.4 for pendimethalin (Alavanja et al. 2004; Beane Freeman et al. 2005; Bonner et al. 2010; Lee et al. 2004a; Lerro et al. 2015b; Rusiecki et al. 2006; Samanic et al. 2006).

Thyroid cancer Searching the role of pesticide exposures in the incidence of thyroid cancer by this review resulted in 4 cohort studies, one of which found a SIR of 1.2 for the risk in farmers who had been occupationally exposed to any kind of pesticides, while the others reported thyroid cancer risk estimates of 1.6, 2, and 4.8 for occupational exposure to alachlor, malathion, and atrazine, respectively (Freeman et al. 2011; Lee et al. 2004b; Lerro et al. 2015a; Pukkala et al. 2009).

Skin cancer Two separate case–control studies estimated skin cancer ORs of 2 and 2.2 in association with occupational use and indoor use of pesticides, respectively (Fortes et al. 2007; Segatto et al. 2015). Occupational exposure to specific classes of pesticides was evaluated in three cohort studies reporting elevated melanoma risk estimates, including 1.6 for acetochlor, 2.4 for maneb, 2.4 for parathion, and 1.7 and 4.1 for carbaryl (Dennis et al. 2010; Lerro et al. 2015b; Mahajan et al. 2007).

Disease-based evidence on neurotoxicity of pesticides

Alzheimer

Alzheimer disease is an increasing age-related neurodegenerative disease which has been shown to be associated with exposure to pesticides. Herein, six studies including three cohort, two case–control, and an ecological studies have been reviewed on the relation between Alzheimer and pesticide exposures (Table 2). The longitudinal and prospective analysis of exposures associated with incidence of Alzheimer diseases by the cohort studies revealed 1.4 and 2.4 times higher risk in people occupationally exposed to any pesticides, while exposure to organophosphorus and organochlorine compounds was shown to increase the risk by 1.5 times. In addition, an Alzheimer RR of about 4.3 in association with exposure to fumigants and defoliants had been previously reported by a cohort study (Baldi et al. 2003b; Hayden et al. 2010; Tyas et al. 2001). A GIS-based ecological study indicated that the prevalence of Alzheimer disease in people living in the areas having higher pesticide usage was two times higher than that of the others (Parron et al. 2011). Two separate case–control studies evaluated pesticide exposures in cases of Alzheimer disease and their matched controls, and the results of the first one indicated an OR of about 1.1 for the risk in relation to pesticides and fertilizers, while the second one showed that the blood level of DDE is positively associated with risk of Alzheimer disease evidenced by an OR of about 4.2 (McDowell et al. 1994; Richardson et al. 2014).

Parkinson

Because of approximate similarity between the pathophysiology of Parkinson disease (PD) and toxicity of pesticides, there have been a huge body of epidemiological and experimental evidence on the role of pesticide exposures in the development of PD. Herein, the results of 33 epidemiological human study comprised of 26 case–control, 5 cohort,

Table 2 Neurotoxicity of pesticides evidenced by disease

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Alzheimer							
McDowell (1994)	CC	258/535		Occup.	Pesticides, fertilizers	1.07 (1.18–3.99)	
Tyas (2001)	Co	694	Questionnaire	Occup.	Fumigants, defoliants	4.35 (1.05–17.90)	
Baldi (2003b)	Pros. Co	1507	Questionnaire	Occup.	–	2.39 (1.02–5.63)	
Hayden (2010)	Pros. Co	3084	Questionnaire	Occup.	–	1.42 (1.06–1.91)	0.02
					OPs	1.53 (1.05–2.23)	0.03
					OCs	1.49 (0.99–2.24)	0.06
Parron (2011)	Ec	17,429	GIS	Env.	–	2.10 (1.96–2.25)	<0.001
Richardson (2014)	CC	86/79	Serum level		DDE	4.18 (2.54–5.82)	<0.001
Parkinson							
Baldi (2003a)	CC	84/252	GIS	Gen.	–	2.2 (1.1–4.3)	
Butterfield (1993)	CC	63/68		Env.	Insecticide	5.75	<0.001
					Fumigants	5.25	0.046
					Herbicides	3.22	0.033
Chan (1998)	CC	215/313	Questionnaire	Occup.	Exposed years	1.05 (1.01–1.09)	0.018
Costello (2009)	CC	368/341	GIS	Env.	Maneb, Paraquat	1.75 (1.13–2.73)	
Dick (2007)	CC	959/1989	Questionnaire	Occup.	–	1.41 (1.06–1.88)	
Dutheil (2010)	CC	101/234	Questionnaire	Occup.	OCs	3.50 (0.90–14.5)	
Elbaz (2009)	CC	224/557	Questionnaire	Occup.	–	1.80 (1.1–3.1)	0.01
Firestone (2005)	CC	250/388	Questionnaire	Occup.	–	2.07 (0.67–6.38)	
Fong (2007)	CC	153/155	Questionnaire	Occup.	–	1.69 (1.07–2.65)	
Frigerio (2006)	CC	149/129	Questionnaire	Gen.	–	2.40 (1.1–5.4)	0.04
Gatto (2009)	CC	368/341	Well water use	Env.	Methomyl Chlorpyrifos Propargite	1.67 (1.00–2.78) 1.87 (1.05–3.31) 1.92 (1.15–3.20)	
Gorrel 1998	CC	144/464	Questionnaire	Occup.	Herbicides Insecticides	4.1 (1.37–12.24) 3.55 (1.75–7.18)	
Hancock (2008)	CC	319/296	Questionnaire	Occup.	–	1.61 (1.13–2.29)	
Manthripragada (2010)	CC	351/363	GIS	Gen.	Diazinon Chlorpyrifos	2.2 (1.1–4.5) 2.6 (1.3–5.4)	
Ritz (2009)	CC	324/334	Questionnaire	Occup.	Paraquat, Maneb	2.99 (0.88–1.02)	
Tanner (2009)	CC	519/511	Questionnaire	Occup.	Pesticides 2,4-D	1.9 (1.12–3.21) 2.59 (1.03–6.48)	
Tanner (2011)	CC	110/358	Questionnaire	Occup.	Rotenone Paraquat	2.5 (1.2–3.6) 2.5 (1.4–4.7)	
Wang (2011a)	CC	362/341	Ambient level	Occup.	Ziram, maneb, paraquat	3.09 (1.69–5.64)	
Zorzon (2002)	CC	136/272	Questionnaire	Env. Occup.	–	2.0 (1.1–3.5) 7.7 (1.4–44.1)	0.0237 0.0212
Ascherio (2006)	Pros. Co	143,325	Questionnaire	Occup.	–	1.7 (1.2–2.3)	0.002
Baldi (2003b)	Pros. Co	1507	Questionnaire	Occup.	–	5.63 (1.47–21.58)	
Kamel (2007)	Pros. Co	55,931	Questionnaire	Occup.	–	2.3 (1.2–4.5)	0.009
Kamel (2014)	CC	89/336	Questionnaire	Occup.	Paraquat Rotenone	4.2 (1.5–12) 5.8 (2.3–15)	
Petrovitch (2002)	Pros. Co	7986	Questionnaire	Occup.	–	1.7 (0.8–3.7)	0.006
Richardson (2009)	CC	50/43	Serum level		β-HCH	4.39 (1.67–11.6)	
McCann (1998)	CC	224/310	Questionnaire	Env.	Rural residency	1.8	<0.001
Lee (2012)	CC	357/754	GIS	Env.	Paraquat	1.36 (1.02–1.81)	
Goldman et al. (2012)	CC	87/343	Questionnaire	Occup.	Paraquat Paraquat (GSTT1*0)	1.5 (0.6–3.6) 11.1 (3.0–44.6)	

Table 2 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Steenland (2013)	CS	400	Questionnaire	Occup.	–	2.57 (0.91–7.26)	
Narayan (2013)	CC	357/807	Questionnaire	Res.	Household pesticide OPs Organothiophosphate	1.47 (1.13, 1.92) 1.71 (1.21, 2.41) 1.95 (1.17, 3.23)	
Brouwer (2015)	Pros. Co	5000	JEM	Occup.	–	1.27 (0.86–1.88)	
James and Hall (2015)	CS	332,971	Groundwater level	Env.	1 μg/L pesticides	1.03 (1.02–1.04)	
Moisan (2015)	CC	133/298	Questionnaire	Occup.	–	2.56 (1.31–4.98)	
Amyotrophic lateral sclerosis							
Bonvicini (2010)	CC	41/82	Questionnaire	Occup.	–	3.6 (1.2–10.5)	
Das (2012)	CC	110/240	Questionnaire	Occup.	Pesticides and insecticides	1.61 (1.27–1.99)	0.03
McGuire (1997)	CC	174/348	Questionnaire	Occup.	–	2.0 (1.1–3.5)	
Morahan and Pamphlett (2006)	CC	179/179	Questionnaire	Overall Occup.	Herbicides/pesticides Herbicides/pesticides	1.57 (1.03–2.41) 5.58 (2.07–15.06)	
Pamphlett (2012)	CC	614/778	Questionnaire	Occup.	Herbicides/pesticides	1.77 (1.30–2.39)	
Qureshi (2006)	CC	95/106	Questionnaire	Occup.	–	↑ risk	0.03
Burns (2001)	Co	1517	JEM	Occup.	2,4-D	3.45 (1.1–11.11)	
Deapen and Henderson (1986)	CC	1136	Questionnaire	Occup.	–	2.0 (0.8–5.4)	
Savettieri (1991)	CC	46/92	Interview	Gen.	–	3.0 (0.4–20.3)	
Gunnarsson (1992)	CC	92/372	Questionnaire	Occup.	–	1.1 (0.2–5.3)	
Chancellor (1993)	CC	103/103	Questionnaire	Occup.	–	1.4 (0.6–3.1)	
Weisskopf (2009)	Co	987,229	Questionnaire	Gen.	–	1.48 (0.82–2.67)	0.0004
Kamel (2012)	AHS (Co)	84,739	Questionnaire	Occup.	OCs	1.6 (0.8–3.5)	
Su (2016)	CC	156/128	Blood level	Gen.	OCs, PCBs, BFRs	5.09 (1.85–13.99)	
Beard (2016)	CC	621/958	Questionnaire	War Field	Agent Orange	2.80 (1.44–5.44)	
Burns (2001)	Co	3/40600	Expert Judgment	Occup.	2,4,-D	3.45 (1.10–11.11)	
Furby et al. (2010)	CC	108/122	Questionnaire	Occup.	–	3.04 (1.19–7.75)	
Malek (2014)	CC	66/66	Questionnaire	Occup.	–	6.50 (1.78, 23.77)	
Yu (2014)	CC	66/66	Questionnaire	Occup.	>30 years exposure	6.95 (1.23–39.1)	<0.05

MA meta-analysis, *CC* case–control, *CS* cross-sectional, *Co* cohort, *Ec* ecological, *Mr* mortality, *Ret.* retrospective, *Pros.* prospective, *Occup.* occupational, *Env.* environmental, *Mat.* maternal, *Pat.* paternal, *Par.* parental, *Res.* residential, *Gen.* general, *GIS* geographic information system, *JEM* job exposure matrix, *OR* odd ratio, *RR* relative risk, *HR* hazard ratio, *PMR* proportional mortality ratio, *SMR* standard mortality ratio, *MRR* mortality rate ratio, *SIR* standard incidence ratio, *ChE* cholinesterase, *OPs* organophosphoruses, *OCs* organochlorines, *2,4-D* 2,4-dichlorophenoxyacetic acid, *β-HCH* beta-hexachlorocyclohexane, *DDE* dichlorodiphenyldichloroethylene

and two cross-sectional analyses have been extracted and reviewed (Table 2). The results of a screening test for neurodegenerative diseases conducted in a population-based sample from Costa Rica implicated on a Parkinson OR of about 2.6 in association with occupational exposure to pesticides, while the other cross-sectional analysis measuring the ground water level of some pesticides, including atrazine, simazine, alachlor, and metolachlor reported an increased risk of PD by 3 % for every 1.0 microg/L of pesticide in groundwater (James and Hall 2015; Steenland et al. 2013). All of five cohort studies included in this review prospectively analyzed the risk of PD in different sized samples of population and reported that occupational exposure to pesticides increases the risk of PD up to a range of 1.3–5.6 times (Ascherio et al. 2006; Baldi et al. 2003b; Brouwer et al. 2015; Kamel et al. 2007; Petrovitch et al. 2002). Among case–control studies which compared the cases of PD with their matched controls from the aspect of pesticide exposures, some generally evaluated the risk in relation to ever used pesticides and reported a total of eleven increased ORs ranging from 1.05 to 2.6 (Baldi et al. 2003a; Chan et al. 1998; Dick et al. 2007; Elbaz et al. 2009; Firestone et al. 2005; Fong et al. 2007; Frigerio et al. 2006; Hancock et al. 2008; McCann et al. 1998; Moisan et al. 2015; Zorzon et al. 2002), while the others whose number is also large enough calculated the risk of the disease in association with exposure to specific types of pesticides. In this regard, elevated Parkinson ORs have been reported for the main classes of

pesticides, including two for insecticides (3.5 and 5.7), two for herbicides (3.2 and 4.1), and one for fumigant (5.2), though exposure to organochlorines and organophosphoruses has been shown to increase the risk of PD as given by ORs of 3.5 and 1.7, respectively (Butterfield et al. 1993; Dutheil et al. 2010; Gorell et al. 1998; Narayan et al. 2013). Specifically, environmental and occupational exposure to paraquat has been linked with the most frequently reported Parkinson ORs including 1.4, 1.5, 1.7, 2.5, 3, and 4.2 and then exposure to the maneb (1.7 and 3) and rotenone (2.5 and 5.8) (Costello et al. 2009; Kamel et al. 2014; Ritz et al. 2009; Tanner et al. 2011). Furthermore, a case–control study comparing the cases of PD with their matched controls regarding the ambient level of pesticides at workplace revealed that combined exposure to paraquat, maneb, and ziram was associated with a threefold increase in the risk of Parkinson (Wang et al. 2011a). Environmental exposure to methomyl, chlorpyrifos, and propargite has been compared between cases of PD and the matched controls by a GIS-based analysis of groundwater, and 1.7- to 1.9-fold elevated risks of Parkinson were estimated for usage of contaminated well water with mentioned pesticides (Gatto et al. 2009). Another GIS-based case–control study has shown that residential exposure to chlorpyrifos and diazinon increased the risk of Parkinson by more than two times (Manthripragada et al. 2010). Occupational exposure to the herbicide 2,4-D has been compared between the cases of PD and matched controls, and 2.6 times increased risk of PD in cases was estimated (Tanner et al. 2009). A 4.4 times elevated risk of Parkinson due to exposure to β-HCH was the finding of another case–control study indicating that β-HCH was more often detectable in the blood of PD cases than control (Richardson et al. 2009).

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive disease of the nervous system presented by muscle weakness for which the role of environmental risk factors specially pesticide exposures has been well studied. Herein, 18 studies including 15 case–control and 3 cohort analyses of the possible link between pesticide exposures and the incidence of ALS have been reviewed (Table 2). A cohort study prospectively evaluated the causes of mortality in relation to the exposures and found out an ALS RR of about 1.5 in people having more than 10 years of regular exposure to pesticides (Weisskopf et al. 2009). In addition, a mortality RR of 3.4 was attributed to the ALS among a cohort of male employees in a 2,4-D-manufacturing plant (Burns et al. 2001). A meta-analysis of the Agricultural Health study data taken from a cohort of 84,739 private pesticide applicators revealed that ALS was associated with occupational exposure to pyrethroid, organochlorines, herbicides, and fumigants evidenced by elevated ORs including 1.4, 1.6, 1.6, and 1.8, respectively, (Kamel et al. 2012). Ten case–control studies included in this review reported the link of pesticide exposures with elevated ALS risk estimates ranging from 1.1 to 6.9 (Bonvicini et al. 2010; Chancellor et al. 1993; Deapen and Henderson 1986; Furby et al. 2010; Gunnarsson et al. 1992; Malek et al. 2014; McGuire et al. 1997; Qureshi et al. 2006; Savettieri et al. 1991; Yu et al. 2014). Three separate studies comparing the cases of ALS with controls regarding occupational exposures, estimated higher risk of the disease relevant to the industrial use of herbicides/pesticides (1.8 and 5.6) and insecticides/pesticides (1.6) (Das et al. 2012; Morahan and Pamphlett 2006; Pamphlett 2012). A case–control study of US military veterans has shown that war field exposure to Agent Orange increased the risk of ALS given by OR of 2.8 (Beard et al. 2016). Furthermore, measuring the blood level of organochlorine pesticides in cases of ALS and their controls revealed that cumulative exposure was significantly associated with ALS evidenced giving an OR of about 5.1 (Su et al. 2016).

