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Neurotoxicology and Teratology 23 (2001) 381–393

NEUROTOXICOLOGY AND TERATOLOGY

Evaluation of acute sensory–motor effects and test sensitivity using termiticide workers exposed to chlorpyrifos

Robert B. Dick[a,*], Kyle Steenland[b], Edward F. Krieg[a], Cynthia J. Hines[b]

[a]*Division of Applied Research and Technology, U.S. Department of Health and Human Services, Public Health Service/Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*

[b]*Division of Surveillance, Hazard Evaluations and Field Studies, U.S. Department of Health and Human Services, Public Health Service/Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA*

Received 23 June 2000; accepted 8 March 2001

Abstract

Sensory and motor testing was performed on a group of termiticide workers primarily using chlorpyrifos-containing products to evaluate both the acute effects from current exposure and sensitivity of the measures to detect effects. The study group comprised 106 applicators and 52 nonexposed participants. Current exposure was measured by urinary concentrations of 3,5,6-trichloro-2-pyridinol (TCP) collected the morning of testing. The mean TCP value for the 106 applicators was 200 μg/g creatinine. Participants received 4–5 h of testing and were evaluated using a sensory–motor test battery recommended by a National Institute for Occupational Safety and Health (NIOSH)-sponsored advisory panel to be appropriate for testing effects from pesticide exposures. Measurements testing olfactory dysfunction, visual acuity, contrast sensitivity, color vision, vibrotactile sensitivity, tremor, manual dexterity, eye–hand coordination, and postural stability were analyzed. Study results indicated limited acute effects from exposure to chlorpyrifos using urinary TCP as a measure of current exposure. The effects occurred primarily on measures of postural sway in the eyes closed and soft-surface conditions, which suggests a possible subclinical effect involving the proprioceptive and vestibular systems. Several other tests of motor and sensory functions did not show any evidence of acute exposure effects, although statistically significant effects of urinary TCP on the Lanthony color vision test scores and one contrast sensitivity test score were found. The visual measures, however, were not significant when a step-down Bonferroni correction was applied. Information also is presented on the sensitivity of the measures to detect effects in an occupationally exposed population using standard error of the parameter estimates.

Keywords: Chlorpyrifos; Sensory–motor; Termiticide workers; Urinary TCP; Test sensitivity

1. Introduction

Conducting research on the health effects of organophosphorus pesticides in humans presents difficult problems. Controlled exposure laboratory studies or independent group studies with double-blind procedures are usually prohibitive because of ethical concerns and if a field study is conducted, securing a worker population exposed to one pesticide is difficult. Another problem is the separation of acute and chronic effects in active workers, because at the time the research is conducted, the participants will have both a current exposure body burden and a past exposure history. Additionally, the selection of tests that have sufficient statistical power and specificity to detect effects at typical occupational exposure levels (i.e., nonpoisoned) is important.

The National Institute for Occupational Safety and Health (NIOSH), with the cooperation of the State of North Carolina and the U.S. Environmental Protection Agency recruited a population of 191 current and former termiticide workers primarily applying chlorpyrifos and 189 controls for a cross-sectional study [52]. From this population of termiticide applicators and controls, a subgroup of applicators who indicated through phone interviews current use of chlorpyrifos-containing products, and a subgroup of controls were preselected to provide urine samples for an assessment of acute effects using a chlorpyrifos-specific urinary metabolite, 3,5,6-trichloro-2-pyridinol (TCP), and to evaluate the sensitivity of a battery of sensory–motor

* Corresponding author. Tel.: +1-513-232-4073; fax: +1-513-533-8596.

E-mail address: maudbay@wans.net (R.B. Dick).

0892-0362/01/$ – see front matter
PII: S0892-0362(01)00143-X

tests to detect effects on the nervous system from exposures to organophosphate (OP) compounds.

Chlorpyrifos is a widely available OP pesticide used for both residential and agricultural pest control. The chemical formula is *O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate. An estimated 18.5 million pounds of the active ingredient chlorpyrifos were consumed in the US in 1995 [51]. The historical use of chlorpyrifos in both agricultural and nonagricultural environments is attributed to its moderate acute toxicity in mammalian species, broad spectrum insecticidal action, and as a replacement for other compounds that were no longer used because of lack of effectiveness or regulatory action [43]. Usage of chlorpyrifos-containing products, however, will drop markedly in the US because of recent pronouncements by the U.S. Environmental Protection Agency to phase out/eliminate the chemical for termiticide, residential indoor, and lawn uses [61].

The signs and symptoms of acute toxic effects from exposure to chlorpyrifos are similar to other OP or cholinesterase-inhibiting compounds and are listed in standard toxicological sources [1,23,28]. Reported long-term nervous system effects from acute poisoning or repeated exposures to OP compounds include impaired memory, attention and concentration, depression, irritability, confusion, speech difficulties, nightmares, mood disorders, delayed reaction times, increased vibrotactile sensitivity, and sensory and motor impairments [5,36,43,53,54,56].

Some OP compounds, may also cause OP-induced delayed neurotoxicity (OPIDN), a neurodegenerative disorder 1 to 4 weeks following a poisoning episode [1,43]. Animal studies, however, show low potential for OPIDN at maximum tolerated doses, and chlorpyrifos is not a potent inhibitor of neurotoxic esterase, which is associated with the occurrence of OPIDN [43]. There have, however, been reports in the literature of possible OPIDN among individuals who received near-lethal doses of chlorpyrifos [2,40]. Whether or not OPIDN can occur in humans from repeated subchronic exposures to chlorpyrifos needs further research [43]. There is some evidence that acute poisoning episodes from exposure to OP compounds, which do not inhibit neuropathy target esterase or produce the classic OPIDN symptoms, may lead to a persistent peripheral neuropathy detectable by neurophysiological tests [53,57].

Upon exposure (e.g., ingestion, inhalation, dermal), the compound is metabolically activated in the liver to the active metabolite, chlorpyrifos oxon, which produces neurotoxicity by inhibiting target esterases in the peripheral and central nervous system. Chlorpyrifos oxon is also detoxified in the liver and plasma to diethyl phosphate and TCP [43]. The main target enzyme is acetylcholinesterase. Similar to other OP compounds, chlorpyrifos binds to the esteratic site causing accumulation of the chemical transmitter acetylcholine at the muscarinic receptors (e.g., parasympathetic autonomic nervous system) and the nicotinic receptors (e.g., parasympathic and sympathetic nervous system and neuromuscular junctions) which results in excess cholinergic stimulation [23]. Acetylcholine is also a neurotransmitter in the brain and chlorpyrifos readily crosses the blood–brain barrier with effects on this structure.

