

Neuropsychologic Effects of Long-Term Exposure to Pesticides: Results from the French Phytoneer Study

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The Phytoneer study investigated a possible association between neuropsychologic performances and long-term exposure to pesticides in Bordeaux vineyard workers, most of whom use fungicides. Among the 917 subjects interviewed from February 1997 to August 1998, 528 were directly exposed to pesticides through mixing and/or spraying (mean exposure duration: 22 years), 173 were indirectly exposed through contact with treated plants, and 216 were never exposed. All subjects performed neuropsychologic tests administered at home by trained psychologists. The risk of scoring a low performance on the tests was constantly higher in exposed subjects. When taking into account educational level, age, sex, alcohol consumption, smoking, environmental exposures, and depressive symptoms and when restricting analysis to subgroups, results remained significant for most tests, with odds ratios (OR) exceeding 2. These results point to long-term cognitive effects of low-level exposure to pesticides in occupational conditions. Given the frequency of pesticide use and the potential disabilities resulting from cognitive impairments, further toxicologic and epidemiologic research is needed to confirm these results and assess the impact on public health. **Key words:** agrochemicals, epidemiology, long-term effects, neuropsychologic effects, pesticides, vineyards. *Environ Health Perspect* 109:839–844 (2001). [Online 14 August 2001]

<http://ehpnet1.niehs.nih.gov/docs/2001/109p839-844baldi/abstract.html>

The use of pesticides has increased dramatically in recent decades, thus improving the quantity and quality of human nutrition but also creating risks for the environment and public health. The acute effects of pesticide exposure are well known, particularly through certification procedures and reports of epidemics of poisoning, occupational accidents, and suicide attempts. However, the long-term effects of pesticide exposure remain controversial (1,2). Besides cancers and reproductive effects, nervous system damage has been reported in terms of peripheral neuropathy and central nervous degenerative disease, with special emphasis on Parkinson's disease (3). Available epidemiologic data on persistent cognitive impairments are still limited. However, in light of the large number of people exposed, the issue must be considered a major concern for public health. First, mild neuropsychologic deficits, even if subtle and subclinical, may be apparent in everyday functioning and may affect quality of life. Moreover, impaired cognitive performances could be predictors of dementia before the clinical diagnosis of the disease (4).

Cognitive impairments have been studied mainly in workers exposed to organophosphates because of the well-known neurotoxic mechanism of these pesticides, which involves acetylcholinesterase. Clinical reports have shown that acute intoxication by organophosphates may be responsible for chronic impairment of cognitive

functions (5). To date, three well-designed studies have assessed neuropsychologic impairments in subjects with a history of acute intoxication from organophosphates (6–8). Despite some limitations caused by population size and exposure assessment, the three studies showed changes in some neuropsychologic performances in subjects with a history of acute intoxication, especially in attention and flexibility, visual memory, visio-motor and motor function, intellectual functioning and abstraction. Their arguments pointed to the delayed neuropsychologic effects of acute intoxication.

Even if some studies did not find positive associations (9,10), the long-term effects of low-level chronic exposure have also been suggested by cross-sectional studies: Cole in Ecuador (11) and Stephens in Great Britain (12) found lower performances for subjects with occupational low-level exposures to organophosphates. A recent study in the Netherlands (13) also found that farmers and gardeners may have a higher risk of developing mild cognitive dysfunction. In the Bordeaux area, a previous study in the elderly found that farm workers had a higher risk of cognitive impairment than subjects who had an intellectual occupation, even after controlling for educational level and other covariates [odds ratio (OR) = 6.1 (95% confidence interval, 3.3–11.4)] (14,15).

Because the Bordeaux area contains many vineyards where pesticides, principally

fungicides, are widely used—which means a high number of workers exposed for long periods in their occupational life—the aim of the Phytoneer study was to assess the cognitive effects of long-term exposure to pesticides in vineyard workers in this area. Neuropsychologic tests were selected according to previous studies to assess memory, abstraction, attention, and speed of information processing.

Material and Methods

Population. Phytoneer is a cross-sectional study based on the population of workers affiliated with the Mutualité Sociale Agricole de Gironde (MSA), the regional branch of the French health and welfare department for agricultural workers.

