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Pulmonary Function and Exercise-associated Changes with Chronic Low-Level Paraquat Exposure

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The present study was undertaken to test the hypothesis that chronic, low-level paraquat exposure causes restrictive lung function with gas transfer impairment. Three hundred thirty-eight Costa Rican farm workers from banana, coffee, and palm oil farms completed a questionnaire, spirometry, and a test of single-breath carbon monoxide diffusing capacity. Subjects 40 years of age or older, without other medical risk factors, completed maximal cardiopulmonary exercise tests. Most (66.6%) were paraquat handlers; 24.8% of handlers and 27.3% of nonhandlers reported current cigarette smoking. In linear regression models, cumulative paraquat exposure was not an independent predictor of V_A, carbon monoxide diffusing capacity, peak oxygen uptake, FVC, or oxygen pulse peak. However, the ventilatory equivalent for CO_2, although within normal range, was significantly higher with increased cumulative paraquat exposure. Oxygen desaturation greater than 5% from rest to peak exercise had an odds ratio of 1.7 (95% confidence interval = 0.9–3.0) with the cumulative paraquat exposure index in models adjusted for age, weight, and smoking status. The association of paraquat exposure with ventilatory equivalent and oxygen desaturation suggests that paraquat may be associated with subclinical gas exchange abnormalities, but overall these findings are consistent with no clinically significant increases in interstitial thickening or restrictive lung disease among this population.

Keywords: agriculture; exercise test; interstitial lung disease; occupational exposure; respiratory function tests

Paraquat (1,1′-dimethyl-4,4′ bipyridinium dichloride), a cationic bipyridylium-class herbicide, is a nonselective and nonsystemic herbicide that has been used widely throughout the world over the past 40 years. The development of this herbicide was considered an important agricultural advance because the chemical is tightly bound to particles in soil and leaves no toxic residue. However, poisonings from acute paraquat exposure have been reported with its use. It is classified as highly toxic based on its inhalation toxicity (EPA toxicity class I), as moderately toxic via the oral route (class II), and as slightly toxic by the dermal route (category III) (1). Since paraquat was introduced into the market, several symptoms have been reported including nail damage, skin burns and rashes, nose bleeding, and pulmonary fibrosis (2–5). Numerous cases of fatal paraquat poisoning have been reported as a result of accidental or intentional (suicidal) ingestion of the compound (6–13). Respiratory failure from adult respiratory distress syndrome (ARDS) is a prominent outcome in fatal paraquat ingestions (14), demonstrating the ability of paraquat in high doses to cause oxidative damage to the lung, pulmonary fibrosis, and respiratory failure. The radiologic appearance in paraquat poisoning begins with air–space consolidation, which then leads to end-stage lung disease with fibrosis (15).

This observation has raised concern that chronic, low-level exposures may result in interstitial fibrosis. However, the occupational hazards presented by chronic paraquat exposure are less well characterized, and epidemiologic studies have not shown consistent pulmonary changes among paraquat handlers (16–20). Castro-Gutierrez and colleagues (19) found that exposure to paraquat, as estimated by skin rash symptoms, was related to self-reported respiratory symptoms (chronic bronchitis and episodic dyspnea with wheezing) but they did not observe an association of paraquat exposure with pulmonary function changes measured by spirometry. A study of 85 Sri Lankan paraquat applicators found no difference in respiratory symptoms, spirometry, or diffusion capacity compared with control subjects comprised of factory workers and general workers (18).

A more recent study by Dalvie and coworkers of 126 paraquat applicators in the Western Cape region of South Africa classified exposure using a job-exposure matrix (20). Dalvie and coworkers did not observe an association of paraquat exposure and respiratory symptoms, spirometry, or carbon monoxide diffusing capacity, but they did report a positive association of chronic paraquat exposure and arterial oxygen desaturation during maximum exercise (20). However, 28% of the saturation traces were considered unreadable, and among those with readable traces the magnitude of the effect was small. Additional limitations from other epidemiologic studies include small sample sizes (16, 21), poor exposure characterization (18, 19), potential for selection bias, insensitive outcome measures (19), and lack of biological measures of exposure (20).

METHODS

Study Population

The Study of Agricultural Lung Disease (SALUD) was conducted at banana, coffee, and palm oil farms throughout Costa Rica. Recruitment focused on these commonly grown commodities in Costa Rica that use similar paraquat application practices. Sixty-two farms were identified on the basis of these criteria. After the initial contact, farms were excluded because they no longer used paraquat (n = 11), or had too few handlers to justify the expense and effort of data collection (n = 25). Among the remaining farms, 22 participated, 2 refused, and 2 were included in pilot study testing.

