Long term respiratory health effects of the herbicide, paraquat, among workers in the Western Cape

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Abstract

Objective—To evaluate the possible effects of paraquat spraying among workers on deciduous fruit farms in the Western Cape, South Africa. Paraquat is a commonly used herbicide world wide and is a well documented cause of pulmonary fibrosis in studies of laboratory animals and in humans after exposure to a high dose (usually accidental or as parasuicide). The respiratory effects of long term, low dose exposure to paraquat have not been fully evaluated.

Methods—A cross sectional study of 126 workers. Administered questionnaires generated information on exposure, respiratory symptoms, and potential confounding variables. Spirometry and gas transfer were measured and chest radiographs performed. Oxygen desaturation on exercise testing was by oximetry during a modified stage one exercise test.

Results—No association was found between long term exposure to paraquat and reported symptoms, spirometry (forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), FEV1/FVC) and gas transfer (TLCO and KCO) or chest radiography. Multivariate analysis showed a significant relation between measures of long term exposure to paraquat and arterial oxygen desaturation during exercise independent of short term exposure.

Conclusion—Previous studies have not shown a significant relation between measures of exposure to paraquat and standard tests of lung function. Arterial oxygen desaturation during exercise represents a more sensitive test. The findings indicate that working with paraquat under usual field conditions is associated with abnormal exercise physiology in a dose dependent fashion independent of recent exposure and acute poisoning events.

Keywords: occupation; exercise test; arterial oxygen desaturation

Regular exposure to a highly toxic compound might present an occupational hazard to workers. This is particularly important in conditions where there is a lack of product knowledge and safety awareness.

Large oral doses of paraquat are often fatal causing lung and kidney damage. Lower doses result mainly in chronic lung injury in laboratory animals. Agricultural occupational exposures have been reported at higher levels than those which cause chronic injury in animals.

Dermal absorption is typically poor in the presence of normal skin but may be enhanced by blistering and ulceration that may be caused by paraquat corrosion.

The mechanism by which paraquat causes injury is usually thought to occur through its reaction with oxygen to form toxic oxygen radicals. High dose pulmonary effects appear gradually over a period of several weeks and evolve from alveolitis to pulmonary fibrosis in both animals and humans.

Of the few epidemiological studies investigating respiratory effects, only Gutierrez et al, (personal communication with C Hogstedt) who investigated workers in a Nicaraguan banana plantation, found exposure to the herbicide to be related to respiratory symptoms. All others, found no associations with symptoms, spirometry, or gas transfer. Earlier study limitations include poor exposure characterisation (duration only, and inability to distinguish long term from acute exposure), and insensitive outcome measurements.

We aimed to investigate the respiratory health effects in the field of long term exposure to paraquat under typical agricultural application conditions, independently of acute exposure or poisoning history, by adding exercise oximetry to the standard battery of pulmonary function tests.

Methods

A cross sectional study of subjects in the Ceres district of the Western Cape Province, South Africa, from 41 deciduous fruit farms which were previously part of an agrochemical neurotoxicity study. All current herbicide sprayers (62) along with 70 controls (workers not currently spraying herbicides) broadly matched for age, overall body size and education were selected, with a view to maximising exposure contrast.

Sample size calculations indicated that these numbers were sufficient to detect clinically notable differences in group outcomes for
exercise oximetry (2% difference) and gas transfer factor (0.4 ml/minute/kPa) at the p=0.05 confidence level with 80% power.

All data were collected at a peripheral primary healthcare centre (Ceres Hospital) during the off season in the last 2 weeks of May 1994. Lifetime exposure questionnaires were administered to trained interviewers. Exposure data were collected for each agricultural job performed. This included type of farming product, employment period and type, exposure mechanism (mixing, tractor spraying, hand spraying, back spraying, repair of spray equipment, gardening), exposure duration (lifetime and previous year), history of poisoning, spray nozzle direction (upwards or downwards), frequency of skin burns and paraquat brandnames.

A paraquat job exposure matrix (JEM) based on that developed in a previous study from participant interview and expert opinion provided the following weights for duration of exposure: mixing indoors 1.0; mixing outdoors 0.8; backspray or handspray 0.7; tractor driving 0.5; repair of herbicide equipment 0.2; gardening 0.05; other exposures 0.05.

