

Author's response to reviews

Title: PM2.5 metal exposures and night heart rate variability: A panel study of boilermaker construction workers

Authors:

Jennifer M Cavallari (jcavalla@hsph.harvard.edu)

Ellen A Eisen (eeisen@hsph.harvard.edu)

Shona C Fang (sfang@hsph.harvard.edu)

Joel Schwartz (jschwartz@hsph.harvard.edu)

Russ Hauser (rhauser@hsph.harvard.edu)

Robert F Herrick (herrick@hsph.harvard.edu)

David C Christiani (dchristi@hsph.harvard.edu)

Version: 2 **Date:** 1 May 2008

Author's response to reviews: see over

April 30, 2008

Philippe Grandjean
David Ozonoff
Editors-in-Chief, *Environmental Health*

Re: MS #6489178671766549

Dear Drs. Grandjean and Ozonoff:

Attached please find the revised manuscript “PM_{2.5} metal exposures and night heart rate variability: A panel study of boilermaker construction workers.” We are grateful for the thoughtful review of our paper and insightful comments. We have carefully considered each suggestion and have incorporated many of them into the revised manuscript.

We have provided a summary of the overall changes to the manuscript as well as point-by-point response to each reviewer's comments. One of the main concerns addressed by two reviewers was the conclusions reached for Vanadium, which had a large a number of samples below the limit of detection and very wide confidence intervals in the exposure-response models. After considering both reviewers suggestions, we removed the Vanadium analysis from the manuscript.

In addition, the editors also requested clarification on how this paper differs from previous investigations among this group of boilermakers. We have clarified the introduction and conclusion to better differentiate the current analysis from our previous work. In brief, the current paper focuses on night HRV as a consequence of workday exposures to metal components of PM_{2.5} exposure. While we previously examined HRV and PM_{2.5} metal exposures, the analysis focused on daytime HRV and found contrary results with linear, rather than inverse, associations between metals and HRV (Magari et. al. 2002). Recently, we identified that nocturnal HRV may best capture the time course of the effects of workday exposures as quantified by total PM_{2.5} mass (Cavallari et al. 2007). The current investigation focuses on the metal components of PM_{2.5} to determine what role composition plays in the cardiovascular response. Unlike our previous investigation of PM_{2.5} metals and HRV, this investigation used night HRV, which may more appropriately account for the time course of cardiovascular responses to metals.

Thank you for considering our article for *Environmental Health*.

Sincerely,

Jennifer Cavallari, ScD, CIH

Reviewer #1:

Review:

The paper poses a question of current interest to the occupational and environmental health communities, i.e., effects of PM constituents on HRV and yields results that may be useful to others concerned about welding fumes in the occupational health community. The relevance to those concerned about the effects of PM constituents in the ambient air is severely limited by the issue of extrapolation to much lower concentrations. Also, as acknowledged by the authors at the top of Page 14, by the chemical form and solubilities of the metals. The metals released by welding are likely to be present as oxides, while those resulting from fossil fuel combustion or smelting are more likely to be sulfates, and therefore more bioavailable. The authors likely have sulfur data from their XRF analyses, and way wish to add some discussion on this issue.

Response: We agree with the reviewer that the high concentrations and unique composition of welding fume exposures may limit the generalizability of our results to air pollution studies. In addition, we acknowledge that while we did not determine the chemical form and solubility of the metal constituents, the welding fume exposures are most likely present as oxides. Within this cohort, the workers were exposed solely to welding fume exposures and were not exposed to fossil fuel combustion materials which may be present as sulfates. Upon the author's suggestion, we investigated the sulfur data from the XRF analysis and found no associations with sulfur content and HRV. We have decided not to include this analysis in the paper, as our original intent was to focus on metal content. We have updated the Discussion section of the manuscript with additional comments on the generalizability of the results and the welding fume composition.

“Therefore, the generalizability of the current study results to ambient air pollution exposures is limited.”

“While the welding fume metals are likely present as metal oxides, the solubility of each element, which may affect toxicity, remains unknown.”

I had no problems with regard to the methods, data soundness, method of presentation, other aspects of the Discussion, and Conclusions. The Title, and Abstract were fine. I found one typo. On Table 5, the Model 4 Heading should have a beta rather than a capital B.

