Original Article

DETERMINATION OF THE AMOUNT OF RESIDUAL PESTICIDES IN THE LIVER TISSUE OF RATS POISONING WITH HALOXYPHOP-R-METHYL AND INDOSACARB PESTICIDES

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Abstract

An aqueous solution of the pesticides Galoxyfop-R-methyl and indoxacarb was administered to laboratory white rats by gavage per os at a dose of the LD50 1/10, and the residue in the liver of rats on the 5th, 10th, 20th, 30th, and 40th days after poisoning. the amount of pesticides was determined using APCI (Atmospheric pressure chemical ionization) method. According to the obtained results, the highest concentration of residual pesticides was determined on the 5th day after rats were poisoned. A HPLC MS (6420) Triple Quad LC/MS (Agilent Technologies, USA) was used for analysis.

Keywords: Galoxyfop-R-methyl, indoxacarb, decapitation, per os, liver, cumulation, residual pesticide, chromatography method.

INTRODUCTION

Pesticides, which are widely used in agriculture, have a negative impact on the environment, enter the living organism in different ways and accumulate in the tissues as residues [5, 7, 9]. It has been proven in experiments that the use of pesticides and accumulation of residues in tissues and organs can lead to death in rats [11].

Pesticides ingested through the consumption of beef as a food product have been found to be carcinogenic in scientific studies. Residual amounts of 22 different chloroorganic pesticides in organs such as liver, kidney, and tongue of livestock were studied in enterprises where meat products are produced in the cities of Mansoura, Zagazik, and Ismailia, Egypt. According to the results, the highest indicator was found in the city of Mansoura, where 152 ng/g (based on lipid weight), kidney 266 ng/g (based on lipid weight), and 488 ng/g (based on lipid weight) of residual pesticides were found in the animal liver. These results were determined using the gas chromatography method [3].

Screening analysis of chemical-toxicological toxic substances is very important, and according to the conclusions of this analysis, the effect of toxic substances on living organisms is determined. Various chromatographic methods are used for the screening analysis of these substances. In particular, D. V. Vedishcheva and others developed the YuSSX method for the determination of imidocloprid, acetamiprid and nicotine insecticides isolated from biological objects (potatoes, cucumbers, tomatoes) and liquids [6]. M.I.Nurmatova and others developed a thin layer chromatographic (TLC) method of isolating imidocloprid and acetamiprid pesticides from biological objects (blood, urine, internal organs) [8]. The sensitivity, reversibility, and specificity of the chromatographic method developed by the authors were studied. The residual amount of imidocloprid, acetamiprid and nicotine pesticides in biological objects was determined by this method. The residual amount of organochlorine and organophosphorus pesticides in samples taken from chicken, pig, and lamb livers was determined using gas chromatography with electron impact ionization tandem mass spectrometry [2].

The purpose of studying the cumulative nature of pesticides is to determine in experiments the nature of the storage of pesticides in the rat liver tissue and its effect on the functional status of a number of enzymes.

Research methods and materials.

We conducted studies to determine the amount of residual pesticides in the liver of rats poisoned with galoxyfop-R-methyl and indoxacarb using YuSSX, mass spectrometry (6420 TripleQuadLC/MS (Agilent Technologies, USA) devices). It was carried out in cooperation with A.A.Mamadrakhimov, an employee of the collective use center" laboratory.

In order to determine the residual amount of haloxyfop-R-methyl and indoxacarb pesticides in the liver using the high-performance liquid chromatography mass spectrometry method, liver samples were taken from rats, and each sample was extracted with an acetonitrile solution containing 0.01 M NSOON in order to dissolve the pesticides in them. received. In order to increase the extraction yield, the samples were kept in an ultrasonic bath for 10 minutes.

