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Urinary and hand wipe pesticide levels among farmers and nonfarmers in Iowa

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In the spring and summer of 2001, as part of a larger study investigating farm family pesticide exposure and home contamination in Iowa, urine and hand wipe samples were collected from 24 male farmers and 23 male nonfarmer controls. On two occasions approximately 1 month apart, one hand wipe sample and an evening and morning urine sample were collected from each participant. The samples were analyzed for the parent compound or metabolites of six commonly used agricultural pesticides: alachlor, atrazine, acetochlor, metolachlor, 2,4-dichlorophenoxyacetic acid (2,4-D) and chlorpyrifos. For atrazine, acetochlor, metolachlor and 2,4-D, farmers who reported applying the pesticide had significantly higher urinary metabolite levels than nonfarmers, farmers who did not apply the pesticide, and farmers who had the pesticide commercially applied (P-value <0.05). Generally, there were no differences in urinary pesticide metabolite levels between nonfarmers, farmers who did not apply the pesticide, and farmers who had the pesticide commercially applied. Among farmers who reported applying 2,4-D themselves, time since application, amount of pesticide applied, and the number of acres to which the pesticide was applied were marginally associated with 2,4-D urine levels. Among farmers who reported applying atrazine themselves, time since application and farm size were marginally associated with atrazine mercapturate urine levels. Farmers who reported using a closed cab to apply these pesticides had higher urinary pesticide metabolite levels, although the difference was not statistically significant. Farmers who reported using closed cabs tended to use more pesticides. The majority of the hand wipe samples were nondetectable. However, detection of atrazine in the hand wipes was significantly associated with urinary levels of atrazine above the median (P-value <0.01).
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Introduction

Farmers are the biggest users of pesticides and one of the most highly exposed groups to pesticides in the US. They applied approximately 1.2 billion pounds in 1999; herbicides accounted for the largest proportion of this amount with approximately 534 million pounds applied (EPA, 2002). They can be exposed through mixing, loading and applying pesticides and from working in treated fields. A wide variety of agricultural pesticides are used on farms including herbicides, crop insecticides, livestock insecticides, fungicides, and fumigants. Crop herbicides are used the most with approximately 50–93% of farmers reporting their use, followed by crop insecticides (48–59%), livestock insecticides (24–37%) and fungicides (11–14%) (Mandel et al., 1996; Alavanja et al., 1996; Reynolds et al., 1998).

Pesticide exposure is thought to be associated with a variety of health effects including cancer, reproductive disorders, neurotoxicity, and endocrine disruption (Maroni and Fait, 1993; Dich et al., 1997; Zahm et al., 1997; Kirkhorn and Schenker, 2002; Richter and Chlamtac, 2002; Alavanja et al., 2004). More specifically, phenoxy herbicides (e.g. 2,4-dichlorophenoxyacetic acid (2,4-D)) have been associated with a number of cancers including soft tissue sarcomas, nonHodgkin's lymphoma (NHL), stomach, colon and prostate; triazine herbicides (e.g. atrazine) have been associated with ovarian cancer; and organophosphate insecticides (e.g. chlorpyrifos) have been associated with delayed neuropathy, chromosome aberrations, central nervous system alterations and NHL (Maroni and Fait, 1993). Further, parental occupation involving pesticide application has been associated with childhood cancers (Daniels et al., 1997; Zahm and Ward, 1998; Flower et al., 2004).

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Several studies have investigated farmer pesticide exposure by measuring dermal exposure to pesticides (Hussain et al., 1990; Calumpang, 1996; de Cock et al., 1998; Stewart et al., 1999a and b; Krieger and Dinoff, 2000). Several studies have also employed biological monitoring of pesticide exposure among commercial and greenhouse pesticide applicators, pest control operators and agricultural workers (Sanderson et al., 1995; Denovan et al., 2000; Hines and Deddens, 2001; Tuomainen et al., 2002; Hines et al., 2003; Coronado et al., 2004). However, little biological monitoring of pesticide exposure among farmers has been conducted. In 1997, Perry investigated atrazine in urine among farm pesticide applicators (Perry et al., 2000). In all, 99 samples were collected within 8 h postapplication, and 37% had detectable levels of the atrazine metabolite deethylatrazine using gas chromatography–mass spectrometry (GC–MS). In all, 50 of these samples were also analyzed using an enzyme-linked immunosorbent assay (ELISA) for the mercapturate metabolite of atrazine with 80% having detectable levels. In 1996, Arbuckle et al. measured the levels of 2,4-D in the semen of 97 farmers (Arbuckle et al., 1999). Approximately 50% of the samples had detectable levels of 2,4-D.

