

Neurologic Function among Termiticide Applicators Exposed to Chlorpyrifos

Kyle Steenland,¹ Robert B. Dick,¹ Ronald J. Howell,² David W. Chrislip,¹ Cynthia J. Hines,¹ Thomas M. Reid,¹ Everett Lehman,¹ Patty Laber,¹ Edward F. Krieg, Jr.,¹ and Charles Knott³

¹National Institute for Occupational Safety and Health, Cincinnati, Ohio, USA; ²Department of Health and Human Services, Raleigh, North Carolina, USA; ³Battelle Memorial Institute, Durham, North Carolina, USA

Chlorpyrifos is a moderately toxic organophosphate pesticide. Houses and lawns in the United States receive a total of approximately 20 million annual chlorpyrifos treatments, and 82% of U.S. adults have detectable levels of a chlorpyrifos metabolite (3,5,6-trichloro-2-pyridinol; TCP) in the urine. The U.S. Environmental Protection Agency has estimated that there are 5,000 yearly reported cases of accidental chlorpyrifos poisoning, and approximately one-fourth of these cases exhibit symptoms. Organophosphates affect the nervous system, but there are few epidemiologic data on chlorpyrifos neurotoxicity. We studied neurologic function in 191 current and former termiticide applicators who had an average of 2.4 years applying chlorpyrifos and 2.5 years applying other pesticides, and we compared them to 189 nonexposed controls. The average urinary TCP level for 65 recently exposed applicators was 629.5 µg/L, as compared to 4.5 µg/L for the general U.S. population. The exposed group did not differ significantly from the nonexposed group for any test in the clinical examination. Few significant differences were found in nerve conduction velocity, arm/hand tremor, vibrotactile sensitivity, vision, smell, visual/motor skills, or neurobehavioral skills. The exposed group did not perform as well as the nonexposed group in pegboard turning tests and some postural sway tests. The exposed subjects also reported significantly more symptoms, including memory problems, emotional states, fatigue, and loss of muscle strength; our more quantitative tests may not have been adequate to detect these symptoms. Eight men who reported past chlorpyrifos poisoning had a pattern of low performance on a number of tests, which is consistent with prior reports of chronic effects of organophosphate poisoning. Overall, the lack of exposure effects on the clinical examination was reassuring. The findings for self-reported symptoms raise some concern, as does the finding of low performance for those reporting prior poisoning. Although this was a relatively large study based on a well-defined target population, the workers we studied may not be representative of all exposed workers, and caution should be exercised in generalizing our results. **Key words:** chlorpyrifos, neurotoxicity, organophosphates. *Environ Health Perspect* 108:293–300 (2000). [Online 18 February 2000] <http://ehpnet1.niehs.nih.gov/docs/2000/108p293-300steenland/abstract.html>

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphate pesticide used in both agricultural and residential pest control. It exhibits moderate acute toxicity, readily inhibiting plasma cholinesterase at low doses and inhibiting red blood cell cholinesterase at high doses (1). Like all organophosphates, chlorpyrifos can cause acute poisoning; well-known symptoms include miosis, increased urination, diarrhea, diaphoresis, lacrimation, and salivation (2).

Chlorpyrifos was first marketed in 1965. Its use has increased rapidly, in part due to the banning of chlordane for termite applications in 1988. The principal manufacturer of chlorpyrifos (Dow Elanco, Indianapolis, IN) has estimated that chlorpyrifos-containing products are applied in and around American homes more than 20 million times a year (3). In a survey of a random sample of U.S. adults ($n = 929$) conducted in the late 1980s and early 1990s, Hill et al. (4) estimated that detectable levels of a metabolite of chlorpyrifos (3,5,6-trichloro-2-pyridinol; TCP) are present in the urine of 82% of U.S. adults, with a mean level of 4.5 µg/L.

TCP is largely specific for chlorpyrifos, although two other less common pesticides, chlorpyrifos-methyl and triclopyr, can also be metabolized to TCP. Agricultural use of chlorpyrifos is also common, accounting for about two-thirds of chlorpyrifos sales (5).

U.S. Environmental Protection Agency (EPA) investigators summarized chlorpyrifos poisoning data based on reports from poison control centers and concluded that "chlorpyrifos is one of the leading causes of acute insecticide poisoning incidents in the United States (6)." U.S. poison control center data for 1993–1994 (6) showed approximately 4,000–5,000 reported cases of unintentional chlorpyrifos exposures per year, with approximately half of these exposures in adults. Approximately 50% of the cases were followed in more detail, and approximately half of these were judged to have symptoms consistent with clinical poisoning (85% with minor symptoms and 15% with moderate or severe symptoms). A subsequent analysis of this same database (3) reported similar numbers. The large number of reported cases is consistent with the widespread use of chlorpyrifos; the data do not suggest that

chlorpyrifos is disproportionately responsible to acute poisonings as compared with other organophosphates.

The potential of chlorpyrifos to cause chronic neurotoxic effects, either because of low level chronic exposure or as sequelae to acute poisoning, is controversial. Based on animal data, chlorpyrifos can cause a mild delayed peripheral neuropathy subsequent to inhibition of neurotoxic esterase (NTE), but only at lethal levels [above the median lethal dose (LD₅₀)] after prophylaxis against cholinergic toxicity (7). There are few epidemiologic data on the subject. There are two case reports of delayed peripheral neuropathy in humans following ingestion of a nearly lethal dose (7,8). Steenland et al. (9) found slower peroneal nerve conduction in 10 men previously poisoned by chlorpyrifos as compared to 90 nonpoisoned subjects; they also observed decreased performance in finger vibrotactile sensitivity and worse self-reported emotional moods in 17 men poisoned by a mixture of organophosphates, including chlorpyrifos. However, no differences were seen on a large number of other neurologic tests.

