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An educational intervention on the risk perception of pesticides exposure and organophosphate metabolites urinary concentrations in rural school children in Maule Region, Chile

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Abstract

Background: Organophosphate (OP) pesticides can be hazardous to human health if not applied with appropriate precautions. There is evidence in the Maule region of Chile that rural schoolchildren are exposed to OP pesticides.

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Conflicts of interest
I have read the journal's policy and the authors of this manuscript have the following competing interests: Dr. Asa Bradman is a volunteer member of the Board of Trustees for The Organic Center, a non-profit organization addressing scientific issues about organic food and agriculture, and is also a member of the USDA National Organic Standards Board. Dr. Bradman also advises organic and conventional food growers and processors on pesticide-related issues (unpaid) and worked with Friends of the Earth on a study examining dietary pesticide exposures. None of the other authors declares any actual or potential competing financial interest.

Publisher's Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade names is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2019.108554.

Objective: To evaluate the effectiveness of an educational intervention on OP exposure and understanding of pesticides and their hazards (risk perception) in two school communities in the Maule Region of Chile during 2016.

Method: We conducted a quasi-experimental study about the effects on OP pesticide exposure of a community outreach and education program (COEP) administered in four 2-h sessions that's included hands-on activities among 48 schoolchildren from two rural schools. The intervention was directed to groups of parents and schoolchildren separately, and aimed to educate them about the risks of exposure to pesticides and their effects on health. We measured 3,5,6-trichloro-2-pyridinol (TCPy), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), malathion dicarboxylic acid (MDA), p-nitrophenol (PNP), specific urinary metabolites of the OP pesticides chlorpyrifos, diazinon, malathion and parathion, respectively, as well as the non-specific diethylakylphosphates (DEAPs) and dimethylalkylphosphates (DMAPs) in 192 urine samples of schoolchildren collected before and after the intervention. The risk perception of school children and their parents was also assessed through a questionnaire before and after the intervention. Generalized Estimated Equations were used to account for each child's repeated measures during four sessions, two in September 2016 (pre-intervention) and two in November 2016 (post-intervention).

Results: The intervention level had significant effect on the risk perception of adults and children, which increased after the intervention. However, the intervention was not associated with reduced of urinary metabolites levels, with no significant differences between the pre and post measures. The detection frequencies were 1.1% (MDA), 71.4% (TCPy), 43.3% (IMPY), 98.96% (PNP), and 100% (DEAPs and DMAPs). Higher DEAPs urine concentrations were associated with eating more fruit at school ($p = 0.03$), a younger age ($p = 0.03$), and being male ($p = 0.01$). DMAPs showed no associations with potential predictor variables (e.g. OPs applied at home, fruit consumption at school, among others). Higher TCPy was associated with attending a school closer to farms ($p = 0.04$) and living in a home closer to farm fields ($p = 0.01$); higher PNP was marginally associated with children younger age ($p = 0.035$).

Conclusion: Environmental exposure to OP pesticides was unchanged even after behavior changes. It is possible that a longer time period is needed to observe changes in both behavior and urinary metabolites. The levels of DEP and DMP metabolites found here are above the reference population of the US, and our findings indicate exposure to a wide variety of OP pesticides. Given that individual-level interventions were not associated with lower exposures, efforts to reduce exposure must occur upstream and require stricter regulation and control of pesticide use by government agencies.

Keywords

Pesticides exposure; Interventions; Children; Risk assessment; Agriculture

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1. Introduction

Organophosphate (OP) pesticides are used in agriculture worldwide to control pest (insects, rodents, fungi and weeds). OP pesticides inhibit AChE, a key enzyme necessary for proper functioning of the central, peripheral and autonomic nervous system (Matthews, 2016; World Health Organization, 2009). Pesticide use in Chile has increased over the last years, and OP pesticides are the most common insecticides used in all regions of the country (Servicio Agrícola Ganadero, 2012). In the Maule Region, some of the most common OP pesticides applied are chlorpyrifos and diazinon (Servicio Agrícola Ganadero, 2012), which are highly or moderately hazardous according to the World Health Organization (WHO) (Matthews, 2016; World Health Organization, 2009).

The effects of OP pesticides have been assessed by several international studies on populations of agricultural workers and their children (Alavanja et al., 2004; Alavanja and Bonner, 2012; Liu and Schelar, 2012; Bradman and Whyatt, 2005; Costa, 2006; Engel et al., 2007; Eskenazi et al., 2008; Garry, 2004; Handal et al., 2007; Jurewicz and Hanke, 2008a,b; Rosas and Eskenazi, 2008; Rothlein et al., 2006a,b; Kamel and Hoppin, 2004). Clinical symptoms can vary from mild to fatal (Matthews, 2016; World Health Organization, 2009). Some chronic effects of OP pesticides in agricultural workers have been observed, including deficiencies in cognition, motor, and sensory domains, and increased neurological diseases (Harrison and Mackenzie Ross, 2016; Joshaghani et al., 2007; Kamel and Hoppin, 2004; Muñoz-Quezada et al., 2016a; Muñoz-Quezada et al., 2016b; Paul et al., 2018; Rothlein et al., 2006a,b; Suratman et al., 2015). Occupational exposure of parents who work in agricultural activities is also associated with greater pesticide exposure in their children (Lu et al., 2004; Naeher et al., 2010; Rodríguez et al., 2006; Valcke et al., 2006; Vida and Moretto, 2007). In children, OP pesticide exposure, especially during the prenatal period, has been associated with poorer psychomotor and mental development (Berkowitz et al., 2004; Eskenazi et al., 2004, 2007, 2008; Garry, 2004; Handal et al., 2007; Huen et al., 2012; Jurewicz and Hanke, 2008a; Jurewicz and Hanke, 2008b; Muñoz-Quezada et al., 2013; Needham, 2005; Rosas and Eskenazi, 2008; Sagiv et al., 2018).

