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STUDY OF THE SAFETY AND HARMLESSNESS OF A DISINFECTANT IN LABORATORY ANIMALS

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Summary. The work aimed to investigate the effect of the disinfectant 'Diolaid' based on sodium chlorite and sodium chloride on acute toxicity indicators, as well as on blood parameters of laboratory animals. The experiments were carried out on 6-month-old clinically healthy male rats (5 groups, 6 animals in each group, n = 30) and female rats (5 groups, 6 animals in each group, n = 30) weighing 200–220 g. The drug was administered to animals intragastrically (by probe) and aerosol treatment of cells with animals was carried out. Separately we studied the skin-irritating and sensitizing action of the disinfectant 'Diolaid' on the groups of clinically healthy guinea pigs and rats weighing 250–300 g by a daily application on their back and sides of different concentrations of the drug for 30 days for 30 min periods. In addition, we tested the effect of 'Diolaid' on nonspecific immune response indicators of these animal species (bactericidal activity of blood serum, level of circulating immune complexes, T and B cells, *etc.*). The work used modern humane methods of care and use of laboratory animals. It was found that after intragastric administration of 'Diolaid', the average lethal dose (LD_{50}) for male rats was 182 mg/kg of body weight, and for female rats it was 170 mg/kg. It has been proven that the drug has a temporary irritating and sensitizing effect and does not adversely affect the parameters of hematopoiesis and non-specific immune response in the form of a 0.06% solution. The research results indicate the low toxicity of the 'Diolaid' drug for laboratory animals and the possibility of its use in low concentrations both for treating cages in the presence of animals and for treating the animals themselves. For disinfection of water during its storage in containers, we used the concentration of the 'Diolaid' drug (by chlorine dioxide) of 0.5–2 mg/l (0.0002–0.0008%), depending on the degree of purity of the water to be treated. Such concentrations ensure compliance of the chlorite residual concentrations with hygienic standards

Keywords: rats, guinea pigs, acute toxicity, irritating and sensitizing effects, immune response

Introduction. *Relevance of the topic*. In the system of veterinary and sanitary measures, disinfection occupies an important place. It is carried out in farms for the prevention and elimination of dangerous infectious diseases of animals and birds, as well as the destruction of the disease-causing pathogens together with opportunistic pathogens in the external environment (Kovalenko and Nedosekov, 2011).

Water purification and disinfection of the water supply system are of great importance in livestock farms, as microorganisms can enter the animals' bodies with drinking water, along with vitamins and other additives (Gebel et al., 2013).

Certainly strict observance of veterinary and sanitary measures requires the use of highly effective, safe, easy to use disinfectants with a wide spectrum of bactericidal action, which are non-toxic, non-carcinogenic, do not cause habituation of the microflora and provide a prolonged effect in the presence of poultry.

Analysis of recent research and publications. Recently, manufacturers offer a wide range of low-toxic disinfectants based on quaternary ammonium and peroxide compounds, aldehydes and dialdehydes, low-molecular organic acids, guanidines and surface-active substances. However, not all disinfectants fully meet expectations. The quality of disinfectants can be affected by a decrease in activity during storage when working solutions come into contact with chemicals, which leads to resistance of microorganisms and carcinogenicity (Addie et al., 2015; Kovalenko et al., 2018; Lineback et al., 2018).

Taking into account the scientific studies and the analysis of active substances for disinfectants, the next development of the authors was a new, environmentally safe, highly effective disinfectant 'Diolaid', which includes sodium chlorite, sodium chloride. These components make it possible to effectively carry out not only comprehensive sanitation of livestock and poultry facilities in the presence of animals and poultry, but also sanitation of water supply (Ge et al., 2021; Ma et al., 2017; Ngwenya, Ncube and Parsons, 2013).

In the conditions of a subchronic experiment on white rats, scientists found that chlorine dioxide in a concentration of 1.35 mg/dm^3 in water does not cause significant changes in blood parameters, lipid peroxidation, as well as structural and functional changes in internal organs in adult animals and in the offspring of females which consumed water containing chlorine

dioxide at the same concentration, except for the stimulation of spermatogenesis and nitric oxide synthase activity in the cellular elements of the spleen (Mokienko et al., 2008).

According to the WHO, the recommended concentration of chlorine dioxide in drinking water has not been established due to its rapid decomposition. The temporarily recommended value for chlorites (0.2 mg/l) provides sufficient protection against the potential toxicity of chlorine dioxide: it has been established that the threshold concentration of chlorine dioxide for the effect on the smell of water is 0.45–0.40 mg/l. An aftertaste with an intensity of 1-2 points has been detected at higher concentrations of this compound in water. The results of chronic experiments on laboratory animals have shown that chlorine dioxide, even in high concentrations — 0.5 mg/kg and 5 mg/kg (or 10 mg/l and 100 mg/l) does not have a pronounced toxic resorptive effect on the body (Mokienko, 2021).

