

Dose-Effect Analyses of Occupational Chlorpyrifos Exposure and Peripheral Nerve Electrophysiology

James W. Albers,^{*,1} David H. Garabrant,[†] Joel L. Mattsson,[‡] Carol J. Burns,[§] Sarah S. Cohen,[¶] Cami Sima,^{||}
Richard P. Garrison,[†] Rudy J. Richardson,[†] and Stanley Berent^{|||}

^{*}Department of Neurology, University of Michigan Health System, Ann Arbor, Michigan 48109-0032; [†]Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109-2028; [‡]Dow AgroSciences, Indianapolis, Indiana 46268; [§]The Dow Chemical Co., Midland, Michigan 48674; [¶]International Epidemiology Institute, Rockville, Maryland 20850; ^{||}Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, Michigan 48109-2028; ^{|||}Department of Psychiatry (Psychology and Neurobehavioral Toxicology Programs), University of Michigan Health System, Ann Arbor, Michigan 48109

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We performed nerve conduction studies (NCSs) on 113 chemical workers, many of whom had occupational exposure to the organophosphorus insecticide chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl]-phosphorothioate), to identify dose effects of subclinical neuropathy. In this masked longitudinal study, we estimated historic and interim chlorpyrifos exposures and measured excretion of 3,5,6 trichloro-2-pyridinol (TCP), a chlorpyrifos metabolite. TCP excretion among exposed workers suggested an estimated daily chlorpyrifos exposure averaging about 576–627 $\mu\text{g}/\text{day}$ and indicated levels approximately 30% (range 0–250%) of the internal dose received by a typical subject exposed during a working day at the threshold limit value of 200 $\mu\text{g}/\text{m}^3$. We modeled NCS results using linear mixed models with repeated measures. Although we found no consistent associations between interim chlorpyrifos exposure and NCS results, we identified several significant associations involving historic chlorpyrifos exposure. Most associations, however, reflected effects at low-exposure levels ($< 20 \text{ mg}/\text{m}^3 \times \text{days}$) without further effects as exposure increased over a 10-fold range (20–220 $\text{mg}/\text{m}^3 \times \text{days}$). This suggested small differences among subjects with low or no chlorpyrifos exposure, rather than a dose-related deterioration among subjects with higher exposures. Two NCS results demonstrating apparent subclinical adverse dose effects showed significant but unexplained interaction with education level. The overall results provide little support for the hypothesis that chronic chlorpyrifos exposures at levels in the range associated with appreciable inhibition of B-esterases produce adverse dose effects on peripheral nerve electrophysiology suggestive of subclinical neuropathy.

Key Words: Chlorpyrifos; insecticides; neurotoxicity; neuropathy; subclinical neuropathy; nerve conduction; peripheral nerve; electrophysiology.

Chlorpyrifos (O,O-diethyl-O-[3,5,6-trichloro-2-pyridyl]-phosphorothioate)-manufacturing workers are not a homogeneous population in terms of exposure. We previously reported that chlorpyrifos workers can be separated into exposure groups reflecting workers with negligible exposure, low-level exposure, or higher levels of exposure based on the urinary excretion of 3-5-6 trichloro-2-pyridinol (TCP), a metabolite of chlorpyrifos (Burns *et al.*, 2006). In addition, we performed masked neurological examinations and nerve conduction studies (NCSs) of selected peripheral nerves and measured plasma butyrylcholinesterase (BuChE) activity and red blood cell acetylcholinesterase (AChE) activity on the same chlorpyrifos-manufacturing workers and on randomly selected chemical workers without current chlorpyrifos exposure (Albers *et al.*, 2004). Thus, questions about the safety of chlorpyrifos in terms of the peripheral nervous system evaluations could be addressed in a unique subpopulation of chlorpyrifos-manufacturing workers who had, on average, many years of exposure to levels of chlorpyrifos sufficient to cause detectable inhibition of plasma BuChE.

The analyses we report were motivated by lingering concerns that chronic, low-level exposure to organophosphorus (OP) insecticides may produce adverse effects on the peripheral nervous system in the form of neuropathy. Within this broader issue, it remains uncertain if prolonged or repeated low-level exposures to chlorpyrifos that do not produce overt cholinergic signs cause neuropathy in humans (Lotti, 2002; Woods and the Working Group on Organophosphates, 1999). Our initial results showed that chronic chlorpyrifos exposure during the manufacturing process, which was sufficient to produce biological effects on plasma BuChE activity in some workers, was not associated with clinically evident or subclinical peripheral neuropathy, either at baseline or after 1 year of additional potential exposure (Albers *et al.*, 2004). In this final part of a series of observations on this selected group of workers, we extend the previous results, which were based on group comparisons of chlorpyrifos workers and referents, and

¹ To whom correspondence should be addressed at Department of Neurology, 1C325/0032 University Hospital, University of Michigan Health System, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109-0032. Fax: (734) 936-5185. E-mail: jwalbers@umich.edu.

describe analyses based on measures of chlorpyrifos exposure over the working lifetime of the subjects as continuous variables. These sensitive analyses permitted us to investigate subclinical dose-effect relations involving chlorpyrifos exposure based on linear mixed models of measures of peripheral nerve electrophysiology, after adjustment for potential confounders.

MATERIALS AND METHODS

The study design, subject description, exposure measures, and statistical analyses have been described previously (Albers *et al.*, 2004). A description of these four areas is presented as follows.