Disease-based evidence on pulmonotoxicity of pesticides

Asthma

The link of environmental and occupational exposures with asthma has long been discovered, and so the role of pesticide exposures in the etiology of the disease has been well studied in both children and adults. Herein, the results of 18 studies including 7 cross-sectional, 7 cohort, and 4 case–control survey on the relation of pesticide exposures with incidence of asthma have been reviewed (Table 3). Among cross-sectional analyses, two have evaluated the risk of asthma in association with occupational and para-occupational exposure to any kind of pesticides and increased risk of the disease has been found (ORs up to 4.6) (Bener et al. 1999; Salameh et al. 2003), while the other study evaluated death data and found an elevated mortality ratio (3.4) associated with asthma in people occupationally exposed to pesticides (Beard et al. 2003). Three cross-sectional studies have measured the serum levels of dioxin, DDE, and cholinesterase enzyme in the participants and reported higher incidence of asthma in exposure to Agent Orange, DDE, and anticholinesterase insecticides, including organophosphoruses and carbamates as evidenced by ORs of 1.6, 3.7, and 1.9, respectively. Albeit, the reported risk of DDE was estimated in combination with polychlorinated biphenyls (PCBs) and HCB in children exposed after birth (Kang et al. 2006; Karmaus et al. 2001; Ndlovu et al. 2014). Further, an asthma prevalence OR of 1.8 in association with

Table 3 Pulmonotoxicity of pesticides evidenced by disease

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Asthma							
Senthilselvan (1992)	CS	1939		Occup.	Carbamate	1.8 (1.1–3.1)	0.02
Kang (2006)	CS	2927	Serum Dioxin	Occup.	Agent Orange	1.62 (1.28–2.05)	>0.05
Bener (1999)	CS	196		Occup.	–	↑ risk	<0.008
Salameh (2006)	CC	262/110	Interview	Occup.	–	4.98 (1.07–23.28)	0.02
Beard (2003)	CS	3983	Death Data	Occup.	–	3.45 (1.39–7.10)	
Hoppin (2008)	Co	25,814	Questionnaire	Occup.	–	1.46 (1.14–1.87)	
Hoppin (2009)	Co	19,704	Questionnaire	Occup.	Coumaphos	2.34 (1.49–3.70)	
					Heptachlor	2.01 (1.30–3.11)	
					Parathion	2.05 (1.21–3.46)	
					Mix (CCl4/CS2)	2.15 (1.23–3.76)	
					Ethylene dibromide	2.07 (1.02–4.20)	
Sunyer (2005)	Co	468	Cord serum	Mat.	DDE	2.63 (1.19–4.96)	
Sunyer (2006)	Co	462	Cord serum	Mat.	DDE	1.18 (1.01–1.39)	
Karmaus et al. (2001)	CS	343	Blood level	Mat.	DDE	3.71 (1.10–12.56)	
Tagiyeva (2010)	Co	13,971	Questionnaire	Par. job	Biocides/fungicides	1.47 (1.14–1.88)	
Salam (2004)	CC	279/412	Interview	Env.	Pesticides	2.39 (1.17–4.89)	
					Herbicides	4.58 (1.58–5.56)	
Salameh (2003)	CS	3291	Questionnaire	Para-Occup.	–	4.61 (2.06–10.29)	
Meng (2016a, b)	CC	60/60	Indoor dust	Res.	DDE	1.82 (1.00–3.32)	0.04
Meng (2016a, b)	CC	620/218	Pooled serum		DDE	1.02 (1.01–1.03)	0.0004
					α-HCH	1.06 (1.02–1.10)	0.001
Ndlovu et al. (2014)	CS	211	Blood ChE	Occup.	ChE inhibitor	1.93 (1.09–3.44)	
Yi et al. (2014)	Ret. Co	111,726	GIS	Occup.	Agent Orange	1.04 (1.01–1.08)	0.015
Hansen (2014b)	Co	965	Mat. serum	Mat.	HCB	1.92 (1.15, 3.21)	
Exacerbated asthma							
Henneberger (2014)	Co	926	Questionnaire	Occup.	Pendimethalin	2.1 (1.1–4.1)	
					Aldicarb	10.2 (1.9–55)	
Chronic bronchitis							
Hoppin (2007)	Co	20,908	Questionnaire	Occup.	Heptachlor	1.50 (1.19–1.89)	
Salameh (2006)	CC	262/110	Interview	Occup.	–	15.92 (3.50–72.41)	<0.0001
Tual et al. (2013)	Co	14,441	Questionnaire	Occup.	–	1.63	<0.05
Valcin (2007)	Co	21,541 women	Questionnaire	Occup.	Dichlorvos	1.63 (1.01, 2.61)	
					DDT	1.67 (1.13, 2.47)	
					Cyanazine	1.88 (1.00, 3.54)	
					Paraquat	1.91 (1.02, 3.55)	
					Methyl bromide	1.82 (1.02, 3.24)	
Yi et al. (2014)	Ret. Co	111,726	GIS	Occup.	Agent Orange	1.05 (1.02–1.08)	0.015
Wheeze							
Hoppin (2002)	Co	20,468	Questionnaire	Occup.	Parathion	1.5 (1.0–2.2)	
					Atrazine	1.5 (1.2–1.9)	
Hoppin (2006a, b)	Co	89,000	Questionnaire	Occup.	Chlorpyrifos	1.48 (1.0–2.2)	0.02
Hoppin (2006a, b)	Co	2255	Questionnaire	Occup.	Chlorimuron-ethyl	1.62 (1.25, 2.10)	
Salameh (2003)	CS	3291	Questionnaire	Res.	–	2.73 (1.85–4.05)	
Xu (2012)	CS	14,065	Questionnaire	Res.	Household use	1.39 (1.08–1.78)	
Gascon (2014)	Co	405	Mat. serum	Mat.	HCB	1.58 (1.04–2.41)	
					DDE	1.35 (1.07–1.71)	

Table 3 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value
Lower respiratory tract infections (LRTIs)							
Sunyer (2010)	Co	520	Mat. serum	Mat.	DDE	2.40 (1.19–4.83)	
Gascon (2012)	Co	1455	Mat. serum	Mat.	DDE	1.33 (1.08–1.62)	
Gascon (2014)	Co	405	Mat. serum	Mat.	HCB	1.89 (1.10–3.25)	

MA meta-analysis, *CC* case–control, *CS* cross-sectional, *Co* cohort, *Ec* ecological, *Mr* mortality, *Ret.* retrospective, *Pros.* prospective, *Occup.* occupational, *Env.* environmental, *Mat.* maternal, *Pat.* paternal, *Par.* parental, *Res.* residential, *Gen.* general, *GIS* geographic information system, *JEM* job exposure matrix, *OR* odd ratio, *RR* relative risk, *HR* hazard ratio, *ChE* cholinesterase, *OPs* organophosphoruses, *HCB* hexachlorobenzene, *α-HCH* alpha-hexachlorocyclohexane, *DDT* dichlorodiphenyltrichloroethane, *DDE* dichlorodiphenyldichloroethylene

exposure to carbamate insecticides has been previously estimated by a questionnaire-based cross-sectional study (Senthilselvan et al. 1992).

Two separate case–control studies reported asthma ORs of 2.4 and 5 in association with, respectively, environmental and occupational exposure to any kind of pesticides, while 4.6 for environmental exposure to herbicides (Salam et al. 2004; Salameh et al. 2006). Recently, Meng et al. measured and compared the concentration of pesticides in indoor dust and blood samples taken from cases of asthma and controls and published two separate case–control studies reporting higher incidence of asthma in association with exposure to DDE (ORs 1.02 and 1.8) and alpha-hexachlorocyclohexane (α-HCH) (OR of 1.06) (Meng et al. 2016a, b).

Hoppin and colleagues have analyzed data from the Agricultural Health Study and once reported an asthma OR of about 1.5 in female farmers exposed to any pesticide, and once again estimated asthma ORs including 2.3, 2, 2.05, 2.15, and 2.1 in male farmers exposed to coumaphos, heptachlor, parathion, mixed CCl4/CS2, and ethylendibromide, respectively (Hoppin et al. 2008, 2009). In addition, the Agricultural Health Study on a cohort of pesticide applicators with active asthma showed that the symptoms were exacerbated due to occupational exposure to pendimethalin (OR: 2.1) and aldicarb (OR: 10.2) (Henneberger et al. 2014). A cohort of Korean Vietnam veterans have been retrospectively evaluated to determine diseases' prevalence by a GIS-based model assessment of exposure, and an asthma OR of 1.04 was estimated for exposure to Agent Orange (Yi et al. 2014). The link of childhood asthma in association with parental occupation has been evaluated by a birth cohort study, and higher incidence of asthma was found in children whose parents occupationally exposed to biocides/fungicides as given by an OR of 1.5 (Tagiyeva et al. 2010). Measuring the cord serum concentration of DDE taken from two cohorts of offspring and their follow-up by Sunyer and colleagues indicated that prenatal exposure to DDE increased the incidence of childhood asthma as the ORs were estimated to be 1.2 and 2.6 (Sunyer et al. 2006; Sunyer et al. 2005). Moreover, the results of a prospective cohort study measuring the maternal serum concentration of organochlorines with 20-year follow-up revealed that prenatal exposure to HCB was associated with an asthma HR of 1.9 in the offspring (Hansen et al. 2014b).

Chronic bronchitis

Regarding the role of pesticide exposures in chronic bronchitis, the results of a case–control plus 4 cohort studies have been reviewed (Table 3). The comparison of occupational exposure to pesticides between cases and controls resulted in a significantly elevated OR of 15.9 for chronic bronchitis (Salameh et al. 2006). Besides, findings from the French AGRICAN cohort study has shown that pesticide poisoning and pesticide exposures among potato farmers were significantly associated with risk of chronic bronchitis (OR 1.6) (Tual et al. 2013). The other three cohort studies determined the risk of chronic bronchitis in association with specified types of pesticides, as a chronic bronchitis OR of 1.5 was estimated for heptachlor usage by pesticide applicators involved in the Agricultural Health Study (Hoppin et al. 2007). Such a risk of chronic bronchitis has also been found by the Agricultural Health Study on non-smoking farm women who had been exposed to dichlorvos, DDT, methyl bromide, cyanazine, and paraquat with respective ORs including 1.6, 1.7, 1.8, 1.9, and 1.9 (Valcin et al. 2007). Further, exposure of a cohort of Korean Vietnam veterans to Agent Orange was shown to be significantly associated with increased risk of chronic bronchitis with estimated OR of 1.05 (Yi et al. 2014).

Wheeze

Wheeze as a typical symptom of the most respiratory disorders has also been the focus of environmental health studies assessing its risk in relation to pesticide exposures. In this regard, the results of two cross-sectional plus 4 cohort studies have been presented in this review (Table 3). Two questionnaire-based cross-sectional analyses have indicated

that residential exposure to pesticides increased the risk of wheeze in children with ORs of 1.4 and 2.7 (Salameh et al. 2003; Xu et al. 2012). Hoppin and colleagues made three separated analyses on data from the Agricultural Health Study and reported that occupational exposure to parathion, atrazine, chlorpyrifos, and chlorimuron-ethyl were associated with increased incidence of wheeze with ORs ranging from 1.5 to 1.6 (Hoppin et al. 2002, 2006a, b). Measurement of maternal serum concentration of persistent organic pollutants (POPs) in a cohort study indicated that incidence of wheeze in the offspring prenatally exposed to DDE and HCB was increased with respective ORs including 1.3 and 1.8 (Gascon et al. 2014).

Low respiratory tract infections (LRTIs)

LRTIs in relation to pesticide exposures have been studied in three separate cohorts of children whose mothers' serum concentration of organochlorines was measured (Table 3), and the results implicated on elevated risk of LRTIs in association with prenatal exposure to DDE (ORs 1.3 and 2.4) and HCB (OR 1.9) (Gascon et al. 2012, 2014; Sunyer et al. 2010).

Disease-based evidence on reproductive toxicity of pesticides

Infertility

There are various types of reproductive disorders in both males and females which may be resulted in infertility or not. Infertility has been defined as the inability to reproduce naturally and has been well studied in relation to the environmental risk factors such as pesticides. The search for the link of human infertility with pesticide exposures has brought totally 9 relevant studies, including two cross-sectional, five case–control, and two cohort analyses in this review (Table 4). One of the cross-sectional studies has measured the level of organochlorines in cord blood of the couples enrolled in a French birth cohort (PELAGIE) and concluded that the time-to-pregnancy increased in association with higher serum concentrations of DDE (Chevrier et al. 2013). The other study is questionnaire based and reported early abortion ORs of 1.4 and 1.5 in the women exposed to phenoxy and atrazine herbicides before conception, while such an exposure to glyphosate and thiocarbamates in women resulted in late abortion ORs of 1.7 and 1.8 (Arbuckle et al. 2001).

There have been two questionnaire-based case–control studies, one of which calculated an infertility OR of about 3 in females occupationally exposed to any kind of pesticides, while increased ORs of 3.3 and 27 were estimated by the other study for female infertility in association with occupational exposure to fungicides and herbicides, respectively (Greenlee et al. 2003; Smith et al. 1997). Two case–control studies published in 2013 compared the blood level of organochlorines between cases of infertile women with controls and cases of endometriosis with controls and reported significantly higher incidence of infertility in association with DDE exposure, while elevated endometriosis ORs of 1.3 and 1.5 were estimated for respective exposure to HCH and mirex (Bastos et al. 2013; Upson et al. 2013). Cases of infertile men were also compared with controls regarding semen concentration of organochlorines, and significantly elevated risk of infertility as well as lower sperm count and motility has been found in association with higher levels of HCH and DDT in the semen (Pant et al. 2007).

Furthermore, there is an infertility-targeted cohort study measuring the maternal serum concentration of DDT and DDE, which has shown increased time-to-pregnancy in daughters of women exposed to DDT (Cohn et al. 2003). Increased time-to-pregnancy in women living in the glyphosate condensed areas has also been reported by a cohort study applying a GIS approach for assessing the exposure to the pesticide in Colombian regions (Sanin et al. 2009).