Response: We have corrected the heading title to a beta.

Reviewer #2:

--Major Compulsory Revisions

1. p5 - methods ' subjects' one subject seem to have contributed 3x to data (and another 2x), disproportionate to other subjects. Though his/her exposure was presumably different on each occasion, it seems that his genetics/other individual characteristics are now disproportionately presented in the resultant data. Seems that he/she should only contribute once to data.

Response: We agree with the reviewer that workers who participated on more than one occasion should not contribute disproportionately to the analysis. Therefore we used mixed-effects regression models to account for multiple participation by some individuals. We describe this in the statistical methods section, and have added an additional sentence to clarify our methods.

“To investigate the association between PM_{2.5} metal exposures and night HRV, we used linear mixed-effects regression models with random intercept and unstructured covariance. The mixed-effects models allowed us to account for the correlated outcomes among workers who participated on multiple occasions.”

2. Seems that the “night susceptibility” concept should be better justified. Seems that main basis for it is one study via same research group and same cohort. (or at least, why not look also at daytime HRV to validate this concept?)

Response: As the reviewer suggested, our concept of night susceptibility is based on results from the one study from our previous research group. In our previous investigation (Cavallari et al. 2007), we found that as compared to day, there were larger particle-related declines in night HRV. As for investigating the PM metal component, daytime HRV and workday metal exposures were previously investigated (Magari et al. 2002). Therefore, we decided to focus solely on night HRV within this analysis. We have changed the language within the manuscript to better reflect how the current investigation fits with our previous investigations and that the night susceptibility hypothesis is limited to only one previous investigation.

3. first paragraph results: “night rMSSD was lower on workdays as compared to non-workdays, although the difference was not statistically significant” – can’t say “lower” and “not statistically significant”. Really, there was just a trend which may be due to chance.

Response: We have updated the text to indicate that the difference was not statistically significant and have removed description of the trend (“lower”).

“Over the 31 measurement occasions, the difference between night rMSSD on workdays as compared to non-workdays was not statistically significant (p = 0.33).”

4. p7 bottom: “we adjusted all models for cigarette smoking by including a dichotomous variable representing smoking status at the time of monitoring” “ it is concerning that you cannot adjust for smoking more quantitatively; that “it is unlikely that the quantity of cigarettes consumed differed between work and non-work periods” does not seem nearly as intuitive as suggested, especially if there are weekday-weekend differences (not clear if non-workday monitoring was on weekends, but regardless assumption might be that smoking very different on days away from work vs days at work)

Response: Unfortunately, we do not have data on the quantity of cigarettes smoked for all workers. In total, there were 6 smokers who were monitored over 9 work and non-work day pairs. We have a semi-quantitative measure of urinary cotinine levels for 8 work and non-work days, and urinary cotinine levels did not differ between the work and non-work afternoons. In addition, the majority (87%) of the monitoring occurred on the weekend and since the workers were allowed to smoke in the workplace, we believe the pattern of smoking, as influenced by nicotine addiction, would not vary largely between work and non-work periods. We have updated the Discussion with the following sentence to support our assumption:

“Semi-quantitative urinary cotinine levels obtained from NicAlert Strips [28] on a sub-set of the smokers confirmed that there was no difference between work and non-work smoking intensity.”

5. first paragraph discussion: “largest declines in HRV were observed per 1 $\mu\text{g}/\text{m}^3$ increase in vanadium, although the confidence intervals were wide, as a number of the vanadium exposures were below the limit of detection.”“same comment as #3 above (i.e. CI also consistent with increases in HRV)

Response: We agree with the reviewer. This topic was also concern to another reviewer and we have updated the manuscript by removing the vanadium analysis.

6. first full para p. 12 “Both the standard and residual models presented an inverse exposure-response relationship for these metals” “ does not seem correct for standard model given wide CIs.

Response: We have removed reference to Vanadium which had wide confidence intervals and a number of samples below the LOD.

7. does “case crossover” best describes study? The non-workday monitoring was under different circumstances that may affect results. Case-crossover would presumably imply crossover between welding/grinding exposure and non-exposure and other factors being equal, which is not the case here. This reflects somewhat incomplete description of overall design “ p5 methods, talks of “non-workday monitoring”, and this might suggest monitoring for metals, but there is no apparent data on that (no table 2 equivalent for non-workday), so

one presumes little metal exposure away from work (I suppose a reasonable presumption, but this should be more explicit).