In Ukraine, the maximum permissible concentration of chlorite anions is 0.2 mg/l, chlorate anions — 20 mg/l (MHU, 2007, 2010; Gosstandart, 1976; MHUSSR, 1991).

An analysis of the market of disinfectants and literary sources indicates that the use of chlorine dioxide in veterinary medicine is almost not used, compared to its spread in human medicine, as well as in related fields (Kovalenko et al., 2018; Mokienko and Petrenko, 2008; Mokienko, Petrenko and Gozhenko, 2006).

The **purpose of the work** and the task of the experiments was to investigate the acute toxicity, skin-irritating and sensitizing effects, as well as the effect on the immunological parameters of the blood of laboratory animals when using the disinfectant 'Diolaid' based on sodium chlorite, sodium chloride.

Materials and methods. The research was conducted in the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) (Kyiv, Ukraine).

The experimental part of the work was carried out taking into account 'Council Directive 2010/63/EU on the Protection of Animals Used for Scientific Purpose' (CEC, 2010), 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (CE, 1986) , and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006). Modern humane methods of care and use of laboratory animals were used in the experiments (Buckmaster, 2012).

Disinfectant 'Diolaid', developed by employees of the SSRILDVSE, consists of Components 1 and 2, which are white powders. pH of a 1% solution is 4.0–6.0. Components 1 and 2 of the 'Diolaid' disinfectant dissolve very well in water without sediment, the working solution of the drug is from transparent to yellowish in color, with a moderate specific smell of chlorine.

Main active substances: component 1 – sodium chlorite (42%), sodium chloride (47%); component 2 — citric acid (95%), adipic acid (3%).

Mass fraction of chlorine dioxide — not less than 1%. To use the 'Diolaid' disinfectant, it is necessary to prepare starting solutions from Components 1 and 2, which are prepared using special equipment for automatic dosing of powdered Components 1 and 2, preliminary dilution and metered introduction of the drug solution with a given concentration of chlorine dioxide into water.

The content of chlorine dioxide in the starting solution is 0.0025% (25 mg/l). The starting solution of the product is a transparent to slightly yellowish liquid with a mild chlorine smell, after diluting it in drinking water, the water remains transparent. Working solutions of the drug for wet and aerosol disinfection of objects are prepared by mixing Components 1 and 2 with drinking water. A mixture of Components 1 and 2 in a ratio of 1:1, 10% of the mixture of components + 90% of the filler (sodium chloride or zeolite or bentonite), respectively, is used for the use of chlorine dioxide aerosol for air disinfection in a small volume of air.

Acute toxicity was studied on clinically healthy male rats (5 groups, 6 animals in each group, n = 30) and female rats (5 groups, 6 animals in each group, n = 30) aged 6 months with a body weight of 190–210 g. The drug 'Diolaid' in different doses: 30 mg/kg, 50 mg/kg, 100 mg/kg, 200 mg/kg, and 300 mg/kg was administered to animals intragastrically using a probe and inhaled, by treating cages. The average lethal dose (LD_{50}) and indicators of acute toxicity were determined following the method of Kerber. In the course of the experiment, animals that died during the experiment and those that survived and were killed, were subjected to a pathological autopsy in order to identify changes in organs and tissues. The classification of substances by toxicity was carried out in accordance with SOU 85.2-37-736:2011 (MAPFU, 2011), and toxicological studies were carried out following the monograph 'Preclinical studies of veterinary drugs' (Kotsiumbas, 2006).

Experiments to determine the skin-irritating effect upon skin application of the disinfectant 'Diolaid' were conducted in compliance with the general rules adopted in sanitary toxicology. For the experiment, healthy animals were selected — guinea pigs weighing 250–300 g. The drug (suspension 1:3) was applied to a pre-shaved site of the animal's skin with an area of 4×4 cm in a dose of 2,500 mg/kg in an open manner, at an ambient temperature of 12–18°C . Laboratory animals were placed in individual fixing cabins and kept for 4 h. After the end of the exposure, the animals were removed from the cabins and the applied drug sample was washed off with warm water and soap. The total duration of animal observation was 2 weeks. On the 1st day after applying the drug sample to the skin, the animals were under the constant observation.

The general state of the experimental animals, their behavioral characteristics, the intensity and nature of motor activity, coordination of movements, reaction to tactile, light and sound stimuli, the condition of the hair coat and mucous membrane, as well as the number of

dead animals in the group were recorded daily. The skin reaction at the application site was evaluated 1 h and 16 h after the end of the exposure.

The functional state of the skin was assessed by the presence of erythema, edema, and visual manifestations of the disease (ulcers, cracks, *etc.*). The degree of expression of signs of irritation was evaluated in points following the requirements of Methodological Guidelines No. 2102-79 'Assessment of the Impact of Harmful Chemical Compounds on the Skin and Substantiation of the Maximum Permissible Levels of Skin Contamination' (MHUSSR, 1980).

