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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Gaseous VOCs rapidly modify particulate matter and its biological effects – Part 1: Simple VOCs and model PM" *by* S. Ebersviller et al.

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The manuscript describes in vitro experiments with a pulmonary cell line (A549) exposed to different kinds of gaseous and particulate model components relevant for ambient conditions. The experiment is very well designed and performed. Especially the systematic approach of investigating cellular response due to gas-phase and particulate phase components separately as well as under combined exposure conditions with gas-phase and particle-phase selective samples is innovative and interesting. Furthermore, the fact that the authors utilize air-liquid interface cell cultures (instead of the traditionally used submerged cell cultures) to examine the toxicity of airborne toxins

under physiologically realistic conditions is essential for the merit of this study. This study provides well-stratified data for studying the interplay between gas phase and particulate phase components in terms of cellular in vitro toxicity. Based on this unique data set it presents direct and convincing evidence for the “effect modification” phenomenon, the enhanced toxicity of PM in the presence of toxic gaseous components. The findings of the authors are innovative and relevant for a deeper understanding of the processes governing the toxicity of ambient particles.

MINOR ISSUES

1) I concur with referee #1 that the authors should discuss the experimental details of the two systems for gas-phase-only (GIVES) and particles-only exposures (EAVES) in more detail. The issue of having negligible particle-related dose in the GIVES and negligible gas-phase dose in the EAVES is the basis for the study. Hence, it requires more rigorous discussion especially with respect to the following aspects:

- Please explain, why the EAVES can be expected to have much less gas-phase deposition than the GIVES, since gas-phase components will be present in both systems and the exposure times are not that much different (4h versus 0.5-1h).

- What are the particle deposition efficiencies in both systems? Typically this will depend on particle size. The geometric number mean diameter of the MOA particles is about 200nm (Fig. 2). BUT mass or possibly even better surface area is likely to be toxicologically more relevant dose metric than particle number. For the relatively broad size distribution presented in Fig 2 the geometric mean surface area and mass diameters will probably lie between 500 and 1000 nm. At this size range not only diffusion, but also sedimentation may play a role in particle deposition. Was this considered?

- This information should then be used to estimate the cell-delivered doses for both gaseous and particle phase components as one of the most important parameters for assessing substance related toxicity.

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2) While the interpretation of the p-Tolualdehyde data with and without MOA particles seems straight forward, the corresponding Acrolein data are much more puzzling as recognized by the authors. The IL-8 response (Fig. 6a) suggests that Acrolein is not significantly taken up by the MOA particles – as suggested by gas-particle portioning theory. On the other hand, Fig. 6b seems to indicate that the MOA particles, which are not toxic per se (see Clean air + MOA control), are substantially more toxic in the presence of Acrolein, which seems to suggest a significant partitioning of Acrolein to MOA. This seems contradictory. Furthermore, when looking at the LDH data (Fig 7) the gas-phase response is significantly reduced in the presence of MOA (Fig 7a) and the Acro+MOA response is larger than the Clean Air + MOA response. Both effects indicate significant uptake of Acrolein by MOA, which – again - is inconsistent with theoretical predictions of Acrolein partitioning. Hence, only the results from Fig 6a seem to agree with partitioning theory.

The authors argue (P. 5084 LL29), that these inconistencies are likely related to the higher sensitivity of LDH to small concentration changes relative to IL-8. Hence, the small amount of PM-adsorbed Acronlein has no effect on IL-8 reduction due to gas-phase Acrolein (Fig. 6a), but a notable effect on LDH (Fig. 7a). But this explanation is not sufficient, since – to my understanding – LDH release is a much more severe insult to the cells than IL-8 release. Hence, LDH should not respond to small amounts of a toxic substance. Thus there should be no response in Fig. 7b. Furthermore, this "supposedly small" amount of Acrolein taken up by MOA induced the most severe IL-8 induction out of all of the cases investigated here (Fig 4a and 6a). Thus I can not see a possible scenario reconciling all of these findings.