Low quality of semen

Some characteristics of the semen are critical determinants of male fertility, and lowered quality of the semen has been studied in association with exposure to pesticides (Mehrpour et al. 2014). Herein, a total of 14 studies including 8 cross-sectional, 5 case–control, and one cohort analyses on the role of pesticide exposure in the lowered quality of the semen have been reviewed (Table 4). Blood concentration of organochlorines was measured in two cross-sectional and one case–control studies, and their results implicated on the lowered quality of the semen characterized by decreased sperm count and motility, decreased volume of ejaculation, oligozoospermia, asthenozoospermia, and Yq deletion in association with exposure to DDE, DDT, and HCH for different values (Aneck-Hahn et al. 2007; Khan et al. 2010; Messaros et al. 2009). The incidence of oligozoospermia, asthenozoospermia, and teratospermia in association with occupational exposure to any used pesticides has also been reported by a questionnaire-based case–control study (De Fleurian et al. 2009). In association with organophosphorus and pyrethroid insecticides, different studies have measured their blood or urine concentration in human and linked their exposure to lowered sperm count and motility, increased sperm DNA damage, and disrupted volume and pH of the semen (Meeker et al. 2009; Perry et al. 2007, 2011; Recio-Vega et al. 2008; Yucra et al. 2008). In addition to DNA damage and low quality of the

Table 4 Reproductive toxicity of pesticides evidenced by disease

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value	Found risk
Infertility								
Bastos (2013)	CC	15/21	Blood level	–	DDE	69 %	0.001	Infertility♀
Arbuckle (2001)	CS	2110	Questionnaire	Mat.	2,4-D & 2,4,5-T	1.5 (1.1–2.1)		Early abortion
					Triazines	1.4 (1.0–2.0)		Early abortion
					Glyphosate	1.7 (1.0–2.9)		Late abortion
					Thiocarbamate	1.8 (1.1–3.0)		Late abortion
Pant (2007)	CC	50/50	Semen level	–	HCH, DDT	–	<0.05	Infertility
						–	<0.05	↓ sperm count ↓ sperm motility
Greenlee (2003)	CC	322/322	Interview	Occup.	Herbicides	27 (1.9–380)		
					Fungicides	3.3 (0.8–13)		
Upson (2013)	CC	248/538	Serum level		HCH Mirex	1.3 (0.8–2.4) 1.5 (1.0–2.2)		Endometriosis
Sanin (2009)	Co	2592	GIS	Res.	Glyphosate	0.15 (0.12–0.18)		↑ TTP
Smith (1997)	CC	281/216	Questionnaire	Occup.		3.02 (1.10–8.29)		
Cohn (2003)	Co	289	Serum level	Mat.	DDT	32 %		↑ TTP
Chevrier (2013)	CS	3421	Cord blood		DDE	–		↑ TTP
Semen disquality								
Swan (2003)	CC	50/36	Urine sample	–	Arachlor	30 (4.3–210)		↓ sperm count, ↓ Sperm motility, ↓ sperm morphology
					Diazinon	16.7 (2.8–98)		
					Atrazine	11.3 (1.3–98.9)		
Aneck-Hahn (2007)	CS	311	Blood level	–	DDE	−0.02	0.001	↓ Sperm motility
						−0.0003	0.02	↓ Ejaculate volume
						1.001	0.03	Oligozoospermia
						1.001	0.02	Asthenozoospermia
Celik-Ozenci (2012)	CS	40	Blood level	Occup.	Abamectin	–		↓ Sperm motility ↓ Sperm maturity
De Fleurian (2009)	CC	314/88	Questionnaire	Occup.		3.6 (0.8–15.8)		Oligospermia, asthenospermia, or teratospermia
Perry (2007)	CS	17	Urine level	Occup.	Pyrethroids, OPs	–		↓ sperm count
Perry (2011)	CC	94/95	Urine level		OPs	1.30 (1.02–1.65)		↓ sperm count ↓ Sperm motility
Ji et al (2011)	CS	240	Blood level		Pyrethroids	−0.27 0.27	<0.001 <0.001	↓ sperm count ↑ sperm DNA damage
Recio-Vega (2008)	Co	52	Urine level	Occup.	OPs	–		↓ sperm count
Khan (2010)	CC	50/50	Blood level	–	HCH	–		↓ sperm count Yq deletion

Table 4 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value	Found risk
Meeker JD et al. (2008)	CS	207	Blood level	–	Pyrethroids	–		↓ sperm count ↓ sperm motility ↑ sperm DNA damage
Messaros (2009)	CS	336	Blood level	–	DDE, DDT	–		↓ sperm count ↓ sperm motility ↑ sperm morphology
Miranda-Contreras (2013)	CS	100	Blood level (ChE activity)	–	OPs, carbamates	–		↑ sperm DNA damage ↓ sperm parameters ↑ FSH, LH
Xia et al. (2008)	CC	376	Urine level	–	Pyrethroids	2.04 (1.02–4.09)	0.027	↓ sperm count
Yucra (2008)	CS	62	Urine level	Occup.	OPs	–		↓ semen volume ↑ semen pH
Birth defects								
Gemmill (2013)	Co	442	GIS	Mat.	Methyl bromide	−113.1 g −0.85 cm −0.33 cm		↓ birth weight ↓birth length Head circumference
Sathyanarayana (2010)	Co	2246	Questionnaire	Mat.	Carbaryl	−82 (−132, −31)		↓ birth weight
Burdorf (2011)	Co	8880	JEM	Mat.	–	2.42 (1.10–5.34)		↓ birth weight
Brender (2010)	CC	184/225	Interview	Mat.	–	2 (1.2–3.1)		Neural tube defects
Brucker-Davis (2008)	CC	56/69	Colostrum	Mat.	DDE			Cryptorchidism
Chevrier C ET AL. 2011	Co	579	Urine level	Mat.	Atrazine	1.5 (1.0–2.2) 1.7 (1.0–2.7)		↓ fetal growth Head circumference
de Siqueira (2010)	Ec	26 states	Pesticide use	Par.	–	–	0.045 0.004	↓ birth weight congenital abnormality
Dugas (2010)	CC	471/490	Interview	Mat.	Insecticides	1.8 (1.06–3.11)		Hypospadias
Perera (2003)	CS	263	Plasma level	Mat.	Chlorpyrifos	–	0.01 0.003	↓ birth weight ↓ birth length
Ren (2011)	CC	80/50	Placental level	Mat.	DDT α-HCH	5.19 (1.70–15.82) 3.89 (1.26–11.97		Neural tube defects
Rocheleau (2009)	MA	9 studies	JEM	Mat.	–	1.36 (1.04–1.77)		Hypospadias
				Pat.	–	1.19 (1.00–1.41)		
Whyatt (2004)	CS	314	Cord blood		Chlorpyrifos/diazinon	–	<0.05	↓ birth weight ↓ birth length
Waller (2010)	CC	805/3616	Surface water	Mat.	Atrazine	1.6 (1.10–2.34)	0.014	Gastroschisis
Michalakis (2014)	CS	29	Hair sample	Par.	DDT HCH DMP	–	0.009 0.037 0.071	Hypospadias
Kielb et al. (2014)	CC	871/2857	JEM	Mat.	–	1.88 (1.16–3.05)		Gastroschisis

Table 4 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value	Found risk
Makelarski (2014)	CC	502/2950	JEM	Mat.	Insecticides + Herbicides	2.1 (1.0–4.1)		Spina Bifida
Jørgensen (2014)	Co	600000	JEM	Mat.	–	1.31 (1.21–1.53)		Cryptorchidism
Carmichael (2013)	CC	690/2195	GIS	Mat.	Aldicarb Dimethoate Phorate	2.69 (1.04–6.96) 2.45 (1.36–4.39) 2.76 (1.19-6.44)		Hypospadias
Changed sex ratio and maturation and hormones								
Tiido (2005)	CS	149	Blood level	Occup.	DDE	1.6 (0.8–2.5)	<0.001	Yq fraction
Tiido (2006)	CS	547	Blood level	Res.	DDE	–	<0.001	Yq fraction
Den Hond (2011)	CS	1679	Blood level	–	HCBDDE	–		Pubertal staging (men)
Meeker (2009)	CS	161	Blood level		Pyrethroids	–	<0.05 <0.03 <0.09	↑ FSH ↓ inhibin B ↓ testosterone
Meeker (2006)	CS	268	Blood level		Chlorpyrifos Carbaryl Naphthalene	–		↓ testosterone, FAI and LH

♂: risk found in male, ♀: risk found in female, *MA* meta-analysis, *CC* case–control, *CS* cross-sectional, *Co* cohort, *Ec* ecological, *Mr* mortality, *Ret.* retrospective, *Pros.* prospective, *Occup.* occupational, *Env.* environmental, *Mat.* maternal, *Pat.* paternal, *Par.* parental, *Res.* residential, *Gen.* general, *GIS* geographic information system, *JEM* job exposure matrix, *OR* odd ratio, *RR* relative risk, *HR* hazard ratio, *ChE* cholinesterase, *OPs* organophosphoruses, *DMP* dimethyl phosphate, *2,4-D* 2,4-dichlorophenoxyacetic acid, *2,4,5-T* 2,4,5-trichlorophenoxyacetic acid, *HCB* hexachlorobenzene, *α-HCH* alpha-hexachlorocyclohexane, *DDT* dichlorodiphenyltrichloroethane, *DDE* dichlorodiphenyldichloroethylene, *FAI* free androgen index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone

sperm, increased levels of the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) have been reported by a cross-sectional study assessing the exposure via the enzymatic activity of acetylcholinesterase and butyrylcholinesterase in the blood (Miranda-Contreras et al. 2013). Swan and colleagues compared cases having low sperm count, motility, and morphology with controls regarding biomarkers of pesticide exposure and found that arachlor, diazinon, and atrazine were more often detectable in the urine of cases than that of controls with respective ORs including 30, 16.7, and 11.3 (Swan et al. 2003). Lowered sperm motility and maturity have also been found in people occupationally exposed to abamectin (Celik-Ozenci et al. 2012).

Birth defects

Birth defects, also known as congenital disorders or anomalies, have different types, e.g., low birth weight and length, cryptorchidism, hypospadias, neural tube defect, spina bifida, gastroschisis, and head circumference, and a substance inducing birth defects is called teratogen. There has been remarkable evidence on the teratogenicity of pesticides in human of which 18 relevant studies including 3 cross-sectional, 8 case–control, 5 cohort, and one ecological have been presented in this review (Table 4). Association of low birth weight with maternal exposure to pesticides, regardless of the type, with an OR of 2.4 has been reported by a JEM-based cohort study (Burdorf et al. 2011). Further, a questionnaire-based analysis of a cohort in the Agricultural Health Study indicated that maternal exposure to carbaryl lowered the birth weight as given by an OR of −82 g (Sathyanarayana et al. 2010). Such a risk plus lower birth length have been linked to maternal exposure to chlorpyrifos and diazinon by two separate cross-sectional studies (Perera et al. 2003; Whyatt et al. 2004). In addition to lower birth weight and length, head circumference has been reported by two cohort studies examining the offspring whose mothers were exposed to atrazine and methyl bromide (Chevrier et al. 2013; Gemmill et al. 2013). In this regard, an ecological study conducted in 26 states of Brazil revealed that there are significant correlations between pesticide use in the agriculture and low birth weight as well as congenital abnormality (de Siqueira et al. 2010).

The risk of neural tube defect has been shown to be doubled due to maternal exposure to pesticides by a questionnaire-based case–control study, while another study comparing the cases with controls regarding the placental level of organochlorines reported that maternal exposure to DDT and α-HCH

is associated with neural tube defect with respective ORs 5.2 and 3.9 (Brender et al. 2010; Ren et al. 2011). Moreover, an almost doubled risk of spina bifida has been estimated for maternal exposure to insecticides and herbicides by a JEM-based case–control study (Makelarski et al. 2014).

A cryptorchidism HR of 1.3 has been estimated by a JEM-based cohort study among the sons of mothers engaged in horticulture and farming (Jorgensen et al. 2014). Further, comparing the cases with controls regarding maternal exposure to organochlorines indicated that DDE had been more often detectable in the colostrum of mothers whose son suffered from cryptorchidism (Brucker-Davis et al. 2008).

Elevated hypospadias OR (1.8) in association with maternal exposure to insecticides was estimated by a questionnaire-based case–control study (Dugas et al. 2010). In addition, the results of a GIS-based case–control study showed that maternal exposure to aldicarb, dimethoate, and phorate increased the risk of hypospadias with respective ORs of 2.7, 2.4, and 2.8 (Carmichael et al. 2013). Michalakis and colleagues have evaluated parental exposure to organochlorines and organophosphates by measuring their level in the hair samples and concluded that chronic exposure of parents to DDT, HCH, and organophosphoruses was associated with higher incidence of hypospadias in the offspring (Michalakis et al. 2014). There has also been a meta-analysis of 9 studies whose results gave elevated hypospadias ORs of 1.4 and 1.2 in the boys whose, respectively, mothers and fathers had been occupied in the jobs dealing with pesticides (Rocheleau et al. 2009).

Gastroschisis, another type of birth defect, has also been linked to prenatal exposure to pesticides by a JEM-based case–control study estimating an OR of about 1.9 (Jorgensen et al. 2014). Further, Waller and colleagues reported that the incidence of gastroschisis increased in the offspring whose mothers were residing in the areas with high concentration of atrazine in the surface water (Waller et al. 2010).

Changed sex ratio, maturation, and hormones

There is sporadic evidence on pesticide-induced sexual dysfunction, some of which relevant to the reproduction have been presented in this review (Table 4). Tiido and colleagues conducted two separate cross-sectional studies examining pesticide exposure via their blood level measurement and concluded that exposure to DDE was significantly associated with the Y-chromosome fraction in human sperm (Tiido et al. 2005, 2006). Higher concentration of DDE and HCB in the blood samples was shown to link with disruption of pubertal staging in men (Den Hond et al. 2011). Regarding sexual hormone alteration, two separate cross-sectional studies carried out by Meeker and colleagues reported an increased level of FSH accompanying a decreased level of inhibin B, testosterone, LH, and free androgen index (FAI) in association with exposure to pyrethroids, chlorpyrifos, carbaryl, and naphthalene (Meeker et al. 2006, 2009).

Disease-based evidence on developmental toxicity of pesticides

Attention deficit hyperactivity disorder (ADHD)

ADHD is a neurodevelopmental disorder manifested by behavioral problems such as attention difficulty, hyperactivity, troubled relationship, and lowered self-esteem. There has been recently ongoing evidence on the role of environmental risk factors such as pesticide exposure in the incidence of ADHD, as such 11 epidemiological studies published during the last decade have been reviewed here. They include 4 cross-sectional, 6 cohort, and a case–control analyses on the link of ADHD with exposure to pesticides (Table 5). Elevated ORs of 1.5 and 5.1 for ADHD have been linked with organophosphoruses by two cross-sectional studies examining the exposure via urine and blood samples (Bouchard et al. 2010; Suarez-Lopez et al. 2013). Comparing the cases of ADHD with control regarding exposure to organophosphoruses indicated that the biomarkers of organophosphoruses were 2–3 times more detectable in the urine sample of the cases than that of controls (Yu et al. 2016). Furthermore, a cohort study measuring the biomarkers of organophosphoruses in the urine samples of the mothers revealed an OR of 1.3 for the risk of ADHD in association with maternal exposure to organophosphoruses (Marks et al. 2010). Another cohort study measuring the urine concentration of organophosphoruses indicated that maternal exposure to chlorpyrifos increased the risk of ADHD in boys and attention deficit (AD) in girls with respective ORs 5.5 and 5.8 (Fortenberry et al. 2014). The link between prenatal exposure to chlorpyrifos and higher incidence of ADHD with a risk estimate of 6.5 was resulted from a cohort study examining the exposure via blood level measurement of the pesticides (Rauh et al. 2006).

In association with pyrethroids, there is a cross-sectional study measuring their biomarkers in the urine and calculated an ADHD risk estimate of 2.4 in relation to exposure to pyrethroids (Wagner-Schuman et al. 2015).

Three separate cohort studies evaluated the cord blood concentration of organochlorines and found a higher incidence of ADHD complications such as irritability and behavioral problems in association with prenatal exposure to DDE (Sagiv et al. 2008, 2010; Sioen et al. 2013). In addition, higher concentration of trichlorophenol has been detected in the urine samples taken from children having ADHD for which an OR of 1.8 has been estimated by a cross-sectional study (Xu et al. 2011).

