Author’s response to reviews

Title: Integrated Exposure Assessment of Sewage Workers to Genotoxicants: an Urinary Biomarker Approach and Oxidative stress Evaluation

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Version: 2 Date: 26 January 2011

Author’s response to reviews: see over
COVER LETTER

Nablus, Palestine, January 26, 2011

Manuscript title: Integrated Exposure Assessment of Sewage Workers to Genotoxicants: an Urinary Biomarker Approach and Oxidative stress Evaluation

Manuscript ID: 1403623043484898

Editors in Chief
BMC Environmental Health

Dear Prof. Philippe Grandjean, University of Southern Denmark; and Prof. David Ozonoff, Boston University School of Public Health

It is a pleasure for us to submit this revised version of our paper entitled “Integrated Exposure Assessment of Sewage Workers to Genotoxicants: an Urinary Biomarker Approach and Oxidative stress Evaluation”.

In the cover letter (see below), we answered the reviewers’ comments as precisely as possible. To make your reading easier, we indicated our new modifications using a different font color “blue”.

We thank you for your attention, which gave us the opportunity to improve the manuscript. We hope it now meets your expectations.

With our kind regards

Hamzeh ALZABADI
Reviewer #1: Nuha El Sharif

-Major Compulsory Revisions

1- In the discussion, the authors presented the study limitations. They mentioned that sample size limited the study findings. Sample size issue: As mentioned previously (Al Zabadi et al. 2008) the authors faced a high non-response rate that decreased the study sample size to 34 underground sewage workers and a control group of 30 office workers, instead of 75 participants in each group. This non-response affected the study type 2 error (#) and decrease it to a much lower level than the authors suggested when they calculated the study sample size, i.e. # =5% and power expectation of 80%). What were the factors that affected the workers' response? How do the authors see that this non-response affects their study power and results? Do they have a description of the non-respondents?

Answer: The Parisians sewage workers are about 400. We firstly excluded the smokers, who represented 45% of the total, to leave about 200 sewage workers. We anticipated recruiting 75 workers comfortably. However, the response rate was indeed much lower than expected. Participation was voluntary. We first needed to set scheduled meetings with the workers to convince them to enrol. Several either refused due to unknown reasons (mainly due to time constraints) or were excluded from the study due to the exclusion criteria that were taken into account (e.g., absence of recent or chronic illness and of medication intake that might interfere with or study outcomes). We also think that the time shortage (very lengthy approval procedures with different local and national committees were required prior to start the inclusion process) and limited resources of the study decreased the response rate to a certain extent.

We agree that a larger sample size would have resulted in more stable results and firm conclusions. However, post hoc calculation of the study power based on this actual sample size (34 and 30 subjects in the two groups, respectively) and distribution of outcome variables, revealed that the study power expectations have not been affected too much, because contrasts between groups were greater than expected.

The explanation and results of this post hoc calculation were added at the end of the discussion section.

-Minor Essential Revisions:

1- Using the word “NOW” at the beginning of some sentences or paragraphs is not needed, such as page 3 paragraph 3 and in page 13.

Answer: This has been corrected and modified in the text accordingly.

2- Results, second line. “The mean ages… “. Authors forget mentioning that between brackets (SD) is standard deviation.

Answer: This has been corrected and modified in the text accordingly.

3- Table 4 page 26 has to be better presented, so please modify its format.

Answer: The format has been modified.
- Major Compulsory Revisions

This study is interesting, since it evaluates genotoxicity potency of urine of subjects exposed to a complex mixture such as PAHs and VOCs. The particular approach that uses comet and MN assays as biomarker of exposure is interesting. However, since MN assay is a biomarker of early effects you could define them simply urinary biomarkers.

Answer: This has been corrected and modified in the text accordingly where appropriate. However, when only genotoxicity tests where used, we maintain biomarkers of exposure, while, as said, it is one main point of the study. MN assay was used as *in vitro* assay in our study; therefore, it is considered as biomarker of exposure here.

1) Did you evaluate cell viability? How many cells remained viable after urine exposure?

Answer: Cell viability was always evaluated. Detailed information was added in the text accordingly. Please see the added text pages 6 to 8.

2) Were the slides examined for severe cytotoxicity prior to comet analysis and were clouds images (indicative of dead cells) excluded from acquisition and analysis?

Answer: Yes. Detailed information was added in the text accordingly. Please see the added text pages 6 and 7.

3) Abstract conclusions. “and suggest a DNA oxidative stress that might increase with work seniority”: This sentence is not supported by the data. There is not a statistically significant difference of mean values of 8-oxodG levels between exposed and control groups (P=0.28 is not near the statistical significance).

Answer: We agree; this sentence is now deleted.

4) Methods page 6, Comet assay: indicate the number of cells used for each exposure, specify the culture medium and how long the cells were grown before the exposure. Indicate if the medium contained also serum and if it was present during exposure.

Answer: Done. This has been indicated and specified in the text accordingly. Please see the added text page 6 and 7.

5) Results, 24hr urinary 8-oxodG: The positive association with the number of working days is not so evident, therefore, since also the difference of mean values of 8-oxodG levels between exposed and control groups is not near the statistical significance, this particular analysis could be eliminated.

