Author's response to reviews

Title: Assessment of the capacity of vehicle cabin air inlet filters to reduce diesel exhaust-induced symptoms in human volunteers

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Author's response to reviews: see over
Comments to Referee 1:

Comments no. 1: “The authors conclude in the start of the discussion that the filters improves “respiratory symptoms”. The term “respirator symptoms” is also used in the methods part of the abstract. That term is somewhat beyond what can be concluded from changes in eye and nasal irritation and bad taste? Usually respiratory symptoms would be associated with the lower airways and elsewhere in the paper “respiratory” is used only in relation to lung function”.

We agree with the reviewer that we had used an expression that was not appropriate and have adjusted the text.

Comment no. 2: “The data on lung function and inflammatory markers presented in table 2 and 3, in particular the statistically significant changes FEV1 and s-ICAM1 related to the filters are a bit difficult to appreciate, It could be helpful to have the delta values or % changes or differences with confidence intervals because as such the pre and post exposure levels give no clue to these differences. Moreover, although these differences are interpreted with commendable great caution by the authors, they fail to recognize that they, even more so with the pairwise comparison despite comparing 4 scenarios where ANOVA or mixed effects based statistical models could be more appropriate, have multiple comparisons with a high risk of type 1 errors”

We agree with the referee that the ICAM1 and FEV1 data are difficult to interpret and have applied great caution. With the perspective of the risk of a type 1 error, the absence of significant effects by unfiltered diesel exhaust and the magnitudes of changes being small, we wanted to avoid over-interpretation of these findings. This has been further adjusted in the manuscript.

To enable a better opportunity for the reviewer and reader of the paper to appreciate the magnitude of differences, we agree that presenting the absolute delta changes is beneficial and have updated table 3. The statistical tests were indeed based on the delta changes (post minus pre exposures) and the net results were then analyzed with the Wilcoxon rank sum test, as the data were not normally distributed. The primary comparisons were between Filtered DE and unfiltered DE, as the potential of the filters to reduce DE
effects was the main focus. Comparisons between Filtered air and unfiltered DE were included for validation of potential filter effects only. We agree that the use of ANOVA or mixed models was possible alternatives of interest, but our statistical advisor suggested the present analyses.

**Comment no. 3:** “filter C and D are not introduced until the discussion in the third paragraph on the selection of filters. This part would be more appropriate in the material and methods section (where 4 filters are briefly mentioned), which would also give a better flow in the paper. RTLF is not defined until the supplement also. In the same context, the data on in vitro effects of materials from filter C and D should be briefly reported in the results section text”

The suggestions for improvement are well appreciated and the manuscript has been updated including additional data on the filters.

**Comment no. 4:** “the PM material for in vitro toxicology should be better reported and characterized. Why is PM1 mass not reported e.g. in Table 1, and the actual size distribution of the aerosols is also lacking – only the filter collection efficiency from the manufacturer, which is not very helpful. This is what the interpretation of the in vitro toxicology is based on and comparisons would be of much more interest if one can combine changes in mass and inherent toxicity per unit mass”

Table 1 has been corrected as the particle mass is indeed PM1. The filter collection efficiency as regards particle mass and number over the respective filters were measured in the study and the data are given in Table 1 and also referred to in the result section. The collection efficiency reported by the manufacturer is only given as an illustration in the online supplement. However, these data were not used within the study proper.

Size distribution data were not given in the original manuscript in order to have it more condensed. We appreciate the opportunity to present also these data and have updated the text and added Figure s3, which adds SMPS data.

**Comment no. 5:** “the extraction protocol need more detail than just methanol and a ca. % yield, or at least references to a similar protocol as used. Of more importance, size distribution of the particles in the RTLF and cell system media should be provided to get an idea of the agglomeration state”

The extraction protocol has been described in detail earlier and the reference has been added (Mudway IS, et al Arch Biochem Biophys 2004, 423(1):200-212). Size distribution was not determined in the cell-based and cell free in-vitro systems at the time point of the investigation, but this is an aspect that deserves further attention in forthcoming studies.

**Comment no. 6:** “In principle cell responses such as release of cytokines should only be considered at subcytotoxic exposure concentrations and thus cytotoxicity should be included. Similarly, combustion PM is known to bind cytokines and this
should also be checked. As this is in the supplement anyway details should not be omitted for space considerations"

The referee is right to point out that the paper may benefit from cell cytotoxicity data, which is now included in the online supplement and figure s4.

Up to concentration of 30 ug/cm² the cells were unaffected and at higher particle concentrations unfiltered DE and particles from filter A had the highest effects on cell viability. Filter B with an active charcoal component showed less cytotoxic effects that were only apparent on the highest PM concentration 100ug/cm². This corresponds well with the IL-8 release data. Particle binding of cytokines should preferably have been investigated, but this was not done at the time of the investigation. It will certainly be included in forthcoming experiments.

