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# Cytogenetic Changes of Cages of the LEK Line at Influence of Pesticide from Group of Synthetic Pyrethroids.

**Valiullin Linar Rashitovich*, Egorov Vladislav Ivanovich, Papunidi Konstantin Khristoforovich, Tremasova Anna Mikhailovna, Saitov Vadim Rasimovich, Sunagatullin Faruk Akhmadullovich, and Shangaraev Nail Gabdulkhaevich.**

Federal Center for Toxicological, Radiation and Biological Safety, Russia, 420075, Kazan, Nauchny Gorodok-2

**ABSTRACT**

Authors have carried out the cytogenetic analysis of cages of the LEK line at influence of pesticide from group of synthetic pyrethroids. Influence of a deltametrin on kulturalno-morphological properties of cages was defined taking into account the following parameters: viability coefficient, proliferation index, cytotoxic index, cytotoxicity index, mitotic index, percent of death of cages. During experiment it is established what deltametrin at impact on cages of the LEK line reduces their viability in comparison with control group. Comparing a mitotic index, at influence of a deltametrin in different concentration, have revealed that increase of a dose of a toksikant lowers proliferative activity of cages and considerably increases cytotoxicity. When studying the percentage of cell death under the influence of different concentrations of deltamethrin is an increase in percent cell death, indicating that the dose-dependent effect of deltamethrin. Comparing morphology of cages in a monolayer, under the influence of a deltametrin in low doses, there were superficial changes of cellular morphology. At increase in doses of a toksikant lack of a monolayer and presence big quantity of the debris indicating high percent of death of cages in comparison with control was observed.

**Keywords:** pesticides, synthetic pyrethroids, deltametrin, lung epithelium cages, proliferation, cytotoxicity.

***Corresponding author***

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## INTRODUCTION

Increase and expansion of scales of economic activity in the conditions of a tekhnogenez are accompanied by increase of comprehensive attention to environmental problems and, in particular, in the agrosphere. In modern conditions the agrosphere most is exposed to negative anthropogenous impact. The increasing technogenic pressure on environment leads to negative impact of various ekotoksikant on an organism of animals and the person [1].

Pesticides – the chemical compounds used for protection of plants against insects and wreckers. Their wrong application can do harm to environment and health of the person. Moreover, these means remain in the soil for many years even after decomposition of an organic product [2, 3].

Special danger to animals and people is represented by circulation of pesticides on food chains with accumulation of the remains in sterns for animals and food of a vegetable and animal origin. According to results of one of the European researches of food of a phytogenesis, about 30% of samples contain the remains of two and more pesticides [4].

In recent years more and more broad application in agriculture is found by the insecticides relating to group of synthetic pyrethroids. Wins first place on scales of use дельтаметрин of which not less than 30% of size of the market of pyrethroids are the share [5].

Thanks to the properties consumption rates of synthetic pyrethroids are very small. Connections these low-resistant, however, when using in agriculture can get to objects of environment, pollute forages and page – agriculture production [6]. Synthetic pyrethroids are widely applied in agriculture to fight against ectoparasites of animals. Often, especially at excess of the recommended doses, they collect in an organism of animals and get to food of the person, such as milk, eggs, meat, and can remain there long enough. Systematic intake of pyrethroids with foodstuff in a human body can lead to developing of allergic diseases, dysbacteriosis, decrease of the activity of protective forces of an organism and other undesirable phenomena [7].

Experimental data demonstrate that only at insects resistance to these preparations is quickly developed, and they are dangerous to farm animals and the person [8, 9, 10, 11, 12].

Studying of biological activity of substances, irrespective of the subsequent purpose of their use, as a rule, at the first stage assumes an assessment of their toxicity. The toxicity assessment methods alternative to classical tests for experimental animals, namely, find for model with use of cultures of cages more and more broad application in biokhimiko-toxicological researches [13, 14]. Such methods allow besides the solution of the ethical problems connected with mass use and death of experimental animals to considerably reduce the price and reduce terms of preliminary research of new chemical preparations, first of all by stages of their preclinical tests. Besides, use of cultures of cages allows to establish nature of biological activity of the studied connections directly at the cellular level [15, 16].

## MATERIALS AND METHODS

In experiment used deltametrin. Purity of a deltometrin has been estimated by means of VEZhH about a mass spetrometriyey (Figure 1). As test object the immortalized culture of cages of an easy embryo of cattle (LEK) was used. Cages were cultivated in the environment of DMEM in the presence of 10% of fetalny veal serum at 37 $^0$C and 5% of $CO_2$. Deltametrin dissolved in mixes DMSO and 96% of alcohol in the ratio (1:1) (10 mM) and and added to the cell culture medium at various concentrations: the first group served as control; in the second group deltametrin applied 0,05 mm/l in a dose; in the third – 0,06 mM/l; in the fourth – 0,07 mM/l; in the fifth – 0,08 mM/l; in the sixth – 0,09 mM/l; in the seventh – 0,1 mM/l.

