ABSTRACT

of the dissertation «Mercury pollution of the Nura River and assessment of its genetic and immunological effects in people living in a mercury-containing territory» of Lyazzat E. Shinetova for the Degree Doctor of Philosophy (PhD) in Biology – 6D060700

The relevance of the research. Scientific and technological achievements and global industrialization contribute to an increase in man-made emissions into the environment. The increasing influence of industrial factors on natural objects has a dangerous effect on living organisms, including humans. Most of the pollutants in the human environment are chemical compounds, or xenobiotics, which, when ingested, cause adverse chemical reactions. The most studied polluting chemicals include hydrocarbons, carbon monoxide and sulfur, nitrogen oxide, formaldehyde, suspended particles of various volumes – dust, PM 2.5, PM 10, nitrogen dioxide, ground-level ozone, hydrogen sulfide, phenol, ammonia and heavy metals. Heavy metals such as mercury, lead, and cadmium occupy a special place among xenobiotics. They are widely used in various types of industrial production and are capable of accumulating in living organisms.

Mercury is an ecotoxicant that causes a wide range of changes in body tissues and has harmful effects on human health. Mercury is widely used in industry, agriculture, and medicine. In the environment, mercury is found in three main forms: elementary, inorganic, and organic, which can easily transform into each other. Mercury is included in the class of “metallic poisons”, and its toxicity depends on the chemical form and routes of entry into the body. Numerous studies have convincingly shown the pathological effects of mercury on the human body and, mainly, on the central nervous system (CNS). There is evidence that it causes damage to genetic material, immune dysfunctions and biochemical disorders.

Elemental or metallic mercury, in the form of vapors, is easily absorbed by the lungs and can linger and accumulate in the lung tissue. Due to its solubility, mercury has a high degree of diffusion and is able to pass through cell membranes, as well as blood-brain and placental barriers and reach target organs. Inorganic mercury, which is a divalent compound, is a toxic type of mercury deposited in human tissues after conversion from other forms. Organic mercury, represented by methyl and ethyl mercury, enters the human body with fish products, meat of marine mammals and mercury-containing vaccines.

A report published by the World Health Organization (WHO) in 2005 noted that the maximum level of mercury in drinking water should not exceed 0.5 micrograms/liter, while the mercury content in the air can reach 10 ng/m3. Taking into account these standards, mercury intake from food is allowed from 2 to 20 micrograms per day. Pacyna and colleagues reported in 2010 that approximately 1,300 tons of mercury were released into the atmosphere in Asia, followed by North America, Europe, the Russian Federation, South America, Africa, and Oceania, each with at least 200 tons.

In Kazakhstan, one of the main sources of mercury pollution of the environment was the Karbid plant located in Temirtau. From 1950 to 1997,

wastewater from the acetaldehyde production plant Karbid was discharged into the Nuru River. The appearance of mercury in the floodplain of the river is mainly due to the fly ash of the power plant. The maximum mercury concentration was detected 15 km below the source of the contamination. The length of the mercury exposure downstream was about 75 km, where it was estimated that about 9.4 tons of mercury were concentrated.

In 2005, a survey was conducted of residents of settlements located in the mercury-affected area along the Nura River. It was found that subgroups consisting of men over 45 years of age, mostly fishermen, have high levels of mercury in their hair. A relationship has been established between the concentration of mercury in hair and the frequency of eating river fish.

Mercury intoxication in humans manifests itself mainly in the form of neurological disorders such as peripheral neuropathy, skin lesions, changes in the functioning of the kidneys, immune system, cardiovascular and reproductive systems. The mechanisms of mercury toxicity include the inhibition of selenium-dependent enzymes, as well as the suppression of the synthesis of catecholamines that cause neurological disorders. Methylmercury binds to plasma cystine, forms complexes with keratin in excrement, tubulins in the brain, and hemoglobin in red blood cells. Recent studies conducted in Kazakhstan and published on the release of mercury into the environment have shown the possibility of its toxic effects on the population, including hundreds of families in the Temirtau region. It should be noted that the objectives of these studies did not include the study of the molecular etiology of toxic damage by mercury. In this regard, in this study, we analyzed the effects of mercury by studying its toxicity, as well as assessed the genetic and molecular effects that may be associated with the induction of toxic effects in the human body.

The purpose of the dissertation research. To assess mercury pollution of the Nura River and the mercury danger to the health of the population living in the *mercury-exposed area*.

Research objectives. Based on the purpose of the study, the following tasks were set:

1. Determine the mercury content in the soil, water, and sediments in the mercury-exposed area.
2. To assess the mercury load on the population living near the polluted water area of the Nura River.
3. To study the prevalence of xenobiotic detoxification gene genotypes (GCLM-588 C/T (rs41303970), GSTM1, GSTT1, GSTP1 Ile105Val (rs1695) among the population living in a mercury-contaminated area.
4. To conduct molecular modeling (in silico) of the interaction of methylmercury ($MeHg^{+}$) with proteins of the xenobiotic detoxification system (GSTP1 and GCLM) to clarify the mechanisms of their binding and confirm the role of these proteins in the biotransformation of mercury.
5. To evaluate the associative relationship between the cytokine profile of blood serum, hypertension and mercury content in urine in people living in a mercury-containing territory.

