ORIGINAL ARTICLE

ASSESSMENT OF THE RISK OF ADVERSE EFFECTS OF DIFFERENT PESTICIDE GROUPS FOR HUMANS CONSUMING APPLES AND GRAPES TREATED WITH PESTICIDES

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ABSTRACT

The aim: To assess the risk of adverse effects of various groups of pesticides for humans, consuming apples and grapes (treated by pesticides).

Materials and methods: The gas-liquid chromatography, high-performance liquid chromatography, atomic absorption spectroscopy and tandem chromatography–mass spectrometry methods were used for the quantitative calculation of pesticides in apples and grapes. The possible intake of pesticides (with mentioned products) and the integral indicator of danger during their consumption were considered, while assessing the risk for the people consuming apples.

Results: It has been proven, that the processes of pesticide decomposition in growing agricultural crops (apples, grapes) occur according to an exponential model. The half-life periods of the studied pesticides in agricultural plants were established. And in terms of stability, the studied substances are moderately stable. An exception is bifenthrin – a persistent pesticide. The calculated risk values of dangerous exposure to pesticides, when consuming apples and grapes, treated with pesticides, were 2-3 orders of magnitude lower than the permitted level, and ranged from 2.0×10 -3 to 7.8×10 -2 . Most pesticides are moderately dangerous, according to the value of the integrated indicator of danger, during the product consumption, except for the kresoxim-methyl and clothianidin, which are not very dangerous.

Conclusions: The obtained results should be taken into account, considering the issue of expanding the pesticides application field, based on the studied substances, and the necessity for monitoring studies.

KEY WORDS: Consuming apples and grapes, The following pesticides, The obtained results

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INTRODUCTION

Apples and grapes are the main sources of vitamins and trace elements, compared to other fruit in Ukraine. This means, that the consumption of these products and their juices gives us the opportunity to obtain unique phytobioactive substances (polyphenols, anthocyanins, lycopene, resveratrol, beta-carotene, quercetin, naringin, nobiletin, caffeic acid, gallic acid, etc.). This group of products provides benefits for physical and mental health, and also takes part in the prevention of various non-communicable diseases (cardiovascular, neurological diseases, obesity, diabetes, osteoarthritis, and some types of cancer) [1, 2].

However, regardless of the benefits we get from eating apples and grapes, there is a possibility of dangerous exposure to pesticides, applied in the cultivation of agricultural crops, including fruit. It is known that pesticides can cause acute and chronic poisoning, being a risk factor for the development of oncological pathology, nervous and reproductive system diseases, as well as endocrine disorders [3, 4].

As of 2022, about 2,450 pesticide formulations have been registered on the territory of Ukraine, 13.4% of these substances are recommended for growing apples and 10.7% - grapes [5]. The number of pesticide formulations proposed for use is constantly increasing, which increases the risk for the population. Therefore, it is relevant to predict the danger, which comes with the consumption of agricultural products, when new pesticides are used.

THE AIM

The purpose of the study is to assess the risk of adverse effects of various groups of pesticides for humans, consuming apples and grapes (treated with pesticides).

MATERIALS AND METHODS

Field studies have been conducted for 10 years on the basis of the Institute of Hygiene and Ecology, valuing the dynamics of the content of different groups of

