ANNOTATION

For the dissertation for the Doctor of Philosophy (PhD) degree
on the educational programm "8D05104 - Genetics" of
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on the theme: «**Influence of the exposure to organichlorinated pesticides on the genetic status of the population in the Almaty region**»

General description of the dissertation.
The research work is dedicated to studying the influence of pesticide pollution on the genetic status and health of people living in the Almaty region, exposed to pesticide pollution for a long time.

Relevance of the research topic.
Results of monitoring in 2011 revealed 64 abandoned pesticide warehouses in the region; the approximate estimation of the content of unowned persistent organic pollutants (POPs) was 6931.4 tons. Due to the privatization of land, information about unutilized pesticides remained unknown. These chemicals have been decomposing in soil for 10 years, contaminating plants and animals, which could affect the population living in these territories.

The toxic effects of these pesticides and their breakdown products on human health have long been confirmed, and their use is prohibited. The dangers of pesticides also increase as initially non-toxic compounds degrade in the soil and form persistent and toxic metabolites, which can then accumulate in foods. Accepted methods for assessing the risks of pesticide pollution are capable of calculating the degree of danger to humans. However, since these methods use average statistical data for the region, and the consumption rates and individual characteristics of each person are different, to fully assess the risk of exposure to pesticides, it became necessary to offer a different approach, taking into account a wide range of individual characteristics of a person.

The purpose of the research.
To assess the risk of long-term exposure to pesticides at the cohort and individual levels among residents of villages in the Almaty region of Kazakhstan.

Research objectives:
1. Cytogenetic analysis of the population living in the pesticide-contaminated areas of the villages of Kyzylkairat, Beskainar, Belbulak, Amangeldy, Enbekshi, Karakestek and Umbetaly.

2. Molecular genetic analysis of human populations for the presence of polymorphisms in genes for xenobiotic detoxification (*GSTM1*, *GSTT1*, *GSTP1*, *CYP1A1*, etc.), DNA repair (*XRCC1*, *XRCC3*, *XPD*, etc.) and antioxidant protection (*SOD1*, *GCLC*, *GCLM*, *GPX4*, *NFE2L3*, etc.).

3. Cohort analysis of food contamination risk for the study villages.

4. Correlation analysis to study the impact of pesticides on health and genetic status of the population.

4. Development of a method for individual assessment of the risk of long-term environmental pollution with pesticides for the genetic status of the population and human health.

The research objects: A mixed population of people in the Almaty region (249 people), exposed for a long time to organochlorine pesticides, living in the villages of Kyzylkaynar, Belbulak, Beskainar, Amangeldy, Enbekshi, Karakastek, Umbetali. A cohort of 343 people from areas not contaminated with pesticides was selected for the control group.

The subject of the research: Genotoxic and toxic effects of long-term exposure to pesticide contamination in the human food chain.

Research methods: DNA isolation, polymerase chain reaction, gel electrophoresis, capillary electrophoresis (fragment analysis), restriction fragment length polymorphism analysis, cultivation of human peripheral blood lymphocytes, routine cytogenetic analysis, whole-genome microarray genotyping, bioinformatics analysis, Spearman rank correlation, personal survey method, estimated daily intake calculation (EDI) for cohort risk analysis, multiple regression method for individual risks.

The scientific novelty of the research.

Exposure to pesticides is known to be associated with numerous harmful effects, the most common of which are reproductive dysfunction and carcinogenesis, less commonly neurodegenerative and cardiovascular diseases, and even genetic damage and the induction of chromosomal changes.

