Part 3. Biosafety

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DETERMINATION OF ACUTE TOXICITY PARAMETERS OF THE DRUG 'MEGASTOP FOR DOGS' ON WHITE RATS AND MICE

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Summary. The experiments were performed on 58 males of nonlinear white rats 3–4 months old and weighing 180–200 g and 64 females of nonlinear white mice 2.5–3 months old and weighing 18–22 g. In the main experiment on rats, six experimental groups were formed, the animals of which were injected intragastrically with the drug 'MEGASTOP for dogs' (by absolute weight) in doses of 1,000.0, 2,000.0, 3,000.0, 4,000.0, 5,000.0, and 6,000.0 mg/kg body weight; in the main experiment on mice, seven experimental groups were formed, the animals of which were administered the drug in doses of 100.0, 500.0, 1,000.0, 1,500.0, 2,000.0, 2,500.0, and 3,000.0 mg/kg body weight. Control rats and mice were injected with 2.0 cm^3 and 0.2 cm^3 of polyethylene glycol-400, respectively. Clinical symptoms of poisoning with the drug 'MEGASTOP for dogs' of white rats (at doses of 2,000.0–6,000.0 mg/kg body weight) and mice (at doses of 1,000.0–3,000.0 mg/kg body weight) were refusals of food and water, loss of coordination, sitting in one place, a dose-dependent increase in depression with subsequent complete depression, lack of response to external stimuli and death on the first or fourth day after administration. During autopsy in rats and mice that died as a result of poisoning with the drug 'MEGASTOP for dogs', we recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; increase in heart volume, atrial blood supply; pulmonary hyperemia; uncoagulated blood; increase in liver volume, dark cherry color, flabby consistency; catarrhal inflammation of the mucous membrane of the small intestine. According to the results of determining the parameters of acute toxicity of the drug 'MEGASTOP for dogs' in the case of a single intragastric injection, LD_{50} for male rats is 3,384.98 ± 444.94 mg/kg, and for female mice — 2,025.88 ± 279.46 mg/kg body weight, which allows to classify it to class IV by the toxicity — low-toxic substances (LD_{50} — 501–5,000 mg/kg) and by the degree of danger to class III— moderately dangerous substances (LD_{50} — 151–5,000 mg/kg)

Keywords: acute toxicity, male rats, female mice, dose, intragastric administration, 'MEGASTOP for dogs'

Introduction. Ectoparasitic and invasive diseases of dogs are widespread both in the world and in Ukraine (Havryk, 2015; Diakou et al., 2019; Hasib et al., 2020), so effective control is possible only with the use of highly effective and available drugs, which determines the relevance of the development of new antiparasitic veterinary drugs.

Besides, it is necessary to rotate (combine) the active substances to prevent their resistance in parasites (Tucker, Kaufman and Weeks, 2019; Klafke et al., 2020; Kumar, Klafke and Miller, 2020). Some of the promising active ingredients of antiparasitic drugs in this regard today are imidacloprid and ivermectin (Sheele and Ridge, 2016; Gomez and Picado, 2017).

Imidacloprid belongs to the group of chloronicotinyl compounds. It blocks postsynaptic cholinergic receptors that are sensitive to nicotine and are located in ectoparasites in the central nervous system. Interruption of nerve impulse transmission leads to paralysis and death of the parasite. Imidacloprid has a low level of penetration through the blood-brain barrier, so it has virtually no effect on the central nervous system of mammals. Ivermectin belongs to the compounds produced by microorganisms of *Streptomyces avermitilis* (Elbert, Nauen and Leicht, 1998).

Ivermectin has a pronounced antiparasitic effect on larval and mature forms of nematodes. The mechanism of action of ivermectin on the parasite is that it stimulates the release of the inhibitory neurotransmitter, gamma-aminobutyric acid, in presynaptic neurons, which binds to special receptors on nerve endings, increasing the permeability of membranes for chlorine ions and blocking the transmission of nerve-muscle pulses. This leads to impaired nerve impulse transmission, paralysis and death of parasites (Laing, Gillan and Devaney, 2017).

Thus, Research and Production Enterprise 'Suziria' LLC offered a new drug — 'MEGASTOP for dogs'. One milliliter of the drug contains active substances:

imidacloprid — 100.0 mg; ivermectin — 25.0 mg, and excipients: N-methylpyrrolidone, dimethyl sulfoxide, polyethylene glycol-400.

