ORIGINAL RESEARCH

Recruiting Strategy and 24-Hour Biomonitoring of Paraquat in Agricultural Workers

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ABSTRACT. The objectives of this study were to recruit agricultural workers in Costa Rica to participate in a 24-hour urine collection for paraquat exposure assessment and to compare the 24-hour sampling to end-of-shift sampling. The authors recruited 187 handlers and 54 nonhandlers from coffee, banana, and palm oil plantations. The completeness of 24-hour urine samples collected (a total of 393 samples) was confirmed by questionnaire and urinary creatinine level. For a subset of 12 samples, the absorbed paraquat level was determined in 24-hours and end-of-shift spot urine samples. The participation rate for handlers was ~90%. The completeness of 24-hour urine collections was verified as the overall average of creatinine levels from 393 urines (1.11 ± 0.50 g/L). A total of 92.4% to 96.7% of urine samples were considered within the acceptable range of urinary creatinine, whereas 94.7% of the samples were described as "complete" from the questionnaire. Measured creatinine correlated well to predicted values (r = .327, p = .0024, 95% CI .12–.51). Detected paraquat levels in spot urine samples had a sensitivity of 96.9% at the high specificity of 100% compared to 24-hour urine

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samples as the gold standard. There was a significant ($p < .0001$) correlation between spot and 24-hour urine paraquat levels ($r = .7825$, 95% CI .61–.88). The recruiting strategy was successful in getting 24-hour urine samples from a farm worker population. Comparison between the paraquat levels in spot and 24-hour urine samples demonstrated that for this compound, end-of-shift spot urine samples would be an appropriate substitute for 24-hour collections.

KEYWORDS. biological monitoring, 24-hour urine, recruiting strategy, paraquat, Agriculture, farm worker

INTRODUCTION

Biological monitoring is an essential component when determining exposure levels and concentrations of target compounds from occupational or environmental exposures.[1–4] When conducting biological monitoring studies, it can be challenging to recruit subjects who will fully comply with sample requirements of the study, and even more difficult when there are education, language, or cultural barriers. For most exposure assessment studies that use biomonitoring, urine is preferred compared to other biological materials (adipose tissues, blood, hair) because it is collected easily and noninvasively.[5,6] Many studies with pesticides have measured the total absorbed dose along with the concentration of parent compound or metabolites in urine collected over select (spot) time periods.[2,4,7,8] Although less commonly used to determine pesticide levels in urine because it requires rigor and planning,[9,10] continuous and complete 24-hour urine collection can yield more accurate results than spot urine sampling because it provides a valid overview of the entire pesticide exposure profile. One of the challenges of 24-hour urine collection, however, is motivating participants and ensuring that they understand that correct collection procedures are crucial for the success of the project; incomplete collection can result in an underestimation of the exposure profiles of the compound of interest.

In the present study, we utilized a culturally sensitive recruiting and education method with farm workers in Costa Rica to obtain 24-hour urine sample collections, which were used to measure exposure to the herbicide paraquat. We used the concentration of urinary creatinine as a useful index for determining the completeness of a 24-hour urine collection,[11,12] mainly because daily excretion of creatinine is kept at a constant level[13] if muscle mass does not change dramatically over short periods of time.[9] Urinary creatinine excretion can also be affected by other factors, such as age, gender, diet, body mass, medication use, physical activity, and renal function.[14–16] Normal ranges for 24-hour urine creatinine concentrations have been reported to be 0.5 to 3.0 g/L,[9,10] 0.3 to 3.0 g/L,[17] and 0.5 to 3.5 g/24 h.[18]

Because 24-hour urine samples can be difficult to collect, we took this opportunity to compare the concentration of paraquat in 24-hour urine samples with more readily obtained end-of-shift spot samples. Paraquat (1,1′-dimethyl-4, 4′-bipyridinium dichloride) is a nonselective and contact herbicide that has been used worldwide for over 40 years. In animals, absorbed paraquat is mainly excreted by the kidney,[19] and almost 100% of absorbed paraquat is excreted in an unchanged form within 24 hours of exposure.[20] Therefore, comparing paraquat concentrations in 24-hour versus spot samples to monitor absorbed paraquat is simpler, because only the parent compound needs to be measured, and the results are not complicated by differences in metabolism among participants.

