Original research

Organophosphate and carbamate insecticide exposure is related to lung function change among smallholder farmers: a prospective study

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ABSTRACT

Introduction and aim Exposure to some insecticides may cause airway obstruction, but existing evidence is limited by cross-sectional designs and inadequate confounder control. We investigated the relation between organophosphate and carbamate insecticides and pulmonary function in a prospective study accounting for important confounders.

Methods In a cohort of 364 smallholder farmers in Uganda (69% women), participants underwent pre-bronchodilator spirometry at baseline (September/October 2018) and at two follow-up visits (November/December 2018 and January/February 2019). Exposure to carbamate and organophosphorus insecticides was assessed using haemoglobin-adjusted erythrocyte acetylcholinesterase (AChE/Hb). Less than 3% of participants were lost to follow-up. We calculated Z-scores for FEV1, FVC and FEV1/FVC using the Global Lung Function Initiative equations. Data were analysed in linear mixed and fixed effect models accounting for family relationships and repeated measures of exposure and outcome.

Results Low AChE/Hb was significantly associated with low FEV1, Z-score in both unadjusted and adjusted analyses. Compared with individuals with AChE/Hb 25.90 U/g (50th percentile, reference), those with lower AChE/Hb 24.50 U/g (35th percentile) had mean FEV1 Z-score 0.045 (0.003 to 0.087) lower, and persons with higher AChE/Hb 27.30 U/g (65th percentile) had a mean FEV1 Z-score 0.043 (−0.002 to 0.087) higher compared with the reference. Similar, but numerically smaller and statistically non-significant effects were seen for Z-scores of FVC and FEV1/FVC.

Conclusion Exposure to organophosphate and carbamate insecticides may lead to lung function decline. Our results add to the growing evidence of health effects in relation to exposure to organophosphorus and carbamate insecticides, underlining the importance of minimising exposure.

Key messages

What is the key question?
► Is exposure to cholinesterase-inhibiting insecticides associated with pulmonary function impairment?

What is the bottom line?
► Low erythrocyte acetylcholinesterase was associated with low FEV1 among farmers, suggesting that subchronic exposure to cholinesterase inhibiting insecticides leads to impairment of lung function.

Why read on?
► We improve on previous studies by using repeated measurements of both exposure and lung function and adjusting for important confounders, thereby reducing the risk of bias in our analyses.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease are major public health challenges, with estimated global prevalence of 273 million and 299 million people, respectively. Some risk factors for these diseases are well-known, including tobacco smoking, genetic susceptibility and air pollution. It has also been suggested that exposure to some classes of pesticides can lead to lung function impairment, both in the general population and among occupationally exposed workers. Acute poisoning with acetylcholine-inhibiting insecticides (organophosphates and carbamates) can lead to respiratory failure, partly due to bronchoconstriction and increased mucus production in the airways. Exposure to organophosphates and carbamates at levels too low to cause acute intoxication might lead to airway obstruction. A recent systematic review and meta-analysis on pesticide exposure and lung function indicated that exposure to cholinesterase (ChE) inhibiting pesticides may reduce FEV1/FVC. However, the confidence in the findings was limited, as most studies were cross-sectional, had inadequate confounder control and did not investigate exposure–response relationships.

Smallholder farmers in low- and middle-income countries can be heavily exposed to pesticides due to a lack of training, unsafe pesticide application practices and limited use of personal protective equipment. If pesticide exposure impairs pulmonary function, such farmers may therefore be...
some of the most affected persons, though few previous studies have focussed specifically on this group. Hence, the objective of this study was to investigate whether subchronic exposure to organophosphate and carbamate insecticides was associated with impaired lung function among smallholder farmers in Uganda, in a short-term follow-up study with abundant information on possible confounders.

METHODS

Study design
The study was conducted as part of the ‘Pesticide Exposure, Asthma and Diabetes in Uganda’ (PEXADU) project, a short-term cohort study among members of two organisations of smallholder farmers from the Wakiso District in central Uganda—one for conventional farmers, and one for farmers working towards organic certification. We recruited participants from these two organisations to minimise confounding from sociodemographic variables while maximising insecticide exposure contrast. Participants were examined at baseline in September/October 2018 (phase 1) and at two follow-up visits in November/December 2018 (phase 2) and January/February 2019 (phase 3). The main insecticide application season in the study area is October/November (personal communication, Aggrey Atuhaire, Uganda National Association of Community and Occupational Health), and we selected the timing of the three rounds of examinations to also maximise contrast in insecticide exposure within the participants.

