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Source: Environmental Health Insights, 11(1)

Published By: SAGE Publishing

URL: <https://doi.org/10.1177/1178630217719269>

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# Plasma Cholinesterase Levels of Nepalese Farmers Following Exposure to Organophosphate Pesticides

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Environmental Health Insights  
Volume 11: 1–4  
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DOI: 10.1177/1178630217719269



## ABSTRACT

**BACKGROUND:** Farmers in developing countries use highly toxic organophosphate pesticides. Thus, the aim of this study was to compare plasma cholinesterase (PChE) enzyme activity before and after exposure to organophosphate pesticides in a real-life setting.

**METHODS:** This was a prospective study conducted on 25 farmers spraying organophosphate pesticide in their farm. The PChE level was measured and clinical signs and symptoms of toxicity were asked before and immediately after spraying.

**RESULTS:** The mean level of PChE before and after spraying was 1.41 and 1.29 IU/L, respectively (8.51% decreased). Farmers reported more clinical signs and symptoms of intoxication after spraying pesticides.

**CONCLUSIONS:** Increase in acute intoxication signs/symptoms and decrease in PChE indicate a serious public health problem among farmers who use organophosphate pesticides. Appropriate training to the farmers is needed to reduce exposure to organophosphate pesticide.

**KEYWORDS:** Cholinesterase, haemoglobin, farmers, organophosphate, acute intoxication, Nepal

**RECEIVED:** November 24, 2016. **ACCEPTED:** June 15, 2017.

**PEER REVIEW:** Four peer reviewers contributed to the peer review report. Reviewers' reports totalled 1467 words, excluding any confidential comments to the academic editor.

**TYPE:** Original Research

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study is supported by the Danish Society for Occupational and Environmental Health and Dialogos and partially

funded by Department of Occupational and Environmental Medicine, Odense University Hospital.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Background

In Nepal, the use of pesticides is increasing by 10% to 20% per year,<sup>1</sup> and approximately 60% of the pesticides were classified as acutely hazardous to health in 2010.<sup>2</sup> In many developing countries, including Nepal, organophosphate (OP) pesticides are widely used in farming<sup>3–8</sup> and therefore threatening the health of farmers.<sup>9</sup> Organophosphates are associated with acute health problems such as nausea, dizziness, vomiting, headaches, abdominal pain, and skin and eye problems.<sup>10</sup> The association between acute health symptoms and OP pesticides has been related to the inhibition of cholinesterase.<sup>11</sup> There are 2 types of cholinesterases (ChEs) present in the body: erythrocyte ChE (acetyl cholinesterase [AChE]), which is attached to erythrocytes and hydrolyses acetylcholine in red blood cells (RBCs), myoneuronal junctions, cholinergic nerve ends, and the central nervous system, and plasma ChE (PChE) also known as butyryl ChE, which is found in plasma and different organs of the body such as liver and muscle which hydrolyses acetylcholine and other choline esters mainly in the plasma.<sup>12</sup> Acetyl cholinesterase inhibition is considered to be a better marker of toxicity, whereas PChE inhibition is more sensitive to exposure to OP pesticides.<sup>13</sup> Plasma cholinesterase measurements have been used successfully as end points in several previous studies of OP-exposed individuals.<sup>3,12,14</sup>

Although the effect of high dose of OP pesticides on PChE level is well known,<sup>11</sup> the effect is still poorly understood among the farmers of developing countries who spray OP

pesticides in their day-to-day life. Even in the United States, Washington and California are the only states currently monitoring ChE of OP and carbamate handlers.<sup>15</sup> In the developing countries, there is no such restricted regulation and monitoring, and the ChE levels are performed only on individuals with exposure to large amounts of toxins either due to suicidal intentions or due to improper use.<sup>15</sup> The evidence on change of PChE due to occupational exposure to the pesticide is almost nonexistent in such settings. Thus, the objective of this study was to compare the mean changes in PChE level before and immediately after spraying OP pesticides in farmer's land, using their spraying equipment and own personal protective equipment (PPE).

## Methods

A prospective pre-post study was designed to conduct this study in Chitwan District of Nepal. First of all, a list of vegetable farmers who used OP pesticides was prepared. Farmers were contacted by telephone and personal visits. Farmers were questioned to determine spraying date, type of pesticide used, and time. Farmers did not spray OP pesticide for 12 hours before the collection of baseline data. On the day of spraying, we visited farmers' houses to observe their activities related to pesticide use. We observed the type of pesticide used during spraying and made sure that they have used at least 1 OP pesticide.