Objects of the research was samples of soil, water, and sediments taken from various problematic areas of the mercury-contaminated territory, samples of peripheral blood, and urine from the population.

The methods of the research: chemical, biochemical, molecular genetic and statistical methods of analysis were used in the work.

The mercury content was analyzed by the cold steam method using a mercury injection flow analyzer FIAS-100 in combination with an atomic absorption spectrometer AAnalyst 300 (PerkinElmer, USA). The genetic analysis was performed by polymerase chain reaction (PCR). Enzyme immunoassay (ELISA) was used to determine the concentrations of a number of biomarkers. Statistical data processing was carried out using Microsoft Excel, SPSS and Jamovi programs.

Scientific novelty of the research. For the first time, the state of mercury pollution in the natural environment of the Nura River basin after clean-up measures has been studied.

For the first time, the state of mercury contamination of soils, water, and sediments after clean-up operations in the Nura River basin has been studied.

For the first time, mercury damage to the population was assessed by the level of its content in the urine of the population living in the mercury-exposed area.

The cytokine profile of blood serum of mercury-exposed individuals has been evaluated for the first time.

The prevalence of xenobiotic detoxification gene genotypes (GCLM, GSTM1, GSTT1, GSTP1) in people living in mercury-contaminated areas near the Nur River has been studied for the first time.

For the first time, molecular docking of methylmercury ($MeHg^{+}$) with detoxification system proteins (GSTP1, GCLM) was performed, which made it possible to clarify the possible binding mechanisms and confirm the role of these proteins in the biotransformation of mercury.

The theoretical significance of the work. The results of the study contribute to the development of modern ideas about the molecular mechanisms of toxic effects of heavy metals, in particular mercury, on the human body. Associations have been established between polymorphisms of genes involved in the detoxification of xenobiotics (GSTM1, GSTT1, GSTP1, GCLM) and impaired immune response in individuals exposed to chronic exposure to low doses of mercury. This allows us to consider these genes as potential molecular genetic markers of sensitivity to adverse environmental factors, as well as elements of pathogenetic mechanisms involved in biotransformation and inflammation. The data obtained expand the theoretical understanding of the interindividual variability of the body's response to exposure to mercury, which is important for developing the concept of personalized environmental risk. The study highlights the importance of genetically determined differences in resistance to toxicants and lays the foundation for further research in the field of environmental genetics and molecular epidemiology. Additionally, the results of the analysis of mercury content in environmental components (soil, aquatic environment, bottom

sediments) have significant theoretical significance for ecotoxicology and hygienic assessment of the environment. A comparison of the detected concentrations with national and international standards (maximum permissible concentrations (MPCs) established by the World Health Organization (WHO), the United Nations Economic Commission for Europe (UNECE), and the Sanitary Rules and Norms of the Republic of Kazakhstan (SanPiN RK)) allowed not only to record the degree of excess, but also to quantify the level of potential threat to public health. This further creates a scientific basis for identifying priority areas of environmental risk, developing criteria for environmental regulation and monitoring strategies.

The practical significance of the work. The identified genetic damages and their relationship to the content of environmental components make it possible to create a diagnostic algorithm for detecting pathological changes in the body, which can later be implemented and used in the practical activities of health authorities in screening populations living in unfavorable ecological regions. The results obtained can be used in the organization of medical and environmental monitoring of the population living in conditions of anthropogenic pollution, as well as in the formation of risk groups for targeted monitoring and prevention of diseases.

The research materials are of practical value for specialists in the field of medicine, toxicology, and environmental hygiene and they can be used in the development of regional health protection programs, sanitary and hygienic measures, as well as in the preparation of training courses for medical and environmental workers.

The results of the work can also serve as a basis for improving regulatory approaches to assessing the risk of exposure to heavy metals on human health and the environment.

Implementation of the research results. The results of this study were implemented in the practical activities of the Department of Ecology in the Karaganda region. As part of the cooperation with this organization, materials were provided, including data on the mercury content in soil, water and sediment samples taken from territories exposed to man-made pollution. The information provided was used in conducting environmental monitoring and developing recommendations to reduce the effects of toxicants on the environment and public health. The fact of practical use of the results is confirmed by the *Act of introducing scientific and technical developments* and best practices into production dated 03/01/2024 (Appendix A). The data obtained formed the basis of analytical materials aimed at strengthening surveillance of polluted areas and substantiating the need for remediation and preventive measures.

The main provisions to be defended.

The mercury content in sediments, soils and water in the area exposed to mercury significantly exceeds sanitary standards, even at a distance from the main source of pollution: at a distance of less than 1 km from the riverbed of the Nura River, the mercury content in soil samples exceeds the maximum permissible concentration by more than 29 times).

Mercury circulation in the body of the population living in the mercury pollution zone is 5 times higher than that of the unexposed population, which is confirmed by analysis of mercury excretion in urine.

