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ENVIRONMENTAL POLLUTION OF SOME FOOTHILL REGIONS OF ARMENIA WITH ORGANOCHLORINE PESTICIDES AND ISSUES OF MORBIDITY

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Abstract

Introduction: *In three foothill regions of Armenia – marzes (provinces) of Lori, Kotayk and Tavush, comprehensive research was conducted to study the total levels of organochlorine pesticides (γ-hexachlorocyclohexane, 4,4-dichlorodiphenyltrichloroethane, 4,4-dichlorodiphenyldichloroethylene, 4,4-dichlorodiphenyldichloroethane) in environmental objects and human biological media (2013-2018).*

An assessment was conducted to evaluate the environmental status and possible adverse health effects of organochlorine pesticides on the population in regions with diverse agricultural activities. The levels of organochlorine pesticides were studied in surface waters, soil, coastal silt, as well as in plant agricultural products in dynamics (spring, summer, fall). The content of organochlorine pesticides in breast milk samples of mothers from studied regions was also determined.

Material and methods: *The determination of organochlorine pesticides was carried out by gas-liquid chromatography with an electron capture detector on a Perkin-Elmer F-17 gas chromatograph (Great Britain).*

Results: *A comparative analysis of the studied regions showed that the levels of organochlorine pesticides in environmental objects did not differ significantly. In all regions, the same pattern was observed – concentrations of organochlorine pesticides increased in the summer-fall period in the following environmental order: surface water - soil - coastal silt.*

In Kotayk marz, compared to Lori and Tavush, residues of dichlorodiphenyltrichloroethane were determined with high frequency in almost all environmental objects studied, mainly in soil samples – up to 60%. The average total content of organochlorine pesticides in agricultural products, as well as in breast milk samples, was significantly higher, p=0.03-0.001 and p=0.0003-0.00003, respectively. Additionally, the highest number of samples showing the simultaneous presence of all studied organochlorine pesticides (3-4 compounds) was noted in Kotayk, accounting for 24%. This indicates the levels of load and a kind of "contamination" of the human organism with these compounds.

Conclusion: *The study results are an important contribution to the available information on organochlorine pesticides. They highlight the importance of conducting regular monitoring of the environment and biomedia to assess the pollution levels with organochlorine compounds. This is crucial for understanding trends in the dynamics of target indicators in certain regions of Armenia.*

Keywords: *organochlorine pesticides, environment, breast milk, morbidity*

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INTRODUCTION

The living environment is one of the factors determining health and well-being. Current stage of social development is characterized by active human intervention in the environment due to the rapid growth of industry, especially the chemical industry, the use of transport, etc., which is the reason for the increase in environmental pollution - one of the most important health problems [*WHO, 2006; Global Chemicals Outlook, 2019*]. The solution to this problem is aimed, first of all, at ensuring safe living conditions and preventing the possible harmful effects of environmental pollution on public health [*Golikov R et al., 2017*]. It is recognized that environmental pollution by various chemicals, including pesticides, play a significant role in changing the nature and structure of morbidity and chronicity of diseases [*Belyaeva N et al., 2007; Kurlyandskiy B et al., 2007; Dunaev V et al., 2008; Mudriy I, 2008; Galani Y et al., 2021; You L, 2024*]. The dominant factor in the development of chronic noninfectious diseases is considered to be not only an increase in the volume of pollution by various chemicals, but also their qualitative change – the emergence of so-called global and "eternal" pollutants, which include persistent organic compounds, pesticides, metals, etc. [*Yablokov A, 2015*].

One of the modern health and environmental problems is environmental pollution with persistent organic pollutants (POPs), to which belong most organochlorine pesticides (OCPs). These compounds are very resistant to degradation processes, have the ability to bioaccumulate and biomagnify, as a result of which they can accumulate in significant concentrations at the highest levels of food chains even at low levels in the environment. They can have a wide range of adverse effects on human health [*Revich B, Shelepchikov A, 2008; Dudarev A et al., 2016; Adenuga A et al, 2022; Liu Q et al., 2024*].

