Exposure to pesticides and health effects in farm owners and workers from conventional and organic agricultural farms in Costa Rica: a study protocol

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Abstract

Background
Pesticide use is increasing in low- and middle-income countries (LMICs), including Costa Rica. This increase poses health risks to farm workers and communities living near agricultural farms. We aimed to examine the health effects associated with occupational pesticide exposure in farm workers from conventional and organic small-scale farms in Costa Rica.

Methods
Fieldwork for our study was conducted between May and August 2016. A total of 300 farm owners and workers from organic and conventional horticultural small-scale farms in the Tapezco river catchment in the Zarcero County, Costa Rica, were enrolled in the study. During the baseline study visit, we administered a structured questionnaire to collect data on socio-demographic, pesticide exposure, and health conditions (e.g., respiratory and allergic outcomes, acute pesticide intoxication symptoms) and a neurobehavioral test battery (e.g., Finger Tapping Test, Purdue pegboard); we measured blood pressure, anthropometry (height, weight, and waist circumference), and erythrocytic acetylcholinesterase (AChE) activity; and we also collected a urine sample. In addition, a functional near-infrared spectroscopy (fNIRS) assessment was conducted with a subset of 50 study participants. During the follow-up study visit (~2-4 weeks after the baseline), we administered the participants a short questionnaire on recent pesticide exposure and farming practices, and collected hair, toenail, and urine samples. Urine samples will be analyzed for various pesticide metabolites, whereas toenails and hair will be analyzed for manganese (Mn), a biomarker of exposure for Mn-containing fungicides. Self-reported pesticide exposures data will be used to develop semi-quantitative exposure models. Exposure-outcome associations will be examined using multilevel linear and logistic models.

Discussion
Our study is one of the first studies to examine differences in health effects of pesticide exposure in farm workers from organic and conventional small-scale farms in a LMIC. We expect that our study will provide critical data on farming practices, exposure pathways, and how occupational exposure to pesticides may affect farm workers’ health. Lastly, we hope that our study will allow us to identify
strategies to reduce pesticide exposure in farm workers and will potentially lay the groundwork for a future longitudinal study of health outcomes in farm workers exposed to pesticides.

**Keywords:** Acetylcholinesterase, agriculture, Costa Rica, farm workers, near-infrared spectroscopy, neurobehavioral outcomes, pesticides, pesticide exposure assessment, respiratory outcomes, TCPy
Background

Pesticides are extensively used in agriculture and control of vector-borne diseases across the globe (Zhang et al., 2011; van den Berg et al., 2012). Current data suggest that pesticide use is increasing globally, with its largest growth in low- and middle-income countries (LMICs) in tropical contexts (FAO, 2017). Notably, registered pesticides are often not assessed in tropical contexts, where decay rates of active ingredients and metabolites of pesticides differ from other settings (Weiss et al., 2016) and regulatory bodies often fail to phase-out harmful pesticides or monitor their safe use (The Lancet, 2017). In LMICs, the widely spread small-scale farming sector frequently struggles to use pesticides in a safe way as there is a general lack of awareness and low risk perception among farm owners and workers (Feola et al., 2010).

Pesticide applicators in small-scale farms are often exposed to pesticides at different stages of the pesticides' handling chain (e.g., storage, mixing, preparation, and application) (MacFarlane et al., 2013; Suratman et al., 2015). Therefore, uncontrolled and uninformed pesticide use can directly expose workers and surrounding communities through drift and pesticide residues in food and drinking water (Deziel et al., 2015). Several studies from LMICs have shown that acute pesticide poisoning represents an important cause of morbidity and mortality among farm workers (Thundiyil et al., 2008). In addition, long-term exposure to pesticides, such as organophosphates and carbamates, has been linked to a broad range of chronic health effects, including impaired neurobehavioral function (e.g., cognitive and behavioral disorders), respiratory problems, obesity, and diabetes (London et al., 1998; Chakraborty et al., 2009; Azandjeme et al., 2013; Tago et al., 2014; Muñoz-Quezada et al., 2016).

