SHORT REPORT

Chlorpyrifos exposure and biological monitoring among manufacturing workers

C J Burns, D Garabrant, J W Albers, S Berent, B Giordani, S Haidar, R Garrison, R J Richardson

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Aim: To use biological monitoring data to evaluate the soundness of job based exposure classifications.

Methods: The authors studied 52 chlorpyrifos manufacturing workers and 60 referent workers to compare chlorpyrifos exposure estimations from job titles and work areas to urinary excretion of 3,5,6 trichloro-2-pyridinol (TCP), a metabolite of chlorpyrifos. Work history records and industrial hygiene monitoring data were used to establish cumulative interim exposure. Chlorpyrifos exposure during the study year was assessed biologically by urinary excretion of TCP.

Results: Exposure as measured by three urinary TCP samples was significantly higher among the chlorpyrifos workers (188 µg/l) than it was for the referent subjects (7 µg/l). Urinary TCP also correlated well with specific exposure categories of negligible (0.73–1.98 mg/m³ days), low (1.99–4.91 mg/m³ days), and moderate (4.92–15.36 mg/m³ days). The weighted Kappa coefficient was 0.80 (95% CI 0.72 to 0.87) for the mean TCP over the study period.

Conclusions: The estimates of chlorpyrifos exposure based on job classifications and industrial hygiene measurements were significantly related to urinary TCP excretion, indicating that the ambient estimates are useful for providing exposure estimates among chlorpyrifos manufacturing workers.

Job titles, tasks, and work areas have been used in epidemiology studies as surrogate indicators of exposure to organophosphorus insecticides. However, biological exposure markers of specific organophosphorus compounds have not been used to confirm or quantify these estimates of workers' exposure. Monthly measurements of butyrylcholinesterase (BuChE, plasma cholinesterase) activity are often collected among chlorpyrifos applicators and manufacturing workers. Whereas the degree of inhibition of BuChE has been demonstrated to correlate with job based exposure categories,¹ BuChE is characterised by a high degree of intra-individual variability.^{2–3} Quantifying the excretion in urine of the principal metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCP) as opposed to enzyme activity is a more specific marker of exposure. The purpose of the current analyses was to use urinary TCP data among chlorpyrifos manufacturing workers to validate the use of job based exposure classifications used in health studies. The present study was conducted as part of a prospective investigation of neurological and neurobehavioural function in chlorpyrifos manufacturing workers.⁴

METHODS

All chlorpyrifos manufacturing workers employed at a Michigan facility on 1 September 1999 were asked to participate. Saran manufacturing workers were chosen as a

referent population. Fifty two of the 66 eligible chlorpyrifos workers (80%) participated and 60 of the 74 (81%) randomly selected Saran workers participated. The referent workers were similar to the chlorpyrifos workers with respect to age (mean 41 years for both), weight (88 kg for both), and education (14 years for both).

Measurements of urinary TCP excretion were collected from each subject on three occasions: the autumn of 1999, the spring of 2000, and the autumn of 2000. Each subject collected a first morning urine sample using a container provided by the company.

Samples were stored in a –20°C freezer until analysis and were then analysed for TCP using GC/MS and HPLC/UV methods.⁵ Total TCP was determined using gas chromatography-negative-ion chemical ionisation mass spectrometry. The samples were acid-hydrolysed to cleave conjugates, extracted into toluene, and derivatised with *N*-Methyl-*N*-[*tert*-butyldimethylsilyl]trifluoroacetamide before analysis.⁵ In addition spike samples, spanning a TCP concentration range of 26–260 µg/l, submitted by the University of Michigan, were analysed to confirm stability and linearity. The limits of detection and quantification for this method were 0.2 and 1.0 µg/l, respectively.