It is recognized that agriculture is one of the main sources of environmental pollution and pesticides, including OCPs, are considered separately among numerous chemical pollutants, taking into account the characteristics of their use, high biological activity, etc. Their use is associated with a number of negative health effects at both high and low levels of exposure, diseases such as cancer, Alzheimer's and Parkinson's diseases, hormonal imbalances, changes in the neuroendocrine, immune systems, metabolism, reproductive and embryonic processes, development of asthma, hypersensitivity, allergies, etc. [*Zastenskaya I et al., 2013; Report of the UN Special Rapporteur, 2017; Kuang L et al., 2020; Hu L et al., 2021*].

Experts recognize that even so-called "safe" levels of organochlorines can have adverse effects and pose a risk to human health [*Porta M et al., 2002*]. Exposure to environmental pollutants occurs through several routes – inhalation, consumption, percutaneous absorption [*Chen M et al., 2015*]. Determination of OCP content in environmental objects, biomonitoring by measuring concentrations in biomedia are widely used in studying load levels and possible risks. Biomonitoring data directly reflects the overall content in the body, possible effects of OCPs/POPs upon entry through all routes. Such data are the most relevant indicator for assessing exposure, especially for bioaccumulative, persistent pollutants that remain in the body for a long time [*WHO Regional Office for Europe, 2015; Chávez-Almazán L et al., 2023; Mironova E et al., 2023; Romanić S et al., 2023; Jonathan J et al., 2024*].

We studied the environmental status and the human organism in some rural foothill regions of Armenia from the standpoint of OCP pollution, since much attention is paid to the development of agriculture in Armenia and it plays an important role in the economic structure of the republic. The study was aimed at comparative assessment of OCPs levels in environmental objects and human biological media, as well as studying their possible adverse effects on public health in regions with various agricultural activities.

MATERIAL AND METHODS

Comprehensive studies were carried out in rural areas of three foothill regions of Armenia (2013-2018) – in the marzes (provinces) of Lori (Spitak, Stepanavan, Tashir districts), Kotayk (Abovyan, Charentsavan, Hrazdan), Tavush (Dilijan, Ijevan, Noyemberyan districts). The selection of regions was justified by some similarity of location (1370-2500 m above sea level), natural and climatic conditions (moderately hot, dry summers, relatively frost-free winters), similar branches of agriculture (production of grain, potatoes, vegetables, live-

stock products, poultry farming), rural population (Fig. 1-2). At the same time, between the regions there are differences that are reflected in the values of gross agricultural output, amounting to 8.7%, 8.5% and 4.5%, respectively, for the regions of Lori, Kotayk and Tavush [*Marzes of the Republic of Armenia, 2023*].

To study the levels of some OCPs – gamma-hexachlorocyclohexane (γ-HCH), dichlorodiphenyltrichloroethane (4,4-DDT), dichlorodiphenyldichloroethylene (4,4-DDE), dichlorodiphenyldichloroethane (4,4-DDD) in environmental objects, monitoring points for sampling were determined for each district of the marz (8-10 points). Samples of surface water (n=243), soil (n=139), coastal sludge (n=135) were taken in dynamics (spring, summer, autumn) in accordance with the seasonality of agricultural work; samples of agricultural products – in summer and/or autumn (n=150) [*ISO, 2016; 2017; Interstate Standard, 2018; 2019*].