Study Design. In a prospective cohort study, we evaluated peripheral nervous system function of chemical workers at the Dow Chemical Company in Midland, Michigan, at baseline and 1 year later. The subjects included chlorpyrifos-manufacturing workers who had known and measurable exposure to chlorpyrifos and chemical workers involved in manufacturing Saran (clear plastic film-wrapping material) who had no current or recent occupational exposure to chlorpyrifos. The referents were chosen to control for potential factors associated with employment in the chemical industry and included to assure that examiners would not presume that all subjects had chlorpyrifos exposure. No subjects had occupational exposure to other OP insecticides or to other known or suspected neurotoxicants. The referents were studied concurrently with the chlorpyrifos subjects, and examiners were masked to group membership and exposure histories. The study was approved by the University of Michigan Institutional Review Board for Human Subject Research and the Dow Human Subject Review Board.

Subjects. Dow employees were eligible for participation if they were between 18 and 65 years of age, male or female, and had no physician-diagnosed condition that made them unable to complete the protocol. No subject was eliminated because of a physician-diagnosed condition. Although this was a healthy group of workers, we were inclusive in our selection process so that potentially vulnerable individuals were not excluded. All eligible chlorpyrifos-manufacturing workers and a random sample of Saran-manufacturing workers were asked to participate. All subjects provided written consent indicating their willingness to participate.

We evaluated 113 subjects, including 53 of 66 eligible chlorpyrifos workers (80%) and 60 of 74 randomly chosen Saran workers (81%). There were 28 female and 85 male subjects. Descriptive characteristics including age, anthropometric, and other data are shown in Table 1. At baseline, chlorpyrifos subjects and referents were comparable in terms of most factors important to the electrophysiological evaluations. The low frequency of existing medical problems was similar in the chlorpyrifos and referent groups, including the distribution of subjects with disorders that could contribute to neuropathy, such as diabetes mellitus (one subject with non-insulin-dependent diabetes in each group). Self-reports of alcohol use for the overall sample averaged one drink (one bottle of beer, four-ounce glass of wine, or 1.5 ounce shot) per day over averaged time intervals (age 15–17, 18–22, 23–30 years, and per decade thereafter). One-year examinations were performed on 111 of the initial 113 subjects (98%); no subject had been diagnosed with a new condition that could potentially contribute to neuropathy.

Exposure Assessment. Ambient chlorpyrifos exposure was estimated using air monitoring data and direct biological measurements. Industrial hygiene records were used to establish estimates of chlorpyrifos exposure during the year between the baseline and 1-year examinations (interim chlorpyrifos exposure) and chlorpyrifos exposure from the time of initial employment to the baseline examination (historic chlorpyrifos exposure). Historical personal air-sampling results of chlorpyrifos exposure were compiled for similarly exposed groups (SEGs) of workers. Geometric mean exposure levels were calculated for each SEG and used to estimate historic chlorpyrifos exposure. Interim and historic chlorpyrifos exposures were calculated by multiplying the exposure

TABLE 1
Descriptive Statistics for the Subject Population at Baseline

Variable	Chlorpyrifos (<i>n</i> = 53), mean ± SD	Referent (<i>n</i> = 60), mean ± SD	<i>p</i> value
Age (years)	41.2 ± 7.5	41.3 ± 8.4	0.92
Height (m)	1.74 ± 0.1	1.75 ± 0.1	0.94
Weight (kg)	88.1 ± 15.0	88.3 ± 20.3	0.96
BMI (kg/m ²)	29.0 ± 4.2	29.0 ± 6.0	0.99
Education level (years)	14.3 ± 2.1	13.6 ± 1.5	0.06
Estimated general ability ^a	106.2 ± 9.2	104.0 ± 9.1	0.21
Anxiety ^b	48.0 ± 11.8	47.8 ± 13.8	0.93
Alcohol use (drinks/day)	0.8 ± 0.9	1.3 ± 1.4	0.19
Smoking (pack-year)	8.2 ± 15.8	5.9 ± 9.5	0.37

Note. *p* value determined by *t*-test for difference in means (adjusted for unequal variance between groups when indicated) or Wilcoxon two-sample test.

^aBlair and Spreen (1989).

^bDerogatis and Spenser (1982).

estimate for each SEG by the number of days worked in the job and summing these products across jobs during the relevant intervals. Interim chlorpyrifos exposure also was assessed biologically by urine TCP, a metabolite of chlorpyrifos. The urine TCP level was reported as a weighted average of four overnight collections obtained during the year, expressed as interim TCP/creatinine (Cr) (μg/g). We also measured plasma BuChE activity and red blood cell AChE activity. Urine TCP excretion was significantly related to inhibition of BuChE but unrelated to inhibition of AChE, suggesting that the measures of internal dose were in the range where appreciable inhibition of B-esterases occurs but below the range where a physiologic effect on AChE exists.