Sensitizing properties of the drug were studied on clinically healthy rats of one group (n = 10). On the right side, for 30 days, a single application of a 0.3% solution of the drug was carried out daily, at the place where the hair was shaved. The skin on the left side served as a control. A single application on it was made with 0.9% physiological solution (Kovalenko and Nedosekov, 2011). Additionally, animal tails were exposed in a flask with disinfectant in the same concentrations for 30 min.

Immunological studies were carried out by the indicators of the opsonophagocytic reaction: phagocytic activity, phagocytic index and macrophage transformation index were carried out following the modified method of V. Yu. Chumachenko.

In addition, we analyzed the indicators of blood serum bactericidal activity, level of circulating immune complex, and also studied the quantitative content of immunocompetent cells in the peripheral blood of rats — T and B cells.

Statistical processing of the results obtained in the course of studies on non-specific immune response was carried out using the software Epitools — Epidemiological Calculators, which is freely available at https://epitools.ausvet.com.au. It was used to estimate confidence limits for the means (Cl) at a confidence level (P = 0.95), and P-Value (a measure of the probability that an observed) was carried out. The estimated confidence limits for the means were calculated taking into account the means value, standard deviations, and sample size. P-Value Definition was conducted with Desired significance level of 0.05.

Results and discussions. The results of the clinical condition after oral administration of a lethal dose of 'Diolaid' on the functional indicators of rats are presented in the Table 1. As can be seen from Table 1, 8 h after the administration of a lethal dose of the drug 'Diolaid' in the stomach, the animals demonstrated a shaky gait, apathy, depression of the central nervous system, immobility, at the same time, the experimental rats showed a reduced reaction to touch, pain interference, grip strength, and decreased respiratory rate. After 24 h, anemia was recorded, the eye slits were narrowed, and the animals tightened their stomachs. The rats died within 3 days.

The pathological autopsy of the dead animals showed that the walls of the abdominal cavity were unchanged, smooth, and moistened; the liver is smooth and shiny, slightly hyperemic on the right side; the lung tissue is pink, hyperemic, unchanged, of uniform consistency; the heart is unchanged.

In the coronary vessels, we observed a slight expansion of the venous sinuses and accumulation of blood. In the place where the probe was inserted into the stomach, mechanical stretching of the walls of the stomach and the adjacent part of the small intestine was noted. The colon was unchanged. However, when the drug was used in a subtoxic dose, these symptoms of rat poisoning disappeared after 48–72 h.

Table 1 — The effect of a subtoxic dose of the drug 'Diolaid' under the conditions of oral administration on the general functional indicators of rats

Indicators	Observation period, h		
	8	24	72
Behavioral responses:			
Motor activity	–1	–1	–1
Arousal	–1	–2	–1
Reactivity	–1	–2	–1
Aggressiveness	–1	–1	–1
Neuromuscular reactions:			
Tremor	0	0	0
Convulsions when walking	0	0	0
Reaction to painful stimuli	–1	–1	0
Grip strength	–2	–1	0
Vegetative responses:			
The size of the eye pupil	unchanged		
Breathing rate	slowed down		
Condition of the hair cover	unchanged		
Color of visible mucous membranes	slight cyanosis		
Frequency of defecation	unchanged		
Amount of feces	slight increase		
Fecal consistency	unchanged		
Urinary frequency	unchanged		
Urine color	unchanged		
Heart rate	unchanged		

Notes: 0 — no effect; '–' — inhibition of the effect.

The results of experiments for the determination of the acute toxicity of the disinfectant drug 'Diolaid' are presented in Table 2. It has been established that when the drug 'Diolaid' was administrated into the stomach of male rats, the LD_{50} was 182 mg/kg of body weight, and for female rats it was 170 mg/kg (the difference is approx. 7.0%).

As shown in Table 2, no rats died when the drug was administered to animals (both males and females) at a dose of 30 mg/kg. At the same time, the use of a large dose (300 mg/kg) caused the death of all animals. Administration of the drug in amounts of 50–100 mg/kg caused the death of one to two animals in the group. Taking into account all of the above, according to the classification of SOU 85.2-37-736:2011 (MAPFU, 2011), the drug is considered moderately toxic.

During the 2-hour exposure of rats in cages treated with different concentrations of the drug (0.06%, 0.1%, and 0.16%), death was not observed among them. According to clinical indicators, increased motor activity was noted during the first day. In the future, the behavior of the animals did not differ from those in the control group.

The results of studies of changes in the non-specific immune response in rats after inhalation treatment of cages with disinfectant 'Diolaid' in different concentrations, are presented in Table 3. The results of studies of immunological indicators of blood in laboratory animals after their treatment with 0.06%, 0.1%, and 0.16% solutions of the drug, showed that after 5 h of the first day in rats under the effect of 0.16% solution, a probable insignificant decrease in phagocytic activity was observed in comparison with the parameters of the control group (p-value < 0.001). However, the following 15 and 30 days of research after treatment with a 0.16% solution and lower concentrations did not reveal any suppressive effect on phagocytic activity in animals.