Study population
We attended the weekly meetings among small groups in each farmers’ organisation and invited all members 18 years or older to participate. Pregnant women were excluded. We compiled a list of 532 persons potentially eligible for participation, and used a pseudo-random number generator to randomise the sequence of persons on the list. Potential participants were invited in sequence by telephone. If a person could not be reached by telephone, or was unable to participate, we excluded that person and called the next person on the list. At baseline, 380 out of 532 individuals came to the examination centre, and after exclusion of ineligible persons, we included 364 participants. We re-examined 356 and 354 subjects in phase 2 and phase 3, respectively (see figure 1).

Outcome assessment
Pulmonary function was quantified by pre-bronchodilator spirometry using a MicroDL spirometer and Spida 5 PC software (Micro Medical, Rochester, Kent, England). Exclusion criteria were myocardial infarction in the last 3 months, angina pectoris, haemoptysis, any surgery in the last 3 months, aortic aneurysm, history of pulmonary embolism, active tuberculosis, other current respiratory infection and severe hypertension (systolic >200 mm Hg or diastolic >120 mm Hg). If standard American Thoracic Society (ATS) criteria for quality of spirometry were not fulfilled after five blows, four additional attempts were provided. After the examination, quality and repeatability of all manoeuvres were assessed by a medical doctor with experience in pulmonary function testing, according to modified ATS criteria (online supplemental appendix 1). Reliable spirometry results were available for 290, 285 and 263 persons in phase 1, phase 2 and phase 3, respectively (see figure 2).

The primary outcomes were Z-scores for forced expiratory volume in the first second (FEV1), forced vital capacity (FVC) and FEV1/FVC, calculated using the Global Lung Function Initiative (GLI-2012) equations, with ‘African-American’ ethnicity
as the reference, while absolute values of FEV₁, FVC and FEV₁/FVC were secondary outcomes. Peak expiratory flow (PEF), instantaneous forced expiratory flow after exhalation of 25%, 50% and 75% of the FVC (FEF₂₅, FEF₅₀ and FEF₇₅), mean forced expiratory flow between 25% and 75% of the FVC (FEF₂₅₋₇₅) and FEF₂₅₋₇₅ Z-score were also secondary outcomes.

Exposure assessment
To quantify exposure to organophosphate and carbamate insecticides, we used capillary blood erythrocyte acetylcholinesterase (AChE) analysed using a point-of-care device (Test-mate ChE Cholinesterase Test System Model 400, EQM Research Inc, Cincinnati, Ohio, USA). The analysis was performed immediately after blood sampling, and according to the manufacturer’s instructions. The primary toxicodynamic mode of action of organophosphate and carbamate insecticides is inhibition of nervous system acetylcholinesterase. The enzyme isoform in erythrocytes (AChE) is readily available for sampling, and can be measured as an expression of exposure in the short to medium term. Our primary exposure metric was AChE activity normalised by haemoglobin (Hb) concentration: AChE/Hb (U/g). Results from the Test-Mate system were recorded on paper and later double-entered using the Open Data Kit (ODK) Collect application.

Confounder selection and assessment
Confounders were defined a priori using Directed Acyclic Graphs and the DAGitty software. The basic set of confounders included age, sex, cumulated lifetime tobacco smoking and cumulated lifetime hours of cooking as a proxy for exposure to biofuel smoke. The extended set of confounders also included body mass index and years of full-time education as a proxy for socioeconomic status. For analyses of absolute spirometric indices, all analyses included sex, age and height as covariates. Tobacco smoking was quantified as pack-years. Biofuel smoke exposure was expressed as ‘cooking years’, defined as the number of years with an average of 2.5 hours of cooking per day. We did not adjust analyses for the specific spirometer used during testing, as calibration check data showed that differences between spirometers were minor and did not explain the observed temporal trends in spirometric results.

Height and weight was measured in a standardised manner using a stadiometer (SM-SZ-300, Sumbow Medical Instruments Co Ltd, Ningbo, China) and medical scale (seca robusta 813, seca GmbH & Co, Hamburg, Germany), respectively. Anthropometric data was recorded directly in ODK Collect.

Subjective covariate information was collected in a structured, questionnaire-based interview. Questions on demographic data and tobacco smoking derived from the WHO

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**Figure 2** Flowchart describing numbers of participants who performed spirometry.
can be described thus:

related to at least one other member of the group. The model 
as a group of participants where all members were genetically 
was allowed to vary between participants. A ‘family’ was defined 
variables. The regression coefficient for the exposure variable 
participant, and fixed effects for the exposure and confounder 

is an error term. In our main fixed effect model, we compared 
results, online supplemental appendix 2.

All outcome variables were analysed on a continuous scale. To 
account for family relationships and repeated measurements of 
both exposure, outcome and covariates, data were analysed in 
a linear mixed effect model with random effects for family and 
and any deviations from the protocol, see online supplemental 
view of all analyses performed (including sensitivity analyses), 
and between families, with less variation within persons. The 
outcome and exposure variables are provided in online supple-
mental appendix 3. Most of the variance was between persons 
and between families, with less variation within persons. The 

reasonable estimate of the mean of between-person 
and between-family variance was 0.12 for FEV1, Z-score, 0.15 
for FVC Z-score, 0.43 for FEV1/FVC Z-score and 0.22 for 
AChE/Hb.

