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CYTOGENETIC ANALYSIS OF THE POPULATION LIVING IN CLOSE PROXIMITY TO THE DESTROYED WAREHOUSES OF BANNED PESTICIDES

Assessment of genotoxicity of pesticides and biomonitoring of their impact on the population living in the area of disposal of endangered stocks of pesticides are of exceptional importance for regulating the ecological state of the environment and protecting the population. The purpose of this study was to assess the level of cytogenetic damage (chromosomal aberrations) in residents of the villages of Karakastek and Umbetala, Zhambul district, Almaty region, located in areas contaminated with pesticides banned for occurrence more than 10 years ago. The soils of the settlements are contaminated with organochlorine pesticides such as aldrin, deldrin, eldrin, chlorobenzilate and DDT. In food, their content does not exceed the MPC. However, there was an excess of MPC for heavy metals – Ni and Co. The residents of v. Karakastek and Umbetali showed a significant excess of the total frequency of chromosomal aberrations compared to the control ($p \leq 0.01$). The occurrence of cases of shift of mutations from the chromatid adrenal glands in the foci of inflammation, the destruction of the nature of chemical mutagenesis. An analysis of the state and dynamics of the level of chromosomal aberrations in the population living in territories contaminated with pesticides can help in developing a rationale for predicting long-term biomedical events and normal control over the health of the population.

Key words: pesticides, persistent organic pollutants (POPs), chromosome aberrations (CA), chemical mutagenesis, genotoxicity.

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Тыйым салынған пестицидтердің жойылған қоймаларына жақын жерде тұратын халықтың цитогенетикалық талдауы

Тұрғылықты адамдарды қорғау және қоршаған ортаның экологиялық жағдайын жақсарту үшін маңызды шешімі бар, ескірген пестицидтер қоры сақталған аймақтарда тұратын тұрғындарға пестицидтердің генотоксиділігін бағалау және олардың әсерінің биомониторингін жүргізу. Бұл зерттеудің мақсаты 10 жылдан астам уақыт бойы қолдануға тыйым салынған пестицидтермен ластанған аумақтарда тұратын Алматы облысы, Жамбыл ауданы, Қарақастек және Үмбеталы ауылдарының тұрғындарының цитогенетикалық зақымдану деңгейін (хромосомалардың аберрациялары) бағалау болды. Елді мекендердің топырағы альдрин, дельдрин, эльдрин, хлорбензилат және ДДТ сияқты хлорорганикалық пестицидтермен ластанған, азық-түлік өнімдерінде олардың мөлшері ШРК-дан аспайды. Алайда ауыр металдар – Ni және Co үшін ШРК артық болатыны анықталды. Қарақастек пен Үмбетәлі ауылдарының тұрғындарында бақылаутобымен салыстырғанда хромосомалық аберрациялардың жалпы жиілігінің айтарлықтай асып кеткенін көрсетті ($p \leq 0,01$). Екі елді мекенде де мутациялар хромосомалықтан хроматидтік өзгеріске қарай ығысу қатынасы химиялық мутагенездің табиғатына байланысты екендігін көрсетеді. Пестицидтермен ластанған аумақтарда тұратын тұрғындардың популяциясындағы хромосомалық аберрация деңгейінің жағдайын және динамикасын талдау ұзақ мерзімді биомедициналық зардаптарды болжау принциптерін әзірлеуге көмектеседі және денсаулық сақтауды басқаруға ықпал етеді.

Түйін сөздер: пестицидтер, тұрақты органикалық ластастауыштар (ТОЛ), хромосомалық аберрациялар (ХА), химиялық мутагенез, генотоксинділік.

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Цитогенетический анализ населения, проживающего в непосредственной близости к разрушенным складам запрещенных к использованию пестицидов

Оценка генотоксичности пестицидов и биомониторинг их воздействия на население, проживающего на территориях захоронения устаревших запасов пестицидов, имеют решающее значение для улучшения регулирования экологического состояния окружающей среды и защиты населения. Целью настоящего исследования была оценка уровня цитогенетических повреждений (хромосомных аберраций) у жителей поселков Каракастек и Умбеталы Жамбульского района Алматинской области, проживающих на территориях, контаминированных запрещенными к использованию пестицидами более 10 лет назад. Почвы поселков контаминированы такими хлорорганическими пестицидами, как альдрин, дельдрин, эльдрин, хлорбензилат и ДДТ. В продуктах питания их содержание не превышает ПДК. Однако, отмечено превышение ПДК по тяжелым металлам – Ni и Co. У жителей пп. Каракастек и Умбеталы отмечено достоверное превышение общей частоты хромосомных аберраций по сравнению с контролем ($p \leq 0.01$). Полученное соотношение сдвига мутаций в сторону хроматидных над хромосомными в обеих поселках, указывает на природу химического мутагенеза. Анализ состояния и динамики уровня хромосомных аберраций в популяции жителей, проживающих на территориях загрязнения пестицидами, может помочь разработать принципы прогнозирования отдаленных биомединских последствий и способствовать управлению здоровьем населения.

Ключевые слова: пестициды, стойкие органические загрязнители (СОЗ), хромосомные аберрации (ХА), химический мутагенез, генотоксичность.

Introduction

It is known that pesticides are widely used in agriculture to control pests and diseases of cultivated plants. Pesticides encompass hundreds of active substances and tens of thousands of preparations of various effects. The vast majority of pesticides belonging to the class of organochlorine compounds are persistent organic pollutants (POPs) that poison target organisms. POPs are not destroyed in the environment for a long time, they are transported through the air and water masses over long distances, far from the original source of pollution. They accumulate in the adipose tissues of living organisms, causing irreparable damage to health. The long-term effects of pesticides, especially at low doses, and their possible synergy with other environmental pollutants and disease vectors have been poorly studied. However, pesticide residues that persist in food may not have toxic or lethal effects, but they reduce disease resistance and gradually accumulate in the body to a dangerous level [1-3].