Our research group conducted an earlier study in the Province of Talca, Chile (Muñoz-Quezada et al., 2012, 2014), with a sample of 190 schoolchildren aged 6–12 years old to examine OP pesticide exposure and associations with environmental and sociodemographic risk factors. Exposure was assessed by measurement of urinary dialkylphosphates (DAPs) metabolites in two periods: summer (period of highest agricultural pesticide use) and autumn (period of lower agricultural pesticide use). Detection frequencies of the non-specific OP metabolites diethylakylphosphates (DEAPs) and dimethylalkylphosphates (DMAPs) in summer were 72.6% and 3.6%, respectively. Detection frequencies in fall for DEAPs and DMAPs were 80% and 18.6%, respectively. We found that the presence of total metabolite concentrations in the urine of schoolchildren was mainly associated with the consumption of fruits harvested using chlorpyrifos and phosmet (apples, tomatoes, and oranges), living near agricultural fields, and the application of OP pesticides (mainly fenitrothion) at home.

Several studies have focused on the importance of training to reduce pesticide exposures and health risks in agricultural populations (Bradman et al., 2009; Farahat et al., 2009; Lehtola et al., 2008; Lu et al., 2008; Mandel et al., 2000; Muñoz-Quezada et al., 2017; Napolitano et al., 2002; Orozco et al., 2011; Ospina et al., 2009; Perry and Layde, 2003 Salvatore et al., 2009; Salvatore et al., 2015). In Chile, training is only provided to workers, primarily those working for large companies or the governmental INDAP (Institute of Agricultural Development), but to date there have been few programs focused on childhood exposure to pesticides in rural areas. A quasi-experimental study in Seattle, Washington, assessed children's longitudinal exposure to OP pesticides after receiving an organic diet intervention in both summer and fall seasons. The authors used a novel design aimed to determine the contribution of the overall dietary intake to the overall OP pesticide exposure. The findings demonstrated that the major source of exposure was the dietary intake (Lu et al., 2008). Also, several studies carried out with parents of children exposed to pesticides have shown that training can lead to changes in the risk perception of exposure to pesticides, reducing risky behaviors (Bradman et al., 2009; Farahat et al., 2009; Napolitano et al., 2002; Salvatore et al., 2009, 2015).

In the present study, we applied methods from prior studies to increase awareness about the risks related to the exposure to OP pesticides and strategies to prevent OP pesticide exposure in parents and schoolchildren (Bradman et al., 2009; Farahat et al., 2009; Mandel et al., 2000; Perry and Layde, 2003; Salvatore et al., 2009, 2015). We evaluated the effect of this intervention by measuring OP pesticide metabolites in the urine of schoolchildren and assessed the risk perception of schoolchildren and their parents (before and after the intervention) in the Maule Region of Chile. We hypothesized that: 1) The children's and parents' risk perception about OP pesticide exposure would increase in the post-intervention period in the group that received the educational intervention; 2) The children's OP pesticide exposure, measured by the urinary metabolites concentrations, would decrease in the post-intervention period in the group that received the educational intervention. This type of intervention has not been implemented in Chile and is novel abroad, since it involves intervening in rural public schools instead of field- or home-based settings.

2. Materials and methods

2.1. Design

We conducted a quasi-experimental study in 2016 that involved a community outreach and education program (COEP) aimed at parents and schoolchildren from two rural communities in the Maule Region located near farms (schools Bu and VP, Fig. 1), and the collection of longitudinal data of children's exposure to OP pesticides (four urinary samples). School Bu is adjacent to farms that use pesticides, and school VP is 200 m from the fields. One school (Bu), shares its courtyard in a common space with adjacent agricultural fields and is separated from fields only by a barbed wire fence, which children sometimes cross to obtain fruit from the orchard. School VP is 200 m from agricultural fields and is not accessible to the school community. In both schools, a group of randomly selected schoolchildren and parents participated in a one-month educational intervention program about OP pesticides exposure and its effects on health (intervention group). Another group of schoolchildren and parents –also randomly selected-participated only in a meeting to discuss the study in general terms (control group). To prevent contamination between groups the interventions were conducted at different times and excluded participation of relatives across groups. Prior to implementation of the intervention, urine samples were collected from children of both schools in two different days within one week (Tuesdays and Thursdays), and the risk perception of the participants (children and one of their parents) about OP pesticides exposure and its effects was evaluated through a questionnaire. After the intervention, the children's urine samples were collected again on two different days within one week to evaluate the effect of the intervention. In this way, each group was their own control.

The measurements and interventions were made during the months of September and November in 2016, corresponding to the periods of low and high agricultural pesticide use in the Maule Region.

2.2. Population and sample

The study population included schoolchildren of both genders from 5 to 13 years of age and their parents from two rural elementary schools of the Maule Region. School VP has a total number of 149 schoolchildren, and is located in a rural area of the Talca county. School Bu has a total number of 127 schoolchildren and is located in a rural area of San Clemente county (Fig. 1). These schools were chosen because they are located in rural areas where, in a previous study, OP pesticide exposure was associated with consumption of vegetables and living near farms (Muñoz-Quezada et al., 2012). The parameters considered to estimate the sample size were taken from an intervention study of Lu et al. (2008), which applied a linear regression with multiple covariates, including the following: level of significance = 0.05, number of covariates = 5, $R^2 = 0.5$, power = 80%. With these parameters, the estimate of the sample size to run the study for each educational community was 20 children and parents (total 40). An oversample of 20% was included in case of possible loss of follow-up of the participants in the project, resulting an estimated total sample of 48 schoolchildren and their respective parents. Participants were recruited and randomly selected for intervention or control groups, in proportion to the number of children and from enrollment lists provided by each of the schools. The study was explained to the parents and children and if they did not agree to participate, a new schoolchild was randomly selected until the sample needed for the study was completed. Final participants included 20 parent-child pairs from VP and 28 from BU. All research procedures were reviewed by the Scientific Ethics Committee of the Maule Health Service. Written informed consent was obtained from parents and all participation was voluntary and confidential.

