Journal of Exposure Science and Environmental Epidemiology (2006) 16, 447–456

www.nature.com/jes

Chlorpyrifos exposure in farm families: Results from the farm family exposure study

BRUCE H. ALEXANDER[a], CAROL J. BURNS[b], MICHAEL J. BARTELS[b], JOHN F. ACQUAVELLA[c], JACK S. MANDEL[d], CHRISTOPHE GUSTIN[c] AND BETH A. BAKER[a]

[a]*Division of Environmental Health Sciences, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA*
[b]*The Dow Chemical Company Midland, Michigan, USA*
[c]*Monsanto Company, St. Louis, Missouri, USA*
[d]*Emory University, Rollins School of Public Health, Atlanta, Georgia, USA*

We used urinary biological monitoring to characterize chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) exposure to farm family members from Minnesota and South Carolina who participated in the Farm Family Exposure Study. Five consecutive 24-h urine samples were obtained from 34 families of licensed pesticide applicators 1 day before through 3 days after a chlorpyrifos application. Daily 3,5,6-trichloro-2-pyridinol (TCP) urinary concentrations characterized exposure profiles of the applicator, the spouse, and children aged 4–17 years. Self-reported and observed determinants of exposure were compared to the maximum postapplication TCP concentration. All participants had detectable ($\geq 1\,\mu g/l$) urinary TCP concentrations at baseline. Applicators' peak TCP levels occurred the day after the application (geometric mean (GM) = 19.0 $\mu g/l$). Postapplication TCP change from baseline in the spouses and children was negligible, and the only reliable predictor of exposure was assisting with the application for children aged 12 years and older. The applicators' exposure was primarily influenced by the chemical formulation (GM = 11.3 $\mu g/l$ for granular and 30.9 $\mu g/l$ for liquid), and the number of loads applied. Repairing equipment, observed skin contact, and eating during the application were moderately associated TCP levels for those who applied liquid formulations. Estimated absorbed doses (μg chlorpyrifos/kg bodyweight) were calculated based on TCP excretion summed over the 4 postapplication days and corrected for pharmacokinetic recovery. The GM doses were 2.1, 0.7, and 1.0 μg/kg bodyweight for applicators, spouses, and children, respectively. Chlorpyrifos exposure to farm family members from the observed application was largely determined by the extent of contact with the mixing, loading, and application process.
Journal of Exposure Science and Environmental Epidemiology (2006) **16,** 447–456. doi:10.1038/sj.jes.7500475; published online 29 March 2006

Keywords: *biomonitoring, pesticide exposure, chlorpyrifos, epidemiologic studies.*

Introduction

Chlorpyrifos (*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate) is an organophosphate insecticide with agricultural and household applications. Estimated annual U.S. domestic use of chlorpyrifos for all purposes was between 13 and 19 million of active ingredient pounds, with 8 and 10 million pounds used for agricultural purposes (Donaldson et al., 2002). Chlorpyrifos is used on a variety of crops, including vegetables, fruit, cotton, tobacco, and corn (United States Environmental Protection Agency (USEPA), 2002). The residential use of chlorpyrifos has been phased out beginning in June, 2000 (USEPA, 2002). The primary toxicological end point of chlorpyrifos exposure is decreased plasma acetylcholinesterase activity. In a study of human volunteers, the no-observed effect level from a single dose was 0.1 mg/kg, and 0.03 mg/kg/day for 28 days of exposure (Nolan et al., 1984). Observable health effects in humans are primarily associated with acute exposures resulting in classic organophosphate poisoning symptoms of tremors, lacrimation, salivation, vomiting, and organophosphate-induced delayed neurotoxicity at very high doses (Albers et al., 1999). The potential for chlorpyrifos-related health effects at subacute exposures is an active topic of research.

The conduct of well-informed risk characterizations and epidemiologic research on any chemical, including chlorpyrifos, requires a clear understanding of exposure determinants and sources of uncertainty. This premise applies to the study of occupationally and non-occupationally exposed

1. Abbreviations: FFES, Farm Family Exposure Study; GM, geometric mean; GSD, geometric standard deviation; μg/kg, micrograms per kilogram bodyweight; μg/kg/day, micrograms per kilogram bodyweight per day; $\mu g/l$, microgram per liter; NHANES, National Health and Nutrition Examination Survey; TCP, 3,5,6-trichloro-2-pyridinol; USEPA, United States Environmental Protection Agency

2. Address all correspondence to: Dr. B.H. Alexander, Division of Environmental Health Sciences, School of Public Health, University of Minnesota, MMC 807 Mayo Building, 420 Delaware Street S.E., Minneapolis, MN 55455, USA. Tel.: +1-612-625-7934.
Fax: +1-612-626-4837. E-mail: balex@umn.edu.
3. Current address: Amgen Global Epidemiology, Thousand Oaks, CA, USA.
Received 1 July 2005; revised 13 December 2005; accepted 15 December 2005; published online 29 March 2006

populations. To help characterize potential exposure to chlorpyrifos from agricultural use, we present biomonitoring data from the Farm Family Exposure Study (FFES) that describe exposure to chlorpyrifos following an application conducted as part of the regular farming operation.