The exposure evaluations have been previously described.^{3–4} In brief, similar exposure groups (SEGs) were created for specific job titles based upon industrial hygiene monitoring data. For each SEG, eight hour time weighted average air samples of chlorpyrifos exposure were identified. The job segment of each participant was assigned the corresponding exposure profile. The geometric mean exposure (mg/m³, milligrams/cubic metre) in each job segment was multiplied by the number of days the job was held, and these exposure duration products were then summed over all jobs held by a participant to derive a cumulative exposure to chlorpyrifos (in mg/m³ days). This was done for all jobs in the study period. Consistent with the previous morbidity studies, each job was categorised as negligible (0.73–1.98 mg/m³ days), low (1.99–4.91 mg/m³ days), or moderate (4.92–15.36 mg/m³ days).^{1–3}

Statistical analysis

The TCP measurements were combined in a weighted average for each subject, with the weights chosen to reflect the number of months each measurement represented. The autumn 1999 and 2000 measures were given a weight of 5.75 months each; and the spring 2000 sample was given a weight of one month, reflecting the likely period of avocational pesticide use.

We compared the mean weighted average TCP excretion between chlorpyrifos and referent subjects using a *t* test and linear regression. We calculated means and ranges for each exposure category. Kappa coefficient (weighted Kappa

Abbreviations: BuChE, butyrylcholinesterase; SEG, similar exposure group; TCP, 3,5,6-trichloro-2-pyridinol

Table 1 Mean TCP excretion by exposure groups defined from work history

Time period	Referent (n = 60) µg/l (range)	Negligible (n = 8) µg/l (range)	Low (n = 18) µg/l (range)	Moderate (n = 26) µg/l (range)
Autumn 1999	5 (ND–20)	20 (3–59)	48 (6–257)	213 (12–604)
Spring 2000	6 (ND–17)	14 (6–32)	50 (1–178)	254 (40–1430)
Autumn 2000	9 (ND–34)	26 (5–92)	99 (ND–699)	450 (9–2390)
Mean TCP*	7 (1–20)	22 (6–72)	72 (10–451)	325 (14–1284)

*Weighted mean over three time periods $((\text{Autumn99} \times 5.75) + (\text{Spring} \times 1) + (\text{Autumn00} \times 5.75) / 3)$
 ND, non-detectable.

statistics) was performed to test for agreement between urinary TCP and the four exposure categories. The Kappa coefficient is appropriate to evaluate the correlation for discrete variables while correcting for chance agreement.⁶ TCP was categorised into four groups in order to get 4×4 contingency tables. TCP for each study period was sorted from lower to higher to reflect the same number of subjects in each exposure group. For example, because the referent group included 60 subjects, the first TCP category included 60 subjects with the lowest values. Similarly, the second, third, and fourth TCP groups included 8, 18, and 26 subjects, respectively. We also calculated the per cent categorical agreement overall and for each study period. The per cent agreement in cells in non-adjacent categories was calculated. All analyses were done using SAS software (SAS version 8.2).⁷

RESULTS

Half of the chlorpyrifos subjects (n = 26) were classified in the moderate exposure category, with the remaining subjects in the low (n = 18) or negligible (n = 8) exposure categories. No subjects were considered to be working in a high exposure job, and no subjects moved across categories during the study period. No acute exposure incidents were known to have occurred during the study period.

The mean weighted average TCP excretion was significantly higher among chlorpyrifos subjects compared to referent subjects, (188 µg/l v 7 µg/l, $p < 0.0001$). Urinary TCP excretion increased significantly as the cumulative chlorpyrifos exposure (in mg/m³ days) increased ($r^2 = 0.41$, $p < 0.0001$). The mean overnight urine TCP excretion levels are shown in table 1, based on the exposure groups as defined by work histories. The referent group and the subjects with negligible chlorpyrifos exposure had the lowest TCP excretion levels, and they showed little variability over the three evaluations (mean 7 and 22 µg/l, respectively). The subjects with low chlorpyrifos exposure exhibited two- to fourfold higher TCP levels than did the subjects classified with negligible chlorpyrifos exposure (mean 72 µg/l).