2.3. Data collection

2.3.1. Dependent variables

Urine collection: Four first morning void urine samples were collected from each of the 48 children, with a total number of 192 samples collected. The first collection period took place at the end of September 2016, with two samples collected on Tuesdays and Thursdays within one week before the intervention. It was followed by a second sampling in November of 2016, again with two samples collected on Tuesdays and Thursdays. The protocol for the urine collection was familiar to both the children and parents. Parents were provided with a prelabeled urine collection containers cups with screw cap lids, and they were instructed to collect at least 25 ml of their child's void. The research staff collected the samples early in the morning, as soon as the children arrived to school, and kept them frozen till its transportation to a University laboratory where the samples were transferred to 10 ml polypropylene test tubes.

Laboratory methods and analysis: Urine samples were aliquoted and frozen at −20 °C and later shipped on dry ice to the U.S. Centers for Disease Control and Prevention (Atlanta, Georgia, USA) laboratory for analysis of 3,5,6-trichloro-2-pyridinol (TCPy, a metabolite of chlorpyrifos and chlorpyrifos methyl), 2-isopropyl-4-methyl-6-hydro-xypyrimidine (IMPY, a metabolite of diazinon), malathion dicarboxylic acid (MDA, a metabolite of malathion) and para-nitrophenol (PNP, a metabolite of parathion and other compounds). The target metabolites were extracted using a semi-automated solid phase extraction method, separated using a reversed-phase high-performance liquid chromatography technique, and detected using tandem mass spectrometry with isotope dilution quantitation. Method details and quality control procedures have been previously described (Davis et al., 2013). We also measured six common dialkylphosphate (DAP) metabolites: dimethyl-phosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP) using a modification of a solid phase extraction-high performance liquid chromatography-tandem mass spectrometry method (Jayatilaka et al., 2017). Limits of detection (LOD) were 0.1 μg/L for TCPy, IMPY, PNP and DAPs and 0.5 μg/L for MDA. Concentrations below the LOD were assigned a value = $\mathrm{LOD}/\sqrt{2}$ (Hornung and Reed, 1990). We did not conduct statistical analyses for MDA, the malathion metabolite, because 99% of samples had concentrations below the LOD; for diazinon (57% of results were below LOD), we dichotomized the concentrations into above and below the LOD. Concentrations were converted to SI units to create molar concentrations and all DEAP (DEP, DETP and DEDTP) and DMAP (DMP, DMTP and DMDTP) metabolites were summed to obtain an aggregate exposure term (i.e., ΣDEAP and ΣDMAP) (Barr et al., 2004). An average of the two urine samples from each child collected before and after the intervention was used for analysis. We collected the whole number of 192 samples expected. However, there were cases in which one of the samples delivered for each period could not be analyzed by the lab (8% of total samples). In those cases, the metabolite concentrations of the other available samples were used for analysis. Creatinine levels were measured at CDC using a colorimetric method. The involvement of the CDC laboratory was determined not to constitute engagement in human subject research.

Evaluation of risk perception: We validated the parents' and children's questionnaires through an inter-judgement agreement and a pilot study, showing internal consistency (Cronbach's Alpha) above 0.70. These instruments were constructed based on questionnaires applied in other studies that evaluated the risk perception of pesticide exposure before and after an educational intervention in agricultural workers (Mandel et al., 2000; Ospina et al., 2009; Perry and Layde, 2003; Salvatore et al., 2009). The parents' risk perception was evaluated through a written questionnaire with 44 closed-end questions (yes or no). The questionnaire took approximately 15–25 min to answer and addressed the following dimensions: knowledge about OP pesticides (e.g. *Have you hear about the following pesticides?*), risk perception about pesticide exposure at home (e.g. *we store the food before applying pesticides in our house)*, risk perception of environmental hazards (e.g. *I am concerned about the burning of empty pesticide containers in the fields to eliminate them*), risk perception of occupational pesticide hazards (e.g. *people can be exposed at work by touching crops after pesticides are applied*) and knowledge about the health impacts of pesticides (e.g. *OP pesticides can harm the health of children*). The total score corresponded to the sum of the points assigned for each question (54 points maximum; some items had a max possible score higher than one point). The higher the score, the greater the understanding of pesticide exposures and health risks. To assess risk perception in schoolchildren, a written questionnaire with 17 closed-end questions was administered. It took 10–20 min to answer and was also validated with similar agreement scores as the parents. The children's risk perception questionnaire addressed the following dimensions: knowledge about OP pesticides (e.g. *Have you heard of pesticides?*), risk perception about pesticide hazards at home (e.g., *Do you know where pesticides are stored in your house?*), risk perception of pesticide hazards at school (e.g., *Do you know if pesticides are applied near your school?*), and understanding of the health risks of pesticides (e.g., *Do you know if pesticides have effects on the human body?*). The total score was based on the sum of the points assigned to each question (maximum 23 points; some items had a max possible score higher than one point). The higher the score, the greater the understanding of risks for OP pesticide exposure and health risks by the schoolchildren.