Methods

Study Population

The Human Subjects Research Committee of the University of Minnesota approved the FFES protocol. The target population was families living and working on an agriculture production operation where pesticides are used routinely. The methods for this study are described in detail elsewhere (Baker et al., 2005) (study protocols are available at www.farmfamilyexposure.org). Briefly, families were identified from lists of licensed pesticide applicators in Minnesota ($N = 25{,}301$) and South Carolina ($N = 10{,}805$). The licensed applicators were randomly ordered and contacted sequentially first by mail, then by telephone for recruitment into the study. Eligibility for the study required that (1) the family lived on a farm, (2) the family consisted of the farmer, a spouse, and at least one child between the ages of 4 and 17, (3) the farmer was planning to personally apply chlorpyrifos as part of the normal operation to at least 10 acres of cropland, some of which had to be within 1 mile, and on a contiguous piece of land with the family home, and (4) the family members were willing to collect 24-h urine samples for 5 days (1 day before through 3 days after the application).

Eligible families that were potentially willing to participate were provided with more information about the study and taken through an informed consent process for enrollment. Once enrolled, a study start date was estimated based on predicted chlorpyrifos application time. To the extent possible, the random order in which the applicators were screened for eligibility was preserved when enrolling the families in the study. The actual date of application was flexible to allow for changes brought on by weather and other needs of the farm. Participating families received an incentive to participate.

All applications were conducted in the 2000 and 2001 growing seasons. Ultimately, 34 applications of chlorpyrifos were observed for this study, of which 28 were located in South Carolina and six were in Minnesota. In Minnesota, chlorpyrifos is predominantly used in a granular formulation during planting. The study limited the number of granular applications to five per state, in order to focus on liquid formulations that were expected to result in greater exposure opportunity.

Data Collection

The farmer and spouse were asked to complete an enrollment questionnaire before and a follow-up questionnaire after the study application. The questionnaires emphasized the following areas: demographics, farm production and practices, pesticide application procedures, use of personal protective equipment, self-reported exposures, activities surrounding the study pesticide application, and potential exposure to the children. The questionnaires were reviewed for completeness and, when possible, the participants were re-contacted and asked to provide missing information.

A research team member observed the chlorpyrifos applications. Observations made included the location and size of the field, proximity to the house, equipment used, chemical name and formulation, methods of mixing, personal protective equipment used, clothing worn, occurrence of spills during the mixing and loading process, accidents, for example, process or equipment malfunction that resulted in exposure, or repairs of the equipment, and presence of children, spouse, or pets during the mixing and application process.

The farmer, spouse, and participating children were instructed to collect all urine into 500-ml high-density polyethylene single-void containers for 5 days; the day before pesticide application through 3 days after pesticide application, hereafter referred to as days -1, 0, 1, 2, and 3. The family was provided with coolers and ice packs or a small refrigerator to store the collected urine samples. The date and time of each void was recorded by the study participants and logged by study staff. Urine specimens were picked up daily during the study period. If one or more of the family appeared to have low volumes or few voids the farmer or spouse were asked to encourage full compliance with the study protocol. The individual urine voids were refrigerated, combined proportionally into 24-h composite samples, and then frozen. The 24-h urine composite samples were timed in 24 h intervals based on the exact starting time of the chlorpyrifos application, that is, the baseline 24-h urine collection (day -1) was the 24 h preceding the start of the application, and days 1, 2, and 3 began 24, 48, and 72 h, respectively, after the beginning of application.

Chlorpyrifos is metabolized primarily to 3,5,6-trichloro-2-pyridinol (TCP), which is excreted in the urine (Nolan et al., 1984). To estimate exposure, the composite urine samples were analyzed for TCP, using a sensitive, selective method developed to measure both TCP and 2,4-dichlorophenoxyacetic acid (Brzak, 2001) (available at www.farmfamilyexposure.org). The analyte was hydrolyzed to the non-conjugated form and extracted into toluene. The organic extracts were treated with *N*-methyl-*N*-(tert-butyl-dimethylsilyl)-trifluoroacetamide (MTBSTFA) to form the tert-butyldimethylsilyl derivatives of TCP. Analysis was accomplished by GC/MS operating in the negative ion chemical ionization mode and quantitation performed using derivatized solvent standards. An isotopically labeled internal standard of $^{13}C_2$,^{15}N-TCP was used in the method. Quantitation and confirmation ions for TCP were m/z 161 and 163,

respectively, with ion m/z 168 used for the internal standard $^{13}C_2$,^{15}N-TCP. The limit of quantitation was 1 μg/l (or 1 part per billion), which was 60 $\times$ the noise for TCP. The measured concentrations of TCP were corrected for laboratory fortification recovery. No correction was made for recovery from field and travel spikes because the recoveries were just over 100% on average and the correction would only marginally decrease the estimated exposure. Creatinine concentration in the composite sample was measured and the total creatinine excreted was estimated based on the total volume of urine excreted in each 24-h period to permit the expression of TCP concentrations standardized by creatinine concentrations in the composite sample (μg/g).

Absorbed dose estimates were calculated to aid risk characterization models. Chlorpyrifos dose with the most public health relevance to farm family members includes all sources of exposure. Accordingly, the dose estimates for this study were based on total TCP excretion over the postapplication study period. The daily total urine volumes were multiplied by the TCP concentration from the composite sample to estimate daily chlorpyrifos exposure on the application day through day 3. A mean elimination rate for urinary TCP was calculated from the applicator's urinary TCP data, using the sigma-minus method (Gibaldi and Perrier, 1975). The total TCP recovered in this 4-day period represented 88% of the total TCP excreted from this chlorpyrifos exposure. Calculated dose values assumed 73.4% of the chlorpyrifos is represented by the TCP in urine (Nolan et al., 1984) and were corrected for the molecular weight difference between TCP and chlorpyrifos.