In the spring and summer of 2001, as part of a larger study investigating farm family pesticide exposure and home contamination in Iowa, urine and hand wipe samples were collected from 24 male farmers and 23 male nonfarmer controls and analyzed for six commonly used agricultural pesticides — atrazine, acetochlor, metolachlor, alachlor, 2,4-D and chlorpyrifos. Urinary levels and hand loadings of pesticides were used as indicators as exposure. The term exposure instead of dose has been used throughout the paper when describing urinary levels since the spot urine samples collected are an indication of exposure and do not necessarily reflect the actual dose the subject has received. The purpose of this paper is to present the urinary and hand wipe pesticide results of the farmers and compare them to the nonfarmers.

The target pesticides in this study were selected because of their extensive use in Iowa agriculture and are among those most commonly used in Iowa (USDA, 2000; Reynolds et al., 1998). The herbicides atrazine, acetochlor and metolachlor were applied to 65%, 42%, and 20%, respectively, of the planted corn acres in Iowa in 1999 — the most recent year of data prior to selecting the pesticides (USDA, 2000). The insecticide chlorpyrifos was applied to 6% of the corn acres planted in Iowa in 1999 (USDA, 2000). In Keokuk County, Iowa, 2,4-D, atrazine, and metolachlor accounted for 50% of the herbicide use reported in the Keokuk County Rural Health Study (KCRHS) and chlorpyrifos accounted for 36% of the insecticide use (Reynolds et al., 1998). Alachlor historically had extensive use in the United States in the late 1980s and early 1990s, but is used less often recently, slipping from the second most used conventional pesticide in 1987 to the 17th most commonly used conventional pesticide in 1999 (EPA, 2002).

Methods

In the spring and summer, 2001, 24 male farmers and 23 male nonfarmers in Iowa were enrolled in a study investigating agricultural pesticide contamination inside homes and family exposure. Participant recruitment has been described previously (Curwin et al., 2002). To be eligible for the study, the nonfarmer had to live in a home on land that was not used for farming, and not be working in agriculture or commercial pesticide application. The farmer had to be using at least one of the six target pesticides. All of the pesticides are corn or soybean herbicides, with the exception of chlorpyrifos which is an insecticide used on corn. Alachlor was not used by any of the farmers and was not detected in any urine samples.

Sample Collection

During May, June, July, and August, 2001, each participant was visited on two occasions. The first visit to a farmer was shortly after an application event (within 1–5 days), with visits to nonfarmers scheduled to coincide with a farmer visit. The second visit was approximately 4 weeks later (average 4 weeks, range 3–5 weeks). Two spot urine samples on each visit were collected from the participants, one in the evening of the day of the visit, and one the following morning. The urine samples were collected in 500 ml nalgene bottles and the participants were asked to store the urine in their refrigerator, or in a cooler with ice packs that was provided. The samples were collected by study investigators the day after the visit and 25 ml aliquots were removed, stored on dry ice and shipped to the laboratory. The total volume of each urine void was recorded.

One composite hand wipe sample was also collected on each visit. The hand wiping method described in Geno et al. (1996) was used to sample for pesticide residue. The method involves wiping one entire hand with a 10 cm × 10 cm Sof-Wick® dressing sponge (Johnson & Johnson, Arlington, TX, USA) moistened with 10 ml of 100% isopropanol, then wiping each finger of the same hand with a second dressing sponge. Investigators wiped the hand by first putting on a clean pair of nitrile gloves. The whole hand was thoroughly wiped with the first moistened sponge. The sponge was unfolded and folded back on itself to present a clean surface and the hand was wiped further. This was repeated on the fingers with the second sponge. Both sponges were placed in the same sample jar for analysis. A second set of sponges was used for the second hand and placed in the same jar. A clean pair of nitrile gloves was worn for each sample collected. Polyurethane foam (PUF) moistened with 6 ml of isopropanol was used in the same manner to sample for 2,4-D. Subjects were either sampled using the Sof-Wick or PUF, but not both. Participants were selected to be sampled with PUF for 2,4-D during recruiting if it was indicated that 2,4-D might be applied.

