Author's response to reviews

Title: Pesticide use, erythrocyte cholinesterase level and self-reported acute intoxication symptoms among vegetable farmers in Nepal: A Cross-sectional Study

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Author's response to reviews: see over
To,
The Editor

Dear Sir/Madam,
Referring to your letter dated August 07, 2014, we thank you for giving us this opportunity to submit our revised manuscript as well as our point-by-point responses to the reviewers comments.
We were grateful to receive the comments and suggestions from the reviewers and have tried to respond to their critiques as well as to follow their advice in the process of revising our manuscript.

Our point-by-point responses to the reviewers follow on the adjoining pages of this letter. The changes made in the manuscripts are presented in red colour.

We hope that the reviewers and you are satisfied with the manuscript in this revised version and that you will find it suitable for publication in your journal.

Yours sincerely,
Dinesh Neupane, corresponding author

On behalf of other Co-authors
Point-by-point response to the Reviewer 1 (Leslie London)

Major Compulsory Revisions

1. One might have expected to see a comparison of symptoms by ChE level. The discussion states that “… a study found that cholinesterase inhibition was associated with symptoms from the respiratory system, eyes and central nervous system among farmers [16]. Thus, the significantly lower AChE level among farmers compared to controls further suggests that the symptoms could partly be due to pesticide exposures.” However, this study had the data to test that association directly so I would expect to see the results of that testing.

The below sentence is added in findings:

Though, participants who reported at least one acute intoxication symptoms had 1.02 (95% CI: -2.06; 0.028) unit less Q level as compared to those who did not report any acute intoxication symptoms, the result was not statistically significant.

The below sentence is added in Discussion

We could not find statistically significant association between reported acute intoxication symptoms (at least one) and decreased Q level. This could be because our exposure measurement was crude, symptoms measurement was for the last month, acetyl cholinesterase level measurement was on the day of data collection and symptoms were self-reported by participants for the last month.

2. Symptoms and Table 4. It isn’t entirely clear to me the symptoms are all equivalent. For example, loss of appetite and back pain are not clearly linked to Cholinesterase inhibition. Was it intentional to include non-pesticide related symptoms as a form of controlling for under-reporting? Or do the authors believe these are pesticide related? If so, I would like to see some motivation in the methods for the choice of these symptoms. The methods are rather thin on describing the symptoms assessed and seem to borrow from an instrument used in Bolivia but it is only in the results that one gets to see what they are and some of the symptoms are rather puzzling. Backache can certainly be associated with farming, but not mediated by pesticide exposure, for example. I would rather call the title of the table ‘Odds Ratio for symptoms…’ and leave the interpretation to the discussion. Please make sure the choice of symptoms and the rationale is clear in the methods.

Response:

Back pain was removed from entire analysis and reporting. Other symptoms are adopted from WHO publication and study from Bolivia though some of them were combined/simplified in order to make questionnaire understandable to them. The following sentences are added in the text:

Posing the following questions to each farmer through an individual questionnaire interview identified self-reported symptoms: “Did you suffer from any of the following symptoms in the last month?” We also asked same question for them whether they experienced such symptoms immediately after spraying pesticides or not. The symptoms included in the questionnaire were: 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 and dry mouth. The World Health Organization has also developed a clinical representation of acute organophosphate and carbamates poisoning [23]. Some of the clinical representations were difficult for farmer’s to report by farmers and therefore such clinical representations have been translated or combined into more understandable terms.

The title of Table 4 revised as: **Odds Ratio for the self reported symptoms among farmers (n=90) as compared to controls (n=90) in the past month**

3. Could the authors elaborate on the statement “The use of dust masks, caps and long-sleeve shirts by the majority of farmers suggests an opportunity to introduce similar but more effective equipment against pesticide exposure…?” If I understand correctly, they are implying that even if dust masks are ineffective (which they are), the fact that farmers are willing to wear them might signal their willingness to adopt other, more evidence-based measures. If so, could the authors make their intention clearer? I feel this is quite an important message and perhaps something unique in this study

Response: We do also feel that this is quite an important message for intervention. The following sentence is modified and added:

The use of dust masks, caps and long-sleeve shirts by the majority of farmers means that farmers are willing to wear them might signal their willingness to adopt other, more evidence-based measures. This could be taken as an opportunity to introduce similar but more effective equipment for prevention of pesticide exposure among farmers.