Prolonged exposure to low doses of mercury leads to an increase in the level of immune markers in the blood serum and the risk of hypertension.

In the group of the mercury-exposed population, the peculiarities of the distribution of genotypes of the polymorphisms of the genes GCLM-588 C/T, GSTP1 Ile105Val were revealed, which indicates their role in the formation of sensitivity to the toxic effects of mercury. The association between deletion of the GSTM1 (-/-) gene and elevated mercury levels in the urine of mercury-exposed individuals confirms the involvement of the GSTM1 gene in the mechanisms of mercury detoxification and reduction of toxic load on the body.

The relationship of the thesis topic with the plans of scientific work. The dissertation work was carried out within the framework of the research in the field of natural sciences "Fundamental research in the field of biomedicine" and is an initiative.

The results of the research.

1. Mercury contamination of soils, water and bottom sediments is observed in all the studied areas adjacent to the Nura River, in the area of exposure to mercury-containing waste; maximum exposure levels were recorded near the village of Gagarinskoye (mercury concentration in water samples of the surface layer of the source is 18 times higher than the maximum permissible concentration in the soil - 29 times, in bottom sediments – 26 times higher than the background level) and the village of Chkalovo (input concentrations are 4 MPC, soil concentrations are 80 MPC, and sediments are 53 times higher than the background).
2. The average group concentration of mercury in the urine of people living in the contaminated area was 15 micrograms/l ±7 micrograms/l, which is higher than in the unexposed group (3 micrograms/l ±1.5 micrograms/l, $p < 0.001$) and confirms the high level of mercury exposure to the body at the present time.
3. Prolonged exposure to mercury leads to impaired immune homeostasis in exposed individuals and is associated with an increased risk of hypertension due to activation of systemic inflammation: increased levels of pro-inflammatory cytokines TNF-α, IL-2, IL-6 and anti-inflammatory IL-10 were detected in the blood serum of 68% of exposed individuals. The concentration of mercury in urine correlates with the serum levels of IL-2 ($\rho=0.598$; $p < 0.001$) and TNF-α ($\rho=-0.564$; $p < 0.001$).
4. Cumulative analysis of genotype combinations showed that the favorable combination of GSTT1+GSTM1+ was twice as rare in the exposed group (12.2%) compared with the control group (33.3%); accumulation of mercury in the body is associated with polymorphisms of the GSTM1 genes: carriers of the deletion genotype (GSTM1–) have higher levels of mercury in the urine (2.91 ± 0.90 mcg/l) compared with individuals with the conserved allele (GSTM1+) (1.49 ± 0.49 mcg/l, $F = 21.51$; $p < 0.05$). This indicates a reduced ability of the body to detoxify mercury in the absence of this enzyme and, as a result, an increased accumulation of the toxicant.

5. The results of *in silico* molecular modeling showed a high affinity of methylmercury to the active centers of the GSTP1 and GCLM proteins, which is consistent with the obtained genotyping data and indirectly indicates the circulation of methylated mercury in the body of the exposed population.

6. A comprehensive analysis of genetic, biochemical and immunological markers revealed a stable relationship between mercury load, polymorphisms of the glutathione system genes and the severity of inflammatory reactions, which allows us to consider a combination of markers - mercury levels in urine, concentrations of cytokines TNF-α, IL-2, IL-6, IL-10, as well as GSTM1 genotypes, GSTT1, GSTP1, GCLM - as promising bioindicators of exposure and risk of health disorders in the population living in conditions of anthropogenic pollution.

Approbation of the Research Results. The results of the study and the main provisions of the dissertation were presented and discussed at international and national conferences.

1. 4th Asian Conference on Environmental Mutagens (ASEM -2014). All India Congress of Cytology and Genetics, CSIR – Indian Institute of Chemical Biology (Калькутта, 2014);

2. Scientific Forum: Medicine, Biology, and Chemistry (Moscow, 2018);

3. Current Environmental Issues — Proceedings of the 8th International Scientific Conference of Young Scientists, Postgraduates, Master's Students, and Undergraduates (Minsk, 2018);

4. Health Risk Analysis – 2023, held jointly with the International Meeting on Environment and Health RISE-2023. The 13th All-Russian Scientific and Practical Conference with International Participation (Perm, 2023);

5. Modern Problems of Ecology and Public Health. The 2nd All-Russian Conference with International Participation dedicated to the 300th Anniversary of the Russian Academy of Sciences and the 5th All-Russian Scientific and Practical Conference of Young Scientists "Ecology and Public Health" (Angarsk–Baikal, 2023).

Publications on the research topic. The main findings of the dissertation research are presented in 14 published works, including 2 articles in journals indexed in the international databases Web of Science and Scopus (Impact Factor – 1.5, Q3; 3.4 – Q1), 4 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education (CQASHE), as well as 8 conference papers and abstracts presented at international scientific conferences.

Dissertation structure. The dissertation consists of an introduction, three main sections, a conclusion, a list of references, and appendices. The main sections of the dissertation are accompanied by tabular and graphical materials for visual representation of the findings. The work is presented on 121 pages and contains 16 tables, 33 figures, and 7 appendices.