Protracted Neurotoxicity from Chlordane Sprayed to Kill Termites

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Over 250 adults and children were exposed to chlordane when the wooden building surfaces and soil around an apartment complex were sprayed in 1987. Two hundred-sixteen adults had neurobehavioral functions measured and completed questionnaires for symptom frequency, mood status, confounding factors, and medical, rheumatic, and respiratory disorders in 1994. Measurements included simple and choice reaction time, balance, blink reflex latency, color vision, cognitive, perceptual motor, memory, and recall functions. We analyzed 216 exposed and 174 referent adults. Age, educational level, weight, height, and gender ratio were similar for the exposed and referent groups. Performance of balance, reaction times, Culture Fair, digit symbol, verbal recall, and trail-making were significantly impaired in exposed persons compared to referents. Mood-state scores were elevated, as were the frequencies of respiratory, neurobehavioral, and rheumatic symptoms. In contrast, long-term memory function was similar in both groups, consistent with its status before exposure. There was no identified bias or confounding factors. Chlordane exposure was associated with protracted impairment of neurophysiological and psychological functions. The central nervous system is the most important target of chlordane cyclohexane insecticides. Human exposure should be prohibited. Key words: balance, choice reaction time, profile of mood states, sway speed, trail-making, verbal recall. Environ Health Perspect 103: 690-694 (1995)

Chlordane was introduced as a pesticide in 1948 and used extensively as a termicide until 1975 when its use was interdicted by the Environmental Protection Agency. For most uses it was banned in 1988 because of human neurotoxicity. Chlordane accumulates in fatty tissues and has been classified as a probable human carcinogen.

In April 1987 the outside wooden surfaces of an apartment complex in Houston, Texas, were sprayed for termites with chlordane in an unknown concentration. Later in 1987 and 1988 chlordane combined with clorpyrifos (Dursban) was again sprayed on these wooden surfaces. The apartment units were tested for chlordane residue in 1990 and 1991, and 85% of 81 samples from wood surfaces were found to have chlordane at 0.5 μg/929 cm² or more.

Indoor concentrations of chlordane were as high as 13.6 μg/929 cm² on wipe samples, and in 24 of the 294 apartments which had air samples, chlorinated insecticides levels were above 0.5 μg/m³ for 8-hr samples. Eight subjects occupying the apartments had elevated levels of chlorinated insecticides in blood or fat; the heptachlor range was 110-186 ppb, oxychlordane was 70-150 ppm, and transnonachlor was 76-200 ppm.

In June–September 1994, 216 adult occupants or former residents of the apartments in question were examined using a pretested neurobehavioral battery and questionnaires. The exposed group of 109 women and 97 men, ages 17–70 years, was compared with 174 unexposed Houston referents consisting of 94 women and 68 men who matched the exposed subjects in age and educational level. We compared neurobehavioral function, pulmonary function and symptomatology, and histories for occupational, personal, and residential confounding factors.

Methods

A cross-sectional design was used. The referents were recruited through networking contacts and newspaper advertisements. The aim was to parallel the gender, age, and years of educational attainment (highest school grade completed) of exposed subjects as well as preferred language, English or Spanish. More referents than exposed subjects had histories of possible chemical contamination from working in refineries, plastics, or electronics industries and from using herbicides. For the oral and written test components, subjects elected testing in Spanish or English. Few referents chose to test in Spanish, and they were older and had fewer years of education than their English-tested counterparts. The referents tested in Spanish were older and had more years of education than the exposed subjects tested in Spanish. Referents were reimbursed for time and mileage. The examiners did not know the subjects’ exposure category during testing. All subjects gave informed consent, and the protocol was approved by the Human Studies Research Committee of the University of Southern California School of Medicine.

Self-administered questionnaires were given to each subject and checked for completion by computer-guided card reading. The questionnaires included the American Rheumatism Association lupus erythematosus questions (1), a standard respiratory questionnaire (2), occupational histories and exposures to chemicals including pesticides and herbicides, tobacco, alcohol and drug use (prescription and illicit), neurologic disorders including unconsciousness, anesthesia, and head trauma, and medical histories (3). The frequencies of each of 35 respiratory, neurologic, and vegetative complaints from daily to yearly or less were recorded by the subject on each questionnaire (3).

The neurophysiological and neuropsychological test battery (Tables 1 and 2) was modified slightly from that used in studies of histology technicians (4,5), firemen exposed to thermolysis products of polychlorinated biphenyls (6), and a solvent-exposed population (3). Alcohol was measured in air expired after a 20-sec breath hold using a fuel cell analyzer.

