Opinion

by Assoc. Prof. Dr. Stela Mironova Statkova-Abeghe
Department of Organic Chemistry, PU «P. Hilendarski»

of a dissertation for awarding for awarding an educational and scientific degree
"PHILOSOPHY DOCTOR"
Area of higher education: 4. Natural sciences, mathematics and informatics
Professional area: 4.2. Chemical sciences
Scientific speciality: „Bioorganic chemistry, chemistry of natural and physiological active substances"
Author: Temenuga Petrova Trifonova
Topic: INVESTIGATION OF PERISTENT ORGANIC POLLUTANTS IN BREAST MILK
Research supervisor: Assoc. Prof. Stanislava Georgieva, Ph.D., MU – Varna

1. General description of the presented materials

By order No. P-109-131/ 01.04.2024 of the Rector of the Medical University - Varna, I have been appointed as a member of the scientific jury in the procedure for the defense of a dissertation work on the topic "RESEARCH OF PERSISTENCY ORGANIC POLLUTANTS IN MOTHER'S MILK" for the acquisition of the educational and scientific degree "PHILOSOPHY DOCTOR" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.2. Chemical sciences, doctoral program - „Bioorganic chemistry, chemistry of natural and physiological active substances". The author of the dissertation work is assistant professor Temenuga Petrova Trifonova - full-time Ph.D. student at the Department of Chemistry of the Faculty of Pharmacy, supervised by Assoc. Professor Stanislava Georgieva, Ph.D., MU - Varna.

The set of papers and electronic materials presented by assistant professor Temenuga Petrova Trifonova are in complete accordance with the Regulations for the development of the academic staff at the Medical University - Varna and include the necessary documents. The documents are correctly formatted and there is no need to reduce the relevant numbers due to overlap in content and absence of required documents certifying the publication.

Temenuga Trifonova was born in 1971. She graduated from the University of Plovdiv with a Master's degree in Chemistry in 1995. She owns a postgraduate qualification in "Sanitary Chemistry" (2002) from the Medical University - Sofia. Her work experience is mainly related to MU - Varna, as an assistant and chief assistant professor for the period 1999 - 2015. Since 2013, he has been a chemist-assistant in the Cyclotron Complex of the "Sveta Marina" UMBAL, Varna. From 2019 to 2023, she is a full-time Ph.D. student in the Department of Chemistry of the Faculty of Pharmacy at the University of Medicine - Varna.

2. Relevance of the topic and appropriateness of the set goals and tasks

The development and validation of a gas chromatographic method for studying the levels of persistent organochlorine contaminants in breast milk contributes to the accumulation of systematic data on the levels of persistent organic pollutants and for the assessment of the exposure of part of the population in Bulgaria. The combination of toxicity, persistence, ability for distribution and bioaccumulation of these pollutants turns them into a potential threat to human health and the environment, which determines the relevance of the topic.

A gas chromatographic method with mass detection was modified and validated for the simultaneous quantitative determination of POPs from the groups of PCBs and POPs in breast milk. The analytical approaches developed in the current dissertation work are both relevant from purely scientific and scientific-applied point of view. The main purpose and scientific

goals are clearly formulated, correctly, expediently and fully correspond to the main topic of the dissertation.

3. Characterization and evaluation of the dissertation work

The dissertation is written on 176 pages, including 37 figures, 24 tables and 7 appendices. 244 literary sources are cited and only 4% of them are in Cyrillic. The research related to dissertation was carried out at MU - Varna. The design of the study is in accordance with the requirements of the Central Ethics Committee and it is based on a protocol developed by WHO/UNEP for the monitoring of POPs from 2017. The guidelines regarding: the number of samples; the selection of donors; collection and storage of samples; the analysis of breast milk for persistent organic pollutants are correctly followed. A total of 29 persistent organochlorine pollutants are investigated. They are from two groups - polychlorinated biphenyls and organochlorine pesticides, selected on the basic data on import and use of pesticides in Bulgaria.