Lists of workers affiliated with the MSA in Gironde were available for the years 1975 and 1995. From these lists it was possible to identify two groups of workers ages 20–35 in 1975: The first group comprised workers *a priori* exposed to pesticides—i.e., employed at that date in vineyards for more than 1,000 hr/year and still employed in vineyards in 1995. It was assumed they had been exposed to pesticides for 20 years or more. The second group comprised workers *a priori* not exposed to pesticides—i.e., not employed in vineyards in 1975 and working in forestry or agricultural cooperatives in Gironde in 1995. These sectors were retained because of the low probability of exposure and the comparability in socioeconomic characteristics with exposed workers.

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We thank the occupational health physicians and secretary of the Association de Médecine du Travail en Agriculture de la Gironde, and the PAQUID research team for their help in collecting the data and their helpful advice.

The Phytoneer study was supported by grants from the French Ministry of Environment, the Mutualité Sociale Agricole, the Agence Nationale pour l'Amélioration des Conditions de Travail, the Conseil Régional d'Aquitaine, the Association Recherche et Partage, the Chambre d'Agriculture de Gironde and the Institut National de la Santé et de la Recherche Médicale.

Received 27 October 2000; accepted 26 February 2001.

Exposure was *a posteriori* checked concerning lifelong occupational contact with pesticides (even wood preservatives). Detailed job calendars and specific questions on pesticides were available for every subject and were reviewed by two investigators.

A first stage determined that according to inclusion criteria, about 1,200 subjects would be eligible in the exposed group. Hypothesizing a 50% participation rate and an equal number of subjects in the nonexposed group, this would lead to detecting a risk of about 2 with an α risk of 0.05 and a β risk of 0.8 if the prevalence of the disturbances in the nonexposed was 5%.

In the nonexposed group, all workers meeting the inclusion criteria were first retained. As systematic annual medical visits were planned according to the sector of activity, several eligible nonexposed workers were expected to attend the annual visit on the same day with the same physician. The extra work for the occupational physicians resulting from the information required for the study could not be managed when more than two workers had to be informed on the same day. In such situations, two workers were randomized for the list of the eligible nonexposed workers to be visited on that day.

Workers were informed by their occupational health physicians during the annual medical visit, which is free and mandatory for all employees according to French occupational legislation. They signed a consent form to take part in the study. They were then visited at home by a psychologist specially trained in neuropsychologic testing. All subjects were interviewed from February 1997 to August 1998. The mean duration of the interview was 90 minutes. Questionnaires included identification data, exposure assessment, neuropsychologic tests, and possible confounding factors.

Exposure assessment. Occupational histories were collected for all subjects on specific questionnaires completed during a face-to-face interview. The first part of the questionnaire collected dates of beginning and end for each job and task, with the name of employer, place of work, and type of activity. One to six calendar periods could be filled in for each subject. In the second part of the occupational questionnaire, accurate questions on occupational exposure to pesticides were asked, specifically the following: "In any of the jobs mentioned in the calendar, have you personally been in charge of mixing, preparing or spraying pesticides?" And "If never personally in charge of treatment, have you ever worked in the vineyard during treatment periods?" Workers answering no to the first question and yes to the second were classified as indirectly exposed to pesticides. Workers answering yes to the

first question were classified as directly exposed. They were also asked to ascertain their direct exposure on the following points: year of first and last exposure; mean duration of exposure per year; tasks performed, including mixing, spraying, and other tasks with direct contact (repairing tractors, handling or washing contaminated containers); and use of protective equipment. All questionnaires were reviewed by investigators with attention to consistency between job calendars and exposure statements. We requested names of pesticides in a pilot stage, but because answers to these questions were not accurate, we sought no further information on this.

Because treatments take place in Bordeaux vineyards from May to August, subjects were classified according to the month when they were interviewed (during treatment periods: yes or no?).

Neuropsychologic tests. The neuropsychologic battery was administered by psychologists at the subject's home during a visit specially planned for the purpose. The five psychologists were trained together, and regular staff meetings were held to standardize the way of passing tests and coding answers. Psychologists knew the job calendar of participants but they were not aware of the research hypothesis. The study team was supervised by a researcher in neuropsychology. Attribution of subjects to a specific psychologist depended only on schedule availability, and procedures remained the same throughout the study. Because vineyard workers are generally low educated, we decided not to use a computer-administered battery and to avoid tests involving alphabetic knowledge.

The neuropsychologic battery administered with standardized material comprised nine tests.