4. The authors argue that 50% of symptomatic farmers seeking health care is low utilization of health care services. This is rather relative. Compared to some studies this is quite high, actually. The impression I get is that farmers in Nepal are actually quite model farmers – they use PPE much more than their colleagues in other countries and seek health care more frequently! (see for example, Lekei et al, 2014). I think this aspect should be examined with a bit more care in the discussion.

Response: We agree with reviewer with the above comment. This is relative. We compared visiting health facilities for being sick in the last month (not only for acute intoxication) between blood donors as compared to farmers. As compared to African study (Lekei), the percentage of farmers who visited health facility was high (50% vs 34%-including pharmacy). However, our purpose of questionnaire was not similar with the Tanzanian study. The below sentence is added/modified in the finding:

Out of 90, 83 farmers (97%) felt sick previous month, and 50% of them visited health care facility. whereas, out of 90 controls, 57 (63%) felt sick previous month, and 65% of them visited health care facility.

5. I am not sure I feel entirely comfortable with the conclusion as stated in the main manuscript. It is different to the conclusion as stated in the abstract (which is more modest). It is true that the study has shown “…Inadequate use of PPE, poor hygienic practices, improper handling of pesticides and the use of moderately toxic pesticides among Nepalese farmers” but I am not sure the analyses presented are sufficient to claim that these factors “…may explain lower AChE levels and increased number of acute intoxication symptoms among Nepali vegetable farmers compared to a control group.” The analyses conducted were...
on the basis of farmer versus control, not on the basis of inadequate PPE use as an association with lower AChE levels, etc. If the authors want to claim this conclusion, they have the data to test it, should do the testing and then make the relevant conclusions. There may well be power issues in the results, and then this should be discussed, but I feel the statement as made in the conclusion is not supportable without evidence.

Response:

Considering the power issue, we did not perform analysis among farmers only. The conclusion is revised as per suggestion of reviewer as follows:

Nepalese farmers exposed to pesticides have significantly more symptoms of possible pesticide intoxication than a control group of healthy individuals. A lower mean haemoglobin-adjusted AChE level was seen among farmers compared to the controls. The use of highly toxic pesticides, inadequate use of personal protective equipment and poor hygienic practices might explain the reason for higher symptoms of pesticide intoxication and a lower AChE level among farmers as compared to blood donors. In spite of many years of promoting IPM to Nepali farmers there is still an urgent need for educating farmers in improved pesticide handling techniques and IPM alternatives to protect the health of themselves and their families.

We believe the following findings from our study directly support the conclusion:

1. On the average, farmers reported 4.78 (95% CI: 4.05; 5.52) possible symptoms of acute intoxication in the previous month compared to the controls, who reported 1.58(95% CI: 1.25; 1.92) (p<0.05)
2. AChE, haemoglobin and Q levels were significantly lower among farmers as compared to the controls.
3. When classified according to WHO criteria, 50% of pesticides were classified as moderately hazardous (II); 15% as highly hazardous (Ib) and 13% as slightly hazardous (III) categories. Only 6% of pesticides were unlikely to represent any acute hazard (U).
4. On an average, farmers used about 2 PPE.
5. Of the 5 hygienic practices, farmers on the average followed 3.63 (95% CI: 3.40; 3.86).

Minor Essential Revisions

1. Table 2 has n=90 in the title line but the n implied by the %s in the table is 65. Please clarify this.
Response: Correction is made. The table was prepared based on the available pesticides stored by the farmers during the home visit. Only 64 farmers were storing pesticides at the time of data collection.

