PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs) and DNA damage: A pilot study among roofers in South Florida
AUTHORS	Serdar, Berrin ; Lee, David; Dou, Zihong

VERSION 1 - REVIEW

REVIEWER	Michael D. McClean, ScD Associate Professor, Department of Environmental Health Boston University School of Public Health USA I have no competing interests.
REVIEW RETURNED	09-May-2012

GENERAL COMMENTS	**General:** This paper evaluates biomarkers of PAH exposure and DNA damage in 35 urine samples collected from 18 roofers (18 before-work and 17 after-work). The main findings were that: biomarkers of PAH exposure and DNA damage increased during the work-shift; 1-OHPyr and 2-OHNap were associated with self-reported skin burns; and post-shift DNA damage was associated with glove use and post-shift 1-OHPyr. The authors acknowledge that the small sample size of this pilot study is an important limitation. An additional concern is the focus on self-reported skin burns, which is of questionable importance. It seems that about half of the population “tore off an old roof and worked with hot mix asphalt” whereas the other half “only tore off an old roof”. With one exception, those in the former category appear to be essentially the same group who self-reported skin burns. I assume the results would be about the same if the analysis focused on the 9 who worked with hot mix asphalt rather than the 8 workers who reported skin burns…but then the focus would more appropriately be on ‘working with hot mix asphalt’ rather than on burns. Also, the type of roofs being removed should be considered in the analysis since previous work has shown that this is a major predictor of PAH exposure among roofers.

	Specific: Abstract: Table 4 indicates that skin burns were a significant predictor of 2-OHNap, not 1-OHNap as stated in the abstract. Also, I think the abstract overstates the potential importance of actual skin burns. In this study, it appears that skin burns are likely just a marker for 'working with hot mix asphalt'…whereas other workers only tore off old roofs. P8L53: What types of roofs were being removed? If some were asphalt and some were not, or if some were coal tar and some were not, these are important factors that must be considered in the analysis. If the variability is not sufficient to include in analyses, the information should at least be added to the text. Previous work has shown that this is a major predictor of PAH exposure among roofers. P8L55: 8 roofers reported skin burns, but only 9 roofers worked with hot asphalt during the study day. So with the exception of 1 worker, is skin burns truly the important predictor or is it simply 'worked with hot asphalt during the study day'? P9L13: there is a lengthy discussion of adjustment for creatinine in the results section, which seems out of place. I suggest shortening and moving to methods section. Also, it is confusing that the authors seem to build the case that the WHO guidelines (ie 30-300 mg/dl) are too restrictive and note the DOT guideline of 5 mg/dl as less restrictive yet appropriate…then in the next paragraph they exclude a worker with values of 12.2 and 17.8 mg/dl. I agree these values are low – I'm just unclear about the purpose of the preceding paragraph. P9L51: I believe 'tempered' is intended to be 'tampered' P12L10: Seems possible that 1-OHPyr is correlated with other unmeasured PAHs that are actually (or also) affecting 8-OHdG. The significant of 'gloves' while controlling for 1-OHPyr would seem to support this idea. Seems like it would be appropriate to address this possibility in the discussion. P12L47: Here again, I think this is overstating the potential importance of actual skin burns. In this study, it appears that skin burns are likely just a marker for 'working with hot mix asphalt'. I

	don't believe these results provide evidence that skin burns are affecting absorption, as is speculated here. Tables (in general): suggest presenting fewer significant digits Tables 1: These analyses should be adjusted for urinary creatinine. Since creatinine was observed to be higher after shift than before shift, the difference in biomarkers could in part be explained by decreased hydration. Table 2: Since Table 1 already compares after work to before work, seems like Table 2 should focus on (1) comparing smokers to nonsmokers before work and (2) comparing smokers to nonsmokers after work. For example, what do we learn by comparing smokers after work to nonsmokers before work? Table 5: Seems like 'With skin burn' could be interpreted as 'Removed old asphalt roofs and applied new asphalt roof' whereas 'No skin burn' could be interpreted as 'Removed old asphalt roofs only'. With the exception of one worker, is this basically correct? I'm just not convinced that 'skin burn' is actually the factor that should be the focus in this table (or in the paper).