The questionnaires were administered before (Measure 1, September 2016) and after (Measure 2, November 2016) the intervention. The data from both questionnaires were treated as continuous variables through the sum of the questions' scores.

2.3.2. Interventions—The methodology of the interventions was based on a community outreach and education program (COEP) approach (Farahat et al., 2009; Napolitano et al., 2002; O'Fallon et al., 2000; Salvatore et al., 2015; Srinivasan and Collman, 2005). An educational intervention was given to the intervention group. The control group treatment consisted of participating in a meeting to discuss general topics about the development and planning of the study. The intervention was conducted during four weeks between October and November. For an extensive revision of the content of the intervention, all the material used is open-access and can be found online (Muñoz-Quezada and Lucero, 2017). Also, a description of the topics and contents addressed in each session is included in the Supplementary Table 1.

The educational intervention for parents involved the participation of at least one parent for each child, and it was implemented in 4 sessions of 2 h each. It included the following topics: 1) exposure and health effects of OP pesticides; 2) Correct use of pesticides and prevention measures; 3) demonstration about the cleaning of produce before consumption; 4) strategies to reduce the use of pesticides at home and at work. The intervention involved presentations with video support and discussion groups about the teaching materials. There was no monetary incentive for participation, but participants did receive snacks during the sessions of the workshop. For the children, the intervention was implemented in 3 sessions of 1 h and a half each using hands-on and concrete activities, which made it accessible to a wide range of ages. An inclusion criterion to participate was that the children knew how to read and write and did not have a disability. To hold the children's attention, the planned activities were playful and participatory and the monitors were trained psychologists on intervention with children. Trainings with schoolchildren included the following topics: 1) What are OP pesticides and how they can affect our bodies; 2) How can children and adults be exposed to OP pesticides; 3) How can we prevent the effects of OP pesticides in our home, school and community. During each session, the active participation of children (expression of views and concerns) was permanently promoted and supported by the intervention staff, according to each children's evolving capacity.

2.3.3. Other variables—

Other variables, also assessed by a questionnaire administered to one of the children's parents, included: children's age, sex, and educational level; proximity of the schoolchildren's home to farms; parent's occupation; parents' report of pesticides used at work, school or home; children's consumption of fruits and vegetables at home and school; and produce cleaning practices before consumption.

2.3.4. Analysis plan—

After data preparation (i.e. reviewing lost data, atypical cases, duplicate measurements), an exploratory data analysis was carried out to determine measures of variation, and the distribution of study variables. The dichotomous and categorical variables were expressed as proportion (and percentages) and the continuous variables as measures of central tendency (mean, geometric mean and median) and dispersion (standard deviation and confidence interval). The data was not normally distributed so we used non-parametric tests: Mann-Whitney U for comparison of both the risk perception and the urinary metabolites between intervention and control groups; the Wilcoxon rank sign test was used to compare repeated measures of risk perception and urinary metabolites both between and within groups.

Data of urinary metabolites from the pre-intervention period (two urine samples of September combined) and the post-intervention period (two urine samples of November combined) were used in the Generalized Estimated Equations (GEE) analysis. Also, it was used a GEE model for risk perception analysis. An exchangeable correlation matrix was assumed. Linear regression was used for continuous outcomes (risk perception, and urinary concentrations of DEAP, DMAP, and of biomarkers of chlorpyrifos and parathion), while logistic regression models were used for the diazinon biomarker (above or below LOD). We also determined whether residuals were normally distributed for each model and computed 95% confidence intervals. We conducted sensitivity analyses to evaluate the impact of outliers (none were identified that changed the outcome of statistical models). The statistical package software STATA 13.0 was used for all the analyses. All models for urinary metabolites included urine creatinine in the model to correct for urinary dilution.

3. Results

The mean age of the participating schoolchildren was 9 years old (± 2.2 SD) with a range from 5 to 13 years old. The mean age of parents was 37 years old (± 8.3 SD) with a range from 24 to 53 years old. Table 1 shows the socio-demographic characteristics of the parents and schoolchildren control or intervention group.

Risk perception:

No significant differences in the risk perception associated with pesticide use and exposure between the control group and the intervention group were found before the intervention ($p = 0.2928$). Post-intervention, the risk perception scores improved for schoolchildren who received the educational intervention about pesticide exposure and health risks compared to students who did not receive the intervention (Mean score = 12.7 and 9.4, respectively; p = 0.0047) (Table 2).

Among parents, no significant pre-intervention differences were found between the intervention group and the control group (p = 0.9502). Post-intervention, parents who received the educational intervention also had improved scores on risk perception about the pesticide exposure and health risks compared to parents who did not receive the intervention (Mean score = 47.6 and 42.2, respectively; p = 0.0001) (Table 2).

Reviewing the risk perception measures for the control and intervention groups before and after treatment, we found that although the intervention group of children had higher scores in the risk perception after treatment, there are significant differences in both groups (control group, p = 0.02; intervention group, $p < 0.0001$). In the case of parental risk perception, we found significant differences within the groups only in the intervention group (control group, p = 0.07; intervention group, $p < 0.0001$).

Urinary metabolites:

Table 3 summarizes OP pesticide metabolites concentrations in the urine samples. The concentrations of specific DAPs metabolites are summarized in the Supplementary Table 2. Detection frequencies for MDA, TCPy, IMPY, PNP, and DAPs were 1.1%, 71.4%, 43.3%, 98.96%, and 100%, respectively. There were no significant differences in urinary metabolites concentrations between both groups. Metabolite levels tended to increase in the post intervention period, which coincides with the pesticide applications season.