In our main analysis, FEV1, Z-score was significantly lower 
among individuals with low AChE/Hb, compared with persons 
with higher AChE/Hb, both in unadjusted analyses and adjusted 
analyses including the basic and extended sets of confounders 
(figure 3 and table 3). FVC Z-score and FEV1/FVC Z-score 
showed similar patterns as FEV1, Z-score, but differences were 
numerically smaller and statistically non-significant.

Analyses of absolute lung function measures generally 
confirmed the results for Z-scores, but were only statistically 
significant in unadjusted analyses for FEV1 (table 3 and online 
supplemental appendix 2). Analyses of the remaining secondary 
outcomes (such as PEF and FEF25) were less consistent (online 
supplemental appendix 2).

A number of preplanned sensitivity analyses conducted to 
check the robustness of the main findings generally gave similar 
results as the main analyses (online supplemental appendix 2). However, in an adjusted post-hoc sensitivity analysis that 
included project phase as a categorical variable, no clear 
relationships were seen between AChE/Hb and spirometry Z-scores 
(see online supplemental appendix 2), probably due to the asso-
ciation between phase and exposure seen in table 2. In post-hoc 
analyses stratified by sex, associations between AChE/Hb and 
spirometry Z-scores were only evident for women (online 
supplemental appendix 2).

In the fixed effect model, we did not demonstrate any clear 
association between change in AChE/Hb between phase 1 
and phase 3, and the change in Z-scores of FEV1 and FVC, 
figure 4. We did see a statistically non-significant association 
between decreases in AChE/Hb between phases (corresponding 
to increased exposure) and decreases in FEV1/FVC Z-score, 
but this result should be interpreted with caution due to the 
unclear pattern for Z-scores of FEV1, and FVC. Sensitivity anal-
yses comparing phase 1/2 and phase 2/3 provided inconsistent 
results, online supplemental appendix 2.

Statistical analyses

Data were managed using Python 3 (Python Software Founda-
tion, https://www.python.org/) and Stata 15 (StataCorp, College 
Station, Texas, USA), and analysed using Stata 15. The analysis 
plan was specified a priori and published online.21 For an overview 
of all analyses performed (including sensitivity analyses), 
and any deviations from the protocol, see online supplemental 
appendix 2.

All outcome variables were analysed on a continuous scale. To 
account for family relationships and repeated measurements of 
both exposure, outcome and covariates, data were analysed in 
a linear mixed effect model with random effects for family and 
and any deviations from the protocol, see online supplemental 
appendix 2.

All outcome variables were analysed on a continuous scale. To 
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account for family relationships and repeated measurements of 
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a linear mixed effect model with random effects for family and 
and any deviations from the protocol, see online supplemental 
appendix 2.
DISCUSSION

In our analyses, low AChE/Hb (indicating high exposure to organophosphate and carbamate insecticides) was associated with low values of most spirometric indices. The relationship was numerically largest and statistically significant for FEV₁ Z-score, but similar trends were seen for FVC Z-score and FEV₁/FVC Z-score. This indicates that exposure to organophosphate and carbamate insecticides might impair pulmonary function. Our confidence in the findings are somewhat tempered by the lack of clear exposure-response patterns in the fixed effect model, but the latter model may have been statistically underpowered due to a lower-than-expected within-person exposure variability.

