

## Evaluation of acute toxicity of the "Orgasept" disinfectant

V.L. Kovalenko<sup>1\*</sup>, G.V. Ponomarenko<sup>2\*</sup>, M.D. Kukhtyn<sup>3</sup>, A.P. Paliy<sup>4,9</sup>, O.O. Bodnar<sup>5</sup>,  
H.I. Rebenko<sup>6</sup>, T.G. Kozytska<sup>7</sup>, T.V. Makarevich<sup>8</sup>, O.V. Ponomarenko<sup>2</sup>, A.P. Paliy<sup>9</sup>

<sup>1</sup>State Scientific Control Institute of Biotechnology and strains microorganisms,  
30 Donetska St, Kyiv, 03151, Ukraine

<sup>2</sup>Kharkiv State Zooveterinary Academy, 1 Akademicheskaya St, urban settlement Malaya Danilivka,  
Dergachivsky district, Kharkov region, 62341, Ukraine

<sup>3</sup>Ternopil Ivan Puluj National Technical University, 56 Ruska St, Ternopil, 46000, Ukraine

<sup>4</sup>National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine",  
83 Pushkinska St, Kharkiv, 61023, Ukraine

<sup>5</sup>State Agrarian and Engineering University in Podilia, 13 Schevchenko St, Kamianets-Podilskyi, Khmelnytskyi  
region, 32300, Ukraine

<sup>6</sup>Sumy National Agrarian University, 160 G. Kondratieva St, Sumy, 40021, Ukraine

<sup>7</sup>State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise, 30  
Donetska St, Kyiv, 03151, Ukraine

<sup>8</sup>Odessa State Agrarian University, 13 Panteleimonovskaya St, Odessa, 65012, Ukraine

<sup>9</sup>Kharkiv National Technical University of Agriculture named after Petro Vasylenko,  
44 Alchevskih St, Kharkiv, 61002, Ukraine

Corresponding author e-mails: [kovalenkodoktor@gmail.com](mailto:kovalenkodoktor@gmail.com)<sup>1</sup>, [gpkh1966@gmail.com](mailto:gpkh1966@gmail.com)<sup>2</sup>

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The purpose of the research was to study acute toxicity, irritating and sensitizing effects, biochemical and immunological parameters of animal blood after influence of "Orgasept" disinfectant, consisted from silver nanoparticles, benzalkonium chloride and lactic acid. To determine acute toxicity, 6 months old clinically healthy male rats (5 groups, six rats per each group) and female rats (5 groups, six per each group) with body weight of 180-200 g were used. We determined the average lethal dose (LD<sub>50</sub>) and the main parameters of acute toxicity after Orgasept rats administration in various doses. We found that, at intra-gastrointestinal administration of Orgasept, the LD<sub>50</sub> for male rats was 5000.0±43.0 mg/kg body weight and 5045.0±56.3 mg/kg for the females. We also registered that Orgasept does not have cumulative and sensitizing properties, does not show irritating effect, suppressive effect on growth and development of animals, and does not affect the hemopoiesis. We revealed that Orgasept demonstrated significant effect on nonspecific and specific factors of the organism's protection compared to the formaldehyde.

**Key words:** disinfectant, Orgasept, acute toxicity, laboratory rats, irritating and sensitizing properties.

## Introduction

Disinfection is a leading factor in ensuring the epizootic welfare of livestock and obtaining safe products of animal origin of high sanitary quality (Zavgorodniy et al., 2013; Gosling, 2018). Disinfection technologies are especially important in the prevention and control of vaccine-uncontrolled diseases (Paliy et al., 2015; De Lorenzi et al., 2020), and due to the concentration of a significant number of animals in limited areas when transferred livestock on an industrial scale (Stringfellow et al., 2009; Shkromada et al., 2019).

Recently, disinfection approach has changed due to latest biocidal products (Paliy et al., 2016; Kovalenko et al., 2018; Stegnyy et al., 2019). This is confirmed the emergence of resistant microorganisms to the action of antimicrobial drugs (Huang et al., 2006; Hadzevych et al., 2019), including disinfectants (Kamal, et al., 2019) with a modern understanding of the environmental aspects of the use of antibacterial drugs (Roeder et al., 2010; Jones et al., 2018). The generally accepted classical concept of disinfection, as the destruction or removal of the infective matter, became the basis for the fact that disinfection is understood only as a specific technique of decontamination. But such a definition and understanding of the purpose and objectives of disinfection does not cover modern ideas about its role as an independent scientific discipline and its goals and objectives in the overall set of measures to influence the infectious process (Gebel et al., 2013; Horiuk et al., 2019; Paliy et al., 2020b).