Nolan et al. [41] studied the kinetics of chlorpyrifos in human volunteers using both oral and dermal dosages and determined that urinary TCP was the principle metabolite present in blood and urine with a half-life of 27 h after administration. Fenske and Elkner [24] in a study of five urban pesticide applicators applying chlorpyrifos in subslab and soil injection to houses confirmed that TCP was the principle indicator found in measurable quantities that reflected total absorbed dose from both dermal and inhalation exposures. Preexposure and complete 72-h urine samples demonstrated considerable interindividual variability, but urinary metabolite concentrations collected 24–48 h postexposures were the most highly correlated with total absorbed dose estimates.

Although chlorpyrifos has been marketed since the late 1960s and is in widespread use, human studies of both acute and chronic effects on the nervous system are sparse. One difficulty has been finding populations exclusively exposed to chlorpyrifos. The relevant studies either reported some limited health effects [3,36,53] or no significant health effects [10,11] associated with exposures to chlorpyrifos. None of these studies were very comprehensive in evaluating the effects on the nervous system from exposures to chlorpyrifos and all lacked current measures of exposure and internal dose at the time of testing.

This research is part of a large effort by NIOSH to study the effects of pesticide exposures. The report of the cross-sectional study is available in Steenland et al. [52]. The purpose of this report is to evaluate the sensitivity of several specifically selected measures of sensory and motor functions using urinary TCP as a measure of current exposure to chlorpyrifos. The TCP concentrations provide a measurement of the chlorpyrifos body burden for each participant and by including nonexposed controls with both low- and high-exposed workers, a dose–response range for the detection of acute effects is possible. This paper also presents more detailed analysis and discussion of the acute effects of chlorpyrifos (using TCP measurements) for the sensory and motor outcomes than is available in Steenland et al. [52].

2. Experimental procedures

2.1. Study design

In total, 384 workers from a 12-county area in North Carolina participated in the cross-sectional study. The exposed population consisted of 191 current and former professional termiticide applicators using chlorpyrifos. The control population consisted of 106 same gender friend controls of the applicators and 83 blue-collar workers

employed by the State of North Carolina. All of the nonexposed participants had never worked as a pesticide applicator and had never been poisoned by pesticides. Additional details regarding participant recruitment are available in Steenland et al. [52].

2.2. Subgroup selection and sample collection

Urine samples were collected from a subgroup of the total study population for the acute effects and measurement sensitivity analysis. This subgroup consisted of 106 applicators who reported current termite work with chlorpyrifos-containing compounds (e.g., Dursban TC, Equity, Cyren TC) in phone interviews and 52 nonexposed participants selected at random (47 friend controls and 5 state controls). A questionnaire was administered for demographic variables, health status, job history and for participants to indicate their last day of termite work, the chlorpyrifos product they were currently using and/or had used in the past year, and other pesticide and chemical use. All participants, except three, were male. Participants brought first morning void urine samples to the test site on their scheduled day of testing and from these samples, 25 ml of urine were retained for TCP analysis using a gas chromatographic–mass spectrometric method at a commercial laboratory [42]. Creatinine was used to normalize urinary TCP.

2.3. Test procedures

Test sessions were run on both Saturday and Sunday from May through June in 1998. Reimbursement for complete participation was US$250. All participants were instructed not to drink any alcohol-containing beverages 12 h prior to testing. An alcohol saliva test (Q.E.D.-A150, STC Technologies) was administered prior to testing to confirm abstinence. Five individuals with a blood alcohol concentration greater than 0.03% were not tested and asked to return at a later time. Participants reported to the test site for either a morning test session or an afternoon test session. Sessions lasted 4–6 h. All tests were administered using duplicate test stations and the test administrators were blind to the exposure status of the participants. The study protocol was approved by the NIOSH Human Subjects Review Board and informed consent was obtained from all participants.

Participants also underwent a limited neurological examination that evaluated selected cranial nerves, speech, resting and postural tremor, coordination and strength, sensation (e.g., touch, pain, joint position, vibration), reflexes, station, and gait. No participant was identified by a neurologist as severely impaired or had an abnormality, which would have required removal from the entire study, or a particular test for analysis purposes.

The tests described below were recommended by an NIOSH-sponsored advisory panel to be appropriate tests for detecting both acute and chronic sensory and motor effects from pesticide exposures [16]. The test battery was developed with two purposes: (1) serve as supplemental tests to the more frequently used, but cognitively oriented, automated test batteries like the Neurobehavioral Evaluation System (NES-2); and (2) provide quantitative measures of sensory and motor domains that would be complementary to the standard neurological exam, thereby increasing the possibility of detecting subclinical effects. The selection was based on Refs. [4,16,17,50,58], and previous reports of effects from pesticides and other chemicals on the nervous system [7,8,12,13,15,25,26,29,34,37,38,44–46,48].

2.4. Sensory and motor tests

2.4.1. Sensory functions

2.4.1.1. Olfaction (Cross-Cultural Smell Identification Test). The Cross-Cultural Smell Identification Test [22] consists of 12 micro-encapsulated odorants positioned on brown strips on the bottom of each test page. Participants scratch and sniff the odorant and mark their choice from a set of four alternative odors. Both the number of correct responses and percentile scores were used for analysis.

2.4.1.2. Vision (visual acuity and Functional Acuity Contrast Sensitivity Test — FACT). The visual acuity and contrast sensitivity tests were administered using the OPTEC 1000 (Stereo Optical). Visual acuity (e.g., letters) testing preceded the contrast sensitivity test to document either normal or corrected distance vision and was scored on an eight-point scale from 0 (*20/200*) to 7 (*20/20*). The FACT presents a series of black and white bars in a 1.7° circular patch arranged into eight rows and five columns. Each column represents one of the five spatial frequencies (e.g., 1.5, 3, 6, 12, 18 cycles per degree) lettered A through E and the rows, numbered 2 through 8, present a degree of difficulty (grating patterns decreasing in contrast at 0.15 log units/patch). Reading across each row from left to right, the participants are asked to indicate whether the lines point to the right (+15°), left (−15°), or straight up (0°). Scoring is done by recording the last patch correctly identified by column and row, which produces a visuo-gram that indicates sensitivity at each of the five spatial frequencies tested [30]. The visuo-gram is scaled and produces both a contrast sensitivity score and a percent contrast score. The contrast sensitivity score and the visual acuity score were used for analysis.

