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Farm Family Exposure Study: methods and recruitment practices for a biomonitoring study of pesticide exposure

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Purpose: The Farm Family Exposure Study was initiated to characterize pesticide exposure to farm family members around the time of one pesticide application in a manner that will facilitate exposure assessment in epidemiologic studies of pesticides.
Methods: A sample of farm families with children was recruited by randomly selecting farmers from lists of licensed pesticide applicators in Minnesota and South Carolina. Eligible families were selected from among those who planned to apply one of three chemicals, glyphosate, 2,4-D, or chlorpyrifos, as part of their normal operations. The applicator, spouse, and all children in the family ages 4–17 years were included in the study. The applicator and spouse completed self-administered questionnaires addressing demographics, farming practices and potential exposures to them and their children. Field observers documented the application, recorded application practices, equipment, potential exposures, and the presence of children or spouses in the immediate vicinity of pesticide activities. All study participants were asked to collect each urine void for 5 days, 1 day before through 3 days after the application. Pesticides were measured in 24-h composite urine samples with a one part per billion limit of detection.
Results: Of 11,164 applicators screened, 994 families met the inclusion criteria. Of these, 95 families were enrolled. Enrollees were similar in most characteristics to their peers who were not participants in the study. In total, there were 106 applications, 10 of which involved more than one chemical. This resulted in urinary data for 48 farmers and spouses and their 79 children for glyphosate, 34 farmers and spouses and their 50 children for chlorpyrifos, and 34 farmers and spouses and their 53 children for 2,4-D. Compliance with the 24-h urine collection was particularly good for the adult participants. There were more missing samples for children than for adults, but overall compliance was high.
Conclusion: The Farm Family Exposure Study should provide insights about pesticide exposure under real world conditions and thereby facilitate improved exposure assessment in epidemiologic studies of agricultural populations.
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Introduction

Characterizing exposure is one of the greatest challenges in the study of how adverse health outcomes may be related to pesticides (Blair and Zahm, 1990a, b, 1995; Morrison et al., 1992; Dich et al., 1997; Fleming and Herzstein, 1997; Zahm and Ward, 1998). Epidemiological studies have largely focused on agricultural populations because the likelihood of appreciable pesticide exposure is greater than for nonfarming populations. Agricultural-related exposure to pesticides can occur from both occupational and nonoccupational sources. Farm family members and farm workers may be exposed directly through application through secondary routes such as contaminated clothing, household surfaces, footwear and drift from a pesticide application. Nonoccupational pesticide exposure in children may occur from working or playing in fields following an application, drift from agricultural use, or through the use of pesticides in gardens, homes or on lawns.

Epidemiologic studies have associated several health effects with subacute pesticide exposure, including cancer, reproductive outcomes, immunologic effects, endocrine disruption, and neurologic effects (Weisenburger, 1993; Savitz et al., 1994; Kavlock et al., 1996; Fleming and Herzstein, 1997), but the results have not been consistent. Overall cancer incidence and mortality for farmers or agricultural workers is generally lower than for the general population (Pearce and Reif, 1990; Maroni and Fait, 1993; Blair and Zahm, 1995;

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Alavanja et al., 1996; Sperati et al., 1999), however, some studies have reported an increased risk of leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, soft tissue sarcoma, lip, stomach, skin, prostate, and brain cancer in agricultural workers (Pearce and Reif, 1990; Blair et al., 1992; Maroni and Fait, 1993; Blair and Zahm, 1995; Alavanja et al., 1996; Acquavella et al., 1998; Sperati et al., 1999).

Epidemiologic studies generally rely on self-reported use of pesticides or job title (farmer or pesticide applicator) to differentiate those with or without pesticide exposure. The validity of these approaches has been debated in the epidemiologic literature (Brown et al., 1990; Boyle and Brann, 1992; Correa et al., 1994; Fritschi et al., 1996; Olsen and Bodner, 1996; Dich et al., 1997; Zahm et al., 1997; Acquavella et al., 1998). Farmers may be quite knowledgeable about the chemicals they use but may have difficulty recalling the formulation used and exact amount of pesticide that was applied. Biomonitoring studies, such as the Farm Family Exposure Study, offer the opportunity to assess methods for developing predictors of exposure intensity that are directly translatable to epidemiologic research.