Minor fluctuations determined the indicator of the phagocytic index in rats after treatment of their cages with 0.06%, 0.1%, and 0.16% solutions of the drug for 15 and 30 days in the range from 1.8% to 3.8%, but were within the normal range compared to the control group of animals. The result of studies of the macrophage transformation index on the first day was reliably lower in animals from the group where the cages were treated with 0.16% 'Diolaid' solution (p-value < 0.001). During the following days of the experiment, the indicator was in the same ranges as in the control group of animals. Lower concentrations of the drug did not affect the macrophage transformation index.

It has been established that 0.06%, 0.1%, and 0.16% solutions of the drug 'Diolaid' did not have a suppressive effect on the indicators of the opsonophagocytic reaction in rats, and this is also indicated by the indicators of the content of the circulating immune complex in the blood serum of animals, which characterizes the body's immune response to destruction of toxic products to restore disturbed parameters of hemostasis.

Indicators in experimental groups of rats after their treatment with 0.06%, 0.1% solutions and level of circulating immune complexes in the control group reliably did not differ from the first day of the experiment (p-value < 0.3). Treatment with a 0.16% solution had a negative effect on the animal's body, where it was established that after 5 h of the first day, circulating immune complexes indicators reliably increased by 28.8% compared to similar indicators in control groups of animal (p-value < 0.01). During the next 15 and 30 days of the experiment, the level of circulating immune complexes was within normal limits.

A slight suppressive effect was observed under the effect of 0.1% and 0.16% 'Diolaid' solutions on indicators of bactericidal activity of blood serum and the content of immunocompetent cells in the peripheral blood of rats (T and B lymphocytes).

Table 2 — Study of acute toxicity in rats after intragastric administration of the drug 'Diolaid' by the method of Kerber (n = 6)

Indicators	Drug dose, mg/kg				
	30	50	100	200	300
Males					
Number of animals, indiv.	6	6	6	6	6
of them: survived	6	5	5	3	0
died	0	1	1	3	6
	LD_{50} = 182				
Females					
Number of animals, indiv.	6	6	6	6	6
of them: survived	6	5	4	3	0
died	0	1	2	3	6
	LD_{50} = 170				

Table 3 — Confidence interval (CI) estimate for nonspecific immune response means with a probability (P) of 0.95 (n = 6)

Research period, day	Experimental groups of animals			
	Drug concentration, %			Control
	0.06	0.1	0.16	
Phagocytic activity, %				
1	11.3–12.0	11.5–12.1	10.1–10.5*	11.5–12.2
15	11.7–12.1	11.6–11.9	11.3–11.4	11.3–12.8
30	11.5–12.0	11.8–11.9	11.2–12.0	11.4–12.1
Phagocytic index				
1	4.1–4.4	3.9–4.0	3.7–4.1*	3.8–4.4
15	3.9–4.5	4.1–4.7	3.8–4.4	4.1–4.3
30	3.8–4.5	4.0–4.2	4.0–4.1	4.0–4.2
Macrophage transformation index %				
1	36.1–41.2	35.2–40.5	30.3–35.5*	40.1–43.4
15	38.3–42.1	40.6–42.8	39.8–41.5	41.2–42.2
30	37.5–44.0	41.4–43.4	37.9–42.0	40.4–44.4
Bactericidal activity of blood serum, %				
1	81.0–83.8*	61.3–65.2*	60.1–66.5*	86.2–95.6
15	88.5–91.7	85.7–93.9*	85.1–88.9*	88.8–96.0
30	92.2–98.3	90.4–98.2	98.1–98.1*	94.0–96.5
Circulating immune complexes, CU				
1	10.9–12.1	10.8–12.1	12.5–16.1*	10.5–12.5
15	10.5–12.8	10.7–13.4	12.0–15.0	10.4–12.2
30	10.5–12.2	11.1–12.8	11.3–13.5	10.2–12.8
T lymphocytes (erythrocyte rosette-forming cells)				
1	29.2–32.4*	33.0–34.4*	31.4–32.6*	34.0–36.2
15	33.7–37.9	33.2–38.1	31.6–34.8	34.4–37.5
30	33.3–37.5	34.4–38.2	31.2–34.5	34.5–37.2
B lymphocytes (erythrocyte-antibody-complement rosette-forming cells)				
1	14.4–15.9*	14.8–15.8*	13.3–13.9*	14.2–16.0
15	14.5–15.1	14.9–15.8*	14.5–15.1*	15.1–16.2
30	15.1–15.8	15.1–16.2	13.9–15.0	14.5–16.2

Note. * — the difference between the value of the indicator and the control is probable (p-value is in the range of 0.001–0.05).

On the first day, we noted a suppressive effect on the immune system of rats due to the action of different concentrations of the drug, where a probable decrease in the bactericidal activity of blood serum indicator was established. Especially the 0.16% solution of the drug with a probable decrease of the indicator by 30.3% (p-value < 0.001) compared to the control group. After 30 days of the experiment, indicators of bactericidal activity were within the normal range in all experimental groups.