The Mini-Mental Status Examination (MMSE) was the sum of subscores that measured different cognitive components: orientation to time and place, recording of three words, language, and visual construction. Possible scores ranged from 0 to 30 (16).

The Wechsler Paired Associates Test (WPAT) involved the reading of 10 word pairs. After reading the list, the examiner read the first word of each test pair and the subject was asked to provide the second word. Three learning trials and a delayed recall were performed. Six of the word pairs were easy associates (e.g., "baby-cries") and four were difficult (e.g., "school-grocery"). The easy pairs were given a score of 5 and the difficult ones a score of 10. We considered here the first reading trial with a possible range from 0 to 85 (17).

The multiple-choice element of the Benton Visual Retention Test (BVRT)

consisted of 15 stimulus cards and 15 multiple-choice cards. After presentation of a stimulus card for 10 sec, the subjects were asked to choose the initial figure among four options. Possible total scores ranged from 0 to 15 (18).

The Isaacs Set Test (IST) measured the ability to generate lists of words in four specific semantic categories (colors, animals, fruits, cities) in a limited time. In our study, scores were the number of words to be generated in each category in 60 sec (19).

We used a modified version of the Stroop Test (ST) for this study. A card containing five columns of 10 sets of symbols was presented to the subject. This card was composed of color names (blue, red, yellow, green) printed in contrasting ink. The instruction was to name the ink color and to ignore the meaning of the word. Before starting, standard instructions emphasizing both time and accuracy were given, and an example with five items was administered to the subjects to ensure that instructions were understood. Time to complete the card was recorded (20). Subjects were not advised of their errors. Time was then recorded independently of the errors.

In the Wechsler Similarities Test (WST), the subject had to explain in what way two things were alike (e.g., "orange-banana"). In our study we considered only the first five pairs of the Wechsler Adult Intelligence Scale similarities subtest. A score of two points was given for an abstract generalization and one point for a specific concrete likeness. Possible scores ranged from 0 to 10 (21).

The Zazzo Cancellation Task (ZCT) measured the ability to cross out as fast and as exactly as possible 29 target signs among distracters on a sheet of white paper containing 8 lines of signs. In our study, we considered the time spent to complete the test (22).

In a modified version of the Trail Making Test Part B (TMT-B), the task was to connect alternately red and green circles with numbers in their respective sequence as fast as possible. The test was adapted for a population with a poor alphabetic knowledge. We used colors instead of numbers and letters to adapt the test to our population. Before the test, a pre-test with only six items was administered to ensure that participants understood instructions; if an error was made, it was corrected by the examiner and instructions were given again. During the test administration, the examiner corrected participants' errors only during the first four connections, but not further. We considered the time spent to achieve the task (23).

The Finger Tapping Test (FTT) measured motor speed. The subject had to press on a standardized tool as many times as possible in fifteen seconds (24).

Other variables. The questionnaire also concerned sociodemographic factors (age, sex, educational level, nationality, and the like), habits (smoking, drinking alcohol), potential environmental exposure to pesticides (drinking well water, home fumigation), and other factors affecting neuropsychologic status (depression, psychotropic drugs).

We examined educational level in two classes: subjects with a primary education level and who had not passed the "Certificat d'Etudes" (a certificate formerly given after an examination at the end of primary education in France); and subjects who had passed the Certificat d'Etudes or who had carried on with a secondary, general, or technical education. This choice was justified by results of a previous study that found the primary school diploma to be the best threshold for prediction of cognitive impairment (25).

For alcoholic drinks, questions concerned daily consumption of wine, beer, and aperitifs. In multivariate analysis, we considered a dichotomous variable: regularly drinks alcoholic beverage, yes or no. We evaluated depression with the French version of the Center for Epidemiological Study Depression Scale (26).

We collected detailed history of smoking; people who had not smoked a single cigarette per day for a whole year were considered nonsmokers.

Analysis. Description of subjects, exposure, and neuropsychologic performances and analysis were performed with STATA software (27). We used the usual tests (chi-square, analysis of variance) to compare descriptive characteristics of subjects. The α risk was 5%.