The mutagenic activity of pesticides is one of the most dangerous manifestations of a negative impact on human health and its offspring. Over the past 15 years, there has been a surge in research on the impact of pesticides on human health [4-10]. It has been proven that some pesticides are carcinogens and cause the development of cancer [5,11]. Moreover, pesticides frequently contribute to the onset of allergies, diathesis, and respiratory illnesses [12,13], and are associated with the development of neurodegenerative disorders [5,14-16], such as Alzheimer's disease, Parkinson's disease, or adolescent suicidality. In addition, pesticides have been linked to endocrine diseases [5], male and female reproductive system disorders [17-19], type 2 diabetes mellitus [5], metabolic syndromes and obesity [9], developmental disorders [20], among other deleterious health outcomes. This list is not complete, but rather representative of the range of health effects associated with exposure to pesticides.

The issue concerning the presence and concentration levels of hazardous pesticides within the geographical borders of Kazakhstan remains unresolved. According to UNEP, as a result of the inventory of obsolete pesticides in 2004, more than 1,500 tons of banned, unusable pesticides and their mixtures of unknown composition were found in the Republic, and in 2008 their number reached 10,000 tons [21-25]. According to the Ministry of Agriculture, as of July 2012, about 6931.4 tons of obsolete, banned and unusable pesticides are stored in warehouses in various regions of the republic [26]. Within the framework of the scientific international program, during the inventory process in 2009-2010, 64 warehouses of chemical plant protection products were found in 10 districts of the Almaty region, they accumulated 68443 kg of obsolete and unusable pesticides. These are pesticides: from the class of s-triazine (atrazine, protrazine, propazine, ziazine), organophosphorus (sayphos, metaphos), chlorine-containing (nitrofen and illoxan), fluorine-containing (treflan), thiocarbamate (temic), as well as German and Chinese origin (50 % Thirams and Hataonyag). When determining the content of organochlorine pesticides in the soil around the territories of 64 former pesticide storage facilities in the Almaty region, it was found that the soil of 24 former storage facilities were contaminated with metabolites 2.4 DDD, 4.4 DDD, 4.4 DDT, 4.4 DDE and isomers of α- HCCH, β-HCCH and γ-HCCH. The most polluted regions were Eskildinsky, Talgarsky, Karasaysky, Enbekshikazakhsky districts. The main contaminants were lindane, the β-isomer of HCH and the metabolites 4.4’DDE and 4.4’DDT. In addition to these metabolites, α-HCH, 4,4-DDD, and 2,4-DDD were present in the soil in many regions, and their presence in the soil is unacceptable under government regulations of Kazakhstan [26-28]. It is known that they are highly toxic drugs with marked skin-resorptive toxicity, and are known to induce mutagenic, antimitotic, and embryotoxic effects. [2,5]. Disused pesticides represent a hazardous source of environmental contamination, mandating immediate measures for their identification and subsequent removal.

The Institute of Genetics and Physiology of the Ministry of Education and Science of the Republic of Kazakhstan implemented a program from 2018-2020 aimed at the comprehensive evaluation of the impact of non-utilized and banned pesticides on the genetic status and health of the population of Almaty region (state registration number BR05236379). Within the framework of this program, a set of measures was carried out in 7 settlements of the Talgar region, including a survey of the territories of former warehouses of non-utilized pesticides. As a result, a high level of contamination of soil, water, plant and animal food products with chlorinated pesticides, their breakdown products and heavy metals was found in 6 locations. A low level of somatic health was also found among the population living in close proximity to the pollution sites (a total of 199 individuals, whose biomaterials are stored in the gene bank). It should be noted that the high content of pesticides in food products and a low level of somatic health correlated with a high frequency of chromosomal aberrations (CA) [29]. In 2021-2022, a survey was conducted of 2 locations of former pesticide storage facilities in the villages of Karakastek and Umbetali, Zhambyl district, Almaty region.

The aim of our study was a cytogenetic analysis of the population living in close proximity to sources of pesticide contamination in combination with the determination of the content of non-utilized POPs and heavy metals in the environment, as well as in plant and animal food products.

Materials and research methods

Materials and objects of research

The study was approved by the local ethical commission (Protocol of the Local Ethical Commission of the Republican State Enterprise “Institute of Human and Animal Physiology” SC MES RK No. 6 dated 07.12.2020), which provides for the analysis of the population living in the locations of non-utilized pesticides prohibited for use, and conditionally healthy people.

In order to expand the genetic bank of the population exposed to persistent organic pollutants (POPs), peripheral blood samples were collected from 25 residents of Karakastek village and 25 residents of Umbetaly village in Zhambyl district of Almaty region, who lived in close proximity to the destroyed pesticide warehouses. Prior to participating in the study, informed consent was obtained from all volunteers, who willingly agreed to participate. A questionnaire was administered to collect relevant demographic and health-related information, and 5 mL of peripheral blood was collected from each participant for the preparation of metaphase chromosome slides and subsequent cytogenetic analysis.

Research methods

Chemical analysis of soil, water and plant/animal food samples

Water sampling was performed in compliance with GOST 17.1.5.05-85, which specifies the general requirements for collecting surface and sea water samples, during the spring season. Soil samples were collected from two different horizons (0-5 cm and 5-25 cm deep) in accordance with the methodology that corresponds to the conditions of GOST 14.4.4.02-84 and GOST-29269-91, using the "envelope" method. Food products of plant (apples, pears, tomatoes, cucumbers, bell peppers) and animal (meat, milk) origin were collected from household plots located in the vicinity of former pesticide storage sites (3 samples of each product type from each location). The chemical analysis of the samples was conducted by an accredited testing laboratory of Scientific Analytical Center LLP, as per the terms of the agreement.

Quantitative analysis of environmental samples for the presence of 24 pesticides (hexachlorobenzene (HCB); α, β, γ, δ-isomers of hexachlorocyclohexane (hexachlorane, HCCH); heptachlor; aldrin; heptachlorepoxide; chlordan; endosulfan 1; endosulfan 2; dichlorodiphenyltrichloromethylmethane (DDT); 4,4'-dichlorodiphenyldichloroethylene (DDE); dichlorodiphenyldichloroethylene (DDD); 2,4'-DDD; deledrin; chlorobenzilate; endrin; endrin aldehyde; endosulfan sulfate; dibutylendan; methoxychlor; hexabromobenzene) and eight heavy metals (zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), cobalt (Co), nickel (Ni), and chromium (Cr)) was conducted. The water, soil, and sediment samples were prepared in accordance with GOST 29269-91 "Soils. General requirements for analysis". The chemical reagents used for the analysis were of a purity grade not lower than the criterion of "pure for analysis". The measuring and testing equipment used for the analysis were certified.