There are three limited studies regarding neurologic effects attributable to chronic low-level exposure. Ames et al. (10) conducted a cross-sectional telephone survey of 68 California pet handlers who used chlorpyrifos to treat fleas. These workers reported significantly higher incidences of blurred vision, flushing of skin, and decreased urination, as

Address correspondence to K. Steenland, National Institute for Occupational Safety and Health, Cincinnati (NIOSH), R13, 4676 Columbia Parkway, Cincinnati, OH 45226 USA. Telephone: (513) 841-4431. Fax: (513) 841-4486. E-mail: kns1@cdc.gov

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compared to nonexposed subjects. Two of these symptoms are consistent with chronic poisoning, but the finding for decreased urination is contradictory. Burns et al. (11) studied 496 men potentially exposed to chlorpyrifos during the manufacturing process and 911 matched nonexposed controls. Exposed subjects did not differ from controls for most medical conditions, but they did report significantly more general ill-defined conditions (dizziness, fever, malaise, fatigue), which were more common in subjects with a history of cholinesterase inhibition. This study (11) was limited because the authors relied on medical data obtained at work from subjects who were self-referred and by the lack of data on neurologic outcomes after exposure. Kaplan et al. (12) presented data on a case series of eight subjects who were reportedly exposed to chlorpyrifos and who subsequently exhibited a mild sensory neuropathy and some memory problems; the documentation of exposure in this study is weak.

In light of some suggestive evidence in the literature regarding chronic neurologic effects of exposure and the widespread use of chlorpyrifos in the United States, we conducted a cross-sectional study of neurologic function among current and former termiticide applicators who had used or were using chlorpyrifos; chlorpyrifos has been the main termiticide used in the United States since chlordane was banned in 1988.

Methods

Exposed population. The exposed population in our study was made up of current and former termiticide applicators using chlorpyrifos in a 12-county area of North Carolina; these subjects were identified from lists of professional applicators provided by the state of North Carolina (Department of Agriculture, Raleigh, NC). Because some termiticide applicators used chlordane before 1988, we included the use of chlordane [reported in one study to have delayed neurotoxic effects (13)] in the analyses.

After identifying all pest control operators from 1987–1997 in the 12-county area, we asked pest control companies to determine which employees had ever worked as termiticide applicators. We asked termiticide applicators reported to have ever worked for a year or more to volunteer for the study; subjects were contacted first by mail and then by telephone. Recruiting continued until approximately 200 termiticide applicators had been scheduled for testing.

Participants (exposed and nonexposed) were asked to undergo approximately 6 hr of tests at a central location, within a 2-hr drive for all eligible participants. Testing was conducted on eight consecutive weekends in

1998. All participants received compensation (\$250.00) for their time.

Nonexposed population. Approximately one-half of the exposed subjects were asked to bring a friend of the same sex and approximately the same age (within 5 years) to the testing site. Recruitment of friend controls was stopped when we reached approximately 100 controls. A second nonexposed group was then chosen from lists of blue-collar North Carolina State employees (maintenance workers and corrections officers) living in central North Carolina. We mailed a request for volunteers to 856 blue-collar state workers in four state departments. All volunteers were included if they matched the overall age, race, and sex distribution of the already recruited applicators and if they had never worked with pesticides or been poisoned by pesticides.

The use of two nonexposed groups provided a check on the validity of any possible exposure effect. A true exposure effect would be expected to be seen in relation to both nonexposed groups, barring any selection biases in choosing either group. If consistent discrepancies are found between the two control groups, a systematic selection bias could be present in one of them. The advantage of friend control groups is that subjects are likely to be similar to the members of the exposed group in lifestyle and demographic variables; the potential disadvantage is that subjects may represent a select group, which is more sociable and perhaps more likely to perform better on some of the neurologic tests.

Interview and alcohol tests. All participants provided informed consent and then completed an interview that focused on demographic variables and a complete work history (termiticide use, other pesticide use, solvent use, job history). We asked all participants to refrain from alcohol consumption on the night before testing. We administered an alcohol saliva test on the morning of the test, and any individuals with an alcohol concentration > 0.03% were asked to return at a later time.

Neurologic tests. We conducted neurologic tests for both central and peripheral function; testers were blind to exposure status. All tests had been used previously in other epidemiologic studies (14,15).

Neurobehavioral Evaluation System. The Neurobehavioral Evaluation System (NES) is used primarily to measure cognitive functions (16). This system includes the following tests: *a*) the vocabulary test, which is a measure of education; *b*) mood scales, which measure the participants' self-reported transient states of tension, depression, anxiety, fatigue, and confusion; *c*) the digit span test, which requires memorizing and replicating a series of numbers as fast as possible; *d*) continuous performance, which requires

pressing a key quickly when a certain letter appears in a temporal sequence of letters; *e*) simple reaction time, which measures how fast one can respond to a visual stimulus by pushing a button; *f*) the symbol-digit test, which requires matching digits to symbols as fast as possible following an exhibited matched pattern; and *g*) the pattern memory test, which requires selection of a previously seen pattern out of three similar patterns.

Vibrotactile test. The vibrotactile sensitivity test measures peripheral nerve function (sensory) by testing the sensitivity of the individual to feeling a vibration, both in the finger and the toe (nondominant). The fully automated test (Vibrometry System-Brüel & Kjør, Naerum, Denmark) was conducted at two vibration frequencies (31.5 Hz and 125 Hz) over an intensity range of 90–160 dB.

Arm/hand tremor. The arm/hand tremor test provides one measure of peripheral nerve function (17). We measured tremor using a NIOSH-developed hand-held device that measured tremor via accelerometers (18). The instrument can detect both visible (e.g., 1–6 hertz) and nonvisible (e.g., 7–30 hertz) tremor. Analyses included horizontal tremor and vertical tremor, both averaged over each minute of a 3-min test.

