Author’s response to reviews

Title: Occupational exposure to asphalt mixture during road paving is related to increased mitochondria DNA copy number: a cross-sectional study

Authors:

Yiyi Xu (yiyi.xu@med.lu.se)

Christian Lindh (christian.lindh@med.lu.se)

Bo Jönsson (Bo_A.Jonsson@med.lu.se)

Karin Broberg (karin.broberg@ki.se)

Maria Albin (maria.albin@ki.se)

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Author’s response to reviews:

Dear reviewers,

Thank you for taking time reading the manuscript and giving valuable comments. One major comment from both reviewers was about the statistical analysis, especially the method for comparing PAH exposures in three occupational groups. In the revised version, we used linear mixed model to analyze the differences of PAH metabolites in three groups by taking consideration of pre- and post-working concentrations. The results and conclusion remained the same as in the original submission, i.e. that asphalt pavers showed higher urinary PAH metabolites than the controls. Another comment was that the conclusion was too strong considering the results of the study. We have now modified our conclusion to avoid overstatement. For further comments, see our point by point response below.

The response (with tables) is also attached as supplementary material, since all the tables are not possible to be included here.

Reviewer reports:

Reviewer #1: This is an interesting manuscript describing changes in PAH metabolites, mitochondrial DNA copy number and telomere length over a working week of two groups of asphalt pavers and a group of controls. I only realized at the end of my review that this was already a revision of an original manuscript, so apologies in advance if my comments and suggestions to not tally with those from previous reviewers.
Main comments:

1) Comments have already been made by the previous reviewer about the statistical analyses, but I would like to pick up from there. Given that this is a repeated-measures design (or a growth model with only 2 measurement points), it is unclear to me why the authors did not analyse all data with hierarchical (linear) mixed effects models, which would allow for incorporation of baseline differences between participants and changes in outcomes over time? Instead, an odd mixture of linear regression, linear regression of differences, indeed some mixed models, and non-parametric tests are used (the latter being especially confusing, given that variables were log(e)-transformed to resemble Gaussian distributions). This makes the 'Statistical analysis' section as well as the tables very difficult to follow, while the data are not necessarily optimally used.

Response: In the whole study population, PAH metabolites were measured twice (from pre- and post-working urine samples), while mtDNAcn/TL were only measured once (from post-working blood sample). Only for the 31 workers in the repeated-measures analysis (previously named as nested self-control analysis), mtDNAcn/TL were measured both from pre- and post-working blood samples. Therefore, mtDNAcn or TL in the whole study population could not be evaluated for repeated measurement, and in those cases, linear models were adopted. The reasons that we did not measure mtDNAcn/TL from pre-working samples for all subjects were: 1) mtDNAcn and TL are recognized as early response biomarkers to different exposures, however, we do not anticipate that they alter much within only 4 days of exposure; 2) the measurements among the 31 workers did not show any changes in either mtDNAcn or TL within 4 days of exposure, which was in line with our expectation. Therefore, considering the time and expense of lab analysis, we decided not to measure mtDNAcn or TL in pre-working samples.

The aim of the non-parametric tests was to compare PAH metabolites from pre- to post-working concentrations in each occupational group, however, we realize that this may not be a relevant analysis. Therefore, we changed the method to linear mixed model, and changed Table 2 in the manuscript accordingly (see below). We kept the general linear regression models to assess the associations between exposure indexes, i.e. occupational groups as categorical variables, changes in urinary PAH metabolites (from pre- to post-working) as continuous variables and outcomes (i.e. mtDNAcn/TL) among the whole study population, since only one measurement of outcomes was available.

2) To follow on from point 1, this would also enable assessment of the change over time of outcomes in the controls presented in Table 4.

Response: As mentioned above, mtDNAcn and TL were only measured once for the whole study population. Therefore, we could not assess the changes over time of mtDNAcn and TL in each occupational group. In Table 4, we assess the differences in post-working mtDNAcn/TL across three groups (Table 4 in the manuscript has been revised).
3) Given that the manuscript deals with exposure to PAHs in asphalt paving specifically, and for which smoking is an important confounder, it surprised me that references specifically looking into this were not included (for example, but not excluded to: Agostini et al. OEM 2013; 70(3): 195-202. de Vocht et al. IAOEH 2009; 82(6): 723-33. de Vocht et al. OEM 2009; 66(8): 502-8).