Table 1  Demographics for the study population at baseline

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>AChE/Hb &lt;26.3 U/g</th>
<th>AChE/Hb ≥26.3 U/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n</td>
<td>364</td>
<td>181</td>
<td>183</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>250 (68.7)</td>
<td>132 (72.9)</td>
<td>118 (64.5)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>114 (31.3)</td>
<td>49 (27.1)</td>
<td>65 (35.5)</td>
</tr>
<tr>
<td>Age, years</td>
<td>46.6 (36.7 to 56.5)</td>
<td>46.8 (37.0 to 56.5)</td>
<td>46.5 (36.6 to 56.5)</td>
</tr>
<tr>
<td>Years of full-time education</td>
<td>7.0 (5.0 to 11.0)</td>
<td>7.0 (5.0 to 10.0)</td>
<td>7.0 (5.0 to 11.0)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baganda, n (%)</td>
<td>283 (77.7)</td>
<td>137 (75.7)</td>
<td>146 (79.8)</td>
</tr>
<tr>
<td>Banyarwanda, n (%)</td>
<td>20 (5.5)</td>
<td>15 (8.3)</td>
<td>5 (2.7)</td>
</tr>
<tr>
<td>Banyankore, n (%)</td>
<td>11 (3.0)</td>
<td>5 (2.8)</td>
<td>6 (3.3)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>50 (13.7)</td>
<td>24 (13.3)</td>
<td>26 (14.2)</td>
</tr>
<tr>
<td>Ever smoked tobacco?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>42 (11.5)</td>
<td>17 (9.4)</td>
<td>25 (13.7)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>322 (88.5)</td>
<td>164 (90.6)</td>
<td>158 (86.3)</td>
</tr>
<tr>
<td>Current smoker?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>15 (4.1)</td>
<td>4 (2.2)</td>
<td>11 (6.0)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>349 (95.9)</td>
<td>177 (97.8)</td>
<td>172 (94.0)</td>
</tr>
<tr>
<td>Pack-years (ever-smokers only)</td>
<td>2.1 (0.9 to 6.8)</td>
<td>4.0 (1.2 to 8.4)</td>
<td>1.8 (0.8 to 6.2)</td>
</tr>
<tr>
<td>Grams of tobacco per day for current smokers</td>
<td>1.1 (0.0 to 4.0)</td>
<td>1.9 (0.4 to 6.0)</td>
<td>1.1 (0.0 to 4.0)</td>
</tr>
<tr>
<td>Cooking fuel type in household</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Charcoal, n (%)</td>
<td>61 (16.8)</td>
<td>37 (20.4)</td>
<td>24 (13.1)</td>
</tr>
<tr>
<td>Wood, n (%)</td>
<td>298 (81.9)</td>
<td>142 (78.5)</td>
<td>156 (85.2)</td>
</tr>
<tr>
<td>No food cooked in household, n (%)</td>
<td>1 (0.3)</td>
<td>0 (0.0)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>4 (1.1)</td>
<td>2 (1.1)</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Number of hours cooking in the last week</td>
<td>8.0 (0.3 to 17.8)</td>
<td>8.0 (0.5 to 16.7)</td>
<td>8.0 (0.0 to 20.0)</td>
</tr>
<tr>
<td>Cumulated lifetime cooking (‘cooking years’)</td>
<td>16.7 (0.9 to 44.9)</td>
<td>14.9 (1.2 to 44.7)</td>
<td>17.5 (0.0 to 45.2)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.3 (21.1 to 26.8)</td>
<td>23.2 (21.1 to 26.9)</td>
<td>23.3 (21.5 to 26.5)</td>
</tr>
<tr>
<td>Mean height across all phases, cm</td>
<td>158.9 (154.1 to 164.3)</td>
<td>158.5 (152.7 to 163.2)</td>
<td>159.5 (155.0 to 164.7)</td>
</tr>
<tr>
<td>Ever mixed or applied pesticides?*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>283 (77.8)</td>
<td>140 (77.3)</td>
<td>143 (78.1)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>81 (22.3)</td>
<td>41 (22.7)</td>
<td>40 (21.9)</td>
</tr>
<tr>
<td>Cholinesterase inhibitor insecticides among three most used pesticides, or used in the last week?†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>133 (47.0)</td>
<td>64 (45.7)</td>
<td>69 (48.3)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>114 (40.3)</td>
<td>53 (37.9)</td>
<td>61 (42.7)</td>
</tr>
<tr>
<td>Unknown, n (%)</td>
<td>36 (12.7)</td>
<td>23 (16.4)</td>
<td>13 (9.1)</td>
</tr>
<tr>
<td>Cholinesterase inhibitor insecticides used in the last week?†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, n (%)</td>
<td>22 (7.8)</td>
<td>9 (6.4)</td>
<td>13 (9.1)</td>
</tr>
<tr>
<td>No, n (%)</td>
<td>246 (86.9)</td>
<td>120 (85.7)</td>
<td>126 (88.1)</td>
</tr>
<tr>
<td>Unknown, n (%)</td>
<td>15 (5.3)</td>
<td>11 (7.9)</td>
<td>4 (2.8)</td>
</tr>
</tbody>
</table>

Categorical variables are n (%). Continuous variables are median (IQR). AChE/Hb dichotomised by 26.3 U/g, the median AChE/Hb at baseline. One ‘cooking year’ is defined as cooking for 2.5 hours per day for 365.24 days.