GEE model results:

We used GEE to evaluate key predictor variables (Table 4). Results showed higher concentrations of TCPy with attendance at the School Bu (p = 0.034) and living closer to farm fields (p = 0.013) (Table 4). There was no effect of the intervention on urinary concentrations of the metabolites evaluated for the study.

For children’s urinary diazinon metabolite, we dichotomized the variable because more than half of the measurements were below the LOD (Table 5). The variable fruit consumption at home was not included in this analysis because all the children ate fruit at home.

Children attending the school Bu and boys (compared to girls) had higher concentrations of the diazinon metabolite in their urine (β = 1.32; p = 0.000 and β = 0.46; p = 0.020), respectively. For PNP, only younger age was associated with higher urinary concentrations ($\beta = -0.06$; p = 0.035).

Urinary DEAP concentrations were higher in younger children, boys, and male children consuming more fruit at school (Table 6). No significant associations were observed for DMAPs.

When we evaluated the effects of the intervention on the risk perception of children with a GEE Model, adjusting for age and sex, we observed that the educational intervention results in an increased risk perception of children over time (β = 2.68, p = 0.025). Also, the intervention results in an increased risk perception for parents ($\beta = 1.58$, $p = 0.020$), associated with older age of children ($\beta = 0.46$, $p = 0.002$); female children ($\beta = 2.35$, $p = 0.000$); school ($\beta = 1.48$, $p = 0.016$ and the closest school to farm fields ($\beta = -1.57$, $p = 0.024$).

4. Discussion

Chlorpyrifos, diazinon, and parathion, the parent pesticides for TCPy, IMPY, and PNP, are dangerous or moderately dangerous pesticides according to the World Health Organization (Matthews, 2016; World Health Organization, 2009). In this study, the concentrations of OP metabolites in the urine are relatively low in most children (Table 3) compared to previous studies conducted in Chile. Yet, we frequently detected PNP, a metabolite of parathion and other compounds.

We found that an educational intervention aimed at reducing OP exposure in rural schoolchildren can lead to a greater awareness and knowledge about potential pesticides risks, but did not lead to decreasing child OP pesticide metabolites concentrations. Thus factors beyond the control of individuals, including nearby agricultural use and residues in food, are likely the primary determinants of exposure. These results are generally consistent with other interventions developed with agricultural workers and parents of exposed children (Bradman et al., 2009; Salvatore et al., 2015). Thus, strategies to reduce community pesticide exposures should focus on regulatory changes to reduce pesticide use and encourage adoption of application methods that minimize exposures to residents and children attending nearby schools. This study is the first to include schoolchildren and their parents at the same time, demonstrating increased risk perception can occur in both adults and children exposed to pesticides.

Although the intervention was not associated with reduced OP exposure to the participating children, we did identify predictors of OP pesticide exposure such as age and sex of the children, application of OP pesticides in the home, consumption of fruits at school, and distance of households and school from farms that apply pesticides.

The greater concentrations of OP biomarkers found in San Clemente schoolchildren is possibly related to the school location in the middle of a large agricultural field and next to a farm that applies pesticides. Therefore, the children’s exposures are likely related to environmental exposure; it is noteworthy that these levels are similar to those reported by other studies that have found that the consumption of vegetables, such as apples and oranges, and living near agricultural fields are risk factors associated with the presence of OP pesticide metabolites in urine (Lu et al., 2004; Muñoz-Quezada et al, 2012, 2013).

We also found that concentrations of DEAP metabolites were associated with more fruit consumption at school, younger age, and male sex. The concentrations and detection frequencies of the metabolites of chlorpyrifos and diazinon were inversely associated with distances between schools and homes and farms that apply pesticides.

This study has several limitations. It is possible that the lack of effects of the intervention on child urinary metabolites might be partly related to the fairly short time after the intervention that we measured the metabolites (1 month). However, all of the measured biomarkers have rather short elimination half-lives (48 h), so a month of behavioral changes should be enough to be manifested in the urinary concentrations. Differences in pesticide exposures by season or consumption of different produce treated with pesticides might also overwhelm possibly smaller impacts due to the intervention. Another limitation of this study is that it reports only on the first-year evaluation of risk perception and exposure to OP pesticides in schoolchildren and their parents. We will examine impacts over two years when these data are available.

It is important to highlight information about the detection of para-nitrophenol (PNP) in children's urine. PNP is a metabolite of parathion, methyl-parathion, and nitrobenzene (Centers for Disease Control and Prevention, 2018). Parathion was banned in Chile since the year 1999 (Servicio Agrícola Ganadero, 2017). Nitrobenzene is a highly toxic chemical banned in most European countries and restricted in the USA (Agency for Toxic Substances and Disease Registry, 1990). It is used in some cases for the preparation of pesticides. Our finding that PNP concentrations in urine were significantly correlated with those of the chlorpyrifos metabolite TCPy suggests a common agricultural exposure source. Parathion is one of the more toxic OP pesticides, and repeated exposure can cause damage to the nervous system (Agency for Toxic Substances and Disease Registry, 1990; Fenske et al., 2002; Rubin et al., 2002) and cancer (Alavanja and Bonner, 2012; Calaf and Roy, 2008); nitrobenzene is an endocrine disruptor and affects reproduction (Agency for Toxic Substances and Disease Registry, 1990; UTZ,2015). If exposure is prolonged over time, the exposed population may develop diseases later in life (Singh et al., 2011).

