

## VETERINARY RESEARCH

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**DETERMINATION OF TOXICOLOGICAL CHARACTERISTICS OF A COMPLEX ANTIEMERIC DRUG BASED ON MADURAMYCIN AND NICARBAZINE****Roman Dotsenko, Yevheniia Vashchyk, Andriy Zakhariiev, Andrii Zemlianskyi, Ekaterina Dotsenko, Nataliia Seliukova**

**The aim:** to determine the parameters of acute toxicity of the preparative form of an antiemeric agent based on maduramycin and nicarbazine for white mice, white rats and guinea pigs with a single oral administration.

**Materials and methods.** Determination of acute toxicity of the formulation by oral administration was performed on 48 adult male mice, 48 adult nonlinear male rats, 48 adult male guinea pigs. To conduct an experiment on the principle analogues were formed seven experimental and one control group, 6 animal each. The dose of the formulation was calculated individually based on body weight values. It should be noted that the total volume of the emulsion of the formulation administered orally is not exceeded 1.0 cm<sup>3</sup> per 100 gram b. w.

**Results.** Toxicometric parameters of the formulation were calculated by the method of least squares for probit analysis of mortality curves. It was found that the LD<sub>50</sub> of the preparative form of antiemeric agent for white mice for a single oral administration is 238.05±28.08 mg/kg, LD<sub>16</sub> – 128.71 mg/kg, LD<sub>84</sub> – 347.39 mg/kg, LD<sub>100</sub> – 402, 06 mg/kg body weight, respectively. LD<sub>50</sub> of the preparative form of antiemeric agent for white rats with a single oral administration is 260.51±28.83 mg/kg, LD<sub>16</sub> – 148.39 mg/kg, LD<sub>84</sub> – 372.65 mg/kg, LD<sub>100</sub> – 428.71 mg/kg body weight, respectively. LD<sub>50</sub> of the preparative form of antiemeric agent for guinea pigs for a single oral administration is 275±21.12 mg/kg, LD<sub>16</sub> – 201.74 mg/kg, LD<sub>84</sub> – 348.25 mg/kg, LD<sub>100</sub> – 384.88 mg/kg body weight respectively.

**Conclusions.** According to SOU 85.2-37-736: 2011 "Veterinary drugs. Determination of acute toxicity" preparative form of a complex antiemeric agent based on maduramycin and nicarbazine on the degree of toxicity can be attributed to moderately dangerous substances (3rd hazard class)

**Keywords:** toxicity, mice, rats, guinea pigs, maduramycin, nicarbazine, eimeriosis

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**1. Introduction**

Coccidiosis (eimeriosis) is one of the most common protozoan diseases in poultry, caused by unicellular protozoa of the genus *Eimeria* [1, 2]. When choosing an anticoccidial drug, it should be borne in mind that the tool may be effective against one or more species of parasites, but very few drugs act simultaneously against all types of coccidian [3]. The created preparative form of antiemeric agent on the basis of maduramycin and nicarbazine is a synergistic combination of ionophore antibiotic of maduramycin and synthetic substance of nicarbazine, small concentrations of active substances provide effective antiemeric effect. The combination of maduramycin and nicarbazine in the anticoccidial drug creates a unique benefit [4, 5].

Maduramycin is an eimeriostatic belonging to the group of ionophores. It is active against *Eimeria tenella*, *E. acervulina*, *E. necatrix*, *E. mivati*, *E. maxima*, *E. brunetti*) at the stages of sporozoites, trophozoites and schizonts of the first generation. The mechanism of action of the drug is to disrupt the transport of monovalent Na<sup>+</sup> and K<sup>+</sup> ions across the cell membrane of the

parasite with its subsequent death. Ionophoric antibiotics are products of *Streptomyces spp.* and *Actinomadura spp.* Ionophores form with ions of sodium, potassium, magnesium and calcium lipophilic complexes that easily penetrate cell membranes. As a result, chlorine ions and water penetrate into the cell. There is swelling of cells, mitochondrial functions in coccidia are disturbed [6].