Analysis

An initial exploration of the data involved analysis of scatter plots and measures of central tendency (mean median, range, and geometric mean (GM)) of the daily and the maximum urine TCP concentrations for the applicators, spouses, and children. For concentrations below the limit of detection, the mid-point from 0 to the 1 μg/l limit of detection (0.5 μg/l) was imputed. The distribution of daily concentrations was highly skewed, thus medians, GMs, and geometric standard deviations (GSDs) were used to characterize the central tendency of the exposures. The change from baseline for daily urine concentrations was assessed using a non-parametric sign test.

The GM, GSD, 75th and 90th percentile, and the maximum values for the systemic dose estimates (μg/kg) are presented for applicators, spouses, all children, and children by age category. Statistical differences in the natural log of the systemic dose estimates between these groups were compared in linear models with each family member type entered as a categorical variable. A sensitivity analysis to evaluate impact of incomplete 24-h collections was conducted by assigning the 25th percentile 24-h urine volume for the applicators, spouses, and children aged 4–6, 7–9, 10–12, and 13 and older from all participants in the FFES as the minimum 24-h volume. This analysis will upweight the estimate of TCP excreted over the study period.

Potential determinants of exposure for the spouses and children are described by the GM and SD of the 24-h sample with the highest postapplication TCP concentration. Observed covariates included the closest distance from the field to the house (yards), the number of acres treated, the number of loads, presence of the child or spouse during the application, and observation of opportunity for direct contact with the chemical, for example, physical contact with the chemical, equipment, or treated field. Self-report of washing the clothes used in the application was evaluated as a determinant of exposure for the spouse. To evaluate potential differences by age, the children were stratified at 4–11 and 12 or more years; a cutpoint used in the CDC National Report on Human Exposures to Chemicals (Centers for Disease Control and Prevention, 2003). The potential determinants of applicator exposure, primarily related to application practices, were also evaluated using the maximum postapplication daily concentration. Categories were compared for pesticide formulation, acres treated, loads, observed skin contact, spills or accidents, use of gloves during mixing or application, repair of equipment, tobacco use (smoking or chewing), and eating during the application. Single and multivariable generalized linear models were fit to examine statistical associations for linear and categorical exposure determinants and the natural log of the maximum daily TCP concentration. All analyses were conducted with PC SAS 9.1(SAS Institute Inc., 2003).

Results

All 34 applications were conducted by male applicators (Table 1). The applicator was the father of the children for 33 applications; for one application, the grandfather of the children was the applicator and the children's father assisted with the mixing and loading of the chemical. In all, 50 children participated in the study with ages ranging from 4 to 18. The 18-year-old child was 17 at the time of recruitment, but turned 18 before the application. Six of the applicators had applied chlorpyrifos the week before the on-study application, but none of the spouses or children reported mixing or applying chlorpyrifos before, or during the study period. Five of the spouses and 18 of the children were present at sometime during the application, and an opportunity for direct contact with the chemical, either in the field or through the application process, was reported for one spouse and seven children. Overall compliance with the urine collection protocol was good and represented excretion of the TCP over the 24 h intervals. Three or more voids were collected for more than 80% of the children. The children younger than 12 had lower volumes collected when

Table 1. Characteristics of applicators, spouses, and children of families in the Farm Family Exposure Study that applied chlorpyrifos for the study application.

	Applicators	*Spouse*	*Children*
N	34	34	50
State			
MN	6	6	7
SC	28	28	43
Gender			
M	34	0	31
F	0	34	19
Age			
Mean (range)	46.3 (40–54)	41.9 (30–64)	11.1 (4–18)
Mix or apply chlorpyrifos in previous week			
Yes	6	0	0
No	28	34	50

Table 2. Characteristics of the observed chlorpyrifos application.

	N
Method of application	
Boom spray	24
In-furrow	10
Formulation	
Emulsifiable concentrate	24
Granule	10
Acres treated	
Median	30
Range	5–318
Number of loads	
Median	2
Range	1–14
Gloves used	
Rubber	16
Leather or cloth	4
None	14
Applicator smoked or chewed tobacco during application	
Yes	7
No	27
Ate during application	
Yes	9
No	25
Enclosed cab on tractor	
Yes	19
No	15

compared to the entire FFES population with 43% of the 115 24-h samples in that age group being below 300 ml. In total, 98% of the spouses and 97% of the applicators collected three or more voids for each 24-h sample. More than 600 ml were collected by 80% of the spouses and 90% of the applicators. At least one void was available for all participants for the day before application through the second day after application. One spouse and four children did not collect urine samples on the third day after application

Emulsifiable concentrate-based spray liquids were applied by spray boom, whereas granules were applied in furrow. (Table 2) The observed applications covered between 5 and 318 acres. The minimum target of 10 acres was not met in one case owing to changes in application plans after enrollment. In total, 16 of the applicators (47%) were observed to use chemical-resistant (rubber) gloves during the mixing/loading or the application. Four of the applicators who applied granular formulations used cloth or leather gloves. In general, the gloves were used for mixing and loading, but not always during the application process.

The preapplication GM TCP concentrations were similar for applicators and children: 7.8 and 7.6 μg/l, respectively, but the concentrations per gram of creatinine were slightly higher in the children (4.2 vs. 5.1 μg/g) (Table 3). As expected, the applicators had the largest change from baseline with the highest GM concentrations of TCP being measured on the day after the application. The average difference between baseline and postapplication TCP urine concentration in the spouses and children was indistinguishable.