2.4.1.3. Color vision (Farnsworth D-15 and Lanthony D-15d). The tests selected are hue discrimination or arrangement tests because the observer arranges colored caps in a natural color order. Both tests are extracted from the 85 color cap Farnsworth–Munsell 100 Hue [29] system. These smaller tests (e.g., 15 caps) are more suitable for field testing.

The tests are administered monocularly on a small table covered with black cloth. A desktop fluorescent lamp with

two F15T8/D bulbs (daylight) adjusted 16 in. above the caps provided the standard light source. Participants wore an eye patch over their left eye and were tested for the right eye on the Farnsworth D-15 followed by the Lanthony D-15d. The patch was then placed on the right eye and the test repeated for the left eye. In addition to the standard hue-circle scoring method for each test, which was used to identify congenital color weakness, the Bowman [9] scoring method was used to produce a total color distance score (e.g., perfect score is minimum total distance with caps in correct order) for each eye on both the Farnsworth D-15 and Lanthony D-15d. These scores were used for analysis purposes.

2.4.1.4. Cutaneous sensation–vibrotactile (vibrometry system). An automated measurement device, the vibrometry system (Brüel and Kjær 9627), was used to measure peripheral sensory nerve function. Vibrotactile thresholds are expressed as decibels ranging from 70 to 160 dB (re. 10^{-6} m/s^2). Both the nondominant middle finger and great toe were tested at two frequencies (31.5 and 125 Hz) preceded by two practice trials. Participants wore headphones to mask potential high-frequency noise and placed their forearm on a resting device with the middle finger curled and touching the top of the test post. When vibration was detected, participants depressed a button held in their nontested hand. When vibration was not detected, the button was released. After the finger was tested the toe was tested. Threshold sensitivities were determined using the Békésy method over several trials and the software calculates a mean threshold level and standard deviation. The mean was used for analysis purposes.

2.4.2. Motor functions

2.4.2.1. Tremor (arm–hand tremor test). An NIOSH-developed [27] automated test device was used to test arm–hand tremor. Test procedures and device characteristics are described in a previous publication [19]. Amplitude (e.g., total power, m/s^2) was calculated for both horizontal and vertical movements for 1-min periods during the 3-min test. Measures retained for analysis were: (1) total power, 1–18 Hz; (2) total power, 1–6 Hz; (3) total power, 7–12 Hz; and (4) power at peak frequency, 7–12 Hz.

2.4.2.2. Manual dexterity(grooved peg board). The grooved pegboard test evaluates visual, tactile, and kinesthetic motor control systems. The test consists of a small board containing a 5×5 set of slotted holes angled in different directions. Each peg has a ridge along one side, so that the peg has to be rotated in position for correct insertion. Participants were seated in front of the pegboard and instructed to insert 25 pegs into the 25 holes as fast as they could starting with their dominant hand first. The time in seconds to complete the 25 insertions was recorded. After a short rest break, the nondominant hand was tested. A single composite score consisting of the sum to the time to complete the test, the number of drops, and the number of complete insertions for each hand was calculated and used for analysis.

2.4.2.3. Eye–hand coordination (trail making test). The trail making test is a visuomotor tracking task that evaluates speed, visual scanning, and visual conception. The test consists of two parts, Form A and Form B. Form A consists of 8 consecutively numbered circles on one side of a sheet and 25 consecutively numbered circles on the other side. Form B consists of 8 circles, 4 consecutively numbered and 4 consecutively lettered on one side of the sheet and 25 circles, 13 consecutively numbered (1–13) and 12 consecutively lettered (A–L) on the other side of the sheet. The participants are instructed to use a pencil to connect consecutively numbered circles on Form A. Following completion of Form A, Form B is administered. In Form B, participants alternately connect (1–A–2–B–3–C and so on) numbered circles and lettered circles starting with the practice trial first. Each test is timed and the number of seconds required to correctly connect the circles was the measure used for analysis.

2.4.2.4. Postural stability (sway). Testing was performed on a microcomputer-controlled force platform using protocols established by the NIOSH [18,20,59] and the University of Cincinnati [6,7]. Test conditions are designed to test the three main afferents (e.g., vision, proprioception, and vestibular) responsible for maintaining postural stability, and are defined below. Six test conditions (e.g., four standing on two legs and two on one leg) each lasting 30 s and preceded by one practice trial were used. Participants were instructed to remove their shoes and stand still on the platform, with arms at their sides focusing on a cross on a wall for the two leg conditions. Testing was repeated with eyes closed, and standing on 4-in. thick foam pads, both with eyes open and eyes closed. Single leg testing required participants to stand still on one leg; eyes were open and focusing on the cross was not required. Two measures, sway area and sway length, were used for analysis. Sway area represents the area within the sway path (cm^2), and sway length is the length of the sway path (cm).

2.5. Statistical analysis

Thirty-nine measurements (e.g., mean scores) of the motor and sensory domains are reported in this study. To maximize the potential for detecting a dose–response relationship, all the tests selected produced continuous measures. Nonautomated tests, such as the vision tests, smell test, peg board, and Trails A and B have been traditionally scored using clinical norms, but the frequency of abnormal scores is usually so low on nonsymptomatic populations that the norms are not useful for dose–response analysis. Fortunately, scoring methods have been developed to provide continuous measures for the vision and smell

tests. Tests that are timed, such as the pegboard and Trails A and B tests, also provide useful quantitative measures. All the automated tests (e.g., vibrotactile, postural sway, and tremor) have software that calculates quantitative measurements. Procedures and methodology for the automated tests are available in Refs. [19,20,39]. Scoring methodology for tests not detailed in previous publications is described below.

In the contrast sensitivity test, the last correctly identified patch score (Section 2.4.1.2) for each column and row was converted to a contrast sensitivity value using an SAS program developed by Geller (see acknowledgements). Contrast sensitivity scores are on a log scale ranging from 2 to 300. Color vision tests were initially hand-scored to identify participants with congenital color weakness, which were removed from the color vision analysis. The color vision tests were also scored using the Bowman [9] method, which quantifies the perceived color differences between pairs of caps [29]. A computerized scoring method developed by the NIOSH was used to produce color difference scores for each eye. For the smell test, the number of correctly identified odors was converted to percentile scores using a table of norms based on gender and age. Both the correct number and the percentile scores were used in the analysis.

Some participants had difficulty completing the full 30-s postural stability test when standing on one leg. Using procedures described in Dick et al. [20], new length measures were calculated and the area measures for these participants were dropped. Equipment problems with one of the tremor devices on one test weekend resulted in the loss of tremor data from nine participants and improper test instructions early in the study resulted in the testing of several participants still wearing their shoes. Preliminary analysis showed that wearing shoes had some effect while standing on one leg and was included as a condition in the final models.

