

# Pesticide Risk Perception and Biomarkers of Exposure in Florida Female Farmworkers

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**Objective:** To compare workplace characteristics, workplace behaviors, and the health beliefs of female farmworkers of childbearing age with actual biomarkers of exposure to organophosphate pesticides and to the fungicide mancozeb. **Methods:** Hispanic and Haitian farmworkers between the ages of 18 and 40 years working in nursery or fernery operations were recruited to participate in a cross-sectional survey, examining demographics, work practices, work-related hygiene, and pesticide exposure beliefs. Single-void (spot) urine samples were analyzed for organophosphate and ethylenethiourea metabolites. **Results:** Women in nurseries worried less frequently about the effects of pesticides on their health than those in fernery operations. In summary, organophosphate and ethylenethiourea levels in nursery workers were significantly higher than levels in fernery workers and the control group. **Conclusions:** Results showed that perceived pesticide exposure did not correspond to actual metabolite levels within differing agricultural subpopulations.

Limited studies have assessed the safety of agricultural work-related tasks among female farmworkers of childbearing age, especially as it pertains to perceptions of occupational risk and actual biologic markers of pesticide exposure. Urinary biomarkers of pesticide metabolites have been extensively used to describe patterns of occupational exposure to organophosphate (OP) pesticides. Few studies have used these exposure biomarkers as effectiveness outcomes in intervention studies aimed to decrease exposure. Several published qualitative and quantitative studies have examined farmworkers' perceptions of the risks of pesticide exposure.<sup>1-7</sup> Qualitative studies have provided detailed discussions of farmworkers' perceptions and cultural beliefs about the dangers of pesticide exposure, means of exposure, and whether and how they can protect themselves at the workplace.<sup>1-3,8,9</sup> Quantitative studies have suggested that farmworkers' knowledge of pesticide exposure is related to risk perception; that risk perception is associated with farmworkers' sense of control in the workplace; and that knowledge and control both can increase safety behavior.<sup>4-7</sup> Nevertheless, most studies measuring farmworkers' beliefs and knowledge about pesticide exposure have not related these data to actual measures of pesticide exposure or to behaviors that might predict exposure.<sup>10</sup>

Studies have concluded that moderate to low OP exposure can cause an increase in neurologic symptoms and declines in neurobehavioral performance.<sup>11-13</sup> More recently, the effects of OP exposures on the developing fetus have been reported.<sup>14-20</sup> Fungicides and carbamates have also been shown to be associated

with neurotoxic symptoms.<sup>11</sup> The fungicide mancozeb is one of a group of pesticides known as ethylenebisdithiocarbamates. The fungicide, mancozeb (zinc manganese ethylenebisdithiocarbamate), is metabolized into ethylenethiourea (ETU), a metabolite with known teratogenic, mutagenic, and carcinogenic potential. The State of California and the US Environmental Protection Agency's Toxic Release Inventory lists mancozeb as a carcinogen.<sup>21</sup> The US Environmental Protection Agency lists mancozeb as a probable carcinogen, although the US Toxic Release Inventory also lists mancozeb as a developmental and reproductive toxin. One study has reported that mancozeb and its metabolites are capable of crossing the placental barrier, resulting in genotoxic DNA damage and the initiation of tumors in fetal cells.<sup>22</sup> Although associations between reproductive and development toxicity and exposure to mancozeb have been observed in mouse models,<sup>23-32</sup> no studies have documented levels of ETU among female farmworkers occupationally exposed to this fungicide. Mancozeb has been regarded as a chemical of concern linked to cases of infants born with birth defects to farmworker women in South Florida.<sup>33</sup> Therefore, the research team in the current project decided to test specifically for occupational exposure to mancozeb given its frequent use to control the growth of molds and fungi in the humid environments of Florida ferneries and nurseries.

The following Community-Based Participatory Research project explored work-related occupational and environmental exposures that have the potential to affect the health of female farmworkers of childbearing age. This article examines female farmworkers working in two different agricultural industries in Central Florida—nurseries and ferneries. Although the workplace organization of these industries differs, the workers share similar cultural and socioeconomic characteristics. The purpose of this article was to describe workplace characteristics, workplace behaviors, and female farmworkers' beliefs about exposure to pesticides and measures to reduce their exposure. These factors are then compared with actual biomarkers of exposure to OP pesticides and to the fungicide mancozeb.

## METHODS

### Participant Recruitment

Hispanic and Haitian farmworkers between the ages of 18 and 40 years working in nursery or fernery operations were recruited to participate in a cross-sectional survey. Participant inclusion criteria were as follows: (1) employment in a Florida nursery or fernery operation for at least 6 months before entry into the study; (2) current employment in a Florida nursery or fernery operation; and (3) employment of at least 20 hours per week during the previous 60-day period. We relied on snowball sampling for recruitment at farmworker housing, local churches, schools, area clinics, local ethnic businesses, community events, Farmworker Association of Florida programs, and other community locations frequented by female nursery and fernery workers. A total of 260 women who met the eligibility criteria consented to take part in the study. All study procedures were reviewed and approved by the Institutional Review Board of Emory University.

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## Farmworker Interviews

Trained community outreach workers (*promotoras*) administered a questionnaire requesting information about demographics, work practices, work-related hygiene, and pesticide exposure beliefs. All consents and questionnaires were administered in English, Spanish, or Haitian Creole depending upon the preferences of study participants. Each participant received \$25 and a “Your Work and Your Health” information sheet developed by the Farmworker Association of Florida and the Farmworker Health and Safety Institute to help farmworkers recognize signs and symptoms of pesticide exposure and how to protect themselves.