Children aged 4–11 had modestly higher concentrations than those 12 and older both for the highest detected (Table 4) and the preapplication concentration (8.8 vs. 6.4 μg/l). The older children had a much greater range of postapplication concentrations, primarily owing to a few of them being present, and helping with the application process. The GM calculated doses were significantly higher in the applicators (2.1 μg/kg body weight (bw)) than the spouses (0.7 μg/kg bw). The doses obtained for the spouses were markedly lower than the ones observed in the applicators and younger children and the older children were between the spouses and the younger children. The older children, however, had a wider distribution of doses and the 90th percentile was higher when compared to the younger children (2.8 vs. 2.2 μg/kg bw). This difference is attributable to the children who were exposed while assisting with the application process. The GM dose for the seven children who had direct contact with the process was 1.9 μg/kg bw, and included the maximum of 6.4 μg/kg bw. The sensitivity analysis that set a minimum volume for each 24-h period did not change the overall distribution. The adjusted GMs were 2.3, 0.7, 1.4, and 1.0 μg/kg bw for the applicators, spouses, children aged 4–11, and children aged 12 and older, respectively. The adjusted 90th percentiles increased to 2.4

Table 3. Summary of urine TCP concentrations and TCP per gram creatinine for applicators, spouses, and children by study day.

Study day	*Applicators*		*Spouses*		*Children*	
	μg/l[a]	μg/g[b]	μg/l	μg/g	μg/l	μg/g
Preapplication						
N	34	34	34	34	50	50
Median	6.6	3.9	4.4	3.4	6.5	4.8
Range (min, max)	1.6, 44.1	0.6, 29.6	1.6, 24.1	1.7, 19.3	1.9, 31.5	1.7, 19.8
GM	7.8	4.2	4.7	3.6	7.6	5.1
GSD	2.3	2.3	1.9	1.8	1.8	1.8
Application						
N	34	34	34	34	50	50
Median	12.3	7.1	4.7	3.6	7.3	5.5
Range (min, max)	4.2, 117.8	2.0, 32.8	0.5, 34.7	0.5, 26.6	1.4, 119.5	1.4, 31.8
GM	15.6	7.6	4.6	3.8	7.6	6.0
GSD	2.3	2.3	2.2	2.0	2.1	1.9
p-value[c]	<0.0001		0.85		0.92	
Day 1						
N	34	34	34	34	50	50
Median	15.3	8.6	4.7	4.0	6.7	6.2
Range (min, max)	4.1, 293.0	2.6. 110.7	1.2, 34.7	1.1, 19.1	1.1, 77.1	11.3, 27.4
GM	19.0	10.5	5.0	4.2	7.6	5.0
GSD	2.8	2.6	2.0	2.0	2.2	2.1
p-value	<0.0001		0.80		0.96	6.0
Day 2						
N	34	34	34	34	50	50
Median	13.5	8.3	4.3	4.3	9.0	5.9
Range (min, max)	4.9, 303.5	2.0, 121.1	1.2, 93.4	1.2, 33.1	2.7,48.1	0.6, 21.5
GM	16.4	9.3	4.7	4.2	8.5	5.9
GSD	2.5	2.6	2.2	1.9	2.1	1.9
p-value	<0.0001		0.63		0.21	
Day 3						
N	33	33	34	32	43	43
Median	12.9	7.7	4.9	4.0	8.9	6.7
Range (min, max)	4.8, 178.7	2.5, 78.0	0.5, 57.8	1.8, 30.7	1.9, 38.4	1.6, 23.1
GM	14.4	9.0	5.0	4.5	8.4	6.5
GSD						
p-value	<0.0001		0.73		0.16	

[a]μg/l TCP concentration in urine.
[b]μg/g TCP per gram creatinine.
[c]*p*-value compares application day 0–day 3 to baseline.

and 3.1 μg/kg bw for the younger and older children respectively, but did not change for the applicators and spouses.

There was little discernable variation in the maximum urine TCP concentrations for the spouses in relation to several determinants of exposure (Table 5). The urine TCP concentration appeared modestly higher for applications with 50–75 acres, but the overall association with number of acres was not significant. The same was true for spouses who were observed to be present during the application process. A potential indicator of secondary occupational exposure, laundering the clothes worn during the application, was not associated with exposure; however, the spouse with the highest postapplication concentration (93 μg/l) had no observed or reported evidence of exposure other than washing clothes the day after the application. The GM TCP concentrations also varied little by the selected exposure determinants for children of either age group. The single exception was whether the child was observed to be present during the application, but was limited to the children aged 12 and older. For the 10 children in this category who were, and the 17 who were not present, the respective GM urine TCP concentrations were 20.2 and 8.6 μg/l ($p = 0.02$). There was no difference between the children aged 4 and 11. The

Table 4. Daily maximum TCP concentrations and estimated chlorpyrifos dose from four day TCP excretion for applicators, spouses, and children ages 4–11 and 12 and older.

