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STUDY OF ACUTE TOXICITY "FLYBLOK INSECTICIDAL TAG"

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ABSTRACT

Introduction. This manuscript presents the results of studies acute toxicity of a drug "Flyblok insecticidal tag" (4% s-fenvalerate and 8% piperonyl butoxide). **Materials and Methods**: The studies were carried out on the basis of the Federal State Budgetary Educational Institution of Higher Education "Moscow State Academy of Veterinary Medicine and Biotechnology-MVA named after K.I. Scriabin "and LLC" Research and Development Center Agrovetzashchita". Compounds that are used in veterinary medicine to combat blood-sucking insects. Studies were conducted on 36 white mice weighing 20-25g. and 36 guinea pigs, weighing 350-400g. **Results:** It was found that the average lethal dose of LD_{50} solution for drug impregnation in white mice with intragastric administration is 542.7 ± 283 mg/kg. The LD_{50} value of solution for drug impregnation, when applied to the guinea pigs on the clipped skin in the region of the vertebral column is 1874 ± 185 mg / kg. **Conclusion:** According to the parameters of acute toxicity after intragastric administration to white mice and cutaneous application of guinea pigs to the skin, it was found that a solution for a drug impregnation according to the generally accepted hygiene classification n refers to substances that are moderately hazardous to animal health.

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Introduction

S-fenvalerate is a 2nd generation synthetic pyrethroid insecticide that does not contain a cyclopropane ring in its structural formula. It is a mixture of four optical isomers which have different insecticidal activities. The 2-S *alpha* (or SS) configuration, known as s-fenvalerate, is the most insecticidally active isomer. S- fenvalerate consists of about 23% of this isomer. [1-4].

Piperonyl butoxide (PBO) is a waxy white solid organic compound used as synergist, an inhibitor of carboxyesterases and monooxygenases that destroy pyrethroids in the insect organism, and also improves the penetration of pyrethroids through the insect cuticle [5-8].

The solution of these active pharmaceutical compounds is effective against ectoparasites and affects mainly the mature and nymphal phases of insect development [9-12].

The mechanism of action of S-fenvalerate is to block the exchange of sodium and potassium ions in the presynaptic membrane of the arthropod nerve synapse, which causes excessive release of acetylcholine during the passage of nerve impulses through the synaptic chain, thereby causing paralysis and death of parasitic arthropods, and acts mainly as contact and insecticide possesses repellent properties [13-15].

The piperonyl butoxide included in the preparation is an inhibitor of enzymes (carboxygenase and monooxygenase), and also promotes the active penetration of S-fenvalerate through the insect cuticle [12; 16-18].

Study purpose. Study of acute toxicity of a solution 4% s-fenvalerate and 8% piperonyl butoxide in guinea pigs and white mice with cutaneous and intragastric administration.

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Materials and Method

Ethical approval

The use on laboratory animals and the design of the experiment were carried out in accordance with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes [18]. The personnel involved in the experiment are trained in the proper and humane handling of laboratory animals.

Objects of research

The studies were carried out on the basis of the Federal State Budgetary Educational Institution of Higher Education "Moscow State Academy of Veterinary Medicine and Biotechnology - MVA named after K.I. Scriabin" and Research Center "Agrovetzashchita".

Experimental design

Acute toxicity was studied in white mice and guinea pigs. The initial weight of laboratory animals ranged from: for mice - 20-25g of guinea pigs - 350-400g, respectively.

For research, we used an experimental drug solution "Flyblok insecticidal tag".

Animals were kept in a vivarium according to sanitary rules and regulations.

Clinically healthy animals that were previously quarantined for 7 days in cells in a specially equipped room were selected for the study. The selection of animals in goups was carried out according to the principle of analogues, using age and body weight as a criterion. Individual body mass values did not deviate from the goup average by more than 15%.

Animals were weighed on a precision Pioneer PA4102C balance.

The parameters of acute toxicity were determined on clinically healthy 36 male white mice, animal body weight of 20 - 25 g. and 36 guinea pigs (18 males and 18 females), body weight of animals 350-400 g. Statistical goups consisted of 6 animals. Before the start of the research, the animals were kept on a hungy diet for 4 hours.

When evaluating intragastric toxicity, the test solution was pre-diluted in 0.9% solution sodium chloride and injected into white mice directly into the stomach through a probe.

The drug was administered intragastrically to white mice at doses of 250, 500, 1000, 2000, and 2500 mg / kg, respectively.

Control mice, as a control substance, were injected once intragastrically with 0.9% solution sodium chloride solution in a volume of 0.07 ml, respectively.

When assessing cutaneous toxicity, the test solution was applied to guinea pigs on a pre-clipped back skin area of 4 x 4 cm in doses: 500, 1000, 1500, 2000 and 2500 mg / kg, respectively.

The control animals were applied to a clipped skin with a 0.9% solution sodium chloride in a maximum allowable volume of 1 ml.

A clinical examination of laboratory animals was regularly conducted throughout the study. During the clinical examination, the following parameters were evaluated and entered into the research journal: coordination of movements, the intensity and nature of motor activity, the presence of seizures and tremors, the reaction of animals to physical (light, sound) stimuli, the condition of the skin and coat, the condition of mucous membranes, and the frequency of respiratory movements, heart rate (only in guinea pigs), type and consistency of fecal matter, feed intake, body weight. The timing of the development of intoxication and death of animals was recorded, a macroscopic examination of the internal organs and tissues was carried out after administration / application of the drug (the degee of blood filling, the presence of hemorrhages, ulcerations of the mucous membranes, etc.).

Statistical analysis

Values of LD_{50} and other parameters of acute toxic effects were determined by probit analysis [6], this method is the most accurate for calculating LD_{50}; for a statistical comparison of the obtained LD_{50} values, the parallelism test, the linearity test, and the dispersion equality test were used [19, 20].