Galaxifop-R-methyl and indoxacarb samples (Sinakem, China) were taken as standards, and their 0.1 molar solutions in acetonitrile were prepared. High-performance liquid chromatography mass spectrometry (YuSSX MS) (6420 Triple Quad LC/MS (Agilent Technologies, USA) device was used for quantitative analysis of pesticides in liver samples. APCI (Atmospheric pressure chemical ionization) was used as the ionization method. mass spectrometers were selected as follows:

– Scanning range 50-2200 m/z.

- The obtained results were calculated by comparing the surface of ion fragments [M+H]+=376 for galaxifop-R-methyl and [M+H]+=529 for indoxacarb using the SIM -Single ion monitoring method.

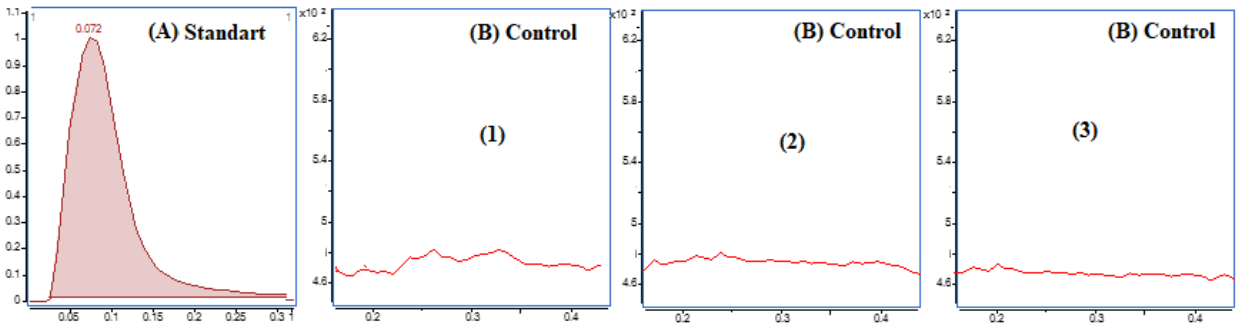
– Gas consumption 4 l/min, gas temperature 3000C, gas pressure in the atomizer 20 psi, evaporator temperature 3000C, capillary voltage 4500 V, fragmentor voltage 30 V.

A complete mass spectrometric analysis of each sample was carried out using the pesticide quantitative analysis (EIC-extracted ion chromatogram) method. The amount of residual pesticides in the liver of experimental rats A. It was conducted based on the methods recommended by Mamadrakhimov [1].

Pesticides galoxyfop-R-methyl and indoxacarb were administered per os to the stomach of rats at a dose of LD50 1/10, and their residual amount in the liver of rats was determined on the 5th, 10th, 20th, 30th, and 40th days after poisoning.

The obtained results and their analysis.

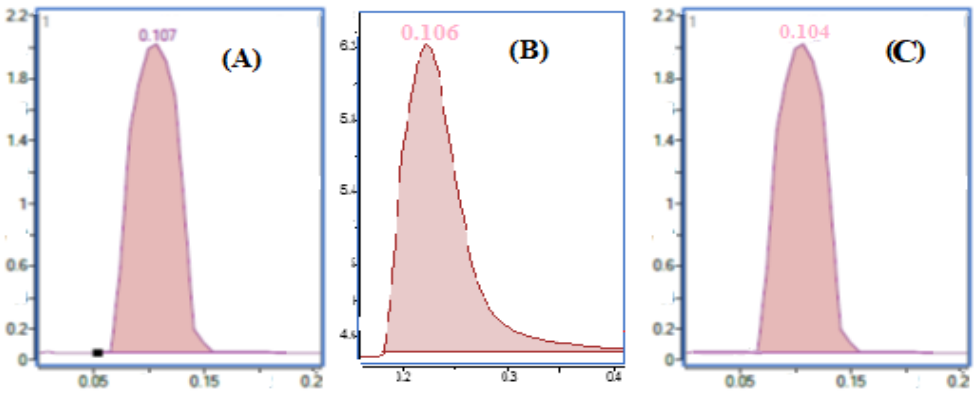
Initially, in our experiments, quantitative chromatographic analyzes of the liver of haloxyfop-R-methyl and control group animals (healthy) were obtained. The results are shown in Picture 1 below.