2. Selection of controls: Blood donors were chosen as having never been occupationally exposed to pesticides and they were matched for sex, age group and district to the exposed group. However, a. Blood donors in other countries may be persons of lower SES if they are paid for donation. This may introduce confounding. Could the authors clarify if this is the case? In the limitations, it is stated that donors are generally more healthy, which may be true in relation to reasons for rejecting donors. But the SES may still play a role.
Response: Selling, buying and storing blood in Nepal is strictly prohibited. The following sentence is added in the limitations: Nepal blood transfusion services are as per WHO advocacy and recommendation, which is based on voluntary non-remunerated regular blood donation, which will minimize the bias that the controls could have a lower socio-economic status.

b. Presumably, the same criteria for exclusion of exposed subjects applied to the controls? 
Response: Not applicable.

c. Age seemed different even though matched (Table 1). Was statistical testing done to confirm no significant difference? 
Response: Yes. We did the test to compare the statistical difference. We found statistically significant difference in mean age between control and farmers. So, age was adjusted in further analysis. To make it clearer, we only matched for 5-year age group. The following sentence is added in method section:

Though we matched for age group, we found statistically significant difference in mean age between control and farmers. So, age was adjusted in further analysis.

3. Table 3 is rather confusing. There are two columns for farmers’ symptom percentage. One appears to be for symptoms in the past month and the other does not have a clear label, but seems to be symptoms after pesticide use. Then, the column for controls has a heading after pesticide use. This must surely be an error and the ‘after pesticide use’ is probably the header for the second farmer column. Can you clarify in the table what is being compared to what? Farmers reporting symptoms in past month to controls reporting symptoms after pesticide use? Or Farmers compared to controls for symptoms in past month. 
Response: Title has been changed as: Table 3: Reported symptoms by farmers (in the last month and immediately after pesticide use) and controls (in the last month). The heading is slightly changed and table is modified.

4. Table 5 – the text cites the ChE and adjusted ChE values as having significant differences but the table doesn’t include the statistical testing – please include a footnote to the table to indicate significance. 
Response: Footnote is added. 
Response: A column with p-value is added in Table 5

5. The authors seem to make a lot of the Haemoglobin level differences. I would avoid making too much of this. Firstly, in my experience, the TestMate OP kit is not a very accurate device for Hb estimation, unless more recent versions have improved. Secondly, if the authors are going to suggest pesticide-related hepaticor renal damage as the reason for the Hb differences, then they have introduced a serious possibility of confounding to their ChE results. The Test mate measures Hb simply to adjust for anaemia and I don’t think it is justified or in the researchers’ interests to treat the Hb results as an outcome. I would delete this paragraph. Need to delete or may put for further explanation.

Response: Amended.

6. A few minor typos have not been corrected
a. A ‘?’ sitting after the p value in line 3 of page 9
Response: Amended

b. In the first paragraph of the discussion, a phrase (“The practice of hygienic measures”) seems to have been left over from a previous edit
Response: Amended

Discretionary Revisions

1. It would be helpful to describe what kind of sickness self-management was practiced?
Response: We do not have further information about that.

2. There were 4 professional sprayers. Presumably, they had the most intense exposure. Was that explored?
Response: Sensitivity analysis was performed by deleting 4 professional sprayers but the overall conclusion remained same.

3. Ability to read is quite a crude measure of education but it is interesting to note that it is associated with PPE usage. Did the authors also explore whether it was related to safety practices (disposal) or storage? There is more recent data (Lekei et al, BMC Public Health 2014; 14:389) suggesting that education is linked to a range of safety practices amongst farmers in Tanzania.
We did not find any statistically significant association between ability to read and correct method of disposal. Though we did not report this information this could be because of majority of messages in pesticide containers are written in Hindi, English or Chinese. Very few pesticides containers are labeled in Nepali language. The following sentence is added in finding section:

We did not find any statistically significant association between ability to read and correct method of disposal.

4. Related to education is knowledge, but a number of studies seem to indicate that for many developing countries, farmers may be aware of risks (have the knowledge) but continue to practice unsafe hygiene measures because of pressures of production and income generation. Was knowledge about risks explored in this study? If so, it would be interesting to see whether the Nepalese farmers are different to their counterparts in many other developing countries and to suggest further research direction as to why this would be the case.
Response: Risk perception was not explored on this study. This is an interesting area to explore for future.

5. The comments about suicide are true. But there is also some literature suggesting that exposure to pesticides, particularly OP’s, may increase the risk for suicide, whether through the use of pesticides as agent or through other methods.
Response: No comments.

6. The limitations:
a. In fact, if controls had undocumented exposure to pesticides, it would only bias the study to the null, so the true measure of effect would have been larger. The authors might want to make that observation.
Response: Controls were not directly exposed to pesticides. They never sprayed pesticide by themselves. So, we believe undocumented exposure to pesticide is very minimal for our control. The below sentence is added in the limitation section:

We could not totally out rule exposure to pesticides among the controls, for instance from food consumption or exposure to pesticide spray field. However, this would lead to bias toward the null.

b. Recall bias – would ‘dummy’ questions have helped to reduce the impact of recall bias?
Response: This might help to reduce the impact of recall bias. However, we cannot completely rule out possible recall bias.

Point by Point Response to Reviewer 2

Page 2
1. Five
Response: Amended

2. exposed-
Response: Not amended. We mentioned that pesticide exposed farmers in the method section.

3. exposed : Not amended. As above

Page 3
1. In the result section, you have clearly written that moderately hazardous pesticides are more in use.
Response: Not amended because both highly hazardous and moderately hazardous pesticides are highly toxic.

3. Limited
Response: Not amended. We think inadequate is ok here.

It provides employment opportunities to 66 percent of the total population and contributes about 39 percent in the GDP.
http://www.doanepal.gov.np/
Response: Amended

6. You should be very careful while citing data. First, it is better to update the recent one. The
second, citation requires at source citation. This 142 g ai/ha is very old data, given by ADB in 1987, but many papers have cited this figure. Here, a.i (active ingredient) is also missing. The write way of citation is 142 g a.i./ha of agricultural land.

Response: Amended

7. hectare
Response amended

9. Cross-Out
Response: amended

Page 4

1. measures
Response: amended

2. Recommended
Response: amended

3. Scientifically it is better to have the same individual tested for AChE at regular time interval rather than having case-control; because the control group may have different social and economic factors than the case group.
Response: We agree with reviewer that it is always important to have baseline information of the participant before exposing to the pesticides. However, in practice this would not be possible. We do also think that non-exposed control group are always important to include from public health perspective.

4. It is good to have district level consumption of pesticides.
Response: We agree with reviewer that it is good to have district level consumption of pesticides. Unfortunately, we could not find reliable source for obtaining such data-

Page 5

1. More information is warranted for the control group. What is their occupation? where they reside? how far is their residence from agricultural field? In Nepal, where agriculture is the main occupation, it is difficult to find 'control' group. Rather i would suggest pesticides applicators and non-applicators; or pesticide users and non-users. Also clear methods for selecting the occupationally exposed farmers is warranted. For the whole district, you may find many farmers engaged in the agriculture and had used pesticides within a year. So better to explore village, VDC, ward, and so on.

Response:
We did not record the information about their occupation. However, we assured that these controls were never used pesticides in their crops. Further, these blood donors were matched for sex, age group and district. In our field experience, we did not have difficulties to find control who never use pesticides and many of them were farmers. We used the term control in epidemiological sense and also mentioned that there could be some problems in the matching. We did not have any plan for stratifying analysis, so we did not analyze the data based on VDC, ward and son on. As our major objective was to see the difference between
long term direct exposure of pesticide compared to never directly exposed group, we believe our methodology does not need to change. We could not totally rule out exposure to pesticide among the controls. However, this would only lead to the bias towards the null.