An analytical procedure developed in the same department of the Medical University - Varna in 2019, according to the analytical protocol based on the European standardized method EN ISO 1528-1996 is successfully applied. A method is developed and adapted for the extraction and determination of lipids by varying the type and volume of extractants and their mixing ratios. The lipid content is determined by the gravimetric method according to BDS (EN ISO 1211:2010).

Chromatographic separation of individual PCBs and POPs is optimized by varying the temperature modes of the gas chromatographic system, and detection of the analytes is performed by optimizing the parameters of mass spectrometer. The validation of the analytical method is performed by determination of the main analytical parameters (according to the requirements of BDS EN ISO/IEC 17025/AC:2006). It is notable that polychlorinated biphenyls and organochlorine pesticides are found in all examined breast milk samples, as well as the increasing the PCBs concentrations of breast milk with increasing the age of mothers.

The obtained results are summarized and systematized by analyzing the dependences between the levels of PCBs and the levels of DDTs and basic demographic characteristics of the participants in the study. The results of the study show a tendency of decreasing the levels of lipophilic pollutants in breast milk during the breastfeeding period. The obtained results confirm that lactation is the main route of excretion of POPs from the woman's body.

The presented experimental results, their analysis and discussion confirm the credibility of the research and support the formulated contributions of the dissertation work. There is no doubt about the personal contribution of the Ph.D. student toward the gained experimental results.

4. Contributions and significance of the research for the science and practice

Scientific, scientific-applied and applied contributions

The systematic examination of individual breast milk samples for residual amounts of persistent organic pollutants from the two groups of polychlorinated biphenyls and organochlorine pesticides is a scientific contribution by development of existing knowledge.

The established relationship between the levels of POPs in breast milk, the individual demographic characteristics and dietary habits of mothers are a scientific contribution with a high potential for practical application.

The assessment made of the actual maternal exposure and potential health risk related to persistent organic pollutants based on their levels in breast milk is a scientific contribution with practical importance.

Two highly efficient gas chromatographic methods with mass detection for the determination of: 15 polychlorinated biphenyls and 14 organochlorine pesticides in breast

milk have been successfully modified and validated. They can be applied for the analysis of various biological samples.

5. Evaluation of publications on the dissertation work

The results achieved in the dissertation work are summarized in three articles, two of them are in leading scientific journals (*International journal of hygiene and environmental health* Q1 and *Journal of IMAB* - Q3) and one in *Bulgarian chemical communications* Q4. In two of the publications, assistant professor Temenuga Trifonova is the first author, which clearly proves her contribution to the achievement of the presented results. The Thesis abstract corresponds to the content of the dissertation, reflecting the main results achieved and described in the dissertation. It is written briefly and analytically, in correct chemical language. Charts and tables reflect the published results. The Thesis abstract is prepared in accordance with the requirements.

CONCLUSION

The dissertation **contains scientific, scientific-applied and applied results, which represent an original contribution to science** and meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of the LDASRB and the Regulations of the Medical University - Varna for DAS. The presented materials and dissertation results **fully correspond** to the minimum national scientometric requirements for the obtaining of educational and scientific degree "PHILOSOPHY DOCTOR" and exceed them by about 70% (50 points).

The dissertation shows that the Ph.D. student Temenuga Petrova Trifonova possesses in-depth theoretical knowledge and professional skills in the scientific fields - "Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances" by demonstrating qualities and skills for independent conducting of scientific research.

In connection with the aforementioned, I confidently give my **positive** assessment of the conducted research, achieved results and contributions, and propose to the honourable scientific **jury to award the educational and scientific degree** "PHILOSOPHY DOCTOR" to assistant professor Temenuga Petrova Trifonova in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.2. Chemical sciences, doctoral program - Bioorganic chemistry, chemistry of natural and physiologically active substances.

29.05.2024 г.

Plovdiv

Member of the scientific jury:

(Assoc. Prof. Dr. Stela Statkova-Abeghe)