For the analysis of pesticides in the samples, gas chromatography and mass spectrometry techniques were utilized. The Agilent 6890N chromatography coupled with an MSD 5975C (USA), Fluorat-02 liquid analyzer, and ACME 9000 HPLC mass spectrometer with UV/VIS detector were used for the analysis. The methods employed in the study adhered to international, Russian, and Kazakh standards, namely: 1) EU standard EN 15662:2008, which outlines the determination of pesticide residues in plant-based food products using GC-MS and/or LC-MS/MS after extraction/separation with acetonitrile and purification using dispersive SPE method QuEChERS; 2) GOST 32689.1-2014, which details multimethods for gas chromatographic determination of pesticide residues in plant-based food products, including general provisions, extraction and purification methods, and identification and result verification; 3) GOST 23452-79, which describes methods for the determination of residual amounts of organochlorine pesticides in milk and dairy products; and 4) GOST 30349-96, which specifies methods for determining residual amounts of organochlorine pesticides in fruits, vegetables, and their processed products.

Cultivation of human peripheral blood lymphocytes and metaphase chromosome preparations

Lymphocyte cultivation and preparation of samples were carried out according to the standard procedure: 0.5 ml of peripheral blood was added to 4.5 ml of the cultivation medium, consisting of 80% HAM medium with glutamine (2 mM), 20% bovine serum, penicillin 100 units/ml, streptomycin 100 units/ml [30]. Lymphocyte division was stimulated with 2% PHA. Cells were incubated at 37°C for 48 hours. For the accumulation of metaphase plates, colchicine at a final concentration of 0.8 µg/mL was introduced into the culture medium 2 hours before fixation. For cytological preparations, the cells were hypotonically treated with 0.075 M KCl at 37°C for 15 minutes, fixed using a methyl alcohol/glacial acetic acid (3:1) mixture, and stained with a Giemsa solution.

Stained metaphase plates were analyzed under microscopes from Leica and Zeiss (Germany) using oil immersion at a magnification of 10x100. Images of metaphase chromosomes were processed with the VideoTestKaryo 3.1 computer program. All types of chromosomal aberrations detected during routine staining of chromosome preparations were taken into account, adhering to standard criteria for the selection and analysis of cytogenetic preparations. For each individual, at least 100 metaphases were studied.

Methods of statistical processing of results

Traditional methods of variation statistics were used in some experiments. Differences were regarded as significant at $p<0.05$. The significance level (P) was determined using Chi2 ($\chi 2$) and Student's t-test.

The calculation of the proportion of cells with chromosome aberrations as a percentage of the total number of analyzed cells was calculated using the formula:

$$P = X/N \times 100\%,$$

where X is the number of detected cells with aberrations,

N is the number of studied metaphase cells.

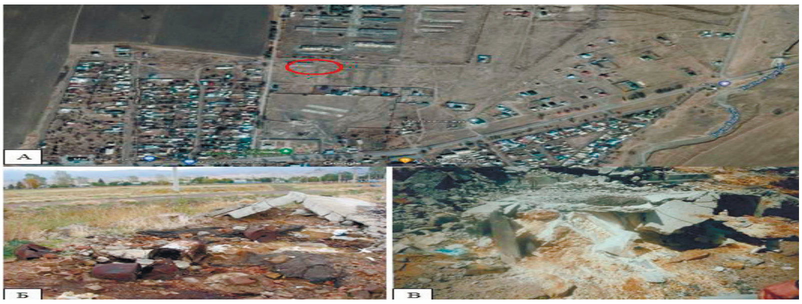
Research results and discussion

Survey of locations of obsolete stocks of pesticides, selection of 2 points

A survey of locations containing outdated pesticide stocks was conducted in the Zhambyl district of the Almaty region. Based on the monitoring results, the two most suitable settlements for analysis were selected: Karakastek village and Umbetaly village.

The warehouse in the village of Karakastek is partially destroyed, the area of the warehouse is 100 square meters. m. GPS coordinates of the warehouse: 43°08’25.7”N – 76°06’14.7”E. Residential houses are located at a distance of 100 m from the warehouse, livestock grazes around the warehouse (Figure 1) and the local population handles/removes parts of the foundation of the building. According to the inventory carried out in 2008, 8 iron containers were found rusty and without a label, and 7 plastic packages torn and without a label. The plastic containers contained a white substance, while the iron containers contained a yellow oily substance. The total amount of pesticides is 10200 kg [29]. No pesticides have been found in the warehouse building at the moment.

In the village of Umbetali, the warehouse area is 100 sq. m., the warehouse building is destroyed. GPS coordinates: 43°16’55.9”N – 76°19’58.9”E. Residential buildings were built around the warehouse, there is a lake nearby where children bathe (Figure 2). According to the inventory data of 2008, the soil surface was contaminated with green substances of unknown origin, old plastic bags from pesticides were lying around [28]. The area is now privately owned. Currently, no pesticides have been found in this area.



