Author’s response to reviews

Title: Longitudinal Follow-up of Health Effects among Workers Handling Engineered Nanomaterials: A Panel Study

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Longitudinal Follow-up of Health Hazards among Workers Handling Engineered Nanomaterials: A Panel Study

Dear Editor and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope to meet with approval. Revised portion is marked in red on the paper. The main corrections in the paper and responds to the reviewer’s comments are as flowing:

Responses to Reviewer’s Comments:

Reviewer #1:
This study investigated the health risk from nanomaterials in populations of nanomaterial workers and unexposed control workers in a prospective follow-up design. A control banding approach is being used to assign relative risk levels to the different sub-populations. This elegantly accounts for the multitude of different nanomaterials in use. The authors assessed a series of physiological and inflammatory health parameters as well as early health response markers. While none of the physiological markers was associated with workplace exposure to nanomaterials, some of the antioxidant markers were changed. This allows the conclusion that the health risk is small. The exposure was able to invoke a mild protective response against oxidative stress but the stress was not strong enough to result in a detectable inflammatory response or any ill-health. Thus, workplace exposures represented by risk levels 1 and 2 seem to be at concentrations that are unlikely to pose any harm to the workers.

Minor essential revisions:

1) It would be useful to discuss the mild antioxidant response to nanomaterial in comparison to other risk factors such as air pollution. Studies in relatively clean countries show that already very low air pollution exposures (from short to long-term) will lead to clear inflammatory responses. No such thing was seen for the nanomaterial workers, which is remarkable. (a side-remark; In follow-up studies, the authors could strengthen this point by assessing air pollution exposure to their population for a more direct comparison).

Response:

Thank you for your important comment. This study adopted panel study design had five repeated examinations and used GEE model to test trend change of health effect markers in the exposed group (RL*Time) compared with the trend change in the control group (Reference group*Time). Environmental epidemiological studies seldom considered appropriate components (time and exposure interaction term) for repeated model to examine the persistent effect or accumulated effect of PM2.5 exposures. Thus, it is only find baseline effect or acute effect in traditional method. Recently, we are going to submit another manuscript to discuss this issue, we suggest the trend change formula in GEE was as follows: Yit =α0 + α1Timeit + β0Zkm + β1ZkmTimeit +γXi0, where Zkm, where Zkm used personal PM2.5.

Moreover, a recent multi-day, full-shift sampling study among 108 U.S. workers presents unrelated evidence between different metrics of carbon nanotubes and nanofibers and clinically relevant outcomes, included lung function, resting blood pressure, resting heart rate, and complete blood count components or pulmonary symptoms [Beard et al., 2018]. We think that the selection of health effect markers (acute or chronic) induced by engineered nanoparticles is also important to affect the final outcomes.

2) Abstract - Conclusion: The results suggest that the existing workplace exposure levels pose "no elevated health risk" to the workers. This is different than the "absence of a hazard". Rather, the hazardous materials seem to be sufficiently contained. I suggest rephrasing and referring to no evidence for elevated health risk to the nanomaterials handling workers" rather than hazard". I recommend to screen also the remainder of the
document to ensure a clear distinction between hazard (the potential to cause harm) and the risk (the possibility of being harmed).

Response:

Thank you for the great effort for reviewing our submitted manuscript. Actually, the terms of health risk or health hazard have clear definition in health risk or hazard assessment. In order to avoid confuse for readers, we have replaced “health hazard” with “health effect”. Please see the conclusion in the Abstract section on page 6, and the revised manuscript for details.

Minor discretionary revisions:

3) The data analysis describes the use of t-test and ANOVA, tests which require approximate normal distribution. It would be helpful if the authors could describe by which means they assessed the distributions before running the tests.

Response:

I am appreciated for your comment. This study did not use t-test and ANOVA approach. Therefore, we deleted the sentence in the method section as “A logarithmic transformation was performed to approximate the normal distribution. Student’s t-test and analysis of variance were used to test the differences among continuous variables”. Moreover, means and standard deviations were used to describe the distributions of continuous variables form Table 3 to Table6, even though Generalized Estimating Equations (GEE) is a semi-parametric approach to longitudinal analysis of categorical response, and can be also used for continuous measurements.

4) There were very few workers in RL3, thus the authors pooled RL2 and RL3. It would be helpful to know if a sensitivity analysis was conducted (i.e. exclude RL3 workers rather than pooling) and what it showed.

Response:

Thank you for your comment. In this study, we adopted 206 exposed workers and 108 controls who participated in no less than 2 examinations in the data analysis. Among them, only 6 exposed workers were in risk level 3. The results of sensitivity analysis presented the similar findings, while we excluded these 6 workers.

5) The conclusion sections make concluding statements about specific measures to keep workplace exposures low, shown in the supplemental data. As this data seems to be central for the conclusions, it would be nice to move that table to the main manuscript.

Response:

Thank you for your great suggestion. We have moved this table to the main manuscript. Please see the table 7.
Responses to Reviewer’s Comments:

Reviewer #2:

This is a thoughtful follow-up of a pioneering effort to study a cohort of workers exposed to engineered nanomaterials.