Influence of a deltametrin on cages was studied by method of cultivation of cages at his presence. After 24 hours of cultivation the cellular layer was investigated by means of the inverted microscope (Nikon Eclipse TS 100) in the following parameters: surface covering percent, form of cages, number of cellular units, quantity of floating cages. Calculation of cages was carried out in Goryaev's chamber. Quantity of living and dead cells estimated by a coloring method trypan blue staining (0,1% solution) [17].

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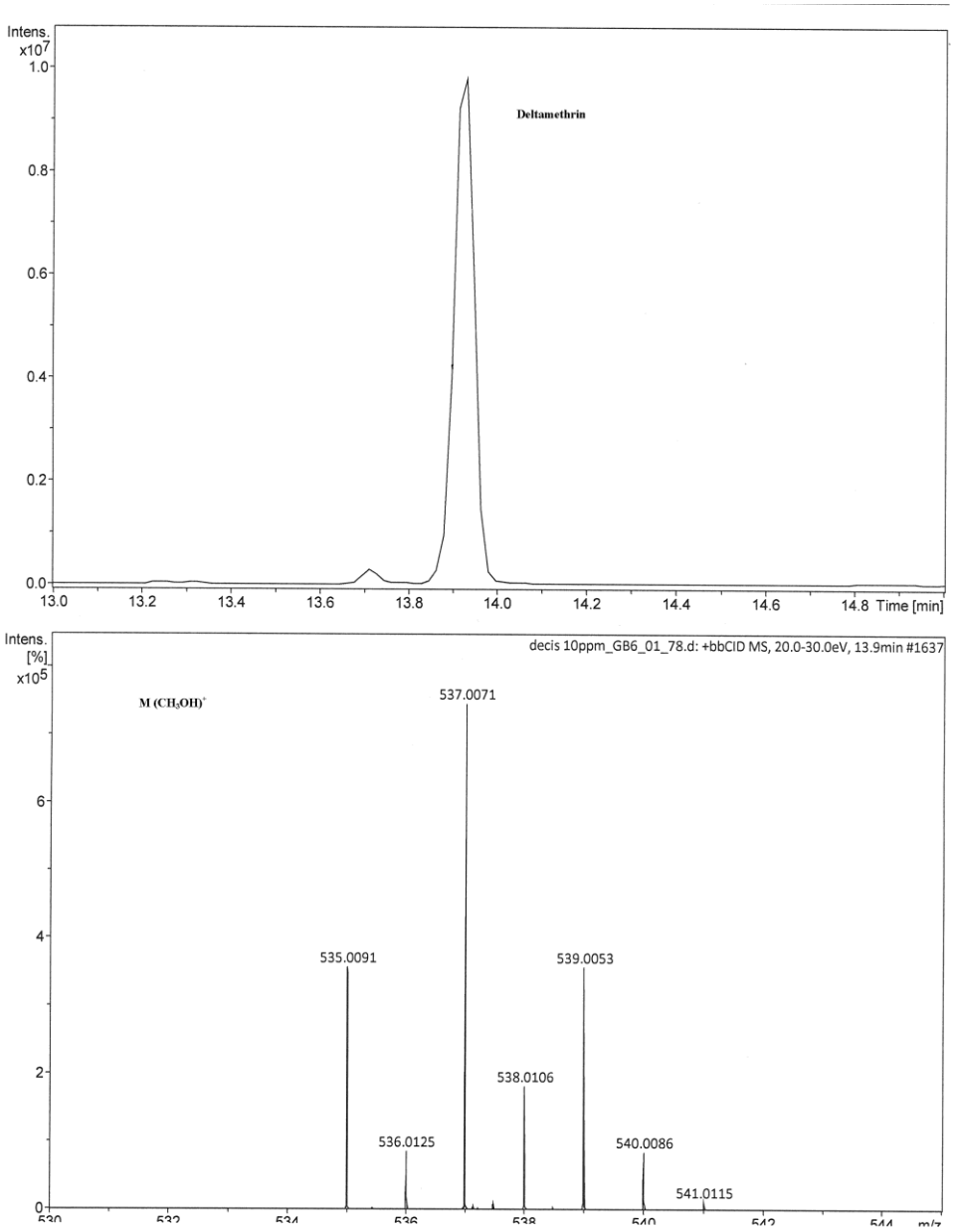