Characterization of pesticide exposures in LMICs is challenging due to the short half-lives of most of these chemicals in the human body, limited availability of biomarkers of exposure, and lack of epidemiological data (Holtman, 2015; Negatu et al., 2016). As highlighted by a recent descriptive review (Carles et al., 2017), most studies in LMICs have relied on pesticide exposures based on self-reported data using knowledge attitude and practice studies. A few studies have generated pesticide exposure matrices, estimating exposure intensity indices using the amount of pesticide used and...
personal protective equipment worn during the applications. However, these exposure matrices are prone to information bias and often lack validation against biomarkers of exposure in humans (e.g., urine, blood) (Holtman, 2015; Negatu et al., 2016; Carles et al., 2017). Several studies have examined the health effects of occupational pesticide exposure in tropical settings (de Araújo et al., 2007; Mohanty et al., 2013; Ramírez-Santana et al., 2015; van Wendel de Joode et al., 2001; Wesseling et al., 2006, 2010, 2013). Nevertheless, to our knowledge, no study has assessed differences in pesticide use and its health effects in workers from conventional and organic farms in LMICs. The focus of this study is to determine whether occupational pesticide exposure (assessed through self-reported data and biomarkers of exposure) affects the health of farm owners and workers from conventional and organic small-scale farms in Costa Rica. Our study is part of a larger research project entitled “Pesticide use in tropical settings” (PESTROP), which aims to integrate this health component with an assessment of pesticide use, analysis of institutional determinants, and pesticide monitoring in surface water within river catchments in both Costa Rica and Uganda.

Methods/design
Objectives and study design
We conducted an epidemiological cross-sectional study of 300 farm owners and workers between June and September 2016 (rainy season, during which the highest pesticide application is expected).

The specific objectives of the project are (Figure 1):

1. to assess occupational pesticide exposure in owners and workers of conventional and organic farms, using self-reported pesticide use data and biomarkers of pesticide exposure (measured in urine, hair, and toenails);
2. to evaluate the association of occupational pesticide exposure (determined using a pesticide exposure matrix and also biomarkers of exposure) with self-reported symptoms of acute intoxication in the last 12 months;
3. to examine the association of occupational pesticide exposure with self-reported respiratory and allergic outcomes in the last 12 months;
4. to evaluate the association between occupational pesticide exposure and cardiometabolic
effects, such as adiposity (determined using weight, height, and waist circumference
measurements) and high blood pressure (measured using a sphygmomanometer);
5. to assess the association of occupational exposure to organophosphates and carbamates with
erthrocytic acetylcholinesterase (AChE) activity (measured in capillary blood);
6. to assess the association of occupational pesticide exposure with neurobehavioral outcomes,
such as working memory, visual perception, and fine motor function (assessed using a
neurobehavioral test battery); and
7. to examine the association of occupational pesticide exposure with changes in brain function
(assessed using functional near-infrared spectroscopy (fNIRS)).

Study area
The study was conducted in the Tapezco river catchment in the Zarcero County, Costa Rica (Figure 2).
This river catchment features approximately 760 small-scale farms with conventional and organic
farming practices (~4 km² of horticultural farms) (INEC, 2015) and has been previously used to
monitor pesticide levels in surface water near small-scale farms. Common crops in the area include
potatoes, tomatoes, cabbage, carrots, and onions (Ramírez et al., 2016).

Study participants’ selection and study visits
Conventional farms in the study area were identified using random Global Positioning System (GPS)
points generated based on small-scale land use data from 2015 (Moraga, 2015; Schaub, 2016). After
a total of 200 GPS points were generated, study staff visited these locations and determined which
ones corresponded to small-scale farms by contacting the farm owners or administrators. When the
GPS point did not correspond to a horticultural farm, the closest small-scale farm within a radius of 1
km was registered; if no farm was nearby, the GPS point was dropped. Organic farms within the
Tapezco river catchment or within 5 km from this area were identified using an existing list provided
by the organic farmers’ association or through identification on site. After organic and conventional farms in the study area were identified, farm owners were briefly
informed about the study aims and procedures (the initial visits to the farms are henceforth called
Information campaign; Figure 3). If they showed interest in participating in the study, basic contact
information was collected in order to schedule a later visit to their farms to enroll study participants. Eligible participants were farm owners, permanent workers, or temporary pesticide applicators, all aged ≥18 years, who owned or worked in conventional farms located in the Tapezco river catchment or in organic farms within or near the catchment area, and who did not have a diagnosis of psychiatric disease or used psychopharmacologic medications. Participants were visited twice during the study duration, either at the farms where they worked or at their homes (Figure 3). The baseline or initial study visits were conducted by two teams of three trained research assistants each and comprised four "stations" (duration ~45 minutes each):