In the abstract and throughout it is important to clearly distinguish between hazards, risks, and effects. A hazard is a source of potential harm. A risk is the probability of harm given exposure. I suggest that instead of hazard you use "potential adverse health effects." These effects have not been validated as health endpoints.

Response:

Thank you for your great effort for reviewing of our submitted manuscript. We totally agree that the terms of health risk or health hazard have clear definition in health risk or hazard assessment. In order to avoid confuse for readers, we have replaced “health hazard” with “potential adverse health effects”. Please see the Abstract section on page 6, and the revised manuscript for details.

The authors should describe how this paper relates to all the other papers on this cohort that they have studied.

1. There have, in fact, been studies of makers of health effects among carbon nanotube exposed workers. These should be discussed in the introduction. Specifically, the following papers deserve mention:


Response:

Thank you for your helpful suggestion. We have added the paragraph in the revised manuscripts as follows: “A recent multi-day, full-shift sampling study among 108 U.S. workers presents unrelated evidence between different metrics of carbon nanotubes and nanofibers and clinically relevant outcomes, included lung function, resting blood pressure, resting heart rate, and complete blood count components or pulmonary symptoms [10]. However, another study in the same research team shows that inhalable Carbon nanotubes and nanofibers structures were associated with matrix metalloproteinase-2 (MMP-2), interleukin-18, glutathione peroxidase (GPx), myeloperoxidase, and superoxide dismutase (SOD) in sputum, and MMP-2, matrix metalloproteinase-9, metalloproteinase inhibitor 1/tissue inhibitor of metalloproteinases 1, 8-hydroxy-2′-deoxyguanosine, GPx, SOD, endothelin-1, fibrinogen, intercellular adhesion molecule 1, vascular cell adhesion protein 1, and von Willebrand factor in blood [11]. It implies
that the selection of health effect markers (acute or chronic) induced by engineered nanoparticles and using which specimens to test is important.”.

2. “Since smoking is a strong confounder, it was forced to be adjusted for every effect marker.” Smoking may affect some markers, but may not affect all markers. Authors need to describe how smoking is associated with exposure to nanomaterials, since a confounder is associated with both the exposure and outcome. I highly recommend providing the results without adjustment for potential confounders or using a stepwise selection process since many covariates may not be confounders.

Response:

Thank you for your great comment. This study first identified potential confounders for each health effect marker by using t-test in compared exposed group and control group. Those variables associated with health effect markers were identified and considered as confounders and adjusted in the GEE model. Please see the footnotes in Tables 3 – 6 for details.

Previous studies mentioned that smoking is a strong factor inducing Inflammation, oxidative damage, cardiovascular markers, genotoxicities, and lung function. We afraid that this strong factor will cause incorrect results. Therefore, we put smoking in the GEE model based on clinic physician's and immunologist’s suggestion.

3. This study includes a wide variety of nanomaterial facilities, and a wide variety of different nanomaterials. Nano silver, silicon dioxide, titanium dioxide, carbon nanotubes, and others likely affect markers of health effects in different ways. By combining many different materials into one exposure, this study introduces a large amount of exposure misclassification which results in bias towards the null. The researchers mention exposure misclassification in the context of not having individual exposure estimates, but the mixing of different types of nanomaterial exposures is the larger contributor to exposure misclassification. The null results should be interpreted with this in mind, and examination of results by facility or materials handled would be informative.

Response:

Thank you for your comment. There is still no standardized method of monitoring exposure to NPs in the field of occupational health. And real-world results showed the levels of exposure are likely mixture or transient or very low. Thus, it is hardly to set up the model for concurrent exposure to varied nanomaterials in order to understand the interaction among different nanomaterials.

In such a context of uncertainty in exposure assessment, this study used the control banding approach in categorizing the risk level of each participant as a surrogate marker of exposure to present potential health effects of any one nanomaterial. The detail information of summary of the most important characteristics of probability scores, severity factors and scores, and CB nano-tool risk level matrix were presented in Supplement Table 1 & 2.
Supplement Table 1 Summary of the most important characteristics of probability scores

<table>
<thead>
<tr>
<th>Variables/scores</th>
<th>30 pts</th>
<th>25 pts</th>
<th>22.5 pts</th>
<th>18.75 pts</th>
<th>15 pts</th>
<th>12.5 pts</th>
<th>11.25 pts</th>
<th>10 pts</th>
<th>7.5 pts</th>
<th>6.25 pts</th>
<th>5 pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated amount of material used</td>
<td>-</td>
<td>&gt;100mg</td>
<td>Unknown</td>
<td>-</td>
<td>11-100mg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0-10mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dustiness/mistiness</td>
<td>High</td>
<td>Unknown</td>
<td>Medium</td>
<td>-</td>
<td>-</td>
<td>Low</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Number of employees with similar exposure</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>&gt;15</td>
<td>Unknown</td>
<td>11-15</td>
<td>-</td>
<td>-</td>
<td>6-10</td>
<td>-</td>
</tr>
<tr>
<td>Frequency of operation</td>
<td>Daily</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Weekly</td>
<td>-</td>
<td>-</td>
<td>Monthly</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Duration of operation</td>
<td>&gt;4hrs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unknown</td>
<td>1-4hrs</td>
<td>-</td>
<td>30-60min</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Supplement Table 2 Summary of the most important characteristics of severity factors and scores