**Figure 1 – Results of VEZhH and mass-spektormetrii deltametrina**

Influence of a deltametrin on kulturalno-morphological properties of cages was defined taking into account the following parameters: viability coefficient – the attitude of living cells towards their general quantity expressed in %; a proliferation index – the relation of number of the grown cages to number sowed; a cytotoxic index – the relation of the living cells which have remained after an exposition with deltametriny to number of living cells in control; a cytotoxicity index – the relation of number initially sowed to number live, remained after an exposition with deltametriny; a mitotic index – the number relation mitotic the sharing cages to the total amount of the counted cages; percent of death of cages – the relation of the dead cages which have remained after an exposition with deltametriny to total number of cages after an exposition with pesticide [18].

## RESULTS OF RESEARCHES

Indicators of toxicity of a deltametrin at impact on LEK line cages within 24 hours are presented in the table.

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**Table – Indicators of toxicity of a deltametrin at impact on LEK line cages within 24 hours**

| Group | Viability coefficient | Proliferation index | cytotoxic effeciency | Cytotoxicity index | mitotic index, ‰ | Percent of death of cages, % |
|---|---|---|---|---|---|---|
| 1. | 95,5±3,7 | 0,80±0,04 | 1,00±0,01 | 0,5±0,01 | 444,4±10,4 | 4,4±0,2 |
| 2. | 81,6±3,5 | 0,42±0,03* | 0,70±0,03* | 0,8±0,02* | 295,7±8,7* | 18,3±1,2* |
| 3. | 75,0±2,6* | 0,24±0,01* | 0,62±0,02* | 1,0±0,02* | 193,5±7,2* | 19,3±1,6* |
| 4. | 72,0±2,8* | 0 | 0,45±0,01* | 1,3±0,04* | 0 | 28,0±1,7* |
| 5. | 72,0±2,5* | 0 | 0,45±0,01* | 1,3±0,04* | 0 | 28,0±1,7* |
| 6. | 64,0±2,6* | 0 | 0,40±0,03* | 1,5±0,05* | 0 | 36,0±2,8* |
| 7. | 60,0±2,3* | 0 | 0,38±0,01* | 1,6±0,04* | 0 | 40,0±2,9* |

*(P<0,05)

According to the table, in the second group decrease of coefficient of viability of cages of the LEK line by 14,6%, in the third group for 21,5% concerning control was observed. In the fourth and fifth groups decrease in coefficient of viability of the LEK cellular line has made 24,6% in comparison with control group. In the sixth group decrease in coefficient of viability of the LEK cellular line has made 33,0%, in the seventh group of 37,2% in comparison with control.

In the second group decline in the index of proliferation in the LEK cellular line for 47,5%, in the third group for 70,0% in comparison with control group was observed. In the fourth, fifth, sixth and seventh groups under the influence of a deltametrin the sowed cages of the LEK line haven't grown, i.e. the index of proliferation is equal to zero.

The cytotoxic index of the LEK cellular line in the second and third groups has decreased by 30,0 and 38,0%, in the fourth and fifth groups for 55,0%, in the sixth and seventh groups the cytotoxic index has decreased by 60,0 and 62,0% respectively in comparison with control.

In the second and third groups increase of an index of cytotoxicity of cages of the LEK line for 60,0 and 100,0%, in the fourth and fifth groups for 160,0%, in the sixth and seventh groups for 200,0 and 220,0% respectively in comparison with control was observed.

The mitotic index of cages of the LEK line in the second group has decreased by 33,5%, in the third group for 56,5% in comparison with control group. In the fourth, fifth, sixth and seventh groups mitotic the sharing cages of the LEK line were found, i.e. this indicator is equal to zero.

The percent of death of cages of the LEK line in the second and third groups has increased in comparison with control by 315,9 and 338,6% respectively. In the fourth and fifth groups increase in percent of death of the LEK cellular line by 536,4%, in the sixth and seventh groups for 718,2 and 809,1% respectively in comparison with control group was noted.

The characteristic of a monolayer of cages of the LEK line in control group is submitted in figure 2.