The drug 'MEGASTOP for dogs' is used for the prevention and treatment of animals affected by fleas (*Ctenocephalides* spp.), acariform mites (*Otodectes cynotis, Notoedres cati, Sarcoptes sapis*), mites of the genus Demodex; prevention of heartworm disease (effective against 3rd and 4th stages larvae of *Dirofilaria immitis*).

The 1st stage of toxicological research of new drugs is to determine their acute toxicity, the purpose of which is to obtain information on the danger of the test substance in short-term conditions and as a result of which it is expected to obtain data on lethal doses and symptoms of acute poisoning.

Therefore, **the aim of the work** was to determine the parameters of acute toxicity of the veterinary drug 'MEGASTOP for dogs' manufactured by Research and Production Enterprise 'Suziria' LLC under conditions of a single oral administration to white rats and mice.

Materials and methods. The experiments were performed on 58 males of nonlinear white rats 3–4 months old and weighing 180–200 g and 64 females of nonlinear white mice 2.5–3 months old and weighing 18–22 g, kept under optimal vivarium conditions (Zapadnyuk et al., 1983; Kotsiumbas et al., 2006; Karkishchenko and Grachev, 2010): room temperature was 18 ± 2 °C, relative humidity 60–70%, lighting cycle day — night, during the experiment, was 10–14 hours, and also a 10-fold change in air volume in the vivarium room per hour was provided.

For feeding rats and mice used complete feed for rodents. The animals had free access to water and food.

Before the start of the study, each animal was weighed. Doses administered were calculated individually according to the weight of each animal, and the volume of drug administered intragastrically at one time did not exceed 2.5 cm^3 and 1.0 cm^3 for rats and mice, respectively. Determination of the dose range for the main experiment was performed in a previous experiment.

In a previous experiment on rats on the principle of analogues control and three experimental groups were formed of four animals each (n = 4). The drug was administered in doses of 1,000.0, 3,000.0, and 6,000.0 mg/kg body weight in absolute weight of the drug once orally using a esophageal gastric tube. Animals in the control group were administered polyethylene glycol-400.

After accounting the results of the previous experiment in the main experiment, six experimental groups were formed, rats which were administered the drug in doses of 1,000.0, 2,000.0, 3,000.0, 4,000.0, 5,000.0, and 6,000.0 mg/kg body weight, as well as the control group, animals which were injected with polyethylene glycol-400 in a volume of 2.0 cm^3 according to the same regulations. There were six animals in each group (n = 6).

In a previous experiment on mice on the principle of analogues control and three experimental groups were formed of four animals each (n = 4). The drug was administered in doses of 200.0, 1,000.0, and 5,000.0 mg/kg body weight in absolute weight of the drug once orally using an esophageal gastric tube. Animals in the control group were administered polyethylene glycol-400.

After accounting the results of the previous experiment in the main experiment, seven experimental groups were formed, mice which were administered the drug in doses of 100.0, 500.0, 1,000.0, 1,500.0, 2,000.0, 2,500.0, and 3,000.0 mg/kg body weight, as well as a control group, the animals of which were injected with polyethylene glycol-400 in a volume of 0.2 cm^3 according to the same regulations. There were six animals in each group (n = 6).

Experiments on animals were carried out in accordance with the rules of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986).

The clinical condition of the experimental animals was observed for 14 days, noting the appearance and development of clinical signs of poisoning, the time of death or recovery to physiological norm. During the clinical examination of rats we paid attention to behavior, reaction to external stimuli, appetite, skin condition, color of mucous membranes, frequency of respiration and defecation, changes in color and consistency of feces, etc. (Kotsiumbas et al., 2006).

After the death of the animals we performed a pathological autopsy. The macroscopic method of research was used to establish pathological changes (Zharov, Ivanov and Strel'nikov, 2003). Pathological autopsy was performed according to the following scheme: at the 1st stage the external inspection was carried out, noting the condition of the coat and mucous membranes; at the 2nd stage we performed autopsy and examination of body cavities and internal organs, such as pharynx, trachea, larynx, heart, lungs, liver, spleen, kidneys, stomach, intestines, noting changes in color, consistency, pattern and shape of organs.