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A total of 77.5% (n = 338) of selected workers participated (Table 1), and refusals to participate were < 2.0%. Two additional workers who completed the interview were excluded because their total work history was less than 1 year.

Data Collection

Data collection included an interviewer-administered questionnaire, pulmonary function testing, and cardiopulmonary exercise testing. All data were collected at the work site with the cooperation of the farm owners and managers. The questionnaire assessed demographic and lifestyle factors, work history, occupational exposures, and the presence of respiratory symptoms. Questions were taken from existing standardized questionnaires where possible (22). The questionnaire was developed in English, translated into Spanish, and underwent an extensive review process by consultants, the field coordinator, and interviewers to ensure that the language was appropriate for Costa Rica.

Pulmonary Function and Cardiopulmonary Exercise Tests

Spirometry and single-breath carbon monoxide diffusing capacity (D_{LCO}) were recorded by a trained technician using the Collins CPL PFT system consisting of a dry-sealed spirometer interfaced to a personal computer (Collins Medical, Inc., Braintree, MA) (23). At least three FVC trials and two D_{LCO} trials, meeting ATS criteria for acceptability and reproducibility, were obtained for each individual (24–26). Normal prediction equations were based on Morris for spirometry (27) and Gaensler for diffusion capacity (28).

Overall, 3.8% of participants did not complete spirometry and 10.9% did not complete D_{LCO} trials. Reasons for failure to complete spirometry included 1.5% unable to perform the necessary breathing maneuvers, 0.3% refusals, and 2.1% for unknown reasons. Reasons for failure to complete diffusion testing included inability to hold breath for 10 seconds (2.9%), refusals (0.9%), lack of gas mixture in the field and subsequent loss to follow-up with test rescheduling (2.9%), and unknown reasons (3.5%).

Pulmonary function tests were individually reviewed and graded for quality, blinded to subject's exposure status (29). Only participants whose spirometry and diffusion tracings met or exceeded ATS criteria were included in analysis. Of the spirometry tests, 98.2% and 97.5% had acceptable FEV_1 and FVC, respectively. Among 305 diffusion tests, 96.1% met acceptability criteria.

Maximal cardiopulmonary exercise tests were performed on a Monark mechanically braked cycle ergometer (Monark Exercise AB, Vansbro, Sweden). A customized exercise protocol consisted of 5 minutes of warm-up cycling at 30 watts followed by 20-watt step increments every minute until subjects reached exhaustion. Measurements were obtained using a Medical Graphics portable exercise system model VO2000 with aerograph software (Medical Graphics Corporation, St. Paul, MN). According to the American College of Sports Medicine (ACSM), maximal exercise testing for men older than 40 years should be performed under physician supervision, even when no symptoms or risk factors are present. Because a physician was not always available during the exercise tests, patients over 40 years of age were excluded.

TABLE 1. WORKER RECRUITMENT AND PARTICIPATION BY CROP

Total number of workers:	Overall 560 % (n)	Banana 356 % (n)	Coffee 163 % (n)	Palm oil 41 % (n)
Ineligible	7.7 (43)	9.3 (33)	4.9 (8)	4.9 (2)
No longer works at farm	14.5 (81)	21.6 (77)	2.5 (4)	0
Refused	1.6 (7)	1.2 (3)	2.6 (4)	0
Nonrespondent	3.6 (16)	2.8 (7)	6.0 (9)	0
Sick/not at work on test day	2.1 (9)	3.3 (8)	0	2.4 (1)
Status unknown	15.1 (66)	10.2 (25)	27.2 (41)	0
Participated*	77.5 (338)	82.5 (203)	64.2 (97)	97.4 (38)

* Excludes those ineligible and no longer working at the farm.

All subjects were screened before exercise testing according to ACSM criteria (30, 31). Resting blood pressure and pulse oximetry measurements using a Quartz Q-400 with reusable finger sensor (Quartz Medical Inc., Louisville, CO) were also taken before the exercise test. One hundred twenty-nine subjects were excluded from exercise testing because of age (33%), prior cardiac disease (4%), back and knee problems (1%), or other unknown reasons (1%). Quality control was performed blinded to subject's exposure status. Breath-by-breath results of each test were averaged every 30 seconds and checked for consistency among variables and across variables with increments in work rates (31). When errors were observed, a revision of the data averaging was performed, and if this was not the cause, an error in the methodology was considered. Six tests were excluded due to significant errors in the methodology.