In animals, ionophore antibiotics can cause electrolyte metabolism when administered in subtoxic or toxic doses, as well as when used in animals whose bodies are very sensitive to these substances. Ionophores affect the early extracellular and intracellular stages of coccidia. According to the literature, the ratio of therapeutic dose to LD<sub>50</sub> in ionophore antibiotics is only 2: 3. Even a slight excess of therapeutic concentration or concentration in the feed leads to negative consequences: reduced average daily gain, there is a loss of appetite. Significant overdose causes a violation of sodium-potassium metabolism, which in turn leads to problems with the cardiovascular system and skeletal muscle. In animals and birds, muscle weakness, incoordination, drowsiness, depression, diarrhea can be observed [7, 8].

Nicarbazine is a synthetic agent that is a complex of 4,4'-dinitrodiphenylurea (DNC) and 2-hydro-4,6-dimethylpyrimidine (HDP), which inhibits energy metabolism in the body of the parasite. Nicarbazine acts mainly at the stage of second-generation schizonts and their merozoites, preventing tissue damage by this stage of coccidian [8, 9].

The study of the toxicity of the developed dosage form with a single administration makes it possible to obtain information on the relationship between dose and systemic and / or local toxicity of the tool. Such data can be used in determining doses in repeated dose toxicity studies. Information on the dose-effect relationship can be obtained from single-dose toxicity studies as part of a pharmacology study or efficacy studies using animal models [10].

The aim of the research to determine the parameters of acute toxicity of the preparative form of an antiemeric agent based on maduramycin and nicarbazine for white mice, white rats and guinea pigs with a single oral administration.

## 2. Materials and methods

Determination of toxicological characteristics of the preparative form of antiemeric agent based on maduramycin and nicarbazine was performed on male mice, male rats and male guinea pigs. Determination of acute toxicity was carried out in accordance with Test No. 401: Acute Oral Toxicity, OECD Guidelines for the Testing of Chemicals [11]. The studies were carried out on the basis of the vivarium of the toxicological monitoring laboratory NSC «IECVM» in 2016–2017.

Determination of acute toxicity of the formulation by oral administration was performed on 48 adult male mice weighing  $20 \pm 2.0$  g. To conduct an experiment on the principle analogues were formed seven experimental and one control group, 6 mice each. The dose of the formulation was calculated individually based on body weight values. It should be noted that the total volume of the emulsion of the formulation administered orally is not exceeded  $0.5 \text{ cm}^3$ . Mice of the experimental groups were administered the preparative form in doses (according to the active substance): Group I – 50 mg/kg, II – 100 mg/kg, III – 150 mg/kg, IV – 200 mg/kg, V – 250 mg/kg, VI – 300 mg/kg, VII – 350 mg/kg body weight, respectively. Animals in the control group under similar conditions were administered sterile water in an amount of  $0.5 \text{ cm}^3$ .

Determination of acute toxicity of the formulation by oral administration was performed on 48 adult nonlinear male rats weighing  $200 \pm 20$  grams. To conduct an experiment on the principle analogues were formed seven experimental and one control group, 6 rats each. The dose of the formulation was calculated individually based on body weight values. It should be noted that the total volume of the formulation administered orally is not exceeded  $2.5 \text{ cm}^3$ . Rats of experimental groups The drug was administered in doses (according to the active substance): Group I – 100 mg/kg, II – 150 mg/kg, III – 200 mg/kg, IV – 250 mg/kg, V – 300 mg/kg, VI – 350 mg/kg, VII - 400 mg/kg of body weight, respectively. Animals in the control group under similar conditions were administered sterile water in an amount of  $2.5 \text{ cm}^3$ .

Determination of acute toxicity of the formulation by oral administration was performed on 48 adult male guinea pigs weighing  $300 \pm 20$  g To conduct an experiment on the principle analogues were formed six experimental and one control group, 6 guinea pigs in each. The dose of the formulation was calculated individually based on body weight values. It should be noted that the total volume of the formulation administered orally is not exceeded  $3.0 \text{ cm}^3$ . Guinea pigs experimental groups were administered the preparative form in doses (according to the active substance): Group I – 150 mg/kg, II – 200 mg/kg, III – 250 mg/kg, IV – 300 mg/kg, V – 350 mg/kg, VI – 400 mg/kg body weight, respectively. Animals in the control group under similar conditions were administered sterile water in the amount of  $3 \text{ cm}^3$ .

The average lethal dose of  $DL_{50}$  was calculated by the method of graphical probit analysis (according to Prozorovsky) [12].