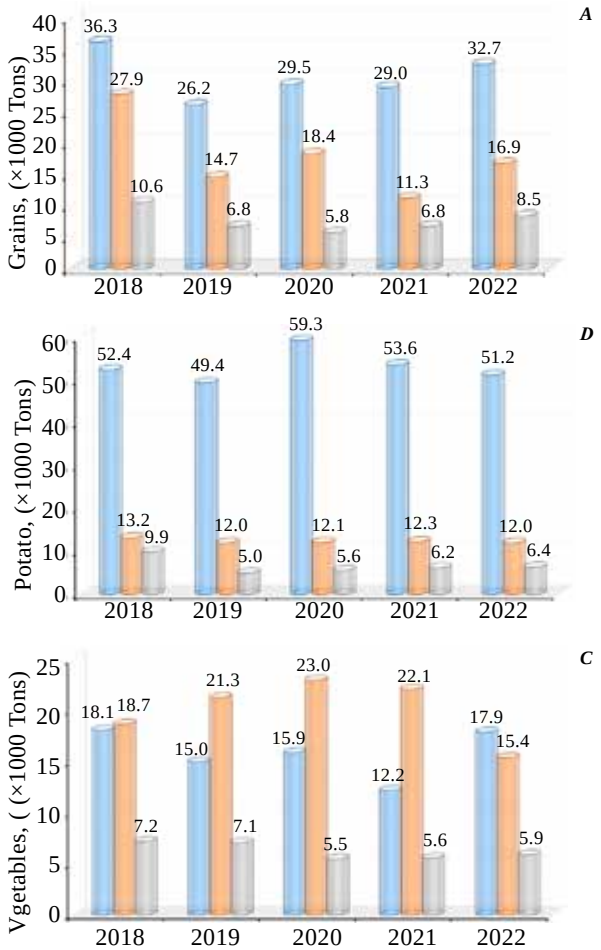

Figure 1. *Gross harvest of some agricultural outputs: Grains (**A**), Potato (**B**) and Vegetables (**C**), in Lori (left columns in each group), Kotayk (middle columns in each group) and Tavush (right columns in each group) marzes (thousand tons).*

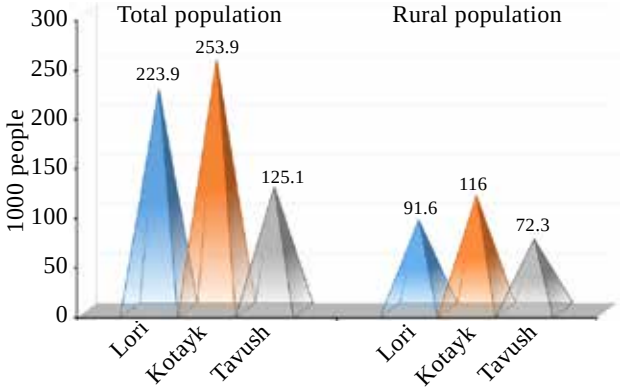

Figure 2. *Rural population in Lori, Kotayk and Tavush, thousand people.*

The content of OCPs was also determined in breast milk samples from residents of the study regions to assess the level of load on the body. The studies were conducted in the medical centers of Lori, Kotayk and Tavush regions among postpartum women who received obstetric care. Breast milk samples (30-50 *ml*) were taken on days 2-3 after birth from postpartum women selected by random sampling, after prior informed consent about the purpose of the study and obtaining the written agreement to participate. Samples were placed in plastic medical containers, which were stored under cold conditions until analytical testing (n=256).

Data from statistical reports of medical centers in the studied regions were copied and analysed in order to study the possible adverse effects of OCP levels circulating in the environment and human biological media (2005-2015). The analysis was carried out on the basis of intensive indicators (moving averages) calculated for the total number of cases among the adult population for certain classes of diseases targeted for OCPs - neoplasms (C00-D48); diseases of the endocrine system, nutritional disorders and metabolic disorders (E00-E90); congenital anomalies (malformations), deformations and chromosomal disorders (Q00-Q99) (*International classification of diseases, 10th revision, 2005*). Analytical incidence curves were constructed using the approximation coefficient (R^2). Trend indicators were calculated using linear, polynomial and other types of approximation. The selection of the appropriate equation was based on the maximum value of the approximation coefficient (R^2 = 0.7-1.0), which served as an additional

criterion in assessing the possible impact of environmental pollution with organochlorine pesticides on human health.