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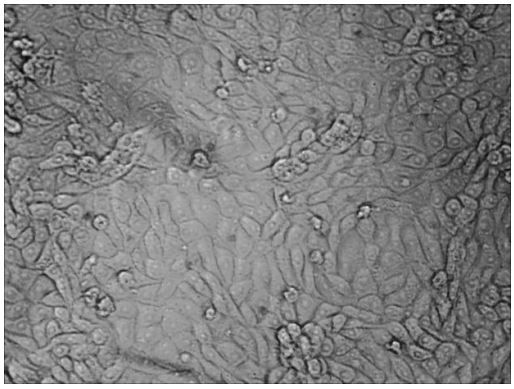

**Figure 2: The characteristic of a monolayer of cages of the LEK line in control group**

In figure 2 the complete monolayer of cages is looked through. Also morphological homogeneity of a monolayer is observed and the plasmatic membrane limiting a cage pays attention, she is visible as the dark line. The kernel in an interfaza stage limited to a nuclear membrane is visible.

Nature of influence of a deltametrin on cages of the LEK line is presented to concentration of 0,05 mmol/l in figure 3.

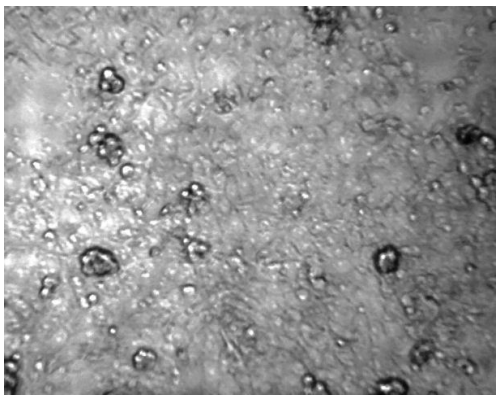

**Figure 3 – The characteristic of a monolayer at influence of a deltametrin in concentration of 0,05 mmol/l on line cages LEK**

The preparation is not expressed by the monolayer, also draws attention to weakly expressed plasma membrane limits the cell, it is represented as a dark line that indicates a weakening of the dynamic processes of efficient attachment, spreading, proliferation, differentiation and renewal of cells in the microenvironment. Nature of influence of a deltametrin on cages of the LEK line is presented to concentration of 0,06 mmol/l in figure 4.

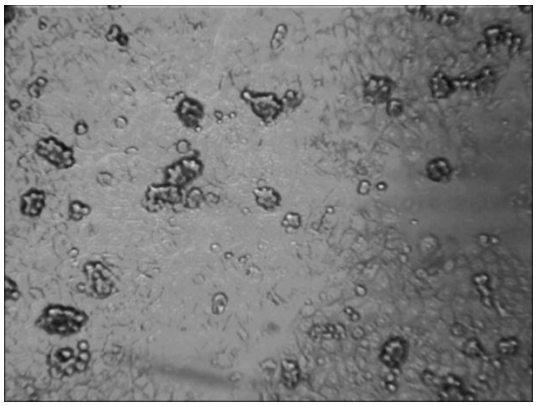

**Figure 4 – The characteristic of a monolayer at influence of a deltametrin in concentration of 0,06 mmol/l on line cages LEK**

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In figure 4 the poor quantity of cages in a monolayer, the majority of which have the changed form that demonstrates suppression of process of division and increase in quantity of the perishing cages, is observed.

Nature of influence of a deltametrin to the line of cages of LEK is presented to concentration of 0,07 mmol/l in figure 5.

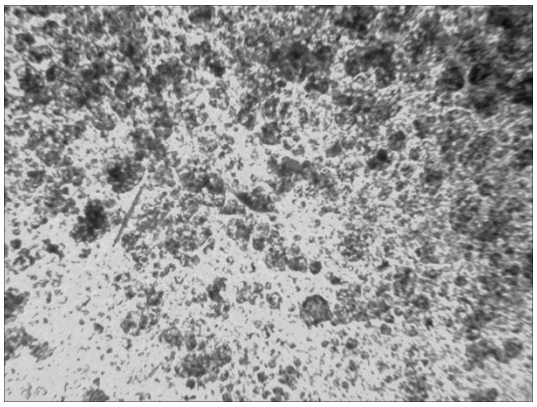

**Figure 5: The characteristic of a monolayer at influence of a deltametrin in concentration of 0,07 mmol/l on line cages LEK**

In the microphoto of figure 5 a large number of fine grains is observed (debris) that testifies to high death rate of cages by apoptosis.

Nature of influence of a deltametrin to the line of cages of LEK is presented to concentration of 0,08 mmol/l in figure 6.