The main independent variable was the urinary 3,5,6-TCP concentration normalized for creatinine. One participant had a concentration approximately 30 times greater than the mean for the entire group and four times greater than the next highest value. Repeat analysis by Pacific Toxicology and follow-up contact by Battelle Centers for Public Health Research and Evaluation, the testing subcontractor, confirmed the possibility of the high exposure, so the data were also analyzed with this participant's value removed.

Correlation analysis was used to identify significant covariates for each of the sensory and motor tests. Covariates that were considered included age, mass, height, mass/height ratio, gender, ethnicity, highest grade completed, tobacco use greater than 6 months, cigarette use greater than 6 months, use of alcoholic beverages, diabetes, high blood pressure, depression, neck or back disorder, neurologic problems, serious injury to nervous system, and carpal tunnel syndrome. Covariates were included in a final model if they were consistently related to test outcomes across condition. For certain tests, variables relevant to a specific measurement outcome (e.g., color weakness, visual acuity) were included in the models.

Linear models were used as final models to test for exposure effects, estimate slope coefficients, and covariate effects. The method of estimation was always restricted maximum likelihood, and in the case of repeated measures, an unstructured covariance matrix was used. The predetermined alpha level for significance was $P \leq .05$. We also calculated an adjusted alpha level using a step-down Bonferroni method [33]. The adjusted levels were based on the number of hypothesis done for a particular test using the final 39 measurements reported. All analysis were performed using SAS Version 6.12 [47].

To evaluate the relative sensitivity of each measurement in the test battery to detect effects within the dose–response range of the study group, a minimum absolute detectable difference (MADD) was calculated using the formula:

$$\text{MADD} = |\beta| = \sigma_\beta(Z_{1-\alpha/2} + Z_{\text{Power}})$$

β is the slope and σ_β is the standard error of the estimate of the slope [49]. The value represents the effect size that is detectable using the sample size of this study at a power of 80%. The MADD values, however, do not represent an "absolute" threshold because it would be possible to detect a significant slope value below the MADD, but at less power.

3. Results

Questionnaire data to confirm the last day of termite work in the past week was complete for 105 out of 106 applicators. Of the 65 respondents indicating recent termite work, 70% ($n = 46$) reported their last day of termite work as Thursday (12.3%), Friday (55.4%), or Saturday (3.1%). The remaining 30% reported Monday (4.6%), Tuesday (18.5%), or Wednesday (6.2%). Forty applicators reported no termite work the past week, but examination of the urinary TCP concentrations revealed that 18 of these 40 applicators had TCP concentrations greater than 20.0 μg/g creatinine, which is above the 99th percentile value (16 μg/g creatinine) reported by Hill et al. [32] from a US adult population. We determined that 20.0 μg/g creatinine was a reasonable cutoff to indicate recent exposure. Seventeen of these 18 applicators who did not do any termite work in the past week reported doing nontermite pest control work and using chlorpyrifos-containing termiticides in the past year. The remaining applicator reported only "other work." Although a specific question relating to use of chlorpyrifos-containing products in nontermite work was not asked, apparently exposures to chlorpyrifos-containing products occurred in nontermite pest control work for some of the applicators.

In addition, 14 of the 106 applicators did not identify a specific chlorpyrifos-containing product that they were

currently using "most often" in the past week, either answering "other," ($n=9$) or leaving the choice blank ($n=5$). Within this grouping, 3 of the 14 applicators had TCP values >20.0 μg/g creatinine, which indicated probable recent exposure to chlorpyrifos or possibly to other compounds which are metabolized to TCP, such as, chlorpyrifos methyl or triclopyr. Exposures to the latter two products were unlikely because they are not commonly used by termiticide workers.

Further examination of the questionnaire from the nine applicators who indicated "other" and the five individuals who left the question "blank," was undertaken to try and identify a termiticide product containing chlorpyrifos or a potential confounding termite product exposure. We were able to verify from nine workers that there was: (1) a chlorpyrifos-containing product used; or (2) no termiticide products used in the past week; or (3) use of a pyrethroid- or imidacloprid-containing product. Pyrethroid compounds have low mammalian toxicity [23], and do not cause accumulation of acetycholine at receptor sites so none of these individuals were removed. No further information could be obtained from the questionnaire by five individuals who left both questions blank, but because they were recruited for using chlorpyrifos-containing compounds, they were also not removed. One worker identified a termite product containing imidacloprid, a nonorganophosphorous nicotinergic compound that causes accumulation of acetycholine at receptor sites. This worker was the only individ-