Response: Thank you for the suggestion. The references mentioned above aimed at better estimation of exposure-lung cancer association through assessing bias from unmeasured confounding factors, such as tobacco smoking. Their results showed that smoking history may not have noticeable effect on the risk estimates of bitumen exposure related lung cancer (Agostini et al. 2013), although the estimated median bias of unmeasured smoking history was about 13% using a Bayesian framework (de Vocht et al. 2009). In our study, smoking was not considered as the main exposure of interest. However, smoking is still considered as an important potential confounder when studying the association between exposure to PAHs in asphalt paving and effects on mtDNAcn and TL. We collected detailed information regarding smoking status and amount in the questionnaire and used such information for adjustments. The results indicated that both smoking status (never-, previous- or current-smoker) and cigarette pack-years caused risk estimates to change more than 10%. According to the criteria of inclusion of adjustment, both variables were included in the fully adjusted models.

References:


4) If I understand it correctly, the control workers worked in green area maintenance performing outdoor work. Presumably this would expose them to significant amounts of vehicle exhaust and other sources of, for example, particulates, which are also associated with oxidative stress. Would this not be a plausible explanation for the lack of observed effects. At present, table 4 indicates that baseline confidence intervals of mtDNAcn and TL basically overlap between conventional paving, CRM, and controls, but the absence of a change over time for the controls prohibits strong inferences about whether observed differences are the results of asphalt paving / PAH exposure, or simply the result of weekend vs. working next to traffic during the week.

Response: Road asphalt paving workers may have co-exposure to particulates from traffic as well as from driving paving machines. To elucidate the associations with PAHs from asphalt mixture, we chose in our study employees working in green area maintenance due to similar working characteristic as road asphalt paving: work outdoors, manual work, possible exposure to particulates, but without exposure to hot asphalt mixture. In our project, we also performed air sampling of about 50 paving workers. The results are included in another paper focusing on air
monitoring and lung function, and the paper currently is under review in OEM (Xu Y et al. submitted). Table A below presents the results excerpted from that paper, and it showed that asphalt workers were exposed to respirable dust, but at moderate levels within the range of other studies involving open area paving workers (Bulter MA et al. 2000, Norseth T et al. 1991, Hicks JB et al. 1995), and with great variations due to outdoor work. We did not perform air sampling for the control workers in this study. Therefore, we could not estimate the particle exposure for the workers and the controls. This is a limitation that we were not able to address any associations between exposures other than PAHs and mtDNAcn or TL. From the available results, we conclude that asphalt workers may experience oxidative stress evidenced by alternation in mtDNAcn; however the effects could not be fully explained by exposure to PAHs from asphalt mixture.

To clarify, Table 4 shows results for post-working mtDNAcn and TL, and baseline mtDNA/TL as this information was not available for all study participants (see comment above). There was no difference in TL between asphalt workers and controls. TL is a marker for chromosomal instability and related to risk of several chronic diseases. The absence of difference here is reasonable since our study participants are healthy, and their PAH exposure levels were not high. We found a difference in mtDNAcn between asphalt workers and controls after controlling potential confounders. MtDNA is a biomarker for oxidative stress and may be more sensitive compared with TL to environmental exposures.

To make Table 4 less confusing, we moved the median values of post-working mtDNAcn and TL to Table 1 and only listed beta estimates and p-values in Table 4 (Page 32).

References:


5) A possible refinement of the analyses which may shed some light on the above, may be to adjust for some measure of 'cumulative paving time'. Depending on the specific job, the company and/or the way days are organized, asphalt paving may be 8-9 hours straight or may involve largely waiting. Presumably some information about this is collected, and if so this would be very informative for interpretation of the results.
Response: In the questionnaire, we asked when the paving work started and ended every working day. The self-reported information showed very similar working hours (from 6-7 am to 4-6 pm) for all asphalt workers. Unfortunately, we did not have detailed information about the waiting time during the working hours. Therefore, it is not applicable to further adjust for ‘cumulative paving time’.

6) Of course, the limited sample size does not help in this respect, but I appreciate that this cannot be changed retrospectively. However, if my above comments are justified, I would suggest weakening the conclusions of the paper.

Response: We appreciate your comment and we have now modified our conclusion to avoid overstatement.

