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REVIEW REPORT

doctoral dissertation of Mrs. ***Faria Khan*** *(M.Sc)*
entitled „Chemical Profiling and Toxicological Assessment of Atmospheric Aerosol Using Human Lung Cells"

1. Legal basis for the preparation of the review

The basis for the review of doctoral dissertation of Mrs. Faria Khan (M.Sc) entitled Chemical Profiling and Toxicological Assessment of Atmospheric Aerosol Using Human Lung Cells is the decision of the Scientific Council of the Institute of Physical Chemistry of the Polish Academy of Sciences with its seat in Warsaw, taken on 19 April 2021. The request for a review is included in the letter of November 22, 2021, signed by the Deputy Director of the Institute of Physical Chemistry of the Polish Academy of Sciences for research, dr hab. Jacek Gregorowicz, institute professor.

2. Assessment of the legitimacy of taking up the topic

With an unspeakable pleasure, I picked up and read the doctoral dissertation of Mrs. **Faria Khan** on determination of the exposure health effects of atmospherically-relevant submicron organic aerosol (OA) by using human lung cell lines. Airborne fine particulate matter of aerodynamic diameters < 2.5 μm (PM2.5) contributes to poor air quality, climatic change and exhibits adverse health effects upon inhalation. Issues of utmost importance in the context of the growing evidence that PM2.5 exposures trigger lung-associated pathologies, including asthma, allergy, chronic obstructive pulmonary disease (COPD) and lung cancer.

A thorough understanding of the formation and health impact of atmospheric particles requires the researcher to be able to combine knowledge from many fields, ranging from chemical and environmental engineering, through analytical and physical chemistry, to ecotoxicology and biochemistry. Dealing with issues of such an interdisciplinary nature is not easy. The researcher is required to be open and the ability to cooperate with researchers from other disciplines. Here, I would like to

emphasize that Mrs. Faria Khan skillfully used the knowledge from these disciplines, striving to effectively achieve her goals. She conducted the research in the Institute of Physical Chemistry Polish Academy of Sciences under the supervision of Prof. Rafal Szmigielski, and Prof. Jason Surratt from the University of North Carolina at Chapel Hill, who are world's leading experts in the field of the atmospheric chemistry. The revied doctoral thesis is an interesting example of the comprehensive research project on deciphering changes in human lung cells at the molecular, cellular, biochemical, and/or genomic levels induced by submicron OA exposures. I do consider the choice of the topic of the research along with hypotheses raised and their forthcoming execution fully justified.

It is very interesting and innovative to select the chemical factors that have been taken into account as sources of human health problems:

- ✓ α-pinene ($C_{10}H_{16}$), an abundantly emitted monoterpene from terrestrial vegetation;
- ✓ Isoprene (C_5H_8) - the most abundant reactive hydrocarbon released into Earth's atmosphere from vegetation;
- ✓ atmospheric-relevant mono-nitrophenols, that are found as trace pollutants in various environmental matrices, including PM2.5, agricultural residues, cloud water, rainwater, wildfires, and industrial waste;
- ✓ four biomass burning aerosol (BBA) components included levoglucosan (LG), 3-nitrosalicylic acid (NS), 4-nitrocatechol (NC), and 4-nitroguaiacol (NG), that are major pollution source, particularly in urban, suburban, and rural areas.

The main objective of the work was to chemically characterize and assess the impact on lung cell systems (A549 and BEAS-2B cell lines) of fine particles originating from four different sources:

- ✓ monoterpene-derived aerosol (SOA) obtained through the ozonolysis of α-pinene;
- ✓ heterogeneously-aged isoprene-derived particulate 2-methyltetrol sulfates (2-MTSs), which are the most abundant particulate organosulfates (OS) detected in ambient PM2.5 and contribute greatly to isoprene SOA,
- ✓ atmospheric-relevant mono-nitrophenols (NPs),
- ✓ other key components of biomass burning aerosol (BBA).

3. Formal evaluation of work

The reviewed dissertation shows a canonical structure. It begins with a well-thought and clearly set research goals. Then, there is a literature part, followed by a block of experimental procedures, and evidently a part devoted to her own research data.

The literature section consists of 20 pages. Here, the author provides basic information on the atmospheric aerosol chemistry, environmental pollution and their health effects. In my opinion, this part of the thesis already proves the author's erudition and her sound knowledge of the subject. The issues discussed are described briefly and clearly, so that the content is understandable to a wide range of readers.

In the next chapter, Mrs. Khan describes in detail how she performed experiments, syntheses and measurements. I have no doubts that the procedures/methods presented here allow for the complete reproduction of experiments by other researchers.

A 181-page chapter *Results and Disscussion* is the very heart of the dissertation, which covers over 70% of the work. It had been divided into four distinct parts.

In the first, Mrs. Khan, describes and discussed toxicological profiling of α-Pinene Ozonolysis SOA and its important atmospheric markers in the lung cells.