According to the results of death, LD_{10}, LD_{16}, LD_{50}, LD_{84}, LD_{90}, LD_{100}, and LD_{50} error were calculated by the method of probit analysis in the modification of Prozorovskiy (Prozorovskiy, 2007). Toxicometric parameters of the drug were calculated by the method of least squares for probit analysis of mortality curves. The percentage of lethality, probits (Y), weights of probits (Z) were established. To plot the abscissa, the values of the drug doses (mg/kg) were plotted, and the value of the effect (%) was plotted on the ordinate axis.

The obtained results were processed by methods of variation statistics using the software package StatPlus v. 5.9.8.5. Data were presented as mean values with a standard deviation of 95% confidence level by the Student's *t*-test (Van Emden, 2019).

Results and discussion. In a previous experiment, rats were administered the drug 'MEGASTOP for dogs' in doses of 1,000.0, 3,000.0, and 6,000.0 mg/kg body weight. Clinical observations showed that intragastric administration of the drug to rats of the experimental group I (1,000.0 mg/kg body weight) 1–2 h after drug administration, caused a slight depression, which persisted during the 1st day after administration, however, the animals ate feed and drank water well. On the 2nd day, the condition of animals in this group returned to normal.

In rats of the experimental group II (3,000.0 mg/kg body weight) 40–60 min after administration of the drug a slight suppression was recorded, which increased during the 1st day after administration, the animals were reluctant to take food and water, moved slowly around the cage, the reaction to external stimuli was reduced. Then in two rats an increase in depression was observed, the animals sat in one place, breathing hard, the fur was puffy. On the 2nd–4th days, the condition of two animals from this group was normalized, and in two animals complete depression was observed, lack of response to external stimuli; and on the 2nd and 3rd day they died (Table 1).

Table 1 — Dynamics of rat death in a previous experiment to determine the acute toxicity of the drug 'MEGASTOP for dogs' (n = 16)

Terms of rat death, in	Groups of rats and doses, mg/kg			
	Control	Experimental		
		I (1,000.0)	II (3,000.0)	III (6,000.0)
4–8 hours	–	–	–	–
1–1.5 days	–	–	–	4
1.5–3 days	–	–	2	–
4–14 days	–	–	–	–
Total died	–	–	2	4

In rats of the experimental group III, which were administered at a dose of 6,000.0 mg/kg body weight, during the 1st day after administration, more and more depression was observed, the animals refused food and water, then loss of coordination was observed, the animals were reluctant to move around the cage, mostly sat in one place. One day after administration, complete depression was observed in animals, rats did not respond to external stimuli, lying on their stomachs. In addition, there was a specific, unpleasant odor, the death of animals occurred within 1–1.5 days after administration of the drug (Table 1).

In the main experiment, rats were administered the drug 'MEGASTOP for dogs' in doses of 1,000.0, 2,000.0, 3,000.0, 4,000.0, 5,000.0, and 6,000.0 mg/kg body weight. During the observation of animals of the experimental group I in 30–60 min after administration of the drug a slight depression was recorded, which persisted during the day after administration, however, food and water during the day the animals took well.

In rats of the experimental group II, the depressed state was observed for 2–3 days after drug administration, appetite was also reduced, but thirst was noted. In one animal of this group increasing depression was observed during 4 days after administration, and on the 4th day it died (Table 2). The picture of poisoning was similar in rats of the experimental group III, but on the 3rd and 4th days one rat died.