A questionnaire was administered to all participants on the first visit and was readministered on the second visit. The questionnaire asked questions about agricultural pesticide use, crops, agricultural practice, and use of personal protective equipment (PPE). Questions were asked about the type of crop, the total size of the crop, the pesticides used on each crop, the number of hours of spraying on each spray day, the number of days the crops were sprayed, who applied the pesticide (the farmer or a custom applicator), the number of acres sprayed, and PPE worn. The questions on pesticide use, crops, and work practices gathered information from the start of the 2001 growing season until the last home visit, and generally reflect the early 2001 growing season among the participants.

Sample Analysis

A 25-ml aliquot from each urine sample was sent to a laboratory at the National Center for Environmental Health for analysis. The samples were analyzed using the method of Olsson et al. (2004). Briefly, a 2-ml aliquot of urine was spiked with isotopically labeled standards, and then diluted with 1.5 ml 0.2 M acetate buffer to which 800 activity units of β-glucuronidase/sulfatase had been added. The solution was allowed to incubate at 37°C overnight to liberate glucuronide- and sulfate-bound conjugates. The hydrolysate was applied to an OASIS® HLB solid-phase extraction cartridge (Waters Corporation, Milford, MA, USA). The SPE cartridge was washed with 2 ml 5% methanol in 1% acetic acid and eluted with 1.5 ml methanol. The methanol was diluted with 2 ml acetonitrile then evaporated to dryness. The residue was reconstituted in 50 μl acetonitrile. Pesticide metabolites were measured in the sample extract using high-performance liquid chromatography–tandem mass spectrometry with atmospheric pressure chemical ionization. A multiple reaction monitoring experiment was used to isolate specific precursor and product ions pairs for each analyte measured. Calibration standards, quality control materials and blank samples were prepared and analyzed concurrently with unknown samples. The concentrations of metabolites of six pesticides — atrazine (atrazine mercapturate), acetochlor (acetochlor mercapturate), alachlor (alachlor mercapturate), metolachlor (metolachlor mercapturate), and chlorpyrifos (3,5,6-trichloropyridinol (TCP)) and 2,4-D (parent 2,4-D) — were calculated using isotope dilution quantification. Urinary creatinine was measured in the urine samples using a commercially available enzyme slide technology (Vitros 250 Chemistry System, Ortho-Clinical Diagnostics). The analytical limit of detection (LOD) varied by analyte (Table 1).

The Sof-Wick sponges were desorbed in their shipping containers with 60 ml of isopropanol, with 0.2 μg/ml 4,4-dibromo-octafluoro-biphenyl internal standard. After tumbling for 1 h, an aliquot of each sample was poured into a GC vial for analysis. Liquid standards were used for quantitation. The PUF sponges were desorbed in their shipping containers with 125 ml of methanol with 0.5% triethylamine, 0.4 μg/ml 2-chloro-5-trifluoromethyl benzoic acid (surrogate standard) and 5 μg/ml bromothymol blue. After tumbling for 1 h, a 4 ml aliquot was blown to dryness under nitrogen. In all, 125 μl of 2,2,2-trifluoroethanol then 250 μl of pentafluoropropionic anhydride was added and the sample was put in an oven at 95°C for 1 h. After cooling to room temperature, 2 ml of 1.0 μg/ml of 4,4,-dibromooctafluorobiphenyl in toluene was added. The solution was extracted two times with pH 7.2 sodium dihydrogen phosphate/sodiumhydroxide buffer, the buffer layer was discarded and the toluene layer was transferred to GC vials containing 200 mg of anhydrous sodium sulfate. The Sof-Wick sponge wipe samples were analyzed using a gas chromatograph equipped with an electron capture detector using a 30 m DB-1701 column programmed from 130 to 270°C. The PUF samples were analyzed using a gas chromatograph equipped with an electron capture detector using a 30 m DB-608 column programmed from 90 to 270°C. The analytical LOD varied by analyte (Table 1).

Table 1. Limits of detection (LOD) for urine and hand wipe samples.

