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Review of the Doctoral Dissertation of Leila Khani Khouzani

The doctoral dissertation of Leila Khani Khouzani entitled *"Functional outcomes of flame retardant action in immune cells"* was carried out under the supervision of Dr. hab. Łukasz Pułaski, Professor at the Institute of Medical Biology of the Polish Academy of Sciences (IBM PAN), in the Laboratory of Transcriptional Regulation at the Institute of Medical Biology, PAS, in Łódź. The dissertation describes research conducted with the participation of the PhD Candidate aimed at elucidating the molecular mechanisms of action of flame retardants on macrophage biology, including biophysical and biochemical changes in their cell membranes, redox balance, and antibacterial potential. The dissertation was presented as a thematically coherent collection of articles, comprising two published papers and two manuscripts which, at the time of preparation of this review, had not yet been published but had been submitted for review in reputable scientific journals. The texts of these publications constitute an integral part of the dissertation and are preceded by a twenty-page short description of the achievement. Statements from all co-authors of the publications were also attached, describing the contribution of each individual. In summary, the dissertation submitted for evaluation meets the formal criteria required for this type of work.

Subject of the Dissertation

The subject matter of the dissertation is timely. The widespread use of flame retardants constitutes a global problem; however, the reported side effects of these compounds on humans are largely descriptive in nature and mainly present observations of changes in physiological and cellular parameters. The evaluated work makes a significant contribution to the development of the discipline, as it focuses on understanding the molecular basis and identifying the mechanisms of action of selected compounds. The choice of macrophages as the subject of study, cells rarely described in the context of the harmful effects of the investigated xenobiotics, also represents an element of novelty and is of particular value due to the key role of these cells in inflammatory diseases, infections, cancer, and tissue regeneration. The obtained results demonstrating that selected brominated and organophosphate flame retardants alter macrophage functions constitute an important scientific discovery. They confirm the need for further research on the immunoregulatory role of flame retardants in the biology of these cells, as well as in other components of the immune system network.

Candidate's Knowledge

Reading the dissertation allows one to conclude that the PhD Candidate possesses extensive theoretical knowledge in biochemistry, immunology, and cell

biology. Undoubtedly, the preparation of the first publication in the series in the form of a review article was an excellent tutoring strategy by the Supervisor and contributed to deepening the Candidate's knowledge. A good understanding of the subject is also evidenced by the description provided in the introduction and the presentation of results obtained during the doctoral studies, indicating that the Candidate is able to identify the most important elements of the work and describe them in a concise and factual manner. An excellent example of the Candidate's knowledge in the subject is the discussion section, in which she refers to various aspects of flame retardant activity, including clinical consequences, while also drawing attention to alternative interpretations of the obtained data and proposing further research perspectives. The critical evaluation of the results constitutes clear evidence of the knowledge acquired by the Candidate during the implementation of her project.

Candidate's Independence

The texts of the attached publications and manuscripts indicate that the Candidate is a mature scientist who is capable of independently formulating hypotheses and then planning and ultimately conducting research to verify them. In the statements attached to each of the included publications/manuscripts, the PhD Candidate's contribution is indicated as including, among others, optimization of research methodology, performance and analysis of experimental results, and preparation and revision of manuscripts.

Originality of the Dissertation

The dissertation constitutes an original solution to the research problem. First, this is evidenced by the choice of macrophages as the experimental model for studies on flame retardants. It is worth emphasizing that in her research the Candidate used numerous cell lines as well as primary cells, with particular attention paid to their polarization, which is a major advantage, as this unfortunately does not constitute a standard in this type of research. Moreover, the work does not replicate established research schemes, but is an original and, most importantly, comprehensive guide to studies on flame retardants, taking into account the appropriate selection of molecules and complementary or alternative research methods that allow for the assessment of both rapid and delayed cellular responses. This approach, differing from those previously applied by researchers, together with the use of macrophage models appropriate to the research needs, enabled the identification of distinct molecular bases of cellular responses to short- and long-term exposure to the investigated xenobiotics. Particularly valuable is the methodological selection of compounds for the study, taking into account various chemical modifications, appropriate control compounds, and the use of concentrations non-toxic to the studied cells.

Critical Remarks / Questions

1. According to the statements attached to each paper, the PhD Candidate had a significant contribution to their preparation; however, for the sake of clarity, it would be worthwhile to specify which particular experiments were performed by her.

2. The Candidate indicates that verification of the effects of flame retardants on macrophage biology should be conducted using in vivo models. At the same time, she critically refers to zebrafish (*Danio rerio*) and rat models described in the literature. Please present a possible scenario for further in vivo studies proposed by the Candidate that, in her opinion, would be optimal.
3. Can the studied flame retardants alter the biology of epithelial cells, which have direct contact with these compounds, and thereby indirectly affect the functions of tissue macrophages? What research model could be proposed to verify this indirect effect on macrophages?
4. In the studies presented in manuscript no. 4, the effect of TCP on redox balance was demonstrated, which consequently reduces the ability of macrophages to eliminate *S. aureus* observed 6 hours after infection. Were attempts made to assess the effect of TCP on long-term (several-day) survival of *S. aureus* inside macrophages? Can a research model be proposed using bacteria less sensitive or insensitive to ROS-mediated killing, as well as a macrophage model in which bacterial elimination is dominated by enzymes and antibacterial peptides?
5. In the studies presented in manuscript no. 4, an effect of TCP on mitochondrial metabolism was demonstrated, could this be relevant in cancer biology and the phenotype of tumor-associated macrophages (TAMs)?

Final Evaluation

In the presented dissertation, the PhD Candidate also summarized her scientific activity, which should be considered impressive at this stage of her scientific career. She listed the number of experimental publications of which she is or will be a co-author, participation in scientific conferences, awards, and scientific internships. This information indicates additional active scientific engagement by the Candidate beyond the implementation of her doctoral research.

In summarizing this review, it is worth emphasizing the well-developed structure of the dissertation. It begins with a thorough analysis of the subject, enabling identification of the research problem and planning of the studies, followed by the formulation of research hypotheses that were verified during the course of experimental work. The Author fully achieved the planned objectives using numerous methods appropriate to the research needs, which demonstrates that she has mastered the necessary research techniques. It is worth highlighting the high scientific value of the conducted studies and their relevance to public health needs. The obtained results are innovative and, crucially, highlight the need for detailed research on flame retardants and indicate the necessity of increasing control over their industrial use in order to protect human health. This work constitutes a very strong contribution that demonstrates the Candidate's knowledge, independence, and thus her scientific maturity. I rate it very highly. The presented doctoral dissertation therefore meets the formal criteria required for this type of work, i.e. the conditions specified in art. 187 ust. 1-2 ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce (t. j. Dz. U. z 2024 r. poz. 1571). On this basis, I am applying to the Scientific Council of the Institute of Medical Biology of the Polish Academy of Sciences to admit Leila Khani Khouzani to the further stages of the procedure for conferring the doctoral degree in the field of medical and health sciences, discipline: medical sciences.

Moreover, I consider the doctoral dissertation worthy of an award due to its high scientific value, originality of the research concept, and the opening of further scientific research perspectives that will enable a reliable assessment of the mechanisms of action of flame retardants on human health.

Prodziekan
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Prof. dr hab. Joanna Kozieł