	Applicator	*Spouse*	*Children*	*Children age 4–11*	*Children ≥12*
N	34	34	50	23	27
Detectable pre-application (%)	100	100	100	100	100
Detectable any day (%)	100	100	100	100	100
Maximum urine TCP					
Median	18.9	6.2	11.2	12.8	10.8
Concentration (μg/l)[a]					
Range	6.0, 303.5	1.7, 93.4	4.3, 119.5	4.5, 38.7	4.3, 119.5
GM	23.0	6.7	12.2	12.7	11.8
GSD	2.7	2.1	2.0	1.8	2.2
Chlorpyrifos dose (μg/kg)[b]					
GM	2.1*,+	0.7	1.0	1.2*	0.9
GSD	2.5	2.0	1.9	1.7	2.2
Maximum	16.3	4.1	6.3	2.5	6.3
90th Percentile	7.4	1.4	2.2	2.2	2.8
75th Percentile	4.6	1.1	1.5	1.7	1.3

[a]Limit of detection was assigned to 0.5 μg/l.
[b]Dose estimated based on excretion of TCP from all sources for days 0, 1, 2, and 3.
*Log dose different from spouse $p<0.05$.
+ Log dose different from children $p<0.05$.

main difference here is that the older children were more likely to be actively assisting with the operation, rather than being a bystander; thus the exposure opportunity was higher.

Compared to other family members, the applicators had much higher overall urine TCP concentrations, which varied substantially by formulation, and the number of loads applied (Table 6). The differences in the concentrations by State are attributable to the formulation, as five of six applications in Minnesota were granular formulation for in-furrow application. The difference in TCP concentration for liquid and granular formulations was large, 30.9 and 11.3 μg/l ($p=0.007$). This is likely attributable to the potential for absorption through skin contact for the liquid formulations. Over half of the applications of chlorpyrifos were conducted with one or two loads, and only four applications had six or more loads. However, the TCP urine concentrations were markedly higher for the four applicators who mixed and loaded chlorpyrifos more than five times, and there was an overall significantly positive increase of urine TCP with the number of loads as a continuous measure, suggesting that exposure opportunity during frequent mixing and loading is an important determinant of exposure. Other covariates associated with higher urinary TCP concentrations were tobacco use during application, repairing equipment, having a spill or accident, and not having a closed cab on the tractor; however, none were significant predictors of exposure. Self-report of equipment repair ($N=19$) was more frequent than observed repair ($N=9$), as the repair may have been repaired after the application was complete and the observer had left the farm. The urine TCP concentrations were modestly lower for those self-reporting repair. One of the farmers observed to repair equipment did not self-report repairing the equipment.

Formulation was clearly a key predictor of exposure in the applicators; therefore, covariates were examined by formulation type (Table 7). In applications with liquid formulations, the number of loads was correlated with urine TCP concentration (p-trend $=0.013$); however, the number of acres applied was not. The covariates of observed skin contact, spills or accidents, repairing of equipment, and tobacco use during application were modestly associated with exposure, but were statistically imprecise. Wearing rubber gloves at some point during the application was associated with lower urine TCP concentrations among those applying the liquid formulation, but the difference was not statistically significant.

Discussion

We found appreciable changes in urinary TCP concentration for applicators and the few children that assisted with the application process. Children had higher pre- and postapplication concentrations when compared to the farm spouses, but overall, children and spouses who did not assist with the application had little, if any, exposure attributable to the application. In the context of exposure assessment for epidemiologic studies or risk characterization, the act of mixing and applying chlorpyrifos will result in exposure for applicators and a minority of family members; however, the

Table 5. Geometric means and standard deviations of the maximum urine TCP concentration from 24-h samples for spouses and children ages 4–11 and 12 and over.

	Spouses			*Children*					
				4–11			12+		
	N	GM	GSD	N	GM	GSD	N	GM	GSD
Total	34	6.7	2.1	23	12.7	1.8	27	11.8	2.2
State									
MN	6	5.7	1.6	1	10.0		6	11.9	1.9
SC	28	6.9	2.2	22	12.9	1.8	21	11.8	2.3
Distance to house (yards)									
<75	9	6.7	1.8	4	12.1	1.9	6	10.2	2.1
75–175	4	5.8	1.5	3	12.7	1.4	3	9.9	2.4
175–600	7	6.3	1.7	3	28.6	1.4	8	13.1	1.9
>600	14	7.1	2.6	13	10.7	1.7	10	12.4	2.6
Acres treated									
<25	13	6.0	1.7	10	15.3	1.7	10	10.1	2.0
25⩽50	12	5.7	1.7	7	10.0	1.8	10	10.8	1.7
50⩽75	6	11.5	3.6	5	11.1	1.8	5	18.5	3.6
75+	3	6.5	1.4	1	20.3		2	12.8	2.5
Loads mixed and applied									
1–2	18	5.5	1.8	12	14.8	1.7	16	10.0	1.9
3–5	12	8.6	2.4	9	9.1	1.6	8	17.3	2.8
6+	4	7.1	2.2	2	24.2	1.3	3	10.4	2.1
Present during application									
Yes	5	8.6	2.4	8	13.1	1.6	10	20.2	2.3
No	29	6.4	2.0	15	12.5	1.9	17	8.6	1.8
Direct contact with process observed									
Yes	0			0			7	21.8	2.7
No	34	6.7	2.1	23	12.7	1.8	20	9.5	1.8
Wash pesticide application clothes									
Yes	27	6.4	2.2						
No	7	7.9	1.5						

GM = geometric mean; GSD = geometric standard deviation.

presence of a family member on the farm at the time of a granular or boom-spray liquid chlorpyrifos application is not a strong predictor of episodic exposure.