- "Station 1" included the administration of the informed consent and a structured questionnaire to collect data on socio-demographic characteristics, occupational history, pesticide exposure at work and at home, medical history including respiratory and allergic outcomes in the last 12 months, and a blood pressure measurement;
- "Station 2" included the administration of a neurobehavioral test battery (e.g., Purdue Pegboard, Finger Tapping Test) and a checklist of acute pesticide intoxication symptoms in the last 12 months;
- "Station 3" included measurements of erythrocytic AChE activity, anthropometry (i.e., height, weight, and waist circumference), and blood pressure (second measurement), and a urine sample collection; and
- "Station 4" included the fNIRS assessment (only completed by a subset of study participants).

The follow-up study visits (duration ~15 minutes, conducted 2-4 weeks after the first visits) were conducted by one teams of two trained research assistants and included the administration of a short questionnaire on recent pesticide exposure (administered to all participants) and farming practices (administered only to farm owners), and collection of toenail and hair samples, and a second urine specimen.

All study instruments were administered in Spanish and research assistants entered data directly into tablets (Samsung Galaxy note 10.1 N8010) via a data entry mask using Open Data Kit (http://opendatakit.org). Questionnaires and other study instruments are available per request with the corresponding author.
Power and sample size calculation
We based our sample size calculation on difference in erythrocytic AChE activity between organic and conventional farmers (Neupane et al., 2014). With a sample size of 300 farmers, the minimum numbers of farms to show a significant effect between the two groups was calculated to be 50 (i.e., 25 conventional and 25 organic farms). In brief, we assumed an average cluster size of six farmers per individual farm, an intraclass correlation coefficient of 0.1, a ratio of standard deviations of 1.5 between exposed and unexposed persons in regard to erythrocytic AChE activity, a significance level of 5%, 80% power, and an effect size of 0.4 (i.e., a difference in the mean of erythrocytic AChE activity between exposed and unexposed workers of \(0.4 \times \sqrt{(1 + 2.25)/2}\) standard deviations).

Interviews and self-report pesticide exposure
Information on socio-demographic variables and occupational exposure to pesticides was collected using structured questionnaires (Table 1). During the baseline visit, study participants were asked about the crops that they had recently worked on, the tasks that they performed, and if they had prepared or applied pesticides while completing these tasks. If they reported preparing or applying one of the 15 pesticides most commonly used in the study area (according to a previous study on pesticide use conducted in the study area, Table 2; Ramírez et al., 2016), detailed data on the mode of application, period, dose, and frequency of pesticide applications, and personal protective equipment use were collected. Participants were also asked about their recent pesticide applications (prior to the collection of each urine sample), previous work in conventional or organic farms, and years of exposure to pesticides during their work life. During the follow-up visit, study participants were asked about changes in their work status and pesticide use since their baseline visit. In addition to the data described above, we collected information on farms’ characteristics (e.g., size, type of crops grown on the farm, farming practices including pest control management, water sources located nearby) using a structured questionnaire that was administered only to farm owners.

Biological sample collection and analyses
Urine samples
Spot urine samples were collected during the baseline and follow-up visits in plastic containers of
100 mL (Vacuette®, sterile). Specimens were stored at 4°C until the end of the fieldwork day. Then samples were aliquoted in 15-mL plastic test tubes (PerformRTM Centrifuge tubes, Labcon®, sterile) and stored at -20°C until their shipment at 4°C to Lund University, Sweden, for analysis. Urine samples will be analyzed for multiple pesticide metabolites including, but not limited to, ethylenethiourea (ETU, metabolite of mancozeb), PTU (propylenethiourea, metabolite of propineb), 3,5,6-trichloropyridinol (TCP, metabolite of chlorpyrifos), 3-phenoxybenzoic acid (3-PBA, metabolite of pyrethroids permethrin, cypermethrin, deltamethrin, and cyfluthrin), and hydroxy pyrimethanil (OHP, metabolite of pyrimethanil). These pesticides and their metabolites were selected because they are among the most commonly used in Zarcero (Ramírez et al., 2016) and for which biomarkers of exposure are available. Briefly, urine specimens will be analyzed by tandem mass spectrometry and high performance liquid chromatography (Ekman et al., 2013; Ekman et al., 2014). The detection limits are 0.08 ng/mL for ETU, 0.1 ng/mL for PTU, 0.03 ng/mL 3-PBA, 0.05 ng/mL for TCP, and 0.1 ng/mL for OHP. In all sample batches, chemical blanks and in-house quality control samples will be included to ensure the quality of all measurements; additionally, the analyses of TCP and 3-PBA are part of the round robin inter-laboratory comparison program (University of Erlangen-Nuremberg, Germany) with results within the tolerance limits. Urine samples will also be analyzed for creatinine and specific gravity, so that sample results can be adjusted for differences in urinary dilution (Barr et al., 2005).