## Urine Collection

Single-void (spot) urine samples were collected from a random sample of 100 study participants stratified across nursery and fernery industries at the time surveys were completed. We also included urine samples from a nonagricultural control group of 30 women who were (1) aged 18 to 40 years; (2) not living within proximity to an agricultural field; (3) not currently employed in agriculture during the last month; and (4) not living with a partner/spouse who was employed in agriculture. All spot samples were collected at approximately the same time of day (6 to 8 PM) and were transported on ice to laboratory facilities, where they were adjusted to pH 3.0, aliquoted into test tubes, and stored at  $-80^{\circ}\text{C}$  until extraction and analysis.

## OP Pesticide Metabolites

Urine samples were analyzed for OP metabolites according to previously published methods.<sup>34–36</sup> Five nonspecific dialkyl phosphate (DAP) metabolites—dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), and dimethyl dithiophosphate (DMDTP)—were analyzed by gas chromatography (Hewlett-Packard model 5890 Palo Alto, CA) equipped with pulsed flame photometric detection. Aliquots of the samples underwent azeotropic distillation with methanol and evaporation under a nitrogen stream. Sample extracts were then derivatized with 2,3,4,5,6-pentafluorobenzyl bromide to convert phosphate acids to esters. All urine samples were measured for creatinine (Cr) concentration (mg%) using a Sigma diagnostic creatinine assay kit and a Spectramax 190 spectrophotometer to identify abnormal samples. Limits of detection (LODs) for the five metabolites were as follows: 5  $\mu\text{g/g}$  Cr for DMP, 3  $\mu\text{g/g}$  Cr for DEP and DMTP, and 2.5  $\mu\text{g/g}$  Cr for DMDTP and DETP. The metabolites were confirmed by gas chromatography/mass spectrometry.

## Fungicide Metabolite

In addition to the DAP metabolites, we analyzed urine samples for ETU, the main metabolite in the fungicide mancozeb. The ETU metabolite is excreted in urine of workers exposed to mancozeb. Urinary ETU was analyzed according to the methods of Aprea et al<sup>37–39</sup> with minor modifications. Ten milliliter of urine was saturated with potassium fluoride and ammonium chloride and extracted with dichloromethane solvent. Sample extracts were purified by the solid-phase extraction technique using Waters C<sub>18</sub> reverse-phase cartridges and silica gel columns. Ethylenethiourea was eluted from the column with (95:5) methanol–dichloromethane mixture, concentrated in a Turbo Vap concentrator and analyzed by a BAS-HPLC instrument, equipped with Waters C<sub>18</sub> Bondapak analytical column and 0.01 M phosphate buffer, pH 7.0 as the mobile phase at 1 mL flow rate. Quantitation was performed with a Shimadzu ultraviolet detector at 235-nm wavelength. The ETU concentration in urine samples was calculated in  $\mu\text{mol/L}$  and in  $\mu\text{mol/g}$  of Cr. The specific LOD for the fungicide ETU was 0.1  $\mu\text{g/g}$ .

## Statistical Analysis

Demographic and work characteristics (eg, age, hours per week working in agriculture, work characteristics, and pesticide exposure beliefs) were summarized with means and standard deviations and analyzed using analysis of variance. Levels of urinary dialkyl phosphate metabolites and ETU metabolite below the LODs were substituted with one-half the square root of the LOD in all analyses. Measures of creatinine-adjusted urinary metabolites were log-transformed to improve symmetry and summarized using the geometric mean (GM) and interquartile range. Total pesticide exposure was estimated by summing the individual exposures to each dialkyl phosphate and then taking the natural log of the total score. We took the *natural log* of each *data* value to create a distribution that was roughly symmetric and normal. Levels of metabolites were compared with self-reported beliefs on risk of pesticide exposure. We examined the relationships between worker age, years of employment in nurseries/ferneries, employment site (Apoka/Pierson), work activities, and overall pesticide exposure. A composite score (exposure score) was obtained by summing reported work activities including planting/potting, cutting/trimming, moving/planting, packing/shipping, unpacking, washing, weeding, grading, transporting, loading, mixing/spraying, cleaning, operating equipment, and supervising. Scores ranged from 0 to 13 with an observed mean of 5.8 ( $\sigma = 2.7$ ). Using multivariate linear regression, we estimated the main and interactive effects between the independent variables (eg, employment site, work practices, and health beliefs) on total pesticide exposure. Data were analyzed using SAS software version 9.3 (SAS Institute Inc, Cary, NC).

## RESULTS

### Participant Characteristics

We recruited 116 female farmworkers employed in nursery operations in Apopka, Florida (mean age = 31.86 years; standard deviation = 6.68), 144 workers employed in fernery operations in Pierson, Florida (mean age = 31.72 years; standard deviation = 5.90) (Table 1), and 30 Hispanic women not employed in agriculture (mean age = 29.83 years; standard deviation = 8.46). Fernery workers were more likely to be employed full-time (61%) than nursery workers (43%). Overall, 73% of all female farmworkers had worked in agriculture longer than 5 years. Nearly all fernery workers (97%) but only 54% of nursery workers reported Mexico as their country of origin. Among the nursery workers, 26% were from Haiti and 16% were from countries other than Mexico. The majority of our sample spoke Spanish (86%), and 12% reported Haitian Creole as their native language. Women from Pierson were more likely to report being married (54%) than women from Apopka (38%). The number of women who reported they were pregnant at the time of study participation was 22 (9%)—7 women in Apopka (6%) and 15 women in Pierson (10%).