Picture 1. Chromatogram of standard (A) haloxyfop-R-methyl 0.01 mg/ml solution and liver sample of rats taken for control (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

From the results presented in Picture 1, it can be seen that the amount of absorption intensity generated for the 0.01 mg/ml solution of the standard haloxyfop-R-methyl was determined to be 0.0001 µg (Picture 1, A). In liver samples taken for control, absorption intensity typical for haloxyfop-R-methyl was not detected (Picture. 1, B 1.2.3).

Liver samples of experimental group animals were taken after 5-, 10-, 20-, 30- and 40-days and chromatographic analyzes were carried out. Picture 2 below shows chromatograms of liver samples taken after 5 days from animals poisoned with a single LD50 1/10 dose of the pesticide haloxyfop-R-methyl.

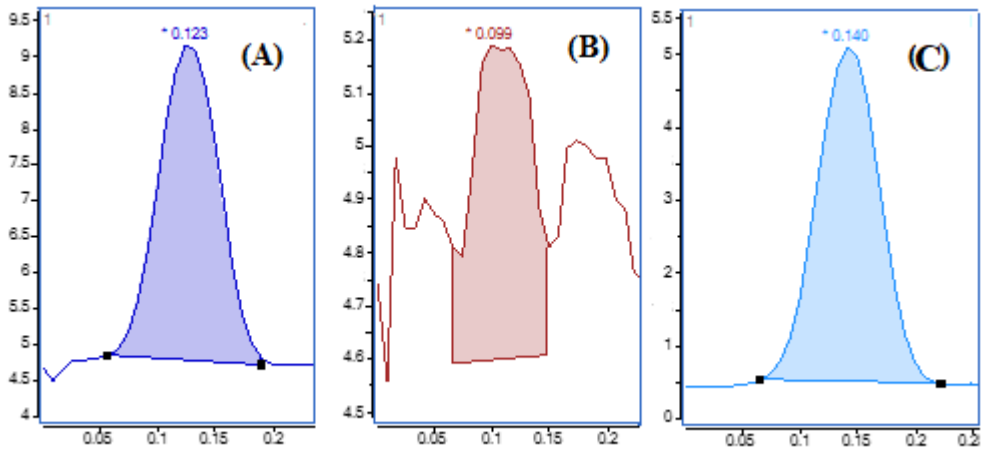


Picture 2. Chromatogram of liver samples from rats (A, B, C) after the 5th day of the experiment (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

As can be seen from the results presented in Picture 2, chromatographic peaks characteristic for haloxyfop-R-methyl were formed in the liver samples. The amount of residual pesticide is estimated by the chromatogram surfaces, i.e. absorption peaks and widths. The obtained results showed that sample 1 had 0.01737 μg (Picture 2, A), sample 2 had 0.01638 μg (Picture 2, B), and sample 3 had 0.01629 μg (Picture 2, C). was found to contain the pesticide haloxyfop-R-methyl.

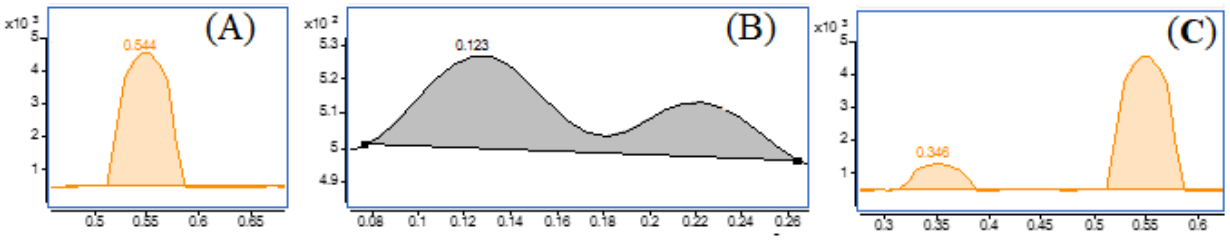
During the research, samples were taken from the livers of animals after 10 days and chromatographic analyzes were performed. The results are presented in Figure 3.

