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.

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

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

**Neurologic assessment**

**Cognitive impairment.** Neuropsychological tests on stan-
dardized 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 hypoth-
thesis. 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 Epidemi-
ologic Studies Depression Scale (16).

**Parkinson’s and Alzheimer’s diseases.** A simple algo-

**rithm 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 neurolo-
gist, 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 classi-
ified by considering the results of jointly available comple-

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

<table>
<thead>
<tr>
<th>Job title</th>
<th>Exposure level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wine grape grower or fruit grower &lt;7 hectares</td>
<td>3</td>
</tr>
<tr>
<td>Worker in wine grape growing or fruit growing</td>
<td>3</td>
</tr>
<tr>
<td>Farmer on &lt;20 hectares</td>
<td>2</td>
</tr>
<tr>
<td>Truck farmer or horticulturist &lt;1.5 hectares</td>
<td>2</td>
</tr>
<tr>
<td>Wine grape grower or fruit grower 7–20 hectares</td>
<td>2</td>
</tr>
<tr>
<td>Wine grape grower or fruit grower 20–40 hectares</td>
<td>2</td>
</tr>
<tr>
<td>Worker in truck farming or horticulture</td>
<td>2</td>
</tr>
<tr>
<td>Poultry or mixed animal breeder &lt;10 hectares</td>
<td>1</td>
</tr>
<tr>
<td>Farmer on 20–40 hectares</td>
<td>1</td>
</tr>
<tr>
<td>Gardener</td>
<td>1</td>
</tr>
<tr>
<td>Carpenter</td>
<td>1</td>
</tr>
<tr>
<td>Farm worker</td>
<td>1</td>
</tr>
<tr>
<td>Herbivore breeder &lt;10 hectares</td>
<td>0.5</td>
</tr>
<tr>
<td>Farmer on &gt;40 hectares</td>
<td>0.5</td>
</tr>
<tr>
<td>Furniture craftsperson</td>
<td>0.5</td>
</tr>
<tr>
<td>Veterinarian</td>
<td>0.5</td>
</tr>
<tr>
<td>Technician in agriculture</td>
<td>0.5</td>
</tr>
<tr>
<td>Fire fighter</td>
<td>0.5</td>
</tr>
<tr>
<td>Farm or forest machine driver</td>
<td>0.5</td>
</tr>
<tr>
<td>Breeding worker</td>
<td>0.5</td>
</tr>
</tbody>
</table>
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,
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.
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 exposure variables.

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) \( (p = 0.02). \)

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
soley 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, Capimme, 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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