I would suggest that the authors admit to this inherent inconsistency of the data and include the following issues in the discussion:

- Could these discrepancies be related to the different exposure times for GIVES and EAVES and hence the differences in cell-toxin contact times (9h incubation time plus 4h and 0.5-1h exposure time, respectively)?

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- Could the fact that exposure F was the only case with RH > 90% (in the chamber), while all other exposures were performed with RH < 75% could have something to do with these “strange” results? In fact the authors hint on potential relevance of RH on P. 5085 LL2, but they fail to relate this statement to this peculiarity in RH conditions.

3) Unlike Referee #1 I did not feel that the concept of the experiment design was described in an unnecessarily repetitive way. Sometimes repeating an important conceptual issue helps understanding the data better. However, I was missing some essential experimental parameters such as:

- Chamber setup: length of sampling tubes, air flow rates and estimated losses in the tubing. Is the chamber convectively mixed to guarantee homogeneous distribution of pollutants?

- At what conditions was the SMPS operated: Sheath, aerosol flow rate, cut-off diameter of inlet impactor? The width of the size distribution indicates that possibly a substantial part of the aerosol mass distribution is beyond the SMPS diameter limit of about 900nm. Was any other sizing instrument such as an aerodynamic particle sizer used to investigate the supermicron size range?

- As a related issue, the authors report both particle mass concentration and size distribution. These values should be combined to estimate the effective particle density to provide evidence that both values are consistent. In fact the authors state that this was done (P 5075 L 8-9), but they never report the results.

4) Biological issues:

- Please provide information on the cell culture conditions such as cell density, culture m edium and conditions. Transwell inserts by Costar were used, but no information is provided regarding type of the wells (6-well, 12-well) and the pore size and material of the perforated membrane of the inserts.

- How long were the A549 cells kept at the air-liquid interface prior to exposure?

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- The cells were incubated for 9h after exposure. For an exposure time of 0.5-1h and 4h in the EAVES and GIVES, respectively, this results in a total toxin-cell contact time of 9.5-10h and 13h. Does this difference in contact time have an effect on the measured response? Did you check varying the incubation time by +/-3h to see how this may affect the measured response? I would expect that for such short incubation times +/-3h this has a significant affect on the measured IL-8 and LDH levels.

- LDH assay: Normalizing the effect level to the control levels without having a positive control is difficult to assess. For instance, if all cells would have been dead (apoptosis) by the time of exposure, there would be no enhancement of the LDH level due to particle exposure. Hence, it would be very helpful to also present some evidence of how viable the cells were at the time of exposure. One way of doing this without using an additional viability test (e.g. WST-1) would be to measure the LDH level of lysed control cells as an upper limit (100%) of the LDH level for complete apoptosis and then report the %-LDH level based on this 100% level. Please offer some information regarding this issue?

- On p.5078 L 15-16 the authors state that it was determined that the presence of PM did not interfere with the ELISA Kit of the IL-8 assay. How was this determined? Was this also investigated for the LDH assay?

- Please report the number of independent exposures (n = ?) and the number of LDH and IL-8 measurements for a given exposure (multiple determination) which were performed.

5) The y-label in Figure 2 reads “Frequency cm-3 / Bin Width / 100”. What are the values (and units) of “bin width”? Without this information the reader can not interpret the number concentration quantitatively. Please present your data in terms of dN/dlogD as is typically done in aerosol science.

Typos/Wording etc.:

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- P.5070: Please use the units “°C” instead of “°”

- P.5070 L 20: When giving the dew point temperatures, please also give a range of corresponding relative humidities.

- Section 2.4 is entitles “Gas phase compositional analysis”, but it also contains information on the condensed phase analysis

- P.5078 L 25: “0.82” should read “0.83” (see line above)

Interactive comment on Atmos. Chem. Phys. Discuss., 12, 5065, 2012.