All study participants had detectable urine TCP concentrations at baseline and postapplication. The children had higher baseline concentrations of this chlorpyrifos metabolite than the spouses, and the 4- to 11-year-old children had, on average, higher baseline concentrations than the older children. Similar findings were reported from the 1999 to 2000 National Health and Nutrition Examination Survey (NHANES), where measurable urine TCP was reported in all children and at least 75% of adults (Centers for Disease Control and Prevention, 2003). The GM TCP concentrations reported from NHANES for children ages 6–11 and 12–19 years were 2.9 and 2.4 μg/l, respectively, and 1.5 μg/l for adults, approximately one-third of the preapplication concentrations we observed. Unlike our study, the NHANES data reflect exposures based on single-void samples collected over different seasons across the country. In contrast, other population-based research has reported urine TCP concentrations closer to the FFES baseline concentrations. In a study of children in Minnesota in 1997, urine TCP concentrations of 7.2 μg/l for urban children and 4.7 μg/l for rural children were observed (Adgate et al., 2001). In a cohort of pregnant mothers enumerated at a large urban hospital between 1998 and 2001, the median concentration of urine TCP were higher than the farm spouses in our study (11.3 vs. 3.5 μg/g creatinine) (Berkowitz et al., 2003). A study of children in families living in a tree fruit production region of Washington State in 1995 had a much smaller

Table 6. Selected determinants of the maximum urine TCP concentration from 24 h samples for applicators.

	N	*GM*	*GSD*
State			
MN	6	12.6	1.8
SC	28	26.2	2.7
Formulation			
Granular	10	11.3	1.8*
Liquid	24	30.9	2.7
Acres treated			
<25	13	22.5	2.7
$25 \leqslant 50$	12	22.0	2.9
$50 \leqslant 75$	6	19.2	3.0
75+	3	43.2	2.5
Loads applied			
1–2	18	17.8	2.6**
3–5	12	22.7	2.3
6+	4	76.1	2.5
Skin contact with chemical observed			
Yes	12	26.2	2.9
No	22	21.5	2.7
Enlosed cab on tractor			
Yes	14	19.6	2.5
No	20	28.9	3.0
Any spillage of chemical or accident[a]			
Yes	9	28.3	3.4
No	25	21.4	2.5
Wore chemical resistant (rubber) gloves			
Yes	16	19.9	2.9
No	18	27.1	2.5
Observed to repaired equipment during application			
Yes	9	31.6	3.5
No	25	20.5	2.5
Reported repairing application equipment			
Yes	19	26.2	2.7
No	15	19.5	2.8
Reported washing application equipment			
Yes	18	24.5	2.5
No	16	21.5	3.1
Smoked or chewed tobacco during application			
Yes	7	41.4	3.4
No	27	19.8	2.5
Ate during application			
Yes	9	20.1	2.3
No	25	24.2	2.9

*$p = 0.007$, **p for linear trend: natural log of the TCP concentration regressed on the number of loads as a continuous variable = 0.003.
[a]Spill or accident in mixing/loading process or during application that might result in exposure.

Table 7. Selected determinants of the maximum urine TCP concentration from 24 h samples for applicators by formulation for application chemical.

	Formulation					
	Granular			*Liquid*		
	N	*GM*	*GSD*	*N*	*GM*	*GSD*
Total	10	11.3	1.8	24	30.9	2.7
Acres treated						
<25	1	8.7	—	12	24.4	2.7
$25 \leqslant 50$	5	12.5	1.8	7	33.1	3.0
$50 \leqslant 75$	3	7.6	1.2	3	48.4	2.0
75+	1	29.2	—	3	52.4	3.4
*Loads mixed and applied**						
1–2	6	10.0	1.9	12	23.8	2.8
3–5	4	13.7	1.9	8	29.3	2.3
6+	0			4	76.1	2.5
Skin contact with chemical observed						
Yes	5	12.5	1.8	7	44.4	2.8
No	5	10.3	1.9	17	27.7	2.7
Any spillage of chemical or accident[a]						
Yes	4	10.7	2.0	5	61.6	2.6
No	6	11.6	18	19	25.8	2.6
Wore chemical resistant (rubber) gloves						
Yes	1	29.2	NA	15	26.9	3.0
No	9	10.1	1.7	9	38.9	2.6
Observed to repair equipment during application						
Yes	3	8.7	1.2	6	60.4	2.7
No	7	12.7	2.0	18	25.6	2.5
Smoked or chewed tobacco during application						
Yes	0			7	41.4	3.4
No	10	11.3	1.8	17	27.4	2.5
Ate during application						
Yes	5	11.9	1.7	4	38.7	2.0
No	5	10.8	2.0	20	29.6	2.9

NA = not applicable.
*p for linear trend: natural log of the TCP concentration regressed on the number of loads of liquid formulation as a continuous variable = 0.013.
[a]Spill or accident in mixing/loading process or during application that might result in exposure.

percent of detectable urine samples in agricultural and non-agricultural families (24% detectable TCP concentrations) with mean concentrations ranging from 4.5 μg/l in the reference children to 6.4 μg/l in the farm-worker children (Fenske et al., 2002). The median values in the Washington study were all non-detectable, but the range for children from agricultural families was up to 100 μg/l. The limit of detection

for TCP was a notable difference between the two studies (1.0 μg/l for FFES and 8 μg/l for Fenske et al.) and likely contributed to the difference in proportion of detectable. The FFES characterized exposure with 24-h urine collections, whereas single-void urine samples were collected in the above-referenced studies. A variety of factors, including hydration status, can influence the concentration of urine over a 24 h period, thus a single-void sample may over- or underestimate TCP levels from a 24-h period, but the extent of that variability is not well characterized.