The study of cellular immunity indicators in rats when using the drug 'Diolaid' showed that after 5 h, a slight inhibition of rosette formation was observed in all experimental groups of animals, regardless of the concentration of the drug, which was characterized by an unprobable decrease in the content of T cells in the peripheral blood. After 15 days, in the group of animals treated with 0.06% and 0.1% solutions of the drug, the relative content of T cells did not differ from the control group and remained so until the end of the experiment.

A study of indicators of the direct immune rosette formation (B cells) showed that the use of a 0.16% concentration of 'Diolaid' reduced the indicators 5 h after inhalation by 6.3%. In the group of animals treated with 0.06% and 0.1% solutions of the drug, the content of B cells did not differ from the control indicators throughout the experiment.

Studying the irritating and sensitizing effects of the drug 'Diolaid' on the skin of laboratory rats for 30 days with 0.06%, 0.1%, and 0.16% solutions, it was established that no skin irritations were detected in the animals at the place of application, which indicates the absence of a negative effect of the drug. Only in the first minutes after application of the concentrate, the animals tried to lick the wetted area of the skin, after that they calmed down and their behavior remained normal. The surface of the skin treated with the disinfectant was unchanged. During a single immersion of the tails of rats in the drug concentrate (exposure time 30 min), increased motor activity was observed in the animals, as a result of the irritating effect. There were no deaths among rats.

The study of the damaging effect on the skin and the development of non-allergic contact dermatitis showed that a single application of 'Diolaid' to the intact skin of the rat backs in the maximum recommended concentration of 1.0% did not cause signs of irritation.

It has been established that 0.06%, 0.1%, and 0.16% concentrations of the drug 'Diolaid' did not cause an irritating and sensitizing effect on rats. At the same time, when the concentrate was applied to the skin of animals, a temporary effect was noted in the form of a change in the behavior of rats.

The research results indicate the low toxicity of the drug 'Diolaid' for laboratory animals and the possibility of its use in low concentrations both for treatment of the cages in the presence of animals and for treatment the animals themselves.

The results of determining the toxicity and establishing the hazard class of the drugs we carried out 5 h after exposure, and then daily for 30 days. During the experiment the death of animals was not observed, the general state was satisfactory, the behavior was without peculiarities, the animals were mobile, the coordination of movements was not disturbed; the animals' skin was smooth and clean. No signs of intoxication were observed; reaction to external stimuli (sound, light, tactile) was normal; the condition of the hair coat and mucous membrane — the fur is neat, dry, the mucous membrane is pale pink, moderately moist.

According to the value of LD_{50} < 2,500 mg/kg, the experimental drug 'Diolaid' when applied once to the skin belongs to the 4th hazard class – low-hazard substances in accordance with SOU 85.2-37-736:2011 'Veterinary Preparations. Determination of Acute Toxicity' (MAPFU, 2011) and GOST 12.1.007-76 'Occupational Safety Standards System. Noxious Substances. Classification and General Safety Requirements' (Gosstandart, 1976).

The results of the study of the skin-irritating effect on guinea pigs after 1 h and 16 h — erythema and edema — 0 points; after 48 h and 72 h — the skin is unchanged, after 96 h and more — the skin of all animals is smooth and clean. According to the results of the study, the skin-irritating effect of the product 'Diolaid' is present, the response assessment — 0 points.

Thus, taking into account the results of the conducted experiments, according to the classification of SOU 85.2-37-736:2011 (MAPFU, 2011), the disinfectant drug 'Diolaid' is considered moderately toxic when administered intragastrically and belongs to the 3rd hazard class; when applied to the skin it belongs to the 4th hazard class — low toxicity.

For disinfection of water when it is stored in containers, drug 'Diolaid' concentrations (by chlorine dioxide) of 0.5–2.0 mg/l (0.0002–0.0008%) are used, depending on the degree of purity of the water to be treated. Such concentrations ensure compliance of the content of residual concentrations of chlorites with hygienic standards. Dosing of the starting solution is carried out using special dosing equipment or manually.

The presented results of our research confirm the information proven in the works of other authors who studied the toxic effect of active substances on the body of laboratory animals (Addie et al., 2015; Kovalenko et al., 2020; Yousef, Abuzreda and Kamel, 2019). Scientists have proven that the acute toxicity of chlorate (LD_{50}), even for two types of short-cycle hydrobionts *D. magna* and *N. spinipes*, is practically identical and amounts to 560 mg/l and 590 mg/l, respectively; chlorate at a concentration of 100–250 mg/l has a probable (P < 0.05) toxic effect on the reproductive parameters of *D. magna* in a chronic experiment.