*Includes both occupational and domestic use of pesticides.
†Data only presented for subjects who have ever mixed or applied pesticides.
AChE/Hb, erythrocyte acetylcholinesterase normalised by haemoglobin concentration; BMI, body mass index.
Table 2  Lung function measures and acetylcholinesterase in each project phase

|---------------------|-------------------------|------------------------|------------------------|--------
| FEV₁ (L)            | 2.36 (1.94 to 2.88)     | 2.33 (1.92 to 2.78)    | 2.28 (1.91 to 2.81)    | −0.04 (−0.05 to −0.03) 
| n                   | 290                     | 285                    | 263                    |        
| FVC (L)             | 2.82 (2.36 to 3.47)     | 2.83 (2.33 to 3.41)    | 2.77 (2.37 to 3.41)    | −0.03 (−0.04 to −0.01) 
| n                   | 288                     | 285                    | 263                    |        
| FEV₁/FVC            | 0.83 (0.80 to 0.87)     | 0.83 (0.79 to 0.87)    | 0.83 (0.79 to 0.87)    | −0.01 (−0.01 to −0.00) 
| n                   | 288                     | 285                    | 263                    |        
| PEF (L/s)           | 6.01 (5.06 to 7.40)     | 6.15 (5.21 to 7.41)    | 5.93 (5.22 to 7.31)    | 0.01 (−0.04 to 0.07)  
| n                   | 290                     | 285                    | 263                    |        
| PEF₁₅ (L/s)         | 5.08 (4.04 to 5.99)     | 4.96 (3.97 to 5.90)    | 4.94 (3.89 to 5.90)    | −0.05 (−0.11 to 0.01) 
| n                   | 290                     | 285                    | 263                    |        
| PEF₁₅ (L/s)         | 3.18 (2.30 to 3.73)     | 2.94 (2.19 to 3.71)    | 2.94 (2.16 to 3.72)    | −0.09 (−0.13 to −0.06) 
| n                   | 290                     | 285                    | 263                    |        
| PEF₁₅ (L/s)         | 0.95 (0.70 to 1.33)     | 0.93 (0.66 to 1.25)    | 0.90 (0.64 to 1.27)    | −0.04 (−0.06 to −0.02) 
| n                   | 290                     | 285                    | 263                    |        
| PEF₁₅ (L/s)         | 2.47 (1.82 to 3.13)     | 2.35 (1.73 to 3.05)    | 2.35 (1.67 to 3.02)    | −0.07 (−0.10 to −0.04) 
| n                   | 290                     | 285                    | 263                    |        
| FEV₁ Z-score        | −0.155 (−0.750 to 0.405) | −0.220 (−0.802 to 0.356) | −0.315 (−0.893 to 0.229) | −0.10 (−0.13 to −0.07) 
| n                   | 290                     | 285                    | 263                    |        
| FVC Z-score         | −0.257 (−0.850 to 0.387) | −0.307 (−0.798 to 0.297) | −0.342 (−0.933 to 0.196) | −0.06 (−0.09 to −0.03) 
| n                   | 288                     | 285                    | 263                    |        
| FEV₁/FVC Z-score    | 0.127 (−0.402 to 0.793) | 0.142 (−0.511 to 0.757) | 0.119 (−0.491 to 0.744) | −0.08 (−0.13 to −0.03) 
| n                   | 288                     | 285                    | 263                    |        
| FEF₁₅ Z-score       | −0.234 (−0.780 to 0.419) | −0.227 (−0.777 to 0.294) | −0.306 (−0.882 to 0.341) | −0.07 (−0.11 to −0.04) 
| n                   | 290                     | 285                    | 263                    |        
| AChE (U/mL)         | 3.10 (2.74 to 3.48)     | 2.98 (2.63 to 3.31)    | 2.88 (2.54 to 3.22)    | −0.12 (−0.14 to −0.10) 
| n                   | 364                     | 353                    | 354                    |        
| Hb (g/dL)           | 11.80 (11.10 to 12.70)  | 11.50 (10.60 to 12.20) | 11.70 (10.80 to 12.40) | −0.13 (−0.18 to −0.07) 
| n                   | 364                     | 353                    | 354                    |        
| AChE/Hb (U/g)       | 26.30 (23.90 to 28.10)  | 26.20 (23.70 to 28.40) | 24.70 (22.30 to 27.20) | −0.74 (−0.85 to −0.63) 
| n                   | 364                     | 353                    | 354                    |        

All variables are presented as median (IQR). Z-scores and FEV₁/FVC are unitless. Δ/phase denotes the mean change in the variable when phase increases by 1. CIs for Δ/phase was calculated in a mixed effect model with random effects for family and person, and fixed effect for phase.

AChE/Hb, erythrocyte acetylcholinesterase normalised by haemoglobin concentration.