The Farm Family Exposure Study (FFES) was initiated to quantify the amount of pesticide absorbed by farm family members around the time of a pesticide application conducted under real-world conditions. The study included three commonly used pesticides: glyphosate, 2,4-D, and chlorpyrifos. In addition, information was also collected on various surrogate measures of exposure, including specific farming activities, self-reported exposure, and trained field staff's recorded observations on the day of application for comparison with self-reported information. The biomonitoring of exposure and absorbed dose will help develop and evaluate epidemiologic exposure models that, by necessity, employ surrogate measures of exposure. Moreover, correlates of exposure for farm families can also be addressed within this study.

The FFES was initiated in 1999 with a pilot study of five farm families, which led to the full study of 95 families during the years 2000 and 2001. This paper presents an overview of the pilot study and provides details about the methods and data and population recruited for the full-scale study, the results of which have been and will be published elsewhere (Acquavella et al., 2004; Mandel et al., 2005).

Methods

Pilot Study

It was uncertain whether farm families would comply with the extensive urine collection planned for the Farm Family Exposure Study. In order to assess likely compliance and to develop methods for conducting a full-scale biomonitoring study in farm families, we conducted a pilot study in 1999 on five farm families in Florida. Study personnel asked county cooperative extension agents in Florida to identify families who would be willing to participate in this pilot study and who actively applied pesticides. The county extension agents identified 789 farm families, from 14 different Florida counties, as possible participants. Study personnel contacted these farm families and after screening 340 families found five farm families who were willing to participate in the pilot study. Eligibility for the pilot study required that: (1) The farmer planned to apply an intended study pesticide (2,4-D, glyphosate, or chlorpyrifos) to at least 50 acres of crop land as a part of their normal operations, (2) the family lived on the farm and included the farmer, a spouse and at least one child between the ages of 6 and 16 years (if there was more than one child, the participating child was selected randomly), (3) the spouse and child did not mix or apply pesticides during the study, and (4) the farmer, spouse and participating children were each willing to collect all their urine in containers for 5 consecutive days.

The pilot study was successful in recruiting farm families to participate in the study and to complete a rigorous protocol requiring 5 days of 24-h urine collections. Three applicators applied glyphosate during the pilot study, one farmer applied 2,4-D and one farmer applied glyphosate and then applied chlorpyrifos 2 days later. The study was well received by participants and the field staff was able to coordinate the delivery and retrieval of questionnaires, urine sampling supplies and collected specimens, and to observe the field application methods and practices of the farmer.

The pilot study also identified several weaknesses that were modified for the full-scale study. The process of selecting farmers based on input from county extension agents did not provide a representative sample; therefore a more systematic selection method of identifying applicators from a statewide list of licensed pesticide applicators was adopted. The pilot study enrolled only one child per family, which was sometimes a source of controversy for participating families. To increase the number of children in the study and reduce potential selection bias, all children in the farm families were eligible for enrollment and the eligible age range for children was increased to ages 4–17 years. Several of the farms initially considered for the pilot study were applying to fewer than 50 acres, but more than 10 acres of land. To better represent farms applying the target chemicals, the full-scale study included farmers applying pesticides to 10 or more acres of cropland. The requirement that the spouse and child not participate in chemical applications was dropped to allow the study to evaluate more real-world exposure scenarios.

FFES Phase Two

The full-scale study was conducted over a 2-year period (2000–2001) in two states: Minnesota and South Carolina. This allowed for a range of agricultural scenarios to be

included in the study. Lists of licensed pesticide applicators in Minnesota ($N = 25,301$) and South Carolina ($N = 10,805$) were obtained from the respective state Departments of Agriculture. The lists were randomly ordered and we contacted applicators sequentially from the randomized lists. An initial solicitation letter was sent to the applicator, followed by a telephone call from a trained interviewer to assess eligibility and willingness to participate in the study. A standardized script and questionnaire were used to conduct the telephone screening. Further tracing was attempted on all applicators not initially contacted, and the interviewers made at least nine attempts at different times of the day to contact each applicator by telephone.