This report describes our strategy for recruiting volunteer agricultural workers in Costa Rica to participate in a 24-hour urine collection for paraquat exposure assessment. Our educational efforts to achieve complete 24-hour urine collection are described, and the completeness of collection assessed with urinary creatinine measurements and questionnaire responses. We also compared absorbed paraquat levels from 24-hour and end-of-shift spot urine samples to determine which sampling method is appropriate for future studies of this type.

MATERIALS AND METHODS

Study Population

Exposure assessment was carried out as part of a cross-sectional epidemiological study on the respiratory health of agricultural workers in Costa Rica.[21] The study was approved by the Institutional Review Board of the University of California, Davis, and the Costa Rica Ministry of Health. The field component was conducted between May and December of 2001. Paraquat handlers are those that mixed, loaded, or sprayed paraquat. One hundred and eighty-seven male handlers from banana, coffee, and palm oil farms, which provide the three main commodities of the country, were recruited for biological monitoring. Of this group, 119 participated (88.8%), with a mean age 36.2 ± 10.8 years, and 15 refused to participate (8.0%). Fifty-four nonhandlers (mean age 35.2 ± 12.2 years) were also recruited. Nonhandlers were plantation workers who were not exposed to paraquat during the biological monitoring period and were employed in duties such as manual weeding, planting, harvesting, and transportation of harvested products from the field.

Recruiting Strategies

Telephone Survey

First, a telephone survey among coffee, banana, and palm oil farms was conducted to obtain information on paraquat application. If paraquat application was confirmed, then the trained exposure assistant asked the farm managers or owners about their interest in participating in the study. The information collected at the participating farms included the following: farm name and location, crop type, farm size, number of total workers, number of pesticide handlers, paraquat yearly spray schedule, paraquat spray methods, work days per week, daily working hours, a contact person, and work start time. Finally, a meeting with the farm owner and/or manager was scheduled during the telephone interview.

Farm Meeting

Through the contact person at the farms, it was possible to obtain updated paraquat application schedules. Furthermore, regular phone calls to the contact person helped in maintaining good relationships with the farms and in gaining their confidence to carry out the study. During the meeting with farm owners or managers, we explained the study purpose and the experimental design, data analysis plan, and confidentiality of both farm and individual worker information. It was also emphasized that there would be no reduction of work hours and no interruption of work schedule with their participation in the study. Another purpose of the meeting at the farm was to review with the owner or manager the information that was acquired by the previous telephone interview. The exposure team then modified their schedule accordingly to meet the actual field situation if the farm information was found to be inaccurate, such as number of pesticide applicators or paraquat application schedule. This meeting was crucial because the farm owners or managers' interest in participating would ensure good collaboration from the farm. The presence of farm managers directly working with farm workers during the meeting was very important for several reasons. For example, it was possible to obtain accurate and specific farm information from farm managers because they were field operators and knew current farm conditions, such as weed type, paraquat application schedule and areas, each worker's personality, or daily/weekly base farm activity. In addition, the biological monitoring time and period was established based on the paraquat application schedule provided by the farm managers. The managers also facilitated scheduling the meeting day, time, and location with farm workers. In general, meetings with farm workers after work hours were avoided because their attention to the explanation of the project details could be very low, which might affect the participation rate.

Meeting with Farm Workers

Field visits were based on paraquat application schedules at the farms. However, each field visit was confirmed by telephone with farm managers prior to the visit because the

paraquat application plan at each farm could change due to weather conditions (rainfall), other farm activities, and/or a shortage of herbicide handlers (e.g., due to illness or job reallocation).

During the meeting with farm workers to recruit volunteers, we organized snack breaks to have the opportunity to speak informally with all farm workers for the purpose of building friendly relationships between the team members and farm workers. This allowed the exposure team to effectively approach the potential participants by knowing critical concerns they would not express otherwise in front of an audience. In this way, farm workers were more willing to share information relevant to the study once they began to know and trust us. In fact, from these informal talks, we realized that the term "paraquat" was not familiar to farm workers in Costa Rica, and we began to use "Gramoxone" instead (a popular brand name for the herbicide). We presented an overview of the study at these meetings, emphasizing the importance of the exposure assessment component to the success of the overall study. We also showed the procedures and equipment used for the 24-hour urine collection to the participants (i.e., personal 4-L containers and carrying bags). We spent some time after the presentation answering questions from the farm workers. At this meeting, we attempted to identify group leaders among the farm workers who might be willing to assist the team by enhancing participation. The help of a group leader among farm workers positively influenced the recruitment; therefore, we dedicated extra time to the leaders to obtain their support.