Table 5 Developmental toxicity of pesticides evidenced by diseases

Study	Type of study	Sample no.	Exposure assessment	Exposure	Associated target	OR/RR/HR (95 % CI)	*p* value	Found disorder related to
ADHD								
Wagner-Schuman (2015)	CS	687	Urine level		Pyrethroid	2.42 (1.06–5.57)		
Bouchard (2010)	CS	1139	Urine level		OPs	1.55 (1.14–2.10)		
Yu (2016)	CC	97/110	Urine level		OPs	Two-threefold	<0.05	
Marks (2010)	Co	323	Urine level	Mat.	OPs	1.3 (0.4–2.1)		
Rauh (2006)	Co	254	Plasma	Mat.	Chlorpyrifos	6.50 (1.09–38.69)		
Suarez-Lopez (2013)	CS	307	Blood ChE	–	OPs (boys)	5.14 (0.84–31.48)		Neurodevelopment
Sioen (2013)	Co	270	Cord blood	Mat.	DDE	9.95 (1.37–72.35)♀	0.023	Behavioral
Sagiv (2010)	Co	607	Cord blood	Mat.	DDE	1.8		
Sagiv (2008)	Co	788	Cord blood	Mat.	DDE	↑ risk	0.03	Irritability
Fortenberry (2014)	Co	187	Urine level	Mat.	Chlorpyrifos	5.55 (−0.19, 11.3)♂ 5.81 (−0.75, 12.4)♀	0.06 0.08	ADHD AD
Xu (2011)	CS	2546	Urine level		Trichlorophenol	1.77 (1.18–2.66)	0.006	
Autism								
Eskenazi (2007)	Co	531	Urine level	Mat.	OPs	–3.5 (–6.6–0.5)		Mental
Braun (2014)	Co	175	Blood, urine	Mat.	Chlordane	4.1 (0.8–7.3)		
Cheslack-Postava (2013)	CC	75/75	Serum	Mat.	DDE	1.79 (0.52–6.21)	0.36	
Roberts (2007)	CC	465/6975	GIS	Mat.	OCs	6.1 (2.4–15.3)		
Keil (2014)	CC	407/262	Interview	Mat.	Imidacloprid	1.3 (0.78–2.2)		
Shelton (2014)	CC	486/316	Questionnaire	Mat. (3rd) Mat. (2nd) Mat. (3rd)	OPs Chlorpyrifos Pyrethroids	2.0 (1.1–3.6) 3.3 (1.5–7.4) 1.87 (1.02–3.43)		
Developmental Delay								
Andersen (2015)	Co	133	Interview	Mat.	–			Neurobehavioral
Bosma (2000)	Co	830	Questionnaire	Occup.	–	2.02 (1.27–3.20)		Cognitive
Ribas-Fitó (2003)	Co	92	Cord blood	Mat.	p,p′-DDE	−3.5 −4.01		Mental Psychomotor scale
Viel et al. (2015)	Co	428	Urine level	–	Pyrethroids		<0.01	Verbal Memory
Zhang (2014)	Co	249	Urine level	Mat.	OPs	−1.78 (−2.12, −1.45		Neurobehavioral
Bouchard et al. (2011)	Co	329	Urine level	Mat.	OPs (DAP)	−5.6 (−9.0, −2.2)	<0.01	IQ
Engel (2011)	Co	404	Urine level	Mat.	OPs			Cognitive
Engel (2007)	Co	311	Urine level	Mat.	Malathion	2.4 (1.55–3.24)		Abnormal reflexes
Young (2005)	Co	381	Urine level	Mat.	OPs (DAP)	4.9 (1.5–16.1)		Abnormal reflexes
Horton (2012)	Co	725	Cord blood	Mat.	Chlorpyrifos	−1.71 (−3.75 to 0.32)		Working memory
Rauh (2011)	Co	265	Cord blood	Mat.	Chlorpyrifos	1.4 2.8	0.064 0.001	Full-scale IQ Working memory

Table 5 continued

Study	Type of study	Sample no.	Exposure assessment	Exposure	Associated target	OR/RR/HR (95 % CI)	*p* value	Found disorder related to
Harari (2010)	CS	87	Interview	Mat.	–	−7.1 (−12.5, −1.6) 5.32 (1.03–27.62) 6.62 (1.02–42.93)	<0.05 <0.05 <0.05	Motor speed Motor coordination Visual memory
Torres-Sánchez (2013)	Co	203	Serum	Mat.	DDE	−1.37 (−2.56 to 0.19 –0.80 (−1.52 to 0.08)	<0.05 <0.05	Cognitive index Memory
Dallaire (2012)	Co	153	Cord blood	Mat.	Chlordecone	−0.19 (−0.35,−0.03) 1.25 (1.07–1.45)	0.02 0.002	Cognitive Motor
			Breast milk	Mat.	Chlordecone	−0.14 (−0.29, −0.01)	0.07	Cognitive

♂: risk found in male, ♀: risk found in female, *MA* meta-analysis, *CC* case–control, *CS* cross-sectional, *Co* cohort, *Ec* ecological, *Mr* mortality, *Ret.* retrospective, *Pros.* prospective, *Occup.* occupational, *Env.* environmental, *Mat.* maternal, *Pat.* paternal, *Par.* parental, *Res.* residential, *Gen.* general, *GIS* geographic information system, *JEM* job exposure matrix, *OR* odd ratio, *RR* relative risk, *HR* hazard ratio, *ChE* cholinesterase, *OPs* organophosphoruses, *DDE* dichlorodiphenyldichloroethylene, *DAP* dialkyl phosphate, *ADHD* attention deficit hyperactivity disorder, *AD* attention deficit, *IQ* intelligence quotient

Autism

Autism has also been recently studied in relation to pesticide exposures for which 6 relevant studies, including two cohorts and 4 case–control analyses, all implicating on maternal exposures, have been brought in this review (Table 5). A GIS-based case–control study reported an autism OR of 6.1 in association with organochlorines, as such a risk with estimated ORs including 1.8 and 4.1 has been, respectively, reported for DDE and chlordane by two blood-measuring studies, one of which was case–control and the other one was cohort (Braun et al. 2014; Cheslack-Postava et al. 2013; Roberts et al. 2007).

Urine sample analysis for biomarkers of organophosphoruses was performed in a cohort of young Mexican–American children, and the results implicated on decreased mental development indices (beta −3.5 points per tenfold increase in prenatal biomarkers of organophosphoruses) at 24 months of age (Eskenazi et al. 2007). In addition, the Childhood Autism Risks from Genetics and Environment (CHARGE) study compared the cases with control regarding residential proximity to agricultural pesticides during pregnancy and reported that exposure to organophosphoruses and pyrethroids during the third trimester and chlorpyrifos during the second trimester of pregnancy was associated with increased risk of autism (ORs ranging from 1.9 to 3.3) in the offspring (Shelton et al. 2014). An autism OR of 1.3 in association with maternal exposure to imidacloprid has also been found by a questionnaire-based case–control study (Keil et al. 2014).

Developmental delay

Developmental impairments, manifested by different features such as cognitive, memory, verbal, visual, behavioral, and motor dysfunctions, have been evaluated in association with pesticide exposures by 14 epidemiological studies, including 13 cohorts as well as one cross-sectional analyses brought in this review (Table 5). The results of a questionnaire-based cross-sectional study implicated on impaired motor speed, motor coordination, and visual memory in association with maternal exposure to any kind of pesticides (Harari et al. 2010). Maternal and occupational exposures to ever used pesticide have also been shown to link with, respectively, neurobehavioral deficits and cognitive dysfunction by two separate cohort studies (Andersen et al. 2015; Bosma et al. 2000). All of the remaining 10 cohort studies have evaluated the risk of developmental impairments in association with maternal exposure to specified classes of pesticides measured in biological samples. Urine sample analysis was performed for detecting biomarkers of organophosphoruses as well as malathion itself by five cohort studies whose results implicated on reduced neurobehavioral development, cognitive development, and intelligence quotient (IQ) as well as increased abnormal reflexes in children having maternal exposure to the mentioned pesticides (Bouchard et al. 2010; Engel et al. 2007, 2011; Young et al. 2005; Zhang et al. 2014). Furthermore, maternal exposure to chlorpyrifos, measured by cord blood analysis in two separate cohort studies, was shown to be associated with lower

working memory and full-scale IQ in childhood (Horton et al. 2012; Rauh et al. 2011).

Regarding developmental impairments in relation to organochlorines, there are two cohort studies on maternal exposure to DDE which has been linked with reduced mental and psychomotor scale, general cognitive index, and memory function (Ribas-Fito et al. 2003; Torres-Sanchez et al. 2013). There is another cohort study analyzing cord blood and breast milk samples and reported both decreased cognitive and motor development due to prenatal and decreased cognitive development due to postnatal exposure to chlordecone (Dallaire et al. 2012).

Disease-based evidence on metabolic toxicity of pesticides

Diabetes

Diabetes has become epidemic because of its prevalent risk factors, including Western diet and physical inactivity in the modern life, though the environmental risk factors, particularly pesticide exposures, have also been linked to its development (Bahadar et al. 2014; Mostafalou and Abdollahi 2012c). Herein, a total of 28 studies including 19 cross-sectional, three case–control, five cohort, and an ecological analyses of the link between human exposure to various pesticides and incidence of diabetes have been reviewed (Table 6). Except one questionnaire-based cross-sectional study estimating gestational diabetes OR of 2.2 in association with occupational exposure to any kind of pesticides (Saldana et al. 2007), the other studies reported the risk of diabetes in relation to specified classes of pesticides. The cross-sectional association of the serum concentration of organochlorines with diabetes and insulin resistance was investigated using data resulted from the National Health and Examination Survey 1999–2002, and adjusted ORs for diabetes and insulin resistance were estimated as 37.7 and 7.5, respectively (Lee et al. 2006, 2007a). Recently, a meta-analysis of 22 studies on the link of exposure to pesticides with incidence of human diabetes resulted in a risk estimate of about 1.7 for organochlorines (Evangelou et al. 2016). Among organochlorines specifically associated with diabetes, the highest number of evidence belongs to the DDE given 11 elevated diabetes risk estimates ranging from 1.3 to 12.7 in 7 cross-sectional, 2 case–control, and 2 cohort studies examining the exposure via blood sample analysis (Airaksinen et al. 2011; Codru et al. 2007; Cox et al. 2007; Lee et al. 2011a; Philibert et al. 2009; Rignell-Hydbom et al. 2007, 2009; Son et al. 2010; Turyk et al. 2009a, b; Ukropec et al. 2010). Furthermore, there has been a published meta-analysis of 18 studies evaluating the incidence of diabetes in relation to DDE, and a significant elevated risk estimate of 1.3 has been analyzed (Tang et al. 2014a). The other organochlorines for which elevated diabetes risk estimates have been found include DDT with 5 reported ORs ranging from 1.9 to 10.6, HCB with five reported ORs ranging from 2.8 to 6.8, trans-nonachlor with 5 reported ORs ranging from 2.2 to 8.1, oxychlordane with four reported ORs ranging from 2 to 6, heptachlor with three reported ORs ranging from 1.7 to 3.1, β-HCH with two reported ORs including 2.1 and 8.2, mirex with two reported ORs including 2.1 and 3.7, aldrin with an OR of 1.5, dieldrin with an OR of 2, chlordane with an OR of 1.6, and alachlor with an OR of 1.3 (Airaksinen et al. 2011; Codru et al. 2007; Cox et al. 2007; Everett et al. 2007; Gasull et al. 2012; Kim et al. 2014; Lee et al. 2010, 2011a; Montgomery et al. 2008; Patel et al. 2010; Son et al. 2010; Starling et al. 2014; Ukropec et al. 2010; Wu et al. 2013). Moreover, a cross-sectional association of occupational exposure to pentachlorophenol with hyperglycemia manifested by fasting blood glucose higher than 100 mg/dl has been found in retired workers of a pentachlorophenol-manufacturing plant (Chang et al. 2012).

Regarding organophosphoruses, there is a questionnaire-based cross-sectional study reporting that occupational exposure to organophosphorus insecticides is significantly associated with hyperglycemia (Malekirad et al. 2013). Parathion, phorate, fonofos, and trichlorfon are organophosphorus insecticides for which, respectively, elevated risk estimates including 1.6, 1.6, 1.6, and 2.6 have been calculated by two separate questionnaire-based cohort studies (Montgomery et al. 2008; Starling et al. 2014).

Occupational exposure to pyrethroids has also been evaluated by two questionnaire-based cross-sectional studies, one of which reported significantly elevated diabetes OR of 1.5, while the other estimated an OR of 18.5 in association with a 5.6 % increase in HbA1c (Hansen et al. 2014a; Wang et al. 2011b).

A cohort study conducted by Montgomery and colleagues indicated that occupational exposure to the herbicide cyanazine is significantly associated with increased risk of diabetes given by an OR of about 1.4 (Montgomery et al. 2008). Occupational exposure to the phenoxy herbicides has also been linked with elevated incidence of diabetes with an estimated HR of 1.6 by a questionnaire-based cohort study (Starling et al. 2014). Furthermore, association of diabetes with exposure to Agent Orange was revealed by a cross-sectional and an ecological study estimating respective ORs of 2.7 and 1.04 among Korean Vietnam veterans (Kim et al. 2003; Yi et al. 2014).

Table 6 Metabolic toxicity of pesticides evidenced by diseases

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value	Found risk
Diabetes								
Lee (2006)	CS	2016	Serum level	–	OCs	37.7 (7.8–182)	<0.001	
Kim (2003)	CS	1378	Military record	–	Agent Orange	2.69		
Yi et al. (2014)	Ec	111726	GIS	Occup.	Agent Orange	1.04 (1.01–1.07)		
Kim (2014)	CS	50	VAT/SAT	–	DDT	9.0 (1.3–62.9)	0.02	
Son (2010)	CC	40/40	Serum	–	Oxychlordane Trans-nonachlor Heptachlor epoxide Hexachloroben-zene β-HCH Mirex DDE DDT	6.0 (1.3–517.4) 8.1 (1.2–53.5) 3.1 (0.8–12.1) 6.1 (1.0–36.6) 8.2 (1.3–53.4) 3.7 (0.9–15.8) 12.7 (1.9–83.7) 10.6 (1.3–84.9)	<0.01 0.02 0.05 0.03 0.02 0.08 <0.01 0.02	
Wang (2011b)	CS	3080	Questionnaire	Occup.	Pyrethroids	1.48 (1.23–1.77)	<0.001	
Chang (2012)	CS	1167	Retired workers	Occup.	Pentachlorophe-nol	7.22 (4.04–12.90)		↑FBG
Malekirad (2013)	CS	374	Questionnaire	Occup.	OPs	–	<0.001	↑FBG
Hansen (2014a)	CS	208	Questionnaire	Occup.	Pyrethroids	18.5 (5.5–62.5)		↑HbA1c
Starling (2014)	Co	13,637	Interview	Occup.	Fonofos Phorate Parathion Dieldrin 2,4,5-T/2,4,5-TP	1.56 (1.11–2.19) 1.57 (1.14–2.16) 1.61 (1.05–2.46) 1.99 (1.12–3.54) 1.59 (1.00–2.51)		
Patel (2010)	CS	503–3318	EWAS	–	Heptachlor epoxide	1.7	<0.001	
Lee (2010)	Nested CC	90/90	Serum level	–	trans-Nonachlor Oxychlordane Mirex	4.8 (1.7–13.7) 2.0 (0.8–5.0) 2.1 (0.8–5.5)	0.06	
Montgomery (2008)	Co	33,457	Questionnaire	Occup. (>100 days)	Aldrin Chlordane Heptachlor Trichlorfon Alachlor Cyanazine	1.51 (0.88–2.58) 1.63 (0.93–2.86) 1.94 (1.02–3.69) 2.47 (1.10–5.56) 1.31 (1.11–1.55) 1.38 (1.10–1.72)	0.08 0.05 0.02 0.02 0.001 0.004	
Lee (2007a)	CS	749	Serum level	–	OCs	7.5 (2.3–23.9)	<0.01	↑HOMA-IR
Saldana (2007)	CS	11,273	Questionnaire	Occup.	–	2.2 (1.5–3.3)		Gestational
Airaksinen (2011)	CS	1988	Serum level	–	Oxychlordane trans-Nonachlor DDE	2.08 (1.18–3.69) 2.24 (1.25–4.03) 1.75 (0.96–3.19)		T2DM
Philibert (2009)	CS	101	Serum level	–	DDE	6.1 (1.4–27.3)		
Ukropec (2010)	CS	2047	Serum level	–	DDE DDT	1.86 (1.17–2.95) 2.48 (1.77–3.48)		
Gasull (2012)	CS	886	Serum level	–	HCB	2.8		
Rignell-Hydbom (2007)	CS	543	Serum level	–	DDE	1.3 (1.1–1.6)		
Everett (2007)	CS	1830	Serum level	–	DDT	2.69 (1.35–5.36)		
Cox (2007)	CS	1303	Serum level	–	Oxychlordane trans-Nonachlor DDE DDT β-HCH	3.1 (1.1–9.1) 2.9 (1.3–6.4) 2.6 (1.2–5.8) 1.9 (1.0–3.7) 2.1 (1.0–4.3)		

