OPEN ACCESS

Original research

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

Martin Rune Hassan Hansen ,^{1,2} Erik Jørs,^{3,4} Anelli Sandbæk ,^{5,6} Daniel Sekabojja ,⁷ John C Ssempebwa ,⁸ Ruth Mubeezi ,⁸ Philipp Staudacher ,^{9,10} Samuel Fuhmann ,¹¹ Torben Sigsgaard ,¹ Alex Burdorf ,¹² Bo Martin Bibby,¹³ Vivi Schlünssen ,^{1,2}

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/thoraxjnl-2020-214609>).

For numbered affiliations see end of article.

Correspondence to

Dr Martin Rune Hassan Hansen, Environment, Work and Health, Danish Ramazzini Centre, Department of Public Health, Aarhus University, Aarhus, Denmark; martinrnehassanhansen@ph.au.dk

Received 30 January 2020
Revised 11 September 2020
Accepted 19 November 2020
Published Online First
21 January 2021

► <http://dx.doi.org/10.1136/thoraxjnl-2020-214609>

© Author(s) (or their employer(s)) 2021. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

To cite: Hansen MRH, Jørs E, Sandbæk A, et al. *Thorax* 2021;**76**:780–789.

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 organophosphate 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 organophosphate and carbamate insecticides, underlining the importance of minimising exposure.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease are major public health challenges, with estimated global prevalence of 273 million and 299 million people, respectively.¹ Some risk factors for these diseases are well-known, including tobacco

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.

smoking,^{2,3} genetic susceptibility and air pollution.^{2,4} It has also been suggested that exposure to some classes of pesticides can lead to lung function impairment, both in the general population^{5,6} and among occupationally exposed workers.⁷ Acute poisoning with acetylcholine-inhibiting insecticides (organophosphates and carbamates) can lead to respiratory failure,⁸ partly due to bronchoconstriction and increased mucus production in the airways.⁸ 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.⁹ 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.⁹

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.^{10–13} 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,¹⁴ 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) criteria¹⁵ 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,¹⁶ with 'African-American' ethnicity

Figure 1 Flowchart of participant recruitment.

Figure 2 Flowchart describing numbers of participants who performed spirometry.

as the reference, while absolute values of FEV_1 , FVC and FEV_1/FVC were secondary outcomes. Peak expiratory flow (PEF), instantaneous forced expiratory flow after exhalation of 25%, 50% and 75% of the FVC (FEF_{25} , FEF_{50} and FEF_{75}), mean forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}) and FEF_{25-75} 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.¹⁷ The primary toxicodynamic mode of action of organophosphate and carbamate insecticides is inhibition of nervous system acetylcholinesterase.¹⁸ 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.¹⁹ 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.²⁰

Confounder selection and assessment

Confounders were defined a priori using Directed Acyclic Graphs²¹ and the DAGitty software.²² 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.²³

Height and weight was measured in a standardised manner²⁴ 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.²⁰

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

STEPS questionnaire,²⁵ while questions on exposure to biomass fuel smoke were adapted from the World Health Survey²⁶ questionnaire. Finally, questions related to pesticide exposure were adapted from an existing questionnaire designed for use among smallholder farmers in low- and middle-income countries.^{13,27} We modified the existing pesticide questionnaire to make it simpler and faster to administer, while still collecting relatively detailed exposure information on, for example, duration and intensity of pesticide usage, and which specific pesticides were used. The interview was conducted in English or Luganda; the Luganda version of the questionnaire was back-translated to English before use. Interviewers entered answers directly into a database using ODK Collect.²⁰

Statistical analyses

Data were managed using Python 3 (Python Software Foundation, <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 participant, and fixed effects for the exposure and confounder variables. The regression coefficient for the exposure variable was allowed to vary between participants. A 'family' was defined as a group of participants where all members were genetically related to at least one other member of the group. The model can be described thus:

$$y = \beta_b \times b + (\sum \beta_{c_i} \times c_i) + \alpha + \tau + \varepsilon$$

y is the outcome. β_b is the regression coefficient for the effect of the exposure variable b on the outcome; β_b is normally distributed in the study population, and each person has his/her own level of β_b . The regression coefficient for the effect of the i^{th} confounder c_i is called β_{c_i} and all members of the population have the same β_{c_i} . Random effects for family and participant are called α and τ , respectively. ε is an error term.