A – GPS coordinates: 43°08’25.7”N – 76°06’14.7”E
B, C – pesticide contamination

Figure 1 – The territory of the former pesticide warehouse in the village of Karakastek Zhambyl district of Almaty region

A – GPS coordinates: 43°16’55.9”N 76°19’58.9”E
B, C – water sampling;
D – polluted area where sheep graze

Figure 2 – The territory of the former pesticide warehouse in the village of Umbetali, Zhambyl district, Almaty region

Determination of POPs degradation products in samples of water, soil and food of plant and animal origin

Chemical analysis of drinking water and natural water showed that the content of pesticides in the samples does not exceed the allowed concentrations. Only a very slight excess of MPC for copper was noted in a sample of natural water from the village of Umbetaly. Chemical analysis of soil samples showed an excess of MPC for a number of pesticides by 2-18 times in both studied settlements (Table 1). Thus, α HCCH, β HCCH, aldrin, deleldrin, 4,4-DDE, 2,4-DDD, endrin, chlorobenzilate, 4,4-DDT and endosulfan 2 were found in soil samples in Karakastek village. In the soil of Umbetali village hexachlorobenzene, aldrin, deleldrin, 4,4-DDE, 2,4-DDD, endrin, chlorobenzilate, 4,4-DDT and endosulfan 2 were found. Also, in soil samples of two studied locations, an excess of the MPC of heavy metals (Zn, Pb, As , Ni, Cu, Cr) by 1.1-21.6 times (Table 2). So, in soil samples from the settlement of Karakastek, the content of zinc exceeds the MPC by 4.5 times, lead by 1.1 times, arsenic by 8.0 times, nickel by 5.4 times, copper by 15.2 times, chromium by 21 times. ,6 times. In soil samples from the village of Umbetali, the content of heavy metals was also increased: zinc (4.9 MPC), arsenic (4.7 MPC), nickel (3.0 MPC), copper (10.2 MPC), chromium (19.7 MPC). Thus, soil samples from the studied settlements are contaminated with both pesticides and heavy metals.

Table 1 – Content of organochlorine pesticides in soil, mg/kg

Sampling locations	MPC / RAC,	v. Karakastek				v. Umbetaly			
Sample type	mg/kg	Soil №1	Soil №2	Soil №3	Mean M±m	Soil №1	Soil №2	Soil №3	Mean M±m
Hexachlorobenzene	0,5	<0,0001	0,05	0,06	0,0363±0,0329	0,05	**0,07**	**0,08**	**0,0664±0,0163**
α-HCH	0,1	**0,66**	0,05	0,04	**0,2521±0,3540**	0,03	0,04	0,08	0,0465±0,0265
γ-HCH (lindane)	0,1	**0,16**	0,06	0,05	0,0941±0,0614	0,06	0,04	0,03	0,0408±0,0144
β-HCH	0,1	**0,36**	**0,13**	0,20	**0,2317±0,1148**	0,04	0,05	0,36	0,1479±0,1853
Heptahlor	0,05	**0,10**	0,02	0,02	0,0458±0,0497	0,02	0,02	0,03	0,0207±0,0042
δ- HCH		<0,0001	0,18	0,17	0,1171±0,1015	0,11	0,06	0,09	0,0865±0,0280
Aldrine	0,0025	**0,02**	**0,06**	**0,07**	**0,0505±0,0272**	**0,06**	**0,03**	**0,04**	**0,0439±0,0133**
Heptachlor epoxide	0,05	0,02	0,01	0,01	0,0122±0,0042	0,01	0,01	0,00	0,0055±0,0023
эндосульфан 1	0,1	0,09	0,03	0,05	0,0548±0,0334	0,02	0,02	0,04	0,0262±0,0116
Keltan		0,02	0,01	0,02	0,0157±0,0044	0,00	0,00	0,02	0,0066±0,0083
4,4- DDE	0,1	**0,36**	0,08	**0,54**	**0,3250±0,2350**	0,10	**0,11**	**0,28**	**0,1659±0,1024**
Chlordane	0,1	0,06	0,01	0,02	0,0304±0,0296	0,01	0,00	0,01	0,0086±0,0045
Dieldrin	0,0005	**0,16**	**0,04**	**0,05**	**0,0817±0,0670**	**0,02**	**0,08**	**0,06**	**0,0511±0,0292**
2,4- DDD	0,1	**0,37**	**0,12**	0,09	**0,1929±0,1516**	0,03	0,04	0,06	0,0451±0,0174
Chlorbezyate	0,02	**0,28**	**0,02**	**0,12**	**0,1396±0,1297**	0,02	**0,02**	**0,05**	**0,0310±0,0191**

Endrin	0,001	**0,36**	**0,01**	**0,32**	**0,2298±0,1903**	**0,05**	**0,17**	**0,09**	**0,1032±0,0609**
4,4-DDD	0,1	0,07	0,01	0,05	0,0425±0,0322	0,01	0,05	0,02	0,0285±0,0175
Endosulfan 2	0,1	**1,59**	**1,73**	**1,73**	**1,6825±0,0838**	**2,16**	**1,00**	**2,38**	**1,8457±0,7380**
4,4- DDT	0,1	<0,0001	**0,72**	**0,40**	**0,3714±0,3590**	**1,10**	**1,34**	**0,40**	**0,9483±0,4934**
Endrin aldehyde		<0,0001	0,08	0,06	0,0444±0,0398	0,03	0,02	0,03	0,0307±0,0054
Endosulfan sulfat		10,93	0,14	0,07	3,7168±6,2493	0,08	0,07	0,04	0,0658±0,0213
Dibutylendan		<0,0001	<0,0001	0,17	0,0561±0,0971	<0,0001	0,02	0,16	0,0623±0,0881
Metoxichlor		0,28	0,19	0,20	0,2250±0,0517	0,88	0,16	0,71	0,5814±0,3752
Hexabromobenzene		0,35	<0,0001	0,61	0,3169±0,3037	<0,0001	1,35	0,27	0,5382±0,7132

Table 2 – The content of heavy metals in the soil

Sampling locations	MPC,	v. Karakastek				v. Umbetaly			
Sample type	mg/kg	Soil №1	Soil №2	Soil №3	Mean M±m	Soil №1	Soil №2	Soil №3	Mean M±m
Zn	23	**116,9**	**94,5**	**96,7**	**102,7000±12,3503**	**84,3**	**102,5**	**148,0**	**111,6200±32,8105**
Cd	0,5	<0,01	<0,01	<0,01	0,0000±0,0000	<0,01	<0,01	<0,01	0,0000±0,0000
Co	5	<0,01	<0,01	<0,01	0,0000±0,0000	<0,01	<0,01	<0,01	0,0000±0,0000
Pb	32*	**44,2**	27,6	30,4	**34,0833**±8,9074	16,2	22,6	22,4	20,3933±3,6417
As	2*	**15,0**	**18,4**	**14,9**	**16,0733±1,9989**	**11,9**	**6,6**	**9,8**	**9,4500±2,6583**
Ni	4	**29,9**	**35,2**	<0,01	**21,7133±18,9915**	<0,01	<0,01	**36,4**	**12,1433±21,0329**
Cu	3	**38,9**	**50,3**	**47,6**	**45,6167±5,9580**	**26,6**	**31,6**	**33,4**	**30,5367±3,5114**
Cr	6	**138,1**	**91,9**	**158,5**	**129,5067±34,1079**	**125,8**	**119,4**	**101,9**	**115,7100±12,3773**
For the elements Zn, Co, Ni, Cu, mobile forms are determined; for elements Cd, Pb, As – gross forms.									

