Author’s response to reviews

Title: Primary DNA Damage and Genetic Polymorphisms for CYP1A1, EPHX and GSTM1 in Workers at a Graphite Electrode Manufacturing Plant

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Author’s response to reviews: see over
Dear Prof. Zauner,

following your recent email, please find attached the revised version of our manuscript. We appreciated the reviewer's positive and constructive comments in relation to our manuscript and our responses to address the reviewer’s comments are detailed below. The paper was also revised to conform to the *BMC Public Health* style requirements.

- As per your instructions we have inserted information about the ethics approval for our study. The following sentence was added in the Methods section:

  The research protocol was approved by the Ethics Committee of the University of Perugia.

**Reviewer # 1**

1) The Referee identified previous studies of relevance, about the influence of *GSTM1* polymorphisms on PAH genotoxicity, that we originally did not cite.

- We have cited the suggested studies (Rojas et al., 2000; Pavanello et al. 2005) and we have added the following sentence in the Discussion section (page 18):

  However, it was reported that DNA damage by benzo(a)pyrene (i.e. benzo[a]pyrene diol epoxide-DNA adducts) in PAH-exposed coke oven workers is influenced by smoking habits and *GSTM1* polymorphisms [63, 64]. Thus, it could be of interest in the future, in workers exposed to high concentration of PAH, to compare damage caused by benzo(a)pyrene (such as BPDE-DNA adducts) and DNA strand breakage (as evaluated with the comet assay) also in relation to genetic polymorphisms.

2) The Referee suggested to reduce the references.

- We have reduced the references from 83 to 66.

3) The Referee suggested to reduce the number of Tables.

- We have removed Table 1 as the same information are also present in the text.
Reviewer # 2

1) The Referee suggested to modify the Title of the article.
   - In this revision, we adopted the Title proposed by the Referee:

   Primary DNA Damage and Genetic Polymorphisms for \textit{CYP1A1}, \textit{EPHX} and \textit{GSTM1} in Workers at a Graphite Electrode Manufacturing Plant

2) The Referee indicated several typing errors and proposed to change some sentences.
   - The errors were corrected and the sentences were modified as proposed by the Referee.

3) The Referee identified a study of relevance, about mortality among workers in a graphite electrode production plant in Italy, that we originally did not cite.
   - We have cited the suggested study (Merlo et al., 2004) and have modified the following sentences in the Conclusion (page 19):

   The results of a cohort study among workers in a graphite electrode production plant in Italy showed an excess of mortality for cancer in these workers with a standardised mortality ratio of 1.27 (CI95% 1.07-1.50) [66]. In conclusion, the main strategy of primary cancer prevention is minimize exposures to recognized genotoxic/carcinogenic risk factors. The outcomes of the present study, together with the results previously published by Marczynski et al. [52], show that molecular epidemiology approaches (\textit{i.e.} cross-sectional studies of genotoxicity biomarkers) can play a role in identifying common genetic risk factors, also attempting to associate the effects with measured exposure data.

4) The Referee suggested to comment 1OHP data in relation to total PAH concentration.
   - We have added the following sentence in the Results section (page 14):

   Urinary 1OHP concentrations found in the present study correlate ($r = 0.732$) with the exposure to total PAH (data not shown).

5) The Referee indicated several redundant parts in the Discussion section.
   - We have eliminated contents that simply re-stated the procedures adopted for statistical analysis. The Discussion is now more concise.

Reviewer # 3

1) The Referee requested the definition of positive control for the comet assay in the Methods section.
   - \textit{In human biomonitoring there are characteristic limitations owing to the fact that there is no possibility to include appropriate positive controls, or to independently reproduce the test. Thus the quality of comet assay results in biomonitoring studies particularly depends on standardized and controlled test conditions.}

   - We have added a paragraph to the Methods to describe the procedure used to control the experimental conditions in the comet assay:

   To control the assay conditions, particularly slides preparation procedure and electrophoresis efficiency, negative and positive internal controls (Jürkat cells, human lymphoblastoid T-cells) were processed in parallel with whole blood samples. Jürkat cells were untreated (negative control) or incubated for 1 h with 1 µg/ml 4-nitroquinoline-$N$-oxide (positive control).
Electrophoresis runs were considered valid only if the internal controls yielded the expected results.

We hope the revised manuscript could be considered worthy of publication in *BMC Public Health*.

Thanking you in advance for your attention, my colleagues and I look forward to hearing from you.

Sincerely,

Massimo Moretti