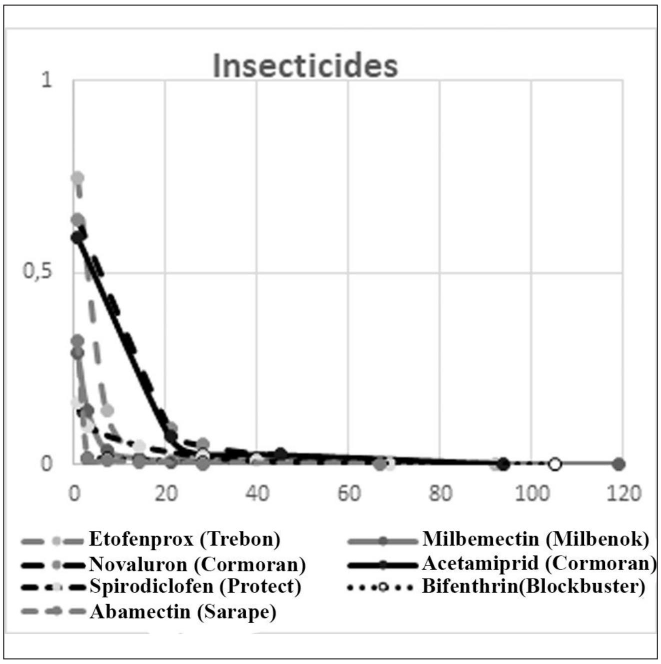

Fig. 1. Dynamics of the content of studied insecticides in apples

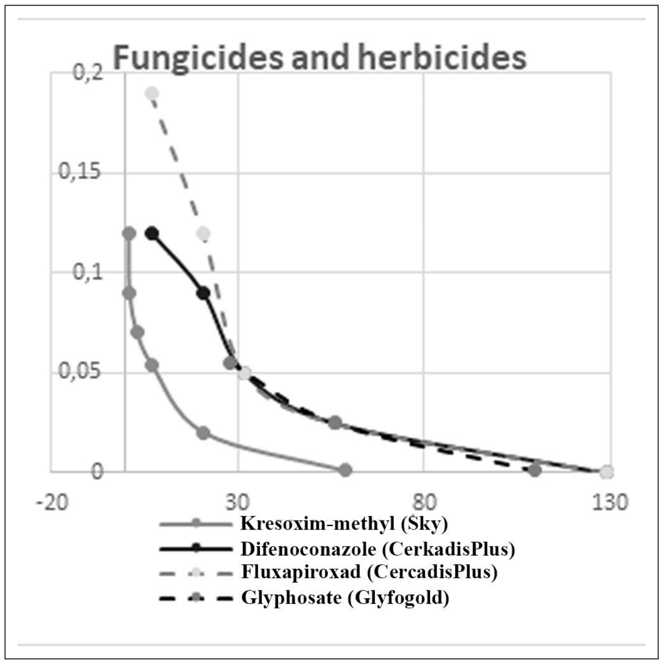

Fig. 2. Dynamics of the content of studied fungicides and herbicides in apples

pesticides in apples and grapes, in different soil and climate regions of Ukraine. The following pesticides formulations, used on apple trees and vineyards, were studied– insecticides Milbanok (active substance (a.s.) milbemectin), Trebon (drug etofenprox), Kormoran (drug acetamiprid, novaluron), Protect (drug spirodiclofen), Blockbuster (a.s. bifenthrin), Sarape (a.s. abamectin), TurboPresto (a.s. clothianidin, lambda-cyhalothrin), fungicides: Lifesul (a.s. sulfur), Bluestar (a.s. copper), Sky (a.s. kresoxim-methyl), Cerkadis Plus (a.s. difenoconazole, fluxapiroxad), herbicides: Zumer (a.s. glyphosate, oxyfluorfen), Glyfogold (a.s. glyphosate) in the industrial sector. The research was performed in the Institute of Hygiene and Ecology of the National Medical University named after O.O. Bogomolets, under accreditation DSTU ISO/IE 17025:2017.

The gas-liquid chromatography, high-performance liquid chromatography, atomic absorption spectroscopy and tandem chromatography–mass spectrometry methods were used for the quantitative determination of pesticides (Table I).

Mathematical modeling of the rate of destruction (the period of decomposition of the substance by 50% (T50), by 95% (T95), the constant of the rate of destruction (K), days) of the studied pesticides was performed using the exponential model, according to the first-order equation, and the calculation of the coefficient of determination (R^2).