REVIEWER	Jon Sobus Physical Scientist US EPA USA I declare that I have no competing interests
REVIEW RETURNED	07-May-2012

THE STUDY	Supplemental documents do not contain information that would be better reported in the manuscript, or raise questions about the work.
GENERAL COMMENTS	General comments: This is a well-written paper that discusses the results of a pilot study of PAH exposures and DNA adduct damage among roof pavers in South Florida. The limitations of the study are clear (i.e., no exposure data and few numbers of subjects/samples) but the authors did a nice job of discussing the implications of these limitations, as well as plans for follow up studies/analyses. Specific comments: Page 2, line 20: you state that the OH-PAHs were measured via LC-MS/MS and that creatinine was measured using HPLC. The text in

	the methods section suggests that both OH-PAHs and creatinine were measured using LC-MS/MS. Perhaps update the text in the abstract to more accurately reflect the methodologies. Page 5, lines 51-53: Given that half the workers were observed in winter months, and the other half in a summer month, did you consider testing for a seasonal effect on OH-PAHS and 8-OHdG? I'm thinking it's possible that the ambient temperature would affect working conditions and potentially worker contact with hot asphalt or other PAH-laden materials. Page 6, line 35: You mention the use of "validated liquid chromatography/tandem mass spectrometry methods." Are you referring to methods that you validated in your lab for this study, or previously published methods? If the latter, please add references. Page 7, line 56: Here you give the LOQ for creatinine in methanol. It would be helpful to also give the estimated LOQ for urinary creatinine in units of mg/dL; this would provide consistency with the values presented in the results section. Page 9, line 47: The two creatinine measurements for this subject are within the acceptable range provided by DoT. You justify the use of the DoT guidance range over the WHO guidance range in the preceding paragraph. Thus, is there some other reason that the samples were not included in your analysis? Page 10, line 35: Is this observed 4.34-fold increase consistent with other studies? In other words, is this an expected response given the potential exposure levels and the time-frame of observation (6 hrs)? Page 14, line 38: According to Table 3, this doesn't appear to be true. It seems as though 1-OHNap and 9-OHPhe were each correlated with 8-OHdG. Tables 1 and 2: The statistical tests used for these tables were not described in the methods section. Please add a brief description with some mention of the cautions of multiple testing given small sample size. Table 3: Please mention in the methods section the statistical test used to generate these coefficients and p-values. Consider mentioning the effect of small sample size (less than 10 when stratified by pre- and post-shift samples) on the p-value estimation. Do you anticipate a lack of statistical power that would affect you interpretation of the results? Is it reasonable to assume a lognormal distribution given that n<10 for each test? Table 6 gives a very nice summary of literature values of 1-OHPyr. A similar table of 8-OHdG values would be informative.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

Page 2, line 20: you state that the OH-PAHs were measured via LC-MS/MS and that creatinine was measured using HPLC. The text in the methods section suggests that both OH-PAHs and creatinine were measured using LC-MS/MS. Perhaps update the text in the abstract to more accurately reflect

the methodologies.
Response: The text has been updated.
Page 5, lines 51-53: Given that half the workers were observed in winter months, and the other half in a summer month, did you consider testing for a seasonal effect on OH-PAHS and 8-OHdG? I'm thinking it's possible that the ambient temperature would affect working conditions and potentially worker contact with hot asphalt or other PAH-laden materials.
Response: We did look at possible seasonal effects in our linear regression analyses. But season was not a significant predictor of any of the urinary analytes. While sampling was done at different months average temperature on study days was similar with a range between 22-29C (including December days).
Page 6, line 35: You mention the use of "validated liquid chromatography/tandem mass spectrometry methods." Are you referring to methods that you validated in your lab for this study, or previously published methods? If the latter, please add references.
Response: The method was validated at Pharmaon laboratories. We modified the text to include more detailed information on validation parameters.
Page 7, line 56: Here you give the LOQ for creatinine in methanol. It would be helpful to also give the estimated LOQ for urinary creatinine in units of mg/dL; this would provide consistency with the values presented in the results section.
Response: Thank you for pointing this out. We modified the text to clarify creatinine LOQ.
Page 9, line 47: The two creatinine measurements for this subject are within the acceptable range provided by DoT. You justify the use of the DoT guidance range over the WHO guidance range in the preceding paragraph. Thus, is there some other reason that the samples were not included in your analysis?
Response: The main reason for excluding these two samples were based on notes taken by the field study team suggesting that the participant may have added water to the samples before providing the sample to the investigators.