Table 6 continued

Study	Type of study	No. of samples	Exposure assessment	Exposure	Target pesticide	OR/RR/HR (95 % CI)	*p* value	Found risk
Codru (2007)	CS	352	Serum level	–	DDE HCB	6.2 (1.8–21.9) 6.8 (2.3–20.3)		
Turyk (2009a)	CS	503	Serum level	–	DDE	3.6	0.009	
Lee (2011a, b)	Co	725	Serum level	–	trans-Nonachlor, DDE, HCB	3.4 (1.0–11.7)	0.03	
Turyk (2009b)	Co	471	Serum level	–	p,p′-DDE	7.1 (1.6–31.9)		
Wu (2013)	Co	1095	Serum level	–	HCB	3.1 (1.3–7.7)		
(Rignell-Hydbom 2009)	Nested CC	39/39	Serum level	–	DDE	5.5 (1.2–25.0)		T2DM
Tang (2014a, b)	MA	18 studies	–	–	DDE	1.33 (1.15–1.54)	0.0007	
Evangelou (2016)	MA	22 studies	–	–	OCs	1.68 (1.37, 2.07)	8×10^{-7}	
Obesity								
Bachelet (2011)	CS	1055	Serum level	–	DDE	1.39 (1.13–1.70)		↑BMI
Jakszyn (2009)	CS	953	Serum level	–	DDE, β-HCH, HCB			↑BMI
Lee (2006)	CS	2016	Serum level	–	OCs		<0.01	↑BMI
Lee (2012)	CS	970	Serum level	–	DDE DDE HCB trans-Nonachlor	1.7 (0.9–3.1)♀ 2.6 (1.1–5.7)♂ 3.4 (1.6–7.5)♂ 2.5 (1.1–5.6)♂	<0.05 <0.01 <0.01 0.04	
Dirinck (2011)	CS	145	Serum level	–	β-HCH		<0.001 <0.05	↑BMI ↑HOMA-IR
Ibarluzea (2011)	CS	1259	Serum level	–	HCB, β-HCH		<0.001	
Lee (2011a, b)	Co	5115	Serum level	–	DDE, DDT		0.05	
Glynn (2003)	CS	205	Serum level	–	DDE β-HCH HCB	3.9 (1.3–6.6) 3.8 (2.1–5.6) 1.1 (0.5–2.3)		

♂: risk found in male, ♀: risk found in female, *MA* meta-analysis, *CC* case–control, *CS* cross-sectional, *Co* cohort, *Ec* ecological, *Mr* mortality, *Ret*. retrospective, *Pros*. prospective, *Occup*. occupational, *Env.* environmental, *Mat.* maternal, *Pat.* paternal, *Par*. parental, *Res*. residential, *Gen*. general, *GIS* geographic information system, *JEM* job exposure matrix, *OR* odd ratio, *RR* relative risk, *HR* hazard ratio, *EWAS* environment-wide association study, *OPs* organophosphoruses, *OCs* organochlorines, *2,4-D* 2,4-dichlorophenoxyacetic acid, *2,4,5-T* 2,4,5-trichlorophenoxyacetic acid, *HCB* hexachlorobenzene, *β-HCH* beta-hexachlorocyclohexane, *DDT* dichlorodiphenyltrichloroethane, *DDE* dichlorodiphenyldichloroethylene, *VAT* visceral adipose tissue, *SAT* subcutaneous adipose tissue, *FBG* fasting blood glucose, *HbA1c* Hemoglobin A1c, *HOMA-IR* homeostatic model assessment—insulin resistance, *T2DM* type 2 diabetes mellitus, *BMI* body mass index

Obesity

Obesity, a condition involving excessive body fat, may not be categorized alone as a disease, but increases the risk of other serious health problems, and recent environmental health studies have shown that obesity may have other risk factors than excess calorie intake and physical inactivity. Exposure to pesticides especially those categorized as persistent organic pollutants has been linked to increased incidence of obesity by epidemiological studies, some of which including 7 cross-sectional and a cohort analyses have been reviewed in this study (Table 6). All of these studies have evaluated exposure to different organochlorine via blood sample analysis of participants, and higher risk of obesity, manifested by increased body mass index (BMI), has been linked with DDE, DDT, HCB, β-HCH, trans-nonachlor, and oxychlordane exposure (Bachelet et al. 2011; Dirinck et al. 2011; Glynn et al. 2003; Ibarluzea et al. 2011; Jakszyn et al. 2009; Lee et al. 2006, 2011b, 2012).

Discussion and conclusion

Our systematic review of 43 human diseases divided into six broad groups of toxicities in association with exposure to pesticides shows that recorded evidence belongs to the, in order from the highest to the lowest, carcinogenicity, neurotoxicity, reproductive toxicity, metabolic toxicity, pulmonotoxicity, and developmental toxicity of pesticides (Fig. 2). Further, carcinogenicity is considered as the most reported toxicity studied in relation to each class of

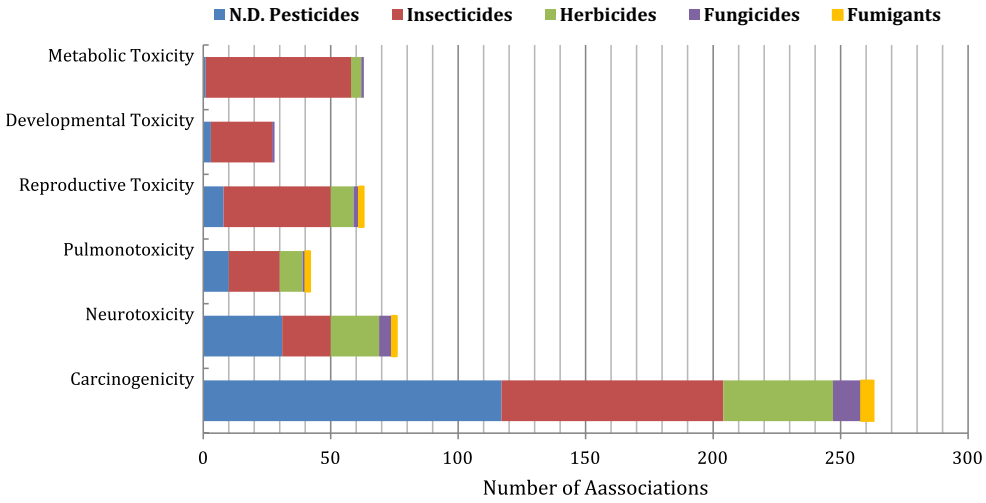

Fig. 2 Schematic diagram showing the weight of evidence on the toxicities of pesticides. *ND* not determined

pesticides, including insecticides, herbicides, fungicides, and fumigants (Fig. 3).

The link of cancer incidence with human exposure to pesticides has been presented based on 28 cites of cancers divided into nine body organ systems among which the most studied associations are related to the malignancies of the hematopoietic system notably leukemia and lymphoma. Brain tumors, prostate cancer, breast cancer, colorectal cancer, pancreatic cancer, and lung cancer have then the most prevalent evidence in association with exposure to pesticides. Among studies focusing on the link of cancers with specified class of pesticides, insecticides have been located in the first place, followed by herbicides, fungicides, and fumigants (Table 7). It should be noted that insecticides and herbicides are generally considered as the most used pesticides which may explain the differences in the rate of their association with cancer incidences (Mostafalou et al. 2013). In the same way, the differences in the prevalence of cancers can exemplify the different degrees of correlation between pesticide exposures and incidence of each site of cancers. Nevertheless, the body of evidence on this link is so huge that the role of pesticides in cancer development cannot be doubted. In addition to epidemiological evidences, such a confirmation can also be derived from experimental studies investigating the mechanisms by which carcinogenicity of pesticides is mediated.

As known, heritable changes in the genes controlling cell cycle, via genetic or epigenetic mechanisms, are responsible for cancer initiation by chemical carcinogens. During the past half century, the progress of molecular biology techniques has made it possible to largely investigate carcinogenicity of widely used chemicals, particularly pesticides. Various pathways leading to genetic or epigenetic alterations of the cell have been explored regarding pesticides tested ex vivo, in vitro, in vivo, and in human. Since genetic damages at the level of both DNA and chromosomes are considered as likely mechanisms of carcinogenicity, the huge body of evidence on genotoxicity of pesticides brought a global concern on carcinogenesis of these chemicals on which human life has become so dependent (Mostafalou and Abdollahi 2012b; Shadnia et al. 2005). In addition to genetic toxicity, epigenetic alterations, including the methylation and acetylation of DNA and its accompanying proteins, histon, have been shown to be induced by some classes of pesticides specially those categorized as endocrine disruptors (Maqbool et al. 2016).

Neurotoxicity has been ordered as the second ranked toxicity of pesticides according to the associations brought in this review, which have been derived from evidence related to the link of pesticides with just three neurodegenerative diseases including Alzheimer, Parkinson, and ALS (Table 8). In comparison with the other toxicities, this can be indicative of a high susceptibility to the neurotoxicity of pesticides, though the incidence of age-related neurodegenerative diseases including Alzheimer and Parkinson itself has been increased, somewhat, due to elevated life expectancy of human in today's world. It should be noted that the primary mechanism of toxicity for the main groups of pesticides, particularly insecticides such as organochlorines, organophosphoruses, and carbamates, is through targeting components of the nervous system (Abdollahi and Karami-Mohajeri 2012; Karami-Mohajeri et al. 2014). However, evidence on the neurotoxicity of pesticides specifically brought in this review shows that the risk of insecticides is somehow similar to that of herbicides among which the most frequently reported evidences are related to the link of paraquat and Parkinson disease. Perhaps, such

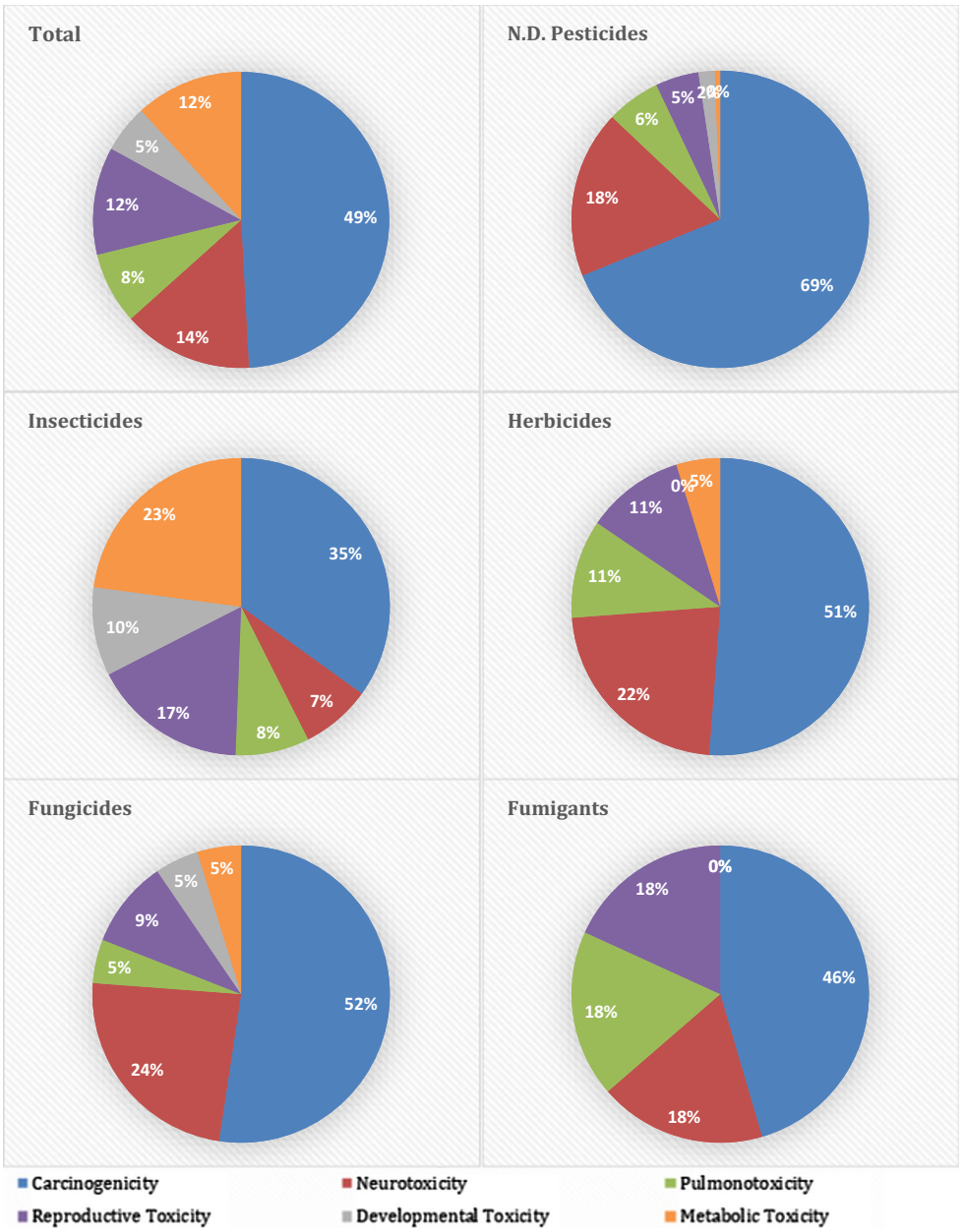

Fig. 3 Schematic charts showing the percent of toxicities attributed to each category of pesticides. *ND* not determined

inspirations to search the link of paraquat with Parkinson by environmental health scientists originate from the use of this herbicide as a drug to induce Parkinson disease in experimental investigating models. In fact, paraquat acts through overproduction of reactive oxygen species which is held in common with toxicity of many other pesticides, and this can give a clue to trace the role of such pesticides in Parkinson disease.

Reproductive and metabolic toxicities have been ordered as the next prevalent toxicities of pesticides in both of which insecticides have adopted the most associations. In these toxicities, the highest share is allocated to the organochlorine insecticides whose physicochemical properties such as high lipid solubility and crossing biological membranes made environmental health scientist much suspicious to their disrupting effects on endocrine and reproductive systems (Table 8). In addition, their dioxin-like biological effect on the nuclear receptors, particularly aryl hydrocarbon receptors, which are involved in the metabolic pathways, has provided enough stimulation to search the role of organochlorine insecticides in the metabolism at cellular or the whole organism level. Although most of the organochlorine insecticides have been widely banned, their ability to persist in the environment for a long period of time made environmentalists to continue their research on the health problems associated with human exposure. For example, lots of evidence on the metabolic disrupting effects of persistent organochlorine

Table 7 Association of cancer incidence with different classes of pesticides

Cancers of	Association with			
	Any pesticide (no.)	Targeted class of pesticides (no.)	Chemical class of pesticides (no.)	Single pesticides
Nervous system	19	Insecticides (1) Herbicides (1) Fungicides (1)	OPs (1) Carbamates (1)	Chlorpyrifos, bufenacarb, paraquat, coumaphos, metribuzin
Digestive system	19	Insecticides (1) Herbicides (2) Fungicides (1)	OCs (1)	Atrazine, 2,4-D, chlordane, trifluralin, methyl bromide Aldicarb, dicamba, imazethapyrc, chlorpyrifos, S-ethyl-N,N-dipropyl, thiocarbamate, trifluralin, acetochlor, HCB, DDT, arsenicals, EPTC, pendimethalin, acetochlor
Hematopoietic system	30	Insecticides (4) Herbicides (5) Fungicides (2) Fumigants (1)	OPs (2) Carbamates (2) OCs (1) Pyrethroids (1) Phenoxy (3)	Crotoxyphos, dichlorvos, famphur, methoxychlor, terbufos, diazinon, Agent Orange, chlorpyrifos, dichlorprop, malathion, diazinon, terbufos, coumaphos, fonofos, carbaryl, lindane, DDT, dieldrin, chlordane, toxaphene, oxychlordane, cis-nonachlor, β-HCH, pentachlorophenol, atrazine, glyphosate, 2,4-D, captan, carbaryl
Bone and soft tissues	7	–	Phenoxyacetic acid (1)	Metolachlor
Urinary system	12	–		Imazethapyr, HCH, DDT
Male reproductive	9	Herbicides (1)	OCs (2) Triazines (1)	Agent Orange, aldrin, malathion (2), fonofos (2), terbufos (2), coumaphos, methyl bromide (3), DDT, carbaryl, chlordecone, ziram, dichlone, azinphos, simazine, maneb, diazinon, parathion, DDE, HCB
Female reproductive	7	–	OPs (1) Triazines (1)	2,4,5-T, captan, DDT (3), DDE (2), DDD, β-HCH (2), HCB, PCTA, diazinon
Head & neck	7	Insecticides (1)	–	Lindane
Lung cancer	4	–	–	Acetochlor, terbufos, dicamba, metolachlor, diazinon, chlorpyrifos, pendimethalin
Thyroid cancer	1	–	–	Alachlor, malathion, atrazine
Skin cancer	2	–	–	Acetochlor, maneb, parathion, carbaryl