The descriptive statistics for the industrial hygiene exposure variables and the biological monitoring results are shown in Table 2. The industrial hygiene data showed a wide range of chlorpyrifos exposures, with substantial differences between the chlorpyrifos subjects and the referents for historic chlorpyrifos exposure (64.16 vs. 0.69 mg/m³ × days; *p* < 0.0001), duration of work in exposed areas (9.72 vs. 0.01 years; *p* < 0.0001), and interim chlorpyrifos exposure (6.13 vs. 0.00 mg/m³ × days; *p* < 0.0001). The subgroup of 32 workers with the most appreciable historic chlorpyrifos exposure (exceeding 20 mg/m³ × day) was exposed on average for 12 years (range of 5–23.7 years). No referents had any identifiable occupational chlorpyrifos exposure during the observation period, but six had past chlorpyrifos exposure ranging from 3.1 to 15.98 mg/m³ × days. These subjects' exposures were included in the present analyses as cumulative exposure was considered as a continuous variable. For most subjects, the interim chlorpyrifos exposures were comparable to yearly exposures experienced previously. The daily TCP/Cr excretion among subjects with occupational chlorpyrifos exposure suggested an estimated daily chlorpyrifos exposure of about 576–627 μg/day, equivalent to approximately 31% (range 0–250%) of the internal dose that would be received by a hypothetical subject exposed during a working day at the threshold limit value (TLV) of 200 μg/m³, calculated as follows. In a worker exposed to chlorpyrifos by inhalation at the TLV of 200 μg/m³, the internal dose would be estimated as 2000 μg/day (200 μg/m³ × 10 m³ air inhaled per work day), or 22.7 μg/kg/day for an 88-kg man (the mean weight in the chlorpyrifos population). The chlorpyrifos workers in our study excreted an average of 192 μg TCP/g Cr, which converts to a daily excretion of 355 μg of TCP per day (assuming excretion of 21 mg Cr/kg/day). Assuming 100% pulmonary absorption of chlorpyrifos and 100% conversion of chlorpyrifos (molecular weight 350.6) to TCP (molecular weight 198.5), this excretion converts to an inhaled dose of 627 μg of chlorpyrifos per day (355 × 350.6/198.5). Inhalation of 627 μg of chlorpyrifos per day is approximately 31% of the 2000 μg/day that would be inhaled at the TLV.

TABLE 2
Descriptive Statistics for Exposure Variables and Biological Monitoring Results

Exposure measure	Chlorpyrifos (n = 53), mean ± SD	Referent (n = 60), mean ± SD	p value
Historic cumulative chlorpyrifos (mg/m ³ × days)	64.16 ± 59.97	0.69 ± 2.51	< 0.0001
Duration of work in exposed areas (years)	9.72 ± 6.12	0.10 ± 0.37	< 0.0001
Interim cumulative chlorpyrifos (mg/m ³ × days)	6.13 ± 3.70	0.00 ± 0.00	< 0.0001
Urine TCP (µg/ml)	0.251 ± 0.399	0.008 ± 0.004	< 0.0001
TCP/Cr (µg/g Cr)	192.2 ± 311.8	6.2 ± 2.2	< 0.0001

Note. p value determined by *t*-test for difference in means (adjusted for unequal variance between groups when indicated) or Wilcoxon two-sample test.

Nerve Conduction Studies. NCSs were performed on the dominant median, ulnar, and sural sensory nerves and the median and peroneal motor nerves using conventional electrodiagnostic medicine techniques. Antidromic sensory nerve action potential amplitude (baseline to negative peak), onset latency, and negative peak latency were recorded from digit II, digit V, and the ankle, respectively. Motor conduction studies recording from the abductor

pollicis brevis and extensor digitorum brevis muscles, respectively. Compound muscle action potential amplitude (baseline to negative peak) and onset latency were recorded, as was the fastest F wave latency (antidromic stimulation at the wrist and ankle) from among approximately 10 trials. Limb temperatures were monitored, and limbs were warmed using an electrical heating pad to maintain temperatures above 32°C (palm) and 31°C (ankle). After inspecting the data to determine if they needed transformation prior to analyses to make them more normally distributed, summary Z scores were calculated for motor nerves and sensory nerves using referent subject baseline results. Amplitude Z scores used distal amplitude results. Conduction velocity Z scores used distal latency, terminal conduction velocity (for sensory nerves), and extremity conduction velocity results (transforming latency measures so that a higher value reflected better performance). The descriptive statistics at baseline for the NCS results are shown in Table 3. Mean values were well within normal limits (Albers *et al.*, 2004) and comparable to those obtained from normal subjects without neuropathy (Albers *et al.*, 1982; Daube, 2005; Greene *et al.*, 1990), normal U.S. military personnel (Letz and Gerr, 1994), and asymptomatic industrial workers (Salerno *et al.*, 1998; Stetson *et al.*, 1992, 1993).

Statistical Analyses and Model Structure. All data were double entered or hand checked against the original records for accuracy. Data were managed in Microsoft Access and Excel, and analyses were performed in SAS, version 8.2 (SAS Institute, Cary, NC). Initial analyses included comparison of clinical, laboratory, and electrophysiological results relevant to the evaluation of peripheral neuropathy at the baseline and the 1-year examinations. All analyses used linear mixed models with repeated measures to account for the longitudinal nature of the data. Each NCS variable was modeled as a function of interim and historic chlorpyrifos exposure, age, and time. Additional covariates considered in the stepwise selection procedure included sex, height, weight,

TABLE 3
Descriptive Statistics for NCS Results at Baseline and Published Normal Values