It is noteworthy that the OP metabolite urinary concentrations presented here for both DEAPs and DMAPs are lower than those reported from a previous study with Chilean schoolchildren in 2010–2011 (Muñoz-Quezada et al., 2012). However, compared to studies in the USA, children in the present study had higher geometric means of urinary DAPs metabolite concentrations than those of CHAMACOS children (Marks et al., 2010) at 5 years (DEAPs = 7.2 nmol/L; DMP = 72.4 nmol/L), and that older children (8–15 years old) in another U.S. population (DEAPs = 11 nmol/L; DMAPs = 41.3 nmol/L) (Bouchard et al., 2010).

In general, the Chilean schoolchildren's metabolite concentrations found in this study are above the 50% of the US schoolchildren population, especially with regard to the DEP and DMP metabolites. This indicates a strong exposure related to OP pesticides such as chlorpyrifos, diazinon, azinphos methyl, fosmet, methamidophos among others, all widely used in the Maule region.

Additional research may assist in better understanding pesticide and community exposures in Chile and provide evidence to inform policies to protect public health and the environment while also supporting farmers in their efforts to grow healthy food. These efforts could involve many stakeholders including government agencies and agricultural companies using pesticides. Many study participants were concerned about pesticide use. Finally, outreach to schools and the general community about pesticide use and risk is important to increase awareness and understanding about steps that can be taken to minimize or prevent exposures. A key recommendation is that children should be prevented from being near agricultural land when pesticides are being applied, or from taking fruit from nearby agricultural plantations, because of the risk that agrotoxins may pose to their health. Another key consideration is that although training may be necessary for reducing pesticides exposure, it is not sufficient because it does not show influence on exposure or dose. The individuals and communities could receive training to use pesticides safely at work or at home, but they have no control of the environment around the agricultural fields. Therefore, training alone is not going to result in reduced pesticide exposure. The reduction in pesticide exposure and dose require measures related to stricter norms about pesticide use and surveillance in Chile. It is important to take more efficient measures in the prevention of exposure of schoolchildren placing protective barriers, prohibiting application during school hours, and banning of the most dangerous pesticides that are still in use in Chile (e.g. methamidophos, azinphos-methyl, methidathion, among others).

Also, the sale of pesticides should be restricted to the common population, and in the case of pesticides for domestic use, regulations should be developed that demand a clear warning label of their health risks, likewise tobacco packages, for example. We also recommend: 1) that applicators obtain a certificate verifying pesticide safety training for the purchase and application of pesticides. 2) Increased support for organic and other strategies to minimize the pesticide use to reduce population exposures from food. 3) That school food suppliers perform at least annual analysis of pesticide residues in fruits and vegetables delivered to the schools and certify that they are free of pesticides.

With regard to environmental exposure, and in addition to the proposals already mentioned above, the reporting, monitoring, and surveillance system must be improved. The health authorities must educate school establishments so that teachers, schoolchildren, and parents can report exposures events to relevant authorities which must respond immediately and apply regulations in a strict manner. To accomplish this, it is essential to have trained inspectors with enough staff to cover the agricultural territory in which agrotoxins are applied as completely as possible. It is also important to carry out annual surveillance of agricultural products for national consumption and from farms near schools and communities that border agricultural land.

Also there are many policy interventions used abroad that could be discussed in the local context in Chile. For instance, the creation of buffer zones where pesticide use would be restricted around schools in agricultural areas should be considered. This approach was recently adopted as a regulation in the state of California, USA (California Department of Pesticide Regulation, 2018). These new rules prohibit pesticide applications using aircraft, air blast sprayers, chemigation, dust or powder, and fumigants within approximately a quarter mile of a school or daycare when children are present, generally 6:00 a.m. to 6:00 p.m. during the school week. This regulation addresses acute pesticide exposures, providing extra safety from pesticide drift (Gunier et al., 2017). Additionally, other policies should address the regulation of overspraying, spray drift, and require notification of spraying, the restriction for the use of pesticides in certain weather conditions, and more education to farmers about application methods and responsible use of pesticides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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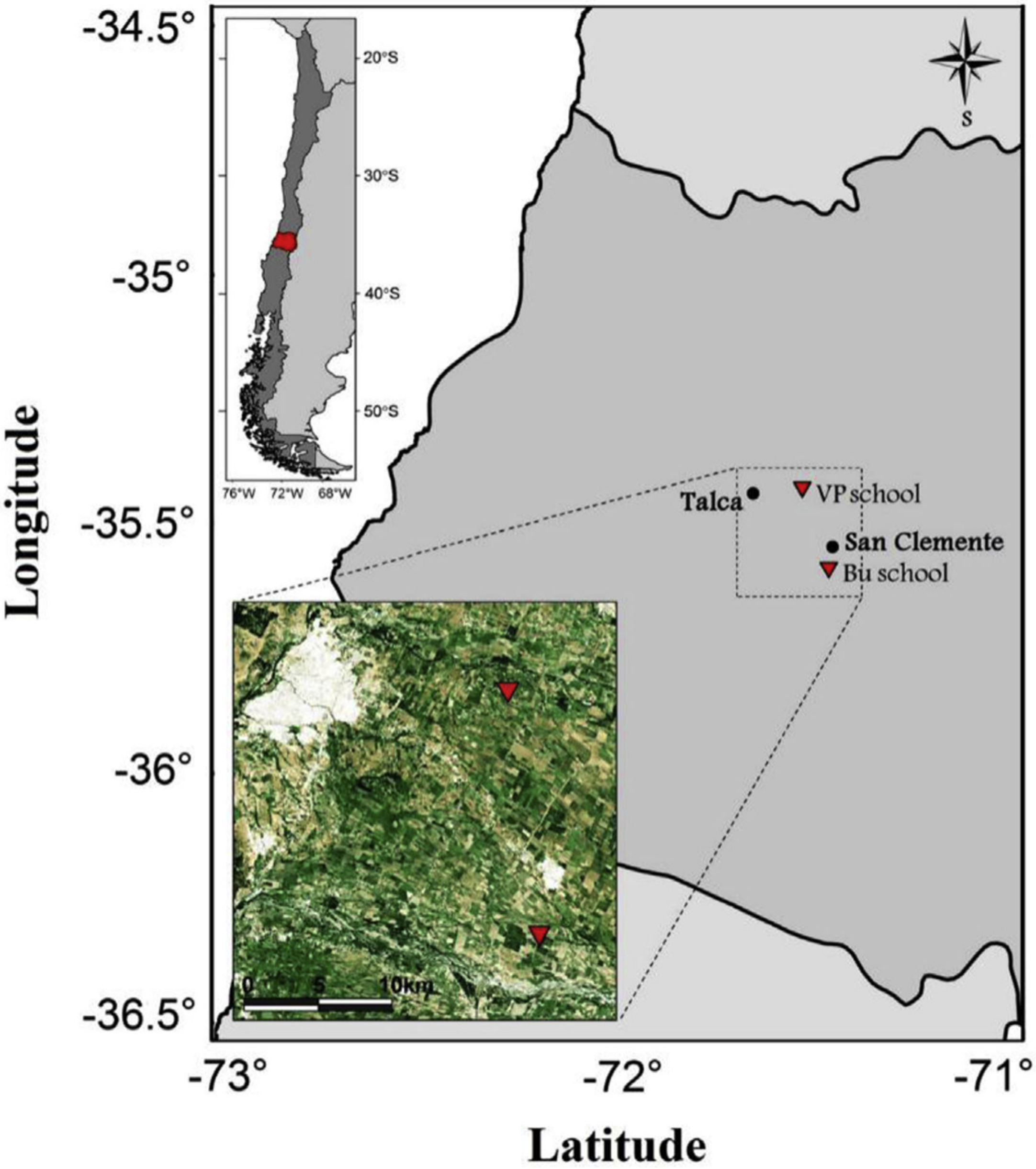