### Work Environment

Previous studies have documented that specific worksite characteristics, such as work tasks, availability of facilities, and adherence to safety procedures, can affect the amount of pesticide exposure that a farmworker experiences.<sup>40</sup> Work tasks in nursery and fernery industries differ. The four most frequently reported work tasks among nursery workers were planting or potting (46.6%), cleaning the worksite (46.1%), cutting or trimming (37.9%), and loading plants (37.1%). Fernery workers reported less variety of tasks; they most often engaged in cutting or trimming (95.5%), and loading ferns (88.9%), with other work activities being reported less than 5% of the time. Mixing and spraying pesticides was reported by only 24 (20.7%) of the nursery workers and 7 (4.9%) of the fernery workers. Table 2 summarizes findings about worksite hygiene from the two groups. Nursery operations were much more likely to provide

**TABLE 1.** Demographic Characteristics of Survey Participants ( $n = 260$ ): Female Farmworkers Working in Nursery or Fernery Operations in Central Florida, April to June 2011\*

	Total Mean N (%)	Nursery Mean N (%)	Fernery Mean N (%)
Age, yr	31.78 ± 6.25	31.86 ± 6.68	31.72 ± 5.90
Race†			
Black	32 (12)	32 (28)	0 (0)
White	54 (21)	20 (17)	34 (23.6)
More than one race	173 (67)	64 (55)	109 (75.7)
Do not know	1 (0.4)	0 (0)	1 (0.7)
Employment status†			
Not employed	8 (3)	4 (3)	4 (3)
Part-time <35 hrs/wk	147 (57)	41 (35)	106 (74)
Full-time >35 hrs/wk	105 (41)	71 (61)	34 (24)
Lifetime in agriculture†,‡			
<5 yrs	71 (27)	52 (45)	19 (13)
≥5 yrs	188 (73)	63 (55)	125 (87)
Country of birth†,‡			
United States	10 (4)	5 (4)	5 (4)
Mexico	201 (78)	62 (54)	139 (97)
Haiti	30 (12)	30 (26)	0 (0)
Other	18 (7)	18 (16)	0 (0)
Native language†			
English	3 (1)	3 (3)	0 (0)
Spanish	224 (86)	80 (68)	144 (100)
Creole	30 (12)	30 (26)	0 (0)
Other	3 (1)	3 (3)	0 (0)

\*Percentages were calculated as a percentage of all respondents in a group who answered each individual question. For nursery workers,  $n = 144$ ; for fernery workers,  $n = 116$ .

†Differences between nursery and fernery participants are significant at  $P < 0.0001$ .

‡One nursery subject was missing from the data.

facilities for its workers than fernery operations. Among nursery workers, 82.8% reported that they always had somewhere to wash their hands at work, compared with only 53.5% of the fernery workers. Nurseries were also more likely to provide water clean enough to drink (73.9% vs 63.6%), and to have bathrooms at the worksite (90.5% vs 29.2%).

### Belief About Pesticide Exposure

Study participants were asked to self-report perceived pesticide exposure (Table 3). Women working in ferneries were much more likely to believe that they had everyday contact with pesticides while at work (74.3% vs 26.7%). Among nursery workers, 37.9% believed they were never exposed to pesticides at work compared with only 1.4% of fernery workers. Women in nursery operations worried much less frequently about the effects of pesticides on their health than in fernery operations.

### Metabolite Results

OP urinary metabolite levels adjusted for Cr are summarized in Table 3. We detected DAP metabolites in more than 90% of the samples in each of our comparison groups. Of the metabolites measured, DMTP and DETP were the most frequently detected. The molar equivalent values of the OP metabolites were summed for

**TABLE 2.** Comparison of Survey Results for Workplace Hygiene: Nursery and Fernery Workers, Central Florida, April to June 2011\*

	Always N (%)	Occasionally N (%)	Never N (%)	P†
Do you have somewhere to wash your hands at the place where you work?				
Nursery workers	96 (83)	20 (17)	0 (0)	<0.001
Fernery workers	77 (54)	40 (28)	27 (19)	
Is there water at your workplace that is clean enough to drink?†,‡				
Nursery workers	85 (74)	21 (18)	9 (8)	0.037
Fernery workers	91 (64)	25 (18)	27 (19)	
Do you usually have a bathroom at the place where you work?				
Nursery workers	105 (91)	11 (10)	0 (0)	<0.001
Fernery workers	42 (29)	67 (47)	35 (24)	
Are you able to use the bathroom at work when you want to?§,				
Nursery workers	82 (71)	34 (29)	0 (0)	0.007
Fernery workers	121 (85)	19 (13)	3 (2)	
How often do you think you have had contact with pesticides while working?				
Nursery workers	31 (27)	41 (35)	44 (20.5)	<0.001
Fernery workers	107 (74.3)	35 (24.3)	2 (1.4)	

\*Percentages reported were calculated as a proportion of all respondents who answered each individual question at each data measurement point. The total number of respondents varies for each question. For both nursery and fernery workers,  $n = 260$ .

†P value reported indicates difference between nursery and fernery workers.

‡Two subjects (one from nursery and the other from fernery) were missing from the data.

