Part 3. Biosafety

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RESEARCH OF TOXICITY PARAMETERS OF DISINFECTANT 'SUN STIM'

Kovalenko I. V. [1], Fotina T. I. [2], Fotina H. A. [2], Berezovskyi A. V. [2], Kovalenko V. L. [3], Fotin A. I. [2], Fotin O. V. [2]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: subotairen@gmail.com
[2] Sumy National Agrarian University, Sumy, Ukraine, e-mail: tif_ua@meta.ua
[3] State Scientific Control Institute of Biotechnology and Strains of Microorganisms, Kyiv, Ukraine, e-mail: kovalenkodoktor@gmail.com

Summary. Acute toxicity of set 'Sun Stim' was determined on laboratory animals in an experimental laboratory research. The disinfectant has high biological activity and low toxicity due to the active substance which is able to show a broad spectrum of activity against microorganisms. Based on the results, DL_{50} of the set for sanitation 'Sun Stim' for female rats 2,000.0 ± 35.0 mg/kg body weight, male — 2,033.0 ± 34.3 mg/kg. Thus, this drug, when injected into the stomach, according to the classification of toxicity in accordance with state standard (GOST 12.1.007-76), is allowed to be classified as III hazard class, low-hazard compounds. The set for sanitation 'Sun Stim' and the substances from which it was made do not have carcinogenic, mutagenic or genotoxic effects.

Keywords: 'Sun Stim', PGMG (polyhexamethylene guanidine hydrochloride), acute toxicity, laboratory animals, disinfectant

Introduction. Sodium hydroxide, formalin, chloramine, etc. are used for pre-incubation eggs processing in Ukraine. These disinfectants are highly effective, easy to use and economical. So they are widely used in poultry farming.

These agents are not expensive, exhibit bactericidal properties, but are toxic and have corrosive and carcinogenic action. For example, disinfectant 'Virkon C' has a high bactericidal effect but if we will use it before incubation of eggs it can causes the death of a part of the embryos from hyperemia and hemorrhages to allantois (Kalyn, 2009).

Currently, the using of disinfectants which based on polyhexamethylene guanidine hydrochloride and quaternary ammonium compounds is very relevant. They can well dissolve, they are colorless, almost odorless, and have highly bactericidal and surface activity, have low toxicity. These disinfectants do not have irritating action and other side effects (Mandygra et al., 2008; Kovalenko, 2011; Kovalenko and Nedosiekov, 2011; Kovalenko et al., 2013; Souza et al., 2015; Addie et al, 2015; Kim et al., 2016).

Disinfectants which based on polyhexamethylene guanidine hydrochloride and quaternary ammonium compounds do not form toxic products. These disinfectants do not inactivate by proteins, stable, nonaggressive. The bactericidal effect of PGMG (polyhexamethylene guanidine hydrochloride) is due to the ability of derivatives of guanidine to bind with cell walls and bacterial membranes.

Derivatives of guanidine can penetrate inside the cell and have the ability to inhibit cellular enzymes. Bactericidal agents with high biological activity and low toxicity, the active substance of which is able to exhibit a wide spectrum of action (bactericidal, antiviral and antifungal), can be offered as an alternative to traditional disinfectant and antibacterial agents (Lysytsya et al., 2015; Kovalenko et al., 2009, 2018).

The aim of our research was to examine the acute toxicity of bactericidal drug based on guanidine groups (PGMG) which is the active substance of set for sanitation 'Sun Stim'.

Materials and methods. We have used the set for sanitation 'Sun Stim' in our experiment. 'Sun Stim' consists of two parts. The first part is an aqueous solution of PGMG which contains 15% active substance. The second part is a mixture of organic (citric, succinic, malic) acid salts. To determine acute toxicity we have used clinically healthy white male rats (5 groups, 6 in each group, n = 30) and white female rats (5 groups, 6 in each group, n = 30), body weight 180–200 g, 6 months of age. We have determined the average lethal dose (DL_{50}) of the set for sanitation 'Sun Stim' depending on the amount of the drug, and we have determined the main parameters of acute toxicity, by using Kerber's and Pershin's methods (Kotsiumbas et al., 2006; Buckmaster, 2012).

Animals had food and water every 4 hours after the administration of the drug. At the same time, we observed the appearance and behavior of animals, the state of wool and mucous membranes, feed using, mobility, rhythm and respiration rate, the occurrence and nature of intoxication. To study the acute toxicity of the set for sanitation 'Sun Stim' laboratory animals were observed daily. Classification of substances by toxicity was made according to the table of toxicity levels with state standard (GOST 12.1.007-76).

To study the cumulative effect of the set for sanitation 'Sun Stim', experimental and control groups of rats (n = 10) were formed. Researches of skin-resorptive action were performed on two experimental and one control groups of rats. Within 15 days, two hours per day, the tails of the rats from the first experimental group were being submerged in a test tube with 0.5%, and the tails of the rats from the second group — in 5% of the 'Sun Stim' solution. Tails of animals from control group were being placed in test tubes with water. The irritating and sensitizing effects were determined on guinea pigs according to generally accepted methods in accordance with valid methodological recommendations 'Toxicological control of new means of animal protection' (Kosenko et al., 1997). Experiments on laboratory animals were conducted in accordance with the requirements of the European Convention for the Protection of Experimental Animals (EC 86/609/EEC) (CEC, 1986).

The results and discussion. The analysis of the indicators in Tables 1 and 2 shows that the toxic effect of the set for sanitation 'Sun Stim' clinically manifested almost equally both in males and females.

