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Doctoral Dissertation Review by Ms. Leila Khani Khouzani

The doctoral dissertation submitted for review by Ms. Leila Khani Khouzani was prepared under the supervision of Dr. Łukasz Pułaski, Professor IBM PAN, from the Laboratory of Transcription Regulation at the Institute of Medical Biology, PAN.

The doctoral candidate is applying for a PhD in medical and health sciences, specializing in medical sciences. The presented dissertation, "**Functional consequences of flame retardant action on immune cells**," is presented in English.

The dissertation is presented in a series of four publications, with additional (i) table of contents, (ii) abbreviations, (iii) theoretical introduction, (iv) objectives and hypotheses, (v) a summary of research techniques, (vi) discussion, (vii) conclusions, and (viii) a summary in Polish and English. Each of the attached publications includes an additional summary of each publication, detailing its significance in the series. Finally, the doctoral student presents a discussion, conclusions, and summary in Polish and English.

Dissertation Topic

Flame retardants are widely used in products that directly affect humans and their surroundings, such as furniture, fabrics, construction materials, and electronics. These substances are not biologically neutral and their negative impact on humans and the environment is known. Epidemiological data indicate their influence on hormone secretion, reproductive and neurodevelopmental disorders, cancer development, and immune system disorders. Flame retardants accumulate in tissues and most likely cross the placenta.

This dissertation focuses on examining the effects of flame retardants on the immune system, taking as its starting point the described effects of some flame retardants on dysregulation of humoral and cellular responses, such as a decrease in circulating antibody levels, altered Th1-Th2 or Th17 lymphocyte ratios, altered T cell maturation, and altered cytokine levels. The author of this dissertation focused on the innate immune response and phagocytic cells such as macrophages.

Toxicological studies suggest that some flame retardants may alter cell membrane properties, and consequently, membrane transport and receptor distribution. Such changes in membrane properties can have a significant impact on the functions of cells such as macrophages through alterations in processes such as endocytosis, pinocytosis, and phagocytosis. Flame retardants can also alter redox homeostasis, consequently modify the release of free radicals and altering macrophage functions, such as the killing of intracellular pathogenic bacteria. However, it should be emphasized that experimental demonstration of the role of flame retardants on macrophages has not been widely demonstrated.

The observed effects of flame retardants on immune cells led to the formulation of the doctoral student's hypotheses and research objectives.

After preliminary evaluations, the doctoral candidate selected 16 compounds for study, which are widely used worldwide as flame retardants. After assessing their effects in *ex vivo* models and cytotoxicity studies, compounds were selected for the studies presented in this dissertation.

The main research hypothesis was that exposure to selected flame retardants could have a significant impact on the structure, signaling, and function of human macrophages. In addition to the main research hypothesis, the author presents several specific research hypotheses:

1. Selected flame retardants may alter the biological properties of macrophage cell membranes

2. Selected flame retardants may modify membrane trafficking in macrophages

3. Selected flame retardants may modify redox homeostasis in macrophages

4. Selected flame retardants may affect the ability of macrophages to eliminate pathogens

The final section of the introductory part briefly discusses the research methodology. The introduction is clear and provides information essential for understanding the content of the dissertation.

The next section of this dissertation consists of four original publications:

1. Cellular and physiological mechanisms of halogenated and organophosphorus flame retardant toxicity. Khani L, Martin L, Pulaski Ł. Sci Total Environ. 2023 doi: 10.1016/j.scitotenv.2023.165272. PMID: 37406685 (IF 8.2, 200 points MNiSW, 30 citations)

2. Tetrabromobisphenol A, but not bisphenol A, disrupts plasma membrane homeostasis in myeloid cell models - A novel threat from an established persistent organic pollutant. Khani L, Studzian M, Martins L, Gorzkiewicz M, Pułaski Ł.Sci Total Environ. 2025 Jan 20;961:178284. doi: 10.1016/j.scitotenv.2024.178284. PMID: 39798458 (IF 8, 200 points MniSW, 2 citations)

3. TBEP and TCP impair metabolic and immune functions in human macrophages: a novel redox related activity with potential immunotoxic consequences; Khani L., Studzian M, Martins L., Pulaski Ł. Submitted to Environment International (July 2025, IF 9,7; 140 points MNiSW)

4. The brominated flame retardant DecaBDE inhibits low-density lipoprotein micropinocytosis in human M2 macrophages **Khani L.**, Studzian M, Martins L., Pulaski Ł. Submitted to Archives of Toxicology (July 2025, IF 6,9; 140 points MNiSW)

In a doctoral dissertation presenting published or peer-reviewed articles or manuscripts, the doctoral student's individual and leading contribution to the work should be highlighted. Each publication is accompanied by a statement from the authors confirming their participation in the work. Therefore, I have a concern regarding the clarification of the doctoral candidate leading role in the experimental part, e.g., which methods were developed by the doctoral candidate and which experiments were performed by her. For example, in publication no. 4, Leila Khani Khouzani's role is defined as "developing research methodology, performing the experiments and data analysis, writing the initial draft, reviewing and editing the manuscript, and data visualization." Co-author Maciej Studzian, in his statement, indicates the identical role of "developing research methodology, performing the experiments and data analysis, writing the initial draft, reviewing and editing the manuscript, and data visualization."