Symptoms reported by the subjects as being treatment-related were described and compared for directly and indirectly exposed workers. We performed univariate analysis to search for an association between exposure and test results and between potential confounders and test results. We used multivariate logistic regressions to test separately for an effect of direct or indirect exposure on the test result with individual confounders. Results of neuropsychologic tests were the dependent variable and were considered as dichotomous variables, because the variables did not follow a normal distribution. In the absence of a normative reference value in such a low-educated population, the performance threshold was the 25th percentile for the distribution of the scores or 75th percentile for the distribution of times. To assess the stability of our results, we also used the 10th percentile in scores and 90th in times. Variables associated with neuropsychologic performances in univariate analysis with $p < 0.25$ were retained in multivariate models. Because difference in educational

level was important between nonexposed and exposed subjects, we controlled with a variable taking into account the school level and the school diploma together; this appeared to be the best predictor of cognitive impairment in a previous study in the Bordeaux area (25). Finally, to consider residual confounding for some variables strongly linked to the outcomes, we restricted our analysis to five subgroups of our population—men, less educated, French, declaring no alcohol consumption, and interviewed outside periods of treatment.

Results

Descriptive Data

Study population. In the *a priori* exposed group, 1,109 workers met the inclusion criteria. Of these, 679 (61.2%) were actually interviewed. For the 430 eligible subjects not included, the following explanations were given: They had moved or an error on the MSA lists was found; they did not attend the annual medical visit during which consent was obtained; they did not give consent for the study; they signed informed consent but could not be contacted by the psychologist.

In the nonexposed group, 1,708 subjects were eligible, among whom one out of four was randomized; 427 subjects were informed and 238 (55.7%) consented to participate.

Thus, from February 1997 to August 1998, 917 subjects were included in the study.

Characteristics of exposure. Analysis of occupational histories and specific questions regarding pesticides showed that among the 917 subjects, 528 (57.6%) were directly exposed through mixing and/or spraying in vineyards, 173 (18.9%) were indirectly exposed as they worked in contact with treated plants, and no contaminating job could be found for 216 subjects (23.5%).

The mean duration of exposure for directly exposed subjects was 22 years. Most were in charge of three types of tasks: mixing, spraying, or other tasks (repairing tractors, handling or washing containers). A minority of exposed workers (12.9%) mixed pesticides but did not spray them.

Changes in pesticide use were observed according to time period. Use of the straddling tractor has become more frequent in recent years (13.8% for jobs before 1970 and 39% after 1990). Manual spraying has tended to disappear (36.7% before 1970 and 19.1% after 1990), and tank size has tended to increase. Workers reported more frequent use of protective equipment in recent periods and shorter annual periods of treatments (mean of 20 days before 1970 and 13 days after 1990).

As the study period was between January 1997 and August 1998, some subjects were

examined during spraying seasons (from May to August in 1997 and 1998). However, most of the workers were examined outside treatment periods (53.6%). Among directly exposed subjects reporting treatment in the current year, 207 (56.6%) were examined between May and August.

Sociodemographic characteristics and potential confounders. Men represented two-thirds of nonexposed subjects, about a half of indirectly exposed people, and 98.9% of directly exposed people (Table 1). The mean age was 50.2 years and was comparable in the three groups. About 15% of the population were not French—mainly Portuguese, Moroccan, and Spanish. This proportion was higher in exposed than in nonexposed ($p < 10^{-4}$) but comparable in directly and indirectly exposed ($p = 0.93$). Educational level was higher in nonexposed people; 83.3% of them had obtained the Certificat d'études versus 40% in directly or indirectly exposed workers. Nationality was closely associated with educational level: 45.6% of the French had a low educational level versus 76.4% of subjects who were not French.

As shown in Table 1, directly exposed workers were more often smokers than nonexposed ($p = 0.004$) and indirectly exposed ($p < 10^{-3}$) subjects. Regarding regular alcohol consumption (including wine, beer, aperitifs), regardless of the quantity, no difference was noted between nonexposed and directly exposed workers. Alcohol consumption principally involved wine (88% of the people consuming alcohol regularly) and to a lesser extent aperitifs and beer. Consumers reported a mean consumption of wine at 0.5 L/day. About 5% of the population were used to drinking water from a well, more often in exposed (6.6%) than in nonexposed subjects (1.9%), with a mean consumption of 1.3 L/day. Psychotropic drug use, home fumigation, and depressive symptoms each concerned less than 10% of the population and were in comparable proportions in the nonexposed, indirectly exposed, and directly exposed groups (respectively $p = 0.36$; $p = 0.72$; $p = 0.89$).

Results on neuropsychologic tests. Data for neuropsychologic tests were missing in fewer than 10% of the 917 subjects.