Hair samples
Hair samples (~20-30 strands) were collected from the occipital region, within 2 mm from the scalp, and using stainless steel scissors, during the follow-up study visit. Hair samples were then stored at room temperature in sterile plastic bags until their shipment to the Federal University of Bahia, Brazil. Hair specimens will be analyzed for manganese (Mn), which is contained in ethylene bisdithiocarbamate (EBDC) fungicides, such as mancozeb. Briefly, the nearest centimeter scalp (proximal end) of hair will be sonicated for 20 min in 0.5% Triton, rinsed five-times with ultrapure water, sonicated for 10 min in 1 N nitric acid, rinsed once with 1 N nitric acid, and then rinsed five-
times with ultrapure water (Eastman et al., 2013). Approximately 10 mg of hair will be digested with 2 mL of concentrated HNO₃ spectroscopic grade acid in a microwave digestion oven (Mars-Express6, CEM, USA). The digested material will be diluted to 10 mL with ultrapure water. Hair samples, certified reference material (Human hair IAEA-086), and reagent blanks will be analyzed using electrothermal atomic absorption spectrometry with Zeeman background correction (Menezes-Filho et al., 2009).

Toenail samples
Toenail samples were collected during the follow-up study visit. Participants were asked to cut their toenails with clean stainless steel nail clippers and put them inside of a sterile plastic bag. Toenail specimens were stored at room temperature until their shipment to Federal University of Bahia, Brazil. Toenail samples will be analyzed for Mn using the same procedure described above for hair sample. Briefly, nails will be washed in a Triton X-100 solution, put in acetone, rinsed repeatedly with ultrapure water, and then dried in an oven. Later, the dried nails will be digested with spectroscopic grade acid in a microwave digestion oven (Mars-Express6, CEM, USA), diluted to 10 mL with ultrapure water, and analyzed electrothermal atomic absorption spectrometry with Zeeman background correction (Mehra and Juneja, 2005; Ayodele and Bayero, 2010). All processed samples and reference material from the International Atomic Energy Agency (IAEA- 085) will be analyzed in duplicates. The analytical limit of detection (LOD) will be set at 0.1 μg/L.

Assessment of health outcomes
Symptoms of acute pesticide poisoning
Participants were administered a checklist of symptoms of acute organophosphate and carbamate poisoning (e.g., excessive salivation, lacrimation, vomiting, diarrhea) during the 12-month period before the baseline study visit. This checklist has been previously used in studies of Latin American farm workers (Rodezno et al., 1995; Catharina Wesseling et al., 2006).

Respiratory and allergic outcomes
A short version of the European Community Respiratory Health Study II (ECRHS) questionnaire (http://www.ecrhs.org/Quests.htm) was administered to study participants in order to identify
respiratory symptoms (e.g., wheezing, shortness of breath, coughing, phlegm), respiratory diseases (e.g., asthma, chronic bronchitis), allergic outcomes (e.g., rhinitis, eczema), and common respiratory hazards such as smoking and pet ownership (Burney et al., 1994). This questionnaire has been previously used in studies of Costa Rican populations (Fieten et al., 2009; Rodríguez-Zamora et al., 2017).

Neurobehavioral outcomes
Study participants were administered the following neurobehavioral tests:

- Animals and words with initial letter “F” (to assess semantic and phonemic verbal fluency) (Ostroski-Solís et al., 1998);
- Digit Span (working memory) (Wechsler, 1981);
- Digit Symbol (visual perception abilities) (Wechsler, 1981);
- Digit Vigilance (sustained attention and psychomotor speed) (Lewis and Rennick, 1979);
- Trails Making Test Part A (executive function) (Reitan and Wolfson, 1985);
- Finger Tapping (psychomotor speed and coordination) (Reitan and Wolfson, 1985);
- Purdue Pegboard (fine motor function) (Costa et al., 1963); and
- Brief Symptom Inventory (behavioral disorders including somatization, obsessive-compulsion, depression, anxiety, hostility, and psychotism) (Derogatis and Spencer, 1982).