Pesticide (urinary metabolite)	Urine LOD (μg/L)	Hand wipe LOD (ng/cm^2)
Acetochlor (acetochlor mercapturate)	0.090	0.36
Alachlor (alachlor mercapturate)	0.600	0.36
Atrazine (atrazine mercapturate)	0.026	23.81
Chlorpyrifos (3,5,6-trichloropyridinol)	0.500	0.12
Metolachlor (metolachlor mercapturate)	0.141	0.36
2,4-dichlorophenoxyacetic acid	0.188	0.71

Data Analysis

The laboratory reported urinary concentrations below the LOD for some of the analytes. Urinary concentrations reported as zero in laboratory reports were replaced with one-half of the LOD. Urinary concentrations reported as nonzero but below the LOD were not modified. Evening and morning concentrations, weighted by sample volume, were averaged and expressed as micrograms of pesticide per liter of urine (μg/l). In addition, evening and morning urinary concentrations were adjusted for varying levels of urine dilution using the associated creatinine concentrations and the averaged adjusted concentration was expressed as micrograms of pesticide per gram of creatinine (μg/g). The urinary concentrations were skewed to the right, therefore, the analysis variables were natural log transformed prior to analysis. In the spray effect analysis, each urine sample was categorized as belonging to a nonfarmer or a farmer where the pesticide was either sprayed by the farmer, sprayed by someone else, or not sprayed prior to the visit. Additional

determinants, such as farm size and use of personal protective equipment, were assessed for significance for selected metabolite levels among farmers. Since each subject was sampled on two visits, mixed-effects models, where subject was treated as a random effect and employing a compound symmetric covariance structure, were used to determine statistical significance. Results are presented as adjusted geometric means by taking the antilog of the adjusted log-transformed means.

Hand wipe results, reported in μg/sample, were standardized to unit area by dividing by 840 cm^2/sample (two hands/sample $\times$ 420 cm^2/hand), assuming the surface area of a hand is 420 cm^2 (EPA, 1997). The percent of hand wipe samples detected above the LOD was computed separately for farmers and nonfarmers. Since less than half of the hand wipe samples were detected above the LOD, only the range of detectable samples was reported. The percent of farmers and nonfarmers with at least one hand wipe sample detected above the LOD were compared using Fisher's exact test. Urinary concentrations and hand wipe levels were compared in a crude analysis due to the small number of detectable hand wipe samples. All statistical analyses were performed using SAS 9 Software (2004) (SAS Institute Inc., Cary, NC, USA).

Results

Pesticide use in this study was described previously (Curwin et al., 2002); 80% of the farmers used atrazine, 56% used 2,4-D, 28% used metolachlor, 20% used acetochlor, 8% used chlorpyrifos. Farmers recorded detailed crop spray information for the period immediately prior to each visit. The number of farms that sprayed a target pesticide prior to the visit is summarized in Table 2 along with information about who sprayed the pesticide (the farmer or a custom applicator). Atrazine was sprayed on crops at 20 farms prior to 24 visits and 2,4-D was sprayed on crops at 15 farms prior to 21 visits. Acetochlor, chlorpyrifos, and metolachlor were sprayed on crops less often, at five farms prior to six visits, two farms prior to two visits, and seven farms prior to eight visits, respectively. The number of days since the pesticide was last applied varied by pesticide and farm-visit. Some visits coincided with spray days, however, a target pesticide could have been sprayed as many as 27 days prior to the visit. There was no difference in the number of days since the pesticide was last applied at visits where the farmer applied the pesticide and at visits where someone else applied the pesticide.

In all, 31 subjects provided both evening and morning urine samples at each of the two visits (four total), 13 provided a total of three urine samples each, and three provided two urine samples. Thus, a total of 169 urine samples, obtained from 24 farmers and 23 nonfarmer controls, were available for analysis. The mean sample volume was 197 ml. Sample volume was similar between farmers and nonfarmers, but evening samples were significantly lower in volume than morning samples (evening mean 161 ml *versus* morning mean 234 ml, P-value <0.0001). Among all participants, metabolites of acetochlor, chlorpyrifos, metolachlor, and 2,4-D were detected above the analytical LOD in more than half of the urine samples while atrazine was detected in only 32% of the combined urine samples (Table 3).

Table 2. Spray practices.

Pesticide	Number of farms that sprayed the pesticide prior to a visit	Number of farm-visits where pesticide was sprayed prior to the visit by[a]	
		Farmer	Custom applicator
Acetochlor	5	4	2
Atrazine	20	15	9[b]
Chlorpyrifos	2	2	0
Metolachlor	7	5	3[b]
2,4-D	15	17	4

[a]Some farms had pesticides both custom applied and applied by the farmer and may have had pesticides applied prior to one or both visits.
[b]For one farm visit the pesticide was applied by a relative of the farmer.