Fig. 1. Map of the study zone.

Map of the locations showing rural VP school near Talca city (regional capital) and Bu school near San Clemente (rural locality) from VI Maule Region of Chile (Inner satellite image credit: Google-TerraMetrics, 2018).

Table 1

Sociodemographic characteristics of home, children and parents from both groups (control-intervention).

	Control (n = 22) median (IQR) n (%)	**Intervention (n = 26) median (IQR) n (%)**	***p*-value (< 0.05)**
Household characteristics			
Distance to farm field			
< 200m	32(73)	38(73)	0.969[a]
≥ 200m	12(27)	14(27)	
Household monthly income *per capita (USD)*	102.12 (80.64–133.69)	92.84 (54.54–148.54)	0.2021[b]
Children			
Age	9.5 (7–11)	9.5(7–11)	0.6443[b]
Sex			
Female	6 (27%)	14 (54%)	0.0630[a]
Male	16 (73%)	12 (46%)	
Education (years)	4.5(2–6)	4(2–5)	0.6890[b]
School			
School Bu	15 (68%)	13 (50%)	0.2030[a]
School VP	7 (32%)	13 (50%)	
Parents			
Age	36(29–41)	39(31–46)	0.7664[b]
Sex			
Women	20 (91%)	24 (92%)	0.8610[a]
Men	2 (9%)	2 (8%)	
Study level parent			
< de 9 years	11(50)	12(46)	0.790[a]
≥ de 9 years	11(50)	14(54)	
Occupation parent			
Non-agricultural	18 (82%)	18 (69%)	0.3160[b]
Agricultural	4 (18%)	8 (31%)	
Partner works in agriculture			
No	4 (18%)	6 (23%)	0.6770[b]
Yes	18 (82%)	20 (77%)	

USD=U.S. dollars.

[a] Values are presented as frequencies (%). Independence χ^2 test was applied.

[b] Values are presented as median and interquartile range (IQR). Independence U Mann Whitney test was applied.

Table 2

Risk perception scores from both control and intervention groups.

	N	Mean ± SD	Median	Min – Max	*p*-value[*] Wilcoxon (Mann-Whitney)
Children					
Pre intervention					
Control	22	7.9 ± 4.2	6.5	2 – 15	0.2928
OP intervention	26	6.2 ± 2.1	7	3 – 10	
Post intervention					
Control	22	9.4 ± 3.3	10	2 – 14	0.0047 [*]
OP intervention	26	12.7 ± 3.2	12	5 – 18	
Parents					
Pre intervention					
Control	22	40.4 ± 4.5	40	30 – 49	0.9502
OP intervention	26	40.4 ± 6.1	41	26 – 50	
Post intervention					
Control	22	42.2 ± 5.1	42	29 – 50	0.0001 [**]
OP intervention	26	47.6 ± 2.7	48	41 – 52	

[*] $p < 0.005$;

[**] $p = < 0.0001$.

Table 3

Urinary concentrations of dialkylphosphates, chlorpyrifos, diazinon, and parathion metabolites in children (pre and post intervention).