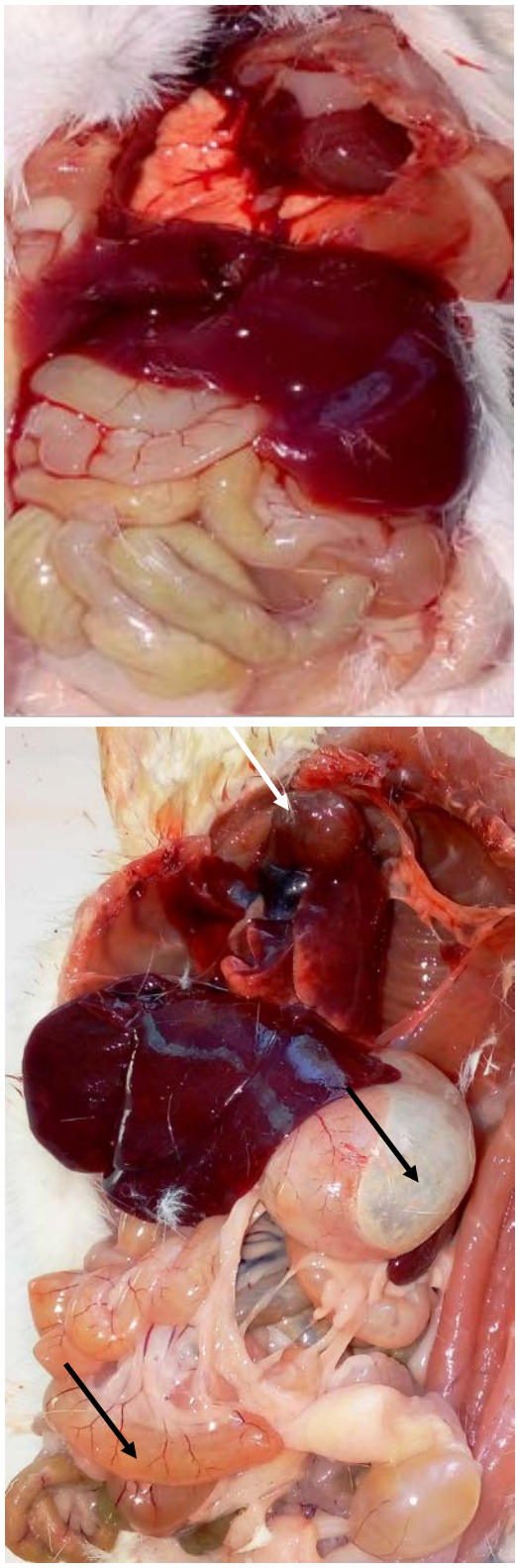
Table 2 — Dynamics of rat death in the main experiment to determine acute toxicity of the drug 'MEGASTOP for dogs' (n = 42)

Groups of rats and doses, mg/kg body weight		Terms of rat death, in					
		1 day	2 days	3 days	4 days	5–14 days	Total died
Control		–	–	–	–	–	–
Experimental	I (1,000.0)	–	–	–	–	–	–
	II (2,000.0)	–	–	–	1	–	1
	III (3,000.0)	–	–	1	1	–	2
	IV (4,000.0)	–	3	1	–	–	4
	V (5,000.0)	4	2	–	–	–	6
	VI (6,000.0)	6	–	–	–	–	6

In animals of the experimental group VI in 30–60 minutes after administration of the drug there was an increase in depression, the animals sat in one place, breathing hard, the hair was puffy. On the 4th day, the condition of two animals from this group was normalized, while in four ones, starting from the 2nd day after administration, complete depression was observed, no reaction to external stimuli; and on the 2nd and 3rd days they died. In rats of experimental groups V and VI during the 1st day after introduction there was an increasing depression, the animals refused food and water, sat in one place, breathed heavily, the coat was puffy. The death of animals occurred on the 1st–2nd day after administration of the drug (Table 2).

After the death of rats we performed a pathological autopsy. During the external examination of the carcasses of the experimental animals, it was found that the fur was disheveled, dull; leaks from the mouth, nasal cavity, eyes, and anus were not observed; however, we noted the pallor of the visible mucous membranes.

At autopsy in rats recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; bloating and drug residues were observed in the stomach; the heart is enlarged, the atria are full of blood; lungs hyperemic, with greater severity in animals of experimental groups V and VI, from pink-marble to dark burgundy, the pattern in the section is blurred; blood is not clotted; liver enlarged, dark cherry color, flabby consistency; spleen and pancreas unchanged; the bladder is filled with urine, the kidneys are light brown, not enlarged; catarrhal inflammation of the mucous membrane was found in the small intestine (Fig. 1).



A

B

Figure 1. Pathological changes in the internal organs of rats under a single intragastric injection of the drug 'MEGASTOP for dogs' (doses of 5,000.0 and 6,000.0 mg/kg body weight): A — control group (liver and intestines without pathological changes); B — experimental group (atria are blood-filled, the liver is enlarged, dark cherry color, bloating in stomach, catarrhal inflammation in the small intestine)

The next step in studying the toxicological characteristics of the drug 'MEGASTOP for dogs' was to determine the average lethal dose and its standard error (LD_{50}, LD_{10}, LD_{16}, LD_{84}, LD_{90}, LD_{100}).

A graphical representation of the dose-effect curve for rats is shown in Fig. 2.