7) The models have been adjusted for 'season'; however, since controls have been measured in different months to the pavers, doesn't this screw up (for lack of a better term) the statistical models in that any differences between the groups would be attributed to the season rather than occupational factors?

Response: We compared the models of PAH exposure (Table 2 in the manuscript) and biomarkers (Table 4 in the manuscript) with and without adjustment for season. The results of this comparison are presented in Table B below. The inclusion of season changed the beta estimates of occupational groups by about 10%, but it did not change the direction of associations or significance. Moreover, the beta estimate of season was close to zero and not statistically significant.

Additionally, we performed sensitivity analysis for PAH exposure and biomarkers by excluding participants investigated during 1) early autumn and winter or 2) winter. The results are presented in Table C below. The results were largely consistent with the results for the whole study population. One exception is that for mtDNAcn, excluding subjects investigated during early autumn and winter changed the beta estimates for CRM asphalt workers (from significantly different from controls to non-significantly different from controls), but it may be due to the smaller sample size.

These sensitivity analyses provided evidence that the difference in PAH exposure and mtDNAcn across groups is due to occupation rather than season. Table C has been added as Additional Table 5 in the revised manuscript.

8) Is it possible, when hierarchical mixed effects models are used as per the above suggestion, to also nest by crew? This may provide some evidence of the impact of job-specific factors such as paving temperature?

Response: The 31 asphalt workers involved in the repeated-measured analysis paved with both conventional and CRM asphalt during different time periods. The time gaps were from one
month to three years. We used linear mixed models to investigate these 31 workers. The change in 1-OH-PYR, mtDNAcn, TL levels were similar when they paved with conventional asphalt to when they paved with CRM asphalt. The only difference was that when the workers paved with conventional asphalt they showed a higher increase in 2-OH-PH than when they paved with CRM asphalt. The results are listed in Table 3 and Additional Table 4.

9) It is very difficult to judge how strong the evidence of association really is, and whether the absence of effects is the result of a true absence or of the limited statistical power. I would suggest to accompany all analyses with 'Bayes factors', which provides some insight into the strength of these.

Response: Bayes factor is an alternative tool for hypothesis testing and model selection. We tried to calculate Bayes factor for part of our analyses, especially the ones with non-significant results. Taken the analysis of TL across three occupational groups as an example (general linear regression model in Table 4), the Bayes factor is 1.28. According to Harold Jeffreys’s evidence scale (H Jeffreys, 1961), 1.28 indicates ‘anecdotal evidence’ (barely worth mentioning) for H1 that TL is different across three occupational groups. This is similar to our results that p>0.3 for TL. However, Bayes factor could not provide evidence for judging if the absence of evidence is true or due to low statistical power. Therefore, we decided not to include it in the manuscript. Furthermore, we were aware of that the low number of CRM workers and few subjects in the repeated-measures analysis, would limit our chance of detecting subtle/minor effects. We have addressed this in the discussion (page 17).

Reference:


Minor comments:

1) I agree with the previous reviewer that 'nested self-controlled study' is not a very helpful name for the study design. I also agree it is not a case-crossover design. Can I suggest to call it a 'repeated-measures study', or something similar, which is much easier to understand then 'nested self-controlled study'.

Response: Thank you for the suggestion. We have changed 'nested self-controlled analysis' to 'repeated-measures analysis'.

2) please remove reference to health effects from the abstract, since the manuscript only deals with biomarkers of exposure and intermediate biological effects.

Response: We have changed the abstract accordingly throughout the manuscript.
3) Why are median presented in all tables, given that analyses were conducted with log(e)-transformed variables to resemble Gaussian distributions?

Response: We realize that the way of presenting the results of biomarkers of exposure is confusing. We have changed accordingly. Median is only used in Table 1, to provide a general idea of the average level in the occupational groups. In Table 2, adjusted means of log-transformed value are reported. In Table 3, adjusted means of Δ log-transformed value are reported.

4) page 14. lines 308-310. Presumably the 'contradictory results' refer to a comparison of this study with previous studies, or does this relate to differences between seasons?

Response: We found a seasonal pattern of urinary PAH metabolites indicating higher PAH exposure in the spring than in the autumn. This is contradictory from other studies that reported higher atmospheric PAHs in autumn/winter than in spring/summer.