Simple reaction time and visual two-choice reaction time were measured with a computerized instrument (7). Body balance was measured with the subject standing erect with feet together. The position of the head was tracked by two microphones from a sound-generating stylus on a headband, processed by a computer, and expressed as mean speed of sway in centimeters per second (8). The blink reflex was measured with surface electromyographic electrodes (EMG) from lateral obicularis oculi muscles bilaterally (9) after tapping the glabella (midline of lower frontal bone) and right and left supraorbital notches with a light hammer which triggered a recording computer. Ten firings of R-1 and the volley of second waves, R-2, were averaged to find the mean response for each site, and failures were recorded (9). Color recognition was measured with the desaturated Lanthony 15-hue test under constant illumination (10) and scored by Bowman’s method (11).

Immediate memory or recall was measured by verbal and visual recall and digits forward and backward from Wechsler’s Memory Scale (12). Culture Fair (battery 2A) was used to test nonverbal, nonarithmetical intelligence based on the selection

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of designs for similarity, difference, completion, and pattern recognition and transfer (13,14), which resembles Raven's progressive matrices (15). Block designs from the Wechsler Adult Intelligence Scale (WAIS) (16) tested constructional, interpretative, and integrative capacity. Digit symbol, also from the WAIS, tested attention and integrative capacity. We used four tests, slotted pegboard, trail-making A and B, and finger-tip number writing, which measures dexterity, coordination, decision-making, and peripheral sensation and discrimination, from the Halstead-Reitan battery (17,18). The vocabulary test was from the multidimensional aptitude battery (19). To profile mood states [profile of mood states; POMS (20)], subjects self-judged their emotional status during the preceding week.

Spirometry was done with subjects standing and using a nose clip on a volume displacement (Ohio) spirometer until two forced expirations agreed within 5%, following the American Thoracic Society conventions (21). Volumes and flows were traced with a digitizer and measured in a computer.

All scores and computed data for sway, blink, and reaction time were entered into a Tri-star 486 EISA bus computer. Descriptive and analytical computations including Student’s t-tests, analysis of variance, and stepwise linear regression model used Stata statistical software. (Stata Corporation, College Station, Texas). Statistical significance was defined as p<0.05 for t-tests and coefficients in regression analyses.

To compare groups of exposed and unexposed subjects, we divided them into tested in English or tested in Spanish for vocabulary and for information, picture completion, similarities, and recall of stories. Spanish-tested subjects were 8 years older than English-tested subjects, a significant difference. Their average educational level was 1.3 years greater than the English-tested subjects, which was not significant. The 138 English-tested subjects had the same gender ratio as the exposed group, an average age of 32.2 years, and an educational level which was not significantly different from exposed subjects. These differences between English-tested and Spanish-tested groups were small, but gender-related differences on certain tests and the larger number of Spanish-tested subjects than referring suggested a need for further statistical analysis. First subjects within language groups were compared. Effects of exposure were also analyzed by covariant analysis using regression equations based on referent subjects for each test. This technique adjusted for the effects of differences in age, educational level, and gender and produced p-values to compare to those from the comparisons of group means.

Modeling to develop the prediction equations for each test used stepwise linear regression after transformation of each dependent and independent variable for maximal linearity (22), graphic methods to study residuals, and a lack-of-fit test (23). These prediction equations will be published later (Kilburn and Thornton, in

### Table 1. Neurophysiological, neuropsychological scores, and profile of mood states in exposed and referent subjects tested in English