Exposure Measure

The primary exposure measure was a cumulative paraquat exposure index that was created using biological monitoring data and included weighting for the type of crop and use of protective equipment. Methodology for the creation of this index is described elsewhere (Lee K, Park E, Stoecklin M, Koivunen M, Gee SJ, Hammock B, Beckett L, Schenker M. Occupational exposure of agricultural workers in Costa Rica to paraquat. Submitted manuscript). The cumulative exposure index was calculated based upon work history reported by each worker, including the handling of paraquat in each job, the length of employment, the type of crop, and the use of protective equipment. In analyses, the cumulative paraquat exposure index was log transformed to improve the fit of the regression models. Values below the limit of detection for paraquat on this index were coded to one before log transformation, resulting in zero values on the log scale. Mean paraquat exposure on this log-transformed index was 1.25, SD = 1.12, and 25th–75th percentile was 0.0–2.25. The log scale exposure index was treated as a continuous variable in multivariate analyses.

Power Calculations

Power and sample size calculations were done based upon the cumulative paraquat exposure measure used in the study. All calculations were based on a two-sided test with $\alpha = 0.05$. With a sample size of 200, we had 80% power to detect an association with cumulative paraquat exposure explaining as little as 3% of the variability in the exercise test outcomes ($\dot{V}O_2$, $\dot{V}E/\dot{V}CO_2$, $\dot{V}O_2$/heart rate [HR]). For analyses of cumulative paraquat exposure with pulmonary outcomes (TLC_{SB}, D_{LCO}, FVC, FEV_1, FEV1/FVC) with a sample size of 300, we had 93% power to detect an additional contribution of as little as 3% to the variation in the outcome. In the logistic regression analysis of the oxygen desaturation data, with 200 subjects we had 81% power to detect an odds ratio of 2.2, assuming 10% of the sample had cumulative paraquat exposure at the mean and the squared correlation of other covariates (age, weight, and smoking) was 0.1.

Statistical Analysis

The primary analytic goal of this cross-sectional analysis was to evaluate the association between paraquat exposure and cardiopulmonary function. Analyses were performed in SAS, version 8 (Cary, NC). Descriptive statistics were performed for outcome and predictor variables, as well as covariates. Histograms and other plots were used to assess the distributions of each variable, and transformations were considered for variables that were not normally distributed.

Linear regression was used to regress pulmonary function and exercise outcome measures on the cumulative paraquat exposure index (30, 31). Initially, age, height, and smoking status were entered simultaneously in models with the pulmonary function outcomes. Models with the exercise test outcomes included age, weight, and smoking status. Other covariates were then added to see if the fit of the model improved with their addition. Covariates were retained in the final models if they changed the parameter estimates from the crude by at least 10% or if they were important for making comparisons to the literature. All comparisons were two-sided at α level = 0.05.

RESULTS

A total of 338 workers from banana, coffee, and palm oil farms in Costa Rica completed the interviewer-administered questionnaire and were included in the study. Mean age of workers was 37 years (SD = 10), and 71% were normal weight (body mass index = 18.5–24.9) (Table 2). A quarter of the subjects reported current smoking. Among current smokers, 46.5% smoked less than five cigarettes per day, and 63% smoked less than half a pack per day in the last 30 days. Median pack-years in the sample was 4.3 (25th–75th percentile = 1.7–12.2).

Workers classified as handlers reported 6 months or more work experience mixing, loading, or applying paraquat; nonhandlers reported no experience with handling paraquat. Sixty-six percent (n = 219) of workers were classified as handlers and 33% as nonhandlers. Handlers reported a mean of 8.5 years' (25th–75th percentile = 2–13 years) experience with paraquat. Handlers were slightly older (38.3 versus 35.6 years) and shorter (167.4 versus 169.1 cm) than nonhandlers, but mean weight among the two groups was comparable. The proportion of current smokers was similar among handlers and nonhandlers, but 19.3% of handlers were ex-smokers compared with 7.3% of nonhandlers ($p = 0.02$). Handlers were less likely to have grown up on a farm ($p = 0.001$). The number of years working at the current farm was similar for handlers and nonhandlers (10.2 years and 9.1 years, respectively).