Eligibility for the Farm Family Exposure Study required that; (1) the family lived on a farm and consisted of the farmer, a spouse and at least one child between the ages of 4 and 17 years, (2) the applicator (applicator had to be a family member living on the farm) planned to apply one or more of the study pesticides, 2,4-D, glyphosate, or chlorpyrifos, during the study period as part of their normal operation, (3) the chemical would be applied to at least 10 acres of land where some of the field was within one mile of 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).

Families that were eligible and interested in the study were mailed a recruitment letter and brochure that explained the study requirements for each family member, the risks and benefits of the study, and that participation was voluntary. A staff member contacted the family 1-week after the brochure was mailed to answer any questions, review the study procedures, and arrange a personal meeting to further discuss the study. At that time, the staff member obtained written informed consent from adult participants and informed assent from children. Upon enrollment a study application date was estimated, however the actual date of application was flexible to allow for changes brought on by weather and other needs of the farm.

With the resources available, the goal was to collect data from 100 pesticide applications equally split between MN and SC and approximately equally split between glyphosate, 2,4-D and chlorpyrifos. The protocol allowed families to participate by applying more than one of the chemicals, either with a mixed application or two applications done on the same day. After the first year of data collection the study was on target to recruit a sufficient number of 2,4-D and glyphosate applications, but needed more chlorpyrifos applications. To obtain sufficient numbers of chlorpyrifos applications, participants who applied 2,4-D or glyphosate in the first year and were planning to apply chlorpyrifos in the second year were recruited for a second application. 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 was predominantly used in a granular formulation during planting. The number of granular applications were limited to five per state to focus on liquid formulations which were expected to result in greater exposure opportunity, particularly secondary exposure. Efforts were made to identify a few applicators planning air-blast chlorpyrifos applications due to the high exposure potential. However, no such planned applications could be identified.

As an incentive for participation, the families received 250 dollars plus fifty dollars for each participating child if all study requirements were completed. Participants who completed part of the study were paid a prorated incentive. The cost of the chemical used in the study application was also reimbursed up to 1000 dollars.

Participating farmers and spouses were asked to complete an enrollment questionnaire to document demographics, farm characteristics, usual pesticide practices, and relevant activities during the week prior to the on-study pesticide application. They were also asked to complete a follow-up questionnaire after the application to document information on the studied pesticide application, personal protective equipment use, and activities of their children that might bring them into direct contact with the pesticide application. The applicator and spouse were required to complete a full set of questionnaires for each pesticide application (unless they applied two pesticides at the exact same time).

The field team collected the enrollment questionnaires on day 0, immediately reviewed each questionnaire for completeness, and asked the farmer or spouse to fill in any blank responses. The farmer and spouse were each given a follow-up questionnaire after the pesticide application. The follow-up questionnaire was collected on the final day of the study period. Again, field staff reviewed the questionnaire for completeness and asked the family to complete any missing information. All questionnaires and field data forms were mailed to the University of Minnesota after field staff completed a QA and QC audit described in the study protocol. The University of Minnesota study staff reviewed the forms for missing information and requested follow-back from the field staff where necessary.

On the day of the application, a member of the study team observed the pesticide application. The observer recorded the location of the field and pesticide mixing site, meteorological conditions, measured proximity of pesticide handling and the application to the house, equipment used, type of crop (year 2 only), chemical name and formulation, methods of mixing, use of PPE, clothing worn, occurrence of spills, accidents, or repairs of the equipment, and the presence of children, spouse, or pets in proximity of pesticide mixing or application activities.