The sequential 5-day 24-h urine samples collected for this study allowed for the detailed characterization of the urine TCP excretion profiles. In the applicators, the maximum postapplication concentrations peaked the day after application; however, there was considerable individual variability. Chlorpyrifos is fairly rapidly metabolized and excreted as TCP, with a half-life of 27 h (Nolan et al., 1984). The FFES data represent exposure scenarios occurring under normal field use conditions, thus the peak postapplication urine TCP concentrations occurring on the day after exposure has implications for biological monitoring of agricultural workers exposed to chlorpyrifos.

As expected, applicators showed an increase in urinary TCP concentration postapplication. Their exposures were moderated by the formulation type and opportunities for direct skin contact with the chemical. While use of both formulations was associated with measurable exposure, the granular formulations were associated with much lower urinary TCP concentrations than liquid formulations. In this study, the apparent primary route of exposure is through direct skin contact; however, respiratory and oral exposure cannot be ruled out. The liquid formulation may more readily absorb through the skin and the process of mixing and loading this formulation creates more opportunities for direct contact. Moreover, the liquid applications are done with a boom sprayer, whereas granular applications were put into the furrow during planting. The aerosolized liquid has much greater chance of coming into direct contact with skin or being inhaled during application. Beyond formulation, the number of loads was the only variable predictive of applicator urine TCP concentrations. Several other covariates were suggestive of an association, all of which implied some direct contact with the chemical, including observed skin contact and repairing equipment during application. Some standard predictors of exposure, including use of protective gloves, having an enclosed tractor cab, and the number of acres applied were not predictive of exposure. Glove use was weakly associated with TCP concentration when the application was made with a liquid formulation. Gloves tended to be worn during mixing and loading, but not always during the application, allowing for exposure opportunity when adjusting equipment, etc. Moreover, all gloves may not be fully chemically resistant to chlorpyrifos, which may increase rather than decrease chance for exposure if the chemical remains in the glove over time. Four of the applicators applying granular chlorpyrifos wore cloth or leather gloves, but the differences in their exposures were minimal. These and other predictors of exposure are used as weighting factors for exposures in epidemiologic studies (Dosemeci et al., 2002). Although limited by the number of applicators and only one application, this study demonstrates the difficulties of quantifying exposure on a population basis when contending with multiple exposure routes. Another complicating factor when applying exposure models is the chemical specific nature of exposures. For example, the exposure profile for chlorpyrifos is vastly different from that of glyphosate, another chemical studied in the FFES. Glyphosate has a much lower prevalence of detectable exposure, both pre- and postapplication, lower urinary concentrations, lower peak urine excretion occurring on the day of application, and a strong relationship between glove use and lower urinary concentrations (Acquavella et al., 2004).

The applicators' TCP concentrations observed in this study were moderate in comparison to other occupational chlorpyrifos exposures. A major non-agricultural use of chlorpyrifos is for termite control. Studies of termite control applicators have shown urine TCP concentrations 15 to 20 times the concentrations (GM 169–262 μg/g creatinine) observed in this study (Hines and Deddens, 2001). Similar concentrations are observed in chlorpyrifos manufacturing workers in 1999 (Albers et al., 1999, 2004). Work-related exposure opportunity is more frequent in these populations than for most farmers. Termite applicators may apply chlorpyrifos on a daily basis. In this study, enrollment data showed that a majority (85%) of the applicators had applied all pesticides 15 or fewer days in the previous year, and no chlorpyrifos applicator made more than 50 pesticide applications (the number of chlorpyrifos applications was not known).

In this study, we estimated total dose from all sources as the metric with the most public health relevance. In doing so, we attributed all TCP excreted postapplication to chlorpyrifos exposure from the farm living and working environment. This, however, is likely to overestimate chlorpyrifos dose as TCP concentrations represent metabolism of absorbed chlorpyrifos, but may also reflect direct exposure to environmental TCP, particularly from the diet (Wilson et al., 2003). The estimated chlorpyrifos doses represent the integrated dose based on excretion of TCP over 4 days. To characterize the risk associated with this 4-day integrated estimated dose, the following must be considered: (1) The dose estimate may represent exposure occurring on a single day, that is, direct exposure on the day of application, or result from direct and indirect exposure over time. (2) The application processes are infrequent over the course of a year or growing season, thus the estimates do not represent daily doses absorbed over these time periods. (3) The route of exposure is likely an undetermined mixture of dermal, oral, and respiratory. The current non-occupational risk assess-

ment for chlorpyrifos by the USEPA is based on plasma and red blood cell cholinesterase inhibition in laboratory animal studies (USEPA, 2000). The toxicological benchmarks for no-observed-adverse effect level (NOAEL) for dietary exposure are 0.5 mg/kg/day (acute) and 0.03 mg/kg/day (chronic). The NOAEL for short- to intermediate-term inhalation exposure is 0.1, and 5 mg/kg/day for dermal exposure, which are also the benchmarks for short-term toxicity end points for workers. Placing the doses, we estimated in the prevailing risk assessment paradigm requires numerous assumptions about route of exposure and frequency of application. However, these data provide a starting point for further risk characterization in farm families based on actual use scenarios.

