Author's response to reviews

Title: Divergent effects of fine concentrated air particles on allergic airway responses are related to particle composition and sources

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Author's response to reviews: see over
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Dear Dr Francesco Forastiere and Environmental Health Editorial Team,

Enclosed please find our revised manuscript entitled “Divergent effects of urban particulate air pollution on allergic airway responses in experimental asthma: a comparison of field exposure studies”, for your consideration for publication in Environmental Health.

The reviewers have provided insightful comments to which we have responded with appropriate changes in the current version of the manuscript. We have also made editorial revisions to align with Journal requirements, including a revised title and other format changes. We believe the revised version is much improved, and look forward to the decision of the editorial team of Environmental Health. Our point-by-point responses are enumerated below.

Authors have no competing interests regarding the work described in the current version of the manuscript. All surviving authors have read the revised manuscript, have agreed it is ready for submission and accept responsibility for its contents.

Sincerely,

James G. Wagner
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This paper describes a study in which the investigators have exposed rats to ambient fine particles at two locations in Michigan by inhalation. Rats were sensitized with ovalbumin to evoke an asthma-like response. Effects such as inflammation and pathology were assessed after 16 hours of an 8 hr exposure and these experiments were part of a larger study in which the role of the physical characteristics and chemical composition is studied.

Although it is well appreciated that studies using concentrator technologies are expensive and time consuming, it is also clear that temporal variations may often exceed those of the spatial variations. In that sense the major criticism I have for this study is that the authors fully rely on the outcomes of single studies per site. Now, when only focusing on a (not defined hypothesis) that not the total mass but chemistry of the particles should be taken as dose metric, the outcomes will indeed underpin such a hypothesis and confirm findings of other studies in North America and Europe that have used the same technologies. However, the authors, most likely attracted or distracted by the large number of exposure variable speculate too much on the role of specific components. Indeed, they do not point to one or more constituents in particular. But, in the end, the outcomes cannot be directly related to the sources of emissions using this very limited number of observations. It would have been much better if repeats at the same sites were performed to extend the conclusions that mass is not the most predictive indicator for respiratory diseases. What was the reason not repeating these studies?

As a consequence, several other aspects of this study may put things a bit out of balance. My suggestion is to put less emphasis on the causal constituents (it is very clear that deposited mass itself does not explain the health effects), and focus a bit more on the underlying mechanism of opposite responses for the two locations.

Unfortunately we did not have the resources (time and money) to conduct repeated 1-day studies at these sites. The current studies were part of a series of CAP exposures at different sites and addressed different aspects of the allergic sensitization and challenge protocol. Only one single-day exposure was scheduled at each site, and more emphasis was placed on repeated exposures (refs 14-17). The focus of field exposures has since turned to understand cardiopulmonary health effects, so similar allergy/CAP studies are not imminent.

The reviewer is absolutely correct that extrapolating the results of a single study to make firm conclusions regarding a causative role for specific PM sources is premature. Indeed it may have distracted from the most novel finding, that the opposite biological responses (promotion vs. inhibition of allergic inflammation) can be induced by the same ambient PM.
mass. In this specific instance we have done our best to provide a detailed characterization of the PM atmospheres associated with these disparate outcomes, and suggest reasonable explanations for the sources of the PM. To ignore these potential sources in our interpretations and discussion would not seem appropriate for this journal. We acknowledge this is a single observation that, while requiring further conformation in other studies, provides an interesting observation for the potential role of specific PM sources in allergy. We have not found similar reports of specific PM-induced inhibition of allergy in the literature. We agree that our some of our conclusions with regard to source-specific effects may be overinterpreted and we have revised several areas of the manuscript to limit the more speculative passages.

Several smaller remarks not necessarily in order of importance
1. Would be nice if the study was based on a clear hypothesis that can be addressed using this study design.

We agree that as presented the study appears to be more exploratory in design. Based on the epidemiological associations with asthma symptoms and airborne PM2.5, our goal was to compare different urban sites that have marked difference in their respective pollution mixture (sources), industrial activity and unique chemical profile. In this case Detroit, with more industrial activities than Grand Rapids, was assumed to have more trace elements than the Grand Rapids site which was in a suburban area near a roadway. Given the disparate sites and PM mixtures, our overarching hypothesis was (as suspected by the reviewer) that the different PM mixtures would elicit different effects in allergic airways. We believed that CAP derived at both sites would worsen allergic airway responses, and that independent of PM mass, responses to Detroit PM would be more pronounced.