Comment no. 7: “The counts and size distribution of particles in the chamber should be given with more detail than just the range from 14 to 660 nm, a figure in the supplemental material and counts in table 1 would be helpful.”

We have now included particle number concentrations in table 1 and added details to the text and added particle size data in the new Figure s3.

Comment no. 8: “The manuscript lacks page and line numbering”

This has been corrected.
Comments to Referee no. 2

Major compulsory Revisions:
“The new generated diesel particles are usually fine particles, like PM2.5 or PM1. Plus, the authors also mentioned that ‘the particle number size distributions in the chamber ranged from 0.014 to 0.660 μm (mobility diameter)’. However, the authors only presented the results of PM10 mass concentration in different exposure scenarios. Thus, the major question is that why the authors chose to present PM10 instead of PM2.5 or PM1. Were number concentration and number size distribution also monitored in different exposure scenarios? Did filters with or without active charcoal have any effect on these two parameters change?”

We appreciate these comments, which were also highlighted by referee no. 1 and have given our response above. The manuscript has been updated with these details and we have also added figure s3 on particle size distributions.

Minor essential revisions

Comment no. 1: In the method part of abstract, the author mentioned ‘Respiratory symptoms and lung function were measured pre and immediately post exposure’ which is inconsistent with the description in the main text quoted as ‘the symptoms were assessed by questionnaire before and every 15 minute throughout the duration of the exposure, and the lung function were performed before and one hour after each exposure”

As given above in the response to Referee 1, this important aspect has been corrected.

Comment no. 2: "It is not correct to say ‘improve health’ or ‘enhance well-being’ as the effect of the filters (the last sentence of the first paragraph of discussion and Conclusion). The filters are only for protection. Recommend to rephrase into something like ‘reduce the adverse health effect of DE”

In accordance with the referee’s recommendation, we have now changed this sentence to “reduce the adverse health effects of DE for both drivers and passengers”.

Comment no. 3: “In the third paragraph of discussion, it was written that ‘Filter A, which was the same filter but without charcoal, was included as reference.’ However, all the comparisons in the paper were only between filtered DE and unfiltered DE. The meaning of having a reference filter A is not clear when there was no comparison between filter A and B”.

In our preceding study (Rudell et al 1999) as well as in the present in-vitro cell investigation, it was indicated that the use of an active charcoal component, as in
Filter B, was highly beneficial. In the human exposure study, we therefore primarily evaluated Filter B, and as a reference included Filter A, which had the same filter medium but lacked the active charcoal component, as regards diminishing effects from unfiltered diesel exhaust.

The statistics were primarily based on the question of each filter being superior to unfiltered diesel exhaust, rather than the comparison between the filters, as this was a secondary issue. We have now added statistical comparison between the filters, which confirmed that Filter B was significantly better than Filter A for a range of parameters. This has been added to the manuscript.

Comment no. 4: “In the fourth paragraph of discussion, the authors wrote as ‘In the present study, the combination filter (B) including active charcoal, .... Both particle number and mass by as much as 75%’. But there is no result about particle number concentration. It would be interesting to include the number concentration changing during different exposures”

The particle number concentration changes have been added in the main text and in Table 1.

Comment no. 5: “The statistical analysis was based on delta changes, so it would be clearer to list delta changes in all the tables”.

Delta values have been added in Table 2 and Table 3.

Comment no. 6: “In table 2 and table 3, it was not clear that in which two exposures were the analysis performed. This should be explicit.”

The primary comparisons were between Filtered DE and unfiltered DE, as the potential of the filters to reduce DE effects was the main focus of the study. Comparisons between Filtered air and unfiltered DE were included for validation of potential filter effects, as regards the active charcoal component. The legends of table 2 and table 3 have been extended regarding these aspects.

Comment no. 7: “In table 3, what is the unit of each biomarker? How are the data given as?”

The unit of each biomarker has been added now in Table 3. For IL-6, TNF-α, and CD 40L the unit was pg/ml whereas for p-selectin and s-ICAM-1 it was ng/ml. Inflammatory markers data were not normally distributed and therefore presented as medians with inter-quartile range. This has been added to the ms.

Comment no. 8: “In the 4th paragraph of results part, the detailed description of the influence of symptoms should be given in Figure 3-5 instead of Figure 2-4”
The reviewer is right and the figures’ numbers have been changed accordingly.

Comment no. 9: “Please pay attention on the use of abbreviation of diesel exhaust in the paper. Some place the authors use DE, some not.”

Discretionary Revisions
All recommendations by the referee have been taken in account, and the text has been changed accordingly.