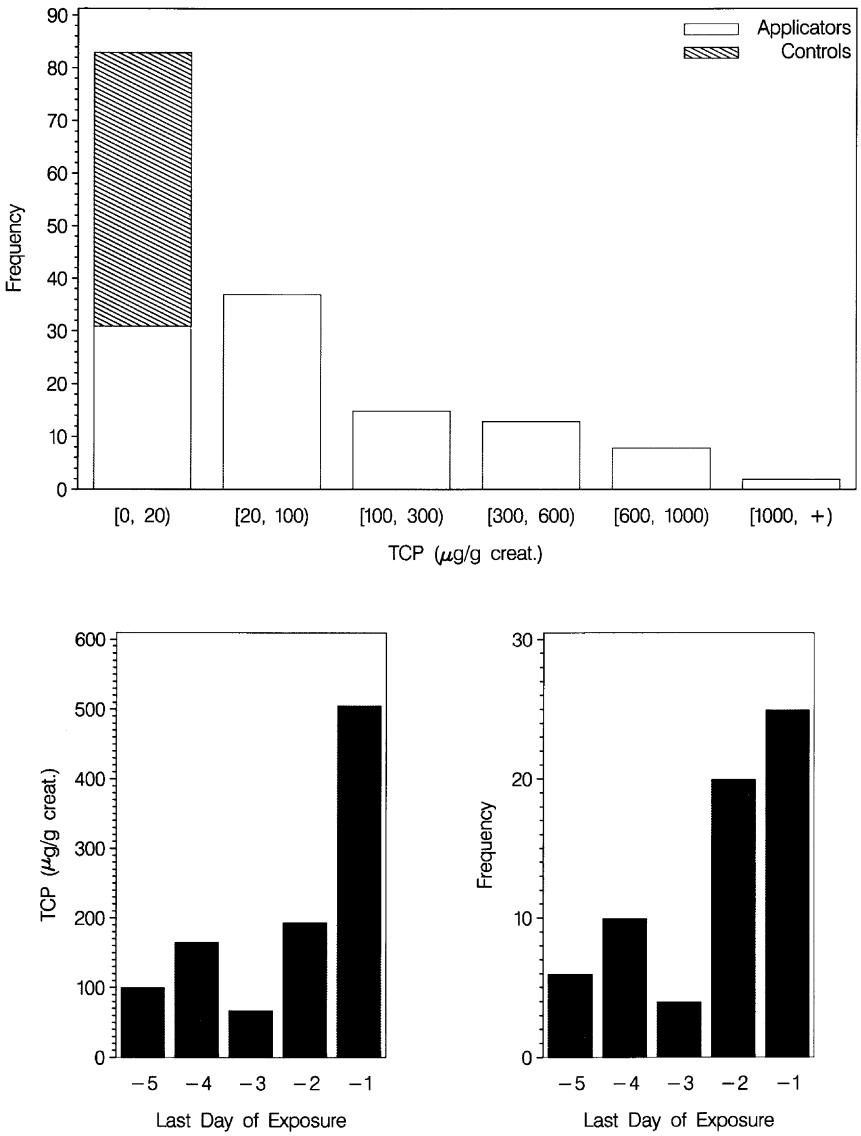

Fig. 1. The top graph shows the urinary TCP (μg/g creatinine) ranges by frequency for all 158 participants; the lower left graph shows the mean urinary TCP (μg/g creatinine) concentrations by day(s) of exposure prior to testing for the 65 participants who indicated recent termite work; and the lower right graph the frequency of last day(s) of exposure prior to testing for the same participants. Square bracket indicates number included; parenthesis indicates number not included.

ual removed from analysis because imidacloprid could mimic the effects of chlorpyrifos on the nervous system.

Fig. 1 graphically illustrates the exposure characteristics of the study group. The top graph shows the urinary TCP ranges by frequency for all 158 participants, the lower left graph the mean urinary TCP concentrations by last day of exposure prior to testing for the 65 participants who indicated recent termite work and the lower right graph the frequency of last day of exposure for the same participants. The last day of exposure has been plotted as 1–5 days prior to testing depending on the participants Saturday or Sunday test day. Table 1 presents the demographic characteristics of the study group, the mean and range of the TCP values, and the percent values of the group for the study covariates. The mean TCP value for the entire study group ($n=158$) was 135 μg/g creatinine, (109 μg/g creatinine with a high participant removed) with a range from 1.1 (i.e., limit of detection) to 4290 μg/g creatinine. The next highest urine value to the maximum value was 1040 μg/g creatinine. Seven values were over 600 μg/g creatinine and the mean for the 106 applicators was 200 μg/g creatinine. The mean for the control participants was 3.3 μg/g creatinine and all controls were <20 μg/g creatinine. Thirty-nine values, including four applicators, were below 3.1 μg/g creatinine, a mean reference range value for adult men and women in the US [32]. The average age of the participants was 39.1, and the most frequent grade completed was equivalent to high school.

Table 2 provides a summary of the data and model results for the measurements used in the study. Listed in the table are the number of participants with useable data for each test measurement, the means, standard deviations, the urinary TCP effect parameter estimates for the tests and measures, and the P values. Also included in Table 2 are the MADD values. Covariates that were used in the final models for each test are listed in the Table 2 key.

The only statistically significant effects related to urinary TCP occurred on one of the color vision tests, one of the contrast sensitivity tests and the postural sway tests. The Lanthony D-15d slope estimates for both the left eye ($b=0.0160$, S.E. $=0.0048$, $t=3.32$, $P=.0011$) and right eye ($b=0.0090$, S.E. $=0.0044$, $t=2.06$, $P=.0410$) showed a significant urinary TCP effect with all participants included. Only the left-eye score ($P=.004$) remained significant with the step-down Bonferroni correction. When the one participant with high TCP concentration was removed, the left-eye slope estimate was not significant (left eye — $b=0.0013$, S.E. $=0.0096$, $P=.8923$). Similarly, the contrast sensitivity slope estimate for left eye–Patch B ($b=-0.0185$, S.E. $=0.0084$, $t=-2.20$, $P=.0297$) showed a significant effect with urinary TCP with all participants but was not significant with the Bonferroni correction

Table 1
Characteristics of the study group

Study variable	*N*	Mean, %	S.D.	Minimum	Maximum
Urinary TCP[a]					
All participants	158	135 μg/g creatinine	384.4	1.1 μg/g creatinine	4290 μg/g creatinine
Applicators	106	200 μg/g creatinine	456.1	1.4 μg/g creatinine	4290 μg/g creatinine
Controls	52	3.3 μg/g creatinine	2.5	1.1 μg/g creatinine	16.1 μg/g creatinine
Age	158	39.1 years	9.0	19.8 years	64.7 years
Grade completed[b]	158	3.9	1.4	2	7
Gender	158	98.1% male		3 females	155 males
Race[c]	106	18.7% nonwhite			
Use of tobacco products 6 months or longer	158	68.9%			
Smoke cigarettes 6 months or longer	158	62.0%			
Ever drink alcohol containing beverages	158	94.3%			
Has a doctor ever told you that you have:					
Diabetes	158	1.8%			
High blood pressure	157	10.1%			
Depression	158	5.1%			
Neck or back disorder	158	20.3%			
A nerve problem or neurologic disorder	158	3.8%			
Serious injury to nerves of arms or legs	158	4.4%			
Carpal tunnel syndrome	158	3.2%			

[a] TCP values are reported three significant figures, one right of the decimal.

[b] Grade: 1 = < 8 years; 2 = 8–11 years; 3 = 12 years or completed high school; 4 = posthigh school training; 5 = some college; 6 = college graduate; 7 = postgraduate.

[c] Race not reported by 52 participants.