§Two cells had expected counts less than 5, so occasionally and never were combined to perform chi-squared test versus always.

|| $n = 1$  fernery subject with missing data.

each subject to determine the total urinary OP metabolite level. The summary metabolite level among nursery workers (GM = 57.29; 95% confidence interval [CI] = 45.65 to 71.89) was significantly higher than levels in fernery workers (GM = 31.40; 95% CI = 26.08–37.82) ( $P < 0.001$ ). Overall, GM concentrations for DMTP, DETP, and summary OP metabolite levels in nursery workers were significantly higher than levels detected in fernery workers and the control group. Although not statistically significant, this trend of elevated summary DAP levels persisted among women who reported mixing or applying pesticides in nursery ( $n = 9$ ; GM = 52.27), compared with fernery workers who mixed or applied pesticides ( $n = 3$ ; GM = 19.91) ( $P = 0.11$ ).

Levels of ETU, adjusted for Cr, are summarized in Table 4. We detected ETU in 100% of the samples in each of our comparison groups. Ethylenethiourea in urine was not correlated with DMP, DMTP, DMDTP, DETP, and DAP summary levels. Nevertheless, there was a moderate correlation between urinary ETU and DEP ( $r^2 = 0.300$ ;  $P < 0.01$ ). Overall, mean ETU levels for nursery workers were three times the mean for fernery workers. Ethylenethiourea was significantly higher among participants from Apopka nurseries

**TABLE 3.** Creatinine-Adjusted Organophosphate Metabolites in the Urine of 97 Female Farmworkers Working in Nursery and Fernery Operations in Central Florida, April to June 2011

Organophosphate Metabolite	Group	N (Detected %)	Creatinine Adjusted, $\mu\text{g/g}$						
			GM (95% CI)	Minimum	Percentile				Maximum
					50th	75th	90th	95th	
DMP	Nursery	47 (29.8)	5.38 (4.31–6.72)	3.54	3.54	6.70	21.70	44.15	50.00
	Fernery	50 (14.0)	4.14 (3.66–4.67)	3.54	3.54	3.54	8.64	13.35	25.00
	Control	30 (20.0)	4.18 (3.57–4.88)	3.54	3.54	3.54	11.38	14.45	15.00
DEP	Nursery	47 (29.8)	3.44 (2.70–4.38)	2.12	2.12	6.10	13.80	25.30	34.00
	Fernery	50 (20.0)	3.01 (2.43–3.74)	2.12	2.12	2.12	10.88	23.85	38.00
	Control	30 (3.3)	2.23 (2.01–2.48)	2.12	2.12	2.12	2.12	5.53	9.70
DMTP	Nursery	47 (89.4)	26.25 (18.29–37.67)	2.12	29.00	59.00	128.00	256.00	300.00
	Fernery	50 (82.0)	11.10 (8.15–15.12)	2.12	12.50	29.00	41.30	61.70	120.00
	Control	30 (90.0)	18.60 (11.73–29.50)	2.12	16.50	48.00	106.50	188.00	210.00
DMDTP	Nursery	47 (10.6)	2.00 (1.79–2.24)	1.77	1.77	1.77	3.71	6.55	9.00
	Fernery	50 (14.0)	1.87 (1.75–2.00)	1.60	1.77	1.77	1.77	3.73	6.70
	Control	30 (3.3)	1.79 (1.74–1.85)	1.77	1.77	1.77	1.77	2.19	2.70
DETP	Nursery	47 (80.9)	7.83 (5.71–10.74)	1.77	9.70	15.00	42.50	79.05	84.00
	Fernery	50 (66.0)	4.35 (3.29–5.74)	1.77	3.85	7.00	16.80	24.90	190.00
	Control	30 (76.7)	5.19 (3.65–7.39)	1.77	5.10	8.90	19.10	48.30	56.00
Summary DAP	Nursery*	47 (95.7)	57.29 (45.65–71.89)	11.3	56.29	101.89	162.63	346.03	391.42
	Fernery	50 (94.0)	31.40 (26.08–37.82)	11.3	29.00	47.02	74.42	115.78	199.55
	Control	30 (96.7)	40.11 (30.36–52.99)	11.3	34.16	57.19	155.33	202.27	219.19

\* $P < 0.001$  for differences detected between nursery workers and fernery workers and the control group.

CI, confidence interval; DAP, dialkyl phosphate; DEP, diethyl phosphate; DETP, diethyl thiophosphate; DMDTP, dimethyl dithiophosphate; DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; GM, geometric mean; N, sample size.

**TABLE 4.** Levels of Ethylenethiourea, Adjusted for Creatinine in the Urine of 100 Female Farmworkers Working in Nursery and Fernery Operations in Central Florida, April to June 2011

Fungicide	Group	N(Detected %)	Creatinine Adjusted, $\mu\text{g/g}$						
			GM (95% CI)	Minimum	Percentile				Maximum
					50th	75th	90th	95th	
Ethylenethiourea	Nursery*	50 (100)	5.69 (4.46–7.26)	1.20	5.71	10.31	15.41	26.78	53.10
	Fernery	50 (100)	1.90 (1.42–2.54)	0.21	1.76	3.30	10.07	13.93	18.22
	Control A	15 (100)	1.69 (1.22–2.36)	0.53	1.67	2.74	3.52	–	4.02
	Control B	15 (100)	0.82 (0.55–1.23)	0.38	0.72	0.87	2.94	–	6.91

\* $P < 0.001$  for differences detected between groups. Control A = Apopka in Lake and Orange counties. Control B = Pierson in Volusia and Putnam counties.