Chemical analysis of food of plant origin (apples, pears, tomatoes, cucumbers, bell peppers) and animal origin (meat, milk), collected from private gardens, orchards of Karakastek and Umbetali residents, who live near former pesticide warehouses, showed that that the content of pesticides in product samples does not exceed the maximum permissible concentrations (Tables 3-4).

However, the products of both plant and animal origin were contaminated with heavy metals, especially Ni and Co. Thus, the content of Ni (maximum concentration limit: 0.5 mg/kg) in samples of cucumbers from the village of Karakastek is 0.64 mg/kg, in apples – 0.10 mg/kg, pears – 1.05 mg/kg, in bell peppers from the village of Umbetali – 1.42 mg/kg. kg.

The content of Co (maximum concentration limit: 0.1 mg/kg) in products from Karakastek is exceeded: in apples 10 times (0.97 mg/kg), pears 12 times (1.27 mg/kg), in tomatoes 4 times (0.43 mg/kg). kg), in cucumbers 7 times (0.66 mg/kg), in bell peppers 12 times (1.17 mg/kg). In products from the village of Umbetala, the concentration of Co is: in apples – 1.83 mg / kg (exceeded 18 times), in pears – 1.32 mg / kg (13 times), in tomatoes – 0.50 mg / kg (5 times), in cucumbers – 0.40 mg / kg (4 times), and in bell pepper – 1.37 mg / kg (14 times).

In two villages, the nickel content in milk and meat samples exceeded the permissible concentration by 3-4 times, and cobalt by 17-18 times.

Table 3 – The content of heavy metals in foods of plant origin, mg/kg

№	Name of the object of analysis	Qty (pcs)	Code	Zn	Cu	Ni	Co	Pb	As	Cd
				mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
		MPC		10	10	0,5	0,1	0,4	0,2	0,03
1	2	3	4	5	6	7	8	9	10	11
	v. Karakestek									
1	Apple (1 site)	5	Б (Я)1	0,33	0,72	0,01	1,01	0,003	<0.001	<0.001
2	Apple (2 site)	5	Б (Я)2	0,36	0,51	0,03	1,01	<0.001	<0.001	<0.001
3	Apple (3 site)	8	Б (Я)3	0,32	0,69	0,02	0,91	<0.001	<0.001	<0.001
Average				**0.34 ± 0.02**	**0.64 ± 0.11**	**0.02 ± 0.01**	**0.97 ± 0.06**			
4	Pear (1 site)	5	Б (Г)1	0,60	1,17	0,26	1,27	<0.001	<0.001	<0.001
5	Pear (2 site)	5	Б (Г)2	0,40	0,83	0,15	1,16	<0.001	<0.001	<0.001
6	Pear (3 site)	4	Б (Г)3	0,47	0,98	0,37	1,38	<0.001	<0.001	<0.001
Average				**0.49 ± 0.10**	**0.99 ± 0.17**	**0.26 ± 0.11**	**1.27 ± 0.11**			
7	Tomato (1 site)	5	Б (П)1	0,12	0,31	0,42	0,40	<0.001	<0.001	<0.001
8	Tomato (2 site)	5	Б (П)2	0,15	0,35	0,49	0,46	<0.001	<0.001	<0.001
9	Tomato (3 site)	5	Б (П)3	0,21	0,33	0,45	0,43	<0.001	<0.001	<0.001
Average				**0.16 ± 0.05**	**0.33 ± 0.02**	**0.45 ± 0.04**	**0.43 ± 0.03**			
10	Cucumber (1 site)	5	Б (О)1	0,18	0,40	0,56	0,56	<0.001	<0.001	<0.001
11	Cucumber (2 site)	5	Б (О)2	0,29	0,54	0,74	0,78	<0.001	<0.001	<0.001
12	Cucumber (3 site)	5	Б (О)3	0,21	0,44	0,62	0,65	<0.001	<0.001	<0.001
Average				**0.23 ± 0.06**	**0.46 ± 0.07**	**0.64 ± 0.10**	**0.66 ± 0.11**			
13	Bulg. pepper (1 site)	5	Б (Б)1	0,33	0,79	0,08	1,12	<0.001	<0.001	<0.001
Prolongation of table										
14	Bulg. pepper (2 site)	5	Б (Б)2	0,44	0,95	0,33	1,34	<0.001	<0.001	<0.001
15	Bulg. pepper (3 site)	4	Б (Б)3	0,31	0,72	0,21	1,03	<0.001	<0.001	<0.001
Average				**0.36 ± 0.07**	**0.82 ± 0.12**	**0.21 ± 0.12**	**1.17 ± 0.16**			