Postural sway. We used the postural sway test (which is analogous to the Romberg clinical test) to measure postural stability during 30-sec tests. We used a microcomputer-controlled force platform (Advanced Mechanical Technology Instruments, Newton, MA) and varied test conditions (eyes open or closed, hard or soft platform, one leg or two legs) (19,20). Because of some initial confusion about the test protocol, 49 subjects (13%) wore shoes during this test; thus, we included a dichotomous variable for wearing shoes in all models. A number of participants failed to complete the full 30-sec test for the one-legged tests (7% left leg, 12% right leg).

Manual dexterity. We used a grooved-pegboard timed test to measure manual dexterity. Participants were required to insert 25 grooved pegs into a board, once with each hand. The test is similar to the Santa-Ana test, but requires more fine psychomotor control.

Eye-hand coordination (two tests, trails A and B). This test is a timed visuomotor tracking task, which requires each participant to use a pencil to connect consecutively numbered or lettered circles as fast as possible.

Vision. Because exposure to pesticides can affect vision (15,21), we performed standard visual tests of acuity and contrast sensitivity (22) using the OPTEC 1000 (Stereo Optical, Chicago, IL), an automated visual test system. Visual acuity was measured on a scale of 0–7 (20/200 to 20/20) with corrected vision (contacts or glasses). Contrast was measured for each eye at five spatial frequencies

with increasing level of difficulty. In the analysis, we tested differences between exposed and nonexposed subjects for visual acuity, for each of the contrast tests, and for the average of the last three (more difficult) contrast tests after restricting the data set to those with good visual acuity (either 6 or 7, 20/30 or better).

Color vision was tested by using two other standard tests, the Farnsworth D-15 and the Lanthony D-15d tests (23); these tests are used to determine congenital and acquired color vision loss, respectively. Each test consists of 15 colored caps that the participant must arrange in order by color, each cap next to the cap closest to it in color. We identified individuals with congenital color vision loss in either one or both eyes using the Farnsworth test and removed them from the analysis of acquired color vision loss based on results of the Lanthony test. Tests were scored using the Bowman method, which produces a quantitative score based on the sum of the color differences of the caps (24).

Olfaction (cross-cultural smell identification test). Recent reviews have indicated possible effects of pesticides on the sense of smell (15,25). In the cross-cultural smell identification test, the participant must scratch and smell the 12 microencapsulated odors and choose the perceived odor from four choices (26). The odors were originally prepared for the University of Pennsylvania Smell Identification Test (UPSIT) (26). We analyzed either the number of odors (out of 12) correctly identified or the age-adjusted percentile of correctly identified smells.

Nerve conduction velocity. We measured nerve conduction velocity (time from stimulation to depolarization) and peak amplitude (the size of the maximal response of the compound action potential) by using surface electrodes in three nerves of dominant limbs (peroneal motor, sural, and ulnar sensory nerves) at 33.0°C for the lower limb and 32.0°C for the upper limb. Motor responses were orthodromic, whereas sensory responses were antidromic. We expected peripheral effects on nerve conduction in the lower limbs with longer nerves (peroneal motor, sural sensory); in some participants the sural response was difficult to measure and the ulnar (in the arm) served as a substitute.

Clinical examination. A brief (15 min) clinical neurologic examination was conducted on all participants by two neurologists; both neurologists were trained to ensure that their methods were comparable. The examination consisted of observation of pupils and eye movement, tremor, coordination, tone, strength, sensation, reflexes, station, and gait. Analyses were restricted to those end points in which more than 10 participants were judged to be abnormal.

Questionnaire. We used a short 24-item self-administered questionnaire to determine neurologic symptoms in the last month (e.g., "in the past month have you experienced trouble remembering," "loss of muscle strength," "numbness or tingling in toes," "lack of coordination or loss of balance"). This is a modified form of the traditional Hogstedt symptom questionnaire (27); each question has five possible ordered responses. Analyses considered either continuous data or dichotomized data (i.e., "not at all" vs. all higher categories combined), depending on the response variability.

Biologic samples: urine and buccal swabs. Applicators who had reported current exposure during a phone interview a few weeks before testing ($n = 105$) collected urine samples (first void) and brought them to the testing site. TCP, a metabolite largely specific to chlorpyrifos (half-life approximately 27 hr), was measured in the urine. We also measured TCP in samples from 52 nonexposed participants. We retained approximately 25 mL urine for analysis of TCP by gas chromatography by a commercial laboratory. The laboratory's limit of detection for TCP by gas chromatography is 2 ng/mL (28). We also measured creatinine.

We obtained buccal cells to determine the genotype of participants with regard to paraoxonase, an enzyme that is instrumental in detoxifying chlorpyrifos (29,30). The gene for paraoxonase at amino acid 54 (chromosome 7) exhibits a polymorphism in humans. Individuals homozygous for methionine, representing about 15% of the population, are likely to exhibit lower serum concentrations of the enzyme (31,32) and would be expected to be more susceptible to neurotoxicity due to chlorpyrifos oxon, the neurotoxic metabolite of chlorpyrifos. We obtained buccal swabs from all of the exposed subjects and from a sample of 55 nonexposed participants to ensure that the exposed and nonexposed subjects exhibited a similar distribution of the polymorphism.

We isolated DNA from buccal swabs using a commercially available kit (Epicentre Technologies, Madison, WI). We then amplified the paraoxonase gene from the DNA by polymerase chain reaction (PCR) using primers specific for sequences in the gene (33). We assayed the amplified paraoxonase gene for differences in DNA sequence at position 54 using a colorimetric method specific for each allele (34).

Data analysis. In our data analysis we focused on comparing results of the neurologic tests of the exposed subjects versus the nonexposed subjects. We used linear regression for this purpose and adjusted for possible confounding variables. We used logistic regression for some outcomes that were

dichotomous or polychotomous (e.g., all clinical examination outcomes, and some vision and symptom outcomes). We also used proportional odds models for some polychotomous outcomes, but these are not reported.