Paraquat's registration for use in the deciduous fruit industry is mainly as a unique application. Concentrations and applications in this sector are relatively homogenous. Lifetime cumulative exposure indices were calculated by the formula below (summing duration in days of exposure weighted by JEM values across all jobs) for herbicide and paraquat. Short term indices were based solely on exposure in the previous year. Absolute long term exposure indices were calculated by subtraction of short term exposure from the long term.

\[
\text{job} = n \sum \text{duration variable} \times \text{weight (JEM)}
\]

Short term and cumulative exposure categorical variables (dichotomised and ordinal) were created with zero exposure as a category as well as the 25th, 50th, and 75th percentiles as cutoff points.

Average lifetime exposure intensities were derived by dividing the respective cumulative exposure indices by the total period of employment for each person.

Lifetime alcohol and tobacco consumption were measured.

A modified vernacular (Afrikaans) version of the American Thoracic Society respiratory symptoms questionnaire was used. A directed medical examination including chest radiographs was carried out to exclude cases of tuberculosis. One subject with tuberculosis was excluded from spirometric testing. Standard posteroanterior chest radiographs were taken by two trained radiographers at Ceres Hospital and were read by NW according to the standards in the International Labour Organisation international classification of radiographs of pneumoconiosis (1980). Spirometry and carbon monoxide gas transfer measurements (Tlco, and Kco) were performed by a respiratory health technologist according to American Thoracic Society criteria with the Medical Graphics PFD/x pulmonary functions test system (Medical Graphics Corporation, USA). The equipment was recalibrated after each work session. Predicted and percentage of predicted values were calculated for spirometry and transfer factor from the European Community for Coal and Steel standard reference values.

A modified standard clinical stage one exercise test was performed by AD and is described elsewhere. This involved the measurement of arterial oxygen saturation by means of earlobe oximetry (Ohmeda Biox 3700) during an incremental exercise test performed to exhaustion on a mechanical Monark cycle ergometer. The oximeter was connected by serial connection to an IBM compatible computer, which captured pulse rate and saturation every 2 seconds. Workloads performed were recorded manually. Computer generated traces of pulse rate and saturation against time were plotted from the recorded values. These were interpreted by the author and verified by a pulmonologist (RR). The method used has been standardised in the Groote Schuur respiratory clinic where patients are routinely monitored with this device. Desaturation was calculated as the difference between resting and maximum exercise levels. Predictions of heart rate and workload used were those of Campbell and Jones.

All observers for each measurement aspect were blind to exposure status and other measurements.

Data were encoded by one person and double punched, and SAS version 6.09 was used for analysis. Multivariate models included a priori variables known or thought likely to be associated with outcomes of interest and variables found to be associated in bivariate analyses with outcome. Significant associations between variables were assessed from multiple regression models and the best explanatory subset of predictor variables was assessed with Mallow’s C. The assumptions underlying linear regression were checked for all models. There was evidence of skewness in the distribution of the residuals, which could not be alleviated by transformation, but it was considered that the robustness of the linear regression F test would accommodate this. Additionally the nature of the potential associations were examined with multiple logistic regression by modelling dichotomised outcome against continuous predictors as well as dichotomised exposure indices.

Results

Of the selected workers 126 (95%), from whom all informed consent was obtained, participated.

Table 1 shows a low education level with 12.7% never attending school. Mean heights and weights were low compared with European Community for Coal and Steel (ECSS) reference populations.

The prevalence of drinkers (92.9%) and smokers (84.8%) was high.

Respiratory symptom prevalences are shown in table 2: 9.5% had a history of lung problems
before the age of 16, and 7.9% reported a past diagnosis of tuberculosis. Only 0.8% reported a past diagnosis of asthma with 2.4% reporting past asthma problems.

The prevalence of past and present radiographic evidence of tuberculosis was 5.6%. Prevalence for small opacities \( \geq 1/0 \) was 7.2% with 1/1 being the highest score. The prevalence of reported tuberculosis or x-ray film, both evidence of tuberculosis was 4%. No significant associations were found between progression of small opacity score and age, weight, height, alcohol, smoking, and exposure on multivariate analysis.

Table 3 shows that lung capacities are consistently 10%–15% lower than ECCS reference values with very similar forced expiratory volume in 1 second/forced vital capacity (FEV₁/FVC) ratios and lung diffusion capacities.