Statistical processing of research results was performed using statistical methods (StatPlus v5 for Windows) with determination of the arithmetic mean (M), the statistical error of the arithmetic mean (m), the probability of the difference (P) between the arithmetic means of the two variation series by the confidence factor for the difference of the means (t). The difference between the two values was considered probable at \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ .

Experiments conducted on animals do not contradict the current legislation of Ukraine (Article 26 of the Law of Ukraine 5456-VI of 16.10.2012 "On protection of animals from cruel treatment") and "General ethical principles of animal experiments", adopted by the First National Congress of Bioethics (Kyiv, 2001), international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes) (Strasbourg, 1985) and 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 [13].

## 5. Research results

The toxicity of the formulation of a complex antiemeric agent, *in vivo*, was studied in white mice by intragastric administration. The degree of toxicity was assessed by changes in the general condition of laboratory animals, took into account behavioral characteristics, response to external stimuli, skin and mucous membranes, feed and water consumption, functioning of organs and systems, recorded the timing of intoxication and death of animals. The experiment lasted 14 days.

Clinical observations have shown that oral administration of the formulation did not cause acute poisoning in mice of experimental group I (Table 1).

The behavior of the animals did not differ from the control group, they responded well to external stimuli, consumed food and water, the mucous membranes were pink, the coat was smooth and shiny. During the observation period, the deaths of experimental animals were not noted. Changes in clinical status were recorded in mice of II – VII experimental groups. Anxiety and diarrhea were noted. In groups II and III one animal died,

in group IV two animals died, in group V three mice, in group VI - four animals, in group VII - all animals died. The next step is to study the toxicological characteristics of the preparative forms was the determination of the av-

erage lethal dose and its standard error ( $DL_{50 \pm m}$ ),  $DL_{16}$ ,  $DL_{84}$ ,  $DL_{100}$ . The average lethal dose of  $DL_{50}$  was calculated by the method of graphical probit analysis (according to Prozorovsky) (Table 2).

Table 1  
Dynamics of death of white mice after intragastric administration of a preparative form of a complex antiemeric agent based on maduramycin and nicarbazine

Animal groups and doses, mg/kg b w		Death terms of animals					
		0–3 h	3–12 h	12–24 h	2 days	3–14 days	All died
I 50	n=6	–	–	–	–	–	–
II 100	n=6	1	–	–	–	–	1
III 150	n=6	–	1	–	–	–	1
IV 200	n=6	1	1	–	–	–	2
V 250	n=6	1	1	1	–	–	3
VI 300	n=6	1	1	2	–	–	4
VII 350	n=6	3	2	1	–	–	6

Table 2  
The results of the calculation of lethal doses of the preparative form of a complex antiemeric agent under conditions of a single oral administration to white mice

Stimulus (Dose)	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
50	0.041	6	3.26	1.53
100	0.166	6	4.03	3.56
150	0.166	6	4.03	3.56
200	0.333	6	4.56	4.56
250	0.5	6	5	5
300	0.666	6	5.43	4.56
350	0.958	6	6.73	1.53
Regression statistics				
$LD_{50}$	238.05	$LD_{50}$ Standard error		28.23
$LD_{10}$	97.91	$LD_{16}$		128.71
$LD_{84}$	347.39	$LD_{90}$		378.20
$LD_{100}$	402.06			

Toxicometric parameters of the formulation were calculated by the method of least squares for probit analysis of mortality curves. Using this statistical method, transformations were performed, as a result of which the original experimental data ( $x_i, y_i$ ) were presented not in the coordinates "x – y", but in the coordinates "f (x) – F (y)", where f (x) and F (y) are such derivatives of x and y, between which there is a linear dependence  $F(y) = a + bf(x)$ . To plot the abscissa, the values of the dose logarithm were plotted, and the values of the statistical function f (x) were plotted on the ordinate axis.

A graphical representation of the curve showing the dose-effect relationship is presented in Fig. 1.

According to research, it was found what  $LD_{50}$  formulation for white micedisposable oral administration is  $238.05 \pm 28.08$  mg/kg,  $LD_{16}$  – 128.71 mg/kg,  $LD_{84}$  – 347.39 mg/kg,  $LD_{100}$  - 402, 06 mg/kg body weight, respectively.

According to research, it was found that oral administration of the formulation complex antiemeric agent based maduramycin and nicarbazine did not cause signs of acute intoxication in rats of the I experimental group (Table 3).