From the results presented in Figure 3, it can be seen that the amount of haloxyfop-R-methyl in the liver samples of animals decreased compared to day 5 (Picture 3.3). In particular, sample 1 has 0.00168 μg (Picture. 3, A), sample 2 has 0.00125 μg (Picture. 3, B), and sample 3 has 0.00184 μg (Picture. 3, C). was found to contain haloxyfop-R-methyl pesticide.



Picture 3. Chromatogram of liver samples from rats (A, B, C) after the 10th day of the experiment (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

From the obtained results, it can be seen that on the 10th day of the research compared to the 5th day, the reduction of haloxyfop-R-methyl in the liver of the animals was significantly observed (Table 1). During the research, samples were taken from the liver of animals after 20 days, and its amount was analyzed by chromatographic method. The results are presented in Picture 4.



Picture 4. Chromatogram of liver samples from rats (A, B, C) after day 20 of the experiment. (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

As can be seen from the results presented in Picture 4, absorption peaks characteristic for haloxyfop-R-methyl were formed in the liver samples of rats even on the 20th day of the study. However, it was found that its amount decreased compared to days 5-10 (Table 1). In particular, 0.00023 µg in sample 1 (taken against 1 g of sample) (Picture. 3.4, A), 0.000151 µg in sample 2 (Picture. 4, B), 0.00014 µg in sample 3 (Picture. 4 , C) was found to contain an amount of haloxyfop-R-methyl.

During the research, samples were taken from the liver of rats after 30 days and the amount of pesticides was determined. The results are presented in Picture 5.

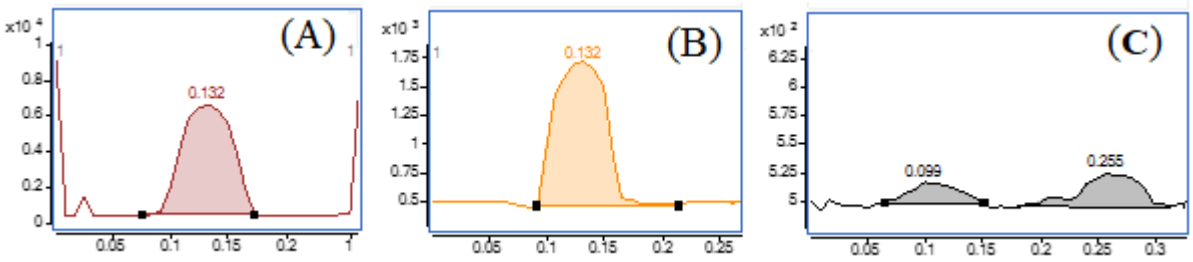
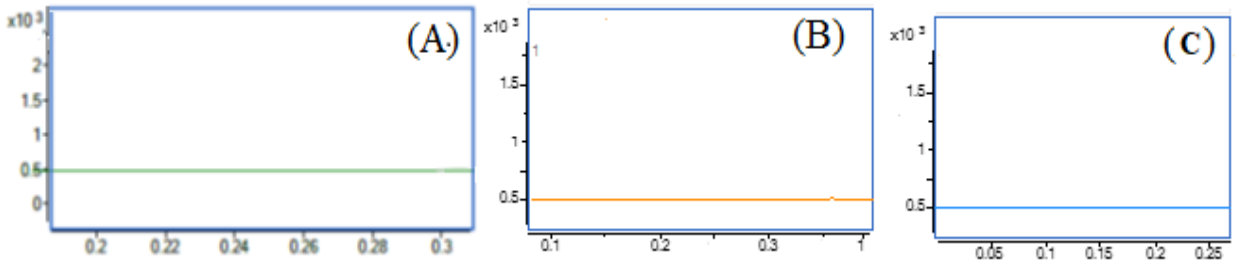