Chromosomal damage associated with pesticide exposure has been identified in several populations. Some researchers have noted significant differences in the incidence of chromosomal aberrations in the study cohorts compared with the control group, but other studies have not confirmed such an association. These contradictions may be due to the fact that unstable chromosomal aberrations (dicentrics, acentrics, interstitial deletions, etc.) are eliminated during cell proliferation. The mutagenic effects of pesticides, measured by the frequency of unstable chromosomal aberrations in blood lymphocytes, can only be detected 2–3 months after exposure to pesticides, before cell division. We believe that in some cytogenetic analyzes of chronically exposed populations, investigators were unable to account for time differences between the date of individual pesticide consumption and the time of blood collection. Therefore, the level of unstable chromosomal aberrations cannot be used as a reliable indicator of health risk.

The mutagenic effect of pesticides can also be influenced by the expression of a number of genes that regulate the metabolism of toxicants and DNA repair. Studies have revealed significant associations of mutations and polymorphisms of genes involved in DNA repair, xenobiotic detoxification and antioxidant protection with the development of a number of multifactorial diseases. Genetic polymorphism in these genes may influence susceptibility to pesticide exposure and serve as a determinant of pesticide toxicity. The genetic status of these genes should be considered when assessing the toxic effects of pesticides in populations exposed over long periods of time to environmental pesticides.

The accepted methods for calculating risks allow us to theoretically assess the short-term and long-term health risks of the population exposed to pesticides, as well as identify the most dangerous groups of pesticides. However, it does not take into account individual variables for each person, but only estimates the average body weight and food intake of the population. Because intake rates and individual characteristics vary from person to person, it was important to assess the contribution of congenital and environmental factors, as well as dietary habits, to assess the risk of pesticide exposure.

In this work, for the first time, for a molecular epidemiological study of a cohort of people exposed to toxic substances for a long time, an individual assessment of the risks of exposure to pesticides on health and genetic status was implemented. This assessment includes many factors identified during the survey of the study group.

Theoretical and practical significance of the research.

The results of the study offer a methodology for a more detailed analysis of the potential medical and genetic risks of pesticide pollution for the population based on individual physiological and life characteristics, such as age, gender, weight, smoking, alcohol consumption, etc.

The results of the study can help improve the existing methodology for assessing the risk of various pollutants, possibly taking into account a wide range of individual characteristics, assessing the threat of toxicants to human health and genetic status, and identifying the most vulnerable groups of the population.

The main provisions for the defense:

1. The research revealed that the level of chromosomal aberrations in the studied villages of Belbulak (3.00±0.33%), Kyzylkairat (2.84±0.37%), Besqaynar (1.88±0.23), Amangeldy (1.96±0.27), Karakastek (1.56±0.25), Enbekshi (3.04±0.33%) and Umbetaly (1.60±0.25) was significantly higher (p<0.0001) than in the control village of Taukaturyk (0.85±0.12%). The nature of chromosomal aberrations indicates the chemical nature of the mutagenic effect.

2. An increase in the frequency of mutant alleles for the genes *XRCC1* Arg399Gln (rs25487), *SOD1* (rs1041740, rs138002121), *CYP1A1* (rs17861084), *CYP2B6* (rs8192718), *CYP2D6* (rs186133763), *GSTP1* (rs1138272) and *GCLC* (rs12524550) compared to average values for a

population of similarly mixed ethnic origin. The frequency of the rare allele of the *GSTP1* gene (rs1871042) for the study sample also exceeded the population average.

1. 3. It has been established that the greatest risk is associated with the consumption of pears, cucumbers, bell peppers, milk and meat, as evidenced by the high content of pesticides of the aldrin, endosulfan and heptachlor group and are characterized by a high hazard index (hazard index of 3 to 20 and higher).

4. The correlation analysis based on data on pesticide content in food products showed a link between the frequency of chromosomal aberrations and the content of pesticides in food products. The analysis identified potentially hazardous groups of pesticides in contaminated products. For all groups of studied pesticides from plant products, the strongest direct correlation between the frequency of aberrations and the accumulation of pesticides is characteristic of pears, tomatoes and cucumbers. The correlation coefficient exceeded 0.9. For products of animal origin, such a relationship was observed only in meat samples (DDT and endosulfan group - Cr 0.84 and aldrin group Cr -0.89; NSV Cr -0.99). For apples, a strong correlation between the frequency of chromosomal aberrations and the accumulation of pesticides was found in the aldrin group (Cr -0.94) and moderately significant for the DDT group (Cr -0.69). In milk samples, a strong correlation was found in the hexachlorobenzene group (Cr -0.99).