In the second part of the *Results and Disscussion* chapter, Mrs. Khan discussed increase the oxidative stress and inflammatory gene responses in human lung cells as a results heterogeneous oxidation products of particulate isoprene epoxydiol-derived methyltetrol sulfates exposure.

In the third part, connected with mono-nitrophenols, analysis for atmospheric prevalence of them and toxicological profiling in the model eukaryotic membranes and human lung cells were done.

In the fourth part a detailed toxicological analysis of four important biomass burning aerosol (BBA) components (a major pollution source, particularly in urban, suburban, and rural areas) in the A549 and BEAS-2B cell lines.

Mrs. Khan's thesis ends with a well-prepared summary of all results obtained and key conclusions followed by a list of acronyms used and a list of references with 395 items. In all parts, Mrs. Khan, well documented her experiments and described results in the Tabels and many Figures.

The only thing that is missing, in my opinion, is a description of how A549 and BEAS-2B cell lines are exposed to toxic substances and a justification for choosing a specific method.

The dissertation is written with understandable and generally correct language. The remarks stated above do not undermine a high substantive value of the

dissertation reviewed and do not disturb my positive opinion on it: this is a role of a reviewer to hunt for the imperfectness and errors.

4. Substantive comments

The range of issues that Mrs. Faria Khan dealt with in her doktoral studies is impressive.

I consider the following to be a significant achievement:

- ✓ three well-established α-pinene SOA tracers (pinic, pinonic, and 3-methyl-1,2,3-butanetricarboxylic acids) contributed ~57% of the α-pinene ozonolysis SOA mass Cellular proliferation, cell viability, and oxidative stress were assessed as toxicological endpoints in this study, but multifunctional hydroperoxides identified in the SOA could have contributed more than these individual secondary organic aerosol tracers to the toxicological changes observed;
- ✓ 2-methyltetrol sulfates (2-MTSs, one of the most abundant atmospheric the isoprene-derived aerosol particles) can undergo further chemical changes in the atmosphere, which leads to the formation of photochemically-aged particles of far more complex chemical compositions. As a results Mrs. Khan gain insights into how these changes might contribute to increased oxidative stress and inflammatory responses in BEAS-2B cells;
- ✓ interesting results involved toxicological profiling of atmospherically relevant NPs (2-nitrophenol, 3-nitrophenol, 4-nitrophenol) using BEAS-2B and A549 cell lines. The exposed cells were analysed for changes in general reactive oxygen species (ROS) and mitochondrial reactive oxygen species (mtROS) to predict altered biochemical pathways at different exposure concentrations and times. This study was concluded by proposing cellular death mechanisms upon exposure to these chemicals;
- ✓ analysis of effects of exposure on four BBA components included levoglucosan (LG), 3-nitrosalicylic acid (NS), 4-nitrocatechol (NC), and 4-nitroguaiacol (NG) on changes in general ROS and mtROS (to predict altered biochemical pathways at different exposure concentrations and times) provide to conclusion proposing cellular death mechanisms upon exposure to these chemicals;
- ✓ The profiling of atmospheric aerosol mixtures and their individual markers from four atmospherically relevant systems provide a comparative toxicology in lung cells (Tab. 5.1). The lowest IC50 value was exhibited by the nitroaromatic compounds (NACs) in the BEAS-2B cells including nitropenols (NPs) and four important BBA components. This was followed by the IC50 of α-pinene

ozonolysis SOA, and the particulate 2-MTSs were found to be safer at the atmospherically-relevant exposure concentrations as their IC50 values were found to be in mg/mL.

The obtained results are very valuable and constitute a significant contribution to the international scientific discussion on the effects of athmospheric pollution on the lung and the understanding of the mechanisms of disease effects.

Based on the results described in doctoral thesis, 5 publications were created, two of them are already published and discussed on scientific forum.
Joining this discussion, I would like to ask PhD student about following issues:

- → How toxic agents have been introduced into lung cell cultures to evaluate their cytotoxic effects on these cells.
- → What are the risks of the way cells are exposed to toxic compounds?

5. Conclusions

To sum up, the doctoral thesis presented to me for review takes up very important research problems. Appropriate research tools were used to solve them and care was taken to ensure that the quality of the results was appropriate.
I would like to emphasize that the doctoral student set an ambitious task for herself, approaching very broadly to toxicological assessment of atmospheric aerosol using human lung cells.
The obtained results make a significant contribution to the area of knowledge that is important for the knowledge and understanding of changes taking place in the air and their impact on human health.

Final conclusion:
Taking into account the groundbreaking results obtained by Mrs. Faria Khan, a good mastery of her 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. Faria Khan to further stages of the doctoral process.

Simultaneously, based on the high quality data, which were published in the top scientific journals, the reviewed work fully deserves distinction. It is worth nothing that comprehensive methodology developed by Mrs. Faria Khan allows for detail profiling of pathophysiological changes in lungs at the molecular and cellular levels after exposure to ambient aerosol, which is a significant element in development of air quality regulations and pollution control strategies.

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