Apart from sex, all independent variables were continuous and were generally modelled using restricted cubic splines with four knots to take into account non-linear exposure–response relationships. However, tobacco smoking was modelled under the assumption of linearity, as the number of smokers was too low to use splines (table 1). We used the `xbc`²⁸ Stata package to plot results for spline variables.

Analyses only included participants with information on all covariates from at least one project phase. The number of observations in each model is available in online supplemental appendix 2. Due to a negligible loss to follow-up (see figure 1), we did not account for it statistically.

To remove the effect of unknown time-invariant confounders, we performed secondary fixed effect analyses focussing on the change in variables within each person between project phases. The model can be written as

$$\Delta y = (\sum \beta_{x_i} \times \Delta x_i) + \alpha + \varepsilon$$

Δy is the change in the outcome between two project phases. Δx_i is the change in the i^{th} independent variable x_i , and β_{x_i} is the regression coefficient for the fixed effect of Δx_i on Δy . All participants have the same β_{x_i} . α is a random effect for family, and ε is an error term. In our main fixed effect model, we compared

phases 1+3. Sensitivity analyses compared phases 1+2 and 2+3, respectively.

RESULTS

Demographics of the study population are provided in table 1, both overall and stratified by AChE/Hb below or above the median at baseline. Two-thirds of participants were women, and most were middle-aged (median age 46.6 years). Few were ever-smokers, and the number of pack-years for ever-smokers was low. The vast majority of the participants used biomass fuels for cooking. Individuals above and below the median AChE/Hb were similar in terms of all included demographics, except for sex, where men had higher AChE/Hb than women. Self-reported use of pesticides, including cholinesterase-inhibiting insecticides, was similar in the two strata of AChE/Hb.

The mean pulmonary function (defined primarily by Z-scores of FEV₁, FVC and FEV₁/FVC) decreased across project phases, and so did AChE/Hb, table 2. The range of observed values showed considerable overlap between phases, and the changes in the means were numerically small, but most were statistically significant. Analyses of variance for both outcome and exposure variables are provided in online supplemental appendix 3. Most of the variance was between persons and between families, with less variation within persons. The ratio of within-person variance to the sum 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 association 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 analyses comparing phase 1/2 and phase 2/3 provided inconsistent results, online supplemental appendix 2.

Occupational lung disease

Table 1 Demographics for the study population at baseline

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

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.

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 2 Lung function measures and acetylcholinesterase in each project phase

	Phase 1 (Sept/Oct 2018)	Phase 2 (Nov/Dec 2018)	Phase 3 (Jan/Feb 2019)	Δ /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	
FEF₂₅ (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	
FEF₅₀ (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	
FEF₇₅ (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	
FEF₂₅₋₇₅ (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	
FEF₂₅₋₇₅ 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.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,^{5 6 30} or depressed cholinesterase activity.^{31–35} Most previous studies were conducted among occupationally exposed workers and farmers, the majority of whom were men.^{30–35} Two studies showed associations in general populations with more balanced gender distributions.^{5 6} Thus, our study differs somewhat from previous studies, as our study population was occupationally exposed and most participants were women, and caution is warranted before directly comparing numerical estimates from the current and previous studies.

A link between exposure and lung function impairment is biologically plausible, as acute intoxication with cholinesterase inhibitor insecticides leads to bronchoconstriction.⁸ 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