The behavior of rats did not differ from the control group: the animals were tidy, active, responded well to light and sound stimuli, the processes of urination and

defecation were normal, respiratory disorders and seizures were not observed, reflex excitability was preserved. In rats of II - VII groups noted depression, animals reacted poorly to external stimuli. In surviving animals, on the third day after administration of the formulation clinical signs of poisoning disappeared. In group II one animal died, in group III two animals, in group IV – three animals, in groups V and VI four rats, respectively, in group VII all animals died.

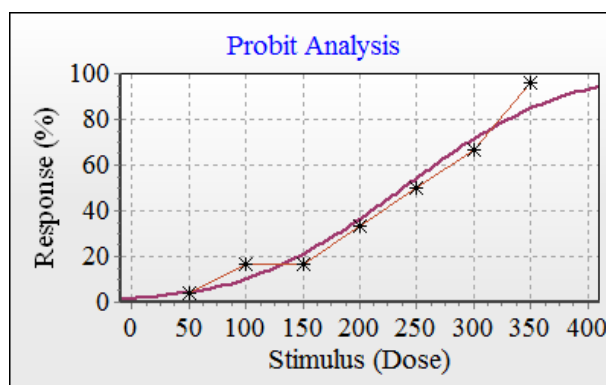


Fig. 1. Mortality curve of white mice under conditions of a single oral administration of a preparative form of a complex anti-emeric agent

Table 3

Dynamics of death of white rats after intragastric administration of a preparative form of a complex antiemeric agent based on maduramycin and nicarbazine

Animal groups and doses, mg/kg b w		Death terms of animals					
		0–3 h	3–12 h	12–24 h	2 days	3–14 days	All died
I 100	n=6	–	–	–	–	–	–
II 150	n=6	1	–	–	–	–	1
III 200	n=6	1	1	–	–	–	2
IV 250	n=6	2	1	–	–	–	3
V 300	n=6	2	2	–	–	–	4
VI 350	n=6	3	1	–	–	–	4
VII 400	n=6	5	1	–	–	–	6

The next step is to study the toxicological characteristics of the preparative forms was the determination of the average lethal dose and its standard error

( $DL_{50 \pm m}$ ),  $DL_{16}$ ,  $DL_{84}$ ,  $DL_{100}$ . The average lethal dose of  $DL_{50}$  was calculated by the method of graphical probit analysis (according to Prozorovsky) (Table 4).

Table 4

The results of the calculation of lethal doses of the preparative form of a complex antiemeric agent under conditions of a single oral administration to white rats

Stimulus (Dose)	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
100	0.041	6	3.26	1.53
150	0.166	6	4.03	3.56
200	0.333	6	4.56	4.56
250	0.5	6	5	5
300	0.666	6	5.43	4.56
350	0.666	6	5.43	4.56
400	0.958	6	6.73	1.53
Regression statistics				
$LD_{50}$	260.52	$LD_{50}$ Standard error		28.95
$LD_{10}$	116.80	$LD_{16}$		148.39
$LD_{84}$	372.65	$LD_{90}$		404.24
$LD_{100}$	428.71			

A graphical representation of the curve showing the dose-effect relationship is presented in Fig. 2.

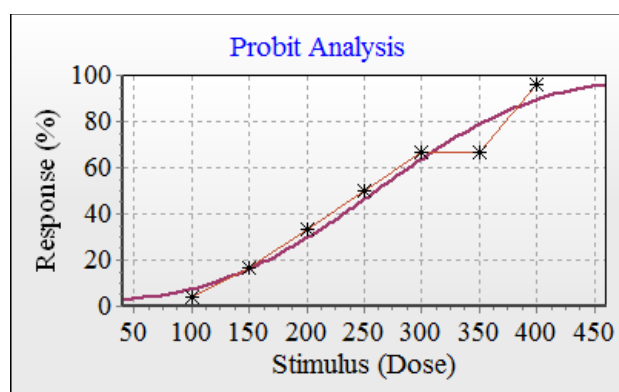


Fig. 2. Mortality curve of white rats under conditions of a single oral administration of a preparative form of a complex anti-emeric agent

According to the results of research, it was found that the  $LD_{50}$  of the preparative form of a complex antiemeric agent for white rats with a single oral administration is  $260.51 \pm 28.83$  mg/kg,  $LD_{16}$  –

148.39 mg/kg,  $LD_{84}$  – 372.65 mg/kg,  $LD_{100}$  – 428.71 mg/kg body weight, respectively.