Answer: We agree; this sentence is now deleted.
6) Discussion, page 10, line 3: Delete “(as biomarker of exposure)” some sentences are not adequately supported by the data. In particular “Interestingly, we found that 24hr urinary 8 oxodG was positively, although weakly ….” This sentence is not necessary. Delete also the other sentence relative to Yoshida results.

Answer: Done and deleted accordingly.

Pag 12, line 7: delete “of exposure”.

Answer: Done and deleted accordingly.

Pag 13, line 27: delete “of exposure”

Answer: Done and deleted accordingly.

Pag 14 line 10: delete all the section relative to the association of 8-oxodG levels with the number of years in the sewage system.

Answer: Done and deleted accordingly.

- Minor Essential Revisions

1) Table 1. population characteristics and exposure factors: Did you ask information about usage of protection equipment? In the Discussion (pag 13) you affirm that 32% of sewage workers declared usage of protection equipment. It would be useful to add also this data on the table.

Answer: Data added in Table 1 as recommended.

2) Table 4. association between exposures workplace toxicants and genotoxicity assays performed on HepG2 cells: It would be better to show “coefficient #” and not B. Moreover I suggest to indicate 95% CI as, for example “-0.17 to 0.23” and not “-0.17-0.23”.

Answer: Showed and indicated in Table 4 accordingly.

3) Figure 2B:
“Comet assay” and “Micronucleus assay” could be the titles of the graphs. “% DNA tail” and “MNi/1000BNed” are related to y axis.

Answer: Done and modified on the Figure 2B accordingly.

4) Figure 2B Legend:
Substitute the first sentence of this legend as: “Effects of 24h exposure to urine organic extract evaluated by comet and micronucleus assay performed on HepG2 cells. A) The bars are related to the means±SD values of % DNA tail obtained by comet assay. (B) The bars are related to means±SD values of MNi/1000 BNed obtained by Micronucleus assay (B). Indicate the number
of experiments. Why do you indicate the number of pairs of positive and negative controls and not the number of experiments?

**Answer:** Substituted and indicated accordingly.

5) Figure 3B:
Add the number of subjects, n; P value and regression equation.

**Answer:** Please note that, according to your recommendations above to delete all data relative to the association between 24h urinary 8-oxodG and number of working years in sewage system in the text, we have also deleted Figure 3B which represents the same idea.

6) Methods. Pag 5:
If you have also asked information about the usage of protection equipment, please specify it at pag 5, line 16.

**Answer:** Done and specified accordingly.

7) Pag. 6:
It is necessary to improve the description of the extraction of the urinary organic fraction.

**Answer:** Done and improved accordingly.

Add a brief description of the used cells. Improve the description of the in vitro exposure conditions.

**Answer:** Done and added accordingly.

8) Results, pag 9 line 17: “The benzene associated ……… ranged from …to ….”

**Answer:** corrected and modified accordingly.
Editorial Team Message

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

The English needs significant grammatical and language work in multiple places e.g on page 3, "..some studies suggest an increase in the incidence" instead of "and increased risk" and in the middle of the page "..through different surfaces" in stead of "different pathways". We strongly suggest asking a native speaker to help with the linguistic editing.

Answer: Corrected and edited accordingly.

On the title page, the heading Title page: and Addresses: should be deleted. The email heading should read Email addresses and at the bottom of the page there should be only the phrase Corresponding author* with the same symbol placed after the corresponding author's superscript number. Throughout the manuscript please use double line spacing and do not use italics, underlining or other fonts; only normal except where requested in the reference section. The headings in the abstract should be above the text and the colons deleted. Please limit if not completely remove the abbreviations in the abstract.

Answer: Done and modified accordingly.

In general the manuscript would also benefit overall, but especially in the Methods section, from fewer headings. General numbers 0-9 should be written out e.g. on page 5 and 10, six months and two to three subjects.

Answer: Done and corrected accordingly.

On page 6 in the third paragraph there is an open square parenthesis, but no closed. Additionally, the first table references is table 2. Tables should be numbered in the order they are referenced and only tables (and figures) that are mentioned and have significance to the findings may be included in the manuscript (material of less importance could be uploaded as additional files).

Answer: Corrected and modified accordingly.
On page 9, instead of writing out percent, the symbol is used repeatedly. The symbol should only be used when quantifying a number e.g. 5% and not in general text. Please spell out. Also, terms like minute and hour should be written out e.g. see page 10.

Answer: Done and modified accordingly.

References in sequential order of three or more should be written as [43-45], see page 12.

Answer: Done and corrected accordingly.

The List of Abbreviations should be formatted in sentence format separating the terms as they are with semi-colons, e.g. MNI: micronuclei; NDI:... Please see the Instructions for Authors for the proper working in the Competing interests section. These headings, like the ones in the abstract should appear above the text and without the colon. Remove the table grid around the reference section, list all the authors for all citations and full page numbers e.g. 211-220. In the tables, all horizontal lines should be visible.

Answer: Done and corrected accordingly.

Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript. Please also ensure that your revised manuscript in all respects conforms to the journal style (http://www.ehjournal.net/info/instructions/). It is important that your files are correctly formatted.

Answer: Corrected accordingly.