We checked demographic variables for potential confounding effects and inclusion in regression models. We constructed a basic model that included variables of *a priori* interest for most tests, i.e., age (continuous), race (white/nonwhite), and education (less than high school, high school, some college, and college graduate), as well as a variable for current smoking, which proved to be predictive for several tests. We did not adjust for sex; there were only six women in the study population, and we found no evidence of gender effects. For tests of peripheral neuropathy (e.g., nerve conduction, vibration sensitivity, tremor, sway, clinical examination), we also routinely included body mass index. We tested other variables, such as hours of sleep the night before, alcohol consumption the night before, current exposure to solvents, and coffee consumption the day of the tests, to explore possible confounding, but these variables were generally not predictive of outcome and were not included in the final models. In addition, we checked tests of peripheral neuropathy for the potential confounding effects of a history of diabetes ($n = 12$), carpal tunnel syndrome ($n = 12$), nerve disorder ($n = 17$), nerve injury ($n = 16$), or back disorder ($n = 66$); these variables in general did not act as confounders.

We tested whether the exposed group differed from both nonexposed groups combined for each outcome and for each control group separately. Results are reported for both control groups combined unless they differed by control groups; in this case, they are reported separately. After comparing the exposed subjects to the nonexposed subjects, we ran further models in which we compared a variety of exposed subgroups to the nonexposed group; these included *a*) workers currently applying termiticide in 1998 ($n = 128$), *b*) workers formerly applying termiticide ($n = 63$), *c*) workers with self-reported poisoning by chlorpyrifos ($n = 8$), and *d*) workers with a susceptible genotype ($n = 18$). Within the applicator group we also tested trends by duration of exposure to chlorpyrifos, chlordane, and other pesticides. We also ran models, after restricting the data to those with TCP values ($n = 157$), in which we determined if higher TCP levels (reflecting recent exposure) were associated with the neurologic outcomes; log of TCP and TCP corrected for creatinine were also considered, but these generally did not improve the fit of the model to the data.

We assessed all linear regression models for model fit (R^2) and checked residuals for

normality; we made log transformations of outcome as needed to normalize residuals. There were a large number of comparisons. We made no formal statistical correction for multiple comparisons (e.g., Bonferroni), but we interpreted the overall results with a view toward consistency and biologic plausibility (35).

Results

Study participants. We identified 3,605 pest control applicators who had been licensed by the state of North Carolina from 1987 to 1997 in the 12-county target area. These employees worked in 246 pest control companies. We contacted 176 of these companies to obtain information about whether their employees had worked as termiticide applicators ("screened" employees). Another 71 companies were out of business and 6 refused to provide information (representing "unscreened" employees). There were 2,917 (81%) screened employees and 688 (19%) unscreened employees. Of the 176 pest control companies contacted, 105 (60%) reported current ($n = 76$) or past ($n = 29$) use of chlorpyrifos-containing pesticides.

Of the 2,917 screened candidates, 383 (13%) were reported to have worked applying termiticides for at least a year. Of these, we were able to contact 239 (62%); we could not locate the remaining 144 (38%), despite extensive efforts. Of the 239 subjects located, 13 reported never having worked as termiticide applicators (5%), 68 refused to participate (28%), 5 were not available during the study period (2%), and 153 were scheduled to be tested (64%).

We then sought other exposed participants for the study among the unscreened group of 688 licensed applicators to reach our target population of 200 termiticide applicators. We tracked 206 individuals in this group; we could not locate 78 (38%), 69 were ineligible (33%), 16 refused or could not participate during the testing period (8%), and 43 (21%) were scheduled.

We tested 193 applicators of the 196 scheduled to be tested; 2 applicators were dropped because they had not worked as termiticide applicators and 1 was dropped because he failed to complete the questionnaire.

We tested 106 "friend" controls who were identified by the applicators, and 83 North Carolina blue-collar state employees.

Table 1 shows the demographic characteristics of the termiticide applicators and the nonexposed participants. Exposed and nonexposed participants were similar, but the exposed subjects had somewhat less education. We found no significant difference in education between applicators and both nonexposed groups combined ($p =$

0.19; chi-square test), but there was a borderline difference between applicators and nonexposed state employees ($p = 0.07$).

Table 2 shows some exposure characteristics of termiticide applicators. Applicators had worked a median of 1.8 years (range 0.1–10.3) applying termiticides, but 90% had also done other pest control work (median 1.6 years; range 0.5–11.3). We tried to restrict our exposed population to those who had worked at least 1 year applying termiticides on the basis of employer reports, but upon detailed interview at the study site, we found that 57 applicators (30%) had worked < 12 months in this field. We then decided to include these subjects and observe the effect of duration of exposure in the analysis. Two-thirds of the applicators had applied termiticides in 1998, whereas the remainder were former applicators. One-third of the applicators had worked with chlordane, which was largely replaced by chlorpyrifos in 1988. TCP levels were quite high in applicators who reported termite work in the last week ($n = 65$) as compared to those who did not ($n = 40$). Some elevated levels in applicators who did not report applying termiticides in the last week resulted from other uses of chlorpyrifos (e.g., on lawns or in interiors of houses). The mean TCP level in 52 nonexposed participants was 6.2 $\mu\text{g/L}$; this is similar to the mean level of 4.5 $\mu\text{g/L}$ observed in a random sample of 929 U.S. adults (4).

There are few data on chlorpyrifos exposure levels in other occupationally exposed workers to compare with data on termiticide applicators. Brenner et al. (36) estimated that time-weighted averaged air levels based on personal samples range from 10 to 120 $\mu\text{g/m}^3$ among chlorpyrifos-manufacturing workers. In work done as part of this study, Hines (37) reported a median full-shift air level of 14 $\mu\text{g/m}^3$ chlorpyrifos for 16 termiticide operators (range 2–126). Fenske and Elkner (38) studied six chlorpyrifos applicators and found

a median air concentration of 10 $\mu\text{g/m}^3$ (range 2–35) over a full shift. These data suggest that termiticide applicators and manufacturing workers may be similarly exposed, although most absorption is probably dermal and comparative air levels may not reflect comparative dermal exposure. In any case, the urinary TCP levels show that the termiticide workers we studied were exposed to levels two orders of magnitude above background, making them a reasonable population in which to study exposure effects.