OPs organophosphoruses, *OCs* organochlorines, *2,4-D* 2,4-dichlorophenoxyacetic acid, *2,4,5-T* 2,4,5-trichlorophenoxyacetic acid, *EPTC* S-ethyl-N,N-dipropylthiocarbamate, *HCB* hexachlorobenzene, *HCH* hexachlorocyclohexane, *PCTA* pentachlorothioanisole, *DDT* dichlorodiphenyltrichloroethane, *DDE* dichlorodiphenyldichloroethylene, *DDD* dichlorodiphenyldichloroethane

insecticides within the context of insulin resistance such as diabetes and obesity have been gathered during the past two decades in which there was no extensive use of these chemicals formally (Karami-Mohajeri and Abdollahi 2011). These metabolic effects of organochlorine insecticides have been attributed to their ability to easily cross through biological membranes and accumulate in adipose tissues which can lead to the inflammation in insulin-responsive tissues (Mostafalou 2016). Furthermore, there is lots of experimental evidence on the role of organophosphorus insecticides in disrupted metabolism of glucose in both insulin-secreting and insulin-responsive tissues which

Table 8 Association of non-cancerous human toxicities with different classes of pesticides

Disease	Association with			
	Any pesticide (no.)	Targeted class of pesticides (no.)	Chemical class of pesticides (no.)	Single pesticides
Alzheimer	3	Herbicides (1) Fumigants (1)	OPs (1) OCs (1)	DDE
Parkinson	17	Insecticides (2) Herbicides (2) Fungicides (1)	OPs (1) OCs (1)	Atrazine, simazine, alachlor, metolachlor, paraquat (7), maneb (3), rotenone (2), ziram, methomyl, chlorpyrifos (2), propargite, diazinon, 2,4-D, β-HCH
ALS	11	Insecticides (1) Herbicides (2) Fumigants (1)	OCs (2) Pyrethroids (1)	2,4-D, Agent Orange
Asthma	6	Herbicides (1) Fumigants (1)	OPs (1) Carbamates (2)	Agent Orange (2), DDE (2), coumaphos, heptachlor, parathion, CCL4/CS2, ethylendibromide, pendimethalin, aldicarb, HCB
Chronic bronchitis	2	–	–	Heptachlor, dichlorvos, DDT, methyl bromide, cyanazine, paraquat, Agent Orange
Wheeze	2	–	–	Parathion, atrazine, chlorpyrifos, chlorimuron-ethyl, DDE, HCB
LRTIs		–	–	DDE (2), HCB
Infertility	1	Herbicides (1) Fumigants (1)	Phenoxyacetic acid (1)	DDE (3), atrazine, glyphosate (2), thiocarbamates, HCH (2), mirex, DDT (2)
Low quality of semen	1	–	OPs (5) Carbamates (1) Pyrethroids (2)	DDT, DDE, HCH, arachlor, diazinon, atrazine, abamectin
Birth defects	6	Insecticides (2) Herbicides (1)	OPs (1)	Carbaryl, chlorpyrifos, diazinon, atrazine (2), methyl bromide, DDT (2), HCH (2), DDE, aldicarb, dimethoate, phorate,
Changed sex functions	–	–	Pyrethroids (1)	DDE (2), HCB, chlorpyrifos, carbaryl, naphthalene
ADHD	–	–	OPs (5) Pyrethroids (1)	Chlorpyrifos, DDE (3), trichlorophenol
Autism	–	–	OCs (1) OPs (2) Pyrethroids (1)	DDE, chlordane, chlorpyrifos, imidacloprid
Developmental delay	3	–	OPs (1)	Malathion, chlorpyrifos, DDE (2), chlordecone
Diabetes	1	–	OCs (3) OPs (1) Pyrethroids (1) Phenoxyacetic acid (1)	DDE (12), DDT (5), HCB (5), trans-nonachlor (5), oxychlordane (4), heptachlor (3), β-HCH (2), mirex (2), aldrin, dieldrin, chlordane, alachlor, pentachlorophenol, parathion, phorate, fonofos, trichlorfon, cyanazine, Agent Orange (2)
Obesity	–	–	–	DDE, DDT, HCB, β-HCH, trans-nonachlor, oxychlordane

The number of associations is brought in the parentheses

OPs organophosphoruses, *OCs* organochlorines, *2,4-D* 2,4-dichlorophenoxyacetic acid, *2,4,5-T* 2,4,5-trichlorophenoxyacetic acid, *HCB* hexachlorobenzene, *β-HCH* beta-hexachlorocyclohexane, *DDT* dichlorodiphenyltrichloroethane, *DDE* dichlorodiphenyldichloroethylene, *DDD* dichlorodiphenyldichloroethane

have been implicated in prediabetic effects of this chemical class of pesticides (Jamshidi et al. 2009; Mostafalou et al. 2012b; Nili-Ahmadabadi et al. 2013; Pakzad et al. 2013; Rahimi and Abdollahi 2007; Teimouri et al. 2006; Pournourmohammadi et al. 2005).

Polmunotoxicity exhibited by diseases such as asthma and chronic bronchitis in association with occupational and environmental exposures has a long history of evidence, and pesticide exposure in this case had a similar pattern. But the association of pesticide exposures with childhood respiratory problems such as asthma and low respiratory tract infections has been recently much considered in regard to maternal or parental exposure to pesticides.

In this context, it should be noted that the link of pesticide exposures with developmental toxicity manifested by ADHD, autism, and developmental delays has been recently evidenced in the population studies concerning maternal or paternal exposure to pesticides in children. Although the lifetime of such an issue is almost short, a remarkable amount of studies have been conducted on the role of parental exposure to pesticides in developmental deficits presented in children. In this regard, most of the associations have been attributed to insecticides particularly organophosphoruses which have been widely used during the last few decades. The proven neural and oxidative stress-induced toxicities of organophosphoruses (Abdollahi et al. 2004a; Akhgari et al. 2003; Bayrami et al. 2012; Ranjbar et al. 2002) have inspired toxicologist to investigate the role of parental exposures in developmental and neuro-developmental disorders such as ADHD and autism, which have recently become prevalent in children, and positive associations have been traced for such risks.

Taken together, going through the mechanistic evidence on the toxicity of pesticides in association with human health disorders clears some common mechanisms, including oxidative stress, mitochondrial dysfunction, inflammatory responses, immune dysregulation, and endocrine disruption (Abdollahi et al. 2004b; Karami-Mohajeri and Abdollahi 2013; Mokarizadeh et al. 2015). In this way, induction of oxidative stress has been much highlighted in the studies focusing on the protective role of antioxidants such as cysteine and selenium against toxic effects of pesticides so that antioxidant therapy has been proposed and investigated for management of pesticide poisoning in human (Fakhri-Bafghi et al. 2016; Mostafalou et al. 2012a; Shadnia et al. 2011). Unveiling the link of oxidative stress with aging and age-related diseases, and inflammation with obesity and metabolic disorders is instances for ongoing exploration on the role of theses mechanisms in rising human diseases (Abdollahi et al. 2014). Given that these mechanisms and the others are gradually scrutinized in the pathology of newly focused diseases, it would be important that future studies pursue the current state of the science on the toxicity of pesticides with systematic approaches coordinated with new discoveries in the modality of human diseases.

Acknowledgments This invited paper is the outcome of an in-house financially non-supported study. The authors wish to thank Iran National Science Foundation (INSF).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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Productie 33

Pesticides and human health

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Pesticides, including herbicides, insecticides, fungicides, fumigants and rodenticides, provide important benefits in public health, food production and aesthetics (http://www.epa.gov/agriculture/ag101/pestbenefits.html). Unlike most other important chemicals, pesticides are designed to impact living systems (http://www.cdc.gov/niosh/docs/81-123/). Consequently there has long been a concern about environmental and human consequences of widespread pesticide use. Carson[1] effectively voiced this concern and documented some problems in her 1962 book, Silent Spring. Global pesticide use increased dramatically between the 1960s and 1990s, and more slowly thereafter, but large increases continue to occur in many developing countries.[2] The estimated worldwide use in 2007 was 5211 million pounds of active ingredients, with herbicides accounting for the major use in agriculture, and about 40% of the use overall.[3] To place this figure in context, it is close to one pound for each of the 6.6 billion people then inhabiting the globe, albeit with unequal distribution.

Human occupational exposure is expected during pesticide production and application, but the general population can also be exposed through drift, contamination of water and food supplies, and biological concentration through the food chain.[4] In addition, pesticide use for vector control and elimination of nuisance pests is an important exposure source for a considerable portion of the world population, and is an especially important source of exposure indoors.[5] These varied pathways have resulted in such ubiquitous exposure that persistent pesticides or their metabolites can be found at low levels in biological tissues of much of the world's population. This includes many who may be especially susceptible to deleterious effects from pesticide exposure, such as children, the elderly, developing fetuses and the immunosuppressed.

How hazardous are pesticides to humans? We really do not know and the answer is most certainly dependent on the specific chemicals and health effects being considered. Pesticides include dozens of chemical families, with hundreds of active ingredients, thousands of different formulations and many known or suspected adverse health outcomes. In addition to the active ingredients, pesticides also contain chemicals known as 'inerts' such as solvents, surfactants, preservatives, which may have toxic actions distinct from the active ingredients.[6] Some contaminants come from the production process. Dioxin, for example, is a contaminant of production of some phenoxyacetic acid herbicides, and is classified as a human carcinogen.[7]

Acute effects from intentional and unintentional pesticide poisoning are well established, but recent worldwide estimates do not exist. Previous estimates of 3 000 000 poisonings and 220 000 deaths worldwide in the 1980s[8] are out of date and are likely severe underestimates given the increase in pesticide use since that time. Scattered, but abundant, case reports and surveys from multiple regions in the world show that acute pesticide poisonings, both occupational and non-occupational, with mild-to-fatal effects, continue to be a major issue and there is an urgent need for a valid global estimate.

For chronic effects, the evidence is even less clear because of the challenge in accurate assessment of exposures during the relevant time period, which may be years before diseases or symptoms develop and only recently have certain health effects, for example, immunotoxicity, endocrine disruption and neurodevelopmental toxicity received much research attention.[9 10] What is clear is that the spectrum of suspected pesticide—chronic human disease associations continues to grow and that human and/or experimental data suggest links between some pesticides and cancer at multiple sites[11 12] and deleterious effects on the immune, nervous, respiratory, endocrine and reproductive systems.[13–15]

Evidence for carcinogenic effects of pesticides comes from experimental and epidemiological studies.[11] Cancers of the lung, prostate, and lymphatic and haematopoietic system have been the sites most frequently associated in epidemiological studies.[11 12] Childhood cancer has also been linked with environmental and parental occupational pesticide exposure.[16] Experimental and mechanistic studies suggest that many pesticides are not mutagenic, but that some may operate through epigenetic mechanisms and at a late stage of the carcinogenic process.[11 12] To date, however, no active ingredient (other than arsenic) has been classified as a definite human carcinogen by an authoritative body. There is a sufficient literature to indicate that there is a need for a chemical by chemical evaluation and the International Agency for Research on Cancer in 2014 announced plans to review of a number of pesticides in the monograph programme.

In addition to cancer, there are several other chronic health effects that may be linked to pesticides. The nervous system is particularly vulnerable to many pesticides of several distinct chemical classes. It is well known that acute poisoning with organophosphates causes long-term neurobehavioral deficits and depression, but health effects from low-dose exposures without clinical poisoning are less clear.[13] A recent meta-analysis of studies investigating low-dose organophosphate exposures found small-to-moderate associations with reduced psychomotor speed, executive function, visuospatial ability, and work and visual memory.[17] Neurodevelopmental effects have been reported in a number of studies of children with prenatal and early childhood exposure to organophosphates.[18] Reviews have also linked organochlorine, organophosphate and other pesticides with Alzheimer, other dementias and amyotrophic lateral sclerosis, with the most consistent results reported for Parkinson disease.[19] There have now been a number of studies reporting joint effects between common genetic variants and an increasing number of pesticides on the risk of Parkinson disease suggesting numerous different mechanistic pathways[20 21] may be involved and that pesticide–gene interactions need to be included in future studies. Recently, pesticides have also been associated with hearing loss,[22] diabetes and obesity,[23] and non-malignant respiratory disease.[24]

Much of the evidence on potential human hazards associated with pesticides has come from studies in developed countries. However, the use has been heavy, and mostly uncontrolled in many

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developing countries,[2 25] which may result in high exposures to large numbers of people and lead to more severe and widespread health effects.

The growth in use of pesticides,[2] the large number of people exposed during application or production and through various environmental exposure pathways, and the possible role of pesticides in the development of many different diseases and ill-health outcomes underscores the need to fully understand the risks associated with use of these chemicals, so that their overall benefits can be appropriately gauged. Also a thorough assessment of potential human health hazards from newly developed pesticides must be undertaken before they are widely used. As with many chemicals of modern society, there has been a tendency to replace the use of pesticides with known hazards by substances for which there is not as much known about their potential health effects, particularly long-term. Future research needs to focus not only on specific active ingredients and various formulations, but also on the possible cumulative and interactive effects from exposure to multiple pesticides over time.[26] To adequately assess the total health burden that might arise from the widespread use of pesticides requires sophisticated research approaches involving multiple disciplines with development of new methods to assess health effects from multiple exposures and sources. In the meantime efforts should be made to inform the public about the potential harm from pesticides exposure, especially vulnerable populations, and to limit or prevent human exposures. Development, strengthening and implementation of sustainable methods in agriculture and vector control are urgently needed to minimise pesticide use globally and, in particular, in developing countries.

Funding AB and LBF were supported by funding of the Intramural Program of the National Cancer Institute, National Institutes of Health. BR was supported by NIH funding from grant# ES10544 and P01ES016732.

Competing interests None.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Blair A, Ritz B, Wesseling C, *et al*. *Occup Environ Med* 2015;**72**:81–82.

Received 17 November 2014
Accepted 7 December 2014
Published Online First 24 December 2014

Occup Environ Med 2015;**72**:81–82.
doi:10.1136/oemed-2014-102454

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Productie 34

ORIGINAL ARTICLE

Exposure to Pesticides and Welding Hastens the Age-at-Onset of Parkinson's Disease

Pierre-Luc Gamache, Ikhlass Haj Salem, Noémie Roux-Dubois, Jacques Le Bouthillier, Ziv Gan-Or, Nicolas Dupré

ABSTRACT: ***Background:*** The age-at-onset (AAO) of Parkinson's disease (PD) is thought to be influenced by environmental factors and polygenic predispositions. Professional exposures to pesticides and toxic metals were shown to be associated with an earlier onset in small sample studies. ***Aim of Study:*** The aim of this study was to confirm the association between professional exposures to pesticides and toxic metals and the AAO of PD, on a larger cohort of patients, defined with a clinic-based ascertainment scheme. ***Methods:*** We used an incident cohort of 290 patients recruited through three designated movement disorder clinics in the province of Quebec, Canada. Patients completed a detailed questionnaire regarding professional exposures to pesticides and toxic metals. We compared the AAO in patients without prior professional exposure ($N = 170$) and those with exposure to pesticides ($N = 53$) or toxic metals through welding ($N = 30$). We further subdivided patients exposed to pesticides according to the frequency and proximity of their contacts. ***Results:*** Patients with prior exposure to pesticides (AAO = 54.74 years) or toxic metals (54.27 years) had a significantly earlier AAO compared to the control group (59.26 years) ($p = 0.003$). In those exposed to pesticides, closer ($p = 0.03$) and more frequent ($p = 0.02$) contacts were negatively correlated with AAO. ***Conclusion:*** Exposure to pesticides and toxic metals were both associated with an earlier onset of PD, an effect that was greater with higher levels of exposure, both in terms of frequency and proximity.