These tests were selected based on previous studies of Latin American populations exposed to pesticides (van Wendel de Joode et al., 2001; Wesseling et al., 2006), administration time, and cultural sensitivity (WHO, 1986). Neurobehavioral assessments were conducted by two trained psychometricians and supervised by a physician with extensive experience on neurobehavioral testing. Quality assurance measures included pilot testing and review of recorded assessments.

Brain function
We employed fNIRS (NIRSport, NIRx Medical Technologies, Los Angeles, CA) to assess cortical function associated with pesticide exposure in a random subsample of 50 study participants (see Baker et al. (2017) for a detailed description of our study methods). Specifically, in this study we hypothesized that pesticide exposure would have a measurable effect on cortical activation or deactivation related to attention and working memory. As these cognitive processes commonly elicit cortical activity within the bilateral dorsolateral prefrontal cortices (Cui et al., 2011; Ehlis et al., 2008; Monchi et al., 2001), we targeted these regions with fNIRS. In order to engage our participants in
tasks that required these cognitive abilities, each participant completed three computer-based tasks that were optimized for neuroimaging applications. These tasks included the Wisconsin Card Sort task (Monchi et al., 2001), Sternberg working memory task (Ehlis et al., 2008), and Go/No-go task (Cui et al., 2011). Each task was conducted on a laptop computer that was dedicated for the fNIRS assessment and was completed on site.

Cardiometabolic outcomes: blood pressure and anthropometric measurements
Systolic and diastolic blood pressure (BP) were measured at two different time points during the baseline study visit using an automatic sphygmomanometer (Advantage 6021N). Height and weight were measured using a portable stadiometer and a digital scale (Tanita BC533, Arlington Heights, IL). Waist circumference was measured using a tape measure Hoechstmass (Hoechstmass Balzer GmbH, Sulzbach, Germany).

Erythrocytic AChE activity
Capillary blood samples were collected at the baseline visit according to the manual of the Test-mate ChE Cholinesterase Test System (Model 400; EQM Research Inc., Cincinnati, OH). Briefly, a small lancet (size 30) was used to collect a small sample of 10µm from the tip of the index finger of each study participant and placed into a capillary tube. Blood samples were analyzed on site for erythrocytic AChE activity and hemoglobin levels using the same collection instrument (EQM Research, 2003).

Statistical analyses
We will explore differences in self-reported pesticide exposures and health outcomes in farm workers from conventional and organic farms. Cumulative pesticide exposure, exposure during the last 12 months, and during the last week will be estimated using exposure intensity scores derived from a semi-quantitative exposure model (based on self-reported data on pesticides, personal protective equipment use, and personal hygiene habits) (Negatu et al., 2016). Exposure intensity scores will be calculated for each chemical family (e.g., organophosphates, carbamates) and active ingredient (e.g., phorate, chlorpyrifos) and then validated against urinary pesticide metabolite concentrations and hair and toenail Mn concentrations. We aim to use multiple-imputation techniques to replace the
metabolite or Mn concentrations below the limit of detection (Lubin et al., 2004). We will fit mixed-effects models to examine the reproducibility of urinary pesticide concentrations by calculating intraclass correlation coefficients (ICC) (Rosner, 2006). If appropriate, we will then average urinary pesticide metabolite concentrations across the repeated samples collected for each study participant. Associations of pesticide exposure with health outcomes of interest will be examined using both exposure intensities and biomarkers of exposure. More specifically, we will fit linear or logistic (depending on the outcome) mixed-effects regression models to explore the association of pesticide exposure with: (i) self-reported symptoms of acute pesticide poisoning in the last 12 months; (ii) self-reported symptoms of respiratory and allergic symptoms and outcomes; (iii) neurobehavioral outcomes and brain activity; (iv) cardiometabolic effects (i.e., obesity and hypertension); and (v) erythrocytic AChE activity. These mixed-effects models will allow us to take into account the correlation between- and within-farms and between- and within-farm workers. We will also fit generalized additive models to examine the non-linearity of the exposure-outcome associations. We will identify potential confounders and known predictors of the health outcomes of interest (e.g., age and education level for neurobehavioral outcomes) using directed acyclic graphs and will include them \textit{a priori} in our regression models. We will assess other potential confounders by adding them, one at a time, to the final models (models with \textit{a priori} covariates). Additional covariates will be possibly included in the final models if they materially changed the magnitude of one or more exposure coefficients (>10%). Missing values (<10%) for covariates will be imputed by randomly selecting a value from the dataset or using multiple imputation techniques (Lubin et al., 2004). Statistical analyzes will be performed using STATA (Stata Corporation, College Station, USA) and R (R Foundation for Statistical Computing, Vienna, Austria).