The response rate was higher in nonexposed people (96.8% or more depending on the test) than in exposed (87.3% or more); the difference could be caused by neuropsychologic difficulties in the exposed.

Figure 1 represents the distribution of neuropsychologic performances according to exposure. Mean scores and times were constantly related to exposure to pesticides with a gradient between directly and indirectly exposed people for several tests. All differences were statistically significant ($p < 10^{-3}$).

Some potential confounders were strongly linked to psychometric tests. Educational level was a major factor, with a risk of scoring a low performance ranging from 2.9 (FTT) to 6.0 (MMSE) for the less educated subjects ($p < 10^{-3}$). The risk of scoring low increased by 6–10% per year of age, and this was statistically significant for all tests. Role of sex appeared significant for most of the tests, except BVRT ($p = 0.2$) and FTT ($p = 0.4$): Men had a 1.7–2.3 risk to score low, compared to women. Regular alcohol consumers had

lower risks than teetotalers; this result was statistically significant for BVRT ($p < 10^{-3}$), WST ($p = 0.005$), ZCT ($p = 0.04$), TMT-B ($p = 0.002$), and FTT ($p = 0.04$). For all tests, the existence of depressive symptoms was associated with lower performances; this result was statistically significant for BVRT ($p = 0.008$), IST ($p = 0.04$), TMT ($p = 0.03$), and FTT ($p = 0.02$). The role of other factors (smoking, drinking of well water, home fumigation, and history of an acute intoxication) was less evident as ORs were moderate (below 2), did not vary in the same

direction between tests, and rarely reached statistical significance.

Multivariate analysis. We performed multivariate analysis, comparing directly and indirectly exposed subjects to nonexposed subjects (Figure 2). Final models took into account different variables according to the test and the group of exposure. Education remained in all final models. Sex remained significant for WPAT, ST, and FTT. Age remained in all final models except MMSE, IST, and WST. Alcohol and depression remained in all final models, but smoking, drinking of well water, and home fumigation did not provide information and were removed from most models. Taking into account confounders, risks of scoring low were significantly higher in directly or indirectly exposed people than in nonexposed for all neuropsychologic tests, except ST and FTT in directly exposed. Most of the risks exceeded 2 and even reached 3.5 for BVRT ($p < 10^{-3}$) and 3.1 for TMT-B in directly exposed. Results for models restricted to subgroups of the population are shown in Table 2. Globally, BVRT and WST were the most constantly affected in directly and indirectly exposed subgroups. With analysis restricted to subgroups, some results were no longer significant, probably because of loss in power, but the strength of the associations changed only slightly. In subjects reporting no alcohol consumption, risks tended to increase and reached 7.04 for BVRT, 5.39 for MMS, and 4.29 for WST in directly exposed—statistically significant for a risk $\alpha < 0.05$, although the number of subjects did not exceed 190 in this subgroup. When using the 10th percentile for scores and 90th percentile for times, results remained significant for most of the tests. Confidence intervals were larger, but strength of the association did not change much. OR significantly exceeded 3 for BVRT, ST, WST, ZCT, and TMT in directly and indirectly exposed subjects. They were between 2 and 3 but were not significant for MME and IST. Results were not significant for WPAT and FTT.

Discussion

In the Phytoneer study, lower neuropsychologic performances were found in people directly or indirectly exposed to pesticides. The same result was obtained when potential confounders were taken into account—age, sex, educational level, alcohol consumption, smoking, environmental exposures, psychotropic drugs, depressive symptoms—and when restricting analysis to homogeneous subgroups.

These findings were coherent in neuropsychologic terms: The most impaired functions were those involved in information

Table 1. Characteristics of subjects according to exposure to pesticides.

Characteristics	No exposure <i>n</i> = 216		Exposed to pesticides				Total exposed <i>n</i> = 701	
	<i>n</i>	%	Direct exposure <i>n</i> = 528	%	Indirect exposure <i>n</i> = 173	%	<i>n</i>	%
Sex								
Male	140	64.8	522	98.9	77	44.5	599	85.4
Female	76	35.2	6	1.1	96	55.5	102	14.6
Age mean (years)	50.0		50.8		50.6		50.7	
Nationality								
French	208	96.3	441	83.5	145	83.8	586	83.6
Others	8	3.7	87	16.5	28	16.2	115	16.4
Educational level								
Lower level	36	16.7	317	60.0	103	59.5	420	60.0
Certificat d'études ^a	180	83.3	211	40.0	70	40.5	281	40.0
Smoking	119	55.3	350	66.5	63	37.1	413	59.0
Alcohol	162	80.6	390	79.1	95	59.7	485	74.4
Well-water drinking	4	1.9	30	5.6	14	7.9	44	6.6
Home fumigation	17	7.9	39	7.4	16	9.4	55	7.9
Psychotropic drugs	14	6.5	42	8.0	18	10.4	60	8.6
Depressive symptoms	12	5.7	32	6.5	9	5.6	41	6.3

^aDegree formerly obtained at end of primary education.