The recruiting procedure consisted of obtaining participant information including name, age, date of birth, job description (handler or control), *cedula* number (social security number in Costa Rica), and number of years working at the current farm. Participants were also assigned a subject identification number and provided with a urine sample collection kit. Informed consent forms in Spanish were obtained from all participants in the final step of the recruiting procedure. We then spent extra time with these participants answering questions to ensure complete understanding of the urine collection procedure, to remind them of the importance of the study, and to encourage a high degree of effort to achieve complete 24-hour urine collections.

Incentives

To reduce the inconvenience and potential embarrassment of carrying the urine container all-day long, we provided the farm workers with a carrying bag for the urine container as part of their sample collection kit, as well as a pen to record voiding times. The subjects kept the carrying bag and pen, as well as other incentives, including an official logo sports cap and T-shirt from the National University of Costa Rica, the collaborating institution in this study.

Preparation for the Field

The exposure assessment team in Costa Rica consisted of a field manager, a graduate student, and a local exposure team assistant hired in Costa Rica. The input of the exposure team assistant was critical to the achievement of the project's goals because he was familiar with the culture, language, and farm locations. During the training period in Costa Rica, there were several simulation tests to improve data collection techniques and increase the team's confidence in successfully completing data collection.

All documents and data collection instruments and instructions were translated into Spanish. These included a form for recording farm information, a description of the study purpose, simple questionnaires, participant's personal information record sheets, field observation sheets, instructions for urine collection, a urine-void (start and end) time sheet, and the consent form. The Spanish written instruction for 24-hour urine collection was printed in color with drawings.

Before departure to the field, the exposure assessment team verified that the equipment and materials to be used, based on a checklist, were in good working condition. Field collection kits to be provided to the participant were prepared. The kit included a 4-L amber-colored urine collection container with molded-in graduations and an opening of 79-mm diameter. To this container was added 12 g of boric acid (H_3BO_3) to decrease contamination of the urine by microorganisms. Containers were placed in a carrying bag with a pen, a self written urine-void time

sheet to record first voiding and last voiding time, and written instructions for the 24-hour urine collection in Spanish. The exposure assessment team usually arrived at the farms at least 2 days prior to a paraquat spray day.

Sampling

The 24-hour urine samples were collected before, during, and after paraquat application days. The exposure assessment team met with the participants each day of the sampling period at the places where the farm workers gathered everyday before beginning fieldwork. The research team arrived at least 30 minutes before the participants began work to answer any questions regarding the 24-hour urine collection, to take the collected urine sample and replace the container with a fresh one for that day's collection. After the first round of 24-hour urine collection, participants were interviewed by the exposure team about job tasks, types of pesticides used, location of loading and mixing of pesticides, and type and use of personal protective equipment (PPE). Participants were also asked if the 24-hour urine collection was accomplished at the time they returned the urine samples. We recorded the answers as "complete collection," "complete except for a few drops," "missing less than a half cup (8oz [237 ml] size)," and "missing more than a half cup." Interviews were restricted to 5 minutes or less based upon permission from the field manager to avoid any conflict with the daily work schedule.

For spot urine samples, 12 paraquat applicators who applied paraquat for 2 consecutive days followed by a nonparaquat handling day and who were participating in 24-hour collections were asked to give spot urine samples at the end of shift. Spot urine samples were prepared for shipping as described below for 24-hour samples.

Sample Preparation and Analysis

Total urine volumes were measured and recorded. After vigorously shaking the urine collection container, the urine of each subject was transferred into five 15-ml clear polypropylene tubes that were immediately placed into a cooler with ice packs until the exposure team left the field for the day. Any remaining urine in the container after transfer was emptied into the toilet at the farm, and containers were washed with water and soap. The empty containers were provided back to the farms on request; otherwise, they were taken to a local recycling company. After returning from the field work, the 15-ml tubes were kept at −20°C at the National University until the samples were airmailed with ice packs to the analytical laboratory located at the University of California, Davis, in the United States. Samples were shipped once a month, with backup samples stored in Costa Rica, to compensate for any loss during shipment.