Table 2
Means, standard deviations, chlorpyrifos urinary TCP parameter estimates, *P* values, detectible difference estimates, and model covariates for the motor and sensory test measures

Test/measurement	*N*[a]	*M*[b]	S.D.[c]	TCP[d]	*P*	Step *P*[e]	MADD[f]	TCPW[g]	*P*	Step *P*	MADDW[h]
Visual acuity[i]	156	6.1	1.3	−0.0001	.662	(0) .662	0.0007	−0.0004	.471	(0) .471	0.0015
Visual contrast sensitivity[j]											
Left eye–Patch A	156	62.9	32.0	−0.0105	.102	(10) .816	0.0179	−0.0184	.157	(10) 1.000	0.0362
Left eye–Patch B	156	101.0	42.3	−0.0185	**.030**	.300	0.0236	−0.0204	.233	1.000	0.0479
Left eye–Patch C	156	122.5	54.6	−0.0005	.958	1.000	0.0275	−0.0088	.657	1.000	0.0559
Left eye–Patch D	156	57.5	37.8	0.0039	.573	1.000	0.0193	−0.0105	.451	1.000	0.0390
Left eye–Patch E	156	19.8	15.4	0.0008	.788	1.000	0.0084	0.0042	.490	1.000	0.0170
Right eye–Patch A	156	56.5	29.4	−0.0062	.304	1.000	0.0169	−0.0134	.276	1.000	0.0343
Right eye–Patch B	156	98.8	43.0	0.0072	.410	1.000	0.0244	−0.0143	.418	1.000	0.0492
Right eye–Patch C	156	123.7	48.7	−0.0150	.090	.810	0.0246	−0.0069	.697	1.000	0.0499
Right eye–Patch D	156	63.5	37.1	0.0095	.155	1.000	0.0186	−0.0086	.522	1.000	0.0375
Right eye–Patch E	156	25.9	16.6	−0.0027	.364	1.000	0.0088	−0.0025	.676	1.000	0.0179
Grooved pegboard[k]											
Dominant hand	157	104.5	20.7	0.0059	.144	(2) .288	0.0113	0.0072	.376	(2) .752	0.0226
Nondominant hand	157	110.6	28.5	0.0032	.590	.590	0.0164	−0.0038	.748	.752	0.0328
Trail making test[l]											
Trails A	157	34.3	11.3	0.0017	.428	(2) .856	0.0061	0.0069	.111	(2) .122	0.0121
Trails B	157	99.1	88.4	0.0092	.590	.856	0.0477	0.0637	.061	.122	0.0945
Vibrotactile[m]											
Finger — 31.5 Hz	157	106.7	8.7	0.0014	.444	(4) 1.000	0.0050	−0.0012	.740	(4) 1.000	0.0101
Finger — 125 Hz	157	105.3	10.2	0.0005	.788	1.000	0.0057	0.0032	.429	1.000	0.0113
Toe — 31.5 Hz	157	112.9	8.9	0.0014	.422	1.000	0.0047	0.0007	.830	1.000	0.0094
Toe — 125 Hz	157	116.5	12.6	−0.0001	.957	1.000	0.0064	−0.0015	.745	1.000	0.0130
Color vision[n]											
Farnsworth — left eye	152[o]	136.7	38.1	0.0047	.575	(4) 1.000	0.0234	−0.0159	.342	(4) 1.000	0.0467
Farnsworth — right eye	151	136.6	37.6	−0.0044	.583	1.000	0.0226	−0.0199	.221	.884	0.0453
Lanthony — left eye	152	83.5	24.3	0.0160	**.001**	**.004**	0.0135	0.0013	.892	1.000	0.0269
Lanthony — right eye	151	79.1	21.2	0.0091	**.041**	.164	0.0123	−0.0067	.440	1.000	0.0244
Olfactory Test[p]											
Number correct	157	10.7	1.3	0.0001	.778	(2) 1.000	0.0007	−0.0006	.284	(2) .344	0.0148
Percentile	157	54.1	32.3	0.0033	.630	1.000	0.0189	−0.0183	.172	.344	0.0375
Postural sway[q]											
HEO-Length	157	34.1	9.2	0.0022	.223	(12) 1.000	0.0051	0.0029	.425	(12) 1.000	0.0102
HEC-Length	157	50.7	18.6	0.0122	**.000**	**.000**	0.0098	0.0117	.095	.855	0.0196
SEO-Length	157	46.0	12.1	0.0051	**.032**	.288	0.0066	0.0049	.297	1.000	0.0132
SEC-Length	156	81.8	27.4	0.0201	**.000**	**.000**	0.0139	0.0204	**.041**	.451	0.0277
LL-Length	155	139.4	41.3	0.0082	.310	1.000	0.0225	−0.0017	.916	1.000	0.0452
RL-Length	154	138.0	44.1	0.0116	.161	.966	0.0231	0.0096	.564	1.000	0.0466
HEO-Area	157	2.6	1.6	0.0002	.572	1.000	0.0009	0.0006	.358	1.000	0.0018
HEC-Area	157	4.2	2.7	0.0011	**.037**	.296	0.0015	0.0020	.062	.620	0.0030
SEO-Area	157	3.7	2.8	0.0005	.396	1.000	0.0016	0.0009	.445	1.000	0.0033
SEC-Area	157	8.5	4.7	0.0035	**.000**	**.000**	0.0025	0.0067	**.000**	**.000**	0.0049
LL-Area	150	9.0	4.3	0.0005	.544	1.000	0.0025	0.0020	.268	1.000	0.0050
RL-Area	140	8.7	4.4	0.0015	.105	.735	0.0025	0.0029	.127	1.000	0.0053
Tremor[r]											
Horizontal 1–18 Hz	148	57.3	5.8	0.0007	.526	(2) 1.000	0.0030	0.0017	.456	(2) .912	0.0064
Vertical 1–18 Hz	148	56.1	4.6	0.0000	.999	.000	0.0026	0.0006	.774	.912	0.0056

(P=.300). Several measures of postural sway showed significant urinary TCP effects, especially in the eyes closed and soft (i.e., foam) conditions. Significant length measurement slopes were found with the hard surface–eyes closed (b=0.0122, S.E.=0.0035, t=3.49, P=.0006), soft surface–eyes open (b=0.0051, S.E.=0.0024, t=2.16, P=.0320) and soft surface–eyes closed (b=0.0202, S.E.=0.0050, t=4.06, P=.0001). For the area measures, significant slopes

occurred in the hard surface–eyes closed ($b = 0.0011$, S.E. = 0.0005, $t = 2.10$, P=.0372) and the soft surface–eyes closed ($b = 0.0035$, S.E. = 0.0009, $t = 3.98$, P=.0001) conditions. After applying the Bonferroni correction, the hard surface–eyes closed length (P=.000), soft surface–eyes closed length (P=.000), and soft surface–eyes closed area (P=.000) measurements were still significant. With the one high TCP concentration value removed, significant slope estimates remained for both the soft surface–eyes closed length measurement ($b = 0.0204$, S.E. = 0.0099, $t = 2.06$, P=.0410) and area measurement ($b = 0.0067$, S.E. = 0.0017, $t = 3.87$, P=.0002). Only the area measurement (P=.000) remained significant after the Bonferroni correction. The hard surface–eyes closed estimates for length ($b = 0.0117$, S.E. = 0.0070, $t = 1.68$, P=.0948) and area ($b = 0.0020$, S.E. = 0.0011, $t = 1.90$, P=.0617) were not significant at P>.05 even before the Bonferroni correction.