To clarify this experimental rationale we have made revisions the abstract and to the last paragraph of the introduction.

2. The fact that particles have been concentrated is actually not that relevant. So it may also be omitted from the title. What is important is that fact that the Harvard Concentrator only concentrates efficiently above 0.18 um. Now, the authors also speculate on the role of ultrafines in their study, but if ambient ultrafines play a significant role, they could have performed the study without a concentrator. Or they should have added a third group namely those that were exposed to air that has passed a size selective inlet but that did not pass the three stage concentrator. Anyhow, give the number of exposure variables and the lack of statistical power due to the lack on the tox parameters, speculations needs to be done with more care (if needed at all)

We have revised the title to remove the reference to “concentrated PM2.5”. We agree that some speculations with regard to ultrafine particles are not strongly supported by the data. It was not our intention to study the effects of the ultrafine fraction and would be better approached with an ultrafine concentrator. We meant to emphasize that there were more smaller particles in the Detroit PM, most notable in both PM fraction 0.18<0.6 micron and in the ultrafine fraction PM<0.18 micron. We have modified the Discussion to remove the emphasis on ultrafine particles.
3. Background section: the authors suggest that there may be a ‘definitive role for PM2.5’. What role do they think of?
We meant to say “definite” (a clearly defined role), but have replaced with “mode of action”. PM is clearly associated with asthma symptoms and severity; the mechanism by which this occurs is not clear.

4. pg 4. CAPS need to be defined (or there term can be avoided). Line 4 from below ‘CAP’ should be CAPs to be consistent.
We have replaced all instances of “CAPS” with “CAPs” and, although commonly used throughout the manuscript, have changed CAPs to PM2.5 or PM in several places.

5. Only 35 LPM of the concentrator is used for whole body exposures. Data are lacking to verify if there was a sufficient air flow in the whole body chambers. Control animals were exposed to clean filtered air, but was the same negative pressure (and RH plus temp) present in the whole body chambers as in that in which rats were exposed to CAPs? If not, to what extend could that have affected the outcomes. An enrichment factor a 30 is very theoretical. Yet table 2 suggest that even higher ratio were obtained, when comparing these values with those obtained outdoors. This needs to be explained (probably due to the averaging time…).

Based on these flow rates we estimated 6-7 air changes per hour in the whole body exposure chambers. Standard operating procedure for exposures is to balance pressures in control and CAPs chambers, in most cases this is around 10mmHg with respect to ambient pressure, and temperature and humidity in chambers are not different from one another. Detailed performance characterization of the concentrator/chambers was reported in Harkema et al. (2004) (Ref 14).

Concentration Enrichment Factors (CEF) for Detroit and Grand Rapids were 29.6 (542/18.3) and 32.2 (519/16.1), respectively, or very close to the expected CEF of 30. Any deviation of CEF from 30 can be due to averaging time as the reviewer points out, but can also be related to ambient CAP and relative humidity. We have added this to the Results section.

6. Some chemical species have been measure on Teflon filters and some of quartz. The latter seem to be filters taken from outdoor PM rather than from a CAPs environment, correct? And what are the implications for using those data to explain the health effects? The main problem is that it is unclear how long ambient PM has been sampled (8 hrs, 24 hrs)?
All the PM data reported in this paper are CAPs from the exposure chamber. We collected the CAPs samples daily during the 8-hour exposure period. The usage of quartz filters has been clarified in the text.

7. With respect to the immunization protocol: was there no need to use an adjuvant?
   In Brown Norway rats, multiple (3) intranasal instillations with OVA is sufficient to sensitize animals without the use of adjuvant. Reference 23.

8. Were the OVA treatments and tissue and BALF collection performed on site in Air Care?
   Ova sensitization and challenge treatment occurred in AirCARE1, but animals were returned to the laboratory for necropsies. We have clarified this in Methods section.

9. Given the history of this group on nasal pathology one would have expected to read about the impact on the nose as well, in particular since a fraction of CAPs will also deposit in the nose. So?
   A complete analysis of nasal tissues from both studies was not conducted. Modest decreases in stored mucous in nasal tissues from rats exposed to Grand Rapids PM were measured, however tissues from the Detroit exposure were not appropriately collected. In previous Detroit CAP studies nasal lesions are enhanced in allergic rats (Ref 14). We expect that nasal lesions would be similar to the exposure- and treatment-related pathology in the lower airways in both studies.

