Author’s response to reviews

Title: Predictors of Beta-Hexachlorocyclohexane Blood Levels among People Living Close to a Chemical Plant and an Illegal Dumping Site

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Response to reviewer

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Predictors of Beta-Hexachlorocyclohexane Blood Levels among People Living Close to a Chemical Plant and an Illegal Dumping Site

Review1.

Predictors of Beta-Hexachlorocyclohexane Blood Levels among People Living Close to a Chemical Plant and an Illegal Dumping Site The goal of this study was to evaluate the determinants of B-HCH blood levels in people living near a chemical plant in Italy. In 1990 two illegal landfills were discovered close to the site of the plant. Subsequently, milk and soil near the site were also contaminated. A surveillance program was established in 2009 to track the potentially exposed population. The present analyses describe the association between consumption of specific foods and body fatness and blood levels of B-HCH.
Major comments:

Introduction: I found this section to be very long and would suggest shortening it. Some of the information could fit in the discussion.

Thanks for the comment. The introduction has been shortened, some parts have been deleted, others have been summarized.

Methods: Some helpful details are not included in the methods.

Was there an incentive to participate in this study?

The participation was voluntary without any incentive. The population was very motivated because they were afraid of contamination and the potential effects on their health. We added this at the end of the section “population under study”

How were participants recruited and by whom?

The subjects included in the list were invited by the personnel of the Department of Prevention of Colleferro. We added this in the section “population under study”

What information is available for the ~13% of participants who did not participate?

No information are available for not responding people, so a comparison with the participants is not possible. We added this statement in the “results” section.

During what time period were the blood samples collected and how and where were they stored prior to laboratory analysis?

Blood samples were collected at the time of the interviews, so in the period 2013-2015. Blood was processed for separating serum and stored at the laboratory of the Department of Prevention in Colleferro at -20°C, until the shipment to the National Institute for Health and Welfare, Department of Health Security, in Kuopio, Finland, which conducted the β-HCH assessment. We added these statements in the β-HCH assessment section.

Are the other contaminants mentioned (lines 157-158) to be included in another similar manuscript? It's a little strange that you mentioned analysing them in the laboratory but then they are not included in this manuscript. If these contaminants are not associated with the chemical plant and dumping site you may want to remove this.

Thanks for the comment, we removed any reference to the other contaminants.

In comparison to the blood B-HCH section, the exposures information (questionnaire) lacks detail. For example, what was the time frame of reference? What instrument was used to collect information on dietary intake and what were the possible responses? Were the exposures collected so that a dose response could be evaluated or were the questions only yes/no?
Thanks for the comment, we added all these information in the questionnaire section.

Line 143-144 - What does this mean? "We also included in the program all the components of an eligible family even though they did not live any longer in the area at risk?"

As the contamination presumably started many years ago, we wanted to be inclusive of all the people potentially exposed, but no longer living in the area. Therefore, we asked all the participants if any member of their family, not included in the list because currently residing elsewhere, was willing to be included in the study. We added this clarification in the “population under study” section.

Line 189, 190 - please define these categories Were there missing data and if so, how were those data handled?

Thanks for the comment. Categories were better defined. We did not have missing data because the questionnaires were administered by doctors and nurses who managed to collect the complete information. Likewise, all the people who agreed to participate in the study gave their consent to the blood tests therefore serum bhch values were available for all of them.

Line 224-226 - The sentence about the crude association doesn't really add anything.

Thanks for the comment. The sentence was deleted.

Discussion: I'm not sure where the genetic factors idea is coming from. That wasn't evaluated in this manuscript.

As this was only a personal suggestion, we deleted all the statements on genetic factors.

Declarations section: Unless that is the style for this journal a number of these items would fit better in the methods section.

Declaration section was redacted according to the journal submission guidelines available at https://ehjournal.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research

Tables/figures: The figures could go into online supplementary material.

According to the journal submission guidelines, we uploaded figures and additional files separately.

The GMR in table 1 would fit better in table 2 because then it is easier to see what happens with the additional variables.

In table 1 we reported the association of each single variable listed with the outcome at study (bhch), separately. By contrast, in table 2 we reported the results from the stepwise selection method, therefore each GMR reflects the effect of the adjustment for the other variables. Moreover, as a result of the variable selection, table 2 includes only some of the variables listed
in table 1. To our opinion, merging two tables which are already so wide to be included as additional file, would not improve the reading of the results.

Minor comments:

Abstract line 31 - high values of B-HCH in [what] among Line 35 and other places in the text: Sacco River

Line 45 mean [blood, plasma or serum?] concentration

Line 50 associated with higher

Line 54 greater contamination among older people and those drinking and washing from private well water and consuming locally produced food

Line 63 The general population

Line 72 comes

Line 79 et al. and in other places in text

Line 83 (BMI) Then use abbreviation thereafter

Line 83 food groups

Line 108-109 Soil analysis indicated contamination was greatest near the river

Line 112 Despite the plant closure

Line 119 cook or wash and among those eating local food

Line 137 This study included

Line 155 participating in the

Line 163 the method

Line 184 after adjusting

Line 233 for drinking, cooking, washing or irrigating. First paragraph in discussion. You have used "general population" four times in a couple of sentences, suggest editing.