Measure	Chlorpyrifos (N = 53), mean ± SD	Referent (n = 60), mean ± SD	p value	Normal limits	Neurologically intact subjects, mean (range)	U.S. Military personnel, mean ± SD	Asymptomatic industrial workers, mean ± SD
Median sensory							
Amplitude (µV)	29 ± 12.8	31 ± 12.3	0.42	> 10.0	32 (15–90)	24 ± 8.6	25 ± 9.3
Terminal CV (m/s)	54.8 ± 6.7	55.3 ± 7.0	0.70	> 50.0		54 ± 6.2	56.0 ± 6.7
Distal latency (ms)	3.3 ± 0.4	3.3 ± 0.5	0.66	< 3.7	3.3 (2.3–3.9)		3.2 ± 0.4
Ulnar sensory							
Amplitude (µV)	27 ± 11.0	28 ± 11.2	0.68	> 10.0	29 (10–75)	22 ± 9.2	23 ± 9.4
Terminal CV (m/s)	57.5 ± 5.5	57.6 ± 5.0	0.88	> 50.0		55 ± 5.9	58.3 ± 4.9
Distal latency (ms)	3.2 ± 0.2	3.2 ± 0.3	0.99	< 3.7	3.3 (2.3–3.7)		3.0 ± 0.2
Sural sensory							
Amplitude (µV)	18 ± 5.9	18 ± 5.9	0.77	> 6.0	17 (5–50)	17 ± 10.7	
Terminal CV (m/s)	50.4 ± 5.0	50.0 ± 5.1	0.77	> 40.0	48 (40–58)	45 ± 4.1	
Distal latency (ms)	3.5 ± 0.3	3.5 ± 0.3	0.38	< 4.2	3.7 (2.5–4.4)		
Median motor							
Amplitude (mV)	9.4 ± 3.1	10.1 ± 3.5	0.27	> 4.2	9.1 (3–20)	10.4 ± 3.5	12.0 ± 3.2
Forearm CV (m/s)	56.6 ± 4.1	57.1 ± 3.3	0.50	> 49.0	54 (48–73)	57 ± 4.3	
Distal latency (ms)	3.4 ± 0.4	3.4 ± 0.6	0.48	< 4.4	3.4 (2.3–4.6)		3.7 ± 0.5
F wave latency (ms)	27.9 ± 2.3	27.7 ± 2.7	0.70	< 31.8	26.4 (22–31)		
Peroneal motor							
Amplitude (mV)	7.2 ± 3.6	7.4 ± 2.9	0.79	> 2.5	6.4 (2–12)	6.9 ± 4.1	
Distal latency (ms)	4.6 ± 0.6	4.5 ± 0.6	0.45	< 6.1	4.9 (2.4–7.0)	47 ± 4.1	
F wave latency (ms)	48.7 ± 4.2	48.7 ± 4.9	0.97	< 56.0	47.4 (38–57)		

Note. p value determined by *t*-test for difference in group means (adjusted for unequal variance between groups when indicated). See text for references for normal limits and normal values. CV, conduction velocity.

body mass index (BMI), skin temperature over the nerve being tested (Letz and Gerr, 1994; Salerno *et al.*, 1998), smoking status (pack-years), alcohol use (drinks/day), alcohol-related behaviors (ever being told of a potential alcohol problem or ever being arrested for driving under the influence of alcohol), estimated general ability (a potential surrogate for socioeconomic status) (Blair and Spreen, 1989), education level, and anxiety level (Derogatis and Spenser, 1982). The latter six covariates were considered because they were believed *a priori* to be potential confounders (Butschek and Miller, 1980; Crawford *et al.*, 1977; Letz and Gerr, 1994). All regressions were performed using interim chlorpyrifos exposure and were repeated using interim TCP/Cr. Using a forward stepwise selection procedure, covariates that had a *p* value < 0.15 were included in the model. Interaction terms between each of the covariates and each of the exposure measures were evaluated, and those having a *p* value < 0.05 were kept in the final model. We examined all covariates for correlations to insure that the linear mixed models did not suffer from problems due to inclusion of highly correlated variables. None of the covariates was correlated highly, with the exception of weight and BMI, $r = 0.86$. Similarly, the correlations between the different exposure variables were not sufficiently high to suggest collinearity issues. For each NCS variable, the "best" model was selected based on the lowest value of the Akaike Information Criterion (AIC). For those models that showed either significant or borderline-significant ($p < 0.10$) effects of historic chlorpyrifos exposure on NCS results, we created models that allowed the effect of historic chlorpyrifos exposure on the NCS variable to be different among three exposure groups (referents, chlorpyrifos subjects who had historic chlorpyrifos exposure less than or equal to $20 \text{ mg/m}^3 \times \text{days}$, and chlorpyrifos subjects who had historic chlorpyrifos exposure exceeding $20 \text{ mg/m}^3 \times \text{days}$). This value was chosen because there was a clear cutpoint between subjects whose historic chlorpyrifos exposure was none/small and those who had appreciable exposure.

RESULTS

Table 4 summarizes the results of the linear mixed models of NCS measures as a function of historic chlorpyrifos exposure, interim chlorpyrifos exposure, age, time, and covariates. Each parameter estimate in Table 4 indicates the change in a nerve conduction measure per unit change in chlorpyrifos exposure. For example, the parameter estimate for median sensory amplitude of -0.028 indicates that median sensory amplitude decreases by $0.028 \mu\text{V}$ for each increase of $1 \text{ mg/m}^3 \times \text{days}$ in historic chlorpyrifos exposure (historic chlorpyrifos exposure of $1 \text{ mg/m}^3 \times \text{days}$ could occur, e.g., by working for 100 days in a setting where the air concentration was at 0.01 mg/m^3). These results show the relationship between chlorpyrifos exposure and changes in nerve conduction measures. The results for age, height, and weight also are shown as these most frequently and most significantly entered into the models. For each NCS measure, the model having the lowest AIC is presented. The results of the analyses among subjects who had historic chlorpyrifos exposure exceeding $20 \text{ mg/m}^3 \times \text{days}$ are shown in italics.

Interim Chlorpyrifos Exposure

A single significant association was identified between the 20 NCS outcomes and interim chlorpyrifos exposure. Median sensory terminal conduction velocity was significantly associ-

ated with the interim TCP/Cr level ($p = 0.05$), suggesting deteriorating performance (decreasing velocity) with increasing interim TCP/Cr level. There was also a single borderline-significant association ($p = 0.08$) between median motor amplitude and interim chlorpyrifos exposure, suggesting improved performance (increasing amplitude) with increasing interim chlorpyrifos exposure. Despite the lack of a significant main effect of interim chlorpyrifos exposure, there were four significant interactions between covariates and interim chlorpyrifos exposure (results not shown). The interactions suggested that subjects who were older, taller, less educated, and had a history of adverse alcohol-related effects were at increased risk for worsening nerve function with increasing interim chlorpyrifos exposure. However, the contradictory findings involving three of these interactions, which showed improving nerve function with increasing interim chlorpyrifos exposure among subjects who were younger, shorter, and more educated, mitigate against a conclusion that these interactions are meaningful.