TABLE 2. CHARACTERISTICS OF COSTA RICAN WORKERS ON BANANA, COFFEE, AND PALM OIL FARMS BY PARAQUAT HANDLING STATUS

	Overall Distribution (*n = 338*)	Handler (*n = 219*)	Nonhandler (*n = 110*)	
Age	% (n)	% (n)	% (n)	p Value*
18–25 yr	15.4 (52)	13.7 (30)	17.3 (19)	
26–35 yr	33.7 (114)	30.6 (67)	41.8 (46)	
36–45 yr	28.4 (96)	32.0 (70)	20.9 (23)	
46+ yr	22.5 (76)	23.7 (52)	20.0 (22)	0.07
Body mass index				
< 18.5	5.4 (18)	6.0 (13)	3.7 (4)	
18.5–24.9	70.5 (234)	70.1 (152)	72.0 (77)	
25–29.9	21.4 (71)	21.2 (46)	22.4 (24)	
⩾ 30	2.7 (9)	2.8 (6)	1.9 (2)	0.80
Education level				
No formal education	4.7 (16)	5.0 (11)	4.6 (5)	
Elementary	83.1 (281)	83.6 (183)	80.9 (89)	
Secondary/ University	12.1 (41)	11.4 (25)	14.5 (16)	0.24
Smoking status				
Never-smoker	59.1 (199)	56.0 (122)	65.4 (72)	
Ex-smoker	15.4 (52)	19.3 (42)	7.3 (8)	
Current smoker	25.5 (86)	24.8 (54)	27.3 (30)	0.02
Grew up on a farm	77.1 (259)	71.6 (156)	88.1 (96)	0.001
Proximity of home to farm				
< 100 m	57.9 (195)	59.4 (130)	54.1 (59)	
100–500 m	25.2 (85)	23.7 (52)	29.7 (32)	
More than 500 m	16.9 (57)	16.9 (37)	16.5 (18)	0.22
Years worked at current farm				
1–4 yr	29.3 (99)	28.3 (62)	29.1 (32)	
5–9 yr	32.5 (110)	32.9 (72)	33.6 (37)	
10–14 yr	16.6 (56)	15.1 (33)	20.0 (22)	
⩾ 15 yr	21.6 (73)	23.7 (52)	17.3 (19)	0.47
Cooking fuel				
Electricity	27.2 (92)	26.0 (57)	28.4 (31)	
Propane gas	61.2 (207)	60.3 (132)	66.1 (72)	
Wood	7.4 (25)	10.0 (22)	2.7 (3)	
Other	4.1 (14)	3.7 (8)	2.7 (3)	0.54
Mean cumulative paraquat exposure (log transformed index)	1.25 (1.12)	1.88 (0.85)	0 (by definition)	

* p Value for chi-square test.

The overall prevalence of reported chronic bronchitis was 7.4%, chronic cough 5.3%, persistent wheeze 7.4%, shortness of breath with wheezing 12.0%, and ever having asthma 3.9%. In logistic regression models adjusted for age, current smokers had significantly higher odds of chronic cough (odds ratio [OR] = 4.0, 95% confidence interval [CI] = 1.5–10.5) and shortness of breath with wheeze (OR = 2.2, 95% CI = 1.1–4.3) compared with never- and ex-smokers as the reference group (Table 3). The odds of asthma, chronic bronchitis, and persistent wheeze were all higher in current smokers, but did not reach statistical significance (Table 3).

Pulmonary function outcome measures were selected *a priori* to be evaluated for association with interstitial or restrictive lung disease. Outcome measures assessed included DL_{CO}, alveolar volume measured with single breath (TLC_{SB}), and peak oxygen uptake ($\dot{V}O_2$) assessed during a maximal exercise test (Table 4). FVC, the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$), O_2 pulse peak ($\dot{V}O_2$/HR), and arterial oxygen desaturation from resting to peak exercise were also examined. Other pulmonary function measurements (FEV_1, FEV_1/FVC, FEF_{25-75}) were evaluated for their distribution and physiologic consistency. Approximately 2% (n = 6) of workers had FVC measures less than 80% predicted, and 3% (n = 10) had FEV_1/FVC results less than 70%. One percent (n = 4) of workers had DL_{CO} values less than 80% predicted.

Paraquat Exposure and Respiratory Symptoms, Pulmonary Function, and Cardiopulmonary Exercise Tests

The cumulative paraquat exposure index was added to logistic regression models after the inclusion of age and smoking status (current versus never/ex-smoker). Each unit increase in the total cumulative paraquat index was associated with a 1.8 increase in the odds of chronic cough (95% CI = 1.0–3.1) and a 2.3 increased odds of shortness of breath with wheeze (95% CI = 1.2–5.1) (Table 3). Increases in the cumulative paraquat index were not significantly associated with chronic bronchitis, persistent wheeze, or ever having a diagnosis of asthma. Models for chronic bronchitis, chronic cough, persistent wheeze, and shortness of breath with wheeze were also examined with asthma added as a covariate, with no change in the association between the paraquat exposure measure and respiratory symptoms (data not shown).