RÉSUMÉ: L'exposition à des pesticides et à des métaux toxiques associés à la soudure diminue l'âge d'apparition de la maladie de Parkinson. ***Contexte:*** Il est courant de penser que l'âge d'apparition de la maladie de Parkinson (MP) est influencé par des facteurs environnementaux et des prédispositions polygéniques. À cet égard, on a montré, dans des études portant sur des échantillons plus restreints, que l'exposition à des pesticides et à des métaux toxiques lors d'une activité professionnelle était associée à un âge d'apparition de cette maladie plus précoce. ***Objectif de l'étude:*** Confirmer cette association à l'aide d'une cohorte de patients plus nombreux, cohorte établie en fonction d'un plan clinique de définition des cas (*clinic-based ascertainment scheme*). ***Méthodes:*** Notre étude a donc reposé sur une cohorte de 290 patients recrutés au Québec au sein de trois cliniques des troubles du mouvement préalablement désignées. Les patients choisis ont alors répondu à un questionnaire complet en ce qui concerne leur exposition à des pesticides et à des métaux toxiques dans le cadre de leur travail. Nous avons ensuite comparé l'âge d'apparition de la MP chez des patients n'ayant pas été exposés à ces éléments (n = 170) à l'âge d'apparition de la MP chez ceux ayant été exposés à des pesticides (n = 53) ou à des métaux toxiques associés à la soudure (n = 30). Plus encore, nous avons subdivisé les patients exposés à des pesticides selon la fréquence et le niveau de proximité de leurs contacts avec ces éléments. ***Résultats:*** L'âge d'apparition de la MP chez les patients préalablement exposés à des pesticides (54,74 ans) ou à des métaux toxiques (54,27 ans) s'est révélé notablement plus précoce en comparaison avec l'âge d'apparition de notre groupe témoin (59,26 ans ; $p = 0{,}003$). Chez ceux ayant été exposés à des pesticides, des contacts plus étroits ($p = 0{,}03$) et plus fréquents ($p = 0{,}02$) ont été corrélés négativement à l'âge d'apparition de la MP. ***Conclusion:*** En somme, l'exposition à des pesticides et à des métaux toxiques a été associée à un âge d'apparition de la MP plus précoce, corrélation qui s'est avérée plus importante avec un accroissement des niveaux d'exposition, qu'il s'agisse de fréquence ou de proximité.

Keywords: Parkinson's disease, Age-at-onset, Environmental factors

doi:10.1017/cjn.2019.248 Can J Neurol Sci. 2019; 46: 711–716

Introduction

Parkinson's disease (PD) is an age-related movement disorder characterized by resting tremor, rigidity, bradykinesia, and postural instability. Its prevalence is dramatically increased after age 60. The etiology of PD is believed to be multifactorial resulting from both genetic and environmental factors, with less than 10% having a causative gene mutation identified.[1,2]

Based on epidemiology and toxicology studies, an important line of research focuses on the role of pesticide exposure associated with the risk of PD.[3–6] Some pesticide families including

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Received January 6, 2019. Final Revisions Submitted July 18, 2019. Date of Acceptance July 21, 2019.

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Table 1: Postulated mechanisms of action of toxic substances associated with an increased risk of PD[5,6]

Environmental risk factor	Presumed mechanism/post-mortem and animal studies observations/imaging characteristics
Pesticides	
Paraquat	Tissue damage by setting off a redox cycle that generates toxic superoxide free radicalsPossible synergism with Maneb
Maneb	Unknown mechanism Potential synergism with paraquat
Rotenone	Mitochondrial toxin. Inhibition of complex I of the electron transport chain. Progressive neurodegeneration of dopaminergic and non-dopaminergic neurons and oxidative damage
Organochlorines (dieldrin and β-Hexachlorocyclohexane)	Impaired mitochondrial function and oxidative stress via reactive oxygen species, leading to cell death in the substantia nigra
Organophosphates	Dopaminergic cell loss and microglial activation
Metals	
Iron	Neuromelanin in substantia nigra binds to iron and produces free radicals, and leads to lipid peroxidation and cell death Auto-oxidation of dopamine in substantia nigra neurons, releasing additional free radicals Postmortem PD brains show an increase in iron levels
Manganese	Degeneration of the globus pallidum mediated by disruption of the mitochondria, leading to apoptosis and cell death via formation of highly reactive oxygen species Magnetic resonance imaging revealing T1 hyperintensity in the striatum and globus pallidus and a normal dopamine transporter scan

herbicides such as paraquat, fungicides, insecticides, or rodenticides containing organochlorides and organophosphates can cause serious damage to the nervous system. They contribute to the development of PD by including oxidative stress, mitochondrial dysfunction, α-synuclein fibrillization, and neuronal cell loss.[5,6] In addition, exposure to metals such as iron and manganese (through welding, battery manufacturing, long-term parenteral nutrition, and IV synthetic drug use) has also been associated with an increased risk for PD.[7–10] Postulated mechanisms of action of toxic substances on the pathogenesis of PD are summarized in Table 1 (based on recent reviews from Nandipati and Litvan[5] and Sánchez-Santed et al.[6]).

Other factors reflecting both genetic and environmental influences on PD phenotype are vascular risk factors (VRFs) and sex. Vascular leukoencephalopathy and VRFs, which are thought to be mediated by genetic predispositions and lifestyle variables, predispose to a more severe PD symptomatology and more prominent cognitive features.[11,12] Women were also found to have a delayed onset compared to men (by about 2 years[13,14]), a difference that could be partly explained by differences in intracerebral estrogen levels, leading to higher striatal dopamine levels. This difference in estrogen levels can be explained by an intrinsic lower postsynaptic dopamine D2 receptor affinity in women[15] and by exogenous factors such as past pregnancies, gynecological surgeries, and hormonotherapy.[16]

Although specific genes have been associated with earlier onset forms of PD,[1] the data available regarding the influence of environmental factors on age-at-onset (AAO) are scarce. Earlier, AAO was observed in 15 former welders[17] and in 188 patients with prior exposure to hydrocarbon.[18] Similar results were obtained in 36 patients exposed to pesticides and/or heavy metals.[19] One study focusing on AAO in relation to multiple environmental risk factors was retrieved.[20] The authors studied 203 sibling pairs with PD and found only one environmental factor that influenced AAO, that is, a history of head trauma. However, the authors point out that their results need to be interpreted cautiously since the high genetic contribution to PD in this sample may have overshadowed potential environmental influences.

The aim of the present study is to confirm the hypothesized relation between environmental risk factors (pesticides and toxic metals exposure through welding) and AAO of PD, in a larger cohort, defined with a clinic-based ascertainment scheme.

Methods

Participants

We used an incident cohort of 290 patients recruited through three designated movement disorder clinics in the province of Quebec, Canada. Every index case was evaluated by a neurologist and met the Ward and Gibb[21] criteria for idiopathic PD. Additionally, patients needed to be dopa-responsive and to have been diagnosed no more than 10 years previously. Informed written consent form was obtained for each participant, and the study was approved by the ethics committee of the recruitment centers.

Data Collection

Participants and their partners completed a detailed questionnaire regarding social, professional, and medical history. The questionnaires included sociodemographic data (age, sex, and education), health outcomes at enrolment, and other conditions likely to impact on health like smoking. Patients were asked about their occupational history, especially those at risk for exposures to pesticides. It comprised specific questions regarding any past job in the following fields: manufacturing, farming, forestry, golf or green space maintenance, automobile, chemical products and pesticides spraying, agriculture products, and welding.[20]

https://doi.org/10.1017/cjn.2019.248 Published online by Cambridge University Press

Table 2: Clinical and demographic features of patient's cohort

Characteristics	Control (*N* = 170) (%)	At-risk job (*N* = 76) (%)	Pesticides (direct contact) (*N* = 20) (%)	Welding (*N* = 30) (%)	Total (N = 246) (%)
Gender male N (%)	92 (55)	69 (91)	16 (80)	29 (97)	167 (65)
Age at diagnosis*	59.8 (.98)	54.7 (1.6)	51.2 (2.1)	54.3 (1.8)	58.0 (.69)
History of living near a farm or vegetable garden	115 (68)	55 (72)	14 (70)	19 (63)	170 (69)
Positive history of smoking	83 (48)	32 (42)	12 (60)	14 (46)	120 (49)
High-school or higher education	134 (78)	47 (61)	15 (75)	18 (60)	30 (65)
VRFs					
DM *N* (%)	13 (7.6)	4 (5.3)	0 (0)	2 (6.7)	11 (15 (6.9)
HTN *N* (%)	22 (12)	43 (57)	5 (25)	9 (30)	57 (26)
MI *N* (%)	15 (8.8)	11 (14)	12 (10)	3 (10)	20 (9.2)
DLP *N* (%)	40 (23)	24 (32)	7 (35)	10 (33)	56 (26)
Stroke *N* (%)	2 (1.2)	2 (2.6)	0 (0)	0 (0)	2 (0.9)
>2 RF *N* (%)	29 (17)	18 (23)	4 (20)	8 (27)	175 (81)

DM=diabetes mellitus; HTN=hypertension; MI=myocardial infarction; DLP=dyslipidemia; RF=risk factor.
*Mean (standard deviation).

For each of these past jobs, information on dates of beginning and end of each activity was collected. A positive occupational history was considered when PD patients reported to have experienced at least 6 months of metals and/or pesticides exposition. Patients were asked if they had been exposed to (or directly manipulated) the following: herbicides, fungicides, insecticides, rodenticides, or other unknown substances of similar nature. They rated the frequency and duration of exposures on a 4-point ordinal scale: less than 1/month for <10 years; less than 1/month for >than 10 years; more than 1/month for <10 years; and more than 1/month for >10 years.

Statistical Analysis

The studied cohort consisted of 290 patients from the Quebec City area in eastern Canada. The final analysis included 256 patients whose status regarding professional exposures was detailed. Among these, 76 reported professional exposures to pesticides, toxic metals or both; 20 confirmed directly manipulating pesticides; 30 confirmed being directly exposed to toxic metals through welding; and 4 of them both manipulated pesticides and were former welders.

The dependent variable was age at diagnosis, which is considered to be the most standardized estimate of AAO, free from recall bias and determined by a neurologist. The main independent variable was prior professional exposure to toxic substances. It was further subdivided into nature of the exposure (pesticides and welding), frequency, and proximity. Analysis was conducted with SPSS 24.0 software (SPSS Inc., Chicago, IL, USA).

Primary Analysis

The primary analysis focused on two groups of patients: patients without professional exposures (Control, $N = 170$) and those exposed to pesticides or welding (All exposures, $N = 76$). The mean age at diagnosis in both groups was compared with an analysis of covariance (ANCOVA), adjusting for potential confounders that were sufficiently documented in our sample: sex, number of VRFs, place of residence (antecedent of living near a farm or vegetable garden – within 1 km for more than 6 months), which increases the risk of indirect exposure to pesticides, and smoking. Significant covariates would be subsequentially included in secondary group comparisons.

Secondary Analysis

In secondary analyses, the All exposures group was further subdivided into two subgroups (Pesticides, $N = 53$ and Welding, $N = 30$). Both groups were treated independently with separate ANCOVAs, since data regarding pesticides exposure or welding were incomplete in some cases and would have reduced the sample size in each group in a combined analysis; moreover, only four patients had antecedents of both welding and exposure to pesticides, rendering a factorial plan futile.

For the Pesticides group, two "exposure–response" models were tested with multiple linear regression analyses, the first with regards to the proximity of exposures (no contact, $N = 170$; at-risk-job, $N = 33$; and direct manipulation, $N = 20$) and, the second, the frequency of exposures (≤ 1 per month, $N = 12$ and >1 per month, $N = 38$), for a minimal duration of six consecutive months were associated to AAO. The frequency of exposure was chosen over the duration given the potential confounding effect of age on duration, with older patients having longer overall working experience. Significant covariates identified in the ANCOVA would be included in the regression models.

Frequency and proximity analyses were not performed in former welders, since they all had high frequency and proximity exposure to toxic metals.

A α-value of 0.05 was used as the signification threshold for all tests.

https://doi.org/10.1017/cjn.2019.248 Published online by Cambridge University Press

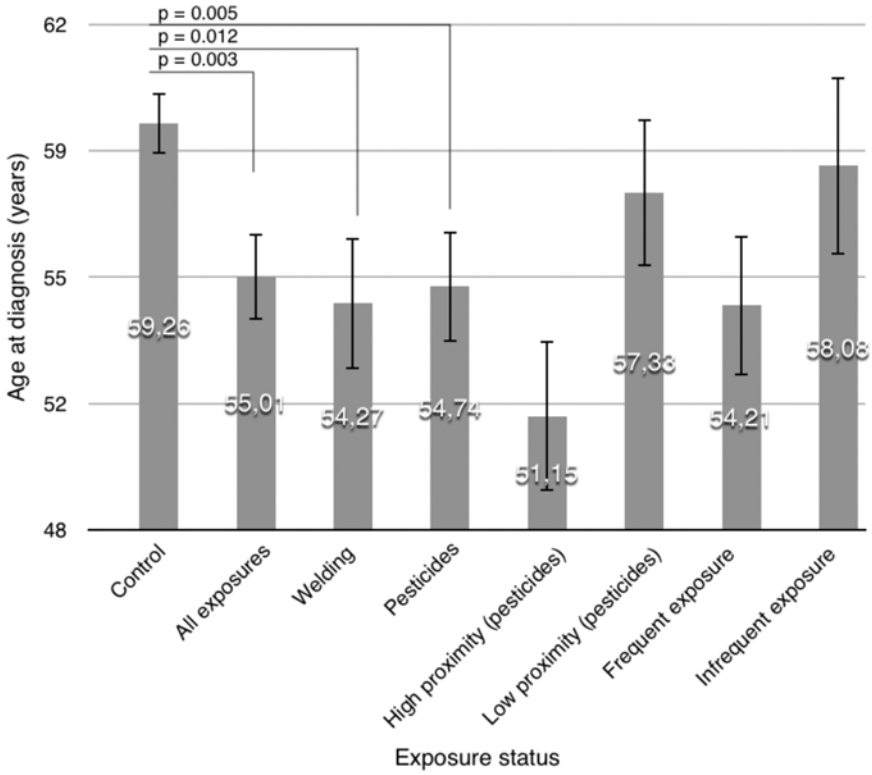

Figure 1: *Age at diagnosis as a function of exposure status. Error bars represent the standard error of the mean. p-values are indicated for all mean comparisons subjected to formal inferential testing.*

Results

Sample Characteristics

The descriptive statistics for the cohort and the subgroups are presented in Table 2. The mean age at diagnosis was 58.0 years (SD = 0.69). The sample included 167 men (65%) and 89 women (35%).

Exposure to Pesticides and Welding Is Associated with Earlier Onset of PD

Homogeneity of the variances among the different cohort subgroups was confirmed with Levene's tests, allowing for the use of parametric tests. Figure 1 presents the mean AAO in the different groups and subgroups. The main ANCOVA included 42 exposed patients and 81 controls in which information about all variables and covariates was sufficient. It revealed a significantly earlier AAO, $F(1, 117) = 9.25$, $p = 0.003$, in exposed patients. The number of VRF was found to be the only significant covariate ($p = 0.0005$; the more VRFs, the latter the AAO).

Subgroup ANCOVAs using the number of VRF as a covariate showed that both pesticides ($N = 65$, $F(1, 231) = 5.76$, $p = 0.017$) and welding ($N = 30$, $F(1, 198) = 17.66$, $p = 0.012$) were associated with earlier onset compared to the control group.

Proximity of Exposures to Pesticides

The mean AAO according to proximity groups is depicted in Figure 1. The linear regression analysis shows that a model including proximity and the number of VRFs significantly predicts AAO ($R^2 = 0.093$, $p = 0.00003$). Proximity is a significant, negatively correlated, independent predictor ($B_s = -0.138$, $p = 0.035$), and the number of VRFs is significantly positively correlated to AAO ($B_s = 0.284$, $p = 0.00002$).

Frequency of Exposures to Pesticides

The mean AAO according to frequency groups is depicted in Figure 1. The linear regression model including frequency and the number of VRFs also significantly predicts AAO ($R^2 = 0.333$, $p = 0.00003$). Proximity is a significant, negatively correlated, independent predictor ($B_s = -0.207$, $p = 0.001$), and the number of VRFs remains significantly positively correlated to AAO ($B_s = 0.266$, $p = 0.00004$).

Discussion

Although there is a clear association in the literature between exposures to pesticides or heavy metals and the risk of PD,[22] how such exposures affect the course of the disease remains unclear. This article reports convincing evidence that occupational exposure to toxic substances influences the AAO of PD, supporting prior evidence of such an association. Exposure to pesticides and welding were both associated with an earlier onset, an effect that was greater with higher levels of exposure, both in terms of frequency and proximity.

Such findings support current – and encourage the development of further – models in which AAO is determined not only by genetic predispositions, but also by exogenous neuronal insults

https://doi.org/10.1017/cjn.2019.248 Published online by Cambridge University Press

over an extended period of time.[2] This represents an important step forward into determining the variables that lead to neuronal death as the brain ages. Environmental exposures have been associated with PD through several mechanisms leading to cellular dysfunction and eventually neuronal death.[5,6] How these insults combine with genetic factors probably represents the key to understanding the AAO of PD.