Each participant was provided with instructions for collecting and storing urine voids. The farmer, spouse and participating children were provided eight wide-mouth 500 ml polyethylene containers for each of 5 days: the day

prior to pesticide application through 3 days after pesticide application, hereafter referred to as days −1, 0, 1, 2, 3. Each void was collected in a separate container. The family was provided with coolers and ice packs or a small refrigerator to store the collected urine samples. The urine specimens were picked up and transported to the field laboratory on a daily basis. Three 24-h composite samples were prepared in the field laboratory with amounts proportional to the volume of each individual void. The composite samples and the remainder of the individual urine voids were then frozen for storage.

The 24-h urine composites were timed in relation to the initiation of pesticide handling activities. The baseline 24-hour urine collection (day –1) included samples collected in the 24 h preceding the start of the next day's pesticide application. The sampling periods for days 0, 1, 2 and 3 included samples collected during the 24-h intervals beginning at the time the pesticide handling began, and at 24, 48, and 72 hours, afterwards. If one or more family members appeared to have low volumes or few voids, the farmer or spouse were asked to encourage full compliance with the study protocol. Once collected by the field teams, all urine samples were composited, stored and analyzed according to Good Laboratory Practices conditions.

Field fortification samples were prepared from urine from the participant providing the most urine on day −1 from selected families. An 1150 g sample of the composite samples was used for the field fortification. If there was not enough total urine from one individual on day −1 to yield 1150 g, additional urine for field fortifications were obtained from another family member's urine on Day −1. In this case, one individual's urine was used to fortify at the low level of the analytes and the other individual's urine were used to fortify at the high level of analyate. The 1150 g of urine was divided as follows: one 200, two 100, and fifteen 50 g aliquots for storage and analysis. Two 50 g aliquots were set aside as controls. Four 50 g aliquots were designated for fortification with glyphosate, 2,4-D, and 3, 5, 6-TCP (two for the low spike and two for the high spike). For each analyte, the low spike level was 10 μg/l (10 times the level of quantification) and the high spike level was 100 μg/l. These control and fortified samples were immediately set on blue ice in a cooler for approximately 24 h at which time all samples were frozen. Travel spikes were prepared in the same manner from three separate sites, but were immediately frozen after fortification and not left on blue ice. In theory, these specimens would provide a zero degradation reference point for each analyte in urine.

The composite samples were analyzed for the specific chemical used in the application. An overview of the methods is presented here and the details of the analytical methods are available at www.farmfamilyexposure.org. Glyphosate samples were analyzed by the Monsanto Environmental Science Laboratory using a previously reported glyphosate method modified for urine (Cowell et al., 1986). The method uses chelation ion exchange for concentration and isolation of glyphosate, followed by quantitation by HPLC with post-column reaction and fluorescence detection. The method has a limit of detection of 1 μg/l (ppb). Final results for glyphosate were corrected for analyte recovery from field and travel spike samples. A sensitive, selective method was developed to determine 2,4-dichlorophenoxyacetic acid (2,4-D) and 3,5,6-trichloro-2-pyridinol (TCP), a metabolite of chlorpyrifos (Brzak, 2001). These analyses were conducted by Morse Laboratories, Inc. in Sacramento, CA, USA. The analytes were hydrolyzed to their nonconjugated forms and extracted into toluene. The organic extracts were treated with *N*-methyl-*N*-(tert-butyl-dimethylsilyl)-trifluoroacetamide (MTBSTFA) to form the tert-butyl-dimethylsilyl derivatives of 2,4-D and TCP. Analysis was accomplished by GC/MS operating in the negative ion chemical ionization (NCI) mode and quantitation performed using derivatized solvent standards. Isotopically labeled internal standards, $^{13}C_6$-2,4-D and $^{13}C_2$,^{15}N-TCP, were used in the method. The limit of quantification was 1 μg/l for each analyte. The results for 2,4-D and chlorpyrifos were corrected for laboratory fortification recovery results. The 2,4-D and chlorpyrifos results were not corrected 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 24-h sample was assessed to evaluate the completeness of the 24-h urine samples and permit expression of analyte concentration per gram of creatinine. The creatinine analysis was performed by Morse Laboratories, Inc., by a spectrophotometric method and using a Kinetic Creatinine Procedure Kit provided by Data Medical Associates (Arlington, TX, USA).