Factors, influenced the transmission of pathogens are soil, animal housing, manure, care tools, transportation, water bodies, animal products, and animal carcasses (Leggett et al., 2017; Paliy et al., 2018b; 2019b). There are potential microbial risks associated with the air in livestock facilities (Abd-Elallet et al., 2009) as well as parasitic insects (Paliy et al., 2018a). In the external environment, many adverse conditions affect the pathogens and cause the death of microorganisms (Haruta & Kanno, 2015). However, all of these factors do not replace or preclude the use of disinfectants to completely disinfect contaminated objects (Paliy et al., 2018c; Paliy et al., 2020; Takagi & Yagishita, 2020; Costard et al., 2009).

High efficiency combined with simplicity and economy of operation promotes wide use of disinfection in animal husbandry and veterinary. Among them, number of disinfectants of various origins and biocidal properties are used in Ukraine. Among the means of physical origin, the most relevant is ultraviolet radiation (Zavgorodniy et al., 2019; Rodionova et al., 2020). The most common

chemicals today are based on glutaraldehyde (Orsi et al., 2010; Paliy et al., 2020c), chlorine-active compounds (Paliy, 2014; Kamal et al., 2019; Paliy et al., 2019a), oxidants (Lineback et al., 2018; Paliy, 2018) and other compounds (Rutala, 1996; Rabenau et al., 2005). Disinfectants are proposed for use in low ambient temperatures (Paliy et al., 2020a). Most existing agents are relatively inexpensive, have a broad spectrum of bactericidal action, but are toxic, can be carcinogenic and have high corrosive activity (Curran et al., 2019; Jin et al., 2020). Currently, the use of bactericidal substances - lactic acid and quaternary ammonium compounds (QACs) in the composition of disinfectants is in great demand. They are well soluble in water, colorless, almost odorless, have high bactericidal and surface activity, combined with low toxicity and no irritating and other side effects (Souza et al., 2015; Addie et al., 2015; Ponomarenko et al., 2017a). They do not form toxic products, are not inactivated by proteins, stable, non-aggressive.

Over the last decade, the use of silver nanoparticles as the main active ingredient in many modern disinfectants has become more widespread (Susan et al., 2009; De Gussemme et al., 2010; Ivask et al., 2014; Lopes et al., 2016). The basis of the antibacterial action of silver is its partial oxidation and release of silver ions, which when interacting with thiol groups of peptidoglycans of the bacterial cell wall and cell membrane proteins, cause cell lysis. Silver ions can also bind to bacterial DNA, preventing its replication and stopping the synthesis of bacterial proteins (Likus et al., 2013). At the nanoscale, silver exhibits extraordinary physical, chemical and biological properties. Due to the pronounced antibacterial activity, nanosilver is used not only for disinfection of liquids, equipment or premises, but also for the treatment of wounds and burns. Thus, the use of nanosilver is becoming increasingly common in human and veterinary medicine, as well as in related fields (Chen, & Schluesener, 2008; Benn et al., 2010). Therefore, the bactericidal agents with high biological activity, low toxicity, and bactericidal, antiviral and antifungal action can be proposed as an alternative to traditional disinfectants and antibacterial agents (Ponomarenko et al., 2017b).

The aim of our research was to study the acute toxicity, irritating and sensitizing effects, biochemical and immunological parameters of animal blood under the action of bactericidal disinfectant Orgasept consisted of silver nanoparticle, benzalkonium chloride and lactic acid.

## Materials and methods

To determine the disinfectant acute toxicity, 6-months clinically healthy male rats (5 groups, six in each group, n=30) and female rats (5 groups, six in each group, n=30) with body weight of 180-200 g were used. Laboratory research was carried out in the Educational and Scientific Laboratory for Molecular Genetic Research Methods named after P.I. Verbitskiy at the Department of Epizootology and Veterinary Management of the Kharkiv State Zoo Veterinary Academy. Depending on the amount of Orgasept administered to rats, the average lethal dose (LD<sub>50</sub>) and the main parameters of acute toxicity were determined using Kerber's method (Kotsiumbas et al., 2006). Classification of substances by toxicity was carried out according to the table of toxicity levels GOST 12.1.007-76. Determination of the irritant action to control the development of contact non-allergic dermatitis was carried out by daily application of the solution on the skin of rat's back for 30 days. In experimental groups (5 rats in each), the recommended concentrations of Orgasept (0.3% and 0.5%) and the non-diluted Orgasept concentrate were used. In control group (n=5), a physiological solution was used. Sensitizing properties of Orgasept were studied in one group with 10 rats. A single application of 0.3% Orgasept solution was administered daily to the right rat side, after shaving of the coat, during 20 days. The skin from rats' left side was a control, where we made one-time application of 0.9% saline solution (Kovalenko & Nedosekov, 2011).

