

REVIEW

by Assoc. prof. Albena Merdzhanova, PhD

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Designated as a member of the Scientific Jury by Order № P-109-131/01.04.2024.

by the Rector of Medical University - Varna

selected to prepare a Review (Protocol №1/10.04.2024)

for the acquisition of the educational and scientific degree "Doctor" in the field of Higher Education 4. Natural sciences, mathematics and informatics, professional field 4.2. Chemical sciences and Doctoral Program: Bioorganic chemistry, chemistry of natural and physiologically active substances

Topic: "Investigation of persistent organic pollutants in breast milk".

Author: Temenuga Petrova Trifonova, full-time PhD student, enrolled by order № P109-84/01.02.2019 at the Department of Chemistry, Faculty of Pharmacy, Medical University - Varna.

Scientific supervisor: Assoc. prof. Stanislava Georgieva, PhD

General information of the procedure

The review of the materials submitted in the competition is based on the requirements of the Act on Development of Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, as well as the Regulations on the Conditions and Procedure for the Acquisition of Scientific Degrees and Holding of Academic Positions and the Qualitative Criteria for the Development of the Academic Staff of Medical University - Varna. No procedural violations, conflict of interest and remarks on the submitted materials were found.

Brief biographical data

Temenuga Trifonova obtained in 1995 a Master's degree in Chemistry from Plovdiv University "Paisii Hilendarski", specialty *Organic Chemistry*. In 2002 she specialized in *Sanitary Chemistry* at the Medical University - Sofia. She has worked as a chemist in the laboratories "Food toxicology" and "Industrial-sanitary chemistry" at the Regional Health Inspectorate - Varna. Currently she works both as an assistant professor at the Department of Pharmaceutical Technologies, Faculty of Pharmacy, Medical University - Varna and as a chemist-assistant at the Cyclotron Complex of the University Hospital "St. Marina" - Varna. She has teaching qualifications, communication skills, teamwork skills and professional experience in the field of non-governmental environmental organizations.

Brief description of the structure of the thesis

The thesis contains 178 pages, including Introduction (2 pages), Literature Review (20 pages), Experimental Section (20 pages), Results and Discussion (60 pages), Conclusions (4 pages) and References (17 pages). The thesis includes 37 figures, 24 tables and 25 pages of appendices (7 figures). 244 references are cited, of which 9 are in Bulgarian and 235 in English. The experimental work was carried out at the Department of Chemistry, Medical University - Varna.

Relevance of the topic

The topic of the peer-reviewed thesis is well chosen, in accordance with the interests of the PhD student and the experience of the supervisor.

Persistent organic pollutants (POPs) are chemical compounds of anthropogenic origin that are toxic and stable to degradation. This type of pollutants has the ability to bioaccumulate in living organisms, including humans through food chains, whereby they significantly increase their concentrations in tissues. The unfavourable combination of toxicity, persistence, ability to spread and accumulation makes POPs a potential threat to human health and the environment.

A valuable tool for assessing human exposure to persistent contaminants is so-called biomonitoring. Biomonitoring data directly reflect the residual amounts of POPs in the human body, taking into account all sources of entry into the body as well as individual specificities in exposure levels, metabolism and excretion rates. Due to the lipophilic nature of POPs, they are studied in biological fluids. Breast milk has a higher lipid content than other biological fluids such

as blood, which has led international experts to recommend it for assessing actual maternal exposure and as a non-invasive method for real and reliable human biomonitoring.

The presence of persistent contaminants in breast milk has been confirmed by a number of international studies, which is why the World Health Organization (WHO), in collaboration with UNEP, is conducting international studies aimed at determining the concentrations of organic contaminants in breast milk, in order to assess exposure and the effectiveness of measures to reduce the use of POPs as set out in the Stockholm Convention.

Data on POPs levels in breast milk of Bulgarian mothers are very limited. The present thesis focuses on new studies of POPs (polychlorinated biphenyls and organochlorine pesticides) levels in breast milk from mothers in the Northeast region of Bulgaria. Therefore, I believe that the peer-reviewed thesis is relevant and would contribute to the accumulation of systematic scientific data on POPs levels, which could be used to assess the exposure of part of the population in this region.

Characteristics and structure of the thesis

The structure of the thesis is balanced and in accordance with the modern requirements for the proportion of parts, without unnecessary theorizing, but with sufficient focus on the results, their statistical treatment and interpretation.

The literature review presents the general characterization and classification of POPs, a detailed description of industrial persistent organic compounds such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs); unintentional by-products such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzodioxins and polychlorinated dibenzofurans, hexachlorobenzene, polychlorinated biphenyls, which are formed as unwanted by-products in the production of chlorinated compounds, in the incomplete combustion of hospital, municipal and hazardous industrial wastes or in landfill fires. Their distribution in the environment is described in detail.

Emphasis is placed on the toxicity of POPs classified by WHO as moderately hazardous to organisms, the manner in which they enter the human body, the maximum permissible levels of residues of DDTs in food products are described in detail, the toxic equivalence factor, the toxic equivalent concentration (TEQ), the ways of assessing the health risk of exposure to complex mixtures of POPs, etc. are commented. Particular attention is paid to the possibility of using breast milk as a bioindicator of POPs, the possibility that lipophilic persistent contaminants present in

maternal adipose tissue may pass along with bioactive components in breast milk is described. It can be assumed that concentrations of toxic substances in breast milk are representative of the levels of these contaminants contained in maternal serum lipids and adipose tissue therefore POP concentrations in breast milk reflect the maternal body burden and can be used as an indicator of total human exposure. International studies of POPs content in breast milk from 45 countries, including Europe and Asia, are presented.

