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