The FFES is unique in its effort to characterize chlorpyrifos exposure for farm families. The intent of the study was to estimate exposures based on usual practice exposure scenarios. The use of five serial 24-h urine collections allowed for more complete characterization of exposure and an estimation of the absorbed dose. While comprehensive as an exposure study, it is not without limitations. Although the compliance appeared to be good, it is likely that some urine collections are not complete. This may underestimate absorbed dose, but not necessarily urine concentration. The potential impact of poor compliance on the systemic dose calculations were described with one sensitivity analysis which assigned a minimum urine volume for each 24-h sample based on the entire FFES. This increase in urine volume increased the estimated dose, but did not change the overall dose distribution or interpretation. The potential for underestimating dose is likely greatest in young children where collection of all urine samples for a 24-h period is difficult. The study was able to characterize exposure based on present practice. However, the extent to which this represents exposure scenarios in the past or future is not clear. It is also based on only one application. While beyond the scope of this study, a relevant question yet to be answered is how much variation does an applicator and the family members experience over one or more seasons.

In conclusion, the chlorpyrifos exposures observed in this study are linked to activities relating to the application of the chemical and its formulation. Overall, the exposures encountered by the farm families are not alarming. Nevertheless, there is potential to reduce non-occupational exposures to all family members by avoiding the immediate vicinity of pesticide mixing and application activities.

Acknowledgements

The FFES was funded through a grant to the University of Minnesota by Bayer, Dow, DuPont, FMC, Monsanto, Syngenta, and the American Chemistry Council. We appreciate the cooperation of participating farm families and advice from sponsoring company scientists and the FFES Advisory Panel, chaired by Dr. Harris Pastides.

References

Acquavella J.F., Alexander B.H., Mandel J.S., Gustin C., Baker B., and Chapman P., et al. Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. *Environ Health Perspect* 2004: 112: 321–326.

Adgate J.L., Barr D.B., Clayton C.A., Eberly L.E., Freeman N.C., and Lioy P.J., et al. Measurement of children's exposure to pesticides: analysis of urinary metabolite levels in a probability-based sample. *Environ Health Perspect* 2001: 109(6): 583–590.

Albers J.W., Cole P., Greenberg R.S., Mandel J.S., Monson R.R., and Ross J.H., et al. Analysis of chlorpyrifos exposure and human health: expert panel report. *J Toxicol Environ Health Part B* 1999: 2(4): 301–324.

Albers J.W., Garabrant D.H., Schweitzer S.J., Garrison R.P., Richardson R.J., and Berent S. The effects of occupational exposure to chlorpyrifos on the peripheral nervous system: a prospective cohort study. *Occup Environ Med* 2004: 61(3): 201–211.

Baker B.A., Alexander B.H., Mandel J.S., Acquavella J.F., Honeycutt R., and Chapman P. Farm Family Exposure Study: methods and recruitment practices for a biomonitoring study of pesticide exposure. *J Expo Anal Environ Epidemiol* 2005: 15(6): 491–499.

Berkowitz G.S., Obel J., Deych E., Lapinski R., Godbold J., and Liu Z., et al. Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. *Environ Health Perspect* 2003: 111(1): 79–84.

Brzak K. Analytical method validation for the determination of 2,4-dichlorophenoxy acetic acid (2,4-D) and 3,5,6-trichloro-2-pyridinol (TCP) in human Urine. *Dow Chemical Company* 2001. Available: http://www.farmfamilyexposure.org/AnalytMeth_24D_TCP.pdf2004].

Centers for Disease Control and Prevention. *Second National Report on Human Exposure to Environmental Chemicals* 2003. Available: http://www.cdc.gov/exposurereport.

Donaldson D., Kiely T., and Grube A. *Pesticide Industry Sales and Usage. 1998 and 1999 Market Estimates*. Office of Pesticides, Prevention, and Toxic Substances, US EPA, 2002. Available: http://www.epa.gov/oppbead1/pestsales/99pestsales/market_estimates1999.pdf (accessed July 14, 2004).

Dosemeci M., Alavanja M.C., Rowland A.S., Mage D., Zahm S.H., and Rothman N., et al. A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. *Ann Occup Hyg* 2002: 46(2): 245–260.

Fenske R.A., Lu C., Barr D., and Needham L. Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. *Environ Health Perspect* 2002: 110(5): 549–553.

Gibaldi M., and Perrier D. *Pharmacokinetics*. Marcel Dekker: New York, 1975.

Hines C.J., and Deddens J.A. Determinants of chlorpyrifos exposures and urinary 3,5,6-trichloro-2-pyridinol levels among termiticide applicators. *Ann Occup Hyg* 2001: 45(4): 309–321.

Nolan R.J., Rick D.L., Freshour N.L., and Saunders J.H. Chlorpyrifos: pharmacokinetics in human volunteers. *Toxicol Appl Pharmacol* 1984: 73(1): 8–15.

SAS Institute Inc. *SAS 9.1 for Windows*. Cary, NC USA, 2003.

United States Environmental Protection Agency (USEPA). *Chlorpyrifos Facts*. US Environmental Protection Agency, 2002. Available: http://www.epa.gov/oppsrrd1/REDs/factsheets/chlorpyrifos_fs.htm, (accessed July 14, 2004).

USEPA. *Chlorpyrifos Revised Risk Assessments*. US Environmental Protection Agency. 2000. Available: http://www.epa.gov/pesticides/op/chlorpyrifos.htm, (accessed July 14 2004).

Wilson N.K., Chuang J.C., Lyu C., Menton R., and Morgan M.K. Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. *J Expo Anal Environ Epidemiol* 2003: 13(3): 187–202.