Line 281 mainly through the food chain
Line 283 Also body fatness seemed to play a role in [Here do you mean because the larger person likely consumes more contaminated foods thus has greater exposure or because the contaminants are stored in adipose tissue? Or both? This comment also applies to the paragraph beginning at line 308].

Line 293 In the literature

Line 307 lower dose

Line 310 body fat

Line 320 participated in the surveillance program were selected because they lived in or owned land in the area near the Sacco River; thus,

Line 322 one another. Usually

Line 331 everybody was

Line 332 the distant past (e.g., ~ how many years) Line 387 expertise

Table 3 omit the word "meat" behind Chicken, Pork, Lamb, Rabbit, Liver.

The comments were considered and amendments or specifications were done in the text.

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Reviewer 2.

This manuscript is to report factors that may influence blood beta-HCH levels among people near the Sacco river. I hope the following comments will help authors improve the manuscript.

Major comments:

1. Description of the study design: The biomonitoring programme seemed to be conducted in 2007 and include two additional phases during 2010-2012 and 2013-2015. Since authors provide little information about the study periods, the enrollment date or the sampling date in the method section, it is difficult for readers to know the data were subsampled from one of the phases of the biomonitoring programme or pooled from all the study phases. According to the results section, it seems like the authors recruited subjects in the second phase of the programme. It's confusing. Authors should provide appropriate description of the study design in the method section.

We apologize for the confusion. As you understand this study reports the results of the second phase of a surveillance program. We added more details at the end of the introduction section.
2. Precision of the analysis: In the results section, authors report the distribution of serum beta-HCH concentrations with different numbers of significant figures. What was the precision and limit of detection/quantitation of the analytical method used for this study? Authors should consider the precision and sensitivity of the analysis to determine the number of significant figures to report the data.

Significant figures were added in the results section.

The limit of detection (3 ng/g lipid given 0.5 % fat content in serum) is quite low considering the median exposure level of the population in the area (71 ng / g lipid) and the number of people with bhch \text{\textless} LOD (7 people out of 602). We believe that this has not altered the results and is negligible. Besides, for subjects with a value lower than the limit of detection, a value equal to LOD/sqrt(2) was used.

3. Statistical modelling: Given authors used multiple linear regression to assess the associations, did authors assess the linear assumption was fulfilled? The serum beta-HCH concentrations had a huge variation (range between 2.21 to 2541 ng/g), for instance, and there were many outliers in the group of age 40-49 years (Figure 2). Did these outliers influence the model performance? How about the co-linearity? Please variance inflation factors (VIF). Authors should also mention about how they selected the current model, i.e. what was the result when using the other stepwise variable selection (forward stepwise, forward-backward stepwise and so on)?

We assessed the linear assumption for linear regression. Bhch variable was skewed and therefore not normal. As a consequence, among several possible transformations, a log transformation was applied as this was the most appropriate. Please find more details in the response to question “3. Line 182: Please give a reason for why log transformation was used.” below.

Bhch is the only continuous variable considered in the analysis. As the reviewer pointed out, its distribution includes some outliers. However since that are not influential points, we did not exclude the extreme values from the analysis.

As expected as a result of a stepwise selection method, mean VIF of the model reported in Table 2 is 1.67, well below 10 which is considered, as a rule of thumb, the threshold for detecting multicollinearity.

No other variable selection methods were used. Backward selection was chosen because it starts from the full model containing all the potential predictors that were reasonably considered to influence bhch accumulation and excludes the least statistically significant.

4. Too much speculations: Authors tend to make speculations with little evidence. For example, in lines 279-282, authors considered the beta-HCH contamination in the Sacco Valley began in the far distant past because the age in the model was a significant predictor. However, authors do not take physiological differences in younger and older people into account. Older people may have lower elimination of the chemical so had the higher blood concentrations.
We tried to clarify better our statement on this

The other example is in lines 302-305. What was the reason for authors to bring up the possible inaccuracy of the well samplings?

We tried to clarify better our statement on this

Discussion about genetic influence (lines 337-338) is the same. Please provide more careful discussion with supporting evidence or literature citation.

We deleted the speculation on genetic influence because not supported by any evidence

Minor comments:

1. Lines 142-144: Provide the justification to choose the specific areas for 'control' population.

We do not understand what the reviewer means with this question. A control population was not mentioned in the document. The population at study consisted of all people residing within 1 km from the Sacco River in the municipalities near the chemical plant.

2. Line 179: Add the information about the statistical software used in this study.

Thanks for the comment. We added the reference to the statistical software used (Stata13.1) in the methods section.