5) page 15. line 349-351. One could infer this, but wouldn't a much more straightforward inference be that there is no association, or that the study is not powerful enough to detect differences?

Response: We have modified the sentence to “One may infer that the short-term PAHs exposure has no effect on TL within four days, or our study was not powerful enough to detect the difference. Yet we could not rule out the possibility that exposure to PAHs may affect TL as a long-term effect”.

6) page 16. line 374. 'Cumulative exposure' (from asphalt paving) is not really taken into account in this study, since exposure to PAHs from paving cannot be distinguished from other sources, while also, given that the biomarkers are lower over the weekend, this implies that the Thursday measurement does not measure all exposure over 4 days (I do not have the metabolism characteristics at hand), so I would suggest to remove or change this sentence.

Response: We have modified the sentence to: ‘The purpose was to evaluate the exposure and effect from the working week rather than for a single working day, and this could supposedly reduce the day-to-day variation.’

Reviewer #2: This study focuses on PAH exposure of asphalt workers in Sweden and tries to relate biomarkers of exposure to two early effect markers. I have several major problems with the design of the study and the statistical analyses used.
First of all, not studying the asphalt pavers and their controls at the same time is a major flaw in the design of the study. Just acknowledging that this is a problem and that it was due to practical reasons is insufficient.

I would like to suggest that the authors at least perform a sensitivity analysis excluding the controls and pavers who were studied in early autumn and winter. The distribution of the remaining workers for all three groups is very similar. Since PAH exposure from road workers will also be determined by air pollution and in particular diesel motor exhausts from nearby traffic and in case of the asphalt workers their machines it is important to control for these exposures if the aim is to see whether CRM asphalt leads to higher PAH exposure that conventional asphalt paving.

Response: During the field work, we tried our best to get as many as controls as the paving workers during the paving season, but we did not succeed. This is a limitation as the season may affect PAH exposures, mainly due to temperature and humidity. In response to the reviewer’s comment, we have now performed a sensitivity analysis for the PAH exposure and biomarkers (mtDNAcn and TL) by 1) excluding the controls and pavers who were investigated during early autumn and winter, and 2) excluding the controls and pavers investigated during the winter (see Table C below).

The sensitive analysis for PAH exposures gave rather similar beta estimates and the same level of significance. Therefore, despite the seasonal differences between pavers and controls, our conclusion that asphalt pavers had higher exposure to PAH than the controls remains. For mtDNAcn, excluding subjects investigated during early autumn and winter changed the beta estimates for CRM asphalt workers (from significantly different from controls to non-significantly different from controls), but it may due to the smaller sample size. If only excluding subjects investigated during the winter the sensitivity analysis did not change the beta estimates nor p-values. Table C has been added as Additional Table 5 in the manuscript.

In our project, we performed air sampling of about 50 paving workers. The results are presented in another paper focusing on air monitoring and lung function, and the paper currently is under review in OEM (Xu Y et al. submitted). The details of air sampling are listed in the response to third comment. Our monitoring showed that the pavers are exposed to these air pollutants at moderate levels within the range of other studies involving open area paving workers (Bulter MA et al. 2000, Norseth T et al. 1991, Hicks JB et al. 1995), and with highly varying concentrations. The limited numbers of air samples, the large variation in particulates and PAHs made it difficult to estimate the air pollutants for all workers, and we therefore did not control for these in the regression models. Moreover, in this project, we also performed asphalt emission analysis in the well-controlled emission lab without other co-exposures, and it showed that paving temperature is a determination of PAH emission in both conventional and CRM asphalt (Nilsson P et al. submitted).

References:


Second I do not understand why the statistical models used differ when comparing PAH metabolites between the groups (controls versus the two asphalt workers groups) and when comparing pre- and post-working levels. Using a non-parametric test for the latter is not needed and too coarse. A linear mixed model with pre and post-working biomonitoring values as dependent variable and a pre/post dummy as explanatory value would be better.

Response: Thank you for the suggestion. We realize that different statistical models made the 'Statistical analysis' section as well as the tables difficult to follow. In the revised version, we used linear mixed model to compare PAH metabolites across three occupational groups and changed the 'Statistical analysis' section and Table 2 in the manuscript accordingly (see below).