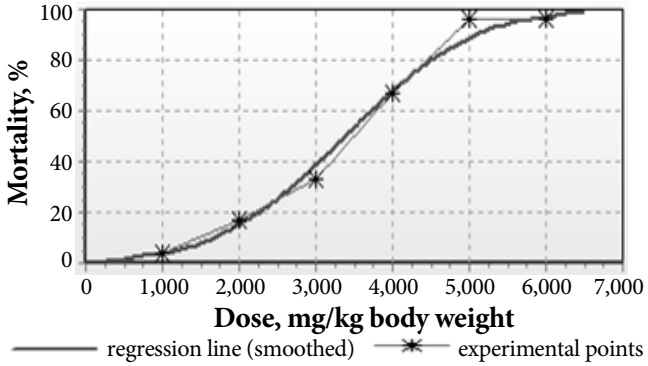


Figure 2. Mortality curve of male rats under conditions of a single oral administration of the drug 'MEGASTOP for dogs' (probit analysis method)

The results of calculating the average lethal dose of the drug for rats under oral administration are shown in Table 3.

Table 3 — The results of the calculation of lethal doses of the drug 'MEGASTOP for dogs' under conditions of a single oral administration to male rats

Dose, mg/kg body weight	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
1,000	0.0417	6	3.2680	1.5359
2,000	0.1667	6	4.0326	3.5653
3,000	0.3333	6	4.5697	4.5697
4,000	0.6667	6	5.4303	4.5697
5,000	0.9583	6	6.7320	1.5359
6,000	0.9583	6	6.7320	1.5359
Regression statistics				
LD_{50}	3,384.98	LD_{50} SE	444.94	
LD_{10}	1,674.12	LD_{16}	2,050.17	
LD_{84}	4,719.78	LD_{90}	5,095.83	
LD_{100}	5,387.18			

According to the results of research, it was found that the LD_{50} of the drug 'MEGASTOP for dogs' under the conditions of its single oral administration to male rats is 3,384.98 ± 444.94 mg/kg, LD_{10} — 1,674.12 mg/kg, LD_{16} — 2,050.17 mg/kg, LD_{84} — 4,719.78 mg/kg, LD_{90} — 5,095.83 mg/kg, LD_{100} — 5,387.18 mg/kg body weight, respectively.

In a previous experiment, mice were injected with the drug 'MEGASTOP for dogs' in doses of 200.0, 1,000.0, and 5,000.0 mg/kg body weight. Clinical observations showed that intragastric administration of the drug to mice of the

experimental group I (200.0 mg/kg body weight) 1–2 h after drug administration, caused a slight depression, which persisted during the 1st day after administration, however, food and water during the day the animals took well. On the 2nd day, the condition of animals in this group returned to normal. Mice deaths were not observed in this group (Table 4).

Table 4 — Dynamics of death of mice in a previous experiment to determine the acute toxicity of the drug 'MEGASTOP for dogs' (n = 16)

Terms of mice death, in	Groups of rats and doses, mg/kg			
	Control	Experimental		
		I (200.0)	II (1,000.0)	III (5,000.0)
4–8 hours	–	–	–	–
9–24 hours	–	–	–	4
2 days	–	–	1	–
3–14 days	–	–	–	–
Total died	–	–	1	4

In mice of the experimental group II (1,000.0 mg/kg body weight) in 40–60 min after administration of the drug a slight inhibition was recorded, which increased during the 1st day after administration, the animals were reluctant to take food and water, moved slowly through the cell, the reaction on external stimuli was reduced. Then one mouse had an increase in depression, the animal was sitting in one place, breathing hard, the fur was puffy. On 2nd–4th days, the condition of three animals from this group was normalized, and one mouse died on the 2nd day after administration (Table 4).

In mice of the experimental group III, which were administered the drug at a dose of 5,000.0 mg/kg body weight, during the 1st day after administration there was an increase in depression, the animals refused food and water, then incoordination was observed, the animals were reluctant to move around the cage, mostly sitting in one place. In 22 h after administration, the complete depression in animals was observed, mice did not respond to external stimuli, lying on their stomachs. In addition, there was a specific, unpleasant odor, the death of animals occurred within 24 h after administration of the drug (Table 4).

In the main experiment, mice were administered the drug 'MEGASTOP for dogs' in doses of 100.0, 500.0, 1,000.0, 1,500.0, 2,000.0, 2,500.0, and 3,000.0 mg/kg body weight. During the observation of animals of the experimental group I no clinical picture of poisoning was observed, the animals were active and took food and water well. In the experimental group II during the day after drug administration there was a decrease in the response to external stimuli, which disappeared during the 2nd day of the experiment. The death of mice in the experimental groups I and II within 14 days was not detected.