Predicting the danger to the population consuming apples and grapes, treated by the chemical protection agents, was performed according to the methods proposed by specialists of the Institute of Hygiene and Ecology [6, 7].

The results were treated statistically, using the package of statistical programs IBM SPSS StatisticsBase v.22 and MS Excel. Descriptive statistics was used in the statistical analysis of the obtained data; comparison of average values of variables was carried out using parametric and non-parametric methods. Differences with a significance level of more than 95% ($p<0.05$) were considered reliable.

RESULTS

The first stage of our research was to determine the organoleptic properties of products, treated by the studied drugs.

Control samples were taken from areas, where untreated apple trees and vineyards were grown.

It was established that the organoleptic properties of the harvested apples and grapes (smell, color, appearance) did not differ from the control samples. The content of the studied active substances in apples and grapes was determined simultaneously with the assessment of organoleptic properties.

The analysis of the obtained results, regarding the content of the residues of the analyzed pesticides in the samples of apples and grapes, showed a gradual decrease in the content of the investigated fungicides and insecticides in the leaves and fruits.

Regarding application of the insecticides Milbenok, Trebon, Kormoran, Protect, Blockbuster, Sarape in the period after the blooming period, it was established that the initial concentrations of milbemectin in leaves were 0.29 mg/kg, apples - 0.037-0.042 mg/kg, etofenprox in apples - 0 ,24-0.14 mg/kg, acetamiprid and novaluron in leaves 0.59 mg/kg and 0.64 mg/kg, respectively, in apples 0.071 mg/kg and 0.094 mg/kg, respectively, spirodiclofen in apples - 0 .16 mg/kg, bifenthrin - 0.017 mg/kg, abamectin in leaves - 0.32 mg/kg, apples - 0.016 mg/kg. In the consequent periods it decreased, and during harvesting, did not exceed the limit of quantitative determination, according to the corresponding analytical method.

Field studies of the fungicides Lifesul, BlueStar, Cerkadis Plus, and Sky showed that the initial concentrations of sulfur in the fruit were 35.5 mg/kg, copper – 1.9 mg/kg, difenoconazole – 0.12 mg/kg, fluxapiroxad – 0, 19 mg/kg, kresoxim-methyl – 0.12 mg/kg. Until the 28th day of the study, the residual amounts of the studied fungicides did not exceed the limit of quantitative determination of the analytical method (Figs 1 and 2).

The studied herbicides were not detected in the fruits during all the periods of the study, which is explained by the method of application of Glifogold and Zoomer - application between the rows of apple orchards.

Field studies of Milbank and Protect insecticides were conducted in vineyards. The initial concentrations of the active substances of the studied insecticides were 0.044-0.051 mg/kg for milbemectin in grape berries, 0.45 mg/kg for leaves, and 0.25 mg/kg for spirodiclofen in berries. The analyzed active substances were determined in an amount below the limit of quantitative determination of the analytical method after 28-40 days of observation.

The initial concentrations of the active substances of the studied fungicides BlueStar and Sky for copper were 2.7 mg/kg, kresoxim-methyl - 0.19 mg/kg in berries, and 1.33 mg/kg kresoxim-methyl in grape leaves. In the subsequent periods of observation, the remaining amounts of the studied compounds were below the limits of quantitative determination of the corresponding method. Residues of the active substances glyphosate and oxyfluorfen herbicides Glyfogold , Zumer were not detected in grapes during all periods of the study, which is explained by the method of plant treatment (treatment between rows of vineyards).

Mathematical analysis of the obtained results of the studied active substances content dynamical pattern, in apples, during treatment by pesticides, made it possible

Table I. Studied pesticides' terms of use general characteristics and analysis methods

Pesticide	Consumption rate, l/ha (kg / t), (multiplicity)	Culture	Application technology	Active substance	MGD [Approval No.]	LQD /LD, mg/kg	
						apples	grapes
Zoomer	3.0 (1)	apple tree, grapes	Application between the rows of apple orchards/ vineyards	oxyfluorfen	GLC [3063-84]	0.04 / 0.01	0.04 / 0.01
				glyphosate	HPLC [363-2002]	0.1 /0.03	0.05 /0.02
Glyfogold	8.0 (1)			glyphosate	HPLC [363-2002]	0.1 /0.03	0.05 /0.02
Milbenok	1.0 (3)	apple tree, grapes	Applied during the growing season	milbemectin	HPLC [1763-2021]	0.02/0.008	0.02/0.008
Sky	0.2 (3) 0.3 (3)			kresoxim-methyl	GLC [205-2000]	0.05/0.02	0.05/0.02
Protect	0.6 (2) 0.4 (2)			spirodiclofen	HPLC [1024-2010]	0.02/0.008	0.02/0.008
Blue star	2.0 (4) 3.0, (4)			copper	AAS [24-97, 527-2004]	1.0/0.3	1.0/0.3
Trebon	0.5 (3)	apple		etofenprox	TXMC [1799-2022]	0.01/0.003	-
Cormorant	0.8 (2)			acetamiprid	HPLC [197-2000]	0.025/0.008	-
				novaluron	HPLC [302-2001]	0.05/0.02	-
Laifsul	6.0 (2)			sulfur	HPLC [292-2001]	5.0/2.0	-
Blockbuster	0.5 (2)			bifenthrin	GLC [6207-91]	0.05/0.02	-
Serkadis Plus	1.5 (3)			difenoconazole	GLC [55-97]	0.05/0.02	-
				fluxapiroxad	HPLC [1514-2018]	0.05/0.02	-
Sarape	1.5 (3)			abamectin	HPLC [1109-2011]	0.005/0.002	-
Harvest Smart	0.00035 (1)	apple	Applied when stored	1-methyl-cyclopropene	GLC [794-2007]	0.01/0.003	-

Notes: 1. MGD – methodical guidelines for the determination of pesticides; 2. LQD – the limit of quantitative determination; 3. LD – limit of detection; 4. HPLC – high performance liquid chromatography; 5. GLC - gas-liquid chromatography; 6. AAS – atomic absorption spectroscopy; 7. TCMS - tandem chromatography -mass spectrometry.

Table II. Degradation indicators of studied pesticides in apples (in the agro-industrial sector) (n=12)

Active substance (Pesticide)	Kinetic equation	K (day $^{-1}$)	T50 (day)	T95 (day)	R^2
		Insecticides			
Milbemectin (Milbenok)	$y = 0.0677e^{-0.059x}$ $y = 0.0714e^{-0.074x}$	0.059 ±0.002	11.8 ± 0.6	51.3±2.5	0.86 0.93
Etofenprox (Trebon)	$y = 0.1299e^{-0.067x}$ $y = 0.0665e^{-0.106x}$	0.086±0.009	8.4±0.9	36.7±3.7	0.78 0.78
Acetamiprid (Cormoran)	$y = 0.3297e^{-0.062x}$	0.062±0.001	11.2±0.2	48.7±0.9	0.94
Novaluron (Cormoran)	$y = 0.4451e^{-0.064x}$	0.064±0.001	10.7±0.2	46.6±0.7	0.98
Spirodiclofen (Protect)	$y = 0.163e^{-0.08x}$	0.079±0.001	8.6±0.1	37.5±0.4	0.98
Bifenthrin (Blockbuster)	$y = 0.0196e^{-0.038x}$	0.038±0.001	18.1±0.1	78.5±0.7	0.99
Abamectin (Sarape)	$y = 0.0401e^{-0.092x}$	0.057±0.001	12.1±0.2	52.5±1.0	0.80
		Fungicides			
Sulfur (Lifesul)	$y = 33.871e^{-0.003x}$	0.028±0.001	24.6±1.0	106.9±4.4	0.70
Copper (Bluestar)	$y = 2.0068e^{-0.047x}$	0.047±0.003	14.7±0.9	64.2±3.8	0.95
Kresoxim-methyl (Sky)	$y = 0.1146e^{-0.076x}$	0.076±0.001	9.0±0.2	138.6±9.1	0.95
Difenoconazole (CerkadisPlus)	$y = 0.3026e^{-0.058x}$	0.058±0.001	11.9±0.1	51.6±0.6	0.97
Fluxapiroxad (CercadisPlus)	$y = 0.406e^{-0.061x}$	0.061±0.001	11.3±0.1	49.1±0.3	0.98
		Herbicides			
Glyphosate (Zoomer)	$y = 1474.2e^{-0.165x}$	0.161±0.002	4.3±0.06	18.6±0.3	0.95
Oxyfluorfen (Zoomer)	$y = 650.96e^{-0.154x}$	0.248±0.002	2.8±0.02	12.1±0.09	0.89
Glyphosate (Glyfogold)	$y = 0.2623e^{-0.048x}$	0.077±0.001	8.9±0.1	38.9±0.3	0.98