Exposure to cholinesterase inhibitor insecticides is the most obvious explanation for depression of AChE/Hb in this population of smallholder farmers. However, we did not see any clear differences in self-reported use of insecticides between persons with AChE/Hb below or above the median at baseline (table 2). The farmers might mainly be exposed in ways on which we do not have information, such as re-entry exposure during work in previously sprayed fields, or through diet. Of note, insecticide exposure is not the only factor that can influence AChE/Hb.\textsuperscript{29} Our results could therefore be confounded, but the problem is likely limited, as demographics between the low and high AChE/Hb groups at baseline were generally similar, and results from unadjusted and adjusted analyses of Z-scores for FEV₁, FVC and FEV₁/FVC were nearly identical. Because of the lack of association between subjective exposure to cholinesterase inhibitor insecticides and AChE/Hb, we did not adjust our analyses for use of other pesticides (such as pyrethroid insecticides, glyphosate and dithiocarbamate fungicides), for which we currently only have subjective exposure information and no biomarkers. Depressed AChE/Hb could therefore be a proxy for exposure to other agrochemicals.

Our findings support previous mainly cross-sectional studies showing associations between decreased pulmonary function and exposure to cholinesterase-inhibiting insecticides, expressed as either urinary metabolites of organophosphates,\textsuperscript{5,6,30} or depressed cholinesterase activity.\textsuperscript{31–35} Most previous studies were conducted among occupationally exposed workers and farmers, the majority of whom were men.\textsuperscript{30–35} Two studies showed associations in general populations with more balanced gender distributions.\textsuperscript{5,6} Thus, our study differs somewhat from previous studies, as our study population was occupationally exposed and showing associations in general populations. A link between exposure and lung function impairment is biologically plausible, as acute intoxication with cholinesterase-inhibiting insecticides leads to bronchoconstriction.\textsuperscript{35} Support is also provided by a double-blind, randomised, placebo-controlled...
### Figure 3
Results from random coefficient model of pulmonary function versus AChE/Hb. Solid line shows lung function parameter relative to value at the median AChE/Hb. Dashed lines show 95% CI. Spline knot location shown by black dots. Histograms show the distribution of AChE/Hb values for observations in the model. Basic covariate set=age, sex, pack-years of smoking, cumulated lifetime hours of cooking. Extended covariate set=basic set+BMI, years of full-time education. AChE/Hb, erythrocyte acetylcholinesterase normalised by haemoglobin concentration.

### Table 3
Summary table of results from random coefficient model

<table>
<thead>
<tr>
<th>AChE/Hb (U/g)</th>
<th>5</th>
<th>35</th>
<th>65</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEV₁ Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.153 (−0.289 to −0.016)</td>
<td>0.050 (−0.091 to −0.010)</td>
<td>0 (ref)</td>
<td>0.048 (0.005 to 0.091)</td>
</tr>
<tr>
<td>Adjusted, basic</td>
<td>0.155 (−0.296 to −0.014)</td>
<td>0.045 (−0.087 to −0.003)</td>
<td>0 (ref)</td>
<td>0.043 (−0.002 to 0.087)</td>
</tr>
<tr>
<td>Adjusted, extended</td>
<td>0.153 (−0.293 to −0.012)</td>
<td>0.043 (−0.085 to −0.001)</td>
<td>0 (ref)</td>
<td>0.040 (−0.005 to 0.085)</td>
</tr>
<tr>
<td><strong>FVC Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.041 (−0.193 to 0.111)</td>
<td>0.019 (−0.065 to 0.026)</td>
<td>0 (ref)</td>
<td>0.019 (−0.029 to 0.067)</td>
</tr>
<tr>
<td>Adjusted, basic</td>
<td>0.038 (−0.192 to 0.016)</td>
<td>0.017 (−0.063 to 0.029)</td>
<td>0 (ref)</td>
<td>0.017 (−0.032 to 0.066)</td>
</tr>
<tr>
<td>Adjusted, extended</td>
<td>0.036 (−0.189 to 0.116)</td>
<td>0.015 (−0.061 to 0.031)</td>
<td>0 (ref)</td>
<td>0.014 (−0.034 to 0.063)</td>
</tr>
<tr>
<td><strong>FEV₁/FVC Z-score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.063 (−0.261 to 0.135)</td>
<td>0.030 (−0.091 to 0.030)</td>
<td>0 (ref)</td>
<td>0.033 (−0.032 to 0.098)</td>
</tr>
<tr>
<td>Adjusted, basic</td>
<td>0.066 (−0.264 to 0.132)</td>
<td>0.020 (−0.080 to 0.040)</td>
<td>0 (ref)</td>
<td>0.020 (−0.044 to 0.085)</td>
</tr>
<tr>
<td>Adjusted, extended</td>
<td>0.058 (−0.254 to 0.139)</td>
<td>0.018 (−0.079 to 0.042)</td>
<td>0 (ref)</td>
<td>0.020 (−0.045 to 0.084)</td>
</tr>
<tr>
<td><strong>FEV₁</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.041 (−0.091 to 0.010)</td>
<td>0.017 (−0.032 to −0.002)</td>
<td>0 (ref)</td>
<td>0.017 (0.001 to 0.033)</td>
</tr>
<tr>
<td>Adjusted, basic</td>
<td>0.042 (−0.094 to 0.011)</td>
<td>0.016 (−0.031 to 0.000)</td>
<td>0 (ref)</td>
<td>0.015 (−0.001 to 0.032)</td>
</tr>
<tr>
<td>Adjusted, extended</td>
<td>0.041 (−0.094 to 0.011)</td>
<td>0.015 (−0.031 to 0.001)</td>
<td>0 (ref)</td>
<td>0.014 (−0.002 to 0.031)</td>
</tr>
<tr>
<td><strong>FVC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.002 (−0.067 to 0.063)</td>
<td>0.004 (−0.024 to 0.015)</td>
<td>0 (ref)</td>
<td>0.005 (0.006 to 0.025)</td>
</tr>
<tr>
<td>Adjusted, basic</td>
<td>0.003 (−0.070 to 0.064)</td>
<td>0.004 (−0.024 to 0.016)</td>
<td>0 (ref)</td>
<td>0.004 (−0.017 to 0.026)</td>
</tr>
<tr>
<td>Adjusted, extended</td>
<td>0.003 (−0.070 to 0.063)</td>
<td>0.003 (−0.023 to 0.017)</td>
<td>0 (ref)</td>
<td>0.003 (−0.018 to 0.024)</td>
</tr>
<tr>
<td><strong>FEV₁/FVC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.007 (−0.019 to 0.005)</td>
<td>0.002 (−0.006 to 0.002)</td>
<td>0 (ref)</td>
<td>0.002 (−0.002 to 0.006)</td>
</tr>
<tr>
<td>Adjusted, basic</td>
<td>0.007 (−0.019 to 0.006)</td>
<td>0.002 (−0.005 to 0.002)</td>
<td>0 (ref)</td>
<td>0.001 (−0.003 to 0.006)</td>
</tr>
<tr>
<td>Adjusted, extended</td>
<td>0.006 (−0.019 to 0.006)</td>
<td>0.002 (−0.005 to 0.002)</td>
<td>0 (ref)</td>
<td>0.001 (−0.003 to 0.005)</td>
</tr>
</tbody>
</table>