In mice of the experimental groups III–V in 40–60 min after administration, dose-dependent depression was observed, which persisted for 2–3 days after drug administration, appetite was also reduced, but thirst was noted. In one animal from the experimental group III and two ones from the experimental groups IV and V within two days after administration, increasing depression and death were observed on the 1st–3rd days after administration. (Table 5).

Table 5 — Dynamics of death of mice in the main experiment to determine the acute toxicity of the drug 'MEGASTOP for dogs' (n = 48)

Groups of mice and doses, mg/kg body weight		Terms of rat death, in				
		1 day	2 days	3 days	4–14 days	Total died
Control		–	–	–	–	–
Experimental	I (100.0)	–	–	–	–	–
	II (500.0)	–	–	–	–	–
	III (1,000.0)	–	–	1	–	1
	IV (1,500.0)	–	1	1	–	2
	V (2,000.0)	1	1	–	–	2
	VI (2,500.0)	2	2	–	–	4
	VII (3,000.0)	6	–	–	–	6

The most pronounced was the poisoning in mice of the experimental groups VI–VII, in 15–30 min after drug administration, an increase in depression was observed, the animals sat in one place, had difficulty breathing, the hair was puffy. The death of animals was observed on 1st–2nd days after administration of the drug (Table 5).

After the death of the mice, a pathological autopsy was performed. During the external examination of the carcasses of the experimental animals, it was found that the fur was disheveled, dull; leaks from the mouth, nasal cavity, eyes, and anus were not observed; however, we noted the pallor of the visible mucous membranes.

At autopsy in mice we recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; bloating and drug residues were observed in the stomach; the heart is enlarged, the atria are full of blood; lungs hyperemic, with greater severity in animals of the experimental groups VI–VII, from pink-marble to dark burgundy, the pattern in the section is blurred; blood is not clotted; liver enlarged, dark cherry color, flabby consistency; spleen and pancreas unchanged; the bladder is filled with urine, the kidneys are light brown, not enlarged; catarrhal inflammation of the mucous membrane was found in the small intestine.

The next step in studying the toxicological characteristics of the drug 'MEGASTOP for dogs' was to determine the average lethal dose and its standard error (LD_{50}, LD_{10}, LD_{16}, LD_{84}, LD_{90}, LD_{100}).

A graphical representation of the dose-effect curve for mice is shown in Fig. 3.

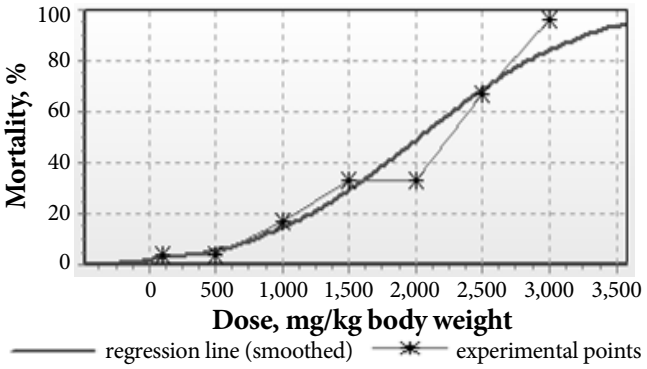


Figure 3. Mortality curve of female mice under conditions of a single oral administration of the drug 'MEGASTOP for dogs' (probit analysis method)

The results of calculating the average lethal dose of the drug for mice under conditions of a single oral administration are shown in Table 6.

Table 6 — The results of the calculation of lethal doses of the drug 'MEGASTOP for dogs' under conditions of a single oral administration to female mice

Dose, mg/kg body weight	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
100	0.0417	6	3.2680	1.5359
500	0.0417	6	3.2680	1.5359
1,000	0.1667	6	4.0326	3.5653
1,500	0.3333	6	4.5697	4.5697
2,000	0.3333	6	4.5697	4.5697
2,500	0.6667	6	5.4303	4.5697
3,000	0.9583	6	6.7320	1.5359
Regression statistics				
LD_{50}	2,025.88	LD_{50} SE		279.46
LD_{10}	785.05	LD_{16}		1,057.79
LD_{84}	2,993.98	LD_{90}		3,266.72
LD_{100}	3,478.02			

According to the results of research, it was found that the LD_{50} of the drug 'MEGASTOP for dogs' under the conditions of its single oral administration to female mice is 2,025.88 ± 279.46 mg/kg, LD_{10} — 785.05 mg/kg, LD_{16} — 1,057.79 mg/kg, LD_{84} — 2,993.98 mg/kg, LD_{90} — 3,266.72 mg/kg, LD_{100} — 3,478.02 mg/kg body weight, respectively.