The geometric mean urine metabolite concentrations for the pesticides in farmers and nonfarmers, respectively, were 0.12 and 0.015 μg/l atrazine mercapturate, 0.16 and 0.17 μg/l acetochlor mercapturate, 0.17 and 0.17 μg/l metolachlor mercapturate, 1.7 and 0.29 μg/l 2,4-D, and 3.6 and 3.3 μg/l 3,5,6-trichloropyridinol. However, for all pesticides except chlorpyrifos, farmers who applied the pesticide had significantly higher urinary metabolite levels than nonfarmers, farmers who did not apply the pesticide, and farmers who had the pesticide commercially applied (Table 3). Generally, there were no differences in urinary pesticide metabolite levels between nonfarmers, farmers who did not apply the pesticide, and farmers who had the pesticide commercially applied. The same patterns hold when considering creatinine-adjusted urinary pesticide concentrations (Table 4).

Information was available on several potentially important determinants of exposure including the amount of pesticide applied, the number of acres sprayed, farm size, and cab type. However, due to the small sample size only a limited analysis was performed for 2,4-D and atrazine (not shown). Among farmers who reported applying 2,4-D themselves, time since application, amount of pesticide applied, and the number of acres to which the pesticide was applied were marginally associated with 2,4-D urine levels. Among farmers who reported applying atrazine themselves, time since application and farm size were marginally associated with atrazine mercapturate urine levels. Only two of the

Table 3. Urinary pesticide metabolite concentrations.

Pesticide	Subject	Spray group[a]	n[b]	% ≥LOD	Urinary metabolite concentration (μg/L)			
					GM[c]	GSD	Adjusted GM[d]	95% CI[d]
Acetochlor	Nonfarmer	Not sprayed	45	96	0.17	1.4	0.17[e]	0.13–0.22
	Farmer	Not sprayed	41	51	0.11	2.7	0.11[e]	0.084–0.15
	Farmer	Sprayed by others[f]	2	50	0.30	6.0	0.28[e]	0.085–0.90
	Farmer	Sprayed by self	4	100	8.0	10	7.2	3.0–17
Atrazine	Nonfarmer	Not sprayed	45	7	0.015	1.6	0.015[e,g]	0.0099–0.021
	Farmer	Not sprayed	23	35	0.043	7.1	0.044[e]	0.026–0.073
	Farmer	Sprayed by others[h]	9	33	0.032	4.9	0.035[e]	0.016–0.078
	Farmer	Sprayed by self	15	100	1.2	3.0	1.1	0.60–2.2
Chlorpyrifos	Nonfarmer	Not sprayed	45	89	3.3	3.2	3.3	2.2–5.2
	Farmer	Not sprayed	45	89	3.5	3.5	3.6	2.3–5.5
	Farmer	Sprayed by self	2	100	5.9	5.3	4.2	0.88–20
Metolachlor	Nonfarmer	Not sprayed	45	89	0.17	1.2	0.17[e]	0.12–0.24
	Farmer	Not sprayed	39	33	0.11	2.0	0.14[e]	0.10–0.19
	Farmer	Sprayed by others[h]	3	33	0.097	1.7	0.14[e]	0.071–0.26
	Farmer	Sprayed by self	5	100	4.7	8.7	0.80	0.44–1.5
2,4-D	Nonfarmer	Not sprayed	45	69	0.29	3.6	0.30[e]	0.18–0.50
	Farmer	Not sprayed	27	70	0.48	4.1	0.54[e]	0.29–1.0
	Farmer	Sprayed by others[f]	4	100	1.6	7.3	1.7	0.43–7.2
	Farmer	Sprayed by self	16	94	13	7.1	11	5.1–24

n = number of samples; LOD = limit of detection; GM = geometric mean; GSD = geometric standard deviation; CI = confidence interval.
[a]Spray group indicates whether the pesticide was sprayed prior to visit 1 for visit 1 urine samples and between visit 1 and visit 2 for visit 2 urine samples.
[b]Includes visit 1 and 2 urine samples. Concentrations reported as zero in laboratory reports were replaced with $\frac{1}{2}$ LOD prior to analysis. Evening and morning urine concentrations were weighted by volume and averaged to produce a summary concentration for each visit.
[c]Summary concentrations were natural log transformed prior to analysis.
[d]Adjusted geometric means and confidence intervals obtained from the antilog of the least-squares-adjusted means and confidence intervals obtained for the log-transformed variables.
[e]Significantly lower than the adjusted geometric mean for farmers who self-sprayed the pesticide (Tukey–Kramer adjusted P-value <0.01).
[f]Custom applicator.
[g]Significantly lower than the adjusted geometric mean for farmers who did not spray the pesticide (Tukey–Kramer adjusted P-value <0.01).
[h]Custom applicator or a relative of the farmer.