In 1–3 hours after oral administration of the drug in a subtoxic dose to laboratory animals, dyspnea and suppression of the central nervous system were observed. Most of them have died during the first day.

Based on the results, DL_{50} of 'Sun Stim' for female rats is 2,000.0 ± 35.0 mg/kg body weight, for male is 2,033.0 ± 34.3 mg/kg. Thus, this drug, when injected into the stomach, according to the classification of toxicity in accordance with state standard (GOST 12.1.007-76), is allowed to be classified as III hazard class, moderate hazardous substance. Further survived animals observation showed that their moving reaction was suppressed over the next 24–72 hours (Table 3). In addition, the experimental rats showed a marked reduction in moving activity, anxiety, reactivity and aggressive, moving disorders, reduced reaction to the touch and pain, irritation, and a decrease in the respiration rate.

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Table 1 — Expression of acute toxicity of set for sanitation 'Sun Stim' in male rats

Indexes	Dose of the drug, mg/kg				
	1800	1900	2000	2100	2200
Number of animals:					
total	6	6	6	6	6
survived	6	5	4	2	0
died	0	1	2	4	6
Z		0.5	1.5	3.0	5.0
D		100	100	100	100
DZ		50	150	300	500

Table 2 — Expression of acute toxicity of the set for sanitation 'Sun Stim' in female rats

Indexes	Dose of the drug, mg/kg				
	1800	1900	2000	2100	2200
Number of animals:					
total	6	6	6	6	6
survived	6	4	4	1	0
died	0	2	2	5	6
Z		1.0	2.0	3.5	5.5
D		100	100	100	100
DZ		100	200	350	550

Table 3 — Influence of subtoxic dose of the set for sanitation 'Sun Stim' at oral administration on the general functional characteristics of experimental rats

Indexes	Time of observation, hours		
	6	24	72
Reactions in behavior:			
anxiety	–3	–2	–1
reactivity	–3	–2	–1
aggressive	–3	–3	–2
Neuro muscular reactions:			
tremor	0	0	0
cramps while walking	–3	–3	–1
reaction to pain stimuli	–2	–1	–1
power of catch	–2	–1	0
Vegetative reactions:	unchanged		
pupil size	slow		
conditions of wool cover	disheveled		
color of the mucous membranes	cyanotic		
number of faecal masses	slight increased		
consistency of fecal masses	semi liquid		
frequency of urination	slight increased		
the color of urine	unchanged		
heart rate	unchanged		

Notes: 0 — the effect is absent; '–' — braking effect.

Daily, within 30 days, submerging of rats' tails in a 5% 'Sun Stim' solution caused an increase of tails' volume and

an increase in the amount of leukocytes in the blood. Significant changes in biochemical parameters in serum were not detected. (Table 4).

Table 4 — Hematological indices of peripheral blood of white rats at 30-days application of 5% solution of the set for sanitation 'Sun Stim' on the tails' skin

Hematologic indices	Experimental group			Control group
	back-ground	on the 15th day	on the 30th day	
Number of erythrocytes, 10^{12}/l	7.1 ± 0.3	6.9 ± 0.12	6.8 ± 0.2	6.8 ± 0.2
Hemoglobin content, g/l	156.3 ± 4.0	148.0 ± 4.6	157.3 ± 9.3	153.3 ± 2.6
Color index, conditional units	0.63 ± 0.01	0.6 ± 0.01	0.63 ± 0.03	0.63 ± 0.03
Number of white blood cells, 10^9/l	9.6 ± 0.4	10.3 ± 0.6	8.9 ± 1.8	9.1 ± 1.0
Segment-nuclear neutrophils, %	21.6 ± 1.4	18.5 ± 0.5	20.6 ± 2.3	15.6 ± 4.1
Palatine-neutrophils, %	0.3 ± 0.3	0.5 ± 0.5	0.33 ± 0.33	0.3 ± 0.3
Lymphocytes, %	71.0 ± 1.5	73.0 ± 1.0	71.3 ± 3.2	77.3 ± 5.7
Monocytes, %	4.6 ± 0.8	3.3 ± 0.3	3.3 ± 0.8	3.9 ± 1.0
Eosinophils, %	2.3 ± 0.3	5.3 ± 1.7	4.6 ± 0.3	1.6 ± 0.6

At studying possible irritating or damaging effects of the skin and the causing contact non-allergic dermatitis, it was found that a single application of 'Sun Stim' on undamaged skin of the back of white rats in most significant recommended concentration of working solutions (2%), have not caused signs of skin irritation.

Non-diluted concentrate of the 'Sun Stim' caused irritation from insignificant to moderate (2–3 points). A single application of 'Sun Stim' on 2/3 of the skin surface of the tails of white rats did not lead to the development of irritative skin reactions.

At determining the teratogenic effect in experiments with rats and rabbits at feeding disinfectant in doses of 2.5 and 1.5 mg/kg of body weight it was found that the drug does not have teratogenic effect.

At determining carcinogenic, mutagenic or genotoxic effects of the set for sanitation 'Sun Stim' it was found that the set and the substances from which it was made do not have carcinogenic, mutagenic or genotoxic effects.

Conclusion. Based on the results, DL_{50} of the set for sanitation 'Sun Stim' for female rats 2,000.0 ± 35.0 mg/kg body weight, male — 2,033.0 ± 34.3 mg/kg.

Thus, this drug, when injected into the stomach, according to the classification of toxicity in accordance with state standard (GOST 12.1.007-76), is allowed to be classified as III hazard class, low-hazard compounds.

The set for sanitation 'Sun Stim' and the substances from which it was made do not have carcinogenic, mutagenic or genotoxic effects.

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