Creatinine Measurement

Creatinine concentrations were measured in triplicate using a 96-well plate format kinetic assay (Sigma Diagnostics, St. Louis, MO) at the University of California, Davis. Frozen urine samples were thawed and vortexed. A 100-µl urine aliquot from each sample was diluted 10-fold with deionized, distilled water for creatinine determination. Each sample was run against an independent 1 to 10 mg/dl calibration curve. The resulting values were generally within 5% but never differed more than 10%. Further dilutions were prepared as necessary to bring samples within the calibrated range.

Validation of Urinary Creatinine Excretion Using a Formula to Predict Creatinine Values

To determine 24-hour urine collection completeness, creatinine levels in collected urine can be compared to predicted values from several statistical models of 24-hour creatinine excretion.[16,22] Of the participants, 84 subjects provided their height and weight, which were used to calculate their predicted 24-hour urinary creatinine excretion. The formula used for men with milligrams of creatinine suggested by Fournier and Achard[22] is:

$$\text{Urinary creatinine (mg/24 h)} = [28 - (0.2 \times \text{age})] \times \text{body weight}$$

The measured urinary creatinine values were compared to the predicted creatinine values calculated by the formula.

Analysis for Paraquat

Paraquat concentration in all urine samples were determined by enzyme-linked immunosorbent assay (ELISA).[23] Briefly, paraquat was purified on an ion exchange solid-phase extraction column to eliminate potential compounds that would interfere in the analysis, including boric acid. The paraquat was eluted using 1 M ammonium chloride in 50% methanol. The eluant was diluted and analyzed. Spiked urine samples with known concentrations of paraquat were purified with each sample set for recovery and quality assessment. For the ELISA, 96-well microtiter plates (Nunc, Roskilde, Denmark) were coated overnight with paraquat coating antigen in carbonate-bicarbonate buffer at pH 9.6. The next day, the plate was washed with phosphate buffered saline, pH 7.5, containing 0.05% Tween 20 (Sigma Chemical). Fifty microliters of each sample or standard was pipetted into wells in triplicate. The standards consisted of paraquat dichloride (0 to 3.2 ng/mL) in phosphate-buffered saline containing 0.05% Tween 20 and 0.05% bovine serum albumin (Sigma Chemical). To each well was added 50 µl of paraquat polyclonal antibody diluted 1/10,000, and the plate was incubated at room temperature for 20 minutes. Unbound antibodies were washed away, and bound antibodies were detected by adding 100 µl of anti-rabbit immunoglobulin G (IgG)–horseradish peroxidase conjugate to each well and incubating for 40 minutes. One hundred microliters of substrate solution was added resulting in a blue color. The enzyme reaction was stopped by the addition of 2 M sulfuric acid. The plates were then read in a microplate reader (Molecular Devices, Sunnyvale, CA) at 450 nm. Paraquat concentrations in the samples were determined by comparison of the absorbance to the absorbance obtained for a known standard using the software package Softmax Pro (Molecular Devices).

The limit of quantitation of the developed method was 2 ng/mL. The intra-assay coefficient of variation was always <15% and in most cases <5%. The interday precision during 4 different days did not exceed 13%. The average recovery for concentrations between 0.4 and 90 ng paraquat/ml was 95.2%. The method was validated by analysis of a set of both spiked and field-collected urine samples and comparison to liquid chromatography-mass spectrometry method. The linearly regressed data showed a good correlatio, with an R^2 of 0.945 and a slope of 0.94 and intercept of 1.23, showing a slight tendency for underestimation by ELISA. Spiked urine samples with known concentrations of paraquat were purified with each sample set for recovery and quality assessment. All samples were analyzed in a blind fashion and several studies conducted to assure data quality, including sample storage stability, stability of extracts, effects of sample shipment, and repeatability.[23]

Statistical Analysis

Pearson correlation coefficient was applied for statistical analysis (GraphPad Prism version 4; San Diego, CA). The test was two-tailed. A p value less than or equal to .05 was considered statistically significant.