Mean percent predicted was in the normal range for all of the spirometry and diffusion capacity outcome measures. A comparison of spirometry and diffusion capacity outcomes between

TABLE 3. ADJUSTED ODDS RATIO AND 95% CONFIDENCE INTERVAL FOR SELF-REPORTED RESPIRATORY SYMPTOMS ASSOCIATED WITH SMOKING AND CUMULATIVE PARAQUAT EXPOSURE

	Current Smokers*† OR (95% CI)	Cumulative Paraquat Exposure‡ OR (95% CI)
Ever had asthma	2.6 (0.8–7.9)	1.6 (0.9–3.0)
Chronic bronchitis	1.7 (0.7–4.1)	1.3 (0.9–2.0)
Chronic cough	4.0 (1.5–10.5)	1.8 (1.0–3.1)
Persistent wheeze	1.4 (0.6–3.5)	1.1 (0.7–1.6)
Shortness of breath with wheeze	2.2 (1.1–4.3)	2.3 (1.2–5.1)

Definition of abbreviations: CI = confidence interval; OR = odds ratio.
* Logistic regression models adjusted for age.
† Never-smokers and ex-smokers as the reference.
‡ Logistic regression models adjusted for age and current smoking.

TABLE 4. PULMONARY FUNCTION AND CARDIOPULMONARY EXERCISE MEASURES

	n	Handler Mean (SE)	Nonhandler Mean (SE)	p Value
Pulmonary function measures				
FVC, % predicted	317	101.3 (0.8)	101.6 (1.1)	0.83
FEV_1, % predicted	319	106.0 (0.8)	105.3 (1.1)	0.61
FEV_1/FVC, %	314	81.8 (0.4)	81.4 (0.6)	0.54
FEF_{25-75} L/s, % predicted	314	106.8 (2.0)	105.3 (1.1)	0.55
TLC_{SB}, % predicted	291	95.3 (0.7)	95.2 (1.0)	0.92
DL_{CO}, % predicted	291	112.9 (1.3)	115.0 (1.8)	0.35
Cardiopulmonary exercise measures				
$\dot{V}O_2$ peak, L/min	200	3.1 (0.1)	3.2 (0.1)	0.19
Resting Sp_{O_2}, %	206	98.5 (0.1)	98.3 (0.1)	0.34
Peak Sp_{O_2}, %	203	96.1 (0.2)	96.5 (0.3)	0.31
Sp_{O_2} (start-max), %	203	2.3 (0.2)	1.8 (0.3)	0.16
Peak HR, beats/min	200	187.4 (0.6)	189.4 (0.8)	0.04
$\dot{V}E/\dot{V}CO_2$	200	28.0 (0.2)	27.5 (0.3)	0.17
O_2 pulse peak per kg weight, ml/beat/kg	200	47.4 (0.8)	49.0 (1.1)	0.24

Definition of abbreviations: DL_{CO} = single-breath carbon monoxide diffusing capacity; FEF_{25-75} = mean mid-expiratory flow; HR = heart rate; Sp_{O_2} = oxygen saturation measured by pulse oximetry; TLC_{SB} = total lung capacity from single breath; $\dot{V}E/\dot{V}CO_2$ = ventilatory equivalent for CO_2.

handlers and nonhandlers revealed no statistically significant differences in mean percent predicted for any of the measures (Table 4). Similarly, there was no significant difference in mean values for the cardiopulmonary exercise testing outcome measures (Table 4).

We tested the association of pulmonary function with cumulative paraquat exposure in regression models after inclusion of age, height (or weight) and smoking status. TLC_{SB}, DL_{CO}, and $\dot{V}O_2$ all showed very small negative coefficients for cumulative paraquat exposure that were not statistically significant (Table 5 and Figure 1). In addition, cumulative paraquat exposure was not an independent predictor of other pulmonary function parameters, including FVC, FEV_1/FVC, and FEF_{25-75}. Among the other exercise outcome measures, the parameter estimate for $\dot{V}O_2$/HR was small and nonsignificant. The total cumulative paraquat exposure index showed a statistically significant increase in $\dot{V}E/\dot{V}CO_2$ for each unit increase in exposure, although paraquat exposure accounted for a small portion of the overall variance (Table 5 and Figure 1). These associations were also examined in models with paraquat exposure measured as a dichotomous variable (handler versus nonhandler) and quartiles of the cumulative exposure index. Regression models with these exposure variables produced similar results as with the cumulative exposure index (data not shown).

