Reviewer's report

Title: Repeated measures of inflammation, blood pressure, and heart rate variability associated with traffic exposures in healthy adults

Version: 1 Date: 11 May 2015

Reviewer: Ling Liu

Reviewer's report:

(Also please see the attachment).

Title of the manuscript: Repeated measures of inflammation, blood pressure, and heart rate variability associated with traffic exposures in healthy adults.

Authors: Mirowsky, Jaime E. et al.

Journal: Environmental Health

The authors used randomized cross-over design to study cardiovascular and respiratory measurements in three sites that had varying traffic and pollution levels. They also studied associations between particulate air pollutants and health measures. The randomized crossover design is powerful, as noted by the authors, in that it “allowed for each subject to act as their own control thereby reducing within-subject covariates.” Noise is a major concern in this study. Although the authors did not have a complete set of noise data, they analyzed cortisol levels to try to evaluate the stress levels at three study sites.

Major compulsory Revisions:

1. The goal of this study is to evaluate the health effects of traffic. The authors used repeated measures ANOVA to compare health measures at three sites where traffic density and air pollution levels tend to be different (but the differences were not statistically different). However, the repeated measures were done on 3 different days (with a washout period of #2 weeks in between) when temperature and humidity etc. might be quite different. Temperature and humidity may affect cardiovascular and respiratory function measurements, and thus may confound the traffic effects. Therefore, repeated measures ANOVA may not be an appropriate statistical method to evaluate the traffic effects, since it does not adjust for potential confounders or effect modifiers. Mixed-effects regression models have been used in situations like this in other studies in which site was an indicator variable and temperature, humidity, day-of-the-week etc. were included as co-variates (Dales R, et al. 2013. Acute changes in lung function associated with proximity to a steel plant: a randomized study. Environ Int 55: 15-19). The authors may even be able to adjust for the distance each participant traveled to the study site, and the walking pace, by including them as a co-variates. But I do not recommend including pollutants as co-variates in the model, because they may be highly correlated with the site and so not independent.
2. Page 14: In Discussion, the authors state that “This present study is unique in its use of multiple locations and diverse outcomes”. The authors ran many models to calculate the associations of particle constituents with many health measures. With so many models done, some statistically significant associations may have occurred by chance - the multiple comparison fallacy. Suggest the authors discuss how they dealt with this issue. There are also statistical methods available to reduce the possibility of spurious relationships due to multiple comparisons (e.g. familywise error rate procedures).

3. Page 18: In Conclusion, the language in the first sentence “We have shown that acute physiological and biological changes can occur in a healthy population following a 2 hr walking exposure to near-walkway traffic” seem a bit too strong, given that most of the physiological functional and protein biomarkers had no associations or moderate associations with the site, and some of the associations were counterintuitive (e.g. significantly reduced blood pressure). Also the influence of noise cannot be ruled out, although one may argue that noise is also part of the traffic pollution.

Minor Essential Revisions

4. Page 4, 1st paragraph, in the sentence “…as well as only requiring only a small amount of blood for the analysis…” Please remove one “only”.

5. Page 7, in Exposure Assessment, air samplers were located at one monitoring station while participants walked along the road, correct? How far did participants walk away from the monitoring station at each site?

6. Page 7, 3rd paragraph, what is “A-weighted noise levels”?

7. Page 10, 2nd paragraph, in the sentence “Repeated measures ANOVA was used to assess for statistical differences among baseline health values at the 3 exposure sites”, how was “baseline” defined? Was “baseline” the pre-exposure measures at each site?

8. Page 12: Many results for three sites were not presented: spirometry, cortisol, CRP, SAA, DBP, LF HRV etc. These are important results relevant to the objective of this study. If space is an issue, suggest including the data in “Supplementary files”.

9. Noise is a big concern in a study like this one. Since noise level was measured only once at SF site, it could not be adjusted for in models. The authors determined cortisol levels in one summer to try to evaluate the stress levels at three study sites. The authors reported no significant difference in cortisol levels among three sites. Please present the results for three sites in the paper.

10. Page 14, sentence “although wind direction could also play a role”. Please present wind data to back this up.

11. Page 16, 1st paragraph, “and remaining serum in IL-1# associated with PM10 [36], ambient air [39], and diesel [37]...” Do you mean “ambient air pollutants”?

12. Figure Legend and the figure: Is “baseline” the same-day pre-exposure values? Please clarify. Since the unit is % change, there is no need to have units
such as ppb, mmHg, in the figure.

13. Table 3: Were pollutant concentrations among three sites statistically significant?

14. Table 5: Since the associations were expressed as % change per unit of pollutant, having other units (e.g. ppb, ng/ml, mmHg) in the table is confusing. Suggest removing these units.

Discretionary Revisions

15. The expression “directly following exposures” is confusing in some sentences, such as the sentence “An approximate 5% decrease in DBP was also observed directly following all exposures…”, as if there were indirect observations. Suggest using “immediately” instead.

16. Page 4, a large part of the second paragraph talks about the methods and the merits of the study. Suggest moving it to “DISCUSSION”.

17. Page 16, a discussion on enhanced vagal tone indicated by decreased rMSSD. I wonder whether reduced blood pressure in more polluted site might also be attributable to enhanced vagal tone?

18. Page 18, 1st paragraph, about participants’ workload, suggest also mentioning that walking pace among three sites were not statistically significant (additional file #1).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests.