Chemical Engineering & Applied Chemistry
UNIVERSITY OF TORONTO

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To the Thesis Review Committee,

This letter is in evaluation of Mrs. Faria Khan's doctoral thesis, titled "**Chemical Profiling and Toxicological Assessment of Atmospheric Aerosol Using Human Lung Cells**". Overall the thesis describes a body of work that substantially advances our knowledge in atmospheric chemistry and health effects. The work is novel, rigorous and comprehensive. In particular, the thesis uses a broad array of biological tools to probe the health impacts of specific aerosol systems, yielding important insights that have far reaching implications.

Overall, the thesis work is substantial and impressive. Organic aerosol is a major component of atmospheric particulate matter, and has important effects on air quality and climate change. One of the current topics in this field is how organic aerosol, whether it is formed from atmospheric oxidation (known as secondary organic aerosol, or SOA) or from emission sources such as wood burning, can affect human health when inhaled. There has been some indication that SOA or biomass burning aerosol is particularly toxic, but there is very little understanding as to why that is the case. The thesis therefore deals with an important topic in studying the links between specific components in SOA or biomass burning aerosol and their effects when exposed to human lung cells. The thesis is comprised of 4 studies, each dealing with a different aerosol system, ranging from simple (α-pinene oxidation products or nitro-phenols) to complex (biomass burning aerosol) mixtures. Cytotoxicity is measured in most of the studies as the first indication of toxicity, but each chapter proceeds to probe the mechanisms further, such as oxidative stress pathways and genotoxicity, by employing the appropriate tools. There are also attempts to link specific chemical components to toxicity, which are partially successful, but also provide directions for future research.

My questions and comments below are in the order as they appear in the thesis chapters. More detailed comments can be found in the marked up version of the thesis.

- Chapter 1 describes an in-depth literature review about SOA chemistry and health effects. I would suggest formulating specific hypotheses and/or research questions to help articulate the knowledge gaps and link them to the objectives addressed by each chapter.
- Chapter 2 outlines the experimental methods used to generate and analyze SOA, to perform in vitro cellular exposures and to measure a wide array of biological outcomes. The broad suite of tools employed in the thesis is impressive. It may be useful to add a few details for each technique to explain why that specific measurement is needed for the big picture. This will help tie the chapter contents together.
- Chapter 3.1 investigate specific chemical components in a-pinene SOA and showed that known stable chemical tracers are not particularly toxic. Rather, it is likely organic hydroperoxides that are responsible. This is a nice study that looks as specific compounds, and the conclusion about reactive organic peroxides being a source of toxicity is

convincing, especially when considering other evidence in the literature. Some minor comments are noted on the annotated PDF.

- In Chapter 3.2, the aging of 2-methyl tetrol sulfate, an important trace compound from isoprene SOA was studied, and the effects on cell toxicity, gene expression of inflammatory markers and oxidative response were investigated. Overall, heterogeneous oxidation of 2-MTS makes the aerosol more toxic, and this trend has been shown in many other aerosol systems. This chapter shows that aging affects a broader set of outcomes that other studies have shown for other system, but it remains unclear what chemical components are responsible. The PCA was not very informative, beyond the point of showing the same message: PC1, which explains almost all of the variance, is simply an indicator of aging.
- Chapter 3.3 deals with exposure to nitrophenols (NPs) and is the most comprehensive in terms of biological outcomes examined. My main question is about the mechanism for particulate NP exposure. Here NPs are dissolved into solution and then exposed to cells, but in airway exposure, at least some, if not all, of the NPs are likely still in particulate form. I am curious how this model exposure represents real-life exposure, and if the dissolution of NP, particle morphology and phase during the particles' time at the air-liquid interface would affect the biological pathways. The fundamental study about interactions with biomimetic membrane is very interesting, and makes me wonder if the NPs can actually enhance the toxicity of other components also present in PM by increase their uptake.
- Chapter 3.3 also opens up the question about why NPs can initiate these effects. It seems that the NO2 functional group plays an important role. There are also differences between the structural isomers (2-NP is very different from 3-NP and 4-NP). What is it about nitrophenols that cause it to oxidative, membrane-disrupting etc.? What would happen if phenols (without NO2) are exposed in the same manner?
- In Chapter 3.4, toxicity of biomass burning aerosol components, including levoglucosan and nitroaromatic compounds, was evaluated. This chapter provides a very detailed profile of each compound's toxicity, including effects on membrane potential (a proxy for cellular ROS defense) and cell death mechanism (apoptosis vs. necrosis) that were not studies in other systems. Nitro-catechols were found to be highly toxic, and likely dominated that toxicity in a mixture of these BBA components. The general comment here is that the chapter reads like a detailed report, and, as an atmospheric chemist, wonder why there is a difference in toxicity. Similar the comment on Chapter 3.3, it would be useful to know if there is anything that can be learned about the mechanism of action for these compounds.
- Chapter 3.4 is particularly interesting, as the detailed results are consistent with some conjectures made by an earlier paper (Tuet et al. 2019) that levoglucosan itself is not toxic, but may be correlated with compounds that are highly toxic. I am curious to see more discussion in this context.
- Chapter 4 recaps most of the results and contains a limited discussion of the future research directions. I recommend expanding the recommendations and discuss them more in the context of the thesis work. More specifically, it would be nice to see what new questions are opened by the key results in this thesis, rather just generic discussion that is essentially "more work on the same topic". To me, the thesis work is very interesting and will enable a lot of new questions for years to come. It would be nice to see the thesis articulate what these new directions could be.

The remarks stated above do not undermine a high substantive value of the dissertation reviewed and do not disturb my positive opinion on it. I raise these points merely for discussion and for my

own curiosity, and these questions are inspired by the interesting observations and conclusions from the thesis.

Taking into account the groundbreaking results obtained by Mrs Khan, a good mastery of the work technique and correct interpretation of research data, herein I conclude that her doctoral dissertation meets all conditions specified in Article 187 of the Act of July 20, 2018 Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended), and thus I am pleased to cast my vote to the Scientific Council of the Institute of Physical Chemistry Polish Academy of Sciences for the admission of Mrs. Khan to further stages of the doctoral process. I look forward to attending the final defense and wish to commend Mrs. Khan on her excellent work.

Simultaneously, based on the high quality data, which were published in the top scientific journals, the reviewed work fully deserves distinction. It is worth stressing that the comprehensive methodology developed by Mrs. Khan allows for detailed profiling of pathophysiological changes in lungs at the molecular and cellular levels after exposure to ambient aerosol, which is a significant element in releasing pollution control strategies.

Sincerely,

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