TABLE 5. ASSOCIATION OF CUMULATIVE PARAQUAT EXPOSURE WITH PULMONARY AND CARDIOPULMONARY EXERCISE OUTCOME MEASURES IN COSTA RICAN FARMWORKERS

		Cumulative Paraquat Exposure		
	n	β (SE)	p Value	R^2
Pulmonary function measures*				
FVC	317	−0.038 (0.026)	0.16	0.422
FEV_1	319	−0.011 (0.02)	0.62	0.481
FEV_1/FVC	314	0.43 (0.29)	0.14	0.180
FEF_{25-75}	314	0.063 (0.059)	0.28	0.206
TLC_{SB}	291	−0.036 (0.035)	0.31	0.386
DL_{CO}	291	−0.36 (0.30)	0.23	0.216
Cardiopulmonary exercise measures†				
$\dot{V}O_2$ peak	200	−0.034 (0.039)	0.38	0.307
$\dot{V}E/\dot{V}CO_2$	200	0.49 (0.20)	0.02	0.032
$\dot{V}O_2$/HR	200	0.039 (0.24)	0.87	0.095

* Linear regression models adjusted for age, height, and current smoking.
† Linear regression models adjusted for age, weight, and current smoking.

Logistic regression models, adjusted for age, weight, and smoking status, were used to assess the association of paraquat exposure with 5% or greater decrease in oxygen desaturation during the cardiopulmonary exercise test (ΔSp_{O_2}). Each unit increase in the total cumulative paraquat exposure index was associated with a 1.7 increase in the odds of a 5% or greater decrease in oxygen desaturation (95% CI = 0.9–3.0).

DISCUSSION

We examined the association of long-term paraquat exposure with sensitive measures of cardiopulmonary function including DL_{CO}, TLC_{SB}, FVC, $\dot{V}O_2$ peak, $\dot{V}E/\dot{V}CO_2$, $\dot{V}O_2$/HR, and ΔSp_{O_2}. The study population included a large number of agricultural workers from coffee, banana, and palm oil farms in Costa Rica. Costa Rica is a country that uses paraquat for weed control, with heavy use of the product, especially on banana plantations. In selecting farms for the study, we attempted to identify all coffee, banana, and palm oil farms using paraquat in Costa Rica. A small number of farms refused participation in the study, and 17.7% of farms contacted reported that they no longer applied paraquat. An additional 40.3% of farms were excluded from the study because the farms were small with too few handlers. Although workers at these farms could make an important contribution to the study, scheduling the field team and conducting testing on less than five handlers at a farm would have posed severe logistical and cost constraints. Handling practices, levels of exposure, and respiratory health of workers on these smaller farms could be different than the larger farms included in the study. Therefore, it is important to consider this factor in generalizing these results to other farms in Costa Rica or elsewhere.

This study population had a similar age distribution to banana workers in Nicaragua (19) and South African agricultural workers (20), but the prevalence of current smoking was much lower in our sample (26% in Costa Rica, 66% in Nicaragua, and 84% in South Africa). In addition, median pack-years were higher in the South African workers (7.5) than in our Costa Rica sample (4.3). According to a WHO report, smoking prevalence in Costa Rica is estimated to be 20–29% (32), which is consistent with our agricultural worker sample.

There has been great variability in the prevalence of self-reported respiratory symptoms across studies of paraquat handlers. Castro-Gutierrez and coworkers reported overall prevalence of 14% and 15.7% for chronic bronchitis and shortness of breath with wheeze, respectively, probably reflecting in large part, higher smoking prevalence (19). The prevalence of self-reported respiratory symptoms was much lower in this investigation. Dalvie found that 7.9% of workers reported chronic cough and 7.9% reported chronic bronchitis (20), which are similar to those for Costa Rican workers in the present study. Dalvie and colleagues also reported a higher percentage of smokers, but a low level of smoking (20). However, it is difficult to compare symptom prevalence for studies done with different study instruments, population groups, local conditions, and work histories.

We observed a weak association of respiratory symptoms and cigarette smoking. Although all respiratory symptoms had an