Notes: 1. K - pesticide degradation rate constants; 2. T50 - half-life of pesticide; 3. T95 - the period of destruction of the pesticide by 95%; 4. R2 – coefficient of determination.

to establish the constants of the rate of degradation (K, days), the half-life period (T50), and the destruction of 95% (T95) of the active substances in apples (Table II).

Degradation indicators of the studied pesticides in apples, grown in orchards in the industrial sector, showed that the value of the coefficient of determination (R^2) was ranged within 0.70 - 0.99, which indicates a reliable relationship between the selected variables, and the selected exponential model is chosen , when modeling the results of the dynamical pattern of the studied pesticides' content.

Mathematical modeling of the obtained results of the studied pesticides' content dynamical pattern showed that the half-life (T50) of most of the analyzed insecticides was 8.4-12.1 days, organic fungicides 9.0-11.9 days, herbicides 2.8-8.9 days, which indicates their moderate persistence in grown agricultural crops (3rd class of danger, according to Sanitary Rules and Standards (DsanPiN) 8.8.1.002-98).

The insecticide bifenthrin and inorganic fungicides (copper and sulfur) had a T50 of 14.7-24.6 days, which made it possible to classify the analyzed compounds as persistent (3rd class of danger).

Similar studies were conducted, when pesticides were used in vineyards. The concentrations of the analyzed groups of pesticides in the initial period of the study were for milbemectin 0.45 mg/kg, spirodiclofen – 0.25 mg/kg, copper – 2.7 mg/kg, kresoxim-methyl – 1.33 mg/kg, glyphosate – < 0.05 mg/kg, oxyfluorfen - <0.04 mg/kg, when carrying out natural experiments in the agro-industrial sector (Table II).

It was found, that during growing of the vineyards, the concentrations of the studied groups of pesticides decreased. The process of pesticide decomposition in grapes, according to an exponential model (determination coefficient (R^2) = 0.92-0.99) (Table III), which is similar to the dynamical pattern of pesticides decomposition in apples.

Table III. Indicators of degradation of studied pesticides in grapes (n=12) (in the agro-industrial sector and personal subsidiary farms)

Active substance (drug)	Kinetic equation	K (day^{-1})	T50 (day)	T95 (era)	R^2
		Insecticides			
Milbemectin (Milbenok)	$y = 0.1441e^{-0.071x}$ $y = 0.0774e^{-0.065x}$	0.068 ±0.001	10.3 ± 0.6	44.6±1.0	0.92 0.98
Spirodiclofen (Protect)	$y = 0.2766e^{-0.084x}$	0.084±0.001	8.2±0.1	35.6±0.4	0.95
		Fungicides			
Copper (Bluestar)	$y = 2.8401e^{-0.035x}$	0.035±0.003	20.1±0.8	87.4±3.7	0.99
Kresoxim-methyl (Blue Sky)	$y = 0.8788e^{-0.098x}$	0.098±0.002	7.0±0.1	30.6±0.5	0.98
		Herbicides			
Glyphosate (Zoomer)	$y = 11.651e^{-0.075x}$	0.085±0.001	8.4±0.9	36.3±3.9	0.95
Oxyfluorfen (Zoomer)	$y = 6.5372e^{-0.074x}$	0.082±0.008	8.5±0.8	37.1±3.3	0.97
Glyphosate (Glyphogold)	$y = 20.981e^{-0.084x}$	0.077±0.008	9.3±1.2	40.4±4.9	0.99