associations were significant: 2,4-D levels with number of acres sprayed ($n=13$, $r=0.58$, P-value $=0.038$) and the amount of pesticide applied ($n=12$, $r=0.60$, P-value $=0.041$). Farmers who reported using a closed cab tended to have higher urinary metabolite levels for 2,4-D and atrazine, but not significantly so. However, farmers who reported using a closed cab when applying pesticides tended to have larger farms, apply more pesticide and to a greater area.

A total of 94 hand wipe samples were collected. A total of 73 were analyzed for acetochlor, alachlor, metolachlor, atrazine, and chlorpyrifos, while the remainder ($n=21$) were analyzed for 2,4-D. A majority of the hand wipe samples were below the LOD for pesticide residue (Table 5). None of the hand wipe samples had detectable 2,4-D residues. For the remaining pesticides, the farmers had more detectable hand wipe samples than the nonfarmers, however, only acetochlor and atrazine were statistically significant. A simple analysis was conducted to see if detectable hand wipe samples were associated with higher urinary pesticide levels for acetochlor, atrazine, and chlorpyrifos (Table 6). The other pesticides had too few detectable hand wipe samples to be included in the analysis. No association was seen with acetochlor and chlorpyrifos. For atrazine, having a detectable hand wipe was associated with having a urinary metabolite level above the median. Further analysis on hand wipe samples could not be conducted due to the small number of detectable samples.

Discussion

Farmers are exposed to pesticides by directly handling and applying the pesticides. Farmers who reported applying a pesticide themselves had significantly higher pesticide urinary concentrations than farmers who had the pesticide applied by a commercial applicator or relative. It appears that merely

Table 4. Urinary pesticide metabolite concentrations adjusted by urinary creatinine.

Pesticide	Subject	Spray group[a]		Urinary metabolite (μg/g)			
			n[b]	GM[c]	GSD	Adjusted GM[d]	95% CI[d]
Acetochlor	Nonfarmer	Not sprayed	45	0.14	1.8	0.13[e]	0.095–0.19
	Farmer	Not sprayed	41	0.073	2.9	0.079[e]	0.055–0.11
	Farmer	Sprayed by others[f]	2	0.27	5.5	0.24[g]	0.070–0.84
	Farmer	Sprayed by self	4	4.6	10	3.0	1.1–8.2
Atrazine	Nonfarmer	Not sprayed	45	0.012	2.2	0.012[e,h]	0.0076–0.018
	Farmer	Not sprayed	23	0.029	6.3	0.030[e]	0.018–0.051
	Farmer	Sprayed by others[i]	9	0.025	4.9	0.030[e]	0.013–0.067
	Farmer	Sprayed by self	15	0.74	2.8	0.64	0.33–1.3
Chlorpyrifos	Nonfarmer	Not sprayed	45	2.5	2.6	2.5	1.8–3.6
	Farmer	Not sprayed	45	2.2	2.9	2.3	1.6–3.2
	Farmer	Sprayed by self	2	3.1	7.3	2.7	0.67–11
Metolachlor	Nonfarmer	Not sprayed	45	0.14	2.0	0.14[e]	0.094–0.19
	Farmer	Not sprayed	39	0.077	2.2	0.094[e]	0.065–0.14
	Farmer	Sprayed by others[i]	3	0.085	1.6	0.066[e]	0.031–0.14
	Farmer	Sprayed by self	5	2.9	9.4	0.76	0.38–1.5
2,4-D	Nonfarmer	Not sprayed	45	0.24	3.5	0.24[e]	0.14–0.39
	Farmer	Not sprayed	27	0.37	4.0	0.42[e]	0.23–0.77
	Farmer	Sprayed by others[f]	4	0.81	5.7	0.76[g]	0.20–2.9
	Farmer	Sprayed by self	16	7.9	6.9	6.7	3.1–14