Figure 5. Chromatogram of liver samples from rats (A, B, C) after the 30th day of the experiment. (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

The obtained results revealed that after the 30th day of the study, absorption peaks typical for haloxyfop-R-methyl were formed in animal liver samples (Picture 5). The decrease in absorption intensity in these spectra is due to the decrease in the amount of haloxyfop-R-methyl. In particular, 0.0000094 µg in sample 1 (taken against 1 g sample) (Picture. 5, A), 0.000153 µg in sample 2 (Picture. 5, B), 0.000176 µg in sample 3 (Picture. 5 , C) was found to contain an amount of haloxyfop-R-methyl (Table 1).

During the research, the amount was determined by taking samples from the liver of animals after 40 days. The results are presented in Picture 6.



Picture 6. Chromatogram of liver samples from 3 rats (A, B, C) after day 40 of the experiment. (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

As can be seen from the results presented in Picture 6, no absorption peaks characteristic of haloxyfop-R-methyl were formed in the liver samples of the animals after the 40th day of the study (Picture 6, A, B, C). Results showed no residual levels of haloxyfop-R-methyl in animal liver samples after 40 days. The average values obtained based on the characteristic absorption peaks for galoxyfop-R-methyl are shown in Table 1 below.

Table 1 Amount of residual pesticides in the liver of rats poisoned with Galaxifop-R-methyl (LD501/10)

Experimental groups	Galaxiphop-R-methyl residue, amount (μg) per 1 gram of sample (n=3).				
	Days				
	5	10	20	30	40
Control	-	-	-	-	-
Galoxyfop-R-methyl	0.01741	0.00159	0.000138	0.0000164	-

The obtained results showed that the highest amount of residual pesticides in the liver of rats was determined on the 5th and 10th days after poisoning. It was observed that this indicator decreased on the 20th and 30th days after poisoning. By the 40th day of the study, no accumulation of residual pesticides was observed in the liver of rats. In our next experiments, chromatographic analysis of the residual amount of indoxacarb pesticide in liver tissue was performed depending on the dynamics of 10-40 days.

In subsequent experiments, the residual amount of indoxacarb pesticide in the liver tissue of rats was determined. Initially, quantitative chromatographic analyzes of indoxacarb and control group animals (healthy) were obtained. The results are shown in Picture 7 below.

As can be seen from the results presented in Picture 7, the amount of indoxacarb in the absorbance intensity generated for the 0.01 mg/ml solution was found to be 0.0001 μg (Picture 7, A). In control liver samples, absorption peaks typical for indoxacarb were not detected (Picture. 7, B).

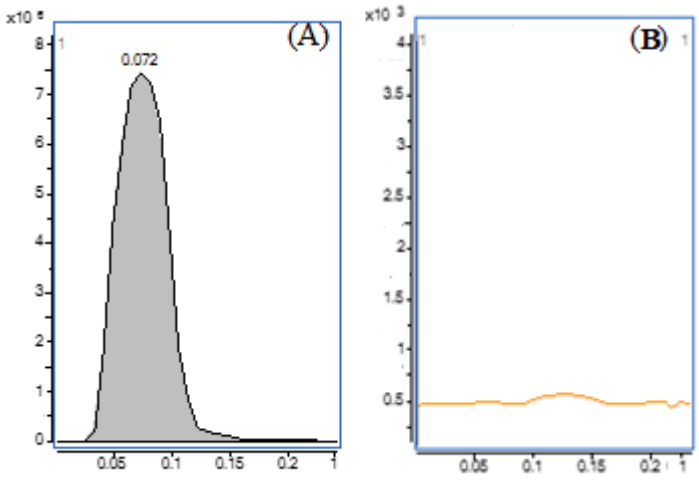
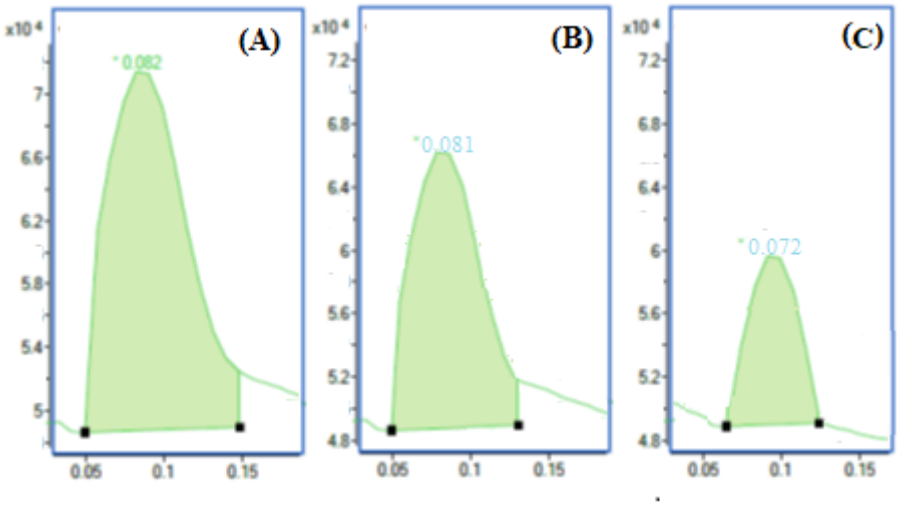


Figure 7. Chromatogram of rat liver sample obtained for standard (A) indoxacarb 0.01 mg/ml solution and control (B) (concentration of substance on ordinate axis and time (min) on abscissa axis).

Liver samples of animals of the experimental group were taken 5, 10, 20, 30, 40 days after poisoning and quantitative chromatographic analyzes were carried out. In studies, animals of the experimental group were poisoned with the pesticide indoxacarb at a dose of the LD501/10. Liver samples from animals after day 5 are shown in Picture 8. The results show that absorption peaks typical for indoxacarb were produced in the liver samples.

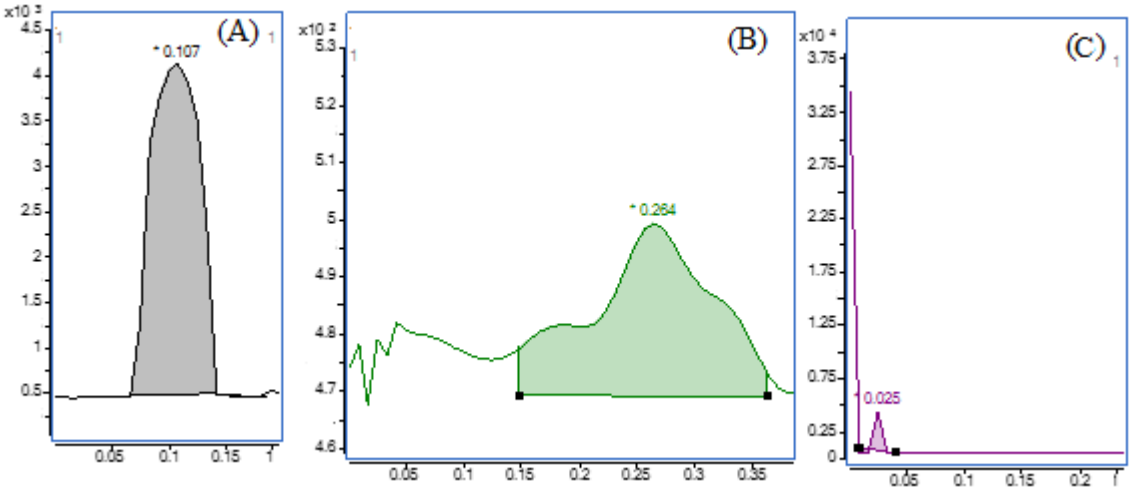


Picture 8. Chromatogram of liver samples from rats (A, B, C) after the 5th day of the experiment (concentration of the substance on the ordinate axis and time (min) on the abscissa axis).