| Parameter | Referents (n = 127) | Exposed (n = 104) | Student’s t-test | Covariant p |
|-----------|---------------------|-------------------|-----------------|-------------|
| Age (years) | 32.2 (11.3) | 32.9 (11.4) | 0.6629 |
| Education (years) | 12.0 (2.4) | 11.5 (2.1) | 0.0892a |
| Height (cm) | Females/males | 77/61 | 58/46 | |
| Weight (kg) | Males | 173.8 (7.0) | 173.4 (8.4) | 0.8033 |
| Grip, right (kg) | Females | 160.7 (7.0) | 160.1 (7.6) | 0.6660 |
| Grip, left (kg) | Males | 49.8 (8.7) | 49.8 (8.7) | 0.9990 |
| Grip, left (kg) | Females | 33.1 (8.2) | 30.7 (6.3) | 0.0836 |
| Visual refractive latency (msec) | Eyes open | 0.82 (0.20) | 0.95 (0.35) | 0.0003* |
| Gaze | 1.26 (0.35) | 1.48 (0.60) | 0.0005* |
| Color score lanthony | 11.6 (3.3) | 11.9 (1.5) | 0.0956 |
| Blink reflex latency (msec) | Right | 14.5 (2.4) | 13.5 (2.3) | 0.0038* |
| Glandular tap | Left | 14.3 (2.5) | 13.2 (2.3) | 0.0015* |
| Recall (Wechsler) | Immediate | 10.5 (4.2) | 8.7 (3.7) | 0.0010* |
| Story 1 | Delayed | 8.3 (4.5) | 6.7 (3.7) | 0.0008* |
| Story 2 | Immediate | 9.4 (4.0) | 8.2 (3.9) | 0.0034* |
| Visual recall | Delayed | 8.3 (4.2) | 6.6 (4.1) | 0.0093* |
| Digit span | Forward | 6.8 (1.5) | 6.7 (1.7) | 0.5983 |
| Backward | 4.4 (1.4) | 4.3 (1.5) | 0.4405 |
| Cognitive function | Culture Fair A score | 28.1 (7.7) | 25.4 (8.1) | 0.0079* |
| Vocabulary score | 18.5 (8.3) | 16.0 (7.7) | 0.0254* |
| Digit symbol score | 57.5 (15.0) | 48.5 (15.0) | 0.0006* |
| Perceptual motor speed | Pegboard dominant score | 72.4 (27.6) | 76.1 (20.5) | 0.2597 |
| Trail-making A score | 37.3 (17.1) | 44.7 (19.4) | 0.0027* |
| Trail-making B score | 79.8 (37.7) | 33.1 (37.1) | 0.0092* |
| Sensory-interpretive | Fingerwriting errors | Right | 2.4 (3.4) | 2.9 (3.5) | 0.3309 |
| Left | 2.0 (3.2) | 2.4 (3.4) | 0.3707 |
| Long-term memory | Information score | 14.6 (6.1) | 13.1 (5.9) | 0.0537 |
| Picture completion score | 13.7 (4.0) | 13.2 (3.9) | 0.3627 |
| Similarities score | 17.7 (6.1) | 16.8 (5.9) | 0.1909 |
| Profile of mood states score | Tension | 32.3 (38.8) | 20.2 (43.6) | 0.0001* |
| Depression | 10.9 (11.3) | 18.5 (8.0) | 0.0001* |
| Anger | 10.8 (9.6) | 18.2 (11.0) | 0.0001* |
| Vigor | 17.1 (6.4) | 12.4 (5.7) | 0.0001* |
| Fatigue | 8.1 (6.3) | 14.2 (7.3) | 0.0001* |
| Confusion | 7.7 (5.4) | 12.9 (6.0) | 0.0001* |

*Stories combined.  
aNo prediction model.  
*Statistically significant.
Results

The 104 English-tested, exposed subjects had the same mean age and educational level; 55% were women and 45% were men (Table 1). Weights were slightly greater in the exposed group for both men and women but were not statistically significant, and heights were virtually identical (Table 1). In contrast, the Spanish-speaking referents were significantly older than the exposed subjects, but the educational level and heights and weights were not different. Using the older Spanish-speaking referents without age adjustment would narrow the apparent exposure differences.

Neurophysiological Tests

The neurophysiological tests of English-tested, exposed subjects showed a significantly slowed simple reaction time (average 414 msec in exposed versus 309 msec in referents). There was a similar significant difference in choice reaction time (639 msec in exposed versus 564 msec in referents). Balance measured as sway speed with eyes open was significantly different between exposed and referent subjects (0.95 cm/sec versus 0.82 cm/sec, respectively), as was sway speed with eyes closed (1.48 cm/sec versus 1.26 cm/sec). The color score on the Lanthony desaturated hue test was not significantly higher in exposed subjects. Blink reflex latency (Table 1) was shorter in the exposed subjects compared to referents for supraorbital taps right and left but not for glabellar taps recorded on right and left. Grip strength was not significantly different in men or women across groups. The same differences were found across groups for those tested in Spanish except for blink reflex latency (Table 2).

There were statistically significant exposure coefficients for simple and choice reaction time, balance measurements, and between English- and Spanish-tested exposed subjects compared to referents. Blink latency after supraorbital tap was significantly dependent on exposure in English-tested subjects. These differences confirmed those found when unadjusted means were compared.