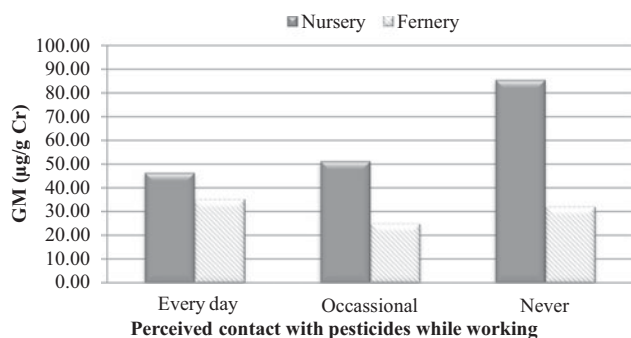
CI, confidence interval; GM, geometric mean; N, sample size.

(GM = 5.69) and controls from Apopka (GM = 1.69) than among participants from Pierson (Fernery workers GM = 1.90; controls = 0.82;  $P < 0.001$ ). A total of 31 (11.9%) women in this study reported mixing or spraying pesticides—24 (20.7%) nursery workers and 7 (4.9%) fernery workers. We did not detect a significant difference in mean concentration of ETU within or between counties for women who applied/mixed pesticides compared with women who did not apply/mix pesticides.

Elevated trends in summary DAP and ETU levels persisted among the 5 nursery workers who reported they were pregnant (GM<sub>summary DAP</sub> = 95.2 and GM<sub>ETU</sub> = 7.79) compared with 13 pregnant fernery workers (GM<sub>summary DAP</sub> = 27.5 and GM<sub>ETU</sub> = 1.68).

### Actual Versus Perceived Pesticide Exposure

Comparisons between perception of pesticide exposure and actual Cr-adjusted GM concentrations of summary DAP metabolites in urine of female farmworkers are shown in Fig. 1. Nursery workers who reported that they never had contact with pesticides in their workplaces had significantly higher levels of OP urinary metabolites (GM = 83.10  $\mu\text{g/g Cr}$ ) than nursery workers who reported they were exposed daily (GM = 46.53  $\mu\text{g/g Cr}$ ) ( $P < 0.05$ ). We detected a marginally significant difference between women in fernery operations reporting daily exposure (GM = 35.16  $\mu\text{g/g Cr}$ ) compared with women who perceived never being exposed to pesticides (GM = 24.99  $\mu\text{g/g Cr}$ ) ( $P < 0.10$ ). A similar trend was apparent in ETU metabolite concentrations. Mean ETU levels were



**FIGURE 1.** Comparisons of perception of pesticide exposure to actual creatinine-adjusted geometric mean concentrations of summary organophosphate metabolites in urine of female farmworkers, April to June 2011.

significantly higher in nursery workers who reported never being exposed to pesticides (GM = 8.72 µg/g Cr) than nursery workers who reported everyday exposure (GM = 8.61 µg/g Cr) ( $P < 0.05$ ).

The only significant relationship that we observed in the multivariate analysis was between the employment site (ie, nursery vs fernery) and total pesticide exposure. The mean total exposure was significantly higher ( $t = 3.76$ ;  $P < 0.001$ ) in Apopka than in Pierson (4.0 vs 3.5, respectively).

## DISCUSSION

The literature is replete with research documenting concerns of pesticide exposure among farmworkers. Nevertheless, a few studies have compared farmworkers' perceptions of exposure with biomarkers of actual exposure. This study results show that perceived pesticide exposure did not correspond to summary OP urinary metabolite levels within differing agricultural subpopulations. The significance of our findings demonstrates that risk assessment of pesticide exposure among farmworkers may not correspond with actual biomarkers of exposure.

Other researchers have documented that perceptions of environmental and occupational health issues among farmworkers may differ by certain demographic characteristics, particularly age and ethnicity.<sup>3,9</sup> Although this study focused on female farmworkers, there were differences between the two communities. The fernery workers were entirely Hispanic in origin, whereas a substantial number of workers in the Apopka nurseries were Haitian. Risk perceptions did not differ between these two ethnic groups in our nursery workers. Likewise, the age distribution was similar in both communities, and younger workers did not differ significantly from older workers. In earlier focus groups with these female worker populations,<sup>41</sup> nursery workers reported that they did not believe they were being exposed to pesticides in the type of tasks they performed—such as repotting, weeding, and loading plants. Fernery workers, however, indicated that they frequently have contact with the crop during their work, and the nature of their work tasks confirms this. They often work surrounded by tall plants and have full bodily contact with wet ferns in the morning because they have to bend over and thrust their arms into the plants to cut them. When they carry the bunches of fern fronds to load them onto a trailer, they must stack and hold them close to their bodies. Both worksites have periods in which pesticide application reentry signs are in place, although women in the nurseries were more likely to report that they mixed or applied chemicals in their workplace.

Concentrations of DMTP and DETP were the most commonly detected urinary OP metabolites in both nursery and fernery workers. According to the Centers for Disease Control and Prevention National Health and Nutrition Examination Survey (NHANES), par-

ticipant levels for both metabolites in this study were much higher than levels reported for other women living in the United States. Levels of DETP (GM = 4.76 µg/g Cr) and DMTP (GM = 9.68 µg/g Cr) for female farmworkers in our sample were much higher than the 95th percentile levels for other women (GM<sub>DETP</sub> = 2.57 and GM<sub>DMTP</sub> = 6.57, respectively) in the US population reported in the 2003 to 2004 NHANES. ETU levels (GM = 5.69 µg/g Cr) in this subsample of female nursery workers were especially perplexing compared with nondetect levels of ETU in other nationally representative women and Mexican Americans reported in NHANES. Urinary ETU levels for women in this study were in the range of those reported in studies carried out in other workers occupationally exposed to mancozeb,<sup>42,43</sup> but higher than those reported in nonoccupational populations.<sup>37,44,45</sup> We did not observe an association between workplace practices/personal characteristics and pesticide exposure levels in our sample of women working in agriculture.