Notes: 1. K - pesticide degradation rate constants; 2. T50 - half-life of pesticide; 3. T95 - the period of pesticide destruction by 95%; 4. R2 – coefficient of determination.

Table IV. Risk assessment of pesticides adverse effects on human health when consuming apples and grapes treated by them

Active substance	PDD, mg/kg	T_{50} in plants, day [1]	T_{50} in plants, day [2]	average consumption of fruits, berries, g/day	IIPS		R
					value, points [1/2]	class [1/2] _	
milbemectin	0.003	11.0 ±0.4* //	5.1 ±0.1		3+2 /2 +2=7 /7	3/3 _	2.6×10^{-2}
etofenprox	0.003	8.4 ±0.9* //	2.1 ±0.1		3+2 /1 +2=7 /6	3/3 _	2.6×10^{-2}
acetamiprid	0.010	11.2 ±0.2 //	7.5 ±2.3		2+2 /2 +2=6 /6	3/3 _	2.0×10^{-2}
novaluron	0.010	10.7 ±0.2 //	6.7 ±2.8		2+2 /2 +2=6 /6	3/3 _	3.9×10^{-2}
1-methylcycloprene	0.0009	-	-		-	-	4.3×10^{-2}
spirodiclofen	0.001	8.4 ±0.1	10.0 ±0.1		4+2 /2 +2=8 /8	3/3 _	7.8×10^{-2}
bifenthrin	0.020	18.1 ±0.1 //	8.9 ±4.1		2+3 /2 +2=7 /6	3/3 _	3.9×10^{-2}
difenoconazole	0.002	11.7 ±0.1	12.2 ±6.8	164	3+2 /2 +2=7 /7	3/3 _	2.0×10^{-1}
fluxapiroxad	0.020	11.3 ±0.1	10.0 ±0.6		2+2 /2 +2=6 /6	3/3 _	9.8×10^{-3}
abamectin	0.0002	12.1 ±0.2	9.5 ±4.7		4+2 /2 +2=8 /8	3/3 _	3.9×10^{-1}
glyphosate	0.010	7.7 ±0.7	9.9 ±4.3		2+2 /2 +2=6 /6	3/3 _	1.2×10^{-1}
kresoxim-methyl	0.100	8.0 ±0.5	7.1 ±1.6		1+2 /2 +2=5 /5	4/4 _	2.0×10^{-3}
oxyfluorfen	0.003	5.7 ±1.3	3.1 ±1.1		3+2 /1 +2=7 /6	3/3 _	1.3×10^{-1}
clothianidin	0.080	7.9 ±0.2 //	14.3 ±8.0		1+2 /2 +2=5 /5	4/4 _	2.4×10^{-3}
lambda-cyhalothrin	0.003	7.6 ±0.1 //	19.9 ±14.8		3+2 /3 + 2 =7 /8	3/3 _	1.3×10^{-2}

Notes: PDD - permissible daily dose, mg/kg; IIPS – an integrated index of product safety; 1 – according to research data in the soil and climatic conditions of Ukraine; 2 according to the data of studies in countries of the EU [9]; * - differences are reliable between the persistence of pesticides in the soil and climatic conditions of Ukraine and EU countries according to the Student's criterion at $p \leq 0.05$; // - differences are reliable according to the z -criterion at $p \leq 0.05$; R - risk values.