Determination of OCPs in samples of environmental objects, agricultural products, and breast milk was carried out by gas-liquid chromatography with an electron capture detector on a "Perkin-Elmer F-17" gas chromatograph (UK) [*Klisenko M et al., 1992*]. The analyses of OCPs were performed in two replications. The detection limit for γ-HCH and 4,4-DDE was 2.0 *ng/L, ng/kg*; 4,4-DDT and 4,4-DDD 4.0 *ng/L, ng/kg*. The total concentrations of OCPs studied was calculated by summing γ-HCH + 4,4-DDT + 4,4-DDE + 4,4-DDD according to seasons and studied objects. The average annual OCP values for each marz were also calculated as well. This approach was taken due to the similarity of the toxicity of these compounds. As it is known they all belong to the same chemical group of organochlorine origin and have a similar impact on the human organism.

The obtained data were statistically processed using modern programs (Excel, SPSS 16.0, etc.), as well as methods of parametric and nonparametric statistics. To assess the difference in mean values the Student's test (t-test): two- sample assuming unequal variances was used. The criterion for the significance of statistical indicators was the probability of a type I error of no more than 5% ($p < 0.05$) [*Glantz S, 1998; Dawson-Saunders B, Trapp R, 2001*].

RESULTS

Comparative analysis of the total annual average concentrations of OCPs in environmental objects: surface waters – 0.12±0.007 μg/l, 0.12±0.009; 0.14±0.01 μg/l, soil - 6.10±0.48 μg/kg; 5.80±1.02 μg/kg and 5.30±0.39 μg/kg and coastal sludge – 4.51±0.50 μg/kg; 4.28±0.35 μg/kg and 4.77±0.41 μg/kg, respectively, for the regions of Lori, Kotayk and Tavush, as well as the total values separately by season (spring, summer, autumn) showed that these levels did not differ significantly by marzes. At the same time, in the Kotayk marz, unlike Lori and Tavush, residues of DDT were determined with high frequency in almost all environmental objects studied, mainly in soil samples – up to 60%.

In comparative assessing the concentration of OCPs by environment objects, it was revealed that their total average annual concentrations in soil and