Response: We agree with the author that the use of the term “crossover” may be misleading. Therefore, we have removed the term crossover from the manuscript and refer to the study as a panel study with baseline monitoring. We do not have metal exposures during the non-work period. However, as compared to workday welding fume exposures, non-workday exposures are likely to be negligent. By assuming non-workday metal exposures are zero, after adjusting for non-workday HRV, we present a conservative estimate of the effects of workday metals. In fact, the actual effects may be larger than reported, yet given the small concentration of ambient metals, we believe any difference would be negligible. We have updated the discussion with the following text:

“While particle metal exposures were not monitored during non-work, as compared to workday welding fume exposures, the non-workday ambient metal exposures are likely to be negligible.”

--Minor Essential Revisions

1. no comma after “twenty-six” in abstract-methods.

Response: We have removed the comma.

--Discretionary Revisions

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

1. abstract: “research priorities have shifted towards identifying the responsible PM characteristics” “ this seems a bit over-generalized.

Response: We have updated the abstract and introduction as follows:

“To better understand the mechanism(s) of particulate matter (PM) associated cardiovascular effects, research priorities include identifying the responsible PM characteristics. “

” As we seek to better understand the mechanisms, research priorities include identifying the PM characteristic(s) responsible for the observed cardiovascular health effects”

2. top of p6 “but 81% of the observations occurred within the same week.” Not clear what the point of this is (is this “good”?). Don't see where the value of this is discussed later.

Response: We have added a sentence to the discussion to address the value of having the non-work and work monitoring within the same week.

“Since 81% of the non-workday monitoring occurred within the same week as workday monitoring, we were able to control for longer term time trends such as season.”

Reviewer #3:

3. Are the data sound and well controlled?

Overall, the data presented appear sound and well-controlled. One concern about the data presented is that the authors' previous related report from this cohort of boilermaker welders (manuscript reference 10) used data from 36 subjects where the current manuscript reports data from only 26. Why is there a difference in subject number? What process was used to exclude subjects for the current manuscript?

Response: The difference in participants is because elemental analysis of PM_{2.5} samples by XRF began in 2004. We have updated the manuscript to describe the difference in the number of participants between the two analyses with the following sentence:

“From 1999 to 2006, we recruited 36 boilermakers at an apprentice welding school to participate in extensive ambulatory electrocardiogram (ECG) monitoring over two 24-hour periods on both a workday and a non-workday. In 2004 through 2006, in addition to ECG monitoring, 26 boilermaker welders were monitored for workday PM_{2.5} exposures which were then analyzed by x-ray fluorescence for elemental content.”

4. Does the manuscript adhere to the relevant standards for reporting and data deposition?

See concern expressed above. In addition, while the authors do state that they have previously reported (in reference 10) an association of total PM_{2.5} with a decline in night rMSSD on p. 11 of the Discussion section, they should also note that this association has been previously reported when they first present this finding on p. 9 of the Results section so that the reader is quite clear that this is not a new finding.

Response: We have updated the Results section with the following sentence which includes reference to our previous findings:

“Mixed model regression analyses for total PM_{2.5} exposures were consistent with our previous findings [10]; after adjusting...”

6. Do the title and abstract accurately convey what has been found?

The title should be changed so there is less confusion about whether the cohort studied here is different than the one reported in reference 10. Reference 10 is entitled “Night heart rate variability and particulate exposures among boilermaker construction workers.” This manuscript’s title should be changed to “PM_{2.5} metal exposures and night heart rate variability: A panel study of boilermaker construction workers.”

Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

See suggested revision re: title above.

Response: We have updated the title as suggested.

The authors need to discuss in the Methods section why they only report the results of analysis of only one index of HRV, rMSSD, when their previous report (reference 10) from the same data set used SDNN (SD of normal-to-normal intervals over entire recording), and SDNN(i) (SDNN for all 5-min segments) as well.

Response: We have updated the methods section with additional discussion of the use of only rMSSD for the current analysis.