The compilation and recording of opsonophagocytic reaction with the study of phagocytic activity, phagocytic index and absolute complete phagocytosis of rat blood were performed according to V. V. Chumachenko modified method (Buchko, 2015). The modern human methods of laboratory animals care were used (Buckmaster, 2012). Data in the tables were presented like means and standard deviations

## Results and discussion

When applied Orgasept to the rats, we did not see any change in the behavior of animals, or in any functional features. In addition, the absence of harmful effects of the drug Orgasept was evidenced by the results of the pathological and anatomical section of the animals slaughtered at the end of the experiment. The results of pathoanatomical studies did not show any cumulative properties of the disinfectant Orgasept.

According to the results of studies of irritating action, it was found that when using the working concentrations of the drug Orgasept there was no irritation of the skin or any other organs and tissues, indicating no negative effect of the drug on living organisms. We found that only in the first minutes after application of the solution the animals attempted to lick the wetted area of the skin, then calmed down and the behavior remained adequate. The surface of the treated with disinfectant area of the skin had a weak hyperemia for two hours. After washing the disinfectant with water, skin damage, edema, and hyperemia were not detected. Therefore, the analysis of the results of studies has helped to find out that the working concentrations of the drug Orgasept do not cause irritating and sensitizing effects, the disinfectant has no cumulative properties.

Initial results presented in Table 1. Further analysis revealed that after intragastric administration of the drug "Orgaspet" the LD<sub>50</sub> for male rats was 5000.0±43.0 mg/kg body weight, and for male rats – 5045.0±56.3 mg/kg.

**Table 1.** Acute toxicity of Orgasept in rats under the intragastric administration (n=6)

Characteristics	Dose of the preparation, mg/kg				
	4800	4900	5000	5100	5200
	Males				
Number of animals, heads	6	6	6	6	6
survived, heads	6	5	4	2	0
died, heads	0	1	2	4	6
	LD <sub>50</sub> = 5000.0±43.0				
	Females				
Number of animals, heads	6	6	6	6	6
survived, heads	6	4	4	1	0
died, heads	0	2	2	5	6
	LD <sub>50</sub> = 5045.0±56.3				

Changes in the non-specific resistance of animals under the action of the drug Orgasept were studied in the directions of comparative study of the influence of disinfectants of formaldehyde and Orgasept on nonspecific and specific factors of protection of the organism depending on the concentration and exposure of their application. During the research of the facilities where laboratory animals were kept, they were treated with preparations of formaldehyde and Orgasept. We considered that non-specific resistance of the organism regards activity and intensity of phagocytosis, namely the parameters of opsonophagocytic response were the characteristic features (Table 2).

**Table 2.** Influence of disinfectants on the immune status of rats (n=6)

Period of investigation, days	Groups of experimental animals						
	Orgasept doze, %			Formaldehyde doze, %			Control
	0.5	1.5	3.0	0.5	1.5	3.0	
	Phagocytic activity, %						
1	12.0±0.32*	9.2±0.39*	9.9±0.85*	11.0±0.60*	9.8±0.70*	9.5±1.10*	12.5±0.31
15	11.7±0.33	11.5±0.54	10.2±0.75	11.1±0.13	10.1±0.56	10.1±0.54	12.4±0.89
30	12.1±0.44	11.9±0.33	10.6±0.42	11.9±0.54	11.8±0.62	10.0±0.56	12.1±0.32
	Phagocytic index						
1	4.3±0.25	3.8±0.56	3.8±0.42	3.2±0.11	3.1±0.04	2.1±0.10	4.5±0.22
15	4.1±0.58	4.2±0.74	4.2±0.78	3.6±0.74	3.2±0.23	3.1±0.15	4.3±0.13
30	4.4±0.75	4.2±0.19	4.0±0.45	4.0±0.41*	3.5±0.11*	3.5±0.21*	4.4±0.35
	Macrophage transformation index (MTI, %)						
1	41.5±4.11	38.1±3.45	31.0±2.44	32.0±2.42	31.2±2.23	28.0±0.05	41.2±2.80
15	42.0±2.31	41.9±2.45	40.8±2.76	35.0±2.20	34.1±2.03	31.0±1.87	42.5±2.01
30	43.1±3.77	42.2±2.01	40.1±3.24	40.2±3.41	35.5±3.15	35.1±3.90	43.2±3.15

\* p≤0.05 to control.