Historic Chlorpyrifos Exposure

Three out of 20 NCS outcomes (median motor forearm conduction velocity, median motor F wave latency, and summary Z score for sensory conduction) showed significant associations with historic chlorpyrifos exposure. All three suggested an adverse effect (decreasing velocity, increasing F wave latency, and decreasing summary Z score) with increasing historic chlorpyrifos exposure. Three additional NCS outcomes (median sensory amplitude, ulnar sensory terminal conduction velocity, and summary Z score for motor conduction) showed borderline-significant associations ($p = 0.09$, $p = 0.06$, and $p = 0.08$, respectively) with historic chlorpyrifos exposure. All three suggested adverse effects (decreasing amplitude, velocity, and summary Z score) with increasing historic chlorpyrifos exposure. In the analyses of subjects with historic chlorpyrifos exposure levels exceeding $20 \text{ mg/m}^3 \times \text{days}$, the parameter estimates for the median sensory amplitude, ulnar sensory terminal conduction velocity, median motor forearm conduction velocity, and the summary Z score for motor conduction were appreciably smaller and none remained significant or borderline significant. The parameter estimates for median motor F wave latency and the summary Z score for sensory conduction were of similar magnitude to those found in the full study population but no longer significant.

Two of the three significant associations between a NCS outcome and historic chlorpyrifos exposure showed a significant interaction between education and historic chlorpyrifos exposure. Subjects who were more highly educated showed increasing median F wave latency and decreasing summary Z score for sensory conduction (both indicating deteriorating electrophysiological performance) with increasing historic chlorpyrifos exposure, whereas subjects with less education

TABLE 4

Parameter Estimates and *p* Values for Longitudinal Analyses of Nerve Conduction Measures Modeled as a Function of Historic Chlorpyrifos Exposure, Interim Chlorpyrifos Exposure or Interim TCP/Cr Level and Covariates (*n* = 113)

	Historic chlorpyrifos exposure			Interim chlorpyrifos exposure or interim TCP/Cr level†		
	Parameter estimate	(95% CI)	<i>p</i> value	Parameter estimate	(95% CI)	<i>p</i> value
Median sensory						
Amplitude (μv)	−0.028 <i>−0.0035</i>	(−0.060 to 0.0044) <i>(−0.060 to 0.067)</i>	0.09 <i>0.91</i>	−0.013 <i>−0.0069</i>	(−0.23 to 0.20) <i>(−0.22 to 0.21)</i>	0.90 <i>0.95</i>
Terminal CV (m/s)	−0.014 <i>−0.012</i>	(−0.038 to 0.011) <i>(−0.059 to 0.034)</i>	0.27 <i>0.60</i>	−0.0032† <i>−0.0032†</i>	(−0.0064 to −0.00004)† <i>(−0.0064 to 0.0000)†</i>	0.05† <i>0.05†</i>
Distal latency (ms)	0.00076 <i>0.00055</i>	(−0.00078 to 0.0023) <i>(−0.0026 to 0.0037)</i>	0.33 <i>0.73</i>	0.0023 <i>0.0024</i>	(−0.0073 to 0.012) <i>(−0.0072 to 0.012)</i>	0.63 <i>0.62</i>
Ulnar sensory						
Amplitude (μv)	−0.029 <i>−0.031</i>	(−0.065 to 0.0072) <i>(−0.098 to 0.037)</i>	0.12 <i>0.37</i>	−0.12 <i>−0.11</i>	(−0.43 to 0.18) <i>(0.42 to 0.19)</i>	0.43 <i>0.46</i>