10. Check vendor/supplier of chemicals and methods
    The identity and accuracy of supplier information has been checked.

11. Were gaseous monitored at the breathing zone of the animals?
    Due to the limited flow that is available for the CAPs sampling, gaseous sampling was completed using ambient air. As reported in Harkema et al., (2004), some of the reactive gaseous species (e.g., ozone) are normally stripped out by the concentrator, and it is likely that the animals were exposed to lower concentrations than what we reported in this paper. We only used the gaseous pollutant data to characterize emission sources, and did not associate them with the health effects of the animals in this paper.

12. If space is becoming an issue, the section on sources (pg 15-16) can be deleted. The Discussion of sources describes the meteorological conditions (wind) and geographically identified PM emission sources (roadway, sludge incinerator and oil refinery) that impacted the site during the hours of exposure. While this could be edited to a shorter version, we don’t feel the length of the manuscript is excessive.
13. Pg 18 Even if Zn, V and Ni can be identified as the compounds mostly responsible for the observed responses in rats, a better explanation on how that connects with studies from the East coasts may be given. We only point out that both our study and 3 epidemiology studies found associations of asthma symptoms with similar trace elements. Having no further explanation than these associations, we have removed this statement from the manuscript.

14. Are there signs that inflammasomes play a role in the effects that were noted? We do not have any data that specifically address the role of the inflammasome in these studies. While there is much interest in PM-induced activation of the inflammasome by our group, this would focus more on the adjuvant effects of PM during the allergen sensitization phase of the protocol and not during exacerbations of challenge. The (NLRP3) inflammasome may be activated in our exposed animals, and one simple approach would be to assess IL-1b expression and production in lung tissues. This single endpoint would be suggestive, but not sufficient to conclude a role for inflammasome activation however. In vitro approaches using these PM samples may be a more a direct approach to study this possibility.

15. As the authors have stated, endotoxins may also have affected the outcomes. Yet, they were not able to measure this before the submission of the manuscript. Meanwhile, have they done this and would it be that crucial that such data has to be presented anyhow? We do not think we can reliably assess the PM-endotoxin content since filters have not been stored in freezers which would have ensured that no contamination or bacterial growth has occurred.

16. pg 10 Once again, the notion that the effects were associated with motor vehicle emissions is far to speculative and cannot be based on just experiments with actually only 3 data points on a dose response curve irrespective the metric of exposure. We cannot identify a passage on Pg10 that mentions motor vehicle emissions. However we have tempered our comments in the Conclusions section and in other parts of the manuscript in this regard.
Wagner et al Divergent effects of fine concentrated air particles on allergic airway responses are related to particle composition and sources

General

In short, the authors contend that air quality standards based on particle speciation and sources may be more relevant than particle mass to protect from PM exposure.

The study was well designed; paper is clearly written and well organized. Both the title and abstract accurately convey the major findings. Studies from this laboratory use a mobile laboratory with particle concentrators to conduct real time inhalation exposure of laboratory animals to concentrated air particles with the goal of determining the relations between PM and health outcome. These controlled studies with environmentally relevant exposures provide much needed data and stimulate hypotheses related mechanisms of particle induced lung injury/allergy in normal and allergic animal models and humans.

The questions posed by the authors are not new but their data are important because they provide a means to compare and understand specific exposure sources and the role of particle composition, size mass in allergic and particle induced lung injury. The methods used to address the questions are appropriate given the identical exposure regimens and the similarity of PM (on a mass concentration basis) collected in two different regions presumably impacted by different emission sources.

Minor Compulsory

1. “The inhibition of allergic inflammation by CAPs may be mediated as immune depression of airway macrophages and epithelium to appropriately respond to allergic and inflammatory stimuli other studies by”

……. alternative explanation for the inhibitory effects of the Grand Rapid particles is discussed in the study of J Wan and D Diaz-Sanchez (2006 journal of immunology) ---suggest that if phase II enzyme induction occurs (presumably resulting from exposure to PAH/organics) enhanced IgE production in B cells (by diesel exhaust particles) can be blocked which protects or ameliorates the otherwise proallergic effects of DEP. (Wagner et al argue that Grand Rapid site is impacted by mobile and diesel sources)

This is certainly a plausible mechanism, especially since we measured a decrease in OVA-specific IgE in serum of allergic rats exposed to Grand Rapids CAPs compared to allergic rats exposed to filtered air. These data are not shown because we did not conduct a similar analysis in Detroit to make a meaningful comparison. This mechanism might also underlie the inhibition of allergic responses by exposure to whole diesel exhaust we have previously published. We have added a brief revision in the Discussion with regard to IgE production and diesel and include the Diaz-Sanchez reference.