The results of toxicological studies have shown that oral administration of the formulation complex antiemeric agent did not cause a picture acute poisoning in guinea pigs of the first experimental group. The behavior of the animals did not differ from the control group, they responded well to external stimuli, consumed food and water, the mucous membranes were pink, the coat was smooth and shiny.

Changes in the clinical condition were registered in guinea pigs of groups II – VI. Depression, signs of diarrhea were noted. In animals that survived, on the third day after administration of the drug clinical signs of poisoning disappeared. In group II one animal died, in group III two animals died, in group IV - four animals, in group V five guinea pigs, in group VI all animals died (Table 5).

According to the results of research, it was found that the  $LD_{50}$  of the preparative form of a complex antiemeric agent for guinea pigs with a single oral administration is  $275 \pm 21.12$  mg/kg,  $LD_{16}$  – 201.74 mg/kg,  $LD_{84}$  – 348.25 mg/kg,  $LD_{100}$  – 384.88 mg/kg body weight, respectively (Table 6).

Table 5  
Dynamics of death of guinea pigs after intragastric administration of a preparative form of a complex antiemeric agent based on maduramycin and nicarbazine

Animal groups and doses, mg/kg b w		Term of death of animals					
		0–3 h	3–12 h	12–24 h	2 days	3–14 days	All died
I 150	n=6	–	–	–	–	–	–
II 200	n=6	1	–	–	–	–	1
III 250	n=6	2	–	–	–	–	2
IV 300	n=6	3	1	–	–	–	4
V 350	n=6	3	2	–	–	–	5
VI 400	n=6	5	1	–	–	–	6

Table 6  
The results of the calculation of lethal doses of the preparative form of a complex antiemeric agent under a single oral administration to guinea pigs

Stimulus (Dose)	Percentage (%)	N	Probit (Y)	Weighting factor (Z)
150	0.041	6	3.26	1.53
200	0.166	6	4.03	3.56
250	0.333	6	4.56	4.56
300	0.666	6	5.43	4.56
350	0.833	6	5.96	3.56
400	0.958	6	6.73	1.53
Regression statistics				
LD <sub>50</sub>	275	LD <sub>50</sub> Standard error		21.14
LD <sub>10</sub>	181.10	LD <sub>16</sub>		201.74
LD <sub>84</sub>	348.25	LD <sub>90</sub>		368.89
LD <sub>100</sub>	384.88			

A graphical representation of the curve showing the dose-effect relationship is presented in Fig. 3.

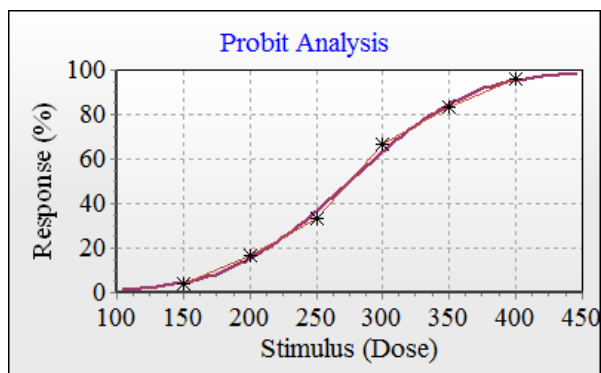


Fig. 3. Mortality curve of guinea pigs under conditions of a single oral administration of a preparative form of a complex anti-emeric agent

Thus, according to SOU 85.2-37-736: 2011 "Veterinary drugs. Determination of acute toxicity" [14] preparative form of a complex antiemeric agent by the degree of toxicity can be attributed to moderately dangerous substances (3rd hazard class).

## 5. Discussion

Determination of acute toxicity is the first stage in the study of the toxic properties of the Drugs. The purpose of which is to obtain information about the dangers of the investigational drug in the conditions of short-term action and, as a result, provides for obtaining data on the lethal dose and the clinical picture [15].