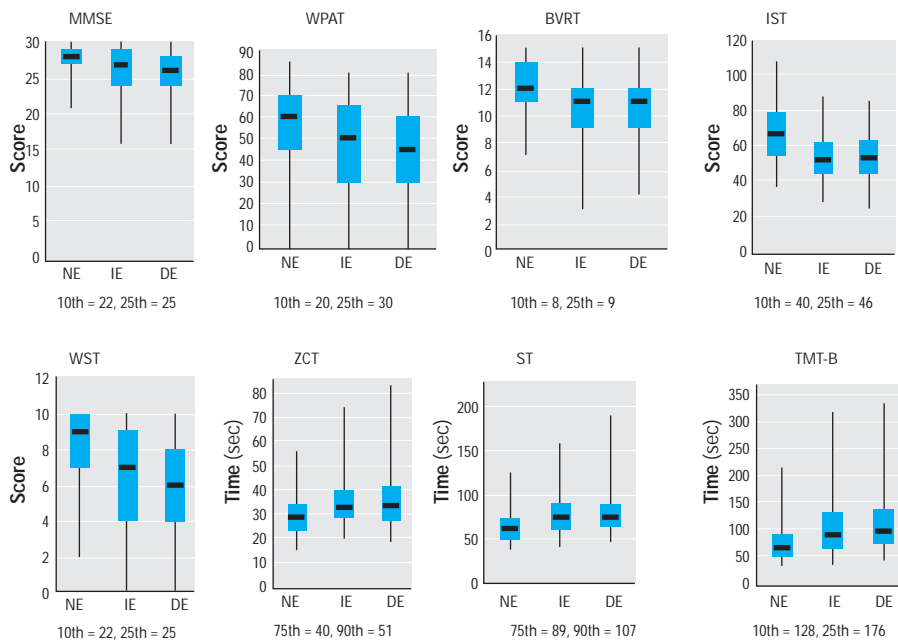


Figure 1. Distribution of neuropsychologic performances according to occupational exposure to pesticides. Abbreviations: DE, direct exposure; IE, indirect exposure; NE, no exposure. Box plots present the 10th, 25th, 50th, 75th, and 99th percentiles of the distribution of scores and times. Percentiles used as thresholds in multivariate analysis (10th and 25th percentiles for scores, 75th and 90th percentiles for times) are presented under each figure. All mean scores and times of exposed workers differed significantly ($p < 10^{-4}$) from nonexposed.

selection (selective attention) and information processing (working memory) as well as associative memory, verbal fluency, and abstraction. Indeed, the association was strongest for the BVRT, a test involving both selective attention and working memory: Directly exposed subjects had a risk of 3.5 of scoring low on that test, compared to nonexposed subjects. A similar result was found with the TMT-B (OR = 3.1), which explores selective attention and flexibility. The two other timed tests (IST, ZCT) were also affected and clearly indicated a slowing of information processing. ST was not significantly associated when the 75th percentile threshold was considered, but showed a significant risk of 3 for the 90th percentile. Such a process is observed in the early stages of dementia. It is noteworthy that FTT, a simple motor time test, was not affected—in contrast with other timed tests exploring speed of information processing.

The deficits were not greater for the directly exposed than for the indirectly exposed. A quick look at this result could suggest that there is no exposure–response trend. However, the assumption that indirect exposure is associated with lower individual cumulated exposure might be a simplification. Indeed, if mixing, loading, and spraying are responsible for peak exposure, longer time spent on re-entry tasks (growing, harvesting,

and the like) might exacerbate a person's lifelong exposure. Our results do not provide information on the respective effects of repeated exposure to low levels (in re-entry tasks) and infrequent high levels (during mixing, loading, and spraying).