The indicator of our study of the average lethal dose is 180 mg/kg of the weight of laboratory animals and it corresponds to an effective bactericidal concentration 0.06% (approx. 100 mg/l) of the drug 'Diolaid', which is consistent with the data of the scientists (Daniel et al., 1990), LOAEL (lowest-observed-adverse-effect level) is

25 mg/l (1.9 mg/kg/day), NOAEL (no-observed-adverse-effect level) (Bercz et al., 1982) as a more stringent standard at the level of 30 mg/l (3.5 mg/kg/day). In mice (Moore and Calabrese, 1980), which received drinking water with chlorine dioxide at a dose of 11.7 mg/kg/day (*i.e.* about 100 mg/l) for 30 days, there were no changes in hematological properties.

The same inconsistency was reflected in the regulation of chlorine oxide, chlorites and chlorates in different countries. For example, in the USA the standard for chlorine dioxide and chlorite in bottled water is 0.8 mg/l and 1.0 mg/l, in Germany the maximum limit for chlorite is 0.2 mg/l, in our country the former USSR standard of 20 mg/l remains, and in Italy, which is the leader in changes to chlorine dioxide for water treatment, neither the reagent itself nor its disinfection by-products are standardized at all (Mokiienko et al., 2006).

The main application of sodium chlorite is the generation of chlorine dioxide. Chlorine dioxide, derived from sodium chlorite under certain conditions, is approved by the US Food and Drug Administration (EPA). The EPA has set a maximum drinking water contamination level of 1 mg of chlorite per liter (Haruta and Kanno, 2015; Lin et al. 2018).

Conclusion. According to the results of studies of the effect of the drug 'Diolaid' on the body of laboratory rats, it was established that for intragastric administration of 'Diolaid', the average lethal dose (LD_{50}) for male rats was 182 mg/kg of body weight, and for female rats — 170 mg/kg. The drug exhibits a temporary sensitizing and skin-irritating effect only in the form of a concentrate. During the inhalation of the drug 'Diolaid' in the form of a 0.06% solution, no violations of non-specific resistance indicators were registered in animals.

Based on the results of the analysis, it was established that the disinfectant drug 'Diolaid' according to the classification of SOU 85.2-37-736:2011 is considered moderately toxic and belongs to the 3rd hazard class, when applied to the skin it belongs to the 4th hazard class — low toxicity, which allows it to be used in the presence of animals.

Prospects for further research. To test the drug 'Diolaid' in broiler chickens in production conditions.

References

Addie, D. D., Boucraut-Baralon, C., Egberink, H., Frymus, T., Gruffydd-Jones, T., Hartmann, K., Horzinek, M. C., Hosie, M. J., Lloret, A., Lutz, H., Marsilio, F., Pennisi, M. G., Radford, A. D., Thiry, E., Truyen, U., Möstl, K. and European Advisory Board on Cat Diseases (2015) 'Disinfectant choices in veterinary practices, shelters and households: ABCD guidelines on safe and effective disinfection for feline environments', *Journal of Feline Medicine and Surgery*, 17(7), pp. 594–605. doi: 10.1177/1098612X15588450.

Bercz, J. P., Jones, L., Garner, L., Murray, D., Ludwig, D. A. and Boston, J. (1982) 'Subchronic toxicity of chlorine dioxide and related compounds in drinking water in the nonhuman primate.', *Environmental Health Perspectives*, 46, pp. 47–55. doi: 10.1289/ehp.824647.

Buckmaster, C. (2012) 'Shifting the culture of lab animal care', *Lab Animal (NY)*, 41(7), p. 205. doi: 10.1038/laban0712-205.

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) (2010) 'Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes', *The Official Journal of the European Communities*, L 276, pp. 33–79. Available at: http://data.europa.eu/eli/dir/2010/63/oj.

Daniel, F. B., Condie, L. W., Robinson, M., Stober, J. A., York, R. G., Olson, G. R. and Wang, S.-R. (1990) 'Comparative subchronic toxicity studies of three disinfectants', *Journal - American Water Works Association*, 82(10), pp. 61–69. doi: 10.1002/j.1551-8833.1990.tb07038.x.

Ge, Y., Zhang, X., Shu, L. and Yang, X. (2021) 'Kinetics and mechanisms of virus inactivation by chlorine dioxide in water treatment: A review', *Bulletin of Environmental Contamination and Toxicology*, 106(4), pp. 560–567. doi: 10.1007/s00128-021-03137-3.

Gebel, J., Exner, M., French, G., Chartier, Y., Christiansen, B., Gemein, S., Goroncy-Bermes, P., Hartemann, P., Heudorf, U., Kramer, A., Maillard, J.-Y., Oltmanns, P., Rotter, M. and Sonntag, H.-G. (2013) 'The role of surface disinfection in infection prevention', *GMS Hygiene and Infection Control*, 8(1), p. 10. doi: 10.3205/DGKH000210.

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].

Haruta, S. and Kanno, N. (2015) 'Survivability of microbes in natural environments and their ecological impacts', *Microbes and Environments*, 30(2), pp. 123–125. doi: 10.1264/jsme2.ME3002rh.

Kotsiumbas, I. Ya., Malyk, O. H., Patereha, I. P., Tishyn, O. L. and Kosenko, Yu. M. (2006) *Preclinical studies of veterinary drugs [Doklinichni doslidzhennia veterynarnykh likarskykh zasobiv]*. Lviv: Triada plus. ISBN 9667596648. [in Ukrainian].

Kovalenko V. L. and Nedosiekov V. V. (2011) *Methodical Approaches to the Control of Disinfectants for Veterinary Medicine [Metodychni pidkhody kontroliu dezinfikuiuchykh zasobiv dlia veterynarnoi medytsyny]*. Kyiv. [in Ukrainian].

Kovalenko, V. L., Kovalenko, P. L., Ponomarenko, G. V., Kukhtyn, M. D., Midyk, S. V., Horiuk, Yu. V. and Garkavenko, V. M. (2018) 'Changes in lipid composition of *Escherichia coli* and *Staphylococcus areus* cells under the influence of disinfectants Barez®, Biochlor® and Geocide®', *Ukrainian Journal of Ecology*, 8(1), pp. 547–550. doi: 10.15421/2018_248.

Kovalenko, V. L., Ponomarenko, G. V., Kukhtyn, M. D., Paliy, A. P., Bodnar, O. O., Rebenko, H. I., Kozytska, T. G., Makarevich, T. V., Ponomarenko, O. V. and Palii, A. P. (2020) 'Evaluation of acute toxicity of the "Orgasept" disinfectant', *Ukrainian Journal of Ecology*, 10(4), pp. 273–278. doi: 10.15421/2020_1982.

Lin, W., Niu, B., Yi, J., Deng, Z., Song, J. and Chen, Q. (2018) 'Toxicity and metal corrosion of glutaraldehyde-

didecyldimethylammonium bromide as a disinfectant agent', *BioMed Research International*, 2018, p. 9814209. doi: 10.1155/2018/9814209.

Lineback, C. B., Nkemngong, C. A., Wu, S. T., Li, X., Teska, P. J. and Oliver, H. F. (2018) 'Hydrogen peroxide and sodium hypochlorite disinfectants are more effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilms than quaternary ammonium compounds', *Antimicrobial Resistance and Infection Control*, 7(1), p. 154. doi: 10.1186/s13756-018-0447-5.

Ma, J.-W., Huang, B.-S., Hsu, C.-W., Peng, C.-W., Cheng, M.-L., Kao, J.-Y., Way, T.-D., Yin, H.-C. and Wang, S.-S. (2017) 'Efficacy and safety evaluation of a chlorine dioxide solution', *International Journal of Environmental Research and Public Health*, 14(3), p. 329. doi: 10.3390/ijerph14030329.

MAPFU (Ministry of Agrarian Policy and Food of Ukraine) (2011) *SOU 85.2-37-736:2011. Veterinary Preparations. Determination of Acute Toxicity [SOU 85.2-37-736:2011. Preparaty veterynarni. Vyznachennia hostroi toksychnosti]*. Kyiv: Ministry of Agrarian Policy and Food of Ukraine. [in Ukrainian]

MHU (Ministry of Health of Ukraine) (2007) *Methodological Recomendations 'Sanitary and Epidemiological Supervision of Water Disinfection in Systems of Centralized Household Drinking Water Supply with Chlorine Dioxide' (MR 2.2.4-147-2007): approved by the order of the Ministry of Health of Ukraine of 30 July 2007 No. 430' [Metodychni rekomendatsii 'Sanitarno-epidemiolohichnyi nahliad za znezarazhuvanniam vody u systemakh tsentralizovanoho hospodarsko-pytnoho vodopostachannia dioksydom khloru' (MR 2.2.4-147-2007): zatverdzheno nakazom Ministerstva okhorony zdorovia Ukrainy vid 30 lypnia 2007 r. № 430]*. Available at: https://zakon.rada.gov.ua/rada/v0430282-07#o12. [in Ukrainian].

MHU (Ministry of Health of Ukraine) (2010) 'State Sanitary Norms and Rules "Hygienic Requirements for Drinking Water Intended for Human Consumption" (SSanR&N 2.2.4-171-10): approved by the order of the Ministry of Health of Ukraine of 12 May 2010 No. 400' [Derzhavni sanitarni normy ta pravyla "Hihiienichni vymohy do vody pytnoi, pryznachenoi dlia spozhyvannia liudynoiu" (DSanPiN 2.2.4-171-10): zatverdzheno nakazom Ministerstva okhorony zdorovia Ukrainy vid 12 travnia 2010 r. № 400], *Official Herald of Ukraine [Ofitsiinyi visnyk Ukrainy]*, 51, art. 1717. Available at: https://zakon.rada.gov.ua/laws/z0452-10#n25. [in Ukrainian].

MHUSSR (Ministry of Health of the Union of Soviet Socialist Republics) (1980) *Assessment of the Impact of Harmful Chemical Compounds on the Skin and Substantiation of the Maximum Permissible Levels of Skin Contamination: methodological guidelines No. 2102-79 approved by the USSR Deputy Chief Public Health Officer of 11 November 1979 No. 5793-91' [Otsenka vozdeystviya vrednykh khimicheskikh soedineniy na kozhnye pokrovy i obosnovanie predel'no dopustimykh urovney zagryazneniy kozhi: metodicheskie ukazaniya № 2102-79, utverzhdennye Zamestitelem Glavnogo Gosudarstvennogo sanitarnogo vracha SSSR 11 noyabrya 1979 g.]*. Moscow: Ministry of Health of the USSR. [in Russian].

MHUSSR (Ministry of Health of the Union of Soviet Socialist Republics) (1991) *Maximum Permissible Concentrations (MPC) and Approximate Permissible Levels (TAC) of Harmful Substances in the Water of Water Bodies of Domestic Drinking and Cultural and Community Water Use: approved by the order of the Ministry of Health of USSR of 11 July 1991 No. 5793-91 [Predel'no dopustimye kontsentratsii (PDK) i orientirovochnye dopustimye urovni (ODU) vrednykh veshchestv v vode vodnykh ob"ektov khozyaystvenno-pit'evogo i kul'turno-bytovogo vodopol'zovaniya: utverzhdeno prikazom Ministerstva zdravookhraneniya SSSR vid 11 iyulya 1991 g. № 5793-91]*. Available at: https://zakon.rada.gov.ua/laws/v5793400-91. [in Russian].

Mokienko, A. V. (2021) *Chlorine Dioxide: Applications in Water Treatment Technologies [Dioksid khlora: primenenie v tekhnologiyakh vodopodgotovki]*. 2nd ed. Odessa: Feniks. ISBN 9789669286468. Available at: https://repo.odmu.edu.ua/xmlui/handle/123456789/10872. [in Russian].

Mokienko, A. V. and Petrenko, N. F. (2008) 'Hygienic estimation of virulicide action of chlorine dioxide and its relation to prior enteroviruses of drinking water and waster waters' [Hihiienichna otsinka virulitsydnoi dii dioksydu khloru po vidnoshenniu do priorytetnykh enterovirusiv pytnoi vody ta stichnykh vod], *Achievements of Biology and Medicine [Dosiahnennia biolohii ta medytsyny]*, 2, pp. 52–57. Available at: http://biomed.odmu.edu.ua/?p=5544&lang=en. [in Ukrainian].

Mokienko, A. V., Petrenko, N. F. and Gozhenko, A. I. (2006) 'Toxicologo-hygenical estimation of chlorine dioxide as facility of the disinfection of water (the review of the literature and result of the own studies)' [Toksikologo-gigienicheskaya otsenka dioksida khlora kak sredstva obezzarazhivaniya vody (obzor literatury i rezul'tatov sobstvennykh issledovaniy)], *Modern Problems of Toxicology [Sovremennye problemy toksikologii]*, 4, pp. 44–49. Available at: http://medved.kiev.ua/web_journals/arhiv/toxicology/2006/4_2006/str44.pdf. [in Russian].

Mokienko, A. V., Petrenko, N. F., Gozhenko, A. I. and Nasibulin, B. A. (2008) 'Chlorine dioxide and drinking water. Validation of harmfulness (report 3). Estimation of chlorates signification as chlorine dioxide derivatives' [Dioksid khlora i pit'evaya voda. K obosnovaniyu bezvrednosti (soobshchenie 3). Otsenka znachimosti khloratov kak proizvodnykh dioksida khlora], *Modern Problems of Toxicology [Sovremennye problemy toksikologii]*, 3, pp. 28–32. Available at: http://medved.kiev.ua/web_journals/arhiv/toxicology/2008/3_2008/str28.pdf. [in Russian].

Moore, G. S. and Calabrese, E. J. (1980) 'The effects of chlorine dioxide and sodium chlorite on erythrocytes of A/J and C57L/J mice', *Journal of Environmental Pathology and Toxicology*, 4(2–3), pp. 513–524. PMID: 7462915.

Ngwenya, N., Ncube, E. J. and Parsons, J. (2013) 'Recent advances in drinking water disinfection: successes and challenges', in Whitacre, D. M. (ed.) *Reviews of Environmental Contamination and Toxicology*. New York: Springer, pp. 111–170. doi: 10.1007/978-1-4614-4717-7_4.

VRU (Verkhovna Rada Ukrainy) (2006) 'Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' [Zakon Ukrainy № 3447-IV vid 21.02.2006 'Pro zakhyst tvaryn vid zhorstokoho povodzhennia'], *News of the Verkhovna Rada of Ukraine [Vidomosti Verkhovnoi Rady Ukrainy]*, 27, art. 230. Available at: https://zakon.rada.gov.ua/laws/3447-15. [in Ukrainian].

Yousef, M. I., Abuzreda, A. A. and Kamel, M. A. E.-N. (2019) 'Neurotoxicity and inflammation induced by individual and combined exposure to iron oxide nanoparticles and silver nanoparticles', *Journal of Taibah University for Science*, 13(1), pp. 570–578. doi: 10.1080/16583655.2019.1602351.