Author's response to reviews

Title: Association of traffic-related hazardous air pollutants and cervical dysplasia in an urban multiethnic population: a cross-sectional study

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Author's response to reviews: see over
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Philippe Grandjean, MD, DMSc  
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Dear Dr. Grandjean,

My colleagues and I are pleased to submit our revised manuscript entitled “Association of traffic-related hazardous air pollutants and cervical dysplasia in an urban multiethnic population: a cross-sectional study” for consideration in *Environmental Health*.

We appreciate the time and effort taken by the reviewers to assist us in clarifying and strengthening the manuscript. We have addressed the reviewers’ comments and made the appropriate changes to the text of the paper. Additionally, we have provided a point-by-point response with our submission.

With best regards,

Philip Lupo, PhD

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Authors’ Response to Reviewers

Reviewer 1

Major Compulsory Revisions

**R1C1:** In the introduction please make it clear whether the authors are searching for cofactors that in addition to HPV increase the risk of cervical dysplasia/cervical cancer (even though the study design is not sufficient for this kind of analysis given the cross sectional design) or merely is studying associations between some factors and cervical dysplasia/cervical cancer. The associations could very easily be explained by an increased risk of being HPV positive, which is known to be a necessary cause for cervical cancer. E.g., when mentioning sexual behavior it is rather obvious that this is found associated with cervical cancer due to the risk of HPV (and assumable not anything else). This is crucial for the entire paper – I recommend to revise the paper with this in mind – including the abstract. The aspect of HPV cofactor is only mentioned in the last sentence in the conclusion, which is a petty, as the data material allow to restrict the study population to high-risk HPV positive women only (which will be the most appropriate definition of the study population).

Response: We agree with the reviewer and have revised the manuscript accordingly. Key revisions include a more thorough assessment of HPV status in our modeling strategy and in the text of the manuscript (including the Abstract). While we opted to include women who were HPV negative, we also restricted the analysis to HPV positive women and found our results to be similar. This has been noted in the Results section. Finally, we have included more information in the Discussion on the limitations of study design.

**R1C2:** In the method section information about how the study participants are selected is needed. Information is needed about HPV testing. Definition of cervical dysplasia (and lack of cervical dysplasia for the controls) is needed.

Response: The Methods section has been modified to include these details. In addition, information about HPV testing and our case definition for cervical dysplasia have been clarified in the Methods section.

**R1C3:** It the mother study is focusing on cervical cancer it is surprising that there is no questionnaire data about sexual behavior and cervical cancer screening history/history of Pap smears. Especially the latter is relevant for a comparison between cases and controls (table 1)

Response: Information on the risk factor questionnaire, which includes items on behavior and lifestyle factors, has been included in the text. We have also provided additional references related to the parent study in the manuscript. Finally, we have noted in the Methods section that all the subjects included in this analysis have a history of screening (i.e., pap smears).

**R1C4:** The distribution of HPV types between cases and controls (especially the unknown category) is important for the reader (could be added to table 1).

Response: We have added this information to Table 1.

**R1C5:** Please also discuss the influence of cervical cancer screening and HPV infection on the results (equally distribution between cases and controls).

Response: We agree with the reviewer and have included additional information on cervical cancer screening and HPV infection in the Methods (including supplemental analyses), Results, and Discussion sections of the manuscript.
Minor Essential Revisions

R1C6: Please be very precise when reporting results from table 2 (it is unclear why table 2 has 3 decimals and the results are only presented with 2 decimals – please be consistent)

Response: The results on the distribution of hazardous air pollutant concentration levels have been modified in the Results section to have a consistent format with that of Table 2.

R1C7: The SD in the sentence “The mean (SD) completed years of education was 13.3. (3.1) years” seems high compared to table 1. Could this be a typing mistake?

Response: The typing error has been corrected in the Results section, paragraph 1.

R1C8: It seems conflicting when writing “All subjects were infected with HPV” and the authors continue with “information on HPV status was not available for 69 subjects”. Please clarify these sentences.

Response: We have excluded those missing information on HPV infection from the analysis (please refer to the response to R1C1), and these sentences have been modified to reflect this in the Results section.

R1C9: Please be more precise in the wording when reporting continuous results.

Response: We have removed the results using the continuous variable based on the comments by Reviewer 2.