Results

The results obtained with oral intragastric administration of a drug solution "Flyblok insecticidal tag" (4% s-fenvalerate and 8% piperonyl butoxide) for white mice at doses of 250 ... 2500 mg / kg are shown in table 1.

Table 1. The results of a study of acute toxicity after a single intragastric administration of a drug solution "Flyblok insecticidal tag" to white mice.

Dose	the number of mice in the study	The number of dead mice after a single intragastric administration (day)								result
		1	2	3	4	5	6	7	14	
250	6	0	1	0	0	0	0	0	0	1/6
500	6	1	1	1	0	0	0	0	0	3/6
1000	6	2	1	1	0	0	0	0	0	4/6
2000	6	2	2	0	0	0	0	0	0	4/6
2500	6	4	2	0	0	0	0	0	0	6/6
Control Dose	6	0	0	0	0	0	0	0	0	0/6

As follows from the table, the introduction of the test drug in all test doses led to the death of animals. The highest dose of 2500 mg / kg resulted in the death of 100% of the mice. In the control goup of animals to which the control substance was dermally applied (0.9% solution sodium chloride) at the maximum allowable volumes, there were no signs of intoxication and death.

The calculated toxicological parameters of the drug solution "Flyblok insecticidal tag" for white mice are shown in table No.2.

Table 2. Parameters of acute toxic effects of a drug solution "Flyblok insecticidal tag" for white mice.

The lethal dose	LD_{10}	LD_{16}	LD_{50}	LD_{84}	LD_{90}
drug solution "Flyblok insecticidal tag"	149± 101	199± 83	542,7± 283	1477± 232	1974± 287

Analyzing the results, we came to the conclusion that a dose of 250 mg / kg with intragastric administration to white mice should be considered tolerable. Doses in the range of 500 - 2000 mg / kg are lethal. A dose of 2500 mg / kg in the dosage form is absolutely lethal (leading to the death of 100% of laboratory animals).

It was found that the average lethal dose of LD_{50} for white mice with intragastric administration is 542.7 ± 283 mg / kg;

A single intragastric administration of the test solution to white mice promotes the development of hepato and nephrotoxic effects.

The results obtained with cutaneous application of the test solution to guinea pigs in doses of 250 ... 2500 mg / kg are shown in table 3.

Table 3. The results of a study of acute toxicity after a single intragastric administration of drug solution "Flyblok insecticidal tag" to guinea pigs.

Dose	the number of guinea pigs in the study	The number of dead guinea pigs after a single cutaneous application of the test solution (day)								result
		1	2	3	4	5	6	7	14	
250	6	0	0	0	0	0	0	0	0	0/6
500	6	0	0	0	0	0	0	0	0	0/6
1000	6	0	0	0	0	1	0	0	0	1/6
2000	6	0	0	1	2	0	0	0	0	3/6
2500	6	0	2	3	1	0	0	0	0	6/6
Control Dose	6	0	0	0	0	0	0	0	0	0/6

As follows from the table, the cutaneous application of the test solution in doses of 500 and 1000 mg / kg did not lead to the death of guinea pigs.

All subsequent doses caused the death of guinea pigs. A dose of 2500 mg / kg resulted in the death of 100% of guinea pigs in the studied group of animals (table 3).

In the control group of animals to which the control substance (0.9% solution sodium chloride) was applied to the skin in the maximum permissible volumes, there were no signs of intoxication and death.

When opening dead guinea pigs, the following was noted: hemorrhage in the chest cavity, the lungs were filled with blood. The liver is enlarged, the edges are round, at the place of application of the drug microgranulomas on the surface of the liver and spleen, blood vessels of the mesentery and hemorrhages in the skin are noted.

The calculated toxicological parameters of a drug solution "Flyblok insecticidal tag" for white mice are shown in table No. 4.

Table 4. Parameters of acute toxic effects of a drug solution “Flyblok insecticidal tag” for guinea pigs.

The lethal dose	LD_{10}	LD_{16}	LD_{50}	LD_{84}	LD_{90}
drug solution “Flyblok insecticidal tag”	1479±321	1559±284	1874±185	2251±350	2374±447

It was found that doses of the test solution below 1000 mg / kg for cutaneous application to guinea pigs should be considered safe.
Doses greater than 1500 mg / kg should be considered lethal. A dose of 2500 mg / kg should be considered absolutely lethal. The LD_{50} value for cutaneous application to a clipped skin in the spinal column of guinea pigs is 1874 ± 185 mg / kg.

Conclusion

According to the parameters of acute toxicity after intragastric administration to white mice and application of guinea pigs to the skin, it was found that the test drug solution “Flyblok insecticidal tag” (4% s-fenvalerate and piperonyl butoxide), according to the generally accepted hygienic classification according to Occupational safety standards system. Harmful substances. Classification and general safety requirements (State standard GOST 12.1.007-76) refers to hazard class 3 - substances moderately hazardous to animals.

Authors’ Contributions

SVE, FIV and AAD formulated the exper-imental design, AVM and ESE did experimental work at the laboratory. AVM drafted the manuscript. ESE and did data analysis under the guidance of SVE and SVSh. All the authors read and approved the final version of the manuscript.

Confirmations

The work was performed at the Department of Veterinary Physiology, Pharmacology and Toxicology of Animals of the Federal State Budgetary Educational Institution of Higher Professional Education "Moscow State Academy of Veterinary Medicine and Biotechnology named after Academician K.I. Scriabin ”with the support of the Center for Pharmacy Research «AVZ Animal Health». Between 20th December 2017 and 10 th February 2018.
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Competing Interests

The authors declare that they have no competing interests.

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