All data collection forms were returned to the University of Minnesota where a receipt control file tracked the data collection. Data were entered into an electronic database and then hand checked against the original for accuracy. The urine sample forms were checked to evaluate correct timing of the composite urine samples and whether the correct voids were included in the composite. The measured distance of the field to the house, the pounds of active ingredient applied, number of acres treated, number of loads, and multiple chemical applications were cross checked with written notes and diagrams. The pounds of active ingredient used in the application were estimated from the reported chemical application rate, chemical label information, and the number of acres treated. The opportunity for dermal contact with pesticides was abstracted from all available research notes. Of particular interest for the applicator was the potential for direct contact with pesticides during mixing and loading or while repairing or adjusting the equipment. For the spouse and children, we abstracted any indication of direct contact with the application process, the applied field, and other potential direct contact with the pesticide, in concentrate or dilute formulation.

Results

There were 25,301 and 10,867 licensed pesticide applicators in South Carolina and Minnesota, respectively, of whom 7630 and 3534 were screened. The screening and recruitment process is summarized in Table 1. A majority of applicators contacted were ineligible: 5352 (70%) in South Carolina and 2324 (66%) in Minnesota. The most frequent reasons for ineligibility were not living on a farm in that state and not having eligible children living on the farm. In South Carolina, 480 (6.3%) of the applicators were determined to be eligible and 514 (14.5%) were eligible in Minnesota. In all, 61 (13%) of the eligible South Carolina families and 63 (12%) of the eligible Minnesota families declined to collect 24-h urine voids for the 5 days during the study. The demographic differences between participants and eligible nonparticipants were relatively small (Table 2).

In all, 45 families in Minnesota and 50 families in South Carolina were enrolled and completed the study. Some families applied a mixture of more than one chemical during a single application or made more than one application of different chemicals. In total, there were 116 chemical treatments resulting from 106 applications (some applications involved treatment with more than one pesticide at a time). A total of 23 families in South Carolina and 25 families in Minnesota applied glyphosate. In all, 17 families in South Carolina and 17 families in Minnesota applied 2,4-D. A total of 28 families in South Carolina and six families in Minnesota applied chlorpyrifos. There were 79 children in families that applied glyphosate, 53 children for 2,4-D families, and 50 children for chlorpyrifos families (Table 3).

The method of application was predominantly ground boom, the most common way to apply these chemicals (Table 4). Two applicators applying 2,4-D also applied some of the chemical with a hand-wand. In all, 10 applications of granular chlorpyrifos were applied "in furrow" during planting.

Overall, the compliance with the 24-h collections appeared good (Table 5). Spouse, applicators and children were asked to collect complete 24-h urine samples for 5 days around the time of pesticide application. Applicators turned in 99.6% of their 24-h urine collections and spouses turned in 99.4% of their 24 h urine collections. Children were more likely to have missing 24-h samples; however, 99.1% of the required 24-h urine collections in children aged 4–11 years were completed while 97.6% of the 24 h urine collections in children age 12–18 years were completed. Many of the missing 24-h urine collections were from the day before application or several days after application. Only one 24-h urine collection was missing for the applicators, two for the spouses and twelve for the children. The median weight in grams of the 24-h urine samples for the applicator, spouse, children aged 4–11 years and children aged 12 years and over were 1131 (range 238–3344), 988 (range 63–2858), 464 (range 6–2215), and

Table 1. Summary of Farm Family Exposure Study screening and recruitment process in by state, 2000–2001.

	South Carolina		*Minnesota*	
	N	*%*	*N*	*%*
Applicators screened	7630		3534	
Unable to contact	882	11.6	273	7.7
Refusals	1859	24.4	696	19.7
Called office to refuse participation	353	4.6	230	6.5
Would not participate in screening call	1350	17.7	439	12.4
Hung up	156	2.0	27	0.8
Determined eligible	480	6.3	514	14.5
Completed study	50	0.7	45	1.3
Eligible but did not complete study	369	4.8	406	11.5
Not willing to collect urine for 5 days	61	0.8	63	1.8
Determined ineligible[a]	5352	70.1	2324	65.8
Not living on farm in state	1627		341	
No adult male living on farm	18		4	
No adult female living on farm	268		169	
No children between the ages of 4 and 17 years living on farm	1892		961	
Will not be personally applying 2,4-D, glyphosate or chlorpyrifos to 10 acres or more	345		308	
Fields more than 1 mile from home	80		107	
Not a licensed pesticide applicator	330		286	
Does not live on farm	1627		350	
Retired or no longer farming	280		55	

[a]Numbers do not add up to total ineligibles because some families were ineligible for multiple reasons.