4. Discussion

Several tests of sensory and motor functions were administered to a subgroup of termiticide workers and controls whose urinary TCP concentrations were measured on the day of testing. To construct a dose–response curve for the detection of primarily acute effects, the subgroup consisted of control participants not exposed to chlorpyrifos, currently employed workers who indicated within the past week, in either termite or nontermite work, using a chlorpyrifos-containing product, and workers either currently or formerly employed who had not used a chlorpyrifos-containing product in the past week. Urinary TCP concentrations for the subgroup ranged from 1.1 to 4290 μg/g creatinine. The maximum concentration (one worker) was approximately four times greater than the next highest concentration (1046 μg/g creatinine), which required the data to be analyzed a second time with the high exposed worker removed. Overall, the TCP concentrations were below concentrations where experimental dosage data in humans has shown evidence of plasma cholinesterase (ChE) inhibition. Using data from previous reports [14,41,60], we calculated that a urinary TCP concentration of 461 μg/g creatinine would approximate an intake dosage equivalent to 0.03 mg/kg/day of chlorpyrifos. This concentration (0.03 mg/kg/day) represents the No Observable Adverse Effects Level (NOAEL) for plasma ChE inhibition [60]. Twelve participants had urinary TCP concentrations (>461) above the estimated NOEL on the day of their testing.

Although the intent of the recruitment efforts was to find applicators currently using chlorpyrifos-containing termiticides for the full week prior to testing, these efforts were not 100% successful. In general, participants with the highest values indicated their last termite treatment work was 1–2 days prior to testing. Several participants indicated a last day of exposure greater than 3 days prior to testing. There were, however, concentrations greater than 100 μg/g creatinine from some of these latter participants and also from some participants who did not indicate a current termite treatment work exposure during the previous week. While these values may be reflective of excretion rates for chlorpyrifos upon cessation of exposure, examination of the questionnaire results also revealed that there was a possibility of exposure occurring in nontermite pest control work

Notes to Table 2

[a] Number of participants with useable data for the analysis; TCP study population.

[b] Mean value for the measurement; TCP study population.

[c] Standard deviation; TCP study population.

[d] Parameter estimates (e.g., slopes) for exposure effect using the urinary TCP (e.g., 3,5,6-TCP normalized for creatinine) for all participants. Expected direction for all estimates is positive except visual acuity and contrast sensitivity, which are negative.

[e] Corrected P values using a step-down Bonferroni method. The number of statistical hypotheses used in the correction is listed in parenthesis with the first measurement for each test.

[f] Minimum Absolute Detectable Difference [MADD] (e.g., ±) at power = 0.80.

[g] Parameter estimate for urinary TCP effect without one participant with a high value (TPCW).

[h] MADD (e.g., ±) (only MADDW) at power = 0.80 without one participant with a high value.

[i] Visual acuity scores were on a scale of 1–7, 7 = 20/20, 6 = 20/30, and so on to 20/200. Age was a covariate in final model but N.S.

[j] Each patch lettered A through E represents a different spatial frequency; the contrast score is a log value (see text). Spatial frequency and visual acuity were significant covariates.

[k] Score represents the sum of the time (s) to complete test, number of pegs inserted, and number dropped. Age, grade completed, and neurologic disorder were used in final model; only age was significant.

[l] Score represents the time (s) to complete the test. Age and grade completed were N.S. in the final model.

[m] Measurement units are expressed as intensity (dB) at each frequency (Hz) tested. Age significant in the final model.

[n] Scores for the Farnsworth D-15d and Lanthony D-15d were calculated using the Bowman [9] method. Age and color weakness used as covariates, but age was only significant in the model for the Lanthony D-15d test.

[o] The N for the color tests is reduced; participants indicating congenital "color weakness" were removed.

[p] Number of correct odor items identified out of 12 items presented. Age was a significant covariate for number correct. Percentile scores are age-adjusted.

[q] HEO = Hard surface–eyes open; HEC = Hard surface–eyes closed; SEO = Soft surface–eyes open; SEC = Soft surface–eyes close; LL = Standing on left leg; RL standing on right leg. Length measurement units are in centimeters (cm) and area measurement units are in square centimeters (cm^2). Age, height, and mass were significant covariates, but not all covariates were significant for each measure. Age was the most consistent (nine conditions), followed by height (five conditions). Mass was significant on three conditions.

[r] Measurement units are log power values (m/s^2) for the amplitude of tremor in the second minute only, of the 3-min test session.

performed by these participants where chlorpyrifos-containing products may have been used.

The questionnaire data were only partially useful in establishing a chlorpyrifos-containing product as the "most used" termiticide in some of the 14 participants who had indicated "other products" or left questions "blank." In summary, of the 106 applicators recruited for the study, the use of chlorpyrifos-containing termiticides within the last week or within the last year as "most used" was established for 96 applicators. One individual was eliminated from data analysis because of the use of a non-OP compound that could have mimicked OP effects.

A large number of applicators ($n = 23$) indicated use of chlorpyrifos-containing termiticides at the time of recruitment, but did not use such products the week prior to testing. This inflated the number of applicators at the low end of the exposure range with urinary TCP concentrations below our cutoff indicator for recent exposure (i.e., urinary TCP concentrations < 20.0 μg/g creatinine). Future studies of this type should use a shorter time frame (e.g., 1 week) for final selection of participants for current exposure measurements than the 3 weeks used in this study, because of possible changes in applicator work practices.

Thirty-nine measurements from eight tests are reported from this study group. With such a large number of measurements subjected to statistical analysis, the possibility of a spurious result (e.g., Type I error) is raised requiring some correction method. The authors believe, however, that strict application of a correction method could lead to insufficient power (e.g., Type II error) to detect exposure effects and possibly overconservative for health effects research. Therefore, we have presented the data with unadjusted P values and adjusted P values, which will allow readers some latitude in interpreting the significance of the results.

The Lanthony color vision test, which is an indicator of possible acquired color vision loss, and the visual contrast sensitivity test showed a urinary TCP effect only when the one participant with the very high urinary TCP concentration was included in the analysis. The left-eye score value still remained significant with the Bonferroni correction. Subsequent analysis with the high value removed did not produce any statistically significant scores. Further research would be needed to confirm possible acute exposure effects on color vision and contrast sensitivity, given this possibly isolated result. Results from the measures taken with the postural sway test, however, were more consistent. More measures were affected, and some effects were still statistically significant after the Bonferroni correction. When the one high urinary concentration was removed, two measures were still significant and one remained after the Bonferroni correction.