Table 4 shows exercise test results. Ninety (72%) of 122 saturation traces were considered readable. The group of subjects with unreadable traces did not differ significantly from the group with readable traces for age, years of schooling, maximum heart rate, smoking history, alcohol consumption, and lifetime exposure to paraquat, but was found to be significantly shorter and lighter (p<0.05). This group had significantly lower prevalences of past chest illnesses and colds (p<0.05). Desaturation prevalences were 73.3% at 1%, 32.2% at 4%, and 18.8% at 5% cut off levels. Maximum workloads attained strongly suggest that subjects were exercising at their true maximal potential.

Table 5 shows the exposures in the study sample. The median number of jobs per worker was 2 (range 1–10); the median number of jobs involving exposure to paraquat was 1 (range 0–4). Of the 68 workers who reported exposure to paraquat, 83.5% were current applicators. The average number of years employed in exposed jobs in this particular group of workers was 11.5 years (range 1–47). No subject reported having been previously poisoned by paraquat. Only four subjects, of whom three had interpretable saturation traces in exercise testing, reported a history of skin burns (back, hands, or other) due to paraquat use. The mean (SD) desaturation in these three subjects was 4.67% (5.03%).

None of the reported respiratory symptoms was significantly related to exposure to paraquat, neither were they consistently related to any of the other predictors in models which included age, weight, height, education, smoking, and alcohol.

Height, weight, and age were significant predictors explaining most of the variance for most respiratory function outcomes and were the variables included in the models with the lowest Cₚᵢ. Alcohol (adjusted \( \beta=5.4\times10^{-6} \pm 2.5 \times 10^{-6} / \text{g} \), \( SE(\beta)=2.5\times10^{-6} / \text{g} \), p=0.03) was found to be a significant predictor of diffusion capacity and was included in the model with the lowest Cₚᵢ. Smoking was not a consistent predictor and exposure did not significantly affect respiratory function.

Table 6 summarises the full multiple linear regression model results for the relation between exercise oxygen desaturation and long term exposure to paraquat, with total average intensity of exposure, which had the strongest effect, as an exposure index. Long term exposure to paraquat (partial \( r²=0.0652; p=0.019 \)) and weight (partial \( r²=0.0431; p=0.027 \)) were significant predictors of exercise oxygen desaturation although they explained only a small proportion of the variance.
Table 6  Two multiple linear regression models for the effect of total average intensity of exposure to paraquat on exercise oxygen desaturation

<table>
<thead>
<tr>
<th>Predictor</th>
<th>β</th>
<th>SE(β)</th>
<th>p Value</th>
<th>Partial ( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full model (n=90, ( C_p=7.000 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.0350</td>
<td>0.0303</td>
<td>0.252</td>
<td>0.0007</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>−0.0629</td>
<td>0.0276</td>
<td>0.027</td>
<td>0.0431</td>
</tr>
<tr>
<td>Smoke (pack-years)</td>
<td>0.0067</td>
<td>0.0178</td>
<td>0.710</td>
<td>0.0012</td>
</tr>
<tr>
<td>Alcohol (kg)</td>
<td>−6.74×10^{-1}</td>
<td>1.55×10^{-4}</td>
<td>0.666</td>
<td>0.0026</td>
</tr>
<tr>
<td>DIFPHR (beats/min)</td>
<td>0.0149</td>
<td>0.0175</td>
<td>0.395</td>
<td>0.0140</td>
</tr>
<tr>
<td>Intensity of exposure to paraquat (days/y)</td>
<td>0.0194</td>
<td>0.0088</td>
<td>0.0190</td>
<td>0.0652</td>
</tr>
<tr>
<td>Model ( R^2=0.1222 ) (DF=6, ( p=0.090 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Best model resulting from ( C_p ) procedures (n=90, ( C_p=0.894 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>−0.0514</td>
<td>0.0260</td>
<td>0.051</td>
<td>0.0408</td>
</tr>
<tr>
<td>Intensity of exposure to paraquat (days/y)</td>
<td>0.0186</td>
<td>0.0075</td>
<td>0.015</td>
<td>0.0611</td>
</tr>
<tr>
<td>Model ( R^2=0.1019 ) (DF=2, ( p=0.010 ))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DIFFHR=difference in heartbeats.