Therefore, the drug 'MEGASTOP for dogs' in terms of toxicity can be classified as class IV — low-toxic substances (LD_{50} 501–5,000 mg/kg) (Kotsiumbas et al., 2006), and by the degree of danger to class III — moderately dangerous substances (LD_{50} 151–5,000 mg/kg) by GOST 12.1.007-76 (Gosstandart, 1976).

Conclusions. 1. Clinical symptoms of poisoning of white rats and mice with the drug 'MEGASTOP for dogs' were refusal of food and water, incoordination, sitting in one place, dose-dependent increase in depression with subsequent complete suppression, lack of response to external stimuli and death on the 1st–4th days after administration.

2. During autopsy in rats and mice killed by poisoning with the drug 'MEGASTOP for dogs', we recorded pallor of the mucous membranes of the mouth, trachea, pharynx, and esophagus; bloating and drug residues were observed in the stomach; the heart is enlarged, the atria are full of blood; lungs hyperemic, with greater severity with increasing dose, from pink-marble to dark burgundy, the pattern in the section is blurred; blood is not clotted; liver enlarged, dark cherry color, flabby consistency; spleen and pancreas unchanged; the bladder is filled with urine, the kidneys are light brown, not enlarged; catarrhal inflammation of the mucous membrane was found in the small intestine.

3. According to the results of determining the parameters of acute toxicity of the drug 'MEGASTOP for dogs' in the case of a single intragastric injection of LD_{50} for male rats is 3,384.98 ± 444.94 mg/kg, and for female mice — 2,025.88 ± 279.46 mg/kg body weight, which allows to classify it by the toxicity to class IV — low-toxic substances (LD_{50} 501–5,000 mg/kg) and by the degree of danger to class III — moderately dangerous substances (LD_{50} 151–5,000 mg/kg).

References

CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: https://conventions.coe.int/treaty/en/treaties/html/123.htm.

CEC (The Council of the European Communities). (1986) 'Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes', *The Official Journal of the European Communities*, L 358, pp. 1–28. Available at: http://data.europa.eu/eli/dir/1986/609/oj.

Diakou, A., Di Cesare, A., Morelli, S., Colombo, M., Halos, L., Simonato, G., Tamvakis, A., Beugnet, F., Paoletti, B. and Traversa, D. (2019) 'Endoparasites and vector-borne pathogens in dogs from Greek islands: Pathogen distribution and zoonotic implications', *PLOS Neglected Tropical Diseases*, 13(5), p. e0007003. doi: 10.1371/journal.pntd.0007003.

Elbert, A., Nauen, R. and Leicht, W. (1998) 'Imidacloprid, a novel chloronicotinyl insecticide: Biological activity and agricultural importance', in Ishaaya, I. and Degheele, D. (eds.) *Insecticides with Novel Modes of Action*. Berlin, Heidelberg: Springer, pp. 50–73. doi: 10.1007/978-3-662-03565-8_4.

Gomez, S. A. and Picado, A. (2017) 'Systemic insecticides used in dogs: potential candidates for phlebotomine vector control?', *Tropical Medicine and International Health*, 22(6), pp. 755–764. doi: 10.1111/tmi.12870.

Gosstandart (The USSR State Committee of Standards) (1976) GOST 12.1.007-76. *Occupational Safety Standards System. Noxious Substances. Classification and General Safety Requirements [Sistema standartov bezopasnosti truda. Vrednye veshchestva. Klassifikatsiya i obshchie trebovaniya bezopasnosti]*. Moscow: Izdatel'stvo standartov. [in Russian].

Hasib, Y., Kabir, H., Barua, S., Akter, S. and Chowdhury, S. (2020) 'Frequency and prevalence of clinical conditions and therapeutic drugs used in dog and cat at Teaching Veterinary Hospital, Chattogram Veterinary and Animal Sciences University', *Journal of Advanced Veterinary and Animal Research*, 7(1), p. 156–163. doi: 10.5455/javar.2020.g405.