\textbf{Discussion}

We conducted the study fieldwork between May and August 2016 and successfully enrolled 300 study participants on 90 farms. Over all farms we had 113 farm owners (as some share the ownership of the farm property) and 187 farm workers. Notably, most of the farm workers that we enrolled in our study were informal immigrants (64% from Nicaragua) that worked for short periods...
of time. Nevertheless, we only had a 5% loss to follow-up of study participants between the baseline and follow-up study visits (conducted 2-4 weeks apart). During the implementation of the study we observed, however, that farms were in average smaller than expected (average of about 3 farmers there were not as many organic farms in the study area as anticipated (only 10 farms out of 25 expected). Further investigations revealed that many organic farm owners had recently started using synthetic pesticides due to the increasing costs of growing organic produce and getting certified as organic producers. Given the limited number of organic farms in the study area, we decided to include all farm owners and workers from organic farms located in the study area or within 5 km from it. To reach the targeted sample of 300 participants, we had to enroll more conventional farm owners and workers than what we had expected.

Our study has several limitations. First, its cross-sectional design will prevent us from identifying causal associations of pesticide exposure with health effects of interest. Second, we will not be able to exclude the possibility that there is recall or information bias, especially when relying on self-reported exposure to pesticides. In our study population, this bias may have worsened under the following conditions: (i) most study participants had a relatively low educational level; (ii) pesticide use varied by crop and season; and (iii) some farm owners did not communicate the specific pesticides that were been used in their farms to their employees. Therefore, validation of self-reported pesticide exposure data against biomarkers of exposure is extremely important. Third, we observed some significant differences between owners and workers from conventional and organic farms (e.g., seasonal workers, differences in country of origin and education level) that could potentially confound the exposure-outcome associations. Hence, it was crucial to also collect detailed information on confounding factors and predictors of the outcomes of interest. The limitations of the present study are offset by notable strengths, including (i) the quantification of pesticide metabolites and Mn concentrations in different biological matrices, which will allow us to validate the exposure information collected via questionnaires (or at least data from recent exposures given the relatively short half-lives of the biomarkers of exposure); (ii) the comparison of workers and owners from conventional and organic farms using comprehensive questionnaires on
occupational pesticide exposure; and (iii) the assessment of health outcomes using internationally standardized tests that will allow for direct comparison of the results from this study to those from studies of other populations.

Our study is one of the first studies to examine the health effects of exposure to a wide range of pesticides in Latin American workers from conventional and organic farms. Ours is also the first epidemiological study to examine the association of pesticide exposure with changes in brain function in farm workers. We expect that our study will provide critical data on how occupational exposure to common pesticides may affect farm workers’ health. Lastly, we hope that our study will allow us to identify strategies to reduce pesticide exposure in farm workers and will lay the groundwork for a future longitudinal study of health outcomes in farm workers exposed to pesticides.

**Abbreviations**

3-PBA: 3-phenoxybenzoic acid  
AChE: Acetylcholinesterase  
EBDC: Ethylene bisdithiocarbamate  
ECRHS: European Community Respiratory Health Study II  
ETU: Ethylenthiourea  
fNIRS: Functional near-infrared spectroscopy  
GPS: Global Positioning System  
ICC: Intraclass correlation coefficient  
LMICs: Low- and middle-income countries  
PTU: Propylenethiourea  
OHP: Hydroxy pyrimethanil  
TCP: 3,5,6-trichloropyridinol

**Declarations**

**Ethics approval and consent to participate**

All study materials and procedures were approved by the human subjects committee of the Universidad Nacional in Costa Rica (UNA-CECUAN-ACUE-04-2016) and Ethical Board of the Ethikkommission Nordwest- und Zentralschweiz in Switzerland (EKNZ-UBE 2016-00771). Written informed consent was obtained from all study participants at enrollment. Study results will be communicated back to participants and stakeholders at restitution workshops organized in Zarcero (Restitution workshops; Figure 3).