Neuropsychological Tests

Both immediate and delayed verbal recall using the Wechsler stories were significantly lower in the exposed group of both English-tested and Spanish-tested subjects (Tables 1 and 2). Visual reproduction or picture recall was not significantly different in any exposed group compared to referents. Digit span forwards and backwards was judged not to differ, although the Spanish-tested exposed group differed for digits forward, the less sensitive test. The cognitive functions domain showed a significant difference for Culture Fair A, with a mean of 25.4 in the English-tested exposed subjects versus 29.5 in the referent group and was also significantly lower, 17.7 compared to 21.2, in the Spanish-tested exposed subjects.
Vocabulary showed statistically significantly lower scores in exposed subjects (16.0 versus 18.5 in English-tested and 11.6 versus 17.6 in Spanish-tested). Digit symbol score differences between exposed and referents were statistically significantly lower for subjects in both language groups. The covariant comparisons with exposure as a coefficient confirmed these statistically significant differences except for vocabulary in English-tested subjects.

In regard to perceptual motor speed, the difference between exposed and referent subjects for grooved pegboard with the dominant hand was not significant by comparison of means, but covariant analysis revealed a significant difference between women in both language groups but not between men. There were statistically significant differences for trail-making A and trail-making B; the numeric differences were large (7.4 sec longer for the English-tested exposed and 22 sec longer for Spanish-tested exposed trail-making A; 16 sec longer for English-tested exposed and 13 sec longer for Spanish-tested exposed for trail-making B). Thus, the age- and educational-level-adjusted comparisons confirmed the differences found in the comparison of means.

The long-term or crystallized memory tests, consisting of information, picture completion, and similarities showed no significant differences between English-tested exposed and referent subjects, consistent with the hypothesis that they were members of the same population before exposure. The Spanish-tested exposed subjects showed significant differences from matched referents in means for two of three tests, information and picture completion. These differences were confirmed by covariant analysis, which suggested that age, education, and gender were not responsible. Fingertip writing errors showed small differences which were not statistically significant in either English-tested or Spanish-tested subjects.

Affective status as revealed by POMS score showed a large exposure difference of approximately 40. The English-tested group mean was higher by about 20 (Table 1) compared to Spanish referents (Table 2) which was statistically significant. There were differences for tension, depression, anger, fatigue, and confusion which were all significantly higher and vigor was significantly lower in the exposed versus the referents.

Symptom Frequency

The frequency of 35 symptoms, including skin and nails, chest, throat, eyes, central nervous system, indigestion, loss of appetite, and swollen stomach were all more common in exposed subjects (p = 0.0001), except for loss of consciousness in the English-tested exposed subjects versus the comparable referent group (p = 0.08). Thus, members of the exposed group had significantly greater frequency of 34 of 35 common symptoms (data not shown).

Respiratory symptoms were also compared (data not shown). Allergy history, the production of phlegm, chronic bronchitis by Medical Research Council criteria, wheezing with and without shortness of breath, asthma, and shortness of breath at rest, walking, and climbing stairs were all significantly more common in exposed subjects than in referents. Pulmonary function studies of exposed subjects showed average values of 97% forced vital capacity, 91% forced expiratory volume, 91% of forced expiratory flow (25–75) and 83% predicted for forced expiratory flow (75–85), but were not significantly different from values for referent subjects.

Duration of residence after the 1987 incident was examined for effect as a continuous variable against each test and was not ever a significant factor.

Confounding Factors

More referents than exposed subjects had worked in industries with exposure to neurotoxins (petroleum refineries, plastics, and electronics). However, testing of these possible confounding variables by covariant analysis did not detect a specific exposure which contributed significantly to the differences among Spanish-tested subjects. In no instance did the exposed group have greater proportional representation in occupations where there were toxic exposures.

Medical histories showed similar prevalences of the childhood diseases mumps, chicken pox, and measels, and there were no differences for neurological or psychiatric disease diagnoses between exposed subjects and referents of either language group. Exposed subjects had more hours of general anesthesia and significantly more children with birth defects, but the referent group had more head injuries. Also, the exposed group had significantly more angina pectoris and kidney disease, but no differences for myocardial disease, lupus erythematosus, and cancer.