Havryk, K. A. (2015) 'Influence of demodicosis and otodectosis pathogens on blood serum biochemical parameters of sick dogs' [Vplyv zbudnykiv demodekozu ta otodektozu na biokhimichni pokaznyky syrovatky krovi khvorykh sobak], *Scientific Bulletin of Veterinary Medicine [Naukovyi visnyk veterynarnoi medytsyny]*, 1, pp. 68–71. Available at: http://nbuv.gov.ua/UJRN/nvvm_2015_1_17. [in Ukrainian].

Karkishchenko, N. N. and Grachev, S. V. (eds.) (2010) *The Guide to Laboratory Animals and Alternative Models in Biomedical Researches [Rukovodstvo po laboratornym zhivotnym i al'ternativnym modelyam v biomeditsinskikh tekhnologiyakh]*. Moscow: Profil'. ISBN 9785903950102. [in Russian].

Klafke, G. M., Moreno, H. C., Tidwell, J. P., Miller, R. J., Thomas, D. B., Feria-Arroyo, T. P. and Pérez de León, A. A. (2020) 'Partial characterization of the voltage-gated sodium channel gene and molecular detection of permethrin resistance in *Rhipicephalus annulatus* (Say, 1821)', *Ticks and Tick-Borne Diseases*, 11(3), p. 101368. doi: 10.1016/j.ttbdis.2019.101368.

Kotsiumbas, I. Ya., Malyk, O. H., Patereha, I. P., Tishyn, O. L., Kosenko, Yu. M., Chura, D. O., Kotsiumbas, H. I., Piatnychko, O. M., Brezvyn, O. M., Zasadna, Z. S. and Chaikovska, O. I. (2006) *Preclinical Studies of Veterinary Drugs [Doklinichni doslidzhennia veterynarnykh likarskykh zasobiv]*. Lviv: Triada plus. ISBN 9667596648. [in Ukrainian].

Kumar, R., Klafke, G. M. and Miller, R. J. (2020) 'Voltage-gated sodium channel gene mutations and pyrethroid resistance in *Rhipicephalus microplus*', *Ticks and Tick-Borne Diseases*, 11(3), p. 101404. doi: 10.1016/j.ttbdis.2020.101404.

Laing, R., Gillan, V. and Devaney, E. (2017) 'Ivermectin — old drug, new tricks?', *Trends in Parasitology*, 33(6), pp. 463–472. doi: 10.1016/j.pt.2017.02.004.

Prozorovskiy, V. B. (2007) 'Statistic processing of data of pharmacological investigations' [Statisticheskaya obrabotka rezul'tatov farmakologicheskikh issledovaniy], *Psychopharmacology and Biological Narcology [Psikhofarmakologiya i biologicheskaya narkologiya]*, 7(3–4), pp. 2090–2120. Available at: https://www.elibrary.ru/item.asp?id=11691694. [in Russian].

Sheele, J. M. and Ridge, G. E. (2016) 'Toxicity and potential utility of ivermectin and moxidectin as xenointoxicants against the common bed bug, *Cimex lectularius* L.', *Parasitology Research*, 115(8), pp. 3071–3081. doi: 10.1007/s00436-016-5062-x.

Tucker, N. S. G., Kaufman, P. E. and Weeks, E. N. I. (2019) 'Identification of permethrin and etofenprox cross-tolerance in *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae)', *Pest Management Science*, 75(10), pp. 2794–2801. doi: 10.1002/ps.5391.

Van Emden, H. F. (2019) *Statistics for Terrified Biologists*. 2nd ed. Hoboken, NJ: John Wiley & Sons. ISBN 9781119563679.

Zapadnyuk, I. P., Zapadnyuk, V. I., Zakhariya, E. A. and Zapadnyuk, B. V. (1983) *Laboratory animals. Breeding, Keeping, Use in the Experiment [Laboratornye zhivotnye. Razvedenie, soderzhanie, ispol'zovanie v eksperimente]*. 3rd ed. Kiev: Vishcha shkola. [in Russian].

Zharov, A. V., Ivanov, I. V. and Strel'nikov, A. P. (2003) *Autopsy and Pathomorphological Diagnosis of Animal Diseases [Vskrytie i patomorfologicheskaya diagnostika bolezney zhivotnykh]*. Moscow: KolosS. ISBN 5953200927. [in Russian].