AChE/Hb	U/g	19.79	24.50	25.90	27.30	32.61
	Percentile	5	35	50	65	95
FEV ₁ Z-score	Unadjusted	−0.153 (−0.289 to −0.016)	−0.050 (−0.091 to −0.010)	0 (ref.)	0.048 (0.005 to 0.091)	0.126 (0.007 to 0.244)
	Adjusted, basic	−0.155 (−0.296 to −0.014)	−0.045 (−0.087 to −0.003)	0 (ref.)	0.043 (−0.002 to 0.087)	0.116 (−0.004 to 0.236)
	Adjusted, extended	−0.153 (−0.293 to −0.012)	−0.043 (−0.085 to −0.001)	0 (ref.)	0.040 (−0.005 to 0.085)	0.108 (−0.013 to 0.228)
FVC Z-score	Unadjusted	−0.041 (−0.193 to 0.111)	−0.019 (−0.065 to 0.026)	0 (ref.)	0.019 (−0.029 to 0.067)	0.036 (−0.096 to 0.169)
	Adjusted, basic	−0.038 (−0.192 to 0.116)	−0.017 (−0.063 to 0.029)	0 (ref.)	0.017 (−0.032 to 0.066)	0.034 (−0.098 to 0.167)
	Adjusted, extended	−0.036 (−0.189 to 0.116)	−0.015 (−0.061 to 0.031)	0 (ref.)	0.014 (−0.034 to 0.063)	0.024 (−0.107 to 0.156)
FEV ₁ /FVC Z-score	Unadjusted	−0.063 (−0.261 to 0.135)	−0.030 (−0.091 to 0.030)	0 (ref.)	0.033 (−0.032 to 0.098)	0.104 (−0.074 to 0.282)
	Adjusted, basic	−0.066 (−0.264 to 0.132)	−0.020 (−0.080 to 0.040)	0 (ref.)	0.020 (−0.044 to 0.085)	0.075 (−0.100 to 0.249)
	Adjusted, extended	−0.058 (−0.254 to 0.139)	−0.018 (−0.079 to 0.042)	0 (ref.)	0.020 (−0.045 to 0.084)	0.079 (−0.095 to 0.253)
FEV ₁	Unadjusted	−0.041 (−0.091 to 0.010)	−0.017 (−0.032 to −0.002)	0 (ref.)	0.017 (0.001 to 0.033)	0.039 (−0.005 to 0.083)
	Adjusted, basic	−0.042 (−0.094 to 0.011)	−0.016 (−0.031 to 0.000)	0 (ref.)	0.015 (−0.001 to 0.032)	0.037 (−0.008 to 0.082)
	Adjusted, extended	−0.041 (−0.094 to 0.011)	−0.015 (−0.031 to 0.001)	0 (ref.)	0.014 (−0.002 to 0.031)	0.034 (−0.011 to 0.079)
FVC	Unadjusted	−0.002 (−0.067 to 0.063)	−0.004 (−0.024 to 0.015)	0 (ref.)	0.005 (−0.016 to 0.025)	0.006 (−0.050 to 0.063)
	Adjusted, basic	−0.003 (−0.070 to 0.064)	−0.004 (−0.024 to 0.016)	0 (ref.)	0.004 (−0.017 to 0.026)	0.006 (−0.052 to 0.063)
	Adjusted, extended	−0.003 (−0.070 to 0.063)	−0.003 (−0.023 to 0.017)	0 (ref.)	0.003 (−0.018 to 0.024)	0.002 (−0.055 to 0.059)
FEV ₁ /FVC	Unadjusted	−0.007 (−0.019 to 0.005)	−0.002 (−0.006 to 0.002)	0 (ref.)	0.002 (−0.002 to 0.006)	0.006 (−0.005 to 0.017)
	Adjusted, basic	−0.007 (−0.019 to 0.006)	−0.002 (−0.005 to 0.002)	0 (ref.)	0.001 (−0.003 to 0.006)	0.005 (−0.006 to 0.016)
	Adjusted, extended	−0.006 (−0.019 to 0.006)	−0.002 (−0.005 to 0.002)	0 (ref.)	0.001 (−0.003 to 0.005)	0.005 (−0.006 to 0.016)

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.

Z-scores: 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; BMI, body mass index.

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 $\Delta(\text{AChE}/\text{Hb})$. Dashed lines show 95% CI. Spline knot location shown by black dots. Histograms show the distribution of $\Delta(\text{AChE}/\text{Hb})$ values for observations in the model. Basic covariate set= Δage , $\Delta\text{pack-years}$, $\Delta(\text{hours of cooking in the last week})$. Extended covariate set=basic set+ ΔBMI . AChE/Hb, erythrocyte acetylcholinesterase normalised by haemoglobin concentration.

human trial on the pulmonary effects of the cholinesterase inhibitor medicine pyridostigmine in healthy volunteers. After administration of 60 mg of pyridostigmine, FEV_1 decreased on average 130 mL, strongly correlated with the degree of acetylcholinesterase inhibition that ranged from ~20% to ~40%.³⁶ In the present study, median AChE/Hb was 26.3 U/g at baseline. While AChE/Hb has a wide normal range,¹⁷ 26.3 U/g 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.¹⁷ Hence, the degrees of cholinesterase inhibition in our study population and in the previous experimental study are of the same order of magnitude.