Several factors could have attributed to the differences found in the pesticide urinary biomarkers. A cross-sectional approach using collection of a single-spot urine sample does not provide a complete picture of how pesticide levels can vary over different growing seasons and during different points in a workweek. Both mancozeb and OP pesticides are metabolized rapidly by the body. Although urinary measures are considered a sufficient marker of pesticide exposure,<sup>46</sup> exposures have been shown to vary during different seasons in berry and pome fruit, and among other types of farmworkers and their children.<sup>35,47,48</sup> Foliage crops and ferns differ from fruit crops in that there are not specific growing times in which pesticides are more heavily used (eg, during the blossoming period for pome fruit).

The levels of OP metabolites observed in these two Florida worker communities can be compared with other reported levels in agricultural communities. During the same year as the current study, male and female nursery workers were studied in Oregon. All metabolites for both these studies were analyzed in the same laboratory with identical analytical techniques.<sup>34–39</sup> The summed total of the five DAP metabolites for female nursery workers in our Oregon sample ranged from 11.36 to 424.11 µg/g Cr, with a mean of 74.89 and a median of 68.31. Results of comparison tests showed comparable summary DAP levels in the Oregon nursery workers (GM = 74.89; CI = 70.67–79.36) compared with summary levels detected in Florida nursery workers (GM = 56.96; CI = 52.19–62.16) ( $P < 0.08$ ).

Interviews with workers did not indicate seasonal variation in pesticide application or reentry restriction enforcement. The fact that both OP pesticide and fungicide levels were elevated in the nursery workers indicates that community, rather than individual, factors are influential. Likewise, the finding that nonagricultural controls in the Apopka community also had relatively higher urinary levels of both fungicides and OPs may indicate that there are environmental factors specific to the wider physical community at play, including household proximity to farmland, pesticide drift, and take-home exposure pathways.<sup>49–52</sup>

Another explanation for the variation in these occupational communities' perceptions of and actual exposures to pesticide may be related to the correlation between exposure perception and the practice of safety behavior, described in other studies.<sup>4,5</sup> External factors can also influence farmworkers' perceptions of exposure. For example, one study with male and female nursery and fernery workers in the same communities as the this study has demonstrated that certain workplace practices, such as the posting of required application warnings or the workers' perceptions that their boss thinks safety practices are important, can lead to an increase in a specific safety behavior such as hand washing.<sup>40</sup> Together, these studies indicate that low levels of exposure perception or lack of other reinforcing workplace safety measures may lead to workers practicing less safety behavior. Assumedly, safety behavior promoted by regulation

such as the Environmental Protection Agency's Worker Protection Standard is designed to minimize pesticide exposure levels. If nursery workers are not practicing certain recommended safety behaviors because of lower risk perceptions or other external variables, they may be experiencing higher levels of exposure.

In fact, one workplace behavior unique to ferneries may also contribute to lower levels of exposure. As previously described, fernery workers often come into full bodily contact with the waist-high ferns they are harvesting. Previous focus groups with male and female fernery workers describe that they are concerned about being wet while they work. They described many health problems associated with being wet at the worksite, including weak bones; aching backs, joints, knees, and hands; pneumonia; and allergies. They also believed that being wet made them more vulnerable to pesticide exposure and absorption because drifting pesticides or residue could stick to and dry on their skin and clothing.<sup>2</sup> In these cases, workers do not believe that merely wearing the recommended long pants and long-sleeved shirts provides protection because it is their clothing that becomes wet. Therefore, fernery workers have adopted the practice of tying large plastic bags, such as those used for garbage collection, around their waists to keep their pants from getting wet. It is possible that by protecting their bodies from the wet conditions, fernery workers are also protecting against pesticide exposure. Likewise, because clipping ferns involves thrusting their hands into ground foliage containing sharp sticks, insects, and sometime snakes, fernery workers wear protective gloves more often than their nursery counterparts. Future studies will explore to what extent the use of plastic bags and gloves was protective in this population of workers.

Our findings are subject to several limitations. First, the analysis of only one spot urine sample presents a less than optimal characterization of pesticide exposure that may be occurring over time. DAP and ETU metabolites measured in this study most likely represent a partial view of the total mix of pesticide exposures received by this agricultural sample. Exposures to other classes of pesticides and herbicides certainly occur, and the total exposure to all classes of agricultural chemicals is not quantified by our methods. Furthermore, the sources and routes of exposure to OP compounds cannot be identified by measurement of urine DAPs, which provide an integrated indicator of exposure to various OP compounds via ingestion, inhalation, and dermal exposure. Farmworkers often have little to no information on the types of chemicals used on the plants they are harvesting.<sup>2</sup> In this Community-Based Participatory Research project, we asked the workers to write down the names of pesticides that they observed in their work settings. Among their notes we found reference to the generic and trade names for mancozeb and several OP chemicals. This grassroots attempt to identify pesticide use provided valuable information on potential pesticide exposures in Florida, a state that has no pesticide reporting requirements or records available for the public.

Future studies will incorporate a longitudinal design of workers in Florida agriculture. Many in this population are not highly migratory, giving assurance that documentation of exposure levels over time is feasible. The protective value of wearing plastic sheeting around the torso needs further investigation. Environmental sampling surveillance is needed to document the levels in the nurseries and fields and dermal sampling to demonstrate the effectiveness of this protective gear from both moisture and chemical exposure.