Terminal CV (m/s)	−0.018 <i>−0.0048</i>	(−0.037 to −0.0010) <i>(−0.042 to 0.032)</i>	0.06 <i>0.80</i>	0.090 <i>0.10</i>	(−0.11 to 0.28) <i>(−0.98 to 0.30)</i>	0.91 <i>0.32</i>
Distal latency (ms)	0.00056 <i>−0.00017</i>	(−0.00032 to 0.0014) <i>(−0.0016 to 0.0019)</i>	0.21 <i>0.84</i>	0.0013 <i>0.0019</i>	(−0.0073 to 0.010) <i>(−0.0068 to 0.011)</i>	0.76 <i>0.66</i>
Sural sensory						
Amplitude (μv)	−0.0082 <i>−0.023</i>	(−0.031 to 0.015) <i>(−0.067 to 0.020)</i>	0.48 <i>0.29</i>	0.090 <i>0.074</i>	(−0.20 to 0.38) <i>(−0.22 to 0.36)</i>	0.53 <i>0.62</i>
Terminal CV (m/s)	−0.0050 <i>−0.011</i>	(−0.019 to 0.009) <i>(−0.037 to 0.015)</i>	0.48 <i>0.39</i>	0.058 <i>−0.0021</i>	(−0.16 to 0.28) <i>(−0.23 to 0.22)</i>	0.60 <i>0.99</i>
Distal latency (ms)	−0.00005 <i>0.0012</i>	(−0.00098 to 0.00088) <i>(−0.0005 to 0.0030)</i>	0.92 <i>0.18</i>	0.0080 <i>0.010</i>	(−0.0042 to 0.0020) <i>(0.0022 to 0.023)</i>	0.20 <i>0.11</i>
Median motor						
Amplitude (mv)	−0.0061 <i>0.0024</i>	(−0.018 to 0.0056) <i>(−0.020 to 0.025)</i>	0.31 <i>0.83</i>	0.11 <i>0.11</i>	(−0.014 to 0.23) <i>(−0.012 to 0.23)</i>	0.08 <i>0.08</i>
Forearm CV (m/s)	−0.013 <i>−0.0024</i>	(−0.024 to −0.0010) <i>(−0.024 to 0.019)</i>	0.03 <i>0.82</i>	0.085 <i>0.078</i>	(−0.071 to 0.24) <i>(−0.080 to 0.237)</i>	0.28 <i>0.33</i>
Distal latency (ms)	0.00098 <i>0.00075</i>	(−0.00081 to 0.0028) <i>(−0.0028 to 0.0043)</i>	0.28 <i>0.41</i>	0.0048 <i>0.0050</i>	(−0.0076 to 0.017) <i>(−0.0075 to 0.017)</i>	0.44 <i>0.43</i>
F wave latency (ms)	0.0068 <i>0.0060</i>	(0.00044 to 0.013) <i>(−0.0060 to 0.018)</i>	0.04 <i>0.32</i>	0.0091 <i>0.0072</i>	(−0.044 to 0.063) <i>(−0.047 to 0.061)</i>	0.74 <i>0.79</i>
Peroneal motor						
Amplitude (mv)	0.00044 <i>0.0065</i>	(−0.010 to 0.011) <i>(−0.014 to 0.027)</i>	0.93 <i>0.54</i>	−0.016 <i>−0.015</i>	(−0.11 to 0.073) <i>(−0.10 to 0.75)</i>	0.72 <i>0.75</i>
Distal latency (ms)	0.00087 <i>−0.0023</i>	(−0.0014 to 0.0032) <i>(−0.0068 to 0.0022)</i>	0.45 <i>0.31</i>	−0.0066 <i>−0.0091</i>	(−0.029 to 0.016) <i>(−0.032 to 0.013)</i>	0.56 <i>0.42</i>
F wave latency (ms)	0.0075 <i>−0.0056</i>	(−0.0038 to 0.019) <i>(−0.028 to 0.017)</i>	0.19 <i>0.62</i>	−0.047 <i>0.048</i>	(−0.16 to 0.068) <i>(−0.16 to 0.068)</i>	0.42 <i>0.42</i>
Summary Z scores						
Motor amplitude	−0.0020 <i>0.0025</i>	(−0.0075 to 0.0035) <i>(−0.0081 to 0.013)</i>	0.46 <i>0.64</i>	0.033 <i>0.035</i>	(−0.017 to 0.084) <i>(−0.016 to 0.086)</i>	0.19 <i>0.18</i>
Motor conduction	−0.0089 <i>−0.0015</i>	(−0.019 to 0.0011) <i>(−0.022 to 0.019)</i>	0.08 <i>0.88</i>	0.025 <i>0.024</i>	(−0.055 to 0.105) <i>(−0.057 to 0.10)</i>	0.54 <i>0.59</i>
Sensory amplitude	−0.0048 <i>−0.0039</i>	(−0.011 to 0.0013) <i>(−0.016 to 0.0084)</i>	0.12 <i>0.53</i>	0.013 <i>0.012</i>	(−0.042 to 0.068) <i>(−0.044 to 0.067)</i>	0.63 <i>0.68</i>
Sensory conduction	−0.010 <i>−0.012</i>	(−0.020 to −0.00044) <i>(−0.030 to 0.0044)</i>	0.04 <i>0.15</i>	−0.016 <i>−0.0022</i>	(−0.15 to 0.12) <i>(−0.16 to 0.12)</i>	0.81 <i>0.76</i>