By genotyping the paraoxonase gene in 184 termiticide applicators (7 samples could not be genotyped), we determined that 18 (10%) were homozygous for the gene which is responsible for potentially producing a low level of paraoxonase; these applicators might be expected to be more susceptible to the effects of chlorpyrifos. A sample of 52 nonexposed participants showed a similar proportion of homozygotes (9%).

Nerve conduction. There were no significant differences (at the $\alpha = 0.05$ level) between applicators and the combined nonexposed group for peroneal, ulnar, or sural nerve conduction velocity or amplitude. When compared to each nonexposed group separately, the only significant finding was that the applicators had significantly less ulnar amplitude ($p = 0.03$) than state employees. We found no significant differences in subgroups of applicators (current applicators, former applicators, susceptible genotype, previously poisoned) from the two nonexposed groups combined, with the exception of the eight poisoned men who had significantly more ulnar amplitude. Currently exposed applicators had significantly lower ulnar amplitude ($p = 0.03$), but significantly faster sural nerve conduction ($p = 0.05$), than state employees. We found no significant trends among applicators by duration of exposure to either chlorpyrifos, chlordane, or other pesticides, nor was there any trend found by the level of TCP in the urine. Using models for

Table 1. Demographic characteristics of study participants.

Characteristic	Applicators ($n = 191$)	Nonexposed friends ($n = 106$)	Nonexposed NC workers ($n = 83$)
Mean age (SD)	39.3 (9.4)	38.0 (9.7)	42.6 (8.7)
Education (%)			
Less than high school	12.6	15.2	4.6
High school	33.5	35.8	43.3
Some college	44.5	33.0	40.1
Completed college	9.4	16.0	12.0
Mean body mass index ^a (SD)	28.7 (5.7)	28.4 (5.4)	29.3 (5.7)
Percent current smokers	36.1	49.0	42.6
Percent current alcohol drinkers	37.2	32.1	33.7
Percent with history of OSE	36.1	50.0	51.8
Percent with current OSE	19.9	30.2	33.7
Diabetic	6	2	4
Race (% white)	81.6	81.6	86.8

Abbreviations: NC, North Carolina; OSE, occupational solvent exposure.

^aBody mass index = weight (in kilograms)/height (in square meters).

nerve conduction velocity, we were able to explain approximately 10% of the variance.

Sway, tremor, and vibrotactile sensitivity. We conducted 12 sway tests comparing exposed to nonexposed subjects for length or area of sway under increasingly difficult conditions: standing on a hard surface with eyes open and closed, standing on a soft pad with eyes open and closed, and two one-legged tests (eyes open, hard surface). The length and area of sway were correlated, with a Spearman correlation coefficient of approximately 0.60–0.70 for the 12 tests.

Exposed subjects differed significantly from nonexposed subjects only for one test—the length of sway on a hard surface with eyes open ($p = 0.04$). This effect was more pronounced when applicators were compared to nonexposed friends ($p = 0.05$) than when they were compared to nonexposed state employees ($p = 0.20$). Separate comparisons of the exposed group to each nonexposed group revealed that the exposed subjects also had more length of sway than friend controls on a hard surface with eyes closed ($p = 0.07$), more area of sway than friend controls on a hard surface with eyes closed ($p = 0.10$), and more length of sway than state employee controls while standing on the left leg only ($p = 0.01$). The R^2 for these models ranged from 0.07 to 0.29, increasing with increasing difficulty of the test.

The TCP level significantly predicted the length of sway for the test conditions with eyes closed (hard surface, $p = 0.03$; foam pad, $p = 0.01$) and area of sway for eyes closed on a foam pad ($p = 0.02$). The findings for length of sway, however, were no longer significant after one participant with a high TCP level was removed from the analysis ($p = 0.18$ for both hard surface and foam pad). The R^2 for these models was 0.20–0.30.

An increased area of sway for a subgroup of the exposed group, the eight men who reported past poisoning with chlorpyrifos, was borderline significant for two conditions: right leg ($p = 0.10$), and soft platform with eyes open ($p = 0.09$). We found no associations with susceptible genotype. When we compared applicators exposed before 1998 to the nonexposed subjects, we found two significant associations for more area of sway (hard platform with eyes open, $p = 0.03$; soft platform with eyes open, $p = 0.05$) and one significant association for increased length of sway (hard platform with eyes open, $p = 0.10$). The duration of chlorpyrifos exposure was significantly correlated with length of sway for the right leg ($p = 0.04$) and was correlated near significance with sway on a hard surface with eyes closed ($p = 0.08$).

No exposure variables were significantly related to vertical tremor, horizontal tremor, or the two combined, during any 1-min period or over the whole 3-min test. The R^2 values for the models for tremor were very low, averaging only 1–2%.

Exposure did not decrease vibrotactile sensitivity for either finger or toe (each was tested at two different vibration frequencies, 31.5 and 125 Hz). Exposure was associated with a significantly increased sensitivity for the toe tested as 125 Hz. TCP was not a predictor of vibrotactile sensitivity. Former applicators performed significantly better than nonexposed subjects for the one-toe test, as did the group with a susceptible genotype. The R^2 values for the vibrotactile models ranged from 0.05 to 0.18.