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 FEV_1 , there was an estimated difference of 78 (9 to 148) mL in FEV_1 between the 5th and 95th percentiles of AChE/Hb, after adjustment for the basic set of confounders. While this is below the Minimal Clinically Important Difference in FEV_1 of 100 mL in pharmacological trials,³⁷ it is a relevant difference at population level. In comparison, a systematic review on the pulmonary effects of outdoor air pollution found that each $10 \mu\text{g}/\text{m}^3$ increase in short-term exposure to $\text{PM}_{2.5}$ was associated with a -7.02 (-11.75 to -2.29) mL change in FEV_1 .³⁸

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.²¹

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%,³⁹ and 56% of the labour time in Ugandan agriculture is provided by women.⁴⁰ 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 FEV_1 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.¹⁷

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

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 GLI-2012 equations¹⁶ 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,²¹ 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.¹⁴ 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.

Author affiliations

¹Environment, Work and Health, Danish Ramazzini Centre, Department of Public Health, Aarhus University, Aarhus, Denmark

²National Research Centre for the Working Environment, Copenhagen, Denmark

³Department of Occupational and Environmental Medicine, Odense University Hospital, Odense, Denmark

⁴Occupational and Environmental Medicine, Department of Clinical Research, University of Southern Denmark, Odense, Denmark

⁵General Practice, Department of Public Health, Aarhus University, Aarhus, Denmark

⁶Steno Diabetes Center Aarhus, Aarhus, Denmark

⁷Uganda National Association of Community and Occupational Health, Kampala, Uganda

⁸School of Public Health, Makerere University, Kampala, Uganda

⁹Eawag, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

¹⁰Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Zurich, Switzerland

¹¹Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

¹²Department of Public Health, Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands

¹³Biostatistics, Department of Public Health, Aarhus University, Aarhus, Denmark

Acknowledgements We wish to thank the participants, as well as our collaborators from the Uganda National Association of Community and Occupational Health, Caritas Uganda, Wakiso District Farmers Association, the Agency for Integrated Rural Development and the Diálogos Foundation. We are thankful for the efforts of the field team: Amusa Wamawobe, Betty Kateregga, Brenda Wagaba, Evans Twin, Grace Lubega, Jonathan Mugweri, Imelda Namatovu, Joviah Gonza, Lydia Yariwo and Timothy Masaba. Finally, we are grateful to Dr Wajid Abbas Hassan Hansen for her assistance with spirometry quality control.

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; supervision. DS: conceptualisation; methodology; resources; writing—review and editing. JCS: conceptualisation; methodology; writing—review and editing. RM: conceptualisation; methodology; writing—review and editing. PS: conceptualisation; methodology; writing—review and editing. SF: conceptualisation; methodology; writing—review and editing. TS: methodology; writing—review and editing. AB: methodology; writing—review and editing. BMB: methodology; writing—review and editing. VS: conceptualisation; methodology; writing—review and editing; supervision; funding acquisition. All authors have reviewed and approved the final manuscript.

Funding This project was supported by grants from Aarhus University Research Foundation (project number 81231) and the National Research Centre for the Working Environment (project number 10322). Neither of the funders played any role in study design, data collection/analysis, drafting of the manuscript or the decision to publish.

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 HS234ES).