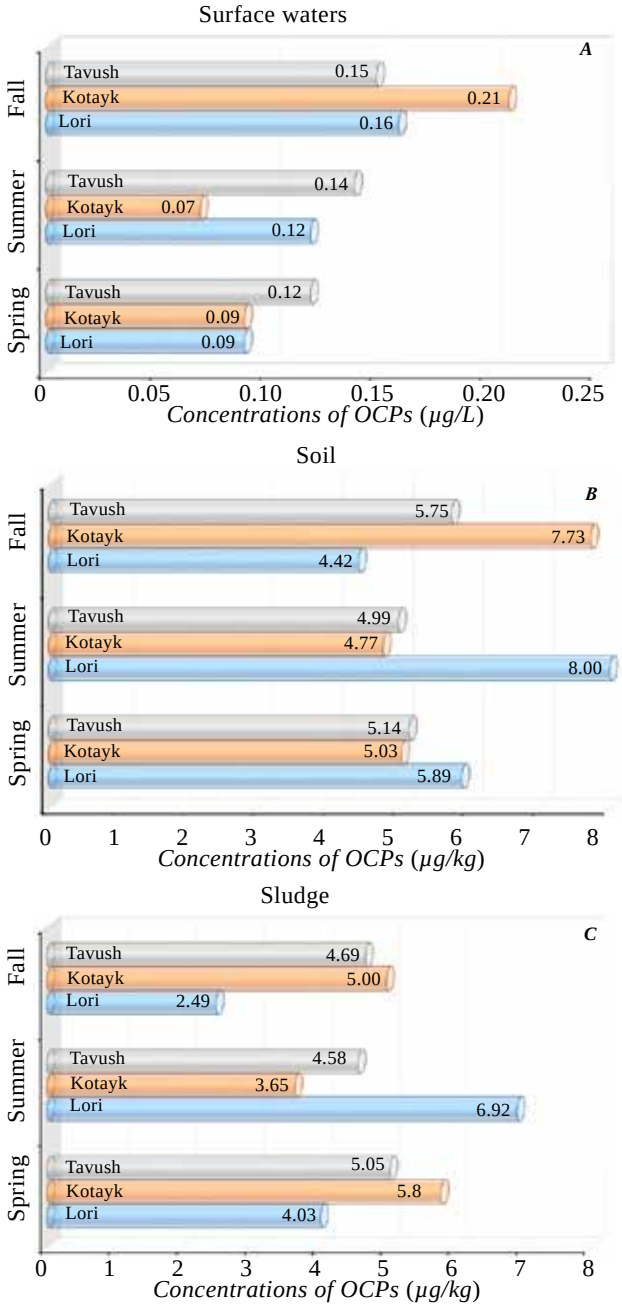

FIGURE 3. *Average total concentrations of OCPs in samples of surface water (A), soil (B) and coastal sludge (C) of Lori, Kotayk and Tavush marzes by seasons.*

sludge samples were significantly higher than in surface waters with a predominant increase in the summer-fall periods: p = 0.01-0.00001 (Fig. 3, 4).

The data obtained made it possible to clearly reflect the dynamics of the distribution of pollutants in environmental objects. Thus, according to the research results obtained, the following pattern was identified regarding the increase in the level of pollution in various environmental objects: surface water - soil - coastal sludge, i.e. during all study periods, OCPs levels determined in soil and sludge

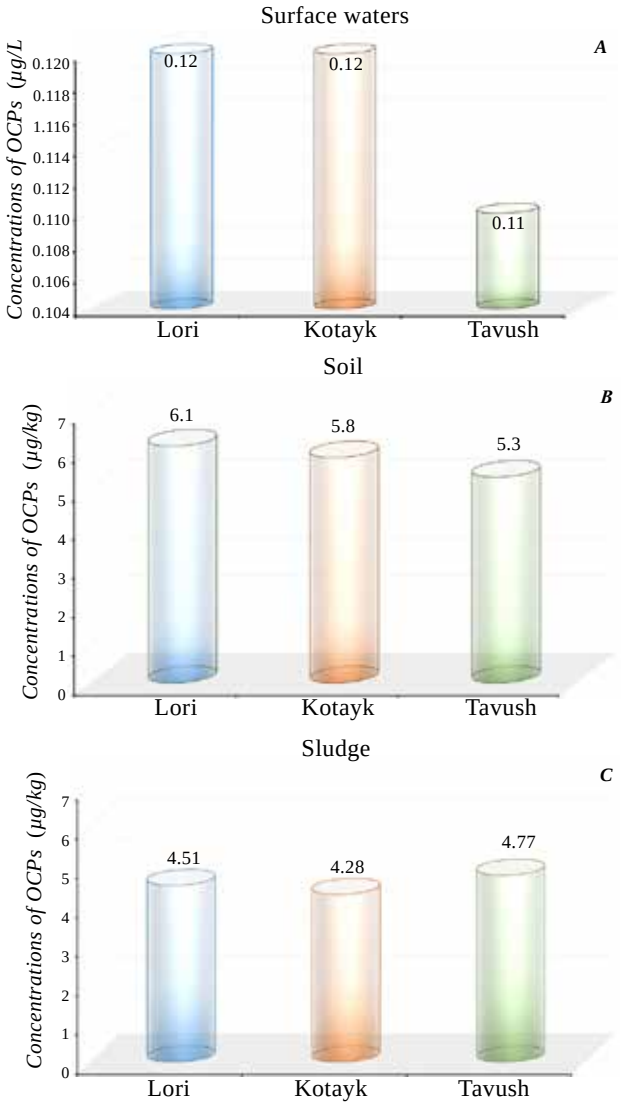

FIGURE 4. *Average annual total concentrations of OCPs in samples of surface water (A), soil (B)and coastal sludge (C) of Lori, Kotayk and Tavush marzes.*