Tapes were analyzed in the time domain, and indexes including the square root of the mean of the sum of the squared differences between adjacent NN intervals (rMSSD), the standard deviation of all NN intervals over the entire period (SDNN), and the mean of the standard deviations of all NN intervals for all 5-min segments (SDNN_i) were calculated over the 7-hr night period (00:00 to 07:00). The current analysis is restricted to the rMSSD measure, which based on our previous analysis, best captures cardiovascular autonomic changes following PM_{2.5} exposure [10].

While the design of using individuals as their own controls in a crossover design of work and non-work time does control for time-invariant individual factors, there were five subjects with hypertension and five with “cardiac compromises.” Hypertension and ischemic heart disease are known to affect HRV. Were there differences in HRV responses to PM_{2.5} and PM_{2.5} metal exposures in these groups compared to the rest of the subjects. In addition, anti-hypertensive or cardiac medication use might vary over the study period. The authors should address these points.

Response: We agree with the author that both cardiac compromises as well as hypertension may be effect modifiers of the HRV-metal association. However, small sample size limits our ability to investigate these potential effect modifiers. We also agree that medication use may be a time-varying factor. In total, only 3 individuals

reported medication use including one on statins, one on ace inhibitors and one on both. When we removed these 3 individuals from the analysis, we observed less than a 10% change in the effect estimates and an increased statistical significance in the Mn residual models. We have updated the Discussion of the manuscript as follows:

“While the majority of the participants reported no health compromises, there were 8 who reported either or both hypertension or cardiac compromises and of these 3 individuals reported medication use, which could also vary with time. However, when we restricted the cohort to exclude the individuals reporting medication use, the manganese effect persisted in magnitude and increased in statistical significance. Due to small sample size, we were unable to investigate the potential modifying effects of hypertension or cardiac compromises.

Reviewer #4:

Major Compulsory Revisions

The one scientific issue that I've identified is with the analysis and discussion of vanadium exposure and effects. Twenty-one of the 31 samples were below the limit of detection. The range of detectable samples was very low and the concentrations did not correlate with any of the other metals or with PM. It is likely that these measures are just “noise.” I think the paper would be stronger without including the regression results for vanadium because the exposure measures for vanadium are so limited. The authors do discuss non-detects for vanadium as a limitation of the study, but given the limited data, I don't think the regression results make sense. The discussion and conclusions related to vanadium are not supported by the data.

Response: After careful consideration, we agree with the reviewer's suggestion and have removed the vanadium analysis from the manuscript.

Minor Essential Revisions

I have a few editorial corrections which I've noted in the manuscript.

Page 7, Line 6: "Sample values below the LOD were utilized as reported." It is not clear what this means.

Response: We have clarified the methods with the following sentence:

“Samples below the LOD were assigned the value of the LOD.”

Page 9, Line 19: "In order to try and apportion..." should be changed to In order to try to apportion...

Response: We have incorporated the above suggestion.

Page 10, Lines 9-11: "Holding each metal exposure constant, ..." This is true for most of the metals, but does not appear to be true for Mn.

Response: We have clarified the presentation of the results with the following sentence:

“In the residual model, holding each metal exposure constant, we observed consistent declines in HRV with increasing total PM_{2.5} exposure, with the exception of manganese, the associations were statistically significant (Table 5, Model 4).”

Page 11, Lines 10-12: "As we hypothesized, unlike our previous study that evaluated daytime HRV over work (Magari 2002), using the ..." should be changed to (Magari 2002), this study used the susceptible night period and we observed...

Response: We have incorporated the above suggestion.

Page 12, Lines 22-23: "This suggests that when metal content is held constant, there remains a total PM_{2.5} exposure effect." It appears that this is not true for manganese.

Response: We have revised the section to more clearly reflect the lack of statistical significance for PM_{2.5} in the manganese residual model.

“Yet, metal content does not completely explain the declines in HRV observed within this cohort. An inverse association was observed with PM_{2.5} in the residual models adjusted for individual metals, although PM_{2.5} was not significant in the manganese model. For these metals, this suggests that when the metal content is held constant, there remains a total PM_{2.5} exposure effect.”

Page 12, Line 26: "...silicon exposures and night rMSSD, we observed a ..." should be changed to ...silicon exposures and night rMSSD and observed a ...

Response: We have incorporated the above suggestion.