5. Assessment of individual individual indicators of the formula for individual health risk assessment revealed that the most significant factors influencing health are the excess of pesticides in food (33%, influence coefficient = 0.8601) and the state of the xenobiotic detoxification system (26%, coefficient influence = 0.5485). Factors influencing the risk of mutations were the level of food contamination with pesticides (29%, influence coefficient = 0.8569), smoking (26%, influence coefficient = 0.9268) and the functional state of the DNA repair system (24%, influence coefficient = 1.1351).

Main research results and conclusions:

1. A cytogenetic analysis was carried out for 191 people, of which 141 were from five villages of the Talgar district (the villages of Beskainar, Kyzylkairat, Amangeldy, Belbulak, Enbekshi), and 50 from the villages of the Dzhambyl district (the villages of Karakastek and Umbetaly). Another 50 people from the village of Taukaraturik were taken as controls. A significant increase in the frequencies of aberrations in the studied settlements compared to the control was shown. All studied villages are characterized by a predominance of chromatid type mutations, which indicates the chemical nature of the mutagenic effect.

2. In the overall cohort, the studied frequencies of alleles of genes involved in the biotransformation of organochlorine pesticides were at the expected levels characterizing mixed Asian-European populations. Increased frequencies of mutant alleles were observed for *XRCC1* Arg399Gln (rs25487), *SOD1* (rs1041740, rs138002121), *CYP1A1* (rs17861084), *CYP2B6* (rs8192718), *CYP2D6* (rs186133763), *GSTP1* (rs1138272), and *GCLC* (rs12524550) compared to the average values for population of equally mixed ethnic origins. The frequency of the rare allele of the *GSTP1* gene (rs1871042) for the study sample was also slightly higher than the population average. The frequency of the *GCLC* polymorphic variant (rs524553) was lower than the population average. Molecular genetic analysis revealed an increased frequency of non-functional alleles of glutathione-S-transferases M1 and T1, which may have an impact on the decrease in detoxification functions of genes and the health status in the study population.

3. During the personal survey, 151 people were interviewed in detail, each of whom lived in close proximity to pesticide warehouses. From each of the respondents, data was obtained on individual dietary habits (the amount and type of food consumed per day), as well as information on chronic diseases, bad habits and sources of food supply. According to the study, people's eating habits vary by village, which can make it difficult to calculate actual risks using known averages. The medical status of individuals was assessed using both a clinical examination and a questionnaire. Cardiovascular diseases (hypertension, ischemia, atherosclerosis, etc.) predominated (38 people, 25.17%). In second place in terms of prevalence were chronic respiratory diseases, identified in 10 respondents (6.62%). Five people had a history of cancer (3.31%), isolated cases of kidney disease,

thyroid disease and diabetes were recorded, and nineteen people (12.58%) had various health complaints.

4. The correlation analysis revealed potentially dangerous groups of pesticides and contaminated products. The high frequency of chromosomal aberrations (CA) in human lymphocytes significantly correlates with the content of DDT pesticides in apples, pears, cucumbers, tomatoes and meat. Pesticides of the hexachlorobenzene group showed a high association with the induction of CA in populations consuming apples, pears, tomatoes, cucumbers, meat and milk. Pesticides of the HCH group in food products revealed a correlation with the induction of CA in human lymphocytes for apples, pears, cucumbers, tomatoes, and bell peppers. A significant correlation has been established between the increase in the frequency of CA and the content of aldrin group pesticides in apples, pears, tomatoes, cucumbers, meat and milk. The content of endosulfan group pesticides in food products revealed a correlation with the frequency of CA in human lymphocytes for pears, cucumbers, tomatoes and bell peppers, as well as meat from the village of Beskainar. The presence of heptachlor group pesticides in food products revealed a correlation with the induction of CA in human lymphocytes for pears, apples, tomatoes and cucumbers. For products of animal origin, a significant moderate correlation of the heptachlor group was observed only for meat from Amangeldy and Kyzylkairat. These data highlight potential associations between the consumption of specific food products and the presence of certain groups of pesticides, contributing to a better understanding of the factors influencing chromosomal aberrations in the study population.