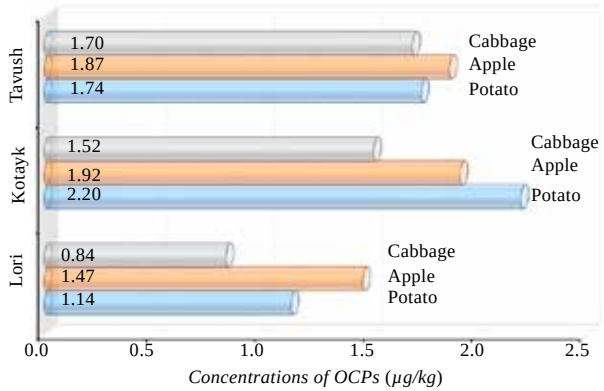

FIGURE 5. *Average total concentrations of OCPs in samples of plant products (potatoes, cabbage, apples) of Lori, Kotayk and Tavush marzes*

exceeded the concentrations determined in surface water samples, which is very typical for organochlorine compounds.

Significantly higher concentrations of OCPs in soil samples can be explained not only by their possible use in the recent past, but also by the fact that the soil is a kind of "depot" for their accumulation, and activation of translocation processes may occur during agricultural work.

The results showed that OCP residues were also found in vegetables and fruits (apples, cabbage, potatoes, etc.), and significantly higher total concentrations were recorded in Kotayk region compared to Lori and Tavush regions. Thus, in samples of potatoes and cabbage, as well as apples, these values were: 2.20±0.25 µg/*kg*, 1.14±0.07 µg/*kg*, 1.74±0.23 µg/*kg* and 1.52±0.07 µg/*kg*, 0.84±0.15 µg/*kg*, 1.70±0.79 µg/kg; 1.92±0.14 µg/kg, 1.47±0.14 µg/kg, 1.87±0.35 µg/kg, respectively, for agricultural products and marzes, p=0.033-0.004. It should be noted that the determined levels of OCPs both in the environmental objects and crops did not exceed the established hygienic standards (Fig. 5).

To study the levels of OCPs load on the organism in breast milk samples of residents of the studied marzes, both their total content and the number of simultaneously present OCPs were determined (Fig. 6).

According to the data obtained, in breast milk samples the highest total levels of OCPs were observed in Lori and Kotayk marz, which was significantly higher compared to Tavush marz – 3.40±0.26 µg/l, 4.06±0.40 µg/l and 2.38 µg/l. ±0.11 µg/l, respectively (p=0.0003-0.00003).

Studying the degree of pollution of biological

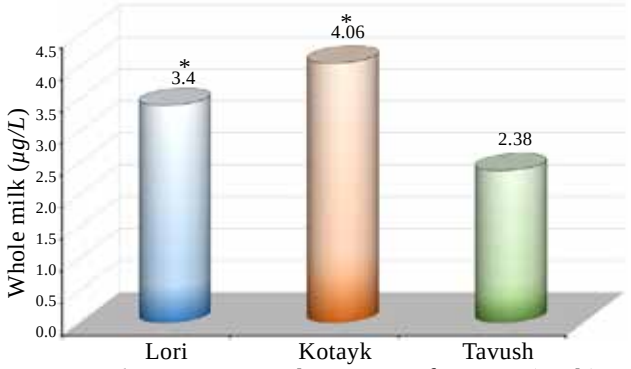

FIGURE 6. *Average total content of OCPs (µg/l.) in breast milk samples of residents of Lori, Kotayk and Tavush marzes, *- the differences are significant with the Tavush region, p=0,0003-0,00003.*

environments, i.e. the number of simultaneously detected pollutants in breast milk samples showed that the content of 2 OCPs (γ-HCH, DDE/DDT) was most often determined - in 57-65% of samples, the presence of 3 OCPs (γ-HCH, DDE, DDT) was detected with less frequency – in 13-18% of samples. At the same time, the largest number of breast milk samples with the presence of all studied OCPs (4 compounds) was observed in the Kotayk region (6%). The presence of only one OCP (γ-HCH) was more common in samples from Lori marz (30%) compared to Kotayk and Tavush marz (19%).