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## REFERENCES

- Snipes SA, Thompson B, O'Connor K, et al. "Pesticides protect the fruit, but not the people": using community-based ethnography to understand farmworker pesticide-exposure risks. *Am J Public Health*. 2009;99(suppl 3):S616–S621.
- Flocks J, Monaghan P, Albrecht S, Bahena A. Florida farmworkers' perceptions and lay knowledge of occupational pesticides. *J Community Health*. 2007;32:181–194.
- Salazar MK, Napolitano M, Scherer JA, McCauley LA. Hispanic adolescent farmworkers' perceptions associated with pesticide exposure. *West J Nurs Res*. 2004;26:146–166; discussion 167–175.
- Arcury TA, Quandt SA, Russell GB. Pesticide safety among farmworkers: perceived risk and perceived control as factors reflecting environmental justice. *Environ Health Perspect*. 2002;110(suppl 2):233–240.
- McCauley LA, Sticker D, Bryan C, Lasarev MR, Scherer JA. Pesticide knowledge and risk perception among adolescent Latino farmworkers. *J Agric Saf Health*. 2002;8:397–409.
- Vaughan E. Chronic exposure to an environmental hazard: risk perceptions and self-protective behavior. *Health Psychol*. 1993;12:74–85.
- Vaughan E. Individual and cultural differences in adaptation to environmental risks. *Am Psychol*. 1993;48:673–680.
- Quandt SA, Arcury TA, Austin CK, Saavedra RM. Farmworker and farmer perceptions of farmworker agricultural chemical exposure in North Carolina. *Hum Organ*. 1998;57:359–368.
- Hofmann JN, Crowe J, Postma J, Ybarra V, Keifer MC. Perceptions of environmental and occupational health hazards among agricultural workers in Washington state. *AAOHN J*. 2009;57:359–371.
- Quandt SA, Hernandez-Valero MA, Grzywacz JG, Hovey JD, Gonzales M, Arcury TA. Workplace, household, and personal predictors of pesticide exposure for farmworkers. *Environ Health Perspect*. 2006;114:943–952.
- Kamel F, Hoppin JA. Association of pesticide exposure with neurologic dysfunction and disease. *Environ Health Perspect*. 2004;112:950–958.
- Brown TP, Rumsby PC, Capleton AC, Rushton L, Levy LS. Pesticides and Parkinson's disease—is there a link? *Environ Health Perspect*. 2006;114:156–164.
- Jayasinghe SS, Pathirana KD, Buckley NA. Effects of acute organophosphorus poisoning on function of peripheral nerves: a cohort study. *PLoS One*. 2012;7:e49405.
- Samarawickrema N, Pathmeswaran A, Wickremasinghe R, et al. Fetal effects of environmental exposure of pregnant women to organophosphorus compounds in a rural farming community in Sri Lanka. *Clin Toxicol (Philadelphia, Pa)*. 2008;46:489–495.
- Venerosi A, Ricceri L, Scattoni ML, Calamandrei G. Prenatal chlorpyrifos exposure alters motor behavior and ultrasonic vocalization in CD-1 mouse pups. *Environ Health*. 2009;8:12.
- Whyatt RM, Garfinkel R, Hoepner LA, et al. A biomarker validation study of prenatal chlorpyrifos exposure within an inner-city cohort during pregnancy. *Environ Health Perspect*. 2009;117:559–567.
- Bradman A, Castorina R, Barr DB, et al. Determinants of organophosphorus pesticide urinary metabolite levels in young children living in an agricultural community. *Int J Environ Res Public Health*. 2011;8:1061–1083.
- Quiros-Alcala L, Alkon AD, Boyce WT, et al. Maternal prenatal and child organophosphate pesticide exposures and children's autonomic function. *Neurotoxicology*. 2011;32:646–655.
- Rauh V, Arunajadai S, Horton M, et al. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. *Environ Health Perspect*. 2011;119:1196–1201.
- Slotkin TA. Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity? *Reprod Toxicol (Elmsford, NY)*. 2011;31:297–301.
- EPA Mancozeb Facts. Prevention, pesticides, and toxic substances (7508C). Available at [http://www.epa.gov/oppsrrd1/REDs/factsheets/mancozeb\\_fact.pdf](http://www.epa.gov/oppsrrd1/REDs/factsheets/mancozeb_fact.pdf). Published September 2005. Accessed January 7, 2013.
- Shukla Y, Arora A. Transplacental carcinogenic potential of the carbamate fungicide mancozeb. *J Environ Pathol Toxicol Oncol*. 2001;20:127–131.
- Ivanova-Chemishanska L, Vergieva T, Antonov G, Mirkova E. Study on the long-term effects of some pesticides. *J Hyg Epidemiol Microbiol Immunol*. 1980;24:295–302.
- Teramoto S, Saito R, Shirasu Y. Teratogenic effects of combined administration of ethylenethiourea and nitrite in mice. *Teratology*. 1980;21:71–78.
- Khera KS. Reduction of teratogenic effects of ethylenethiourea in rats by interaction with sodium nitrite in vivo. *Food Chem Toxicol*. 1982;20:273–278.