Page 10, line 35: Is this observed 4.34-fold increase consistent with other studies? In other words, is this an expected response given the potential exposure levels and the time-frame of observation (6 hrs)?
Response: 8-hydroxydeoxy guanosine is a widely accepted marker of oxidative DNA damage, however there are still some uncertainties regarding its use in population based studies. Several studies have linked increased levels of urinary 8-OHdG to PAH exposures, but a dose-response relationship is still not well established. It is also important to note that 8-OHdG is a measure of total oxidative DNA damage (and of DNA repair). There have been controversial results regarding 8-OHdG levels in urine and PAH exposures. It is possible that there may be other factors creating oxidative damage that were not accounted for in this study. However, due to the lack of sufficient studies comparing urinary 8-OHdG before and after exposure to PAHs, we cannot say that our results are typical or expected for this population. To provide an overview of some observed values, we added Table 7 and a new section in the discussion. In a study among workers exposed to gasoline, highest levels of urinary 8-OHdG were seen in the late evening hours, with a slight decrease the next morning suggesting a rapid elimination within 24 h (Nilsson et al 1996). Thus, we believe that our timing of sample collection is acceptable.
Page 14, line 38: According to Table 3, this doesn't appear to be true. It seems as though 1-OHNap and 9-OHPhe were each correlated with 8-OHdG.
Thank you for pointing this error. We corrected this statement in the text.

Tables 1 and 2: The statistical tests used for these tables were not described in the methods section. Please add a brief description with some mention of the cautions of multiple testing given small sample size.
Thank you for catching this. We modified the text to describe all statistical methods used for our analyses and also the potential influence of single observations may have on the results due to small

sample size.

Table 3: Please mention in the methods section the statistical test used to generate these coefficients and p-values. Consider mentioning the effect of small sample size (less than 10 when stratified by pre- and post-shift samples) on the p-value estimation. Do you anticipate a lack of statistical power that would affect you interpretation of the results? Is it reasonable to assume a lognormal distribution given that n<10 for each test?
We modified the text to present statistical methods. Our results with the nonparametric tests were very similar to those obtained using parametric tests (we have also added text describing these). We agree that there is a great concern for biased estimates if the sample size is small. Biomarker data in this pilot study were evaluated for normality using the Shapiro-Wilks test. Our analyses revealed that urinary measurements of PAH metabolites and 8-OHdG fit a normal distribution after the natural logarithmic transformation.

Table 6 gives a very nice summary of literature values of 1-OHPyr. A similar table of 8-OHdG values would be informative.
Table 7 was added to present literature values of 8-OHdG.

Reviewer 2:

The authors acknowledge that the small sample size of this pilot study is an important limitation. An additional concern is the focus on self-reported skin burns, which is of questionable importance. It seems that about half of the population “tore off an old roof and worked with hot mix asphalt” whereas the other half “only tore off an old roof”. With one exception, those in the former category appear to be essentially the same group who self-reported skin burns. I assume the results would be about the same if the analysis focused on the 9 who worked with hot mix asphalt rather than the 8 workers who reported skin burns…but then the focus would more appropriately be on ‘working with hot mix asphalt’ rather than on burns. Also, the type of roofs being removed should be considered in the analysis since previous work has shown that this is a major predictor of PAH exposure among roofers.

Response: This is an important point and we realize that we did not provide sufficient clarification in the manuscript. Among the 19 roofers included in this pilot study, 9 reported having skin burn due to contact with hot asphalt (this was incorrectly written as 8 before). Among those 9 roofers, 5 reported working with hot asphalt that day - but 4 roofers reported not working with hot asphalt. Regardless of their work with hot asphalt, none of the workers reported injury or skin burn occurrence on the study day. Our findings on skin burn may reflect increased exposures to PAHs during hot asphalt work, however, 'work with hot asphalt' was not a significant predictor in any of the regression models. Thus, it is possible that skin burn here reflects an increased absorption through injured skin. Alternatively, skin burn could also be a cumulative marker of exposure through work with hot asphalt, with the assumption that incidence of skin burn is correlated with number of hours of hot asphalt work.
Almost all roofers reported removing roofing and 11 reported applying new roofing. We agree that the type of the roof removed might have significant effects on the results. Unfortunately, all of the roofers reported removing roof that contained coal tar. Thus, we were not able to investigate the contribution of coal tar roofing.
We added a new section under discussion to clarify these points.