n = number of samples; LOD = limit of detection; GM = geometric mean; GSD = geometric standard deviation; CI = confidence interval.
[a]Spray group indicates whether the pesticide was sprayed prior to visit 1 for visit 1 urine samples and between visit 1 and visit 2 for visit 2 urine samples.
[b]Includes visit 1 and 2 urine samples. Concentrations reported as zero in laboratory reports were replaced with $\frac{1}{2}$ LOD prior to analysis. Evening and morning urine concentrations were adjusted by creatinine concentration and averaged to produce a summary concentration for each visit.
[c]Summary concentrations were natural log transformed prior to analysis.
[d]Adjusted geometric means and confidence intervals obtained from the antilog of the least-squares-adjusted means and confidence intervals obtained for the log-transformed variables.
[e]Significantly lower than the adjusted geometric mean for farmers who reported self-applying the pesticide (Tukey–Kramer-adjusted P-value <0.01).
[f]Custom applicator.
[g]Significantly lower than the adjusted geometric mean for farmers who reported self-applying the pesticide (Tukey–Kramer-adjusted P-value <0.05).
[h]Significantly lower than the adjusted geometric mean for farmers who did not apply the pesticide (Tukey–Kramer-adjusted P-value <0.05).
[i]Custom applicator or a relative of the farmer.

having a pesticide applied to a crop does not elevate a farmer's exposure over farmers who do not apply the pesticide at all or nonfarmers. The key determinant of a farmer's exposure appears to be actually applying the pesticide. Denovan et al. (2000), in a study investigating atrazine exposure among commercial applicators, found significantly higher parent atrazine in the saliva of applicators on days they applied atrazine *versus* days they did not apply atrazine.

While it is expected that a farmer who applies a pesticide himself would have more exposure to that pesticide than farmers who do not apply the pesticide or nonfarmers, it is also expected that a farmer who has a pesticide commercially applied would also have higher exposures than farmers who do not have that pesticide applied or nonfarmers. This was not the case in this study. Farmers who had pesticide commercially applied to their crops had exposure levels similar to nonfarmers and farmers who did not have that pesticide applied. It is unclear why this was so, but perhaps in the case of corn and soybean crops — the crops grown by farmers in this study, little contact is made with the treated crops after application, and therefore little opportunity exists for exposure.

The hand wipe samples were largely nondetectable even for the farmers despite the fact that hand exposure can account for a substantial portion of dermal exposure (Hussain et al., 1990; Tuomainen et al., 2002). 2,4-D was not detected in any of the samples. Only the acid or amine forms of 2,4-D were analyzed. It is possible that only the ester forms of 2,4-D were applied in this study. However, the lack of detectable samples is likely the result of using PUF as the sampling media for the 2,4-D sampling. PUF did not hold the isopropanol well, and was the reason why only 6 ml of isopropanol was added to the PUF instead of the 10 ml

Table 5. Hand wipe concentrations.

Pesticide	Subject	Hand wipe concentration (ng/cm^2)[a]					
		n[b]	n > LOD (%)	Range[c]	N	n > LOD (%)[d]	P-value[e]
Acetochlor	Nonfarmer	34	2 (5.9)	0.36–0.48	17	2 (12)	
	Farmer	39	9 (23)	0.71–480	20	9 (45)	0.037
Alachlor	Non-farmer	34	0 (0)	—	17	0 (0)	
	Farmer	39	2 (5.1)	1.2–1.2	20	2 (10)	0.49
Atrazine	Nonfarmer	34	0 (0)	—	17	0 (0)	
	Farmer	39	11 (28)	24–4300	20	9 (45)	0.0015
Chlorpyrifos	Nonfarmer	34	4 (12)	0.36–0. 99	17	4 (24)	
	Farmer	39	8 (21)	0.36–19	20	7 (35)	0.50
Metolachlor	Nonfarmer	34	0 (0)	—	17	0 (0)	
	Farmer	39	5 (13)	2.4–6000	20	4 (20)	0.11
2,4-D	Nonfarmer	12	0 (0)	—	6	0 (0)	
	Farmer	9	0 (0)	—	5	0 (0)	—

n = number of samples; LOD = limit of detection; N = number of subjects.
[a]Measured concentration (μg/sample) standardized to unit area ($\mu g/cm^2$) by dividing by 840 cm^2/sample (two hands/sample × 420 cm^2/hand).
[b]Includes visit 1 and 2 hand wipe samples.
[c]Range of detectable samples.
[d]N > LOD gives the number of subjects with one or more detectable hand wipe concentrations over the two visits.
[e]P-value from Fisher's exact test for farmer versus nonfarmer.