RESULTS

A total of 16 farms participated in the study, including 5 banana farms, 10 coffee farms, and 1 palm oil farm. One hundred and eighty-seven farm workers who were classified as paraquat handlers were recruited for the biological monitoring component of the study (Table 1) as well as an additional 54 nonhandlers. The largest number of subjects was from the coffee farms. The overall refusal rate and rate of subjects sick or not working on the study day were low (8.0% and 2.1%, respectively). Subjects (N = 49, 26.2%) having other jobs, such as insecticide spraying or hand weeding on the sampling day, were excluded. Among the paraquat handlers, a total of 119 subjects (88.8%) participated. Participation rate at banana and palm oil farms was 100% but was 83.5% at coffee farms. This was due to the exposure team not having the primary responsibility for the recruiting procedure at one coffee farm, resulting in 11 handlers refusing to participate.

To determine the completeness of the 24-hour urine collections, we compared the answers on a short questionnaire with the urinary creatinine

TABLE 1. Paraquat Handler Recruitment and Participation Rate by Crop

Total Number of Paraquat Handlers	Overall (*N* = 187) % (*N*)	Banana (*N* = 28) % (*N*)	Coffee (*N* = 140) % (*N*)	Palm Oil (*N* = 19) % (*N*)
Refusal	8.0 (15)	—	10.7 (15)	—
Sick/not at work on test day	2.1 (4)	3.6 (1)	2.1 (3)	—
Other jobs on test day	26.2 (49)	10.7 (3)	32.9 (46)	—
Participated*	88.8 (119)	100 (24)	83.5 (76)	100 (19)

*Excluded those sick/not at work and doing other jobs on test day.

levels measured. A total of 393 24-hour urine samples from 173 participants were collected. Although we anticipated collecting three samples per participant, the number of samples collected on the day prior to paraquat spraying and on the day after spraying was less than on the spraying day because handlers were often applying 6 days per week, and it was difficult to collect urine samples during the weekend. The overall average creatinine level was 1.11 (± 0.50) and ranged from 0.13 to 3.48 g/L (Table 2). In our study, 92.4% (*N* = 363) of the urine samples were in the acceptable range of urinary creatinine (0.5 to 3.0 g/L).[9,10] If normal references of urinary creatinine were employed (0.5 to 3.5 g/24 h[18] and 0.3 to 3.0 g/L[17]), 96.7% (*N* = 380) of the urine samples fell within these ranges.

The results from the questionnaire indicated that 94.7% (*N* = 372) of the samples were "complete urine samples," 1.8% (*N* = 7) of the samples were "complete except for a few drops," 0.3% (*N* = 1) of the samples were "missing urine less than a half cup," and 3.3% (*N* = 13) of them were "missing urine more than a half cup" (Table 3). The mean urinary creatinine level for subjects who reported a complete urine collection on the questionnaire was 1.10 (± 0.49) g/L (Table 3). When we dichotomized completeness of urine collection by merging the responses "complete urine samples," "complete

TABLE 2. Overview of Creatinine Levels in All Urine Samples

Creatinine Level (g/L)	Frequency Rate (%) (Urine Samples/Total)	Mean (± SD)	Median	Range
Overall	100 (393/393)	1.11 (± 0.50)	1.09	0.13–3.48
0.5–3.0	92.4 (363/393)	1.15 (± 0.43)	1.12	0.50–2.98
<0.5	6.9 (27/393)	0.35 (± 0.10)	0.34	0.13–0.49
>3.0	0.8 (3/393)	3.22 (± 0.22)	3.10	3.09–3.48

SD, standard deviation.