According to the presented data, the degree of contamination of human biological media was more higher in the Kotayk marz, where the largest number of pollutants (3-4 OCPs) were simultaneously determined in 24% of breast milk samples, compared to the Lori and Tavush marzes, 12.5% and 16.5%, respectively (Table).

In order to study the possible adverse effects of OCPs levels circulating in the environment and human biological media, disease prevalence indicators (moving averages) were calculated and analysed for some targeted classes of diseases among the adult population of the studied marzes (C00-D48, E00-E90, Q00-Q99). The dynamics of morbidity indicators for classes C00-D48 "Neoplastic diseases" and E00-E90 "Diseases of the endocrine system, nutritional disorders and metabolic disorders" for Kotayk and Lori marzes was similar - characterized by an increase in cases of diseases, R^2 = 0.9865 and R^2 = 0.9928; R2=0.9307 and R^2=0.9451, respectively, for marzes and classes, in contrast to Tavush marz, where a decrease in values was recorded - R^2=0.7619 and R^2=0.2307.

In the class "Congenital anomalies" (Q00-Q99), in all studied marzes there was generally a downward trend in indicators (Fig. 7).

TABLE

Number of breast milk samples with the simultaneous presence of several OCPs (%) in Lori, Kotayk and Tavush marzes.

OCPs (%)	Regions		
	Lori	Kotayk	Tavush
1	29.5	19.3	18.8
2	58.0	56.6	64.7
3	12.5	18.1	13.0
4	0.0	6.0	3.5

DISCUSSION

Analysis of the results showed that in the studied marzes, the actual levels of OCPs circulating in environmental objects did not differ significantly from each other. However, in the Kotayk marz, unlike Lori and Tavush, DDT residues were determined with high frequency in almost all studied environmental objects, mainly in soil samples - up to 60%. The average content of OCPs in breast milk samples of Kotayk residents was significantly higher than in Tavush marz - 4.06±0.40 μg/l and 2.38±0.11 μg/l, respectively (p=0.0003-0. 00003). In this marz, the largest number of breast milk samples was observed with the simultaneous presence of all the studied OCPs - in almost a quarter of the samples (24%) 3-4