26. Khera KS. Ethylenethiourea: a review of teratogenicity and distribution studies and an assessment of reproduction risk. *Crit Rev Toxicol*. 1987;18:129–139.
27. Daston GP, Yonker JE, Powers JF, Heitmeyer SA. Difference in teratogenic potency of ethylenethiourea in rats and mice: relative contribution of embryonic and maternal factors. *Teratology*. 1989;40:555–566.
28. Lentz-Rizos C. Ethylenethiourea (ETU) in relation to use of ethylenebis-dithiocarbamate (EBDC) fungicides. *Rev Environ Contam Toxicol*. 1990;115:1–37.
29. Saillenfait AM, Sabate JP, Langonne I, de Cauriz J. Difference in the developmental toxicity of ethylenethiourea and three N,N'-substituted thiourea derivatives in rats. *Fundam Appl Toxicol*. 1991;17:399–408.
30. Tsuchiya T, Nakamura A, Iio T, Takahashi A. Species differences between rats and mice in the teratogenic action of ethylenethiourea: in vivo/in vitro tests and teratogenic activity of sera using an embryonic cell differentiation system. *Toxicol Appl Pharmacol*. 1991;109:1–6.
31. Iwase T, Yamamoto M, Shirai M, et al. Time course of ethylene thiourea in maternal plasma, amniotic fluid and embryos in rats following single oral dosing. *J Vet Med Sci*. 1996;58:1235–1236.
32. Ohta T, Tokishita S, Shiga Y, Hanazato T, Yamagata H. An assay system for detecting environmental toxicants with cultured cladoceran eggs in vitro: malformations induced by ethylenethiourea. *Environ Res*. 1998;77:43–48.
33. Calvert GM, Alarcon WA, Chelminski A, et al. Case report: three farmworkers who gave birth to infants with birth defects closely grouped in time and place—Florida and North Carolina, 2004–2005. *Environ Health Perspect*. 2007;115:787–791.
34. Muniz JF, McCauley L, Scherer J, et al. Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. *Toxicol Appl Pharmacol*. 2008;227:97–107.
35. Lambert WE, Lasarev M, Muniz J, et al. Variation in organophosphate pesticide metabolites in urine of children living in agricultural communities. *Environ Health Perspect*. 2005;113:504–508.
36. Rothlein J, Rohlman D, Lasarev M, Phillips J, Muniz J, McCauley L. Organophosphate pesticide exposure and neurobehavioral performance in agricultural and non-agricultural Hispanic workers. *Environ Health Perspect*. 2006;114:691–696.
37. Aprea C, Betta A, Catenacci G, et al. Reference values of urinary ethylenethiourea in four regions of Italy (multicentric study). *Sci Total Environ*. 1996;192:83–93.
38. Aprea C, Betta A, Catenacci G, et al. Urinary excretion of ethylenethiourea in five volunteers on a controlled diet (multicentric study). *Sci Total Environ*. 1997;203:167–179.
39. Colosio C, Fustinoni S, Birindelli S, et al. Ethylenethiourea in urine as an indicator of exposure to mancozeb in vineyard workers. *Toxicol Lett*. 2002;134:133–140.
40. Mayer B, Flocks J, Monaghan P. The role of employers and supervisors in promoting pesticide safety behavior among Florida farmworkers. *Am J Ind Med*. 2010;53:814–824.
41. Flocks J, Kelley M, Economos J, McCauley L. Female farmworkers' perceptions of pesticide exposure and pregnancy health. *J Immigr Minor Health*. 2012;14:626–632.
42. Steenland K, Cedillo L, Tucker J, et al. Thyroid hormones and cytogenetic outcomes in backpack sprayers using ethylenebis(dithiocarbamate) (EBDC) fungicides in Mexico. *Environ Health Perspect*. 1997;105:1126–1130.
43. Aprea C, Sciarra G, Sartorelli P, Mancini R, Di Luca V. Environmental and biological monitoring of exposure to mancozeb, ethylenethiourea, and dimethoate during industrial formulation. *J Toxicol Environ Health Part A*. 1998;53:263–281.
44. Colosio C, Visentin S, Birindelli S, et al. Reference values for ethylenethiourea in urine in Northern Italy: results of a pilot study. *Toxicol Lett*. 2006;162:153–157.
45. Castorina R, Bradman A, Fenster L, et al. Comparison of current-use pesticide and other toxicant urinary metabolite levels among pregnant women in the CHAMACOS cohort and NHANES. *Environ Health Perspect*. 2010;118:856–863.
46. Saieva C, Aprea C, Tumino R, et al. Twenty-four-hour urinary excretion of ten pesticide metabolites in healthy adults in two different areas of Italy (Florence and Ragusa). *Sci Total Environ*. 2004;332:71–80.
47. Coronado GD, Igoren EM, Griffith WC, Faustman EM, Thompson B. Organophosphate pesticide exposure among pome and non-pome farmworkers: a subgroup analysis of a community randomized trial. *J Occup Environ Med*. 2009 [Epub ahead of print].
48. Arcury TA, Grzywacz GJ, Isom S, et al. Seasonal variation in the measurement of urinary pesticide metabolites among Latino farmworkers in Eastern North Carolina. *Int J Occup Environ Health*. 2009;15:339–350.
49. Lu C, Fenske RA, Simcox NJ, Kalman D. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. *Environ Res*. 2000;84:290–302.
50. Ward MH, Nuckols JR, Weigel SJ, Maxwell SK, Cantor KP, Miller RS. Identifying populations potentially exposed to agricultural pesticides using remote sensing and a Geographic Information System. *Environ Health Perspect*. 2000;108:5–12.
51. Curl CL, Fenske RA, Kissel JC, et al. Evaluation of take-home organophosphorus pesticide exposure among agricultural workers and their children. *Environ Health Perspect*. 2002;110:A787–A792.
52. Lee S, McLaughlin R, Harnly M, Gunier R, Kreutzer R. Community exposures to airborne agricultural pesticides in California: ranking of inhalation risks. *Environ Health Perspect*. 2002;110:1175–1184.