TABLE 3. Relationship Between Questionnaire and Urinary Creatinine Level

Questions in Questionnaire	Frequency Rate of Answer		Urinary Creatinine Level (g/L)			
			Mean	SD	Median	Range
Complete	94.7%	(372/393)	1.10	0.49	1.08	0.13–3.48
Complete except for a few drops	1.8%	(7/393)	1.37	0.35	1.23	0.78–2.33
Missing ≤ half cup	0.3%	(1/393)	1.10	—	1.10	1.10
Missing ≥ half cup	3.3%	(13/393)	1.38	0.70	1.12	0.63– 2.98

except for a few drops," and "missing urine less than a half cup" from the questionnaire into the "complete" category (resulting in the response "missing urine more than a half cup" equaling the "not complete" category), 380 urine samples were included as complete 24-hour urine collection (Table 4). Among the 380 samples, 350 samples (92.1%) were in the acceptable urinary creatinine range (0.5 to 3.0 g/L), with an average of 1.15 (± 0.42) g/L. Furthermore, 96.8% (N = 368) and 96.6% (N = 367) fell within the normal reference of urinary creatinine, reported to be 0.5 to 3.5 g/24 h[18] and 0.3 to 3.0 g/L,[17] respectively.

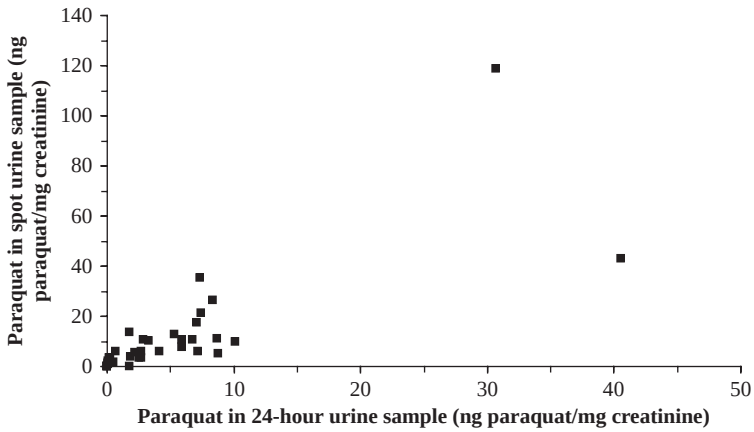
Eighty-four subjects who participated in the epidemiologic study[23] provided their weight and height. The mean age of these participants was 34.9 (± 10.6) years, and 75% were of normal weight (body mass index = 18.5–24.9). The mean measured urinary creatinine level for these 84 subjects was 1.18 (± 0.41) g/L or 1.24 (± 0.36) g/24 h. The mean predicted creatinine excretion value for the 84 subjects determined by the predicted creatinine formula was 1.37 (± 0.23) g/24 h. Correlation (Pearson's r) between measured creatinine and predicted creatinine was .327 (p = .0024, 95% CI .12–.51).

Twelve paraquat applicators provided end-of-shift spot and 24-hour urine samples over 3 days that were used to determine urinary paraquat levels. Urinary paraquat levels were adjusted for urinary creatinine levels. Compared to 24-hour urine paraquat levels (gold standard with specificity of 100%), detected paraquat levels in spot urine samples had a sensitivity of 96.9%. There was a significant (p < .0001) correlation between end-of shift spot and 24-hour urine sample paraquat levels (Pearson's r = .7825, 95% CI .61–.88) as presented in Figure 1.

TABLE 4. Distribution of Urinary Creatinine Level From the Questionnaire When Questions "Complete," "Complete Except A Few Drops," and "Missing Less Than Half Cup" Were Treated As Complete 24-Hour Urine Collection Samples

Creatinine Level (g/L)	Frequency Rate (Urine Samples/Total)		Mean	SD	Median	Range
Overall	100 %	(380/380)	1.11	0.49	1.08	0.13–3.48
0.5–3.5	92.1 %	(350/380)	1.15	0.42	1.12	0.50–2.64
<0.5	7.1 %	(27/380)	0.35	0.10	0.34	0.13–0.49
>3.5	0.8 %	(3/380)	3.22	0.22	3.10	3.09–3.48

FIGURE 1. Relationship of absorbed paraquat levels between end-of-shift spot and 24-hour urine samples.