Specific:
Abstract: Table 4 indicates that skin burns were a significant predictor of 2-OHNap, not 1-OHNap as stated in the abstract. Also, I think the abstract overstates the potential importance of actual skin burns. In this study, it appears that skin burns are likely just a marker for ‘working with hot mix asphalt’…whereas other workers only tore off old roofs.

Response: The abstract has been modified.

P8L53: What types of roofs were being removed? If some were asphalt and some were not, or if some were coal tar and some were not, these are important factors that must be considered in the analysis. If the variability is not sufficient to include in analyses, the information should at least be added to the text. Previous work has shown that this is a major predictor of PAH exposure among roofers.

Response: All roofers reported removing roofs that contained coal tar. This has been added to the text.

P8L55: 8 roofers reported skin burns, but only 9 roofers worked with hot asphalt during the study day. So with the exception of 1 worker, is skin burns truly the important predictor or is it simply 'worked with hot asphalt during the study day'?

Response: Please see our response above. We regret a typo in the text, since 9 roofers had skin burns. However, 4 of these 9 roofers did not work with hot asphalt on the study day. Work with hot asphalt during the study day was not a significant predictor in any of the models.

P9L13: there is a lengthy discussion of adjustment for creatinine in the results section, which seems out of place. I suggest shortening and moving to methods section. Also, it is confusing that the authors seem to build the case that the WHO guidelines (ie 30-300 mg/dl) are too restrictive and note the DOT guideline of 5 mg/dl as less restrictive yet appropriate…then in the next paragraph they exclude a worker with values of 12.2 and 17.8 mg/dl. I agree these values are low – I'm just unclear about the purpose of the preceding paragraph.

Response: The main reason for excluding these two samples were based on concerns raised by the field study team suggesting that the participant may have added water to the samples before providing the sample to the investigators. We revised the text and moved to the methods section.

P9L51: I believe 'tempered' is intended to be 'tampered'

Response: The text has been modified.

P12L10: Seems possible that 1-OHPyr is correlated with other unmeasured PAHs that are actually (or also) affecting 8-OHdG. The significant of 'gloves' while controlling for 1-OHPyr would seem to support this idea. Seems like it would be appropriate to address this possibility in the discussion.

Response: Thank you for pointing this out. We have added this to the discussion.

P12L47: Here again, I think this is overstating the potential importance of actual skin burns. In this study, it appears that skin burns are likely just a marker for 'working with hot mix asphalt'. I don't believe these results provide evidence that skin burns are affecting absorption, as is speculated here.

Response: We modified the text.

Tables (in general): suggest presenting fewer significant digits

Response: We modified our tables presenting biomarker levels.

Tables 1: These analyses should be adjusted for urinary creatinine. Since creatinine was observed to be higher after shift than before shift, the difference in biomarkers could in part be explained by

decreased hydration.

Response: Creatinine itself has some variation due to individual differences. In this population, we observed that race and BMI significantly altered urinary creatinine levels (added to the text) with African Americans having higher creatinine levels. Thus, routine creatinine adjustment for these tables would introduce error into our analyses. The contribution of creatinine on urinary levels was investigated as an independent variable in linear regression models (evaluated along with other potential variables such as race and BMI).

Table 2: Since Table 1 already compares after work to before work, seems like Table 2 should focus on (1) comparing smokers to nonsmokers before work and (2) comparing smokers to nonsmokers after work. For example, what do we learn by comparing smokers after work to nonsmokers before work?

Response: Table 2 provides a stratified view of the effects of occupational exposure in relation to cigarette smoking. We can see that smokers have high values of PAH biomarkers before the work, sometimes even exceeding those of nonsmokers after work. This table presents the contribution of smoking on biomarker levels in comparison to work.

Table 5: Seems like 'With skin burn' could be interpreted as 'Removed old asphalt roofs and applied new asphalt roof' whereas 'No skin burn' could be interpreted as 'Removed old asphalt roofs only'. With the exception of one worker, is this basically correct? I'm just not convinced that 'skin burn' is actually the factor that should be the focus in this table (or in the paper).
Response: Among those workers who had skin burn, 3 did not apply new roof. And among those who did not have skin burn, 7 did apply new roofing.

VERSION 2 – REVIEW

REVIEWER	Jon Sobus Physical Scientist US EPA USA No competing interests
REVIEW RETURNED	22-Jun-2012 - The reviewer completed the checklist but made no further comments.