1	2	3	4	5	6	7	8	9	10	11
	v. Umbetaly									
1	Apple (1 site)	5	Г (Я)1	1,04	1,19	0,11	1,99	<0.001	<0.001	<0.001
2	Apple (2 site)	5	Г (Я)2	0,98	0,90	0,10	1,67	<0.001	<0.001	<0.001
3	Apple (3 site)	5	Г (Я)3	1,01	1,48	0,09	1,83	<0.001	<0.001	<0.001
Average				**1.01 ± 0.03**	**1.19 ± 0.29**	**0.10 ± 0.01**	**1.83 ± 0.16**			
4	Pear (1 site)	5	Г (Г)1	0,30	0,76	1,05	1,38	<0.001	<0.001	<0.001
5	Pear (2 site)	5	Г (Г)2	0,37	0,90	1,22	1,26	<0.001	<0.001	<0.001
6	Pear (3 site)	5	Г (Г)3	0,24	0,61	0,88	1,32	<0.001	<0.001	<0.001
Average				**0.30 ± 0.07**	**0.76 ± 0.15**	**1.05 ± 0.17**	**1.32 ± 0.06**			
7	Tomato (1 site)	5	Г (П)1	0,19	0,18	0,25	0,67	<0.001	<0.001	<0.001
8	Tomato (2 site)	5	Г (П)2	0,12	0,23	0,33	0,33	<0.001	<0.001	<0.001
9	Tomato (3 site)	4	Г (П)3	0,05	0,12	0,17	0,50	<0.001	<0.001	<0.001
Average				**0.12 ± 0.06**	**0.18 ± 0.06**	**0.25 ± 0.08**	**0.50 ± 0.17**			
10	Cucumber (1 site)	5	Г (О)1	0,11	0,21	0,29	0,29	<0.001	<0.001	<0.001
11	Cucumber (2 site)	5	Г (О)2	0,14	0,23	0,46	0,45	<0.001	<0.001	<0.001
12	Cucumber (3 site)	2	Г (О)3	0,14	0,33	0,46	0,45	<0.001	<0.001	<0.001
Average				**0.13 ± 0.02**	**0.26 ± 0.07**	**0.41 ± 0.10**	**0.40 ± 0.09**			
13	Bulg. pepper (1 site)	4	Г (Б)1	1,48	1,39	1,42	1,37	<0.001	<0.001	<0.001
14	Bulg. pepper (2 site)	4	Г (Б)2	1,52	1,68	1,36	1,50	<0.001	<0.001	<0.001
15	Bulg. pepper (3 site)	4	Г (Б)3	1,45	1,10	1,48	1,24	<0.001	<0.001	<0.001
Average				**1.48 ± 0.04**	**1.39 ± 0.29**	**1.42 ± 0.06**	**1.37 ± 0.13**			

Table 4 – The content of organochlorine pesticides in food of animal origin

№	Name of the object of analysis	Quantity	Unit	Zn	Cu	Ni	Co	Pb	As	Cd
				mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
MPC				**10-Meat// и 1-Milk**	**5,0-Meat// 0,5-Milk**	**0,5-Meat// 0,1-Milk**	**0,5-Meat// 0,1-Milk**	**0,05-Meat// 0,05-Milk**	**0,5-Meat// 0,05-Milk**	**0,05-Meat// 0,01-Milk**
	v. Karakestek									
1	milk (1 site)	1L	mg/kg	0,83	1,83	1,28	1,63	<0.001	<0.001	<0.001
2	milk (2 site)	1L	mg/kg	0,76	1,79	1,01	1,87	<0.001	<0.001	<0.001
3	milk (3 site)	1L	mg/kg	0,91	1,81	1,55	1,40	<0.001	<0.001	<0.001
Average				**0.83 ± 0.07**	**1.81 ± 0.02**	**1.28 ± 0.27**	**1.63 ± 0.24**			
4	meat (1 site)	300 g	mg/kg	1,11	1,42	1,11	1,69	<0.001	<0.001	<0.001
5	meat (2 site)	300 g	mg/kg	1,04	1,54	1,31	1,82	<0.001	<0.001	<0.001

6	meat (3 site)	300 g	mg/kg	1,07	1,65	0,92	1,57	<0.001	<0.001	<0.001
Average				**1.07 ± 0.04**	**1.54 ± 0.11**	**1.11 ± 0.19**	**1.69 ± 0.12**			
	v. Umbetaly									
10	milk (1 site)	1L	mg/kg	0,82	2,04	1,12	1,86	<0.001	<0.001	<0.001
11	milk (2 site)	1L	mg/kg	1,04	2,17	1,11	1,99	<0.001	<0.001	<0.001
12	milk (3 site)	1L	mg/kg	0,98	1,90	1,12	1,67	<0.001	<0.001	<0.001
Average				**0.95 ± 0.12**	**2.04 ± 0.14**	**1.12 ± 0**	**1.84 ± 0.16**			
13	meat (1 site)	300 g	mg/kg	0,61	1,66	1,22	1,93	<0.001	<0.001	<0.001
14	meat (2 site)	300 g	mg/kg	0,72	1,53	1,15	1,75	<0.001	<0.001	<0.001
15	meat (3 site)	300 g	mg/kg	0,49	1,59	1,18	1,61	<0.001	<0.001	<0.001
Average				**0.61 ± 0.11**	**1.59 ± 0.06**	**1.18 ± 0.04**	**1.76 ± 0.16**			

***Questioning and collection of biosamples of* the population living near the centers of pesticide contamination to replenish the gene bank**

To determine the impact of long-term pesticide pollution on the genetic status of the population, peripheral blood samples were collected from people living in close proximity to the former pesticide warehouses in the village of Karakastek (25 people) and the village of Umbetali (25 people). When collecting the material, voluntary informed consent to the study was issued and at the same time a detailed questionnaire was conducted, including both personal data and information on health status, pedigrees, consumption of food of plant and animal origin. Table 5 shows representative data for the studied cohorts. According to the questionnaire data, the ethnic composition of the studied groups is not very diverse and includes mainly representatives of Kazakh nationality. The average age in the studied groups is in the range of 40-50 years. Personal data of people were also analyzed in relation to medical status. We also used the ROFES device to assess the current state of health (see section 2.2.2). The measurement results were evaluated according to a five-point rating system. Table 6 presents data on the types of diseases found in residents of the studied settlements. It was found that the largest percentage of people with chronic diseases is represented among the surveyed residents of the village of Karakastek (100%), and 28% of them have arterial hypertension. In addition, there was an increased percentage of diseases of the genitourinary system, gastrointestinal tract and vascular diseases. Also, people with cancer were found in both cohorts.