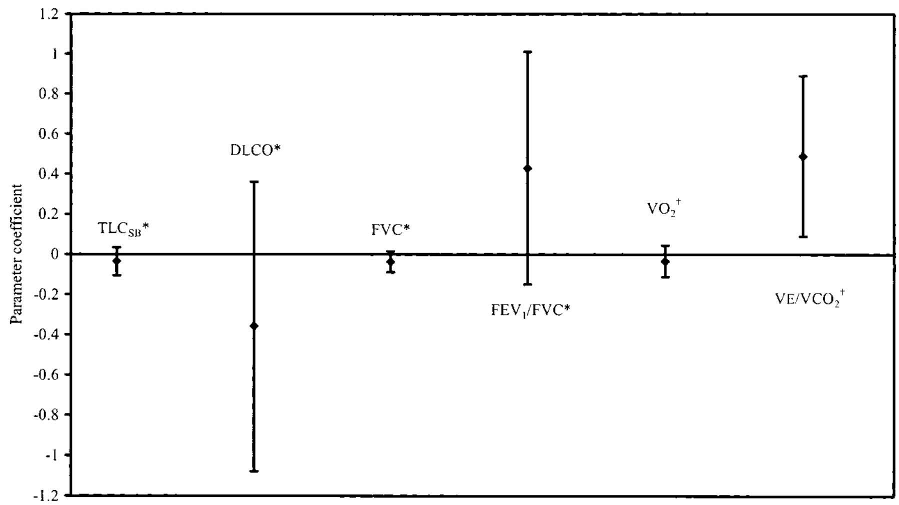

Figure 1. Parameter coefficients and 95% confidence intervals for cumulative paraquat exposure with selected pulmonary and cardiopulmonary exercise outcome measures in Costa Rican farm workers. *Linear regression models adjusted for age, height, and current smoking. †Linear regression models adjusted for age, weight, and current smoking.

increased odds ratio with cigarette smoking, the increases were statistically significant for only chronic cough and shortness of breath with wheeze. The most likely explanation for the lack of a strong association between smoking and self-reported respiratory symptoms is that this was a young population and most of the smokers were light smokers. Forty-seven percent smoked fewer than five cigarettes per day, and only about 13% of current or ex-smokers had ten or more pack-years of smoking.

Importantly, participation in this study did not vary by completion of different test components. There were very high completion rates for spirometry (96%) and diffusion testing (90%). The distribution of participants completing spirometry and diffusion testing was similar across age group, education level, smoking status, and body mass index. Exercise testing was limited to participants 40 years of age and younger, following the recommendation of the ACSM for exercise testing when a physician is not present during the test. There were no differences by smoking status and body mass index among those who completed exercise testing and those who did not. We also examined completion rates of test components by self-reported respiratory symptoms. The proportion of workers reporting respiratory symptoms among those completing spirometry, diffusion, and exercise testing was similar to the overall prevalence of self-reported respiratory symptoms. The high completion rate and absence of differences between subjects completing and not completing pulmonary function tests makes a response bias due to differential completion of pulmonary function tests unlikely.

We observed a significant independent association of shortness of breath with wheeze with cumulative paraquat exposure and a small increase in chronic cough with paraquat exposure. Small increases that were not statistically significant were observed for asthma, chronic bronchitis, and persistent wheeze. Although Hoppin and coworkers reported an association between paraquat and wheeze, multiple exposures and the absence of temporal data limited their ability to measure any independent effect of paraquat on wheeze (33). Additional study with repeated measures of respiratory symptoms and airflow in relation to work activities and physical exertion would be useful to explain this observation.

We selected the pulmonary function measures for this study to optimally address the study hypothesis that chronic low-level paraquat exposure is associated with impaired pulmonary function and interstitial lung disease. To achieve this goal we conducted spirometry, $D_{L_{CO}}$, and cardiopulmonary exercise testing. Although spirometry is the most reliable and well described test of pulmonary function, and most commonly used for occupational lung disease studies, it has limitations when used to evaluate interstitial or restrictive lung disease (34). The FVC has greater variability than the FEV_1, and may be decreased because of either restrictive or obstructive lung disease. Restrictive lung function may be inferred by a reduced FVC and a normal or increased FEV_1/FVC, but this measurement is considered less specific than measures of alveolar volume or total lung capacity (35). Two standard pulmonary function tests for interstitial lung disease, TLC_{SB} and $D_{L_{CO}}$, are more sensitive and specific than spirometry, but they evaluate lung function at rest and thus are not as sensitive as measurements at maximal exercise (34). Finally, we measured cardiopulmonary function during maximal exercise on a cycle ergometer. This system permitted us to measure oxygen uptake and other measures of gas exchange during exercise, and not to rely on the less sensitive and less specific pulse oximetry.

Abnormalities of oxygen saturation with exercise are correlated with $D_{L_{CO}}$ at rest in interstitial lung disease (36). Oxygen desaturation at maximal exercise was considered to be less reliable than the other measures because measurement errors were felt to be common due to movement artifact and other technical problems, and because it was a less specific measure of interstitial disease than oxygen uptake (37–39). When it is clinically significant, reduced oxygen saturation may also result in reduced oxygen delivery via reduced O_2 content and by hypoxic vasoconstriction. We measured oxygen desaturation in part because it had been reported by Dalvie and coworkers (20). Inefficient ventilation (increased $\dot{V}_E/\dot{V}_{CO_2}$) may also be observed in interstitial lung disease (34).