Table 7  Summary of estimated adjusted regression coefficients for predicted exercise oximetry in multiple regression models

<table>
<thead>
<tr>
<th>Exposure</th>
<th>( \beta )</th>
<th>SE(( \beta ))</th>
<th>( p ) value</th>
<th>Partial ( r^2 )</th>
<th>Model ( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative exposure to paraquat</td>
<td>0.00114</td>
<td>0.00056</td>
<td>0.0464</td>
<td>0.0474</td>
<td>0.106</td>
</tr>
<tr>
<td>Intensity of exposure to paraquat</td>
<td>0.01941</td>
<td>0.00812</td>
<td>0.0190</td>
<td>0.0652</td>
<td>0.122</td>
</tr>
<tr>
<td>Short term exposure to paraquat</td>
<td>0.01198</td>
<td>0.00826</td>
<td>0.1506</td>
<td>0.0250</td>
<td>0.084</td>
</tr>
<tr>
<td>Cumulative exposure to herbicide</td>
<td>0.00116</td>
<td>0.00316</td>
<td>0.0391</td>
<td>0.0508</td>
<td>0.109</td>
</tr>
<tr>
<td>Intensity of exposure to herbicide</td>
<td>0.01938</td>
<td>0.00810</td>
<td>0.0192</td>
<td>0.0650</td>
<td>0.123</td>
</tr>
<tr>
<td>Absolute cumulative exposure to paraquat</td>
<td>0.00126</td>
<td>0.00358</td>
<td>0.0504</td>
<td>0.0458</td>
<td>0.104</td>
</tr>
<tr>
<td>Absolute intensity of exposure to paraquat</td>
<td>0.01984</td>
<td>0.00849</td>
<td>0.0219</td>
<td>0.0624</td>
<td>0.120</td>
</tr>
</tbody>
</table>

Each row represents a separate independent exposure regression model which includes that exposure and other variables in table 6. Paraquat and herbicide exposure intensity as well as cumulative paraquat and herbicide exposure are significant predictors (\( p<0.05 \)). Absolute long term measures are also strongly associated. Short term exposure to paraquat is not a significant predictor (\( p=0.1506 \)). Average intensity was the strongest predictor.

The unadjusted regression coefficients were: weight=−0.0459% desaturation/kg (\( R^2=0.0326 \), \( p=0.0887 \)); exposure to paraquat intensity=0.0176% desaturation/JEM days/y (\( R^2=0.057 \), \( p=0.0323 \)).

Exposure and weight were also the only variables selected in stepwise procedures and in the model with the lowest \( C_p \) (table 6).

Similar results were found for all long term exposure measures (table 7). Table 7 shows the estimated regression coefficient for multiple regression models including each of the exposure variables and other potentially explanatory variables in table 6. The linear regression exposure results were also supported by logistic regression results based on dichotomised outcomes.

Table 8 indicates the prevalence of clinically notable desaturations, the mean desaturation and the variation that occurs at different exposure levels.

Short term exposure weakened the effect of lifetime exposure by a small degree and slightly reduced the proportion of the variance explained when both variables were forced in full models, for example \( \beta \) (exposure to paraquat intensity)=0.0180; \( p=0.062 \); partial \( r^2=0.042 \) when the full model includes short term exposure to paraquat, compared with \( \beta \) (exposure to paraquat intensity)=0.0194; \( p=0.019 \); partial \( r^2=0.065 \) when excluding short term exposure to paraquat. With stepwise procedures, and in the model with the lowest \( C_p \), only lifetime exposure was selected. Short term exposure was, however, not significant when included as the only exposure variable in the model (table 7). Also, because table 7 shows that the effect of long term exposure remained after subtracting the short term component (absolute long term exposure), long term exposure can be considered to have an effect which is independent of short term exposure.

Excluding those that reported the use of other herbicides as well as paraquat (n=37) from the study sample, does not weaken the association between long term exposure to paraquat and exercise desaturation. Reported asthma and tuberculosis prevalences did not have an effect on exposure-outcome relations when included in the models.

The correlations between general herbicide and paraquat, between intensity and cumulative, and between total and absolute long term exposure indices were high (>0.78). All these correlations, as well as that between short term and long term cumulative exposure to paraquat (\( r=0.39 \)) were significant (\( p=0.0001 \)). Long term exposure indices were not included simultaneously in modelling.

There were three outliers, but they were not influential.