**Consent for publication**

Not applicable
Availability of data and material
Not applicable

Competing interests
The authors declare that they have no competing interests

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Authors' contributions
“MSW is the principal investigator of the PESTROP project, responsible for the overall study coordination. AMM is the project leader of the PESTROP project in Costa Rica and, together with SF, responsible for the design of the research protocol, supervision of the field work and the overall analysis of data. JB and RG developed and executed the protocol for fNIRS analysis. PS, FW, CS, RILE, FR contributed to the development of the study protocol, questionnaire design and field work. CL, JAM have contributed to improve of the study protocol and will conduct the analysis of the human samples. SF, in close collaboration with AMM and MSW, drafted this manuscript. All authors provided comments on the draft and have read and approved the final version of it.

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## Tables

**Table 1.** Information collected during the baseline study visit using structured questionnaires.

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<tr>
<td>Housing characteristics</td>
<td>Years living in the current house, number of bedrooms, number of people living in the house, water source, pet ownership, and farm animals living in or next to the house</td>
</tr>
<tr>
<td>Work history</td>
<td>Age when started working in agriculture, age at first contact with pesticides, past jobs and current jobs in agriculture</td>
</tr>
<tr>
<td>Pesticide use</td>
<td>Pesticide use (Table 2) during the last 12 months and last week, mode of application, frequency of pesticide applications, use of personal protective equipment, personal hygiene habits</td>
</tr>
<tr>
<td>Residential pesticide use</td>
<td>Indoor or outdoor pesticide use in the home (e.g., pyrethroids, herbicides)</td>
</tr>
<tr>
<td>Medical history</td>
<td>Respiratory and allergic symptoms, acute pesticide intoxications during the last 12 months, other illnesses</td>
</tr>
</tbody>
</table>
Table 2. Most frequently used pesticides (active ingredients) in agricultural farms in the Zarcero County, Costa Rica.

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Commercial names</th>
<th>Chemical subgroup</th>
<th>Group of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorothalonil</td>
<td>Bravo®, Bravonil®, Knight®, Talonil®, Thalonex®, Folio Gold®, Odeon®</td>
<td>Chloronitriles</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Benfuracarb</td>
<td>Oncol®</td>
<td>Carbamates</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>Dithane®, Mancol®, Ridomil®, Titan®</td>
<td>Dithiocarbamates</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Boscalid</td>
<td>Bellis®, Endura®</td>
<td>Pyridinecarboxamides</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>Afin®, Cozaid®, Crotonox®, Carbendazina®</td>
<td>Benzimidazoles</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Acephate</td>
<td>Acefate®, Orthene®, Yuca®</td>
<td>Organophosphates</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Phorate</td>
<td>Forato®, Thimet®, Thimetoato®, Timefor®</td>
<td>Organophosphates</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Fenamiphos</td>
<td>Fenemiphos®, Nemacur®</td>
<td>Organophosphates</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Agromil®, Batazo®, Baygon®, Lorsban®, Solvent®, Terminator®, Swat®</td>
<td>Organophosphates</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Atila®, Evigras®, Ranger®, Round Up®</td>
<td>Glycine derivatives</td>
<td>Herbicide</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>Carbodan®, Curator®, Furadan®</td>
<td>Carbamates</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>Best®, Cascabel®, Cipermetrina®, Combat®, Cruz Verde®, Tigre®, Excalibur®</td>
<td>Pyrethroids</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Propamocarb</td>
<td>Acrobat CT®, Previcur®, Proplant®, Prevalor®</td>
<td>Carbamates</td>
<td>Fungicide</td>
</tr>
<tr>
<td>Paraquat</td>
<td>Gramoxone®, Preglon®®, Rafaga®</td>
<td>Bipyridiliums</td>
<td>Herbicide</td>
</tr>
<tr>
<td>Proprineb</td>
<td>Antracol®, Inicol®, Taifen®</td>
<td>Dithiocarbamates</td>
<td>Fungicide</td>
</tr>
</tbody>
</table>

Modified from: Ramírez et al., 2016.
Pesticides are ordered from most frequently use to the least frequently used.
Figures

Figure 1. Aims (bold text), research design details and tools used () in the study conducted in Zarcero County, Costa Rica, 2016.
Figure 2. Study area (Tapezco river catchment) with GPS locations of the 90 farms that were included in the study, Zarcero County, Costa Rica, 2016. Shape files provide by Moraga (2015)
Figure 3. Diagram of the fieldwork set-up in the Zarcero study, Costa Rica, 2016.