Table 5 – Ethnicity, age, and sex of the individuals in surveyed cohorts

Settlement	Number of individuals	Ethnicity, ind. (%)	Males, ind. (%)	Females, ind. (%)	Years of birth (mean age)
v. Karakastek	25	Kazakh – 24 (96%) Other – 1 (4%)	4 (16%)	21 (84%)	1939-1997 (50,48±3,14)
v. Umbetaly	25	Kazakh – 25 (100%)	10 (40%)	15 (60%)	1954-1999 (40,84±2,91)

Table 6 – Medical state of the individuals in surveyed cohorts

Diagnosis, number of individuals (%)	Locality	
	v. Karakastek	v. Umbetaly
CVD	2 (8%)	4 (16%)
DM	1 (4%)	2 (8%)
AH	7 (28%)	2 (8%)

Bronchitis	1 (4%)	-
Psoriasis	-	1 (4%)
GIT	3 (12%)	-
Diseases of the genitourinary system	4 (16%)	-
Diseases of the thyroid gland	3 (12%)	4 (16%)
Vascular diseases	3 (12%)	1 (4%)
Oncological diseases	1 (4%)	1 (4%)
Total	25 (100%)	9 (36%)
Notes – CVD – cardio-vascular diseases, DM – diabetes mellitus, AH-arterial hypertension, GIT – Diseases of the gastrointestinal tract		

Population cytogenetic analysis

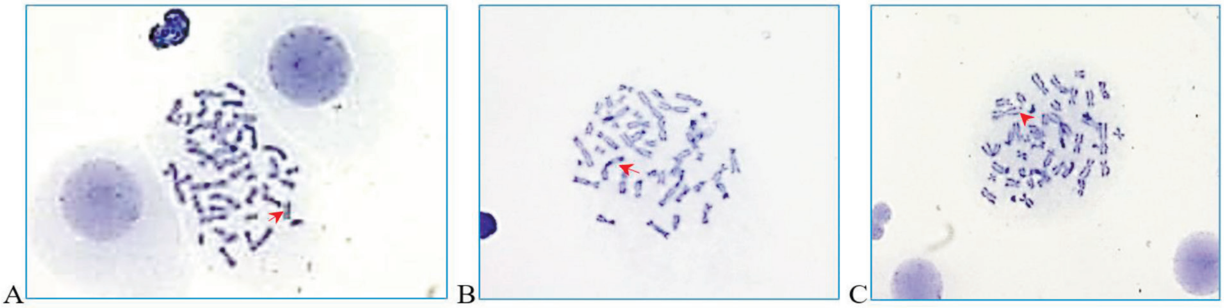
From the heparinized blood samples of 50 people (the village of Karakastek and the village of Umbetali, Zhambyl district, Almaty region, collection 2021), living in close proximity to pesticide-contaminated areas, metaphase chromosomes were prepared for cytogenetic analysis. When studying the frequency of chromosome aberrations, 8270 metaphase plates from 50 people were analyzed. Table 7 shows the results of cytogenetic analysis and reflects representative data for the studied cohorts.

Table 7 – The frequency of chromosome aberrations in residents of the studied settlements

Cohort	**Karakastek**	**Umbetaly**	**Taukaratyryk (control)**
Serveyed persons (males/females)	25 (4/21)	25 (10/15)	30 (12/18)
Average age	50,48±3,16	40.84±2.91	52.30±2.34
Number of studied metaphases	2500	4200	6108
Frequency of aberrations	0,02	0.018	0.008
Freequency of aberrations,%			
Chromatid gaps	0	0	0
Chromatid breaks	0,72±0,17	0,84±0,18	0.61±0.09
Single fragments	0,52±0,15	0,68±0,16	0.04±0.02
Chromatid type aberration frequency	**1,32±0,23** (p=0.01)**	**1,48±0,24****(p=0.002)	**0.65±0,10**
Chromosomal gaps	0	0	0
Chromosomal breaks	0,53±0,12	0,06±0,04	0.09±0,04
Paired fragments	0,08±0,06	0,20±0,09	0
Dicentrics	0,04±0,04	0	0
Rings	0	0	0,01±0,01
Chromosomal exchanges	0	0	0,01±0,01
Chromosome type aberration frequency	**0,40±0,13**	**0,28±0,011**	**0,19±0,05**
Total aberrations	39	44	52
The number of cells studied	**1,56±0,25** (p=0.01)**	**1,76±0,26**(p=0.002)**	6108
The number of cells with aberrations	2500	2500	52
The frequency of cells studied	39	40	0,85±0,12
Aberration frequency	**1,56±0,25** (p=0.01)**	**1,60±0,25**(p=0.009)**	**0.85±0.12**
The ratio of chromosomal and chromatid aberrations	**1:2.9**	**1:2.8**	**1.3:1**
Note: t>3, P<0,001 -*** high significance, t>2,5, P<0,01 -** moderate significance, t>2,0, P<0,05 -* low significance, t<2,0, P>0,05 – not significant			

As a comparison cohort (control), we used previously obtained data on cytogenetic analysis of residents of the village of Taukaraturik from an ecologically favorable region of the Almaty region [21].

When analyzing the spectrum of structural disorders of chromosomes, both the overall frequency of chromosome aberrations and their type (chromosomal and chromatid) are determined. Aberrations of the chromosomal type were represented by pair breaks and fragments, dicentrics and rings, chromatid type – by single breaks and fragments (Figure 3).



A) Karakastek – sample code PS-12, chromatid gap, x1000;
B) Karakastek – sample code PS-3, chromosome break, x1000;
C) Umbetaly – sample code PS-28, single fragment, x1000

Figure 3 – Typical aberrations of chromosomes in the studied cohorts

The residents of Karakastek and Umbetali showed a significant frequency of chromosomal aberrations compared to the control ($p \leq 0.01$). There are no significant differences between Karakastek and Umbetali in the overall level of chromosomal aberrations and in individual types of mutations. Significant differences ($p \leq 0.01$) compared with the control were noted in relation to the overall frequency of aberrations of the chromatid type, but not of the chromosomal type (table 7).