5. A cohort analysis of short-term and long-term risks found that the greatest risk is associated with the consumption of pears, cucumbers, bell peppers, milk and meat, since these products contained unacceptably high levels of pesticides such as aldrin (HQ = 20%, HI > 20%), endosulfan (HQ = 4%; HI = 14%) and heptachlor (HQ = 4%; HI = 9%). In the village of Enbekshi, the hazard ratio exceeds 20%, especially in bell peppers and milk, indicating a high long-term risk of exposure to pesticides.

6. A variant of calculation of individual risk assessment indicators is proposed taking into account a wide range of individual characteristics of a person, including calculation of the degree of excess of the actual daily intake (EDI) compared to the acceptable daily intake (ADI) of a specific type of pesticides for each individual. An assessment of individual indicators of the individual health risk assessment formula revealed that the most influential factors on health are the excess of pesticides in food (influence coefficient = 0.8601) and the state of the xenobiotic detoxification system (influence coefficient = 0.5485). T-test confirms the significance of excess of both pesticides and heavy metals in calculating health risks (T3 = 2.562 (>2.263), T4 = 2.962 (>2.263)). The most significant factors influencing the risk of mutation were the level of food contamination with pesticides (influence coefficient = 0.8569), smoking (influence coefficient = 0.9268) and the functional state of the DNA repair system (influence coefficient = 1.1351). However, the reliability of the state of the DNA repair gene factor was not confirmed (T5 = 1.905 (<2.693)), while the pesticide pollution factor was confirmed (T2 = 3.028 (>2.693)).

Personal contribution of the author.

The author was directly involved in all stages of the study: formation of aims and objectives, project development, collection of biomaterial, DNA extraction, conducting an individual survey of the population, determination of single nucleotide polymorphisms, formation of a database, statistical data processing, interpretation of the results and formulation of conclusions, development of an individual risk assessment method, conceptualization, original draft writing of publications and thesis for domestic and foreign journals.

Connection with the plan of basic scientific work. The study was carried out within the framework of the projects BR05236379 "Comprehensive assessment of unutilized and banned pesticides impact on genetic status and health of population of Almaty region" and AP09260631 "Study of gene polymorphisms responsible for antioxidant protection and immune response in individuals exposed by pesticides for a long time".

Approbation of work.

The research results were presented at scientific conferences:

1. The 46th FEBS Congress "The Biochemistry Global Summit" (Lisbon, Portugal, July 9-14, 2022)

2. The 47th FEBS Congress "Together in bioscience for a better future" (Tours, France, July 8-12, 2023)

3. BIO Web of Conferences "International Scientific Forum "Modern Trends in Sustainable Development of Biological Sciences" (Almaty, Kazakhstan, 27-28 March, 2024)

The main results of the study were presented annually at scientific seminars of the Laboratory of Molecular Genetics of the Institute of Genetics and Physiology (IGP), and were also presented at an interlaboratory seminar of the Institute of Genetics and Physiology. The results of the PhD work are included in the annual research reports for the project BR05236379 (2018–2020) and the grant project AP09260631 (2021–2023).

Publications.

10 scientific papers have been published on the research topic, including:

3 articles in scientific publications included in the 1-2 quartiles by impact factor in the Web of Science database; 3 articles in domestic journals recommended by the Committee for Quality Assurance in Science and Higher Education, 3 abstracts for international conferences, and 1 methodological manual.