	Period	Control Group (n = 22)				Intervention Group (n = 26)				
		Geometric Mean (Geometric SD)	Min-Max		% above the LOD	Geometric Mean (Geometric SD)	Min-Max		% above the LOD	*p*-value* Mann Whitney *U*
TCPy µg/L	Pre	1.53(6.36)	< LOD	10.80	88.10	0.80(2.97)	< LOD	5.76	81.63	0.0092
	Post	1.40(5.3)	,LOD	15.3	71.43	1.23(3.82)	< LOD	31.8	63.83	0.1420
IMPY µg/L	Pre	0.12(2.6)	,LOD	1.91	31.82	0.10(−1.3)	< LOD	0.234	5.77	0.0009
	Post	0.50(2.8)	,LOS	3.21	80.49	0.30(0.7)	< LOD	2.75	67.35	0.0815
PNP µg/L	Pre	1.00(2.1)	0.17	5.10	100	0.74(1.36)	< LOD	2.35	98.08	0.0183
	Post	1.27(2.4)	0.20	7.39	100	1.13(1.94)	0.18	5.49	100	0.5756
ΣDEAP nmol/L	Pre	16.46(60.14)	1.11	182.63	100	10.80(38.59)	0.86	119.29	100	0.1208
	Post	24.80(70.54)	3.78	467.94	100	29.20(98.07)	3.89	658.21	100	0.6067
ΣDMAP nmol/L	Pre	47.58(145.85)	6.18	438.3	100	22.53(79.12)	1.49	457.29	100	0.0050
	Post	69.72(265.48)	7.49	943.93	100	91.90(357.00)	3.22	1878.75	100	0.3504
Creatinine mg/dL	Pre	61.01(122.04)	9.3	162.9	100	48.49(96.05)	3.26	131.98	100	0.0776
	Post	92.50(170.09)	34.81	345.88	100	93.54(172.79)	24.34	384.11	100	0.7269
Creatinine nmol/L	Pre	539.96(917.61)	82.0	1441.9	100	429.13(722.21)	28.04	1167.96	100	0.0776
	Post	818.62(1278.92)	308.05	3060.88	100	827.86(1299.21)	215.39	3399.20	100	0.7269

TCPy = Chlorpyrifos and chlorpyrifos methyl metabolite; IMPY = Diazinon metabolite; PNP=Parathion and other compounds metabolite; ΣDEAP = sum of diethyl alkylphosphates; ΣDMAP = sum of dimethyl alkylphosphates; LOD = limit of detection (0.1 µg/L).

Table 4

Results of GEE model of the chlorpyrifos metabolite in urine before and after the intervention.

TCPy in urine (μg/L)	**β coefficient**	***p*-value**	**CI 95%**	
Age of children	−.23	.088	−.40	.03
Sex of chidren[a]	.38	.528	−.80	1.56
School[b]	1.30	.034	.10	2.50
OPs were applied at home[c]	.48	.351	−.52	1.48
Fruit consumption at school[d]	.39	.500	−.74	1.53
Intervention[e]	−.62	.309	−1.81	.57
Distance of home less than 200m from farm fields[f]	1.68	.013	.37	3.03
Creatinine mg/dL	.02	.000	.01	.03

TCPy = chlorpyrifos and chlorpyrifos-methyl metabolite.

[a] Sex: 0 = girl; 1 = Boy.

[b] School: 0 = School VP; 1 = School Bu.

[c] OPs were applied at home: 0 = No; 1 = Yes.

[d] Fruit consumption at school: 0 = No; 1 = Yes.

[e] Intervention: 0 = No; 1 = Yes.

[f] Distance of home less than 200m from farm fields: 0 = equal to or greater than 200m; 1 = Less than 200m

Table 5

Results of GEE model of the diazinon metabolite in urine dichotomized before and after the intervention.

IMPY (dichotomized)[g]	**β coefficient**	***p*-value**	**CI 95%**	
Age of children	−.19	.020	.01	.02
Sex of chidren[a]	.46	.020	−.35	−.03
School[b]	1.32	.000	.59	2.04
OPs were applied at home[c]	− .64	.068	− 1.34	.04
Fruit consumption at school[d]	−.46	.246	− 1.24	.31
Intervention[e]	−.39	.254	− 1.06	.28
Distance of home less than 200m from farm fields[f]	−.28	.462	− 1.04	.47
Creatinine mg/dL	.02	.000	.01	1.71

IMPY: Diazinon metabolite.

[a] Sex: 0 = girl; 1 = Boy.

[b] School: 0 = School VP; 1 = School Bu.

[c] OPs were applied at home: 0 = No; 1 = Yes.

[d] Fruit consumption at school: 0 = No; 1 = Yes.

[e] Intervention: 0 = No; 1 = Yes.

[f] Distance of home less than 200m from farm fields 0 = equal to or greater than 200m; 1 = Less than 200m

[g] Diazinon metabolite: 0 = No; 1 = Yes.

Table 6

Multiple linear regression model GEE of DEAP metabolites in urine before and after the intervention, adjusted by variables of interest.

ΣDEAP metabolite in urine (nmol/L)	**β coefficient**	***p*-value**	**CI 95%**	
Age of children	− 4.95	.037	− 9.60	−.31
Sex of chidren[a]	26.16	.012	5.79	46.53
School[b]	− 8.31	.431	− 29.02	12.39
OPs were applied at home[c]	16.60	.077	− 1.82	35.04
Fruit consumption at school[d]	24.01	.026	2.86	45.15
Intervention[e]	11.97	.259	− 8.76	32.51
Distance of home less than 200m from farm fields[f]	12.97	.268	− 10.00	35.95
Creatinine mg/dL	.53	.000	− 66.04	40.98

[a] Sex: 0 = girl; 1 = Boy.

[b] School: 0 = School VP; 1 = School Bu.

[c] OPs were applied at home: 0 = No; 1 = Yes.

[d] Fruit consumption at school: 0 = No; 1 = Yes.

[e] Intervention: 0 = No; 1 = Yes.

[f] Distance of home less than 200m from farm fields: 0 = equal to or greater than 200m; 1 = Less than 200m