<table>
<thead>
<tr>
<th>Variables/scores</th>
<th>10 pts</th>
<th>7.5 pts</th>
<th>6 pts</th>
<th>5 pts</th>
<th>4.5 pts</th>
<th>4 pts</th>
<th>3 pts</th>
<th>2.5 pts</th>
<th>0 pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanomaterial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface chemistry</td>
<td>High</td>
<td>Unknown</td>
<td>Medium</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>Particle Shape</td>
<td>Tubular/fibrous</td>
<td>Unknown</td>
<td>Anisotropic</td>
<td>-</td>
<td>-</td>
<td>Compact/spherical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle Diameter</td>
<td>1-10nm</td>
<td>Unknown</td>
<td>11-40nm</td>
<td>-</td>
<td>-</td>
<td>&gt;41nm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solubility</td>
<td>Insoluble</td>
<td>Unknown</td>
<td>soluble</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Unknown</td>
<td>-</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Reproductive Toxicity  - - Yes - Unknown - No
Mutagenicity  - - Yes - Unknown - No
Dermal Toxicity  - - Yes - Unknown - No
Asthmagen  - - Yes - Unknown - No
Parent Material

Occupational exposure limit  <10μg/m3 Unknown 10-100μg/m3 - - 101-1000μg/m3
Carcinogenicity  - - - Yes Unknown - No
Reproductive Toxicity  - - - Yes Unknown - No
Mutagenicity  - - - Yes Unknown - No
Dermal Toxicity  - - - Yes Unknown - No

4. Tables 3 - 6 should include which covariates are included in the models.

Response:

We have added the footnotes for all covariates in each analytical Model. Please see Tables 3 – 6 for details.

5. No rationale is provided for using control bands rather than estimating exposure using a job exposure matrix. Control bands incorporate information on toxicity which could increase exposure misclassification compared to a traditional exposure assessment. Especially since nanomaterial toxicity is not well understood in humans, it seems more informative to just use exposure or exposure potential.

Response:

Thank you for the comment. Since there is still a lack of consensus on equipment and methodology for personnel sampling of engineered nanoparticles, this study used the control banding nanotool risk level matrix that was proposed by Dr. Paik and his colleagues to categorize the risk level of each participant as a surrogate marker of exposure.
Although nanomaterial toxicity is not well understood in humans, it was a lot of accumulated evidence in animal models. This evidence makes us unable to rule out toxic effects. The nanomaterial toxicity severity score in this study was based on the toxicity summary tables of a review document in order to obtain consistent scores.

6. This study was designed as a repeated measures panel study. With this design, people are measured at multiple points in time, so changes within the same person over time can be modeled, as well as differences between people. So for models of changes within the same person over time, time-invariant confounders do not need to be adjusted. It seems that in this paper the results were mostly focused on comparisons of groups of control bands, but I think that the more important interpretation if the intra-variability among individuals over time.

Response:

I am apologized for unclear presentation and explanation in GEE models. Indeed, this study focuses on the trend change of health effect markers in the exposed group (RL*Time) compared with the trend change in the control group (Reference group*Time). Thus, two models in GEE approach were presented. One is based on the risk level (Risk Level 1*Time vs. control*Time, and Risk Level 2*Time vs. control*Time) in Model1 and another is based on continues risk levels (Risk Level*Time) for dose-response relationship of change of markers in Model 2. Although time-invariant confounders were not change within the same person over time, it still affects the main effect in the baseline in the GEE model. All tables have been revised. Please see Table 3-6 for details.

7. Healthy worker survivor bias was not discussed and may be an important limitation, if workers with higher exposures were less likely to participate in additional follow-up exams.

Response:

Thank you for your insight suggestion. We have added this limitation in our revised manuscript. The healthy worker effect may reduce the health outcomes and underestimate the effect of NPs exposure, if workers with high exposures were not participate in follow-up exams. However, this study collected workers’ specimens during company annual health examination period, it can avoid workers absent from work without permission and without a reasonable excuse.

Editorial Comments:

1. Page 7, Line 6: "consuming" should be changed to "consumer"

2. Page 7, Line 32: "Most the documents" should be changed to "Most of the documents"

3. Page 9, Line 3: "Control banding…” should be the start of a new paragraph
4. Page 10, Line 57: "Among them, 39 plants have been site-visited and invited" should be "Among them, 39 plants were visited and invited"

5. Page 11, Line 7: "We have performed five repeat examinations during the four-year." should be "We performed five repeat examinations during the four years."

6. Page 16, Line 6: "participated" should be "participating"

7. Page 25, Line 22: "fail" should be "failure"

8. Page 26, Line 9: "dramatic" should be "dramatically"

Response:

Thank you for your great suggestion. We have revised the manuscript and have made the correction. Please see the revised manuscript for details.