3. Line 182: Please give a reason for why log transformation was used.

A transformation was needed due to the skewed distribution of the variable. Several possible transformations were considered (cubic; square; square root; log; 1/(square root); inverse; 1/square; 1/cubic) and the log transformation resulted to be the most appropriate in terms of performance and interpretability of results. Please find below a plot (command gladder in Stata13.1) representing the histograms of the transformed variables and the normal curve corresponding to mean and variance of the transformed variable.

4. Line 186-187: Provide references for each factor.

Done

5. Table 1: Since blood testing was not done for individuals below 6 years, age class 0-18 should be 6-18.
6. Figures: Provide the graphics with better resolution.

7. English can be improved with a native check.

Reviewer 3.

The paper "Predictors of Beta-Hexachlorocyclohexane Blood Levels among People Living Close to a Chemical Plant and an Illegal Dumping Site" (n. ENHE-D-19-00021) reports on a relevant case-study of human exposure to β-HCH contamination due to past local production and illegal disposal in the Sacco River Valley (Central Italy). As the other POPs, its long persistency in the environment and its high bioaccumulation capacity and toxicity makes this pollutant particularly harmful for both ecosystems and human health. Results of epidemiological surveillance programs such those presented in this study are therefore valuable. Overall, the manuscript is well-written and results were clearly described and discussed.

I suggest to check English since I found some errors/typos in the text (e.g., "comes" in line 72, "aspects" in line 133,"years old" in line 141, "variables" in line 241, "consumption of food" in line 287, "accumulation of β-HCH" in line 317) and to check the use of acronyms such as HCH, BMI, … (after the first citation in the text an acronym should be used throughout the manuscript).

Done

Comments relative to each section of the paper are reported in the following.

Background

- (lines 59-62) Despite lindane, technical-grade HCH was previously used as pesticide. The production and use of these two commercial products in Italy as well as in other countries around the world have led not only to local but also to global contamination. I suggest to mention also technical-grade HCH in your introduction and to add some references on the detection of HCHs in the environment.

Thanks for the comment. We added a statement on HCH technical grade and added some references.

- (lines 64-71) The second sentence ("Among the isomers of HCH…") sounds as a repetition of the previous one. I suggest to reorganize the text summarizing the information reported in both the sentences.
- (lines 72-78) I suggest to remove the sentence "The primary source of information..." since it does not contain relevant information and to add information on the toxicity of β-HCH (see IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 113. Lyon, 2018).
- (lines 79-91) This paragraph appears as a list of findings from different author, i.e. Devoto et al. investigated…., Brauner et al. examined… . I think that the writing of this part of the paper can be improved. - (lines 92-98) I suggest to remove the text from line 92 to line 95 ("A Ministerial Decree...") as its content is not relevant and not adequately connected with the previous part. Specify if also the HCH use has been banned (not only the production). Please, correct the subject relative to "has been included in the list of the POPs". - (lines 115-116) This sentence can be removed and the references can be moved to the previous one. - (lines 127-129) Please, specify the differences between the two phases of the program if they are relevant, otherwise remove the sentence.

All the section were amended following the suggestion of the reviewers

Methods

β-HCH assessment

I suggest to remove all the parts referred to the analysis of the other POPs since they were not considered in this study. Report just the values of difference between measured and reference concentrations, CV-%, Z, LoQ referred to β-HCH. Otherwise, the results of some other organochlorine pesticides produced and used in Italy, like DDT, may be included in the work as a comparison.

Thanks for the comment. We removed any reference to other contaminants, reporting only values for β-HCH

Discussion

- (lines 269-278) I suggest to change POPs with HCHs in the first line, since other POPs were extensively used in the mentioned time span.

Done

Overall, I suggest to increase the number of references to extend the comparison of the β-HCH levels you found with those recorded in other geographical areas and context, and to consider the possible sanitary risk associated with them.

We added a paragraph in the discussion to mention other European studies on this issue

- (lines 298-307) Even if β-HCH water concentration was decreased below the normative standard, β-HCH concentration in the biota could keep at harmful levels due to the
bioaccumulation property of this pollutant. Water monitoring is not adequate for lipophilic compounds and the normative standard could be not enough reliable and protective.

We thank the reviewer for this clarification, but the private well waters were very important in this area because they were widely used not only for irrigation but also for domestic use. We agree that water monitoring may not be an adequate protective measure for the population, however as a precautionary measure any use of water from private wells in the area was banned in 2006 and a Surveillance programme of the population was established to evaluate the burden of the contamination.

Conclusions

Please, specify "The study indicates that β-HCH contamination of general population living near a chemical plant closed in the '80 and an illegal dumping remediated in the past decade was greater...".

Done

Keywords I suggest to change some keywords, for example substituting "β-Hexachlorocyclohexane" with human exposure.

Done