Originality of the Dissertation

Publication No. 1 is a review article and constitutes a very extensive analysis of the literature on flame retardant toxicity. I agree with the doctoral candidate's statement that preparing this work laid the conceptual foundation for a whole series of studies presented in subsequent publications. The analysis is very extensive and critical; systematizing issues scattered throughout the literature on the presented topic. Of note is the graphical editorial aspect of this publication (prepared by the co-author), which greatly facilitates understanding the mechanisms of retardant effects on cells.

Publication No. 2 concerns the effect of halogenated flame retardants on the properties of cell membranes. The study compares the effect of the well-known endocrine disruptor bisphenol A with its counterpart tetrabisphenol A. The primary conclusion from the published experiments was the confirmation of the thesis about the biophysical mechanism of the potential immunotoxicity of hydrophobic flame retardants, associated with membrane disruption and direct interaction with its components. A valuable result was the detection of different effects of the tested compounds on outer membranes and organelle membranes.

Publication No. 3 tested the hypothesis that flame retardants can modulate membrane properties and influence their function related to lipid transport in a cellular model. It was shown that some compounds impaired LDL uptake by reducing pinocytosis by disrupting the regulatory cascade in the cell. The tested retardants did not impair endocytosis, phagocytosis, or cell viability. The obtained results indicated a role for regulating the expression of specific genes in macrophages involved in the pinocytosis process.

Publication No. 4 describes tests of the effect of flame retardants on macrophage activity against pathogenic bacteria. The doctoral candidate demonstrated that the tested compounds disrupt the redox balance, which affects macrophage functions related to killing intracellular pathogens.

The main goal of the dissertation was to determine the immunotoxic effects of flame retardants at the level of molecular interactions, cellular response, and their effect on function. The PhD candidate demonstrated that selected flame retardants impair macrophage function through distinct mechanisms that modulate innate immunity. The work involved examining biophysical membrane parameters, membrane metabolism, redox balance, and the elimination of a selected pathogen (Staphylococcus aureus). The PhD candidate's summary included the following conclusions:

1. The range of immunotoxic properties and induced effects between the tested flame retardants was smaller than expected, based on knowledge of the complex immune system.

2. The PhD candidate identified three new mechanisms of potential immunotoxicity.

3. The observed effects of flame retardants on cells corresponded to concentrations found in highly polluted environments, rather than typical everyday use concentrations.

4. The study observed a relationship between the hydrophobicity/hydrophilicity of flame retardants and the cellular structures they affect.

5. Compounds such as TBBPA interact differently with inner and outer cell membranes.

6. Some effects of flame retardants may affect cells indirectly by regulating signaling pathways.

7. Selective inhibition of LDL uptake via pinocytosis does not affect general macrophage metabolic disorders and has the potential to influence foam cell formation. This observation requires further investigation.

8. From a toxicological perspective, the specific consideration of increased ROS production as harmful is not justified in the case of immune cells, as illustrated by the immunotoxicity of TCP mediated by inhibition of ROS production.

9. Inhibition of ROS and ATP production in the absence of phagocytic properties leads to impaired pathogen killing by macrophages and may have protective properties for pathogens.

Critical Comments/Issues to be Discussed During the Defense

The doctoral candidate's thesis focused on examining the effects of flame retardants on various aspects of cell membrane function in macrophages. From a microbiologist's perspective, I was most interested in the effect of flame retardants on pathogen-host interactions between macrophages and *Staph. aureus*.

Could I ask the doctoral candidate to design/propose a series of experiments on the role of flame retardants on the interactions between phagocytic cells and other bacteria, including intracellular pathogens? What mechanisms, besides altered ROS and ATP levels, could be disrupted in the interactions between macrophages and pathogens?

Final Assessment

The doctoral dissertation submitted for evaluation represents an original solution to a scientific problem. The doctoral student demonstrated theoretical knowledge in the field of her research and the ability to conduct scientific research and solve scientific problems.

Based on the evaluation of the dissertation of Ms. Leila Khani Khouzani, I can state that the submitted dissertation meets the requirements for doctoral dissertations specified in the Act of 20 July 2018, "Law on Higher Education and Science" (Journal of Laws of 2018, item 1668, as amended).

Based on the above, I request that Scientific Advisory Board of the Institute for Medical Biology of Polish Academy of Sciences admit Ms. Leila Khani Khouzani, to the next stages of the proceedings for the award of a doctoral degree in the field of medical and health sciences, in the discipline of medical science

Warszawa 11.01.26