Clinical examination. The clinical examination results showed no significant differences between exposed and nonexposed subjects for abnormal upper extremity tremor (95 were abnormal with exposed and

nonexposed combined); abnormal toe, finger, wrist, ankle, or knee sensitivity to vibration (134, 54, 11, 66, and 43 were abnormal, respectively); abnormal sensation in response to a pinprick in the arm or leg (24 and 11 were abnormal, respectively); abnormal gastroc soleus reflex (16 were abnormal), or abnormal Romberg test (12 were abnormal). The majority of subjects had at least one abnormality ($n = 208$). Overall, the exposed subjects had a significantly lower probability of any abnormality than the nonexposed subjects ($p = 0.006$), a finding that was consistent for each individual nonexposed group. On the other hand, the average number of abnormalities (for each subject) did not differ significantly between the exposed and nonexposed groups ($p = 0.44$).

The TCP level was not a significant predictor of any clinical outcomes. The eight men who reported chlorpyrifos poisoning had decreased wrist sensitivity to vibration ($p = 0.003$) and decreased sensitivity to leg pinprick ($p = 0.002$). The 18 exposed men with the susceptible genotype had increased odds of an abnormal gastroc soleus reflex ($p = 0.01$) and an abnormal Romberg test ($p = 0.04$); the latter finding was not seen on the analogous computerized sway tests. Currently exposed applicators had borderline higher odds of an abnormal leg pinprick test ($p = 0.08$), whereas formerly exposed applicators had borderline higher odds of an abnormal gastroc soleus reflex.

Visuomotor tests: trails and pegboard.

The exposed group did not perform as well as the nonexposed group in the pegboard test for the dominant hand ($p = 0.07$). This effect was apparent when the applicators were compared to state employee controls ($p = 0.005$) but not when applicators were compared to nonexposed friends ($p = 0.43$). Results were similar for the nondominant hand, with respective p -values of $p = 0.09$ (vs. both nonexposed groups), $p = 0.02$ (vs. state employees), and $p = 0.70$ (nonexposed friends). The eight applicators with self-reported chlorpyrifos poisoning had a significant decrease in performance on the pegboard test for the nondominant hand ($p = 0.01$) and a borderline decrease in performance for the dominant hand ($p = 0.09$). Former applicators performed less well for the dominant hand test ($p = 0.01$). No other exposure variables showed an effect for the pegboard tests. The R^2 values for these tests were approximately 0.15. Exclusion of seven outliers (scores > 200) for the nondominant hand significantly improved the fit.

There were no associations between any exposure variable and either the trail A or trail B test. The R^2 values for these two models were 0.16 and 0.32 respectively.

Table 2. Exposure characteristics of termiticide applicators ($n = 191$).

Characteristic	Value
Applied termiticide in last week (n)	76 (40%)
Median number (range) termite jobs in last week if any	4 (1–41)
Applied termiticide in current year (n)	128 (67%)
Ever applied chlordane (n)	65 (34%)
Median years (range) applied chlordane ($n = 65$)	4.5 (0.5–28.5)
Mean years (SD) applied chlordane ($n = 65$)	7.0 (6.3)
Median years (range) applied chlorpyrifos ($n = 191$)	1.8 (0.1–10.3)
Mean years (SD) applied chlorpyrifos ($n = 191$)	2.4 (2.2)
Median years applied other pesticides ($n = 173$)	1.6 (0.5–11.3)
Mean years (SD) applied other pesticides ($n = 173$)	2.5 (2.5)
Median (range) TCP level in urine ($\mu\text{g/L}$), termiticide applied in last week ($n = 65$)	172.7 (5.7–13,009)
Mean TCP level (SD) in urine ($\mu\text{g/L}$), termiticide applied in last week ($n = 65$)	629.5 (1,636.6) ^a
Median (range) TCP level in urine ($\mu\text{g/L}$), no termiticide applied in last week ($n = 40$) ^b	28.1 (1–946)
Mean TCP level (SD) in urine ($\mu\text{g/L}$), no termiticide applied in last week ($n = 40$)	119.0 (196.) ^a
Mean TCP level (SD; $\mu\text{g/L}$) in nonexposed subjects ($n = 52$) ^c	6.2 (6.1) ^a
Self-report of chlorpyrifos poisoning (n)	8

^aThe creatinine-corrected values for applicators exposed in the last week, applicators not exposed in the last week, and the nonexposed were 331, 55, and 3 $\mu\text{g/g}$ creatinine, respectively. ^bUrine containers were mailed to all applicators who reported applying termiticide when testing was scheduled, several weeks before the test date. However, many applicators to whom we mailed specimen containers did not actually apply termiticide during the week before testing, but a number of these did use chlorpyrifos in non-termite work (lawns or house interiors) during that week. ^cSimilar to the mean level of 4.5 $\mu\text{g/L}$ observed in a random sample of 929 U.S. adults (4).

Neurobehavioral tests. There were no significant differences between the exposed and nonexposed groups on most of the neurobehavioral tests (simple reaction time, symbol-digit, continuous performance, digit span, forward and backward, pattern memory, vocabulary). There was no significant difference in self-reported effort on these tests between the exposed and nonexposed groups. The R^2 values for the exposed/nonexposed models were 5%, 34%, 7%, 12%, 16%, 13%, and 31%, respectively, for these tests. The level of TCP did not significantly predict performance on any of these tests except for the vocabulary test, for which subjects with more urinary TCP did significantly worse ($p = 0.02$). In results of these tests in exposed subjects, we found no significant effects of current versus former exposure; duration of use of chlorpyrifos, chlordane, or other pesticides; or presence of a susceptible genotype. Self-reported poisoning by chlorpyrifos was associated with decreases in performance on the continuous performance test ($p = 0.0001$) and on simple reaction time ($p = 0.06$).