Neuropsychologists trained in such tests used almost the same battery to assess the neuropsychologic outcomes on a cohort of the elderly (28,29). In our study, interviews and tests were conducted by the same interviewers throughout the study. The experience of our neuropsychologists, who have been involved in this kind of research through the Paquid study for 10 years, made it possible to select, adapt, and supervise the most appropriate tests for our population. Psychologists knew the exposure status of participants as they also collected work history. Even if the psychologists were not completely informed of the research hypothesis, we could argue that such knowledge influence response to the tests. However, psychologists were told that the same procedure had to be followed for all interviews, and the questions had a standardized formulation. Less than 10% of workers refused the tests, indicating that the subjects were fully cooperative. Some practical difficulties ensued because the tests were taken at home, often in the evening: Distraction could occur because of phone calls, television, children's shouting, or relatives' prompting, and in some homes interview conditions were poor because of noise or poor light. Psychologists were told to mention every hitch occurring during testing. When coding questionnaires, we reviewed hitches and decided whether or not to keep a result from a test.

Assessment of exposure was based on detailed job calendars reviewed for consistency. Questions on pesticide names, asked in a pilot stage, revealed that workers did not know the names of currently used pesticides, much less the names of pesticides used in the past. This is not surprising considering the

large number of pesticides used during a sole treatment period in vineyards, a number obviously larger over a worker's life. Farm owners are in charge of ordering products and establishing treatment calendars; workers are in charge only of mixing and spraying.

Our analysis took into account several confounders. The effects of some of them were considerable, such as educational level, sex, age, depression, or alcohol consumption. Difference in educational level was important in comparing nonexposed and exposed subjects. Because we could not absolutely exclude a residual effect on tests of the educational level between exposed and nonexposed, we analyzed a homogeneous subgroup of subjects with primary school education but no diploma. Our results were confirmed in these restricted analyses. The effect of alcohol could seem paradoxical because regular consumers appear to be protected from low performances for most of the tests, even in multivariate analysis. Even if underreported, it was comparable in nonexposed and directly exposed subjects. Our finding of better neuropsychologic performances in moderate alcohol consumers is consistent with previous studies (30). Restricting analysis to the subgroup of subjects declaring no alcohol consumption tended to increase the strength of the associations.

The effect of short-term exposure could not explain the associations because being interviewed during treatment periods was not associated with performances in the multivariate models (Table 4).

The confounders we took into account appear to be the most important ones. Even if some were missed, it is unlikely that they could explain the strong associations we found.

The question remains regarding the toxicologic explanation for our epidemiologic findings. In the Bordeaux area context, we believe that special attention should be given to the fungicides widely used in vineyards, because they comprise 80% of pesticides

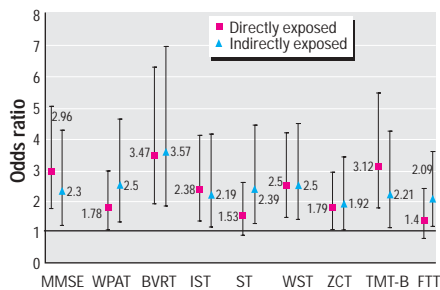


Figure 2. Adjusted risks of low performance to tests for directly and indirectly exposed subjects compared to nonexposed subjects, with 95% confidence interval.

Table 2. Risks of low performance in subgroups of the population (adjusted on age, sex, depressive symptoms, education, alcohol consumption).

Characteristics	MMSE	WPAT	BVRT	IST	ST	WST	ZCT	TMT	FTT
Men (no.)	612	633	648	641	602	649	640	625	646
Directly exposed	2.40**	1.53	2.80**	1.99*	1.27	2.10**	1.61*	3.13**	1.35
Indirectly exposed	1.33	1.53	2.23*	2.21**	1.19	2.41**	1.85*	2.29**	1.87
Less educated (no.)	326	346	357	356	334	357	352	342	356
Directly exposed	2.22*	1.02	2.86**	1.80	1.45	2.30**	1.41	2.22*	1.50
Indirectly exposed	2.12	2.19	4.53**	2.46*	2.03	2.51**	2.18	2.66*	2.61*
French subjects (no.)	673	691	702	701	677	704	695	684	702
Directly exposed	3.05**	1.66*	3.57**	1.99**	1.40	2.20**	1.62*	2.72**	1.52
Indirectly exposed	2.36*	2.48**	4.03**	1.94**	2.03**	2.20**	1.53	1.83*	1.79*
No alcohol consumption (no.)	179	184	190	185	176	189	188	182	189
Directly exposed	5.39**	1.88	7.04**	1.97	2.75*	4.29**	1.83	3.07**	1.81
Indirectly exposed	3.04	2.77	3.09*	2.82*	3.27**	2.70*	1.26	1.23	4.21**
Interviewed outside treatment period (no.)	403	419	428	425	400	428	420	414	425
Directly exposed	2.07**	2.06**	3.58**	2.17**	1.90*	2.29**	1.97**	2.96**	1.33
Indirectly exposed	1.70	1.74	2.70**	1.84	2.96**	2.79**	2.08*	2.19*	1.87*

* $p < 0.10$; ** $p < 0.05$.

used in a preliminary work in Gironde (versus 15% for insecticides and 5% for herbicides). A pilot study (31) conducted on 32 farms in Gironde in 1992 and 1993 showed that organophosphates represent less than 5% of organic pesticides used. On the other hand, dithiocarbamates and Fopel each represent about 40%. To date, most studies on cognitive impairments related to pesticide use have focused on insecticides (32), especially organophosphates, because of their well-known neurologic impact, but they may have done so partly because the existence of biomarkers makes them easier to study. However, hypotheses regarding other products should not be ruled out.

In previous studies (11,12,32) on chronic central nervous system effects of pesticide exposures, arguments were given for cognitive impairments but the design of these studies differed from the Phytoneer. First, exposed people were recruited through poisoning surveillance systems. Such a population is surely not representative of occupationally exposed subjects. Exposure is more comparable to the Phytoneer study in studies by Cole et al. (11), Stephens et al. (12), and Daniell et al. (32), because these considered low-level chronic exposures. Nevertheless, in those studies, exposure seems to be lower: In the study by Daniell et al. (32), the mean duration of exposure was 10 years, with a mean age of 30 years and 10 days of pesticide treatment per year. In Cole et al. (11), the mean duration of exposure was longer (16 years) but the population was also young (mean: 37 years). In such conditions, both the duration and the delay for observing long-term effects might not be optimal. Moreover, in Stephens et al. (12), the characteristics of exposure might be different because they studied not plants but sheep dipping. In these studies, the absence of exposure in control groups was not clearly ascertained: Daniell et al. found that one control out of four had at some time worked with pesticides, and the control group in Stephens et al.'s study comprised quarry workers among whom "several helped on sheep farms during spare time" (12). However, the major problem concerning previous study populations was the sample size, which ranged from 89 (including 49 exposed) (32) to 249 (including 146 exposed) (12). This latter number represents less than a third of the Phytoneer population.

The main confounders (age, educational level, sex) were generally taken into account in previous studies, but other factors, especially depressive symptoms or alcohol, were not or insufficiently considered. Some of these limitations might explain the absence of significant results in Daniell et al.'s study (32). However, in Stephens et al. (12), sheep dippers performed significantly worse than

controls in tests to assess sustained attention and speed of information processing; and in Cole et al. (11), visuo-spatial tests (including BVRT) and tests involving attention (TMT) were the most sensitive to pesticide exposure. These results are fully coherent with ours.

Our findings corresponded to subclinical symptoms. Disturbances appeared to be compatible with occupation, which is not surprising because times were affected more than the scores: Performances of exposed workers were similar to those of nonexposed if the tasks were slowed. This trend should be examined carefully in an occupational health perspective. Indeed, perhaps under certain occupational conditions (speeding up the work, sustained attention for various reasons, such as bad meteorologic conditions or steep slopes), slowing becomes impossible or inefficient, and failure could occur with possible physical risks for workers, especially when driving tractors.

Our results obtained in subjects under 60 raise other major questions: What is the natural history of these disturbances? Is there a possibility of persistence, aggravation, or evolution towards dementia? These questions must be addressed through prospective studies because of the possible major impact on public health.

To our knowledge, Phytoneer is the first study based on a large sample of workers exposed to pesticides that assesses long-term neuropsychologic effects of chronic low-level exposures. It points to possible effects on functions involving selective attention, working memory, associative memory, verbal fluency, and abstraction. These effects remained even after we considered a large number of confounders, and they were strongest in workers directly exposed compared to the nonexposed. They were not explained by current exposure. Further research is needed to appreciate in detail the dose-effect relationship and the natural history of the effects and to provide toxicologic explanations.

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