Third, given that apparently also air monitoring has taken place (reference 14) I do not understand why these levels were not taken into account when trying to explain the biomonitoring levels. Just using three groups is very coarse when one actually has air concentrations levels during the survey.

Response: In our project, we have performed air monitoring, but only in limited numbers of conventional and CRM asphalt workers (see Table D below, taken from another paper focusing on air monitoring and lung function, which currently is under review in OEM). We did not perform air monitoring in the controls. As shown in the table below, the exposure levels were not significantly different between conventional and CRM asphalt. Moreover, levels varied greatly, mainly due to outdoor work and large variations of asphalt mix and paving temperature. Therefore, it was difficult to estimate exposure levels for all workers based on the available observations. Thus, we were not able to address associations between air monitoring measurements and mtDNAcn or TL, and we were not able to include air monitoring data as adjustments, either. In the conclusion of the manuscript, we wrote that ‘asphalt workers may experience oxidative stress evidenced by alternation in mtDNAcn; however the effects could not be fully explained by exposure to PAHs from asphalt mixture’.
Fourth, the authors do not explicitly address the difference between conventional and CRM asphalt workers in terms of the measured biomarkers. This also holds for the conclusion and in the abstract.

Response: We performed analysis of biomarkers in 31 asphalt workers paving with conventional and CRM asphalt during different time periods with at least one month apart (the repeated-measures analysis) and results is listed in Additional Table 4. In the ‘Results’, we wrote: ‘In the repeated-measures analysis, no difference was found in either change in mtDNAcn or change in TL from pre- to post-working among the same workers paving with conventional and CRM asphalt (Additional Table 4).’ (page 12). We have added one sentence: ‘Paving with CRM asphalt did not show higher PAHs exposure or more effects on mtDNAcn and TL compared to conventional asphalt’ in the conclusion in the Abstract. (page 3)

Fifth, the discussion can be considerably shortened. The paragraph on seasonal effects lines 305-313 does not make sense given that biomonitoring levels are compared to air samples without knowing what the air concentrations were during the biomonitoring collection.

Response: Thank you for the suggestion. We have shortened the discussion and taken away the seasonal effect paragraph in the revised version.

Sixth, the discussion on the effect markers (lines 314-359 on page 14-15) is very speculative and in my opinion it makes no sense to compare coke-oven workers with asphalt (bitumen) pavers. The exposure of the latter will be at least one order of magnitude lower. I would like to suggest to shorten this section drastically and not compare asphalt pavers to coke-oven workers.

Response: MtDNAcn is a relatively new biomarker of oxidative stress. The studies about mtDNAcn and environmental PAH exposure are few and the results have been inconsistent. Coke-oven workers are also exposed to PAHs, and in the study (Pavanello S et al. 2013) they used same urinary PAH metabolites (1-OH-PYP) to evaluate the PAH exposure as we did here. Comparing results from different studies focusing on PAH is to give a picture about how the associations were and what factors may cause the inconsistency between studies. However, we realized this part of discussion was too long and we have now shortened it.

Reference:


Some more minor issues

Table 1
Please add the (unadjusted) exposure variables (1-OH-PYR and 2-OH-PH) to Table 1

Response: We have now added the pre- and post-working concentrations of 1-OH-PYR and 2-OH-PH in Table 1.

Table 2

This is very hard to interpret.

- what is the adjusted mean and how is it estimated. It is totally unclear where the adjusted mean stands for. For which age, BMI, smoking and snus status is it estimated?

- the last column should be taken out (see comment on the Wilcoxon test earlier.

Response: Table 2 has been revised accordingly. The adjusted means stand for the estimated marginal means. It is estimated when other covariates were set to their respective average.

Table 3

It is unclear what is presented in the fourth and eighth column. If it is the median difference it is very strange that in both cases the difference is larger than the both the median pre- and post-working. Was the difference not log-transformed? Why are you presenting medians in Table 3 and adjusted means in Table 2? It is very confusing.

Response: Table 3 has been revised. Only adjusted mean, beta estimates and p-values from linear mixed model are now presented.

Table 5

Why is the difference analysis restricted to the nested self-controlled analysis? Why not also for the total group?

Response: Table 5 is the combination of association analyses for all asphalt workers and the workers in the nested self-controlled analysis (called repeated-measures analysis in the revised version). We changed the table layout to make it less confusing.