Note. Additional potential covariates not shown included sex, BMI, temperature over nerve being tested, estimated general ability, education level, anxiety level, alcohol use (drinks/day), alcohol-related behaviors, and smoking pack-years. For each measure, the model having the lowest AIC is presented. The results of the analyses of subjects with historic chlorpyrifos exposures exceeding 20 mg/m³ × days are shown in italics (*n* = 32). CI, confidence interval; CV, conduction velocity.

TABLE 4—Continued

	Age			Height			Weight		
	Parameter estimate	(95% CI)	<i>p</i> value	Parameter estimate	(95% CI)	<i>p</i> value	Parameter estimate	(95% CI)	<i>p</i> value
Median sensory									
Amplitude (μV)	−0.79 −0.84	(−1.00 to −0.57) (−1.057 to −0.62)	< 0.0001 < 0.0001	−0.82 −0.79	(−1.41 to −0.23) (−1.40 to −0.19)	< 0.01 0.01			
Terminal CV (m/s)	−0.30 −0.32	(−0.43 to −0.17) (−0.45 to −0.18)	< 0.0001 < 0.0001				−0.054 −0.051	(−0.079 to −0.030) (−0.076 to −0.026)	< 0.0001 < 0.0001
Distal latency (ms)	0.022 0.022	(0.012 to 0.032) (0.012 to 0.033)	< 0.0001 < 0.0001				0.0030 0.0030	(0.0012 to 0.0048) (0.0011 to 0.0048)	0.001 0.002
Ulnar sensory									
Amplitude (μV)	−0.56 −0.57	(−0.75 to −0.37) (−0.77 to −0.37)	< 0.0001 < 0.0001				−0.045 −0.042	(−0.082 to −0.0073) (−0.080 to −0.0033)	0.02 0.03
Terminal CV (m/s)	−0.19 −0.19	(−0.29 to −0.086) (−0.29 to −0.081)	< 0.01 < 0.01	−0.41 −0.41	(−0.72 to −0.13) (−0.71 to −0.12)	< 0.01 < 0.01			
Distal latency (ms)	0.012 0.012	(0.0065 to 0.017) (0.0061 to 0.018)	< 0.0001 < 0.0001	0.31 0.33	(0.019 to 0.043) (0.020 to 0.045)	< 0.0001 < 0.0001			
Sural sensory									
Amplitude (μV)	−0.27 −0.24	(−0.41 to 0.13) (−0.39 to −0.10)	< 0.001 0.002	−0.58 −0.56	(−1.02 to −0.14) (−1.02 to −0.10)	0.01 0.02	−0.042 −0.043	(−0.072 to −0.012) (−0.073 to −0.013)	< 0.01 < 0.01
Terminal CV (m/s)	−0.10 −0.09	(−0.19 to −0.018) (−0.18 to −0.0039)	0.02 0.04	−0.88 −0.94	(−1.14 to −0.63) (−1.21 to −0.68)	< 0.0001 < 0.0001	0.029 0.030	(0.011 to 0.047) (0.012 to 0.049)	< 0.01 < 0.01
Distal latency (ms)	0.0095 0.0080	(0.0036 to 0.015) (0.0020 to 0.014)	< 0.01 < 0.01	0.055 0.054	(0.039 to 0.072) (0.037 to 0.071)	< 0.0001 < 0.0001			
Median motor									
Amplitude (mV)	−0.075 −0.090	(−0.15 to −0.0026) (−0.16 to −0.014)	0.04 0.02						
Forearm CV (m/s)	−0.16 −0.17	(−0.23 to −0.096) (−0.24 to −0.095)	< 0.0001 < 0.0001				−0.016 −0.016	(−0.029 to −0.0023) (−0.029 to −0.0023)	0.02 0.02
Distal latency (ms)	0.023 0.024	(0.011 to 0.034) (0.012 to 0.036)	< 0.001 < 0.001				0.0022 0.0022	(−0.000054 to 0.0042) (−0.0001 to 0.0044)	0.04 0.04
F wave latency (ms)	0.068 0.071	(0.034 to 0.10) (0.034 to 0.10)	< 0.001 < 0.001	0.41 0.40	(0.31 to 0.51) (0.30 to 0.50)	< 0.0001 < 0.0001			
Peroneal motor									
Amplitude (mV)	−0.040 −0.042	(−0.11 to 0.026) (−0.11 to 0.029)	0.24 0.25						
Distal latency (ms)	0.00053 0.0016	(−0.015 to 0.014) (−0.013 to 0.016)	0.94 0.83	0.033 0.039	(0.0011 to 0.065) (−0.013 to 0.016)	0.04 0.02			
F wave latency (ms)	0.065 0.075	(−0.0059 to 0.14) (0.00073 to 0.15)	0.07 0.05	1.072 1.11	(0.92 to 1.22) (0.95 to 1.26)	< 0.0001 < 0.0001			
Summary Z scores									
Motor amplitude	−0.036 −0.041	(−0.071 to −0.0020) (−0.077 to −0.0050)	0.04 0.03						
Motor conduction	−0.13 −0.14	(−0.20 to −0.066) (−0.21 to −0.070)	< 0.001 < 0.001	−0.44 −0.46	(−0.63 to −0.25) (−0.65 to −0.26)	< 0.0001 < 0.0001			
Sensory amplitude	−0.15 −0.16	(−0.19 to −0.11) (−0.20 to −0.12)	< 0.0001 < 0.0001	−0.17 −0.23	(−0.29 to −0.056) (−0.32 to −0.13)	< 0.01 < 0.0001	−0.012 −0.012	(−0.020 to −0.0037) (−0.020 to −0.0035)	< 0.01 < 0.01
Sensory conduction	−0.18 −0.17	(−0.23 to −0.13) (−0.22 to −0.12)	< 0.0001 < 0.0001	−0.47 −0.46	(−0.57 to −0.37) (−0.56 to −0.35)	< 0.0001 < 0.0001			

showed no effect. The presence of an effect among only highly educated subjects has no physiological explanation. Among the measures for which a main effect was not identified, there were two significant interactions between education and historic chlorpyrifos exposure. For each measure, more highly educated subjects showed deteriorating electrophysiological performance with increasing historic chlorpyrifos exposure, whereas less-educated subjects showed no effect (ulnar sensory amplitude)

or even slightly better performance (median sensory terminal conduction velocity) with increasing exposure.

DISCUSSION

We previously reported that no significant differences existed for any of the clinical or NCS measures based on group

assignment (chlorpyrifos vs. referent), either at baseline, 1 year, or change over 1 year, and that all mean values were comparable to normal values (Albers *et al.*, 2004). The unremarkable results were important in assuring that no clinically important peripheral abnormalities were overlooked, but those data did not exclude the possibility of subclinical adverse dose effects on NCS results. The NCS measures we used are among the most sensitive quantitative indicators of neuropathy and are those favored in most clinical studies in which neuropathy is a primary endpoint, such as the ongoing National Institutes of Health sponsored Epidemiology of Diabetes Intervention and Complications study (Department of Health and Human Services, 2006).

The present analyses identified few dose-effect associations between any of the NCS results and measures of interim chlorpyrifos exposure. The two significant or borderline-significant associations gave conflicting results, one suggesting deteriorating electrophysiological function and the other suggesting enhanced electrophysiological function with increasing interim chlorpyrifos exposure. These conflicting findings of decreasing conduction velocity and increasing amplitude with increasing interim chlorpyrifos exposure are not explained by a neurotoxic effect. A single measure (ulnar sensory terminal conduction velocity) showed a significant interaction between interim chlorpyrifos exposure and alcohol-related behaviors. Subjects with adverse alcohol-related behaviors showed an adverse effect with increasing interim chlorpyrifos exposure, whereas subjects without these behaviors showed no effect. The explanation for this single observation in the absence of a pattern of adverse effects among the other electrophysiological measures is unclear.