There is still, however, a possibility that the results may have been influenced by outliers (e.g., high values) in the distribution of the urinary TCP values. Using regression analysis, we calculated Cook's distance values [55] to determine the influence of individual TCP values on the slopes for each measurement. Only the highest TCP value had a Cook's distance value greater than 1 on any measurements (e.g., postural sway, color vision, smell, Trails A + B), indicating a high influence on the slopes. Because the data are reported both with and without this high TCP value, concerns about the influence of outliers is reduced.

Maintaining postural balance is a dynamic process that demands a complex interaction of peripheral and central nervous system components, specifically, contributions from three major afferent sources: (1) proprioceptive and kinesthetic, (2) vestibular, and (3) visual [21]. The postural sway test conditions used in this study were designed to manipulate input from the various afferents. The hard surface–eyes open condition followed by the hard surface–eyes closed condition removes the visual system, but maintains vestibular and proprioceptive input. The soft surface (i.e., foam)–eyes open condition modifies proprioceptive feedback and the eyes closed condition then removes the visual system, leaving only the vestibular afferents intact. In the present study, the primary urinary TCP effects occurred in the eyes closed conditions, both on the hard surface and the soft surface. This implicates possible effects on both the proprioceptive and vestibular systems.

One of the principle sites of action for organophosphorous compounds is at the neuromuscular junction on the nicotinic receptors affecting the somatic motor nerve fibers. Acetylcholine is the primary neurotransmitter affected. These nerve fibers innervate skeletal muscles, and one of the symptoms of OP compound toxicity is muscle weakness [23]. Acetycholine has also been identified as a neurotransmitter for efferent control in the vestibular labyrinth [31,35]. Japanese researchers [62] have recently reported possible vestibular effects using postural sway analysis in victims of the Tokyo Subway Sarin Poisoning in Japan. Sarin is a potent anticholinesterase OP compound. Effects on the vestibular system from OP pesticides in common use, such as chlorpyrifos, however, have not been reported in the literature.

The strongest possibility for an explanation of the effects from chlorpyrifos detected in this study may be on the chemical neurotransmitters that are important in proper peripheral and central nervous system control of postural sway. However, because of the small number of participants with urinary TCP concentrations indicative of exposures high enough to produce cholinesterase inhibition, such effects are subclinical. The effects on postural stability measured in this study population on postural sway are also primarily acute. Evidence in this study population of any long-term or chronic effects on sway when participants were analyzed by exposure (exposed vs. control) was limited [52]. Only one measure (standing on one leg-area) was statistically significant. There were, however, some significant associations with eight participants reporting past poisoning with chlorpyrifos and in formerly exposed work-

ers [52] on some of the postural sway measures. The lack of relationship between acute effects and chronic effects with OP compounds has been reported in a previous study [54].

A study conducted by Sack et al. [45] found an association with exposure in pesticide-exposed workers both on measures of long-term exposure and recent exposure (e.g., previous year) with postural sway. Significant associations were strongest with the OP compounds. Similar to the findings in this study, the strongest effects were found with the eyes closed and soft surface (e.g., foam) conditions implicating the proprioceptive and vestibular responses.

As mentioned in the Introduction, a significant reason for the recruitment of this study population was to evaluate the sensitivity of the sensory and motor test measures to detect effects from pesticide exposures. We chose an indicator of effect size, the MADD value, for this evaluation. For the most part, the uncorrected statistically significant TCP slopes are greater than the corresponding MADD value, but there are some exceptions (Lanthony right eye, sway-SEO length, and HEC area), because it is possible to have a significant slope value less than the MADD value for the same measurement but at less power. With slopes that were not significantly different from zero, the MADD values were 1.64 to 64 times greater than the absolute value of the estimated TCP slope. Assuming that there are relationships between exposure and measurement to detect, this indicates that a larger sample size, or exposure effect, or a combination of both is needed to detect significant relationships. Additionally, these relationships apply only to compounds such as chlorpyrifos that metabolize to TCP.

Interpretive comparisons of the MADD values are restricted to measurements with the same units. This would limit most comparisons to the multiple measures for the same test, such as the sway length measures (cm), the sway area measures (cm^2), vibrotactile measures (dB), the contrast sensitivity measures which use the same scaled log score, and the tremor measures (m/s^2). For example, examining the six measures of sway length, the two measures taken when participants were standing on either their left or right leg have higher MADD values than the four other conditions tested with participants standing on both legs. Use of the single leg test conditions in future studies with chlorpyrifos may not be necessary for acute effects, because the two leg conditions are more sensitive.

Some tests probably have methodological problems that contributed to high MADD values. The TCP slopes for the grooved pegboard dominant hand measure (0.0059) and Trails B (0.0092) were in the expected direction but the MADD slopes required were greater (0.0113 and 0.0477). The contrast sensitivity test, which had a lot of measures, was similar. When there was some evidence of positive relationship from the TCP slope, the corresponding MADD value for that measure was much greater, except for the one significant measure (left eye–Patch B, uncorrected). The grooved pegboard, trails and contrast sensitivity tests are not automated and require a trained individual to both administer and score the tests. The grooved pegboard and trails tests are timed so there is a motivational component and the contrast sensitivity test requires standard lighting conditions for proper administration. Because these tests showed some response to exposure, they should still be considered for future studies, but with improved methodology.

In summary, the results of this study indicate limited acute effects from exposures to chlorpyrifos using urinary TCP as a measure of current exposure. The mean concentration for the study group, which included both exposed pesticide workers and nonexposed participants was 135 μg/g creatinine; the mean concentration for the applicators was 200 μg/g creatinine. The effects occurred primarily on measures of postural sway. Several other tests of motor and sensory functions did not show any evidence of urinary TCP effects. Evaluation of the sensitivity of the sensory and motor test measures to detect effects from pesticide exposures using an indicator of effect size, the MADD, indicated that for many measures either larger numbers of participants or a greater exposure effect was needed for a significant relationship.

Acknowledgments

The authors would like to thank within the U.S. Environmental Protection Agency, the Office of Pesticide Programs for providing significant funding, the National Health and Environmental Effects Research Laboratory for providing a site to conduct the study, and Dr. Andrew Geller for assistance in scoring the color vision and contrast sensitivity tests. Special thanks to NIOSH staff members Ebben Dowell, Karen Weyer, and Bill Ehling for their assistance in preparing the large data set for analysis and to Steven Brightwell and David Chrislip for monitoring test sessions. Special appreciation is also due to Ron Howell and Carl Falco of the State of North Carolina for recruitment of the participants and to Charles Knott and staff of Battelle Centers for Public Health Research and Evaluation, for the scheduling and testing of participants.

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