Numbers show lung function parameter relative to value at the median AChE/Hb in the analysis, with 95% CI. Absolute lung function measures: All analyses (including unadjusted) include sex, age and height as covariates. Basic covariate set=age, sex, height, pack-years of smoking, cumulated lifetime hours of cooking. Extended covariate set=basic set+BMI, years of full-time education. AChE/Hb, erythrocyte acetylcholinesterase normalised by haemoglobin concentration; BMI, body mass index.
The effect sizes demonstrated in the study were modest, but non-negligible. In the analysis of the influence of AChE/Hb on the absolute value of FEV₁, there was an estimated difference of 78 (9 to 148) mL in FEV₁ between the 5th and 95th percentiles of AChE/Hb, after adjustment for the basic set of confounders. While AChE/Hb has a wide normal range, it is strongly correlated with the degree of acetylcholinesterase inhibition that ranged from ~20% to ~40%. In the present study, median AChE/Hb was 26.3 U/g at baseline. While AChE/Hb has a wide normal range, it is only 84% of the reference value (31.4 U/g) stated by the manufacturer of the device that we used to measure AChE/Hb. Hence, the degrees of cholinesterase inhibition in our study population and in the previous experimental study are of the same order of magnitude.

The strength of this study is the relatively large study population, all recruited from farmers’ organisations, and followed over time to account for both interindividual and intraindividual variability in pesticide exposure. We had low losses to follow-up. Exposure and outcome were objectively determined following established standards, and we accounted for confounders defined a priori based on Directed Acyclic Graphs.

Our study also has clear limitations. Foremost, our sampling strategy was convenience-based, that is, participants were recruited from farmers’ organisations that we presumed a priori to have different mean exposure levels, but similar demographics.

Limited information from each recruitment meeting was available regarding the number and characteristics of farmers who did not wish to participate in our study, and selection bias before baseline is theoretically possible. The proportion of female participants was high (69%), but this does not necessarily reflect imbalanced selection by sex, as the overall proportion of women among individuals economically active in agriculture in Uganda is 50%, and 56% of the labour time in Ugandan agriculture is provided by women. Loss to follow-up between phases was negligible and unlikely to introduce any considerable bias. However, some selection bias could have been introduced by (un)availability of spirometry data for included participants. We conducted a post-hoc analysis limited to spirometric results from phase 1, as Directed Acyclic Graphs showed that in this phase, the potential for selection bias was lower than in the study overall (see figure 2 and online supplemental appendix 2). The association between low AChE/Hb and low FEV₁ Z-score disappeared in this cross-sectional analysis (online supplemental appendix 2). This could indicate that our main analyses are biased by selection processes, but on the other hand, the cross-sectional analysis did not account for the considerable physiological variation in AChE/Hb.