Per cent categorical agreement ranged from 71% to 80%. Kappa statistics paralleled the findings for per cent agreement for autumn 1999 (0.83, 95% CI 0.75 to 0.90), spring 2000 (0.81, 95% CI 0.73 to 0.89), autumn 2000 (0.73, 95% CI 0.63 to 0.81), and the entire study period (0.80, 95% CI 0.72 to 0.87). The urinary TCP moderately agreed with the job exposure classifications when the referent workers were omitted from the analyses, with statistically significant Kappa coefficients of 0.65, 0.65, and 0.44, respectively. We observed increasing variability of urinary TCP with each successive exposure category. However, the per cent non-adjacent misclassification was only 4.5% for the weighted TCP.

DISCUSSION

The job based exposure levels corresponded with increasing TCP excretion. We confirmed that TCP excretion was

significantly higher in chlorpyrifos workers than it was in referent workers. Similarly, TCP excretion progressively increased across exposure category. Notably, the variability of exposure increased with increasing exposure levels. Some of the subjects in the low and moderate exposure categories experienced little occupational exposure as measured by TCP excretion. When conducting specific tasks, workers are required to wear personal protective equipment. The variability in urine TCP excretion may reflect differences among individual work practices, including assiduous adherence to safety procedures when performing defined tasks. Although the overall misclassification bias was likely to be small, these findings suggest that exposure to some individuals may be overestimated when using jobs and departments. Underestimation is less likely.

This study is limited by the pharmacokinetic properties of chlorpyrifos. Chlorpyrifos metabolises to TCP in the body as well as the environment. As a result, we could not attribute the source of TCP uniquely to the parent compound.⁸ Surface wipe testing is regularly conducted at the site to evaluate and stimulate routine housekeeping activities and these determinants indicated that TCP is present in the workplace. Low level occupational exposure to environmental TCP was likely for our chlorpyrifos workers.

Another limitation is the short half-life of TCP in the human body. The metabolite measured reflects exposure experienced within the last 24–48 hours.⁹ The job based exposure categorisation is based on the full scope of normal activities. If a task with high potential for exposure was not undertaken one or two days before data collection, the TCP excretion would not have reflected the previous exposure(s).

The study also had several strengths. We collected urine three times over the course of a year. It was important to use several measurements over time to capture the full scope of potential exposure opportunities. As an example, we found that the lowest TCP excretion value during each collection period was observed in a different individual.

The TCP excretion (7 µg/l) among our referent subjects was consistent with background populations.^{10–11} Our chlorpyrifos workers also exhibited urinary values that are consistent with other occupational groups. The subjects with negligible exposure (mean 22 µg/l) experienced exposures similar to that of US farmers (19 µg/l).¹² The moderate exposed group was comparable with termiticide workers, albeit indicating lower exposure (325 µg/l v 629 µg/l).¹⁰

Causal assessment from epidemiology studies is strengthened from the use of exposure response analyses. Quantification of historical exposure relies upon surrogate exposure estimates based upon work history data. These exposure groups have been used to determine high exposure groups and potentially the employees at highest risk for adverse health effects as result of exposure. Our study demonstrates that the exposure group classification applied to the chlorpyrifos manufacturing cohort in our previously published studies correlates strongly with biological indicators of exposure, thus lending validity to these health studies.

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Authors' affiliations

C J Burns, S Haidar, The Dow Chemical Company, Department of Epidemiology, Midland, MI, USA

J W Albers, S Berent, R J Richardson, University of Michigan, Department of Neurology, Ann Arbor, MI, USA

J W Albers, S Berent, B Giordani, University of Michigan, Department of Psychiatry, Ann Arbor, MI, USA

D Garabrant, J W Albers, S Berent, R Garrison, R J Richardson, University of Michigan, Department of Environmental Health Sciences, Ann Arbor, MI, USA

S Berent, University of Michigan, Department of Psychology, Ann Arbor, MI, USA

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Correspondence to: Dr C J Burns, The Dow Chemical Company, Department of Epidemiology, 1803 Building, Midland, MI 48674, USA; cburns@dow.com

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