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.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Martin Rune Hassan Hansen <http://orcid.org/0000-0001-9681-2393>

Anneli Sandbæk <http://orcid.org/0000-0003-1647-2646>

Daniel Sekabojja <http://orcid.org/0000-0001-8444-778X>

John C Ssempebwa <http://orcid.org/0000-0002-8289-2844>

Ruth Mubeezi <http://orcid.org/0000-0002-1377-0106>

Philipp Staudacher <http://orcid.org/0000-0001-8314-1519>

Samuel Fuhrmann <http://orcid.org/0000-0002-1861-1737>

Torben Sigsgaard <http://orcid.org/0000-0002-2043-7571>

Alex Burdorf <http://orcid.org/0000-0003-3129-2862>

Vivi Schlünssen <http://orcid.org/0000-0003-4915-1734>

REFERENCES

- GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the global burden of disease study 2017. *Lancet* 2018;392:1789–858.
- Eisner MD, Anthonisen N, Coultas D, et al. An official American thoracic Society public policy statement: novel risk factors and the global burden of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;182:693–718.

- 3 Burke H, Leonardi-Bee J, Hashim A, *et al.* Prenatal and passive smoke exposure and incidence of asthma and wheeze: systematic review and meta-analysis. *Pediatrics* 2012;129:735–44.
- 4 Bowatte G, Lodge C, Lowe AJ, *et al.* The influence of childhood traffic-related air pollution exposure on asthma, allergy and sensitization: a systematic review and a meta-analysis of birth cohort studies. *Allergy* 2015;70:245–56.
- 5 Raanan R, Balmes JR, Harley KG, *et al.* Decreased lung function in 7-year-old children with early-life organophosphate exposure. *Thorax* 2016;71:148–53.
- 6 Ye M, Beach J, Martin JW, *et al.* Urinary dialkyl phosphate concentrations and lung function parameters in adolescents and adults: results from the Canadian health measures survey. *Environ Health Perspect* 2016;124:491–7.
- 7 Alif SM, Dharmage SC, Benke G, *et al.* Occupational exposure to pesticides are associated with fixed airflow obstruction in middle-age. *Thorax* 2017;72:990–7.
- 8 Vale JA, Bradberry SM. Organophosphorus and Carbamate Insecticides. In: Brent J, Burkhardt K, Dargan P, *et al.*, eds. *Critical care toxicology*. Cham: Springer International Publishing, 2016: 1–26.
- 9 Ratanachina J, De Matteis S, Cullinan P, *et al.* Pesticide exposure and lung function: a systematic review and meta-analysis. *Occup Med* 2020;70:14–23.
- 10 Oesterlund AH, Thomsen JF, Sekimpi DK, *et al.* Pesticide knowledge, practice and attitude and how it affects the health of small-scale farmers in Uganda: a cross-sectional study. *Afr Health Sci* 2014;14:420–33.
- 11 Okonya JS, Kroschel J. A cross-sectional study of pesticide use and knowledge of Smallholder potato farmers in Uganda. *Biomed Res Int* 2015;2015:1–9.
- 12 Okonya JS, Petsakos A, Suarez V, *et al.* Pesticide use practices in root, tuber, and banana crops by Smallholder farmers in Rwanda and Burundi. *Int J Environ Res Public Health* 2019;16:400.
- 13 Fuhrmann S, Staudacher P, Lindh C, *et al.* Variability and predictors of weekly pesticide exposure in applicators from organic, sustainable and conventional smallholder farms in Costa Rica. *Occup Environ Med* 2020;77:40–7.
- 14 Hansen MRH, Jørs E, Sandbæk A, *et al.* Exposure to cholinesterase inhibiting insecticides and blood glucose level in a population of Ugandan smallholder farmers. *Occup Environ Med* 2020;77:713–20.
- 15 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
- 16 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-Ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40:1324–43.
- 17 EQM Research, Inc. Test-mate Che cholinesterase test system (model 400). Available: <http://www.eqmresearch.com/Manual-E.pdf> [Accessed 21 Jan 2020].
- 18 Casida JE, Durkin KA. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annu Rev Entomol* 2013;58:99–117.
- 19 Lionetto MG, Caricato R, Calisi A, *et al.* Acetylcholinesterase as a biomarker in environmental and occupational medicine: new insights and future perspectives. *Biomed Res Int* 2013;2013:1–8.