Pulmonary function was only assessed by pre-bronchodilator spirometry, as we did not have ethical clearance to administer bronchodilator medicine to participants. Hence, we cannot tell if airway obstruction was reversible.

We could confirm relationships between spirometric results and well-known determinants of pulmonary function, for example, age, sex and height (online supplemental appendix 2), strengthening our confidence in results related to AChE/Hb. Unexpectedly, we found no consistent associations between biomass smoke exposure or tobacco consumption with pulmonary function. The latter is probably due to power issues, as few

Figure 4 Results from fixed effect model of change in pulmonary function and AChE/Hb from phase 1 to phase 3. Solid line shows lung function parameter relative to value at the median Δ(AChE/Hb). Dashed lines show 95% CI. Spline knot location shown by black dots. Histograms show the distribution of Δ(AChE/Hb) values for observations in the model. Basic covariate set=Δage, Δpack-years, Δ(hours of cooking in the last week). Extended covariate set=basic set+ΔBMI. AChE/Hb, erythrocyte acetylcholinesterase normalised by haemoglobin concentration.
of the participants had ever smoked, and our metric for biomass smoke was crude, meaning there is likely bias toward the null. Residual confounding due to the crude exposure metric for biomass smoke is likely limited, as biomass exposure is probably determined by a combination of sex, age and socioeconomic status—variables that we have adjusted for.

The median Z-scores for FEV₁, FVC and FEF₂₅₋₇₅ in the study population were <0 in all phases, while the Z-score for FEV₁/FVC was >0. This could be a result of the harmful influence of various exposures on pulmonary function, or because the GLL-2012 equations do not include data from Uganda. Due to a lack of normal values for the Ugandan population, we used normal values for African-Americans. We analysed the Z-scores as continuous variables, and adjusted analyses of the raw metrics FEV₁, FVC, FEV₁/FVC and FEF₂₅₋₇₅ gave similar results to the analyses based on the corresponding Z-scores. Therefore, the use of African-Americans as reference population is unlikely to pose a threat to the internal validity of our findings.

A considerable number of statistical analyses were conducted. Nevertheless, we do not think that multiple comparisons pose a substantial problem for the study validity, as we decided a priori which covariates to adjust for, which outcomes were primary and secondary, and which analyses were primary, secondary and sensitivity analyses. Furthermore, we see clear and consistent patterns for each exposure–outcome pair in the random coefficient models. We prepublished the analysis protocol in an online repository, and all deviations from protocol are listed in online supplemental appendix 2.

While we believe that our findings are generalisable to small-holder farmers using organophosphate and carbamate insecticides in Eastern Africa, care should be taken before extrapolating the findings to other populations with lower exposure levels, for example, farmers applying pesticides in high-income countries or consumers eating fruits and vegetables with pesticide residues. The PEXADU project population has a relatively high exposure to pesticides due to a lack of training on proper handling of pesticides and poor use of personal protective equipment. Further studies are needed to investigate whether similar effects can be demonstrated in lower-exposed populations.

CONCLUSION

Our main analyses indicate that occupational exposure to organophosphate and carbamate insecticides may lead to lung function impairment, though diverging results from some sensitivity and secondary analyses mean that caution is warranted when interpreting the findings. Nevertheless, our results add to the growing evidence of health effects in relation to exposure to organophosphate and carbamate insecticides, underlining the importance of preventive measures to avoid or minimise exposure.

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Contributors MRHH: conceptualisation; methodology; formal analysis; investigation; data curation; writing—original draft; project administration; funding acquisition. EJ: conceptualisation; methodology; writing—review and editing; supervision. AS: conceptualisation; methodology; writing—review and editing. RO: conceptualisation; methodology; resources; writing—review and editing. JCS: conceptualisation; methodology; writing—review and editing. TM: conceptualisation; methodology; writing—review and editing. PS: conceptualisation; methodology; writing—review and editing. SF: conceptualisation; methodology; writing—review and editing. TS: conceptualisation; methodology; writing—review and editing. AB: methodology; writing—review and editing. BMH: methodology; writing—review and editing. VS: conceptualisation; methodology; writing—review and editing; supervision; funding acquisition. All authors have reviewed and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

Ethics approval The study was carried out in accordance with the Declaration of Helsinki. Participants gave informed consent before inclusion and were financially compensated for lost earnings on examination days. The project was approved by the Higher Degrees Research and Ethics Committee at Makerere University School of Public Health (MakSPH-HDREC, registration number 577) and the Uganda National Council for Science and Technology (registration number HS234E).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Please contact the corresponding author for access to de-identified data from the subset of participants that consented to data sharing. Access requires approval from the MakSPH-HDREC and the Danish Data Protection Agency.

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