Table 2. Demographic characteristics from screening of participants and non-participants in the Farm Family Exposure Study in South Carolina, 2000–2001.

	South Carolina		Minnesota	
	Study participants	Eligible nonparticipants[a]	Study participants	Eligible nonparticipants
Total number of families	50	370	45	406
Plan to apply 2,4-D	26 (52%)	214 (57.8%)	23 (51.1%)	141 (34.7%)
Plan to apply glyphosate	36 (72%)	272 (73.5%)	38 (84.4%)	355 (87.4%)
Plan to apply chlorpyrifos	21 (42%)	100 (27.0%)	4 (8.9%)	36 (8.0%)
Gender of applicator[b]				
Male	49 (98%)	361 (97.6%)	45 (100%)	403 (99.3%)
Female	1 (2%)	9 (2.4%)	9 (0%)	3 (0.7%)
Age range of applicator	30–70	19–88	20–55	19–70
Mean age of applicator	44.3	44.8	44.9	43.6
Mean (range) number of adult males on farm	1.4 (1–4)	1.6 (1–6)	1.2 (1–2)	1.3 (1–6)
Mean (range) number of adult females living on farm	1.5 (1–3)	1.5 (1–8)	1.1 (1–2)	1.2 (1–4)
Mean (range) number of children living on farm between the age of 4–17 years	1.9 (1–7)	2.1 (1–8)	2.0 (1–5)	2.3 (1–7)
Mean Age of children	9.2	10.1	9.9	10.5
Age range	4–17	4–17	4–17	4–17

[a]Nonparticipants were eligible but were not enrolled due to random order or timing of the pesticide application.
[b]Applicator identified on licensing list used to select the family, not always the licensed applicator that made the application.

Table 3. Number of farm families, and number of chemical treatments or applications, and number of children associated with the applications by state in the Farm Family Exposure Study, 2000–2001.

	South Carolina	Minnesota	Total
Total number of farm families	50	45	95
Total number of chemical treatments	68	48	116
2,4-D applications	17	17	34
Glyphosate applications	23	25	48
Chlorpyrifos applications	28	6	34
Number of children enrolled	93	74	167
Number of children for each chemical application	23	30	53
2,4-D applications	11	20	31
Female	12	10	22
Male	38	41	79
Female	14	22	36
Male	24	19	43
Chlorpyrifos applications	43	7	50
Female	17	2	19
Male	26	5	31

739 (range 33–4112), respectively. While there is no sure way to validate the completeness of the 24-h samples only 3% of the 24-h urine samples from the applicators and spouses had with fewer than three voids or contained less than 300 g of urine. Children had up to 18% of the composite samples represented by fewer than three voids or less than 300 g. The proportion of days with fewer than three voids were similar between older (age ≥ 12 years) and younger (age 4–11 years) children. However, 27% of the younger and only 6% of the older had samples of <300 g. We also assessed the completeness of the 24-h urine samples by determining the creatinine concentration in each 24-h urine sample. The median grams of creatinine excreted in the 24-h urine collections was 1.97, 1.11, and 0.76 for applicators, spouses and children, respectively. The range of creatinine excretion was very wide for applicators, spouses and children of both age groups. Expected daily minimum creatinine excretion for adults varies between 0.566 and 1.493 g and maximum ranges from 1.991 to 2.873 (Bingham et al., 1988; Foreman, 2003). As expected the younger children had lower creatinine values than the older children.