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Farmers were also asked to follow the same procedures that they usually did while spraying. The duration of the spraying session depended on the farmers' need to spray in the field on the given day of the visit. Each farmer sprayed as usual with their pesticides, own spraying equipment, and own protective devices.

Farmers using OP pesticides, male sex, and age 25 to 65 years without any previously known history of liver or other chronic underlying diseases. Only men were chosen because women rarely involve on pesticide spraying in the study area. Data on the number of self-reported negative health signs and symptoms, haemoglobin, and PChE levels were collected before and after the farmers' spraying session. An increase in the number of self-reported adverse health symptoms indicates immediate acute intoxication, and a decrease in PChE level indicates exposure to OP pesticide. Posing the following question to each farmer through an individual questionnaire interview identified self-reported adverse health symptoms: 'Do you have any of the following symptoms right now?' (yes or no): nausea, blurred vision, dizziness, skin allergy, excessive salivation, muscle cramp, headache, trembling hands, breathing difficulties, extreme tiredness, vomiting, abdominal pain, loss of appetite, lack of coordination, excessive sweating, difficulty in speaking, dry mouth, and backache.<sup>3</sup>

The PChE level was measured by collecting blood samples. The farmers' fingers were wiped clean with alcohol before spraying with OP pesticides to ensure that no residues remained on the skin. A total of 10 µL of blood from a finger prick on the farmers was put into a capillary tube. Each blood sample was analysed on the spot using a PChE Assay Test Kit (Model 470) at 30°C (<http://www.eqmresearch.com/>).

Statistical analyses were performed in Stata version 14. Data were presented as mean ± SD. A comparison was conducted using the Student paired *t* test. *P* < .05 was considered as statistically significant.

Farmers provided a written informed consent for their participation. Ethical approval (reg. no. 20/2012) of the study was obtained from Nepal Health Research Council.

## Results

A total of 25 male farmers participated in the study. The mean age of the farmers was 44.20 (18–63) years. On an average, they had used pesticide for 9.48 (1–19) years, and they owned a total land of 0.83 (0.168–2.02) hectares. The mean spraying time was 81 minutes ranging from 30 to 240 minutes. The number of farmers wearing personal protective devices was as follows: cap (20), long-sleeved shirts (16), trousers (12), mask (10), gloves (3), boots (2), and sunglasses (2). None of the farmers used all protective devices. All farmers used backpack sprayer.

Table 1 shows the signs/symptoms reported by farmers before and after spraying OP pesticides. Nausea, excessive salivation, and vomiting were not reported either before or after spraying. Blurred vision was reported by 2 respondents before

**Table 1.** Signs/symptoms reported by farmers before and after spraying (N=25).

| VARIABLE               | BEFORE SPRAYING (N) | AFTER SPRAYING (N) |
|------------------------|---------------------|--------------------|
| Abdominal pain         | 0                   | 1                  |
| Blurred vision         | 2                   | 8                  |
| Breathing difficulties | 1                   | 3                  |
| Difficulty in speaking | 1                   | 1                  |
| Dizziness              | 3                   | 3                  |
| Dry mouth              | 1                   | 8                  |
| Excessive salivation   | 0                   | 0                  |
| Excessive sweating     | 1                   | 8                  |
| Extreme tiredness      | 1                   | 7                  |
| Headache               | 0                   | 3                  |
| Lack of coordination   | 1                   | 1                  |
| Loss of appetite       | 0                   | 1                  |
| Muscle cramp           | 1                   | 2                  |
| Nausea                 | 0                   | 0                  |
| Others                 | 1                   | 2                  |
| Skin allergy           | 2                   | 3                  |
| Trembling hands        | 1                   | 4                  |
| Vomiting               | 0                   | 0                  |

spraying but increased to 8 after spraying. The dizziness reported by 3 farmers remained constant after spraying. Skin allergies were reported by 2 farmers and 3 farmers before and after spraying, respectively. The signs/symptoms after spraying increased more than 2-fold.

There was 8.5% decrease in PChE level and 9% decrease in haemoglobin after spraying OP pesticides. The change in haemoglobin before and after spraying OP pesticides is presented in Table 2 with their respective *P* values.