As we mentioned in the selection of participants, only male farmers who owned at least 10 katthas (0.168 hectares) of land, had used pesticides within a year, were engaged in vegetables production, did not have any other profession and did not report any known conditions that could influence on erythrocyte cholinesterase levels (e.g. paroxysmal nocturnal haemoglobinuria, macrocytic anemia, microcytic anemia or use of pyridostigmine) were eligible to be participant. Blood donors who had known paroxysmal nocturnal haemoglobinuria, macrocytic anemia, microcytic anemia or use of pyridostigmine also excluded.

Page 6
1. Add Bharatpur, Chitwan
Response: Amended

2. Delete soon after obtaining the blood
Response: Amended

Page 7
1. 28 types of pesticides in stock at household is worrisome. May be he is a pesticides dealer? if yes, better to remove such outlier.
Response: No. He was a farmer. We are doing this research among commercial vegetable farmers

Page 8
1. under normal use
Response: WHO classification of pesticides is based on under normal use. So, we think it is fine without adding this sentence.

2. This is against the possible health effects of pesticide use or sun exposure? Instead of cap, head cover sounds better.
Response: Farmers replied that they used cap. Regardless of purpose of using cap, it has some protective effect. However, there are many limitations about the cap and mask used by farmers. We have discussed this in the discussion section and also highlighted that this could be an opportunity to introduce more evidence based personal protective measures. We also added detail about personal protective equipment and hygienic practices in the method section.

3. this is the average value through observation or actual practice
Response: This came through by asking farmers what they used while spraying pesticides.

Page 9
1. what are these? i did not see in your text so far!
Response: five hygienic practices are added in the method section( at sample size and
(selection of participants)

2. confusing. 96% wash hand after pesticides application. 72% wash hand after pesticides application before eating. What is the behavior difference here?
Response: These are two different information. 96% wash their hand after finishing pesticides application whereas 72% wash their hand before eating. This could also be in the middle of spraying.

3. change to whole number:
Response: Amended

4. From this paragraph, 50% sold containers, 44% threw in field, and 12% burned; this is more than 100%.
Response: This is correct. Farmers practiced more than one methods of disposal.

5. Delete /bottles/bags/boxes
Response: Not amended. We think to make clear for international audiences, it is important to keep this information.

Page 10
1. why question mark here?
Response: ? mark deleted. It was typo.

2. just remove this paragraph as the data are non-significant, and you have not shown in any Table.
Response: Though the data are not significant. We would like to keep. This information has public health importance. Reviewer 1 also highlighted this finding.

3.4. you add both groups here, but elsewhere you showed data for different groups. try to be consistent.
Response: Amended. The sentence is modified as follows:
Out of 90, 83 farmers (97%) felt sick in the previous months, and 50% of them visited health care facility. Whereas, out of 90 control, 57 (83%) felt sick in the previous months, and 65% of them visited health care facility.

5. Here, the most important is the Q, which is haemoglobin adjusted AChE. So just concentrate on it. Also it is better to explain how it is calculated in the method section. Are you sure that haemoglobin was also statistically different between two groups? interesting!
Response: Though Q is the most important; we followed EQM Manual which also reported three biomarkers. So, we will like to report both results- Yes, we found haemoglobin statistically significant between two groups. As suggested by reviewer 1 we are deleting it from this manuscript. However, we are preparing another manuscript to explore why haemoglobin is lower among farmers as compared to donors. Many earlier articles described about the detail methodology and procedure of AChE measurement via EQM Research Kit, so we are not repeating whole procedure and calculation. We have given the reference about procedure and method in the method section.

6.
Page 11

1. Is there any possibility to run correlation between OP + OC pesticide use versus AChE levels, only for farmers. If you could show the relationship, that would be wonderful.
Response: Because of sample size and power issues, we decided not to report analysis conducted among farmers only. Yes, it was a possibility to run correlation between farmers who stored OP+OC pesticides at the time of data collection versus AChE level.