In Bulgaria, no systematic studies have been conducted on levels of persistent organochlorine contaminants in breast milk or blood plasma.

The only data on concentrations of organic pollutants in breast milk were obtained in an international study coordinated by WHO in 2001-2002 from three regions of the country - an ecologically clean region - Bankya and two regions with different degrees of ecological pollution - Sofia and Blagoevgrad. The results showed that the cumulative concentrations of indicator PCBs and dioxin-like contaminants in breast milk from Bulgaria exceeded the calculated safe levels of these compounds for infants, but were lower than the maximum permissible concentrations.

The aim of this thesis is to investigate persistent organic pollutants (POPs) in breast milk of women from North-eastern Bulgaria and assess the potential health risk. The 6 tasks are clearly formulated in a logical sequence and are consistent with the stated aim.

In the Experimental part, the methodology of the study of POPs in breast milk from voluntary breast milk donors from mothers breastfeeding in the city of Varna and Varna region, as well as from the city of Dobrich and Dobrich region is presented in detail. The rationale for selection of donors in the study, recruitment of participants is described in detail. The study of POPs in breast milk samples was carried out using an applied analytical procedure developed at the Department of Chemistry of MU-Varna, based on the European standardized method EN ISO 1528-1996. The instrumental optimized parameters of the gas chromatographic system for qualitative identification and quantification of PCBs and individual POPs are presented in tabular form.

The validation procedure of the analytical method by determination of basic analytical parameters is described in detail. Classical methods are used for quality control to ensure the reliability of the procedure. Adequate methods for statistical processing of the obtained results are selected.

In the Results and Discussion chapter, the thesis describes in detail the experimental results obtained, but at the same time discusses the changes observed, which shows the systematic approach in the implementation of the research. Data on the content of polychlorinated biphenyls

and of 14 organochlorine pesticides, which are persistent pollutants included in the Stockholm Convention, are presented. The influence of maternal age, body weight order on PCB content in breast milk is thoroughly reviewed and discussed. Similarly, with emphasis on the influence of age and birth order, results for organochlorine pesticide content are presented. The well-structured summaries of the contents of these contaminants after each section show that the PhD student has acquired the analytical and statistical methods of analysis used.

The thesis presented a detailed and insightful assessment of exposure and potential risk to infant health by calculating daily intakes and comparing the resulting data to regulatory standards. The summarized results indicate that infants are not at risk of adverse effects caused by indicator PCBs via breast milk. Hazard quotients, (HQs) were calculated for the sum of DDT, toxic equivalents for dl-PCBs, and specific ratios of POPs in breast milk.

The conclusions (14 items) reflect the experimental results fairly correctly and logically. The conclusions significantly exceed the objectives. I believe they are too many and could be shortened and merged.

Publication activity

Two full-text publications have been published on the topic of the thesis, one full-text publication has been accepted for publication. All publications are in refereed and indexed by Scopus and Web of Science journals (1-Q1, 1-Q3, 1-Q4). As. Trifonova is the first author in two of these publications. Seven participations in scientific forums are presented, of which six are national forums, one international participation - held online.

The scientific activity of the PhD student is in accordance with the Act on the Development of Academic Staff of the Republic of Bulgaria and the Regulations for its application at MU-Varna.

Personal contribution of the PhD student

The thesis ends with 3 contributions of original character and 3 contributions of scientific and applied character. The research has a practical focus related to the modification and validation of a gas chromatographic method with mass detection for the determination of 15 PCBs and 14 organochlorine pesticides in breast milk, which can be applied to the analysis of other biological samples and has allowed the systematic screening of individual breast milk samples for residues of persistent organic pollutants.

Critical comments and recommendations

I have a few critical remarks that do not diminish the quality of the thesis presented. I would recommend that some of the data presented in tables in the Results and Discussion chapter be moved to the Appendices. I consider that the graphs illustrate the results well enough. I recommend as. Trifonova to participate also in international scientific forums where to disseminate the results obtained. Despite the fact that the author states that the conducted researches on the scientific theme are new for Bulgaria, they are primarily of regional importance, which implies their continuation in other more comprehensive studies.

Conclusion

The thesis fully complies with the requirements of the Regulations for the Development of Academic Staff and the minimum science metrics of MU-Varna, and covers the requirements of the PhD program in *Bioorganic Chemistry, Chemistry of Natural and Physiologically Active Substances*. The set goals and objectives have been fulfilled, the described experiment is sufficient in volume, which allows to draw relatively accurate conclusions. The PhD student has acquired and applied highly efficient chromatographic methods, presenting a good analytical approach and appropriate statistical analysis in the presentation of the results.

In view of the foregoing, I give my *positive evaluation* for the presented thesis and propose the scientific jury to vote positively for the award of the Temenuga Trifonova the degree of Doctor of Education and Science in the field of Higher Education **4. Natural sciences, mathematics and informatics**, professional field **4.2. Chemical sciences** and scientific specialty: **Bioorganic chemistry, chemistry of natural and physiologically active substances**

May 29, 2024

Varna

Reviewer:

(Assoc. prof. A. Merdzhanova, PhD)

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