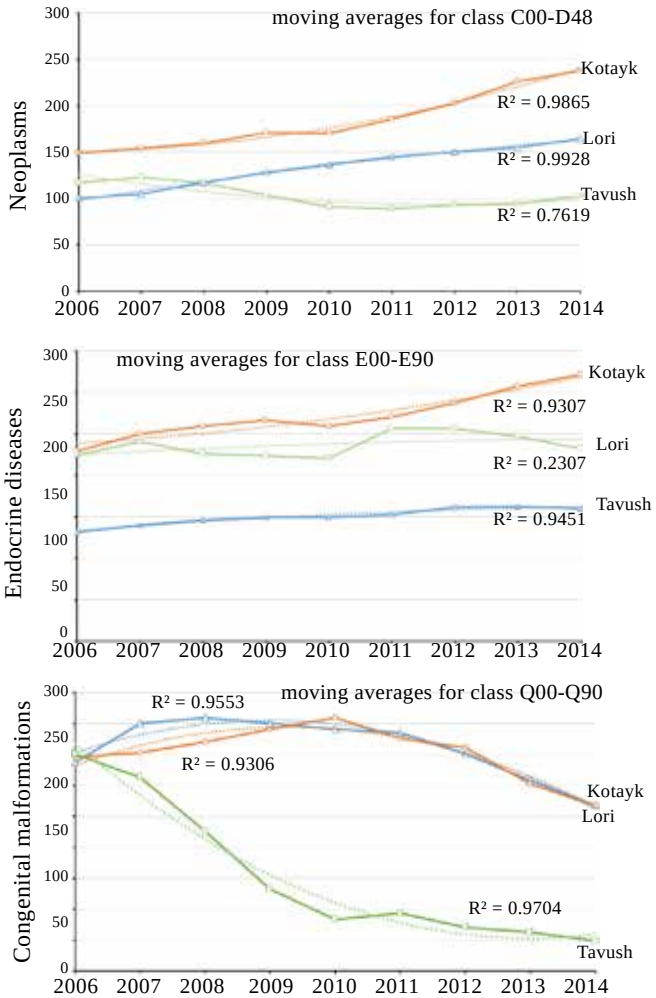

FIGURE 7. *Moving averages for the classes "Neoplasms" (C00-D48) (A) and "Endocrine diseases (the endocrine system, nutritional disorders and metabolic disorders) (E00-E90) (B), "Congenital anomalies" (Q00-Q99) (C)for the adult population of Lori, Kotayk, Tavush marzes (2006-2014).*

compounds were identified, which indicates levels of load and a certain degree of "contamination" of the human organism. OCPs residues in agricultural products sampled in the Kotayk marz were also significantly higher (p = 0.03-0.001).

One of the main routes of exposure to pesticides, including OCPs, is food. These compounds have an exceptional ability to accumulate at higher levels of the food chain and, due to their lipophilic properties, easily accumulate in animal foods rich in fat. However, the residues of OCPs contained in plant products of the Kotayk marz can also be one of the sources of their penetration and accumulation in the body of residents.

An analysis of the incidence rates of some target classes of diseases showed that in classes C00-D48 "Neoplastic diseases" and E00-E90 "Diseases of the endocrine system, nutritional disorders and metabolic disorders" in Kotayk marz there was an increasing trend - R^2 = 0.9865 and R^2 =0.9307 respectively. In the class "Congenital anomalies" (Q00-Q99), a downward trend in incidence was observed in all marzes - Lori, Kotayk, Tavush: R^2=0.9553, R^2=0.9306 and R^2=0.9704, correspondingly.

Thus, a comprehensive analysis of the totality of the results obtained, namely data from monitoring of OCPs in environmental objects and biomaterial of residents of the foothill regions of Lori, Kotayk, Tavush, incidence rates of certain target classes of diseases showed that Kotayk marz is somewhat different from the other studied regions.

However, it is still difficult to talk about some "contribution" of OCPs in terms of their presence in the environment and the human body in Lori, Kotayk, Tavush, since organochlorine pesticides found both in the environment, agricultural products, and in human biomaterial were determined in significantly lower levels when comparing the studied regions with valley ones (Ararat Valley).

At the same time, the categorical exclusion of the possible impact of circulating concentrations of OCPs and their presence in the human body on morbidity rates related, in particular, to classes of diseases targeted for the group of POPs, is somewhat controversial, since these compounds have the ability to have an adverse effect at levels not only not exceeding established hygienic standards, but even at significantly lower levels - at levels of subthreshold, "extra-low" concentrations. Under conditions of prolonged exposure to circulating ultra-low concentrations, organochlorine contaminants can cause various temporarily hidden pathological conditions and disorders of human health, which can subsequently manifest themselves at the population level.

Therefore, the presence of OCPs in the human body is alarming due to their potential adverse health effects. The results of the study are an important contribution to the available information on OCPs and indicate the need for periodic monitoring of the environmental status and human biological media to determine levels of contamination with organochlorine compounds and assess trends in the dynamics of indicators in certain regions of Armenia.

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