The field and laboratory recovery for the analytes indicate that glyphosate alone had lower recoveries (Table 6). Accordingly all glyphosate urine data were adjusted for recovery. The results for 2,4-D and chlorpyrifos suggest that recovery was complete enough to obviate further adjustments to the results.

Discussion

The Farm Family Exposure Study identified and recruited a sample of farm families who applied at least one of three chemicals as part of their normal farming operations. Overall, compliance with the study protocol was good and field observers were able to document the application process

Table 4. Summary of applications methods and formulation by chemical and state in the Farm Family Exposure Study, 2000–2001.

Chemical Glyphosate	Method	Formulation[a]	South Carolina	Minnesota	Total
	Boom sprayer	Emulsifiable concentrate	21	13	33
	Boom sprayer	Solution	3	12	15
2,4-D	Boom sprayer	Emulsifiable concentrate	13	15	28
	Boom sprayer	Solution	4	2	6
	Hand spray[b]	Emulsifiable concentrate	2	0	2
Chlorpyrifos	Boom sprayer	Emulsifiable concentrate	22	2	24
	In furrow	Granular	6	4	10

[a]Formulation reported by observer.
[b]Two applicators used both boom and hand spray (hand boom) in the application.

Table 5. Summary of 24-h urine collections for all study days for all study participants in the Farm Family Exposure Study, 2000–2001.

Age	Applicator		Spouse		Children			
					4–11 (years)		12+ (years)	
	N	%	*N*	%	*N*	%	*N*	%
Participants		106		106	85		82	
Number of 24 h urines expected		530		530	425		410	
Voids (number)								
0 or missing	1	0.2	2	0.4	3	0.7	9	2.2
1–2	14	2.6	11	2.1	64	15.1	55	13.4
3–4	145	27.4	113	21.3	173	40.7	190	46.3
5–6	227	42.8	223	42.1	117	27.5	118	28.8
7–8	121	22.8	152	28.7	61	14.4	32	7.8
9–10	20	3.8	26	4.9	7	1.7	4	1.0
11+	2	0.4	3	0.6	0	0	2	0.5
Weight of 24 h urine (g)								
0 or missing	1	0.2	2	0.4	3	0.7	9	2.2
<300	7	1.3	10	1.9	117	27.3	23	5.6
301–600	38	7.2	80	15.1	154	36.2	112	27.3
601–900	109	20.6	136	25.7	97	22.8	122	29.8
901–1200	137	25.9	119	22.5	28	6.6	75	18.3
1201–1500	111	20.9	94	17.7	17	4.0	35	8.5
>1500	127	23.9	89	16.8	9	2.1	34	8.3
Creatinine (g)								
0 or missing	2	0.4	39	7.4	10	2.4	11	2.7
<0.5	3	0.6	29	5.5	208	48.9	35	8.5
0.5–1	35	6.6	167	31.5	165	38.8	132	32.2
1–1.5	88	16.6	194	36.6	35	8.2	146	35.6
1.5–2	147	27.7	81	15.3	7	1.7	60	14.6
2–2.5	143	26.9	16	3.0	0	0.0	15	3.7
2.5–3	75	14.5	2	0.4	0	0.0	10	2.4
≥3	37	6.9	2	0.4	0	0.0	1	0.2

and opportunities for exposure. The data from this study should contribute to the development of better exposure assessment approaches in epidemiologic studies of pesticides.

As with all pesticide biomonitoring studies, there are some limitations that need to be considered. First is potential selection bias or how well the studied population represents the target population. It is conceivable that study participants were more or less concerned with chemical exposure compared to other farm families and that this could detract from the generalizability of findings from this study. Having

Table 6. Percent recoveries of spiked field fortification and travel spikes by spike concentration for each chemical in the Farm Family Exposure Study, 2000–2001.