DISCUSSION

The recruiting strategy developed in this study was very useful and successful in our effort to collect 24-hour urine samples among a population of farm workers. Where 24-hour urine collections are needed, the time, cost, and logistics involved as well as the time commitment of the volunteer make compliance critical to success. Particular challenges to the 24-hour urine collection were successfully met by our developed recruiting strategies. The first important step was building a relationship between the exposure team and farm managers or owners to obtain their support for arranging a meeting with farm workers and for providing farm information and farm working hours to the research team for recruiting and interviewing. This study demonstrates the importance of involving local leaders in carrying out field epidemiologic or exposure studies conducted by local or international investigators. The local leaders influenced farm workers' decisions during the recruiting process. This issue is of particular relevance among populations with a different cultural background, such as in non–English-speaking communities. High participation in this study may also be attributed to our attention to the potential embarrassment of carrying urine containers throughout the day, which was alleviated by providing a carrying bag. The consistent review of the collection procedures was also a positive outcome of meeting with the participants before work hours. In addition, by answering participants' questions about the study and urine sample collection before work hours, recall bias was decreased.

The participation rate of herbicide handlers in this study was about 90%, which was higher than a study of arsenic determination conducted in Australia[24] that had 100% participation for spot urine collection but 82.5% for 24-hour urine collection. We feel that our developed recruiting strategy had a positive impact on participation, which has been declining in all types of studies over the past 30 years.[25]

Complete 24-hour urine collection may not be an easy task to achieve due to forgetfulness, embarrassment, spills, dropping of the container, difficulty in carrying the container, accidental contamination, and loss of urine during defecation. Assuming urine creatinine is the "gold standard" reference, 24-hour urine collection completeness can be estimated because muscle mass does not change dramatically over short periods of time.[9] A total of 92.4% to 96.7 % of urine samples in this study fell within the normal creatinine ranges of 0.5 to 3.0 g/L,[9,10] 0.3 to 3.0 g/L,[17] and 0.5 to 3.5 g/24 h.[18] The overall mean (± SD) urinary creatinine level from 173 participants (N = 393) was 1.11 (± 0.50) g/L or 1.21 (± 0.45) g/24 h in this study. This is comparable to several studies reporting mean measured 24-hour urinary creatinine levels, including 1.73 (± 0.39) g/24 h from 98 turf applicators in Canada,[10] 1.37 (± 0.47) g/24 h from 15 pesticide applicators in the United States,[26] 1.08 g/L (range 0.96–1.19) from 79 male bus drivers and mail carriers in Denmark,[27] and 1.48 g/L (range 1.45–1.51) from a sample of the general U.S. population (10,610 participants).[28] Exact comparability is not expected because workers in different jobs and countries may have differences in body mass.

The completeness of the 24-hour urine collection based on the questionnaire responses was high (94.7%) in this study. Other studies using self-reporting to check completeness of 24-hour urine collection have had similar or lower success rates; 89% completeness (N = 175) from 196 24-hour urine samples from professional turf applicators in Canada[10] and 78% completeness (N = 145) from 186 24-hour urine samples from bus drivers and mail carriers in Denmark.[27] The quality of 24-hour urine in this study was satisfactory based on urinary creatinine values that had good correlation with the questionnaire data.

It is known that urinary creatinine excretion is affected by several factors, for example, age, gender, diet, body mass, medication use, physical activity, and renal function.[14–16]. To determine predicted urinary creatinine excretion, the formula suggested by Fournier and Achard[22] was applied to 84 subjects with age and weight determinations, and the mean predicted urinary values significantly correlated with measured urinary creatinine values (p = .0024). When we compared absorbed paraquat concentrations in end-of-shift spot and 24-hour urine samples, there was a significant correlation between the

two sampling methods ($p < .0001$). Spot urine sampling is commonly used in occupational health because it is comparatively easy, practical, and simple compared to 24-hour sampling. Detected paraquat levels of spot urine samples had a sensitivity of 96.9% at the high specificity of 100% using 24-hour urine samples as the gold standard; these levels of sensitivity and specificity indicate that our developed recruiting strategy and education for 24-hour urine collection are useful for occupational health studies, but spot urines may be acceptable for some investigations.

The recruiting strategy developed in this study was successful and applicable in conducting a study of biological monitoring with 24-hour urine collection and has important implications for other studies that involve biological monitoring of populations with low-education and cultural differences. Specifically, it is realistic to collect 24-hour urine samples among low-education farm workers and other working populations in developing countries. The advantages of collecting 24-hour urine samples must be considered in light of the specific agents being measured and the additional time and cost involved.

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