R1C10: Table 3: please add the continuous… per…

Response: The continuous results have been removed from Table 3 (refer to the response to R1C9).

R1C11: There might be a typing mistake in table 3/the results section for the 95% CI for benzene aOR high exposure.

Response: Thank you for pointing out this typing error. The results presented in Table 3 have since been updated.

R1C12: Reference 31 and 32 seem old for referencing “epidemiological evidence”.

Response: These references have been updated to include more recent publications.

Discretionary Revisions

R1C13: Instead of using the expression level of education “years of education” could be used, which will be more precise.

Response: “Level of education” has been replaced with “years of completed education” in the Statistical Analysis section, paragraph 1.
Reviewer 2

Major compulsory

**R2C1:** the inclusion criteria are not reported, the HPV status is not report in tables, but only mentioned in the text and it is not possible to understand it is referred to cases or to both cases and controls (in the first case the study is very weak, in the second it can be sound).

Response: The Abstract and the Methods sections have been modified to describe the inclusion criteria of both the cases and controls. In addition, the Methods and Results sections have been modified to clarify the distribution of HPV infection status among the cases and controls (please refer to the responses for R1C1 and R1C2).

**R2C2:** cervical intraepithelial neoplasia usually grows very slowly, furthermore the clinical significance of CIN is more relevant when the lesion is older, i.e. the probability of regression is high for new lesions and lower for long lasting lesions; this means that the lag time is relevant: women exposed for a long time should have a higher risk not only for a dose-response effect, but also because there is a substantial lag time.

Response: The reviewer brings up an important point. Because this is a cross-sectional study it is impossible to evaluate temporality as well as possible lag times between exposure and disease. This has been further discussed as a study limitation in the Discussion section.

**R2C3:** The text has several small errors in reporting, sometimes they make impossible to understand what has been observed (cancer and neoplasia are used I suspect for the same disease; the OR for continuous variables are reported without the unit increase, there is no mention of the distribution and if a transformation was useful)

Response: We have made modifications throughout the manuscript to clarify these points.

Minor essential

Abstract

**R2C4:** define the inclusion criteria of the women, please explain if all the women are HPV positive. this is the most important point of the study design: in the presence of a necessary cause, the risk factors should be clearly divided in those influencing the probability of having the necessary condition (i.e. infection) and those influencing the probability of developing the disease once the necessary cause is present.

Response: This has been clarified along with the other inclusion criteria throughout the manuscript including the Abstract (see responses to R1C1 and R1C2).

**R2C5:** The two analyses, continuous variables and for quartiles, are testing the same concept with two different formulation of the alternative hypothesis. The authors should decide which is the most appropriate and then make their conclusions according to that analysis. the alternative analysis should be presented only to corroborate the results or to confirm the hypothesis of linear trend (if the authors decided to rely on the analysis on continuous variables). My suggestion is to present only the analysis on continuous variables and to dedicate some words to explain the unit increase (without this explanation the OR is meaningless!).

Response: Based on the reviewer’s comment, we have opted to only include the categorical assessment of exposure (quartiles) to be consistent other studies evaluating associations between exposure to hazardous air pollutants and health effects.
Methods

**R2C6:** please define inclusion criteria for the trial. How is it possible that all the women (or only the cases???) were HPV 16 or 18 positive? Was this an inclusion criterion? If it is so, why there are 69 subjects with unknown HPV status? If it is not an inclusion criterion the positivity for HPV16 or 18, how is it possible that you did not find any other high risk HPV? HPV 16 or 18 are responsible of less than 50% of the infections in the general population and of about 2/3 in high-grade CIN.

Response: We have included additional information on the inclusion criteria.

**R2C7:** Please report briefly the treatment and diagnostic management.

Response: This information has been added to the Methods section.

**R2C8:** please define the endpoint (for this study the case) and the non cases: which CIN are included? CIN1, 2 and 3? The inclusion of CIN1 is critical since they are not pre-cancerous lesions. If they are included a sensitivity analysis should be added excluding CIN1.

Response: We agree that the case definition was not clearly stated previously and we have modified the Methods section accordingly. The primary outcome under study is cervical dysplasia, not cervical cancer; therefore, we included all forms of cervical dysplasia, including mild forms of dysplasia, in the case definition. In addition, we modified Table 1 to include the distribution of cervical dysplasia histologic types among the cases included in our analysis. Lastly, as the reviewer suggested, we conducted a sensitivity analysis where cases with mild dysplasia were excluded from the analysis and we found similar effects as seen in the unrestricted analysis which includes cases with mild dysplasia. A description of the results of the sensitivity analysis was added to the Results section.