The analysis of the danger of the investigated pesticides groups, according to the indicator of persistence in grown agricultural crops (grapes), showed that the danger period duration, using pesticides based on copper T50, in the the agro-industrial sector, is 20.1 days, in private households - 16.6 days. According to this criterion, the substance belongs to persistent compounds (2nd class of danger). T50 of milbemectin, spirodiclofen, kresoxim-methyl fungicide, glyphosate and oxyfluorfen herbicides was within the range of 7.0-10.3 days, which stands for moderate resistance in grapes (class 3, according to DSanPiN 8.8.1.002-98) [8].

DISCUSSION

The described methods were used to assess the risk to the population, consuming apples and grapes, treated by the investigated pesticides [6, 7].

At the first stage, the permissible daily intake with food products, and the possible intake of pesticides with the analyzed products, were calculated (Table IV). The calculation results showed that the risk values were in the range from 2.0×10^{-3} to 7.8×10^{-2}, which is much lower than the permissible value.

At the next stage, assessment of the danger to the people, consuming apples and grapes, treated by the studied pesticides groups, was carried out, in accordance with the methods [7]. According to these methods, the assigned points for the PDD of the pesticide, its stability in growing crops and the average daily consumption of apples and grapes, are added (Table IV). The summation of the obtained points for each criterion makes it possible to establish an integrated index of product safety (IIPS), and to establish a class of danger, according to this indicator.

The obtained results made it possible to establish, that most pesticides are moderately dangerous, according to the IIPS indicator, with the exception of kresoxim-methyl and clothianidin, which are not very dangerous (Table IV).

A comparative analysis of the parameters of pesticide resistance in the soil and climate conditions of Ukraine and EU countries showed no differences in pesticide hazard classes, regarding consumption of contaminated apples and grapes [9 - 11].

The obtained data on the resistance of pesticides in vegetative crops showed significant differences ($p\leq0.05$) in T50 for milbemectin, etofenprox, acetamiprid, novaluron, bifenthrin, clothianidin, lambda-cyhalothrin, during the fruit growth, in Ukraine compared to other countries. The specified differences can be caused by soil and climatic conditions, meteorological conditions during the growth of crops, as well as the application rate of the studied pesticides [9 - 11].

CONCLUSIONS

1. It has been proven that the processes of pesticide decomposition in vegetating agricultural crops (apples, grapes) occur, according to an exponential model (the coefficient of determination (R2) – 0.70-0.99).
2. Half-life period (T50, days) of the studied pesticides in agricultural plants was established: milbemectin (11.0 ±0.4), acetamiprid (11.2 ±0.2), novaluron (10.7 ±0.2), spirodiclofen (8.4 ±0 ,1), difenoconazole (11.7 ±0.1), fluxapiroxad (11.3 ±0.1), abamectin (12.1 ±0.2), glyphosate (7.7 ±0.7), kresoxim-methyl (8, 0 ±0.5), oxyfluorfen (5.7 ±1.3), clothianidin (7.9 ±0.2), lambda-cyhalothrin (7.6 ±0.1), etofenprox (8.4 ±0.9) and by stability belong to moderately resistant (class 3), bifenthrin (18.1 ±0.1) – resistant pesticides (hazard class 2), in accordance with DSanPiN 8.8.1.002-98 .
3. The calculated values of the risk of dangerous exposure to pesticides, when consuming apples and grapes, treated by them, were 2-3 orders of magnitude lower than the permissible level, and ranged from 2.0×10^{-3} to 7.8×10^{-2}.
4. It was shown, that according to the value of integrated index of product safety (IIPS), most pesticides are moderately dangerous (class 3), according to the indicator of IIPS, with the exception of kresoxim-methyl and clothianidin, which are slightly dangerous (class 4).
5. The obtained results should be taken into account, when considering the issue of expanding the field of use of pesticides, based on the investigated substances, and the necessity for conducting monitoring studies.

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Conflict of interest:

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