2. “In addition to secondary/transported sulfate, increased concentrations of anthropogenic metals including Pb, V, and Se, suggest that the site was also impacted by emissions from the local industrial sources that we have identified southwest of the exposure study location ..” ....Although the sulfur content and some metals were higher in the Detroit air particles relative to the Grand Rapids particles, indeed there were several other metals (Mn Fe Cu Ba) higher in the Grand Rapid samples.
Elevated concentrations of Fe, Mn, Ba and Cu have been commonly associated with brake wear and urban road dust so the higher concentrations of these trace elements in Grand Rapids are likely to be associated with motor vehicle/diesel due to our proximity (60m) to the roadway. As for Detroit, we have several years of experience, where we have mapped specific industries and know their annual PM emission, where elements such as La, V and Pb can be very industry specific.

3. Although not necessary---- figures 3 and 4 could be combined ---it is not clear why the data from the OVA only exposed rodents could not be pooled (why is this data so variable?) for a more direct comparison of the responses to the CAP Detroit and CAPs Grand Rapid

“Another notable difference between the exposures was that the ultrafine fraction of Detroit CAPs was more than twice as high as that found in Grand Rapids CAPs. 

….Just because (<PM0.18) was twice as high doesn’t (in-of-it-self) does not necessarily “suggest” that impacts from local Detroit combustion sources had stronger impacts than in the Grand Rapid site.

We considered combining the BALF data into one Figure but this would result in 10 separate graphs with uneven rows (2 rows with 3 plots, 2 rows with 2 plots). It seemed better as two figures.

We do not routinely pool data from treated groups, and we use equal numbers of animals per group to keep a balanced study design. We prefer to keep groups from each exposure separate (not pooled), with their respective controls that came from the same batch of animals, same time of year, same OVA prep, etc.

OVA BALF cellularity is within historical ranges, with the more important variable being percentages of each cell type, and percent increase from non-allergic controls.

With regard to the statement on the ultrafine, our assumption is that freshly generated particles from combustion are initially in the ultrafine size range and then “grow” into the accumulation mode to >0.18µm. Hence with more smaller particles found in Detroit CAPs, we assume these were derived from fresh combustion sources. We have reworded this passage for clarity.

4 The studies below seem to contradict the findings by Wagner et al and should be mentioned


“Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice. …..is this because of the exposure regimen or differences in rodent model?


Ambient ultrafine particles provide a strong adjuvant effect in the secondary immune response: implication for traffic-related asthma flares.

Li N, et al Inhale Toxicol. 2007; 19 Supple 1:117-26

Inhalation of concentrated ambient particulate matter near a heavily trafficked road stimulates antigen-induced airway responses in mice exposed to CAPs 50 m downwind of the roadway had, on the average, greater allergic responses and showed greater indications of inflammation than did mice exposed to CAPs 150 m downwind.
The first and last study mentioned above by our colleagues in southern California included PM exposure during the sensitization phase of experimental allergy protocols. The sensitization pathways are quite distinct from the IgE-dependent activation that occurs during the challenge phase that we examined in the current study. In the current manuscript we have not introduced or discussed the role of air pollution during the sensitization phase as was done in these two studies by Kleinman (the last reference is not by Li et al). As such we could not easily add and discuss these references without an additional

The second study above (to which we collaborated during the field exposures, and conducted all the necropsies, evaluation of BALF, gene expression and histopathology) has some key differences from Grand Rapids study. The LA exposure site was more than 400ft from the expressway, with a 5 story storage building blocking both the view and windflow from the highway; the inhalation exposure was to ultrafine (nano) particles; and 3 inhalation exposures were conducted one, four and five days before any allergen challenges. By comparison the current study was conducted 170 ft from the freeway separated only by a cyclone fence, animals inhaled PM2.5, and were exposed only once, approximately 30 minutes before a single challenge. We have mentioned this study in the Discussion.

In summary, the study design and reported findings support further efforts to compare source specific differences/potency -both for a more responsive regulatory perspective but also as a means to better understand the mechanisms underlying particle induced lung injury and the induction of allergic responses in susceptible populations (eg asthmatics).