The absence of an association of $\dot{V}_{O_2}$ peak and cumulative paraquat exposure provides strong pulmonary function evidence that paraquat did not cause clinically significant functional impairment in this population. This result was consistent for all measures of paraquat exposure, providing additional support for the absence of an association between cumulative paraquat exposure and reduced aerobic capacity. The estimated effect size from the regression model was close to zero with a narrow confidence band that included no effect. Although exercise testing was only done on workers 40 and younger, and thus excluded the longest-exposed workers, the spirometry and diffusion measurements completed by the full study population also failed to show an association with any of the paraquat exposure variables, consistent with the absence of an association of paraquat exposure and $\dot{V}O_2$ peak.

Two cardiopulmonary exercise outcomes were associated with cumulative paraquat exposure—$\dot{V}E/\dot{V}CO_2$ and ΔSp_{O_2}. Both of these outcomes were based on the exercise testing, and thus derived from the smaller sample size. $\dot{V}E/\dot{V}CO_2$, although within the normal range, was significantly associated with cumulative paraquat exposure, and in addition, examination of this association using quartiles of cumulative paraquat exposure suggested a dose–response relationship. ΔSp_{O_2} failed to achieve the standard cutoff for statistical significance with cumulative paraquat exposure. It showed a positive association with other paraquat exposure variables of a similar magnitude but these models were not statistically significant.

The finding of oxygen desaturation associated with paraquat exposure is consistent with the observed increase in $\dot{V}E/\dot{V}CO_2$ with paraquat exposure. Together, these two findings suggest that paraquat exposure may be associated with a subclinical abnormality of pulmonary gas exchange in this population (34). It is noteworthy that our finding of oxygen desaturation with maximal exercise is similar to the observation of Dalvie and coworkers, providing additional support for the conclusion that this was not a chance observation (20).

The association of $\dot{V}E/\dot{V}CO_2$ with paraquat exposure may be due to an increase in the ratio of physiologic dead space to tidal volume ($\dot{V}D/\dot{V}T$) associated with paraquat exposure. However, there are alternative plausible explanations for this observation. It is possible that this reflects hyperventilation in response to metabolic acidosis, or it could be due to mechanoreceptor stimulation, although neither of these latter two explanations would account for the difference in $\dot{V}E/\dot{V}CO_2$ associated with paraquat exposure. Thus, whereas the mean $\dot{V}E/\dot{V}CO_2$ of 27.8 (interquartile range 26.1–29.3) reflects normal efficiency of the lungs in this population, the statistically significant association with paraquat exposure may reflect subclinical changes in ventilatory efficiency and should be further investigated.

There was no evidence of chronic airflow obstruction in this population, or of an association with chronic paraquat exposure. The mean FEV_1 % predicted was 105.6, and only two subjects (0.6%) had an FEV_1 less than 80% predicted. Further, none of the measures of airflow obstruction showed an association with chronic paraquat exposure.

We did not perform chest X-rays on the study population because radiography was not available in the remote field setting, and would have been prohibitively expensive to obtain. However, studies of asbestos exposed workers and other subjects with interstitial thickening have shown that chest radiography is a less sensitive and less specific test for mild degrees of interstitial thickening than is pulmonary function testing (40).

Although including cardiopulmonary exercise testing was an important strength of the study, this testing had several limitations. Only workers 40 years of age or older completed exercise testing, so approximately one-third of study subjects did not perform this more sensitive test. This is an important consideration because older workers, especially handlers, will have potentially greater long-term exposure to paraquat. In addition, any interaction with other exposures (e.g., cigarette smoking) would be more easily observed in the older workers. We also used a pulse oximeter to measure oxygen saturation of the blood. An indwelling arterial catheter would provide more accurate oxygen saturation measurements, the determination of alveolar to arterial Po_2 gradient ($P(A\text{-}a)O_2$), and $\dot{V}D/\dot{V}T$; however, an indwelling arterial line is more technically demanding and has more risks. Future studies of paraquat-exposed workers may require an indwelling arterial line to more carefully assess possible gas exchange abnormalities, but such investigations would need to be performed in a pulmonary function laboratory and not in a field setting.

Overall our findings of small changes in $\dot{V}E/\dot{V}CO_2$ at maximal exercise and ΔSp_{O_2} from rest to peak exercise suggest that subclinical function changes may be present, but long-term, low-level paraquat exposure in this population is not associated with clinically significant interstitial lung disease or impairment of gas exchange. We also did not observe changes of chronic airflow obstruction with paraquat exposure, but self-reported respiratory symptoms associated with cumulative paraquat exposure (chronic cough, shortness of breath with wheeze) should be further studied. Studies using daily measurement of symptoms, work load, and peak flow would address the etiology of the reported symptoms, and tests of airway hyperresponsiveness (e.g., methacholine) would be useful in further evaluating the etiology of self-reported respiratory symptoms.

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