



November 14, 2023

Assessment report

for Ph.D. thesis, in doctoral degree program Pharmacology and Toxicology, of Patrícia Alexandra Alves Dias, M.Sc.: "Study of the mechanisms of action of phenolic compounds on the vascular smooth muscle". Supervisor prof. Přemysl Mladěnka, Pharm.D., Ph.D., and consultant assoc. prof. Jana Pourová, Pharm.D., Ph.D.

The reviewing thesis deals with the cardiovascular effects and mechanisms of action of two major categories of phenolic compounds: dietary phenolic metabolites, 3-hydroxyphenylacetic acid (3-HPAA), and bisphenols focused on the newly introduced NextGen bisphenols (bisphenols A, AF, AP, B, BP, C, E, F, G, M, P, PH, S, and Z).

The entire project is fully based on the long-term scientific and research focus of the trainer's workplace. Right at the beginning of the review, it is necessary to state that the work is based on very extensive experimental material, which was part of grant projects implemented at the training workplace with the financial support of several grant agencies. This fact fully confirms the very high professional, methodical, and result level of the assessed work. According to the doctoral student's publication letter, it is also clear that most of the originally obtained results have already been reviewed. I highly appreciate the first authorship of publications with a high impact factor (8,9; 5,7, and 5,6).

The presented Ph.D. thesis puzzled basic *in vivo*, *in situ*, and *in vitro* experimental results of dietary phenolic metabolites 3-HPAA as well as NextGen bisphenols with systematic review focused on the well-known endocrine disruptor bisphenol A (BPA), its novel congeners, and their potentially harmful effects on the cardiovascular system. The Ph.D. student in her thesis designed an extremely wide scale of experimental techniques and methods for validation of the cardiovascular effects and mechanisms of action of phenolic compounds: dietary phenolic metabolites and possible pollutant bisphenols. Applying this attitude, the new original data about vascular bioactivity tested phenols proposed cardiovascular beneficial effects of the small phenolic metabolite 3-HPAA and that most of the NextGen bisphenols showed vasodilatory effects, albeit at much higher concentrations than their typical exposure levels. However, the author proposes that the possible harmful effects of NextGen bisphenols have to be studied in long-term toxicological investigations.

The manuscript is written according to the standard style and format required for a Ph.D. thesis and consists of 111 pages divided into 11 chapters. The integral parts of the thesis are the abstract, list of tables, figures, abbreviations, copyrighted materials, contributions of authors, and list of 242 cited relevant literature sources. The selection of up-to-date references covers all attributions of theoretical reviews, methodologies of experimental and clinical studies, as well as discussions of thesis, and clearly demonstrates the good orientation of the candidate in the field of her study.

In the literature review, the author discusses in detail the issues of cardiovascular physiology and phenolic compounds. I very much appreciate the logical structure and the very clear picture and graphic processing of the data, which makes it very easy to understand a very complex issue. However, the excessive atomization of the text has a somewhat disturbing effect; the theoretical background is divided into 4 main subchapters and 22 related subsections within 24 pages.

In Chapter 3, the chapter 3 Aims of the doctoral thesis is presented as a separate chapter, which only very briefly introduces the reader to the experimental issues of the work presented. Although the publications included as results of the work define the hypotheses, aims, and methods, as a reader, I would have

appreciated much more details that would integrate the entire experimental work and facilitate the understanding of all the experimental data and the structure of the discussion.

The precision and successful experimental work create sufficient original data already published. Because the process of publication in these journals undergoes rigorous reviewing and requires the acceptance of selected assessors and editors, there are no open questions or problems for me.

The Discussion of Ph.D. thesis is the best part of the manuscript. The author successfully recapitulated the most important results and compared them with published ones. The 10 pages of discussion are written very consistently and clearly demonstrate the competence to complete a research project and present the findings in comparison to up-to-date science knowledge, findings, and discoveries.

Conclusions (Chapter 7) are concise and logically formulated and briefly summarize the data from the experimental work. However, I miss the relevant summarization of all results from published data, while the author summarised separately data from each published article in Chapter 5 Commentary on Published Works.

Summarisation:

I have carefully assessed Patricia Alexandra Alves Dias's Ph.D. thesis manuscript and concluded that it is unambiguously suitable for awarding a doctorate of philosophy. My decision is based on the original approach to problems, a clearly stated hypothesis, the aims and tasks of experimental and clinical work, precise experiments and trial realization, as well as the candidate's competence to complete a research project, present the findings, and discuss them within the present status of science. The thesis constitutes the original data and findings that are a distinct contribution to knowledge in the field of cardiovascular systems, with a focus on the bioactivity of phenols. The excellent level of the thesis reflexes in three published papers impacts factors (8,9 5,7, and 5,6). I appreciate the opportunity to consider this work.

Questions:

1. Do you have any idea about whether the effectiveness of 3-HPAA is comparable to that of existing vasoactive drugs?
2. The European Food Safety Authority (EFSA) (2023) has carried out a re-evaluation of risks to public health related to the presence of bisphenol A in foodstuffs. EFSA established a 20,000-fold lowering of the tolerable daily intake for BPA from 4 µg/kg bw per day to 0.2 ng/kg bw per day. EMA is not in agreement with the currently revised TDI. What is your opinion?

Final decision:

Evaluation of Ph.D. thesis of Patrícia Alexandra Alves Dias, M.Sc.: "Study of the mechanisms of action of phenolic compounds on the vascular smooth muscle" (supervisor prof. Přemysl Mladěnka, Pharm.D., Ph.D. and consultant assoc. prof. Jana Pourová, Pharm.D., Ph.D.) - **Pass**.

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