The determination of the toxic properties of the formulation of a complex anti-eimeriosis agent based on maduramycin and nicarbazine began with the determination of LD<sub>50</sub> in white mice, white rats and guinea pigs. LD<sub>50</sub> is one of the main indicators of the toxicity of substances for determining acute toxicity. In the study of acute toxicity of the drug, the following parameters of acute poisoning were determined: doses at which the death of experimental animals occurs, the occurrence and development of clinical signs of poisoning, pathological changes.

According to [6, 9], oral LD<sub>50</sub> for laboratory animals is described separately for maduramycin and nicarbazine. We did not find data on the joint toxicity of the studied coccidiostatics. It is also known that the toxicity of a drug depends on excipients found in commercial products. To clarify this indicator, studies were carried out on laboratory animals.

The study of acute toxicity of the formulation of a complex anti-eimeriosis agent based on maduramycin and nicarbazine showed that clinical signs of poisoning appear in all laboratory animals on average 1–5 hours after its administration.

After administration in laboratory animals, depression, frequent excretion of feces and lack of appetite developed due to the irritating effect of the drug on the stomach. Let's associate these clinical signs with the toxic effect of the drug on the gastrointestinal tract of laboratory animals.

In animals that remained alive during the first day, similar symptoms were observed, namely: general depression, lack of appetite, anxiety. On the second day, the animals showed only a slight depression, which let's



associate with a general disturbance of the physiological state of the animals. On the third day, laboratory animals showed no signs of poisoning, and on the following days their general condition did not differ from that of the animals in the control group.

When comparing our data with the experiments of other authors [9, 16], who investigated the toxic properties of maduramycin and nicarbazine, it should be noted that in the first hours of poisoning, similar clinical signs were observed. But the restoration of the general state of the animals that survived, in our case, took place faster than in the experiments of other authors [17].

After the death of the animals, let's perform a postmortem autopsy [18]. As with most acute poisoning, pathological changes were not pronounced, one can only note swelling of the small intestine and stomach due to local irritation, blood filling of internal organs and blood vessels with uncleaned blood is a characteristic sign of acute poisoning [9, 16]. It should also be noted that according to the data of other authors [9, 19], in conditions of acute poisoning in laboratory animals, pathological changes were expressed, such as congestion in the lungs and liver.

After the end of the experiment, based on the data obtained, the LD 50 was calculated by the probit analysis method, equal for white mice – 238.05±28.08 mg/kg, white rats – 260.51±28.83 mg/kg, guinea pigs – 275±21.12 mg/kg body weight, respectively.

**Study limitations.** The obtained results can't fully characterize all toxicological features of a complex antiemetic agent based on maduramycin and nicarbazine under the conditions of a single intragastric administration.

**Prospects for further research.** In the future, let's plan to conduct studies of chronic toxicity, carcinogenicity and dermal toxicity of the antiemetic formulation based on maduramycin and nicarbazine in laboratory animals.

## 6. Conclusions

The parameters of acute toxicity of the preparative form of a complex antiemetic agent under the conditions of a single intragastric administration for white mice were determined. It was found that LD<sub>50</sub> is 238.05±28.08 mg/kg, LD<sub>16</sub> – 128.71 mg/kg, LD<sub>84</sub> – 347.39 mg/kg, LD<sub>100</sub> – 402.06 mg/kg body weight, respectively.

The parameters of acute toxicity of the preparative form of a complex antiemetic agent under the conditions of a single intragastric injection for white rats were determined. It was found that LD<sub>50</sub> is 260.51±28.83 mg/kg, LD<sub>16</sub> – 148.39 mg/kg, LD<sub>84</sub> – 372.65 mg/kg, LD<sub>100</sub> – 428.71 mg/kg body weight, respectively.

The parameters of acute toxicity of the preparative form of a complex antiemetic agent under the conditions of a single intragastric injection for guinea pigs were determined. It was found that LD<sub>50</sub> is 275±21.12 mg/kg, LD<sub>16</sub> – 201.74 mg/kg, LD<sub>84</sub> – 348.25 mg/kg, LD<sub>100</sub> – 384.88 mg/kg body weight, respectively.

According SOU 85.2-37-736: 2011 "Veterinary drugs. Determination of acute toxicity" preparative form of a complex antiemetic agent based on maduramycin and nicarbazine on the degree of toxicity can be attributed to moderately dangerous substances (3rd hazard class).

## Conflict of interests

The authors declare that they have no conflicts of interest

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