The ratio of chromosomal and chromatid aberrations to a certain extent indicates the type of mutagenic effect. In spontaneous mutagenesis, the frequency of chromosomal abnormalities is lower than or equal to the frequency of chromatid ones. The predominance of chromosome-type aberrations may indicate radiation exposure, which can cause double-strand breaks of chromosomes. A shift towards chromatid mutations, as in the case of the results of a survey of residents of paragraphs. Karakastek (1:2.9) and Umbetali (1:2.8), but not residents of the village of Taukaraturik (1.3:1), testifies in favor of chemical mutagenesis.

Populations who lived in the territory of old buried storage facilities were regularly exposed to pesticides in various doses are of great interest for studying their genetic and cytogenetic status. Chromosome aberrations (CA) are an important tool for assessing pesticide exposure. The genotoxic potential of pesticides is a major risk factor for long-term health effects. The present study aimed to evaluate CA among these populations. It was shown that the frequencies of chromosome aberrations in human lymphocytes from Karakastek significantly exceed the control level (village Taukaraturik) by 0.75 times, and Umbetali – by 0.78 times ($p < 0.01$). These data indicate a higher frequency of aberrations in these cohorts compared to representatives of other previously surveyed villages from the Talgar region [29]. These results are consistent with the results of chemical analysis which showed that the content of pesticide residues in foodstuffs in these villages was significantly lower than in previously studied ones, as well as the frequency of chromosome aberrations. However, the observed shift of mutations towards chromatid over chromosomal in both settlements indicates the nature of chemical mutagenesis. In addition to POPs, Ni and Co contamination in food products was found in both settlements. It is likely that complex interactions between pesticides and heavy metals (i.e., their combined action) has a synergistic effect.

A large number of studies have been conducted regarding chromosome aberrations in connection to pesticides [31-35].

Thus, Bianco, G.E., et al. showed a significant increase in CA in individuals exposed to pesticides compared with the control group (4.20 ± 0.15 vs. 1.00 ± 0.05, respectively; $p < 0.001$), indicating that pesticides are clastogenic agents causing DNA damage [36].

DE, Mañas FJ et al. analyzed chromosomal aberrations, micronuclei and comets in 30 workers in the province of Córdoba. They showed that occupational exposure to pesticides increases CA, MN, and DNA fragmentation biomarkers, which are indicators of damage to genetic material, suggesting that chronic pesticide exposure is a potential health risk for workers [37].

Jelena Pajic, Dubravka et al. considered certain cytogenetic parameters as valuable markers for preventive medical screening, as the degree of cytogenetic damage reflects the cumulative exposure and possible health outcomes associated with chronic occupational genotoxic exposure [38]. Claudia Bolognesi and Nina Holland conducted a systematic review of the use of micronucleus assays and reported positive results for micronuclei associated with pesticide exposure. The range of increasing frequencies of micronuclei (FR=1.2 – 7.6) was associated with the degree, duration and types of exposure. [39].

It is known that under the conditions of anthropogenic load, pregnant women and children are the most vulnerable groups, and that reproductive function and immune defense primarily suffer [40]. Colombian researchers assessed infant exposure to one of the most commonly used pesticides in agricultural areas and showed a trend towards an increase in the frequency of markers of cytogenetic damage in the agricultural area groups compared to the control group [41]. In another study, the effects of exposure to organochlorine pesticides were studied in a group of mother/child couples (N=50) in rural Mexico where pesticides were applied from aircraft. Significantly higher levels of pesticides were found in cord blood than in maternal plasma. There is no increase in the frequency of micronuclei associated with pesticide exposure, but DNA damage assessed by comet analysis was significantly higher in cord blood of newborns than in mothers [42].

One of the most dangerous environmental pollutants are heavy metals that can enter the soil, water bodies and atmosphere as a result of not only natural processes, but also as a result of human activities. Two mechanisms of action of metals predominate: the induction of oxidative damage to DNA and the effect on DNA repair processes, which leads to an increase in genotoxicity in combination with various DNA damaging agents. In such heavy metals as Cd, Ni, Co, Pb, and As, DNA repair processes are disrupted at low, non-cytotoxic concentrations of the corresponding metal compounds [43]. An important role is played by understanding the influence of indirect mechanisms and their combined effects, which can cause genotoxic effects, even if the concentration of individual heavy metals does not exceed the MPC [44].

Morales et al. noted that the number of insertions increases under the influence of heavy metals as a result of a significant effect on DNA repair [45], other scientists have shown the ability of heavy metals to lead to the formation of double-stranded DNA breaks [46]. Lazutka et al. explain that the increase in the frequency of chromosome aberrations depends on the duration of exposure to heavy metals, but not on the duration of exposure to most organic substances and the ability of heavy metals to accumulate in various tissues of the body, and organic substances are usually metabolized and excreted quickly [47]. Existing literature data on the effects of metals give reason to believe that their intake into the body, which exceeds its physiological needs, can pose a serious genotoxic hazard [48]. A number of literary sources show a positive correlation between the degree of mutagenicity of heavy metals and cancer incidence [49-51], which indicates the need to exclude mutagenic factors from the human environment and the need to use preventive measures.

Conclusion

Environmental pollution with obsolete non-utilized pesticides and products of their metabolism through a variety of food chains affects the human body. Studying the health and genetic status of the population in Zhambul district, Almaty region, living in close proximity to the locations of unutilized banned pesticides, we paid attention to the components of the human food chain. The results of our study indicate the need to raise public awareness of pesticide use practices and strengthen food safety control services as a public health measure.

The study of genetic damage at the individual and population level is of great importance for monitoring the effectiveness of individual preventive measures, and in the case of a disease, for monitoring and correcting treatment. Analysis of the state and dynamics of the level of chromosome aberrations in the population living in areas contaminated by pesticides can help develop principles for predicting long-term biomedical consequences and contribute to public health management. To ensure that there is no threat to public health, it is important to involve the regional administrative resource and private landowners in the sharing of unused stocks of banned pesticides, land reclamation, and regulation of the food safety control system against pesticide contamination.

Conflict of interest

All authors have read and are familiar with the content of the article and have no conflict of interest.

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