**R2C9:** In my opinion the principal analyses are those stratified for HPV status (i.e. excluding the HPV unknown).

Response: A sensitivity analysis was conducted stratifying on HPV status and the results were similar. Therefore the final analyses included both HPV positive and negative subjects (excluding those with missing information HPV status) adjusting for the HPV status in the regression models (please refer to R1C1).

Results

**R2C10:** The difference in exposure between the most and the less exposed group is not so high: is it an effect of the distribution (i.e. the centile includes also very high outliers) or of the average of census tract?

Response: The exposure variables are based on the distribution of annual pollutant concentration estimates among the residential census tracts of the control subjects. There is considerable variation in pollutant concentration levels between the exposure categories defined in our analysis, which also includes levels that have previously been shown to be positively associated with adverse health risks (Sexton et al. 2007). The Discussion section was modified to include this point.

**R2C11:** please better describe the cases.

Response: The Results section has been modified to further describe the cases. Specifically we described the distribution of mild, moderate, and severe cervical dysplasia among the cases, as well as described the distribution of HPV infection status among the cases and control groups.

**R2C12:** page 9 second par: please report the unit increase for the continuous variables.
Response: The results for the continuous variables have been removed from the manuscript. Please refer to the response to R2C5.

**R2C13:** The analysis with categorical variables should be presented before to show the prerequisite of the continuous analysis, i.e., a substantially linear effect. After that, the analyses that should be considered for the test of hypothesis should be only the ones on continuous variables: the authors should decide which are the most appropriate analyses and not leave the decision to the readers.

Response: We opted to include only the categorical (quartile) analysis based on the previous literature evaluating hazardous air pollutants and adverse health outcomes.

**R2C14:** Please consider to present the analyses restricted to HPV known and just report the analyses including the HPV-unknown as a secondary analysis.

Response: The analysis has been restricted to include only those who have a known HPV status; those missing information on HPV infection were excluded from the analysis.

**R2C15:** page 9 par 2, line 9: cervical cancer or neoplasia?

Response: This typing error has been corrected in the Results section.

**R2C16:** It could be interesting to see the OR for smoking, age and ethnicity, may be in a supplementary table, just to confirm existing data and to give more reliability to the study.

Response: Per the reviewer’s suggestion, univariable logistic regression analyses were conducted to generate odds ratios to evaluate the associate between cervical dysplasia and demographic variables including age, race/ethnicity, smoking status, and completed years of education. These results were included in a Supplementary Table.

**Discussion**

**R2C17:** Again the authors should decide which is the most appropriate analysis and discuss this one, the other analysis has the meaning of sensitivity analysis (to test the robustness of the results) or to show that testing a linear relationship is a reasonable assumption.

Response: The results for the continuous variables have been removed from the manuscript. Please refer to the response to R2C5.

**R2C18:** discussion pag 10 first par: This is the first place where the authors explain what is the unit for the linear trend OR!

Response: The results for the continuous variables have been removed from the manuscript. Please refer to the response to R2C5.

**R2C19:** clearly report which kind of lesions (this is missing in the whole paper, but it should be reported also in the discussion)

Response: The Discussion section has been modified to include this information.

**R2C20:** Among the etiological hypotheses there are some including the immunological response.

Response: We have added information (and provided a reference) on the link between these pollutants and an altered immune response in the Discussion.
R2C21: limits: please discuss better the problem in assess temporality: the main limit is that the authors do not have the history of exposure.

Response: The reviewer brings up an important point. Because this is a cross-sectional study it is impossible to evaluate temporality. This has been further discussed as a study limitation in the Discussion section.

R2C22: the use of CIN2+ as surrogate outcome for cervical cancer is highly validated but should be discussed; in particular, the validation is for incidence but dealing with prevalence more assumptions are needed.

Response: The Discussion section has been updated to discuss that higher grades of CIN have a higher risk of progressing to cervical cancer.

Conclusions
R2C23: the conclusions are confusing and refer to things that are not mentioned in the introduction or in the discussion (urbanization in Latin America...)

Response: The Conclusions section was modified to briefly summarize the findings of the study.