- 20 Hartung C, Lerer A, Anokwa Y, *et al.* Open data kit: tools to build information services for developing regions. Proceedings of the 4th ACM/IEEE International Conference on Information and Communication Technologies and Development, London, United Kingdom: ACM, 2010:1–12. doi:10.1145/2369220.2369236
- 21 Hansen MRH, Jørs E, Sandbæk A, *et al.* Protocol for statistical analyses of health outcomes in the study entitled "Pesticide exposure, asthma and diabetes in Uganda (PEXADU)". *Zenodo* 2019
- 22 Textor J, van der Zander B, Gilthorpe MS, *et al.* Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol* 2016;45:1887–94.
- 23 Hansen MRH. *Exposure to pesticides in present-day use, diabetes mellitus and lung function impairment*. Aarhus, Denmark: Environment, Work and Health, Danish Ramazzini Centre, Department of Public Health, Aarhus University, 2020.
- 24 World Health Organization. Who steps surveillance manual: the who stepwise approach to chronic disease risk factor surveillance. Available: <https://www.who.int/ncds/surveillance/steps/manual/en/> [Accessed 21 Jan 2020].
- 25 World Health Organization. STEPwise approach to surveillance (STEPS). Available: <http://www.who.int/chp/steps/en/> [Accessed 21 Jan 2020].
- 26 World Health Organization. Questions on cooking practices. Available: http://www.who.int/entity/indoorair/cooking_questions_en.pdf?ua=1 [Accessed 13 Dec 2017].
- 27 Fuhrmann S, Winkler MS, Staudacher P, *et al.* Exposure to pesticides and health effects on farm owners and workers from conventional and organic agricultural farms in Costa Rica: a protocol for a cross-sectional study. *JMIR Res Protoc* 2019;8:e10914.
- 28 Orsini N, Greenland S. A procedure to Tabulate and plot results after flexible modeling of a quantitative covariate. *Stata J* 2011;11:1–29.
- 29 Saldanha C. Human erythrocyte acetylcholinesterase in health and disease. *Molecules* 2017;22:1499.
- 30 Callahan CL, Al-Batanony M, Ismail AA, *et al.* Chlorpyrifos exposure and respiratory health among adolescent agricultural workers. *Int J Environ Res Public Health* 2014;11:13117–29.
- 31 Chakraborty S, Mukherjee S, Roychoudhury S, *et al.* Chronic exposures to cholinesterase-inhibiting pesticides adversely affect respiratory health of agricultural workers in India. *J Occup Health* 2009;51:488–97.
- 32 Fareed M, Pathak MK, Bihari V, *et al.* Adverse respiratory health and hematological alterations among agricultural workers occupationally exposed to organophosphate pesticides: a cross-sectional study in North India. *PLoS One* 2013;8:e69755.
- 33 Abou Salem E, Abdel-Rasoul M, Al-Batanony A. Spirometric changes in relationship to serum cholinesterase levels for workers in a pesticide-processing factory. *Egyptian Journal of Occupational Medicine* 2007;31:63–74.
- 34 Sutoluk Z, Kekec Z, Daglioglu N, *et al.* Association of chronic pesticide exposure with serum cholinesterase levels and pulmonary functions. *Arch Environ Occup Health* 2011;66:95–9.
- 35 Hernández AF, Casado I, Pena G, *et al.* Low level of exposure to pesticides leads to lung dysfunction in occupationally exposed subjects. *Inhal Toxicol* 2008;20:839–49.
- 36 Ram Z, Molcho M, Danon YL, *et al.* The effect of pyridostigmine on respiratory function in healthy and asthmatic volunteers. *Isr J Med Sci* 1991;27:664–8.
- 37 Jones PW, Beeh KM, Chapman KR, *et al.* Minimal clinically important differences in pharmacological trials. *Am J Respir Crit Care Med* 2014;189:250–5.
- 38 Edginton S, O'Sullivan DE, King W, *et al.* Effect of outdoor particulate air pollution on FEV₁ in healthy adults: a systematic review and meta-analysis. *Occup Environ Med* 2019;76:583–91.
- 39 Food and Agriculture Organization of the United Nations. The state of food and agriculture. women in agriculture: closing the gender gap for development, Rome, 2011. Available: <http://www.fao.org/publications/sofa/2010-11/en/>
- 40 Palacios-Lopez A, Christiaensen L, Kilic T. How much of the labor in African agriculture is provided by women? *Food Policy* 2017;67:52–63.