Several significant main effects were identified among measures of conduction velocity, distal latency, or F wave latency and historic chlorpyrifos exposure that suggested an adverse effect of chlorpyrifos exposure on sensory and motor nerves. However, few dose-effect relationships were found among subjects with historic chlorpyrifos exposure exceeding $20 \text{ mg/m}^3 \times \text{days}$. In analyses that spanned a 10-fold historic chlorpyrifos dose range ($20\text{--}220 \text{ mg/m}^3 \times \text{days}$), only two NCS measures (median motor F wave latency and summary Z score for sensory conduction) showed parameter estimates that were of similar magnitude to those found in the models based on the full data set. Neither of these two measures was significant or borderline-significant, and the results are somewhat incongruent as each measure reflects a different physiological component of the NCS evaluation (motor conduction vs. sensory conduction). For the remaining associations, the parameter estimates differed substantially from those found in the models based on the full data set, showing virtually no effect of exposure on NCS results. This last observation suggests that the initial results were explained by small differences among subjects with low chlorpyrifos exposure and referents and not a dose-related deterioration among subjects with higher levels of historic exposure. The lack of adverse NCS effects among

these subjects was not due to outliers or lack of spread in the exposure estimates. Among the 32 subjects whose historic chlorpyrifos exposure exceeded $20 \text{ mg/m}^3 \times \text{days}$, the median exposure was $103.8 \text{ mg/m}^3 \times \text{days}$, with an interquartile range of $52.7\text{--}126.6 \text{ mg/m}^3 \times \text{days}$. There is no clear physiological explanation for the adverse effect of chlorpyrifos at low doses that is not accompanied by evidence of an adverse effect at higher doses over a 10-fold range.

The numerous significant interactions between education level and historic chlorpyrifos exposure were unexpected and intriguing. In general, subjects with more education showed an adverse electrophysiological effect with increasing historic chlorpyrifos exposure, and subjects with less education showed no effect or slightly better performance. Subjects with higher levels of education had the least historic chlorpyrifos exposure, and those with less education had the greatest historic exposure. The explanation for these interactions is unclear. Education level may be a surrogate for socioeconomic status, which has been reported as a predictor of NCS results (Letz and Gerr, 1994). Alternatively, selection into work assignments involving chlorpyrifos exposure may have been based on educational and physical performance.

Alcohol use and diabetes could have influenced our results. However, the number of alcohol drinks per day did not differ significantly between groups (0.8 vs. 1.3 , $p = 0.19$), and the behavioral indicators of alcohol overuse also were similar for both groups. Namely, six chlorpyrifos subjects and eight referents ($p > 0.99$) had ever been told they had an alcohol problem, and three chlorpyrifos subjects and 10 referents ($p = 0.08$) reported an arrest for driving under the influence of alcohol. Moreover, consuming up to six drinks per day did not adversely affect NCS results among 35- to 45-year-old men, a finding consistent with our results based on substantially lower levels of alcohol consumption (Gerr and Letz, 1994). Although inclusion of the two subjects with diabetes could influence our models, the final models were similar, independent of whether or not these two subjects were included or excluded.

There are several reasons for cautious interpretation of the few statistically significant associations we identified between chlorpyrifos exposure and NCS results. Importantly, we were looking for evidence of subclinical neuropathy as no group difference existed between exposed and referents for any of the NCS results (Albers *et al.*, 2004). While the sensitivity of NCS results is laudable in terms of detecting subtle degrees of neuropathy, associations of the type we identified within the normal range may reflect additional unrecognized factors, such as the significant associations involving age and body size identified in our models. Further, none of the statistically significant associations involved the sural NCS measures, measures considered among the most sensitive indicators of sensory neuropathy (Albers *et al.*, 1996; Shefner, 2004). Typically, in the presence of a clinically important sensory or sensorimotor neuropathy, the sural response is unrecordable (Hendriksen *et al.*, 1992). No subject in this study had an abnormal sural response amplitude,

and the mean sural amplitude of 18 μV for the entire study population is indistinguishable from the mean value among healthy adults without neuropathy (Gerr and Letz, 1994; Rivner *et al.*, 2001; Stetson *et al.*, 1992). In the context of suspected neurotoxicity, NCS abnormalities involving conduction velocity or latency measures are poor indicators of axonal loss relative to amplitude recordings (Redmond *et al.*, 1992; Shefner and Dawson, 1990). None of the significant associations we identified involved an adverse effect of chlorpyrifos exposure on an amplitude measure.

In summary, the findings from the present study were not suggestive of causal relationships between exposure to chlorpyrifos over many years during the manufacturing process and subclinical neuropathy. The associations we identified between historic chlorpyrifos exposure and deteriorating NCS results were either present only at low-exposure levels and not accompanied by further effects over a 10-fold range or showed significant interactions (e.g., with education level) that remain unexplained. Moreover, those workers with the highest chlorpyrifos exposures (exceeding $20 \text{ mg/m}^3 \times \text{day}$) showed only minor, statistically nonsignificant relationships between historic cumulative exposure and NCS results.

It could be argued that the results were not unexpected as we studied well-educated workers who were employed in a carefully controlled environment. Nevertheless, the subgroup of workers with the highest chlorpyrifos exposure was exposed regularly for an average of 12 years at levels sufficient to cause chronic, mild inhibition of plasma BuChE. Given the widespread use of chlorpyrifos and concerns that resulting exposures might have adverse peripheral nervous system effects, these workers represent an important group to observe. Our study provides a rare opportunity to correlate accurate low-level exposure data in a well-defined population with sensitive electrophysiological measures.

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done under full compliance with all government policies and the Helsinki Declaration.

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