2. Are you sure that these PPE are used for reducing pesticides toxicity OR these are as usual practices. The main reason for wearing cap may be to reduce sun exposure!
Response: Regardless of the purpose, materials used by farmers such as long-sleeve shirt, glass, cap, boots and mask have some kind of protective effect against pesticide exposure. That is why, we have explained that use of such devices creates an opportunity to introduce more evidence based measures.

3. Long sentence with no punctuation marks! make it clear and concise.
Response: Amended. The sentence is modified and added more as per comments from Reviewer 1.

4. these are easily available at local levels, so no need to introduce.
Response: The sentence is revised.

5. incomplete sentence
Response: the sentence is revised.

6. sentence not clear
Response: The sentence is revised as follow:
For most of the symptoms that might be related to pesticide intoxication, we see more among farmers as compared to the control.

7. observed
Response: see replaced by observed

8. This is very serious fault. In the manuscript, the data are explained as the illness/intoxication were significantly higher for farmers than control, however, the manuscript could not control, in fact, the pesticide-induced acute symptoms. Just the manuscript listed number of illness and compare between two groups. The one group is
farmers, and the another control group is not clearly defined. Response: This is not a case-control study. It is a comparative cross-sectional study. The aim of this study was to report pesticide use, erythrocyte cholinesterase level and self-reported acute intoxication symptoms among pesticide exposed vegetable farmers in Nepal. For the purpose of reporting AChE level and self-reported acute intoxication, comparing with non-expose control group give better understanding. In our study, non-exposure control groups means who never sprayed pesticides in their crops. So, we have included pesticide non-exposed blood donors matched with age group, sex and residence for making better matching. So, we do not think we had any fault on our study design. We are aware that our non-exposure control group may have exposed through other different roots. This will lead to bias towards null. We also mentioned this in our limitation section.

9. unclear Response: This means the higher symptoms among farmers .....
Response: Conclusion is modified as per suggestion of reviewer 1

Page 21
1. You can run t-test to see the group differences on this variable.
Response: Strengthening the Reporting of Observational Studies in Epidemiology (STROBE), we decided not to present p-values in Table1. The below information is provided in STROBE:
Inferential measures such as standard errors and confidence intervals should not be used to describe the variability of characteristics, and significance tests should be avoided in descriptive tables. (Ref: http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.0040297)

2. The same questionnaire were asked for both groups. I am sure that you do have data on control group regarding mean land used, involvement in vegetable farming, and pesticides use. Because most Nepalese do agriculture. Otherwise, you excluded farming population explicitly and taken care of service holders, business man, and so on. So this warrants control group better explanation in the method section.
Response: We partially agree with reviewer that selecting blood donors as a control group may have some biases. We have mentioned that in our limitation. We have similar information about demographic characteristics, sign/symptoms and AChE level for both farmers and control groups. We do not have information about land use and other information about our control. We collected blood donors data randomly and have not explicitly excluded farmers and taken care of service holders, businessman and so on.

Page 22
1. while writing active ingredients and formulation, you should be very careful on consistency. For eg. "ec" is capital, as well as small letter. Formulation is not given for all the pesticides
Response: amended.

2. unit of frequency? number of application per year or per month or per crop season or ........
Response: it is a frequency of the farmers who stored pesticide during the data collection period.

3. could not understand its value:
Response: What we found during collecting data. For example, 76 means 76% of farmers stored Cholopyriphos50% and Cypermethrin 5% EC in their home during our visit.

4. This is under Class II
Response: This has not mentioned in WHO classification(http://www.who.int/ipc/publications/pesticides_hazard_2009.pdf?ua=1) . So, we kept this as non-labeled.
1. Reformat this Table. Place p-value at the last column. Also I am uncomfortable with the last column at present. What is the difference between values between two Farmers column. Just make sure this table is well described.

Response: Table is reformatted.