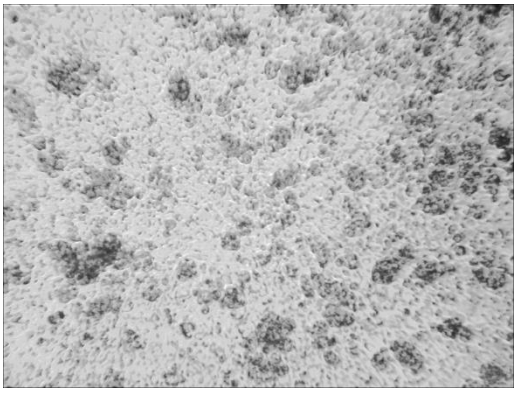

**Figure 6 – The characteristic of a monolayer at influence of a deltametrin in concentration of 0,08 mmol/l on line cages LEK**

In the microphoto 6 it is visible that feature is lack of a monolayer, existence of large cages with dark cytoplasm against a large number of not changed cages of this line.

Nature of influence of a deltametrin to the line of cages of LEK is presented to concentration of 0,09 mmol/l in figure 7.

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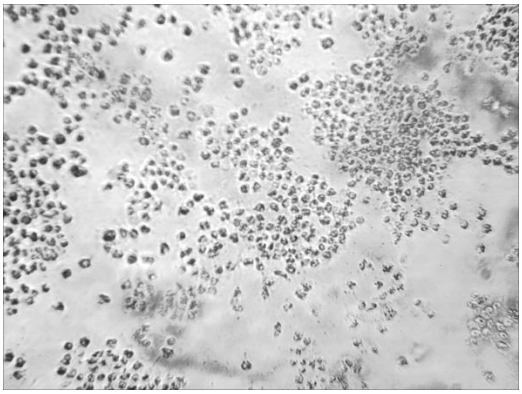

**Figure 7: The characteristic of a monolayer at influence of a deltametrin in concentration of 0,09 mmol/l on line cages LEK**

In the microphoto 7 the poor quantity of cages, the majority of which have the changed form that demonstrates suppression of process of division and increase in quantity of the perishing cages, is noted.

Nature of influence of a deltametrin to the line of cages of LEK is presented to concentration of 0,1 mmol/l in figure 8.

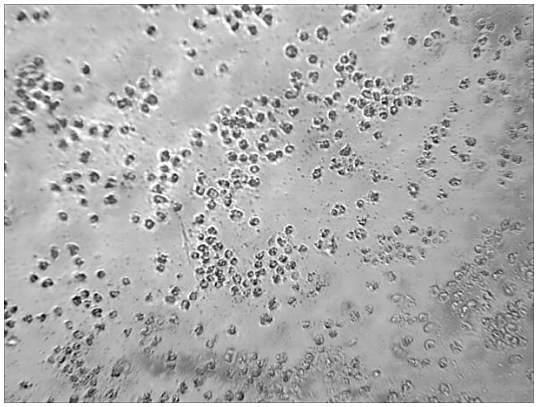

**Figure 8 – The characteristic of a monolayer at influence of a deltametrin in concentration of 0,1 mmol/l on line cages LEK**

In figure 8 it is visible that there is no monolayer and a large number of a debris is revealed what speaks about fast death of cages as a result of influence of a deltametrin.

## CONCLUSION

By results of the conducted researches it is visible what deltametrin in a dose of 0,05 mm/l at impact on cages of the LEK line reduces viability of cages by 14,6%, and in group with application of a deltametrin in a dose of 0,1 mM/l for 37,2% in comparison with control group. Comparing a mitotic index at influence of a deltametrin in doses of 0,05 mM/l and 0,1 mM/l it is possible to draw a conclusion that increase of a dose of a toksikant lowers proliferative activity of cages and considerably increases cytotoxicity (MI of 33,5% – 0,05mM; IP of 47,0% – 0,05mM/l; MI of 0% – 0,1 mM/l; IP of 0% – 0,1 mM/l). Thus, this pesticide considerably influences proliferative activity and toxicity concerning LEK cages. When studying percent of death of cages under the influence of a deltametrin there is an increase of percent of death of cages (315,9% – 0,05 mM/l; 338,6% – 0,06 mM/l; 536,4% – 0,07 and 0,08 mM/l; 718,2% – 0,09 mM/l and 809,1% – 0,1 mM/l) that indicates dozozavisimy effect of this pesticide. Comparing morphology of cages in a monolayer at influence of a deltametrin in a dose of 0,05 mM/l there were superficial changes of cellular morphology. At increase in a dose of a toksikant up to 0,1 mM/l lack of a monolayer paid attention, there was a large number of a debris, pointing to high percent of death of cages (percent of death of cages of 809,1% in comparison with control group). Thus, the obtained data open the mechanism of influence of pesticide from group of synthetic

pyrethroids at the cellular level and prospect of researches on search of anti-pillboxes at poisoning with this toksikant.

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