Of the five components that constitute the mood scales, the exposed subjects reported significantly more fatigue and tension than the nonexposed subjects ($p = 0.0002$ and $p = 0.01$, respectively). The finding for fatigue was significant for the applicators versus each nonexposed group separately, whereas the finding for tension was significant for the applicators versus state employees ($p = 0.007$) but not versus nonexposed friends ($p = 0.14$). The test for anger ($p = 0.07$) was borderline significant (vs. state employee controls, $p = 0.008$). The test for depression was significant for exposed subjects versus nonexposed friends ($p = 0.05$) but not versus state employees ($p = 0.55$). The eight men who reported past chlorpyrifos poisoning reported significantly or borderline significantly more anger ($p = 0.06$), fatigue ($p = 0.02$), tension ($p = 0.01$), depression ($p = 0.06$), and confusion ($p = 0.03$). Those with current chlorpyrifos exposure reported significantly more fatigue ($p = 0.0004$) and tension ($p = 0.02$). No associations were found for genotype, by level of TCP exposure, or by duration of chlorpyrifos exposure. The mood scales were correlated with each other, with a Spearman correlation coefficient usually on the order of 0.50. The R^2 values for mood scales were low, generally approximately 5%.

Symptom questionnaire. The exposed subjects reported significantly more symptoms than nonexposed subjects on 16 of the 24 questions about frequency of symptoms in the last month. The exposed subjects more often reported being tired, dizzy (2 items), confused, less able to remember (3 items),

irritated, and less coordinated, and having a loss of strength in limbs (4 items), headaches, susceptibility to dizziness from chemicals, and less tolerance for alcohol. We found borderline significant findings ($0.05 < p < 0.10$) on reported increased depression, worse concentration, and numbness and tingling in toes. The R^2 values for linear models were low, on the order of 0.05; in the logistic models, few variables other than exposure were predictive of outcome. Several of the symptoms were highly correlated with each other, particularly for three related questions on remembering; two questions about confusion and difficulty concentrating; and four questions on loss of strength in arms, hands, fingers, and legs (pairwise Spearman correlation coefficients 0.40–0.60). Previously exposed applicators were significantly different from the nonexposed subjects on 12 items, whereas currently exposed applicators had nine significant associations. The eight men reporting previous chlorpyrifos poisoning had 10 significant associations (tiredness, memory, irritability, dizziness, understanding written material, sleep problems, arm/hand/leg weakness, and lack of coordination) and 1 borderline significant one (heart palpitations). The group of 18 applicators with genetic susceptibility had a significant association on only one test (lack of coordination), and there were no significant trends associated with TCP. The only symptom showing a significantly increased trend with duration of chlorpyrifos exposure was heart palpitations.

Vision tests. Twenty-four participants were found to have congenital loss of color vision in one or both eyes on the Farnsworth test and were eliminated from the analysis of acquired color vision loss via the Lanthony test.

The Lanthony test showed no difference overall between exposed and nonexposed subjects. However, there was a trend of poorer color vision with increasing levels of TCP for both the right ($p = 0.006$) and left ($p = 0.0008$) eyes. This trend was significant for the right eye only because a single participant with a very high TCP level performed poorly on the test. When we removed this participant from the analysis, the regression coefficient for each eye decreased 25%; the coefficient was no longer significant for the right eye ($p = 0.47$), but it remained significant for the left eye ($p = 0.006$).

There was a correlation of approximately 0.70 between eyes for the Lanthony test, and the regression models explained approximately 12–14% of the variance of the data (R^2) for either eye.

Visual acuity did not differ between the exposed and nonexposed groups. The tests of visual contrast, either overall or restricted to those with good acuity (20/30 or better), did not reveal any significant differences

between exposed and nonexposed groups for either eye, nor was there any trend of decreased performance with increases in the urinary TCP level. We found no associations between visual contrast and exposure for subgroups of the exposed group (former, current, susceptible genotype, poisoned applicators) and no significant trends for contrast with duration of exposure to chlorpyrifos, chlordane, or other pesticides. Linear regression models for acuity and contrast explained only approximately 5% of the variance of the data.

Smell tests. We found no differences in the ability to correctly identify odors between exposed and nonexposed groups; this ability was not correlated with the TCP level. We found no significant associations among subgroups; the eight men who reported previous chlorpyrifos poisoning did marginally better on the smell tests ($p = 0.08$). R^2 values for the linear models were approximately 0.05 for the smell tests.

Discussion

This study is the first large study of clinical and subclinical neurologic effects of participants who have been exposed to chlorpyrifos. This study has a number of strengths, including a thorough evaluation of neurologic function at both the clinical and subclinical level, a well-defined exposed population, and a large sample size.

However, existing “objective” tests for neurologic effects may not be adequate to detect some effects caused by exposure. In our study, exposed subjects reported significantly more symptoms than nonexposed subjects, but relatively few significant differences were found when more quantitative tests were used.

The weaknesses of our study include possible selection biases. We were able to recruit only a minority of the exposed target population, primarily because we could not locate many subjects. However, we tested 69% of eligible applicators whom we contacted. The nature of any possible selection bias is not clear: workers more affected by chlorpyrifos may have preferentially participated; on the other hand, workers most affected by exposure may have left the workforce or been harder to find, and thus did not participate. One strength of our study is that it was population-based and had a defined target population; this tends to decrease selection biases.

We studied termiticide applicators because they are exposed primarily to chlorpyrifos rather than other pesticides. However, we found that termiticide applicators also had substantial exposure to other pesticides including chlordane, which preceded chlorpyrifos as the main pesticide used against termites. Nonetheless, in the analyses we found

that exposure to chlordane or other pesticides was not, in general, associated with effects and therefore was unlikely to confound the analysis of chlorpyrifos.

Our study population was rather young—90% of the applicators were under 50 years of age. If chlorpyrifos causes delayed neurotoxic effects that become apparent only with age, we would have missed such effects.