Discussion

This study is the first to examine the effect of long term low dose exposure to paraquat by exercise oximetry. The main finding is a small but significant effect, independent of short term recent exposure and acute poisoning history and is robust for the use of different exposure indices and modelling techniques. As no effects on any other respiratory variable were detected in our or any other study,\(^{15, 16}\) this suggests that exercise oximetry is a sensitive...
The primary biochemical mechanism of paraquat toxicity is currently thought to occur by the cyclic redox reactions of paraquat with reducing equivalents and oxygen in the cell leading to the consumption of NADPH and the production of superoxides.22 There is still uncertainty about subsequent events. Either the consumption of NADPH could lead to cell death or the production of superoxides could initiate a series of events causing toxicity and fibrosis.

Fibrosis causes a decrease in inspiratory capacity due to stiffening of the lungs. This reduces ventilatory capacity. Ventilation perfusion imbalances are created especially during exercise when the reduced ventilatory reserve prevents sufficient oxygen delivery to blood thus leading to hypoxaemia and desaturation. Pulmonary fibrosis also reduces the lung diffusion capacity leading to hypoxaemia during exercise when red blood cell transit times in the capillaries are not adequate to ensure sufficient oxygenation of blood.

Watters et al23 have shown that changes in alveolar-arterial oxygen tension and changes in saturation measured with ear oximetry correlated better with histopathological and histological changes than subjective clinical assessments, spirometry, diffusion capacity, and radiography in patients with pulmonary fibrosis. Sue et al found spirometry and diffusion capacity at rest to be insensitive predictors of abnormal gas exchange when compared with changes that occurred in arterial blood gases during exercise.24 Powers subsequently showed a high (0.82–0.93) correlation between invasively measured oxygen saturation and pulse oximetry, thus validating the use of oximetry for measuring respiratory health.

The simple linear regression model for desaturation projects that 5000 JEM days of cumulative exposure to paraquat (β=0.001%/JEM day) will produce, on average, 5% desaturation, which is commonly regarded as clinically notable.26 However the low R² in our model is indicative of the substantial (unexplained) variability about this mean level. Indeed although none of the subjects in our study had that level of lifetime cumulative exposure, 19% had clinically notable desaturation relations in such a way as to bias the effect towards the null, except in the case of extreme exposure scenarios which do not apply in this study. The study sample was obtained from farms belonging to two large cooperatives which export fruit to international markets. There is therefore a degree of similarity in conditions of application of paraquat on the different members’ farms minimising variability of exposure due to employer factors.

Biases of reporting and diagnosis are unlikely due to the subclinical nature of the outcomes measured, the complexity of exposure measurement, and the blinded nature of the observation process. It is also difficult for the subject to knowingly interfere with exercise desaturation relations in such a way as to bias the effect away from the null.

The ability of these results to be generalised is limited by the exclusion of women and other farm sectors. An unknown number of women are being employed in work involving agrochemical spraying in South Africa.

An interesting finding was that body weight was an unexpected predictor of exercise desaturation and transfer factor, indicating that low weight (a possible marker of poor socioeconomic status) may be associated with poor respiratory health. Mean heights and weights of the study group were extremely low, indicative of serious childhood problems of nutrition and growth retardation resulting in low weight for age and stunting. This is consistent with the finding in the previous study where low serum
albumin was associated with poorer neurobehavioural function (vibration sense) in a similar group. Bias in either direction could have been introduced by the fact that the group that produced unreadable traces was significantly shorter and lighter.

The prevalence of history of tuberculosis seemed to be similar or lower than that found in industrial settings in Cape Town.10,12 The inconsistent relation between smoking and reported respiratory symptoms and lung function is consistent with earlier studies performed on industrial workers11–12 and might be due to the low level of smoking (average of 7.5 pack-years) despite the high percentage of smokers (54%). Alternatively, there was insufficient contrast among subjects for smoking to have any effect.

The practicability and sensitivity of exercise oximetry in a field setting suggests its value as a respiratory screening tool for the early detection of subclinical respiratory effects in various settings which are undetectable with questionnaires and standard lung function tests.

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Appendix
The following equations were used from Jones and Campbell:23

Maximum predicted heart rate (beats/min) = 210 – (0.65 x age)

Maximum predicted workload (MPW): MPW (kpm/min) = [((60–0.55 x age) x weight) – (3.5 x weight)]/2

Conversion of workload (WL) to oxygen consumption (VO2):

VO2 (ml/min) = (WL x 2) + (3.5 x weight)

Units: WL (kpm/min); age (y); weight (kg).

References
27 Du Toit A. The farm as a family. Paternalism, management and modernisation on Western Cape wine and fruit farms. Stellenbosch: Centre for Rural Legal Studies, 1992.