## Discussion

To our knowledge, this is the first study comparing self-reported acute intoxication symptoms and PChE level before and after spraying OP pesticide on the same day in the developing countries. This study supports the anticipation that the number of self-reported acute intoxication symptoms will increase and PChE levels will decrease among Nepali farmers who are exposed to OP pesticides after spraying on the same day.

Two points may help to improve the understanding of the study results. First, the increase in the number of self-reported adverse health symptoms may be associated with the decrease in the ChE levels. The association between human exposure to

**Table 2.** Comparison of biomarkers before and after spraying (N=25).

|                       | BEFORE SPRAYING | AFTER SPRAYING | P VALUE |
|-----------------------|-----------------|----------------|---------|
| Mean PChE (SD)        | 1.41 ± 0.59     | 1.29 ± 0.54    | .0548   |
| Mean haemoglobin (SD) | 12.59 ± 1.27    | 11.93 ± 1.39   | .0049   |

Abbreviation: PChE, plasma cholinesterase.

OP pesticides and most of its negative health symptoms has been related to the inhibition of ChE.<sup>11</sup> Second, pesticides may enter the body not only through the skin but also through the mouth or nose. Hence, not wearing adequate personal protective devices increases pesticide exposure in the body making the farmers more susceptible to being acutely intoxicated.<sup>16</sup>

The farmers' haemoglobin levels were also provided along with their PChE levels, and it is worth noticing that the haemoglobin levels decreased after OP spraying. A study from India showed that pesticide spraying men had lower RBC counts than their controls,<sup>17</sup> and Nigerian farmers' haemoglobin levels decreased after pesticide spraying in another study.<sup>18</sup> Most of the sprayers and farmers had used OP pesticides. The reduction in the farmers' haemoglobin levels is hardly understood but raises an important research question: 'Can haemoglobin levels be considered as another biomarker for farmers' exposure to OP pesticides?'

Although the decrease in PChE level was only 8.5%, this is a crucial finding. Despite short exposure duration (only about 1 hour) and involving those who have a long history of using OP pesticides, clear differences were shown on PChE level and acute intoxication sign and symptoms before and immediately after spraying OP pesticides. However, due to lack of control group, it was not possible to compare the rate of differences. Future studies are recommended to compare the rate of change of PChE level among those who spray OP pesticides compared with those who do not spray OP pesticide but are exposed to the similar working environment.

This study has several strengths and limitations. The use of paired *t* test helped to minimise the individual differences, resulting in small effects as the amount of random error is small. However, we cannot avoid the possibility of noise because of the differences in the concentration of OP pesticides, use of PPE, individual response to OP exposure, and length of spraying. The timing of the measurement being before and after spraying on the same day ensured that the immediate acute intoxication was captured. Conducting the study under the farmers' natural working conditions enabled realistic outcomes of the study. The farmers only sprayed for a short period, and seeing that OP pesticides have different levels of toxicity depending on the concentration, longer spraying time and information on concentration levels would provide a more differentiated relationship. As farmers had been using pesticides for many years, there would be a residual effect of pesticide exposure at the time of baseline methods. Farmers'

self-reported adverse health symptoms might still be a result of heavy workload and heat stress because these symptoms can be generalised to many kinds of illnesses and PChE levels cannot be a sole biomarker.

## Conclusions

Results of this study support the anticipated outcome that on the same day Nepali farmers are exposed to OP pesticides, their number of self-reported negative health symptoms will increase, and mean level of PChE will decrease. Increase in acute intoxication signs/symptoms and decrease in PChE indicate a serious public health problem. Appropriate training to the farmers is needed to reduce exposure to OP pesticide.

## Acknowledgements

The authors thank Deepak Neupane of Blood Transfusion Service Center, Nepal Red Cross Society for helping to collect and analyse blood samples. They are also thankful to Anshu Varma, Rupesh Gautam, and Shiva Raj Mishra for providing valuable comments on the draft manuscript, assisting on finalising the manuscript, and helping to make an additional analysis. This original article is based on corresponding author's master thesis report entitled 'Pesticide exposure and its health effect among farmers in Nepal' for partial fulfilment of Master of Science in public health at University of Southern Denmark, Denmark.

## Author Contributions

DN, EJ, and LPAB conceived and designed the experiments; contributed to the writing of the manuscript; agree with manuscript results and conclusions; jointly developed the structure and arguments for the paper; and made critical revisions and approved final version. DN analysed the data and wrote the first draft of the manuscript. All authors reviewed and approved the final manuscript.

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