Table 3 provides a summary of all results. We found few significant differences between exposed and nonexposed subjects on the clinical examination, with the exception of 2 (out of 10) tests that indicated increased abnormality for the 8 men who reported previous chlorpyrifos poisoning and 2 tests for the 18 men with a susceptible genotype. We also found no widespread pattern of effects associated with exposure in most other tests. However, there were exceptions to this generally negative pattern. The exposed subjects did not perform as well as the nonexposed subjects on pegboard turning and postural sway tests. Most markedly, the exposed subjects consistently reported more current psychologic and physical symptoms than the nonexposed subjects. The differences between exposed and nonexposed subjects for self-reported symptoms could have been a true effect of exposure. One prior study of chlorpyrifos-exposed subjects in a

manufacturing setting also found significant increases in such ill-defined conditions (11), and one study of men poisoned by chlorpyrifos (in combination with other organophosphates) found more severe self-reported mood states than a nonpoisoned comparison group (9). It is possible that there was a systematic bias of applicators to overreport their symptoms, but we have no evidence to support such a bias. The applicators did not, in general, appear to complain about chlorpyrifos during the testing sessions.

The differences in symptoms were more marked for former rather than current applicators, suggesting a long-term effect. However, these differences were generally not more apparent for those with longer exposure to chlorpyrifos. Future studies should consider the temporal sequence of exposure and any self-reported symptoms; we lacked data on this question because our questionnaire was limited to current symptoms.

We observed a pattern of worse performance on several tests (sway, pinprick sensitivity, self-reported physical and mental symptoms, two neurobehavioral tests) by the participants ($n = 8$) who reported chlorpyrifos poisoning as compared with other applicators. The data on poisoning are limited because of their self-reported nature; these poisonings represent a past acute exposure

accompanied by self-reported symptoms (only one of the eight men who reported poisoning sought medical treatment). Similar findings for self-reported negative mood scales was also reported in a previous study (9) for the subgroup with chlorpyrifos poisoning. Our findings for the eight men who reported chlorpyrifos poisoning suggest that further study of the delayed effects of chlorpyrifos poisoning are warranted.

Applicators with a paraoxonase genotype expected to increase sensitivity to the effects of organophosphates ($n = 18$) showed few positive effects, with the exception of two tests on the clinical examination.

We studied applicators with a relatively short duration of exposure (2 years) to chlorpyrifos, possibly limiting our ability to observe exposure effects. Furthermore, it is possible that exposures were not sufficiently high for an exposure effect to be observed. However, 18% of the exposed subjects had more than 4 years of exposure, and their exposure levels (as indicated by urinary TCP) were two orders of magnitude greater than background levels in the U.S. population (4).

In summary, in this cross-sectional study of workers exposed to chlorpyrifos, we found few exposure-related effects for most tests, including a clinical examination. However, the exposed subjects did not perform as well

Table 3. Summary of tests (p -value) for which exposure effect had p -value ≤ 0.10 .

	6 NCV tests ^a	12 Sway tests	4 VBT tests	10 Clinical tests	2 Pegboard tests	6 NB tests	5 MS tests	24 Symptom tests	ACV loss (2 eyes)	Smell test
Applicators ($n = 191$) vs. nonexposed ($n = 189$)		1 Length sway test (0.04)	1 Toe test, better sensitivity (0.03)	Better odds, no abnormality ($p = 0.0006$)	Dominant hand (0.07)		Fatigue, tension, anger, (0.001, 0.01, 0.07)	16 Tests, (< 0.05 ; dizzy, memory, headaches, fatigue, etc.), 3 tests (< 0.10)		
Poisoned applicators ($n = 8$) ^b	1 Better amplitude (0.05)	2 Area tests (0.09, 0.10)		2 Tests (0.004, 0.003)	Dominant hand (0.01), nondominant (0.09)	1 Test (0.0001), 1 Test (0.06)	5 Tests (0.02, 0.06, 0.06, 0.01, 0.03)	10 Tests (< 0.05), 1 test (< 0.10)		Better (0.08)
Susceptible genotype ($n = 18$) ^b	Velocity: 1 better (0.10), 1 worse (0.10)		1 Better finger test (0.05)	2 Tests (0.04, 0.01)				2 Tests (0.04, 0.07)		
Currently exposed ($n = 128$) or formerly exposed ($n = 63$) ^b		Current: 1 length test (0.10); former: 1 length test (0.04) and 2 area tests (0.03, 0.05)	Former: 1 better toe test, (0.04)	Former and current: 1 test worse for each (0.07, 0.08)	Former: dominant hand (0.01)		Current: 2 tests (0.0004, 0.02)	Former: 12 tests (< 0.05), 1 test (< 0.10); current: 9 tests (< 0.05), 3 tests (< 0.10)		
Trend with duration exposure ^b		2 Length tests (0.04, 0.08)						1 Test (0.01)		
Trend with TCP ^b		2 Length tests (0.03, 0.01)				Vocabulary test (0.02)			Both eyes (0.006, 0.008)	

Abbreviations: ACV, acquired color vision; MS, mood scales; NB, neurobehavioral; NCV, nerve conduction velocity; VBT, vibrotactile. Exposed subjects did not perform as well as unexposed subjects except where noted. We observed no significant differences in the two-arm tremor tests, the trials test, or the vision contrast.

^aVelocity and amplitude in three nerves. ^bPoisoned applicators, susceptible genotype applicators, current or former applicators each compared with nonexposed. Trend with duration for applicators only. Trend with TCP among all participants with TCP data (105 applicators, 52 nonexposed).

as the nonexposed subjects on pegboard turning tests and some postural sway tests. Furthermore, exposed subjects reported more symptoms than nonexposed subjects; this is a cause for concern because previous studies (9,11) lend some support to this finding. There was some suggestion of a pattern of delayed effects for subjects with a past history of poisoning, which is also supported by some previous reports of men poisoned by organophosphates (9,39,40). Although this was a relatively large study based on a well-defined target population, the workers we studied may not be representative of all exposed workers, and caution should be exercised in generalizing our results.

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