Chemical	Spike concentration[a]	*N*	Mean	SD	Min	Max
Glyphosate	10	28	69.2	15.4	33.0	90.9
	100	28	78.0	9.2	55.7	91.9
2,4-D	10	46	101.4	19.1	73.0	137.7
	100	47	101.1	13.4	41.2	121.3
TCP	10	38	105.3	31.6	69.1	291.2
	100	38	101.4	14.0	73.2	170.5c

[a]Concentration in ppb.

the entire list of licensed pesticide applicators to randomize for sequential screening and enrollment helped reduce the potential for bias. Similarly, the presence of an observer during pesticide application activities may influence farmers to adopt safer practices than normal. It is difficult to evaluate such a bias; however, we did observe a range of practices that could lead to exposure, including the use chemical resistant gloves and of incidents of spillage or skin-contact while mixing or applying pesticides (Acquavella et al., 2004; Mandel et al., 2005). Nonetheless, it is possible that the presence of an observer had some influence on pesticide handling practices for at least some of the farmers in our study. The study participants were demographically similar to the eligible nonparticipants, and they were told that we expected them to follow their usual practices. Other than the size of the application (10 acres planned), distance to the house, and type of chemical, no restrictions on the application practice were made. The logistics of conducting a biomonitoring study, with self-reported and observer reported exposure, and handling of specimens in the field and laboratory, presents an opportunity for some error in this study. The effects of these potential errors in handling specimens in the field and laboratory were likely minimized by adherence to Good Laboratory Practices protocols. The process of adhering to GLP protocols, including external data audits, increased the probability that other errors were identified and corrected.

We focused on 24-h urine collections instead of spot urine samples to better characterize exposure. The 24-h values are felt to better represent the individual's pesticide exposure that day and are less likely to be influenced by a change in urine concentration from void to void or a missing urine void or two. Many of the 24-h urine collections appear to be complete but it is difficult to assess whether the entire 24-h urine sample submitted represented the complete urine output for that 24-h period. The children tended to have more missing 24-h urine collections (14) than the applicators (two) or the spouses (three). Younger children tended to have fewer total voids and less total urine weight in grams. If fewer voids or lower volume are an indication of incomplete 24-h collections, it is likely that some of these samples are incomplete. Normal urine excretion in an adult is 1200–1500 ml but may range from 600 to 2500 ml depending on fluid intake (Finnegan, 1998; Wallach, 2000). It is more difficult to estimate a normal range of urine output in children because it depends on their age, kidney function, water and liquid intake and other factors. The daily urine output in a child is approximately 500–1000 ml and in an adolescent 700–1400 ml (Foreman, 2003; Nicholson and Pesce, 2004). Assuming a specific gravity of urine of 1.0, then 600 to 2500 ml of urine output in a normal adult would correlate to 600–2500 g of urine. A urine output of 500–1500 ml in children would correlate with 500–1500 g of urine in children. In all, 91% of the applicator's 24-h urine collections were between 600 and 2500 g and 83% of the spouse's 24-h urine collections contained between 600 and 2500 g of urine. Another gauge of urine output is total creatinine, however, normal ranges for this are also difficult to identify, particularly for children, and large inter- and intraperson variation is expected. For adults the normal range of creatinine excretion is 0.566–2.873 g per day (Bingham et al., 1988). The normal values for children are variable and will depend on age, weight and gender. Normal creatinine excretion for children less than 10 years may be 0.5 grams per day, but older children may excrete more than 1.5 g per day or creatinine excretion may be approximately 10–20 mg creatinine/kg body weight (Bingham et al., 1988; Foreman, 2003). The creatinine excretion results for the Farm Family Exposure Study have a very wide range, with some 24-h samples having unusually high values for the male applicators, and others relatively low values for some applicators, spouses and children. This may be a function of interindividual variation and the completeness of the urine collection. Overall, however, compliance with our urine collection protocol was very good.

In conclusion, the Farm Family Exposure Study successfully recruited farm families to participate in the study and complete a rigorous protocol requiring 5 days of 24-h urine collections. The study was well received by participants, which allowed the study to obtain fairly comprehensive self-reported and observed exposure data to relate to the biomonitoring results. The Farm Family Exposure Study will provide a resource for evaluating models of pesticide exposure for epidemiologic studies and for developing educational materials to promote practices by farm families that should result in reduced pesticide exposure.

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