Lifestyle factors which could cause brain damage or affect neurobehavioral performance such as drug overdoses or alcohol consumption were not significantly different. However, significantly more of the exposed group had never used alcohol (44.9% exposed versus 28.4% referents), and more referents had alcohol overdoses (4.2% versus 0.6% subjects). There were no differences in illicit drug use or in use of prescription tranquilizers; about 5% of both groups stated they currently used drugs. Neurological and psychiatric disorders were rare in exposed and referent groups. In summary, any confounding factors would reduce the difference between the referents and exposed and favor the null hypothesis. However, no possible confounding factor or group of factors was a determining variable for any test score.

Use of the American Rheumatism Association criteria for lupus showed that significantly more of the exposed group had numb and cold fingers, mouth sores, rashse elicited by sunlight, painful breathing (pleuritic chest pain), hair loss, and seizures. In contrast, there were no differences for general rheumatic complaints, anemia, protein in the urine, or seizures. Ten percent of the exposed group had five or more lupus symptoms versus no symptoms reported by the referent group.

Discussion

Examination of subjects exposed in their homes to chlordane as compared to referent subjects showed significant, and we suggest important, impairment of both the neurophysiological and psychological functions including mood states. Accompanying these changes were significant differences in symptom frequency and in respiratory rheumatic and cardiovascular disease symptoms. The most notable changes were slowing of reaction time, balance dysfunction as revealed by increased sway speed, reductions in cognitive function, perceptual motor speed, and immediate and delayed verbal recall. Blink reflex latency was delayed in the English-tested exposed subjects and differences in color discrimination were of borderline significance. The neurobehavioral impairments measured in this environmental epidemiological study were similar to those noted in patients exposed to chlordane at home (Kilburn and Thornton, submitted). These impairments include probably irreversible dysfunction of the brain. Possible effects on trigeminal nerve–pons–facial nerve function were suggested for the first time (Kilburn and Thornton, submitted). Confirmatory studies, including follow-up after removal from exposure, are urgently needed. Meanwhile, chlordane use should be prohibited worldwide.

The exposure of our study group appears to be from indoor air, due to the outgassing of chlordane from the wooden surfaces of the apartment complex. Analyses showed elevated chlordane levels in several exposed subjects’ blood as well as elevated levels on wipe samples and in the air in some of the apartments of the complex.

We have also demonstrated how exposure status (effect) can be examined by covariant analysis to adjust for age and educational level. Educational attainment

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Chlordane is a chlorinated cyclodiene insecticide introduced in 1948 and considered to act as a toxicant with many of the signs and symptoms of poisoning like those produced by DDT (24). One striking difference is the ability of chlordane and other chlorinated cyclodienes to induce convulsions. Other frequent findings are headache, nausea, vomiting, dizziness and chronic, jerking movements (25). Children who had convulsions from chlordane exposure subsequently had learning disorders (26).

It is thought that the cyclodiene interacts with the picrotoxin receptor in the nervous system, releasing excitatory transmitters and interfering with the γ-aminobutyric acid neurotransmission system (27). The primary target appears to be synapses with the highest number of converging presynaptic elements, so the threshold for excitation is lowered and increases the number and frequency of action potentials (28). This is a "kindling" process which potentiates through the nervous system via postsynaptic pathways, producing responses 10-100 times more intense than normal. Also, epoxides are formed by biotransformation of chlorinated hydrocarbons (24). In view of the likelihood that hyperresponsiveness occurs from the blocking of inhibitory activity or the increase of excitation, it is strange that, aside from the symptoms, our chronically exposed subjects showed depression of function, particularly of basic responses like balance and reaction time, trail-making, and Culture Fair. They did show the psychological disorders characterized by anxiety, irritability, and insomnia and motor pathology, which have been previously associated with chlordane exposure.

It is tragic that exposure is still occurring to a material that the National Research Council in 1982 characterized as a hazard at any dose: "it could not determine a level of exposure to any of the [cyclodiene] termiticides below which there would be no biological effect. . . . Every effort should be made to minimize exposure" (29, p. 164). Also, in 1986 the EPA reported that chlordane was the most frequently misused or misapplied of the termiticides. As of 1987 under an agreement with EPA, the manufacturer Velosilco ceased to sell chlordane for consumer use in the United States, although the company was still licensed to export it. It is peculiar, therefore, that in 1988 EPA allowed chlordane to be applied at 150 residences across the country and decided that air monitoring would be done for 2 years to detect whether there were levels in the air (29). It is regrettable that chlordane was applied around and on 30 million or more homes in the United States before the ban. The contemporary problem is illustrated by chlorinated hydrocarbons accounting for 11% of calls about insecticides to Minnesota poison centers compared to 21% about organophosphates in 1988 (30).

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