The obtained results showed that sample 1 had 0.03274 μg (Picture 8 A), sample 2 had 0.03270 μg (Picture 8 B), and sample 3 had 0.03271 μg (Picture 8 B). indoxacarb pesticide was identified.

During the research, chromatographic analyzes were performed by taking samples from the liver of animals after 10 days.

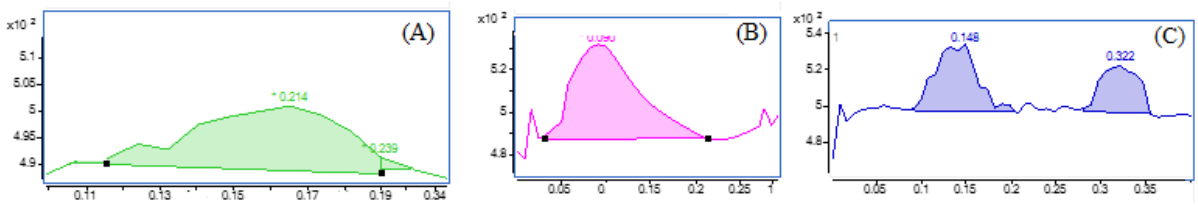
The results are presented in Picture 9.



Picture 9. Chromatogram of liver samples from rats (A, B, C) after the 10th day of the experiment (concentration of the substance on the ordinate axis and time (min) on the abscissa axis).

As can be seen from the results presented in Picture 9, the amount of indoxacarb in the liver samples of the animals decreased compared to the 5th day. In particular, sample 1 had 0.00473 μg (Picture. 9, A), sample 2 had 0.00254 μg (Picture. 9, B), and sample 3 had 0.00292 μg (Picture. 9, C). were found to contain the pesticide indoxacarb (Table 2). As it can be seen from the obtained results, in the 10th day compared to the 5th day of the research, the amount of indoxacarb pesticide in the liver of animals was significantly reduced.

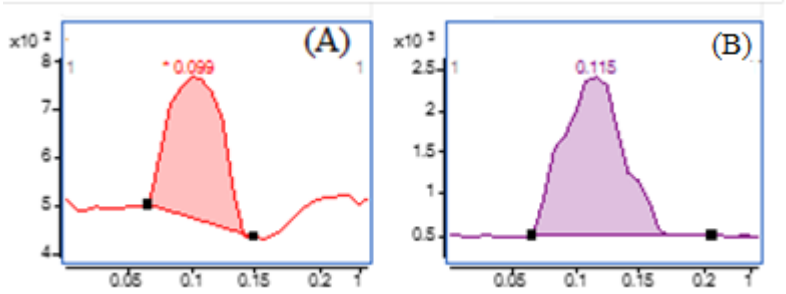
During the research, samples were taken from the liver of animals after 20 days, and its amount was analyzed by chromatographic method. The results are presented in Picture 10.



Picture 10. Chromatogram of liver samples from rats (A, B, C) after the 20th day of the experiment (concentration of the substance on the ordinate axis and time (min) on the abscissa axis).

According to the obtained results, even on the 20th day of the research, absorption peaks typical for indoxacarb were formed in animal liver samples (Picture. 10). However, it was found that its amount significantly decreased compared to 10 days. In particular, 0.000488 µg in sample 1 (taken against 1 g sample) (Picture. 10, A), 0.000484 µg in sample 2 (Picture. 10 B), 0.000409 µg in sample 3 (Picture. 10, C) was found to contain an amount of indoxacarb (Table 2).