According to the results of researches of these indicators in laboratory animals under their treatment with 0.5, 1.5, and 3.0% solution of the drug Orgasept it was found that in 5 hours after treatment with the disinfectant, regardless of its concentration, animals showed a probable decrease in phagocytic activity, especially under the action of 3.0% solution. However, subsequent studies of the activity of neutrophils of peripheral blood in 15 days after treatment of animals indicated its growth in all experimental groups of animals. After 30 days in animals treated with 0.5 and 1.5% solution of the drug Orgasept, phagocytic activity did not differ from those in animals in the control group. In the experimental group of animals treated with 3.0% solution of the Orgasept, the phagocyte activity values remained lower than those in control animals at 0.7%, which showed a somewhat suppressive effect of disinfectant in such a concentration on the body of rats. In this case, the phagocytic index in animals, under the treatment of facilities with a 0.5% solution of disinfectant, did not change, and after 15 and 30 days, even had a slight tendency for unreliable growth in the range from 0.5 to 1.2%. In the group of laboratory animals, which were treated with higher concentrations of the drug – 1.5 and 3.0%, in 15 days after treatment, the characteristics of the phagocytic index were unlikely to increase.

We saw that 30 days after the application of 1.5% solution of Orgasept in the experimental animals, the phagocyte index did not differ from that of the animals in the control group. In animals treated with the 3.0% solution of the drug, the characteristics of the phagocytic index remained below the control by 3.5%, which showed a slight suppressive effect of this concentration of drug on the phagocytic properties of neutrophils of peripheral blood in rats. Therefore, 0.5% solution of the drug Orgasept was optimal for the treatment of animals, because in 15 days the parameters of opsonophagocytic reaction came to the norm. Our studies showed that 0.5% concentration of the drug administered orally at a dose of 1cm<sup>3</sup> did not lead to visible pathological and anatomical changes in laboratory animals. The latter was established at the autopsy after their euthanasia and indicated a low toxicity and harmlessness of the drug in case of application in the working concentrations of the drug. The analysis of the results of morphological, biochemical and immunological studies of blood of rats under the processing with 0.5% solution of disinfectant Orgasept showed that the initial and final indices in animals of the experimental and control groups were within the normal range.

The level of endogenous intoxication of the body of rats (sorption capacity of erythrocytes) of the experimental and control groups during the observation period was stable and amounted to 17.15% on average, which indicates the absence of suppressor action of the disinfectant Orgasept on the antioxidant properties of the animal's organism. Thus, it has been experimentally confirmed that the drug Orgasept has no suppressive effect on metabolic processes in rats. The use of effective and safe disinfectants is of paramount importance in providing the population with quality products of animal origin (Paliy et al., 2017, 2018d). One of the main criteria that determines the feasibility of using disinfectants in practical conditions is their environmental safety and low toxicity to staff and animals (Du et al., 2017; Bondarchuk et al., 2019).

In the development of intoxication of the macroorganism with potent toxic substances there are four periods: the period of contact; hidden period; period of toxic pulmonary edema; period of complications (Presgrave et al., 2008). Substances with a weak cauterizing effect are dangerous, in particular phosphorus chloride and sulfur chloride. Inhalation of phosphorus chloride vapors for several minutes at a concentration of 0.08-0.15 g/m<sup>3</sup> leads to acute poisoning (Wason et al., 1984). The disinfectants belonging to hazard classes 3 and 4 can be recommended for use in various ways of their entry into the body with no or weak local irritant and sensitizing effects (Catlin et al., 2018). Disinfectants containing glutaraldehyde and quaternary ammonium compounds (Lin et al., 2018) are considered safe. High antibacterial activity of nanoparticles, especially against gram-negative and gram-positive bacteria is regarded as a positive effect, but there is a possibility of potential risk in their use in terms of toxic effects (Bąkowski et al., 2018). Nanoparticles have been shown to be toxic to bacteria, algae and invertebrates (Exbrayat et al., 2015). The results of our research generally coincide with the information given in the works of other authors who studied and analyzed experimental data on the toxic effects of silver nanoparticles on the body not only of laboratory animals but also of humans (Armstrong et al., 2013; Kovalenko et al., 2017; Yousef et al., 2019).

## Conclusions

We registered that the Orgasept LD<sub>50</sub> for male rats was 5000.0±43.0 mg/kg body weight and for female rats – 5045.0±56.3 mg/kg. After our experiments, we finally proved that Orgasept belongs to class IV in accordance with the classification of dangerous substances. We also revealed that Orgasept does not have cumulative and sensitizing properties and does not demonstrate an irritating effect.

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