Table 6. Association between hand wipe level and urinary pesticide level.

Pesticide	Hand wipe[b]	Urine level[a]		Total	P-value[c]
		Low (<median)	High (⩾median)		
Acetochlor	Nondetect	9 (35%)	17 (65%)	26	
	Detect	7 (64%)	4 (36%)	11	0.15
	Total	16	21	37	
Atrazine	Nondetect	26 (93%)	2 (7%)	28	
	Detect	0 (0%)	9 (100%)	9	< 0.0001
	Total	26	11	37	
Chlorpyrifos	Nondetect	14 (54%)	12 (46%)	26	
	Detect	3 (28%)	8 (72%)	11	0.17
	Total	17	20	37	

[a]Each subject was categorized as low or high based on the average of their visit 1 and 2 urinary pesticide concentrations.
[b]Each subject was categorized as nondetect (both nondetect) or detect (at least one detect) with respect to hand wipe pesticide concentrations from visit 1 and 2.
[c]P-value from Fisher's exact test.

added to the Sof-Wick. 2,4-D was not detected on any hard surface wipe samples in farm and nonfarm homes using PUF as the sample media despite 100% of the dust samples having detectable 2,4-D residues (Curwin et al., in press).

A sampling efficiency study on the wipe method for this study was not conducted and is a limitation of the study. However, Geno et al. (1996) reported good efficiencies using the method with Sof-Wick sponges. It is possible that poor collection efficiency may have contributed to the low number of detectable samples.

Analysis of determinants of exposure was limited due to sample size. However, an interesting observation is the trend of higher urinary levels of atrazine and 2,4-D in farmers who reported applying these pesticides with a closed cab. The farmers who used a closed cab in this study had bigger farms, and more specifically, the farmers who applied atrazine and 2,4-D applied more of these pesticides and to more acres than farmers who used an open cab. None of the differences were statistically significant, but this may be due to the small sample size. It appears then, that farmers with closed cabs may be potentially more exposed to pesticides than farmers who apply with open cabs, not because of the cab but because they are handling more pesticide. They may have larger crops and therefore are mixing, loading, and applying more pesticide. Further research is needed to investigate this hypothesis.

The low number of detectable samples for the other pesticides where a Sof-Wick sponge was used is likely due to the time since application of the pesticides. As time since application increased, pesticide residue would have been removed from the hands due to absorption, washing and rubbing. The small number of hand wipe samples obtained from farmers who self-applied some of the pesticides (acetochlor, $n=3$; chlorpyrifos, $n=2$; metolachlor, $n=5$; and 2,4-D, $n=3$) prevented an analysis of the time since the pesticide was sprayed and detection of the pesticide in hand wipe samples among the farmers who sprayed. In all, 14 hand wipe samples, however, were obtained from 10 farmers who self-applied atrazine. Atrazine was detected in 10 of these hand wipe samples, which were obtained 0–4 days since atrazine was last applied (median 1 day). Atrazine was not detected in the remaining four hand wipe samples, which were obtained 2–22 days since atrazine was last applied (median 4.5 days). Although the sample size is small, this result is suggestive of a relationship between the number of days since the pesticide was applied and pesticide levels in hand wipe samples. However, regardless of the time since application, detection of atrazine in the hand wipes was significantly associated with urinary levels of atrazine above the median. Others have found positive correlations between pesticide hand exposure and urinary levels among greenhouse pesticide applicators and agricultural workers (Aprea et al., 1994; Tuomainen et al., 2002).

Conclusion

The small sample size and small number of detectable samples for some pesticides limited the analysis of the data. Additionally, the determinants of exposure were self-reported and recall may not reflect actual determinants. Therefore, the trends presented need to be interpreted with caution. Despite these limitations, the data indicate that several factors are involved in determining urinary and hand pesticide levels. Farmers have significantly greater pesticide exposure when applying that pesticide themselves. Having a pesticide applied to a crop by someone else does not elevate urinary pesticide metabolite levels over those of nonfarmers. Among farmers who apply pesticides themselves, time since application, amount of pesticide applied and the number of acres the pesticide is applied to may be associated with urine levels and the use of a closed cab to apply pesticides may increase urinary pesticide metabolite levels, perhaps because the use of this equipment may be associated with greater use of pesticides.

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