During the studies, the amount of residual pesticide was determined in the liver of animals 30 days after poisoning. The results are presented in Picture 11. According to the results of the study, after 30 days, absorption peaks typical for indoxacarb were formed in animal liver samples (Picture 11).

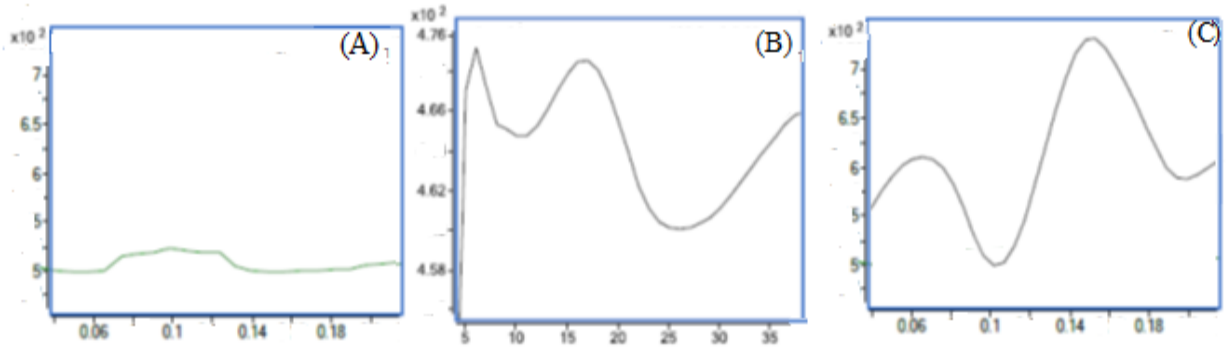


Picture 11. Chromatogram of liver samples from rats (A, B) after the 30th day of the experiment (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

The decrease in absorption intensity in the spectra is due to the decrease in the amount of indoxacarb. In particular, 0.0000155 µg in sample 1 (taken against 1 g sample) (Picture. 11, A), 0.0000136 µg in sample 2 (Picture. 11, B).

During the research, the amount was determined by taking samples from the livers of the animals after 40 days. The results are presented in Picture 12.

During the research, the amount was determined by taking samples from the livers of the animals after 40 days. The results are presented in Picture 12.



Picture 12. Chromatogram of liver samples from 3 rats (A, B, C) after the 40th day of the experiment (concentration of the substance on the ordinate axis, time (min) on the abscissa axis).

As can be seen from the results presented in Picture 12, no absorption fragment specific for indoxacarb was found in animal liver samples after 40 days of studies. The results showed no indoxacarb in animal liver samples after 40 days. The obtained results correspond to the data in the literature. After poisoning rats with karate at a dose of LD501/10, the highest amount of

residual pesticide was detected on the 10th day. The amount of residual pesticide was not detected in the chromatograms 50 days after poisoning [5].

Average values obtained based on absorption peaks characteristic of indoxacarb are shown in Table 2 below.

Table 2 Levels of residual pesticides in the liver of rats poisoned with indoxacarb (LD501/10).

Experimental groups	**The residual amount of indoxacarb, (μg) per gram of sample (n=3)**				
	Days				
	5	10	20	30	40
Control	-	-	-	-	-
Indoxacarb	0.03271	0,00340	0,000460	0,0000146	-

Thus, high residue levels in the liver tissue of rats poisoned with haloxyfop-R-methyl and indoxacarb were detected 5-10 days after poisoning. It was observed that this indicator decreased on the 20th and 30th days after poisoning. The results of the study showed that the residual amount of pesticides administered to rats was not detected in the liver tissue by 40 days. Our results showed that the amount of residual pesticides was found to be higher in the liver of rats poisoned with indoxacarb compared to the liver of rats poisoned with haloxyfop-R-methyl.

Based on our results, it can be concluded that the amount of residual pesticides in the liver of rats affects the biochemical and physiological indicators of liver mitochondria and causes a number of changes[4;5;10].

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