Epidemiological studies are pivotal to identify the biological targets warranting scientists' attention in order to develop prevention strategies and disease modifying therapies in PD.[23] Our ability to target high-risk individuals is desirable since they are the most likely to benefit from eventual therapeutic options[24] and knowing that some of these are at risk of developing the disease earlier might contribute to optimize the timing of interventions.

Validity of the Study

The main findings of this study are consistent with prior reports in the literature, notably with the results of Ratner et al.[19] who used a data collection method similar to ours. The addition of an "exposure–response" profile and controlling for many variables reported to be associated to PD risk increases the robustness of the observed effects.

One strength of this study resides in the clinic-based ascertainment scheme, since PD requires diagnostic expertize to differentiate it from other types of movement disorders (e.g. essential tremor, Lewy Body disease, and cerebrovascular disease). This approach minimized the risk of contaminating the cohort with misdiagnosed patients.

Using a case-only design also takes away several potential sources of bias, notably recall and sensitivity bias. However, such designs are also prone to other forms of biases. Wilk and Lash[25] outlined that differences in AAO may reflect generational trends in the prevalence of exposure to the risk (or protective) factors. One finding that could be attributable to such a bias in this study is the positive relationship between AAO and VRF. Indeed, since VRFs are associated with a clinically more severe PD syndrome,[11,12] one could have hypothesized that it would lead to an earlier onset, which is contrary to the results obtained in the present study. The simple – and probable – explanation for this seemingly paradoxical result is that older patients from our cohort, as is the case in the general population, have more accumulated VRFs acquired in an age-dependent manner.[26] As regards the effect of occupational exposures on AAO, if such a bias was in play, it would have led to its underestimation. Indeed, exposure to occupational factors should be intrinsically lower in young patients (who have less working experience), which might falsely associate lower exposure levels to earlier AAO. The opposite inclination in our results strengthens their validity. Looking at the frequency of exposures instead of their duration also reduced the potential impact of such a bias.

Limitations

The main limitation of the current study is the relatively small sample size and the missing data in some subgroups, which prevented the use of a full-factorial plan. Multiple regression analyses were used in order to underline an "exposure–response" profile according to the frequency and proximity of exposures. Traditionally, such analyses are performed on much larger sample sizes, although some authors found that a very small numbers of subjects per variable allows adequate estimation of regression coefficients, standard errors, and confidence intervals.[27] The small number of women in the exposure groups did not allow to consider stratified analysis to assess if the observed effects are applicable to both men and women. Although previous findings showed no significant difference in mean age of onset of PD among male and female,[19,20] we could not fully exclude the possibility of a small contribution of sex differences to the main effect.

All potential confounders could not be controlled for since the data were either not included in the questionnaires or incomplete (e.g. physical activity, caffeine, specific medication, ...). The missing data in some subgroups limited our ability to test separately the association between exposure to specific pesticides and the AAO of PD. The lack of genetic data that may influence AAO also limits the appreciation of a potential interaction with the studied environmental factors. Moreover, the questionnaire items regarding frequency and proximity to exposure are rather vague, which possibly leads to heterogeneous exposure levels within the different subgroups.

Conclusion

Although this study reinforces the notion that environmental exposures affect the AAO of PD, epidemiological studies on larger cohorts are still warranted in order to better identify, among people with prior exposures, who is at risk of developing PD, and who will do so earlier. Such studies should notably be aimed at identifying which specific pesticides compounds are particularly noxious and determining if their effect depends on individuals' genetic profiles.

Acknowledgements

We are deeply grateful to all patients for their participation in this study. We thank Dr. Myers Richard for kindly providing the questionnaire. Access to the data for this research has been made possible through the *Réseau Parkinson du Québec*, Canada.

Disclosures

Dr. ZG-O reports personal fees from Sanofi Genzyme, personal fees from Lysosomal Therapeutics Inc., personal fees from Idorsia, personal fees from Prevail Therapeutics, personal fees from Denali, and personal fees from Inception Sciences, outside the submitted work. The other authors have no conflicts of interest to declare.

Statement of authorship

1. Research project: Conception: P-LG, ND; Organization: P-LG, ND, ZG-O; Execution: P-LG.
2. Statistical analysis: Design and execution: P-LG; Review and critique: ND and ZG-O.
3. Manuscript preparation: First draft: P-LG; review and critique: All authors.

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https://doi.org/10.1017/cjn.2019.248 Published online by Cambridge University Press

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Disclosure: The authors report no conflicts of interest.

Received May 15, 2006. Accepted in final form July 28, 2006.

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Acknowledgment

The authors thank Drs. K.C. Cheung, K.M. Leung and K.F. Tam for advice and comments.

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Motor neuron disorder simulating ALS induced by chronic inhalation of pyrethroid insecticides

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Some agricultural chemicals are known to be neurotoxic. Pyrethroid insecticides are commonly used as they are highly toxic to a wide range of insects but have low toxicity to mammals. However, overexposure to pyrethroids can induce various neurotoxic symptoms such as numbness, seizure, tremor, and memory impairment.[1] Such symptoms are usually transient, and there are no reports indicating that chronic intoxication from pyrethroid insecticides causes motor neuron damage, although a case of pathologically proven motor neuron death after acute massive ingestion of pesticides containing pyrethroids and organochlorine has been reported.[2] Here, we report a case of slowly progressive motor neuron disease (MND) following chronic exposure to pyrethroids that was indistinguishable from ALS.

Case report. A 44-year-old woman, a food shop proprietor, had been using cans of pyrethroid insecticides containing imiprothrin, phenotorin, D-T80-resmethrin, and D-T80-phthalthrin almost every day for 3 years in an unventilated room. Initially, she experienced tongue numbness, nausea, and rhinitis while using the insecticides. Two years after beginning to use the insecticides, she noticed difficulty lifting heavy objects with her left arm, and her symptoms steadily worsened over the next 8 months. Three months before admission, she developed slurred speech, gait disturbance, and generalized muscle weakness. On admission, neurologic examination revealed dysarthria, nasal voice, and dysphagia with fasciculating atrophied tongue (figure), moderate muscle weakness with fasciculation in both upper limbs, predominantly on the left side, and fasciculation in the trunk and both lower limbs. Jaw jerk was hyperactive, and hyperreflexia was seen in all limbs without pathologic reflexes. Sensory and autonomic systems were all normal.

Needle electromyography (EMG) disclosed widespread giant and polyphasic motor unit potentials, together with fibrillation and fasciculation potentials in all four limbs. Nerve conduction studies showed decreased compound muscle action potential amplitudes without conduction block but normal sensory responses in all limbs. F-wave frequency was decreased at the right median nerve, but F-wave latency was normal. Motor-evoked potentials (MEPs) with transcranial magnetic stimulation were recorded from the abductor hallucis and abductor pollicis brevis muscles. Lower limb MEPs revealed prolonged central conduction time (latency between the cortex and lumbar stimulation) on the left side (26.4 milliseconds, normal <21.04 milliseconds), whereas the right side was normal. Upper limb MEPs were not evoked by scalp or cervical stimulation on either side. Somatosensory-evoked potentials following electric stimulation of the median and tibial nerves were normal.

Two months after cessation of pesticide use, her motor weakness partially ameliorated and fasciculation in all four limbs ceased, leaving tongue atrophy and fasciculation, mild weakness of the upper limbs, and mild generalized hyperreflexia. Acute denervation potentials also disappeared on needle EMG. The subclinical hypothyroidism (thyroid-stimulating hormone 4.60 μIU/mL, free T_4 1.00 ng/dL) seen on admission had improved, consistent with thyroid toxicity by pyrethroids.[3] Seven months after cessation of pesticide use, no exacerbation was apparent.

Discussion. The patient showed upper and lower motor neuron signs in bulbar, cervical, and lumbosacral regions, consistent with clinically definite ALS based on El Escorial criteria. Neurophysiologic studies also indicated both upper and lower motor neuron involvement. Her illness was thought to be caused by pyrethroids for two reasons: 1) The usual manifestations of pyrethroid intoxication, such as tongue numbness, nausea, and rhinitis, preceded the motor dysfunction; 2) the motor symptoms and ongoing denervation potentials partially improved after the cessation of pesticide usage. The case indicates that chronic pyrethroid intoxication may cause an ALS-like disorder in humans, similar to lead[4] and domoic acid[5] intoxication.

Pyrethroids are synthesized from chrysanthemum extracts. They disturb ion channels, such as voltage-dependent sodium channels and voltage-sensitive chloride channels, and readily induce neuronal excitation by current prolongation.[1] Moreover, deltamethrin, one of the pyrethroids, is reported to impair axonal transport and then degenerate axons in rats, with impaired axonal flow causing motor neuron death in various animal models of MND.[1] Although pyrethroids have low toxicity, due in part to their rapid detoxication via ester hydrolysis in mammals,[1] some human populations are thought to be poor metabolizers of pyrethroids, whereas carboxyesterase inhibitors can enhance pyrethroid toxicity.[6] Therefore, chronic exposure to pyrethroids may cause MND through disturbance of either ion channels or axonal flow, especially in poor metabolizers.

MND caused by pyrethroids might be rare, because the heavy exposure to pyrethroids in our patient is an exceptional event in normal everyday life; as well, the population of poor metabolizers is small. Nonetheless, epidemiologic studies have reported associations of MND with agricultural work, during which there can be exposure to agricultural chemicals.[7]

Figure. Atrophy of the tongue in the patient.

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Disclosure: The authors report no conflicts of interest.

Received April 26, 2006. Accepted in final form August 1, 2006.

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3-Methylglutaconic aciduria type I causes leukoencephalopathy of adult onset

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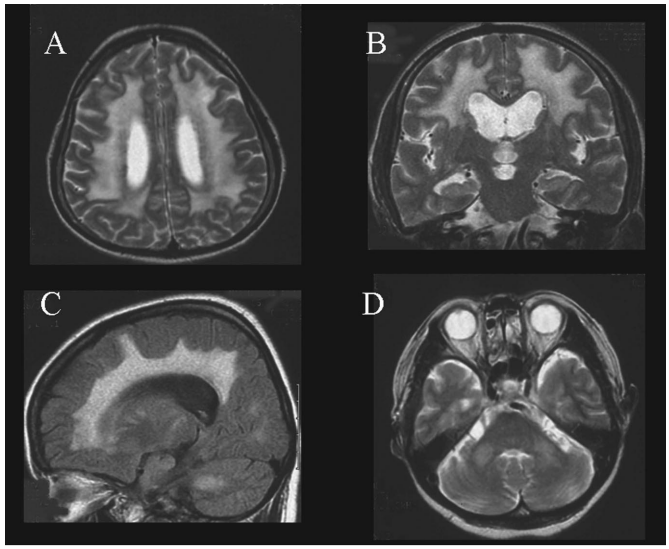
3-Methylglutaconic amino aciduria (MGA) is characterized by an increased urinary excretion of 3-methylclutaconic acid and 3-methylglutaric acid.[1] Four distinct forms are currently recognized: type I (MGA1), 3-methylglutaconyl-CoA hydratase deficiency caused by mutations of the *AUH* gene[1-7]; type II, caused by mutations in the tafazzin gene; type III, occurring in a genetic isolate of Iraqi Jews (causative gene has been recently isolated); and type IV, a heterogeneous group. The symptoms usually appear in the first decade of life.[1-7] We herein report MGA1 presenting with progressive leukoencephalopathy in late adulthood.

Case report. A 55–year-old woman was referred to us for an evaluation of progressive forgetfulness and an unsteady gait since age 53. Urinary incontinence also appeared at age 52. She easily tripped and fell, resulting in a fracture of her right clavicle. Her parents were first cousins. She had no serious illnesses during her childhood, graduated from a junior college, got married at age 23, and gave birth to two healthy children. Her height was 145 cm and weight 48 kg. A neurologic examination showed impaired cognitive functions, hyperreflexia in all limbs with positive Babinski sign, bilateral cerebellar ataxia including dysarthria, and urinary incontinence. She could walk, but had a slight wide-based gait. There was no abnormal eye movement, sensory disturbance, apraxia, or agnosia. The assessment of Wechsler Adult Intelligence Scale showed Verbal IQ 65, Performance IQ 56, and Full-Scale IQ 59.

EKG, complete blood count, and blood chemistry tests including lactate and thyroid function tests were normal. Serologic tests for syphilis were negative. T2-weighted images of brain MRI showed diffuse and symmetric hyperintensity in the cerebral white matter extending into the subcortical U-fibers (figure). Symmetric lesions were also observed in the middle cerebellar peduncle (figure). MR images of the spinal cord were normal. CSF was normal. The nerve conduction velocities and somatosensory and visual-evoked potentials were normal. Urinary organic amino acids analyzed by gas chromatography–mass spectrometry showed increased levels of 3-methylglutaconic acid (relative peak area; 108.1, normal <4.2), 3-methylglutaric acid (12.8, normal <4.5), and 3-hydroxyisovaleric acid (15.2, normal <2.3). This pattern led us to suspect MGA1. We obtained her family's consent to perform a gene analysis. A mutation analysis of the responsible *AUH* gene demonstrated a disease-causing homozygous G→A mutation of the last nucleotide at the splice acceptor site of intron 8, described as IVS8-1G→A.[4,5] After the diagnosis of MGA1, she was placed on a low-protein diet supplemented with 200 g/day of leucine-free milk Her neurologic status was unchanged during follow-up for 1 year.

Discussion. MGA1 is a rare autosomal recessive disorder with only 13 patients reported in the literature as of 2003.[5] 3-Methyglutaconyl-CoA hydratase is encoded in the *AUH* gene.[4] A patient with a nonsense R197X mutation in exon 5 developed speech retardation; and a patient with the current IVS8-1G→A mutation presented with speech retardation and a delay in motor development.[5] The compound heterozygosity for a missense R240V mutation and an insertion M205fs mutation was found in a patient presenting with vomiting, insomnia, irritability, persistent crying fits, self-mutilation, and hepatomegaly and a deletion S27fs mutation in an asymptomatic 2.5-year-old boy.[5] Recently, two new mutations have been identified in MGA1: an IVS9-2A→G mutation in a boy with fever-associated seizures[6] and an IVS1-2A→G mutation in a boy with severe encephalopathy causing spastic quadriplegia and dystonia in the first year of life.[7] As a result, MGA1 can be defined as a heterogeneous disease in both genotype



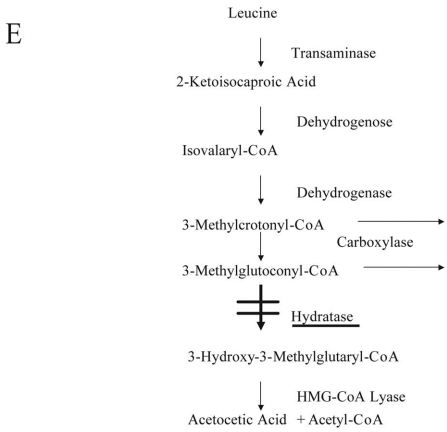


Figure. T2-weighted images of the brain MRI show diffuse and symmetric hyperintensity in the cerebral white matter (A, axial view; B, coronal view; C, sagittal view). Symmetric hyperintensity is also present in the middle cerebellar peduncle (D). Lower panel shows catabolic pathway for leucine (E). The underline (hydratase) shows 3-methylglutaconyl-CoA hydratase.

and phenotype. The demonstration of the IVS8-1G→A mutation in both a young child with speech retardation[4] and our adult patient developing severe dementia and ataxia after a long asymptomatic period suggests that there might be little correlation between the clinical phenotype and mutational genotype in MGA1.

MGA belongs to disorders of leucine catabolism leading to the synthesis of ketone bodies (figure), which are used as alternative substrates to glucose and provide a major source of energy to the brain during fasting. Therefore, energy depletion and the accumulation of neurotoxic leucine metabolites in the brain may cause neurologic and neuroradiologic abnormalities in MGA1. MRI of MG1 at a young age showed that pathologic lesions are restricted to the cerebrum, mainly in the basal ganglia and the white matter.[4,7] In our patient, brain MRI showed more widespread involvement of the cerebral white matter and bilateral involvement of the cerebellar peduncles, implying the relentless progressive nature of the disease and the necessity of treatment with a low-leucine diet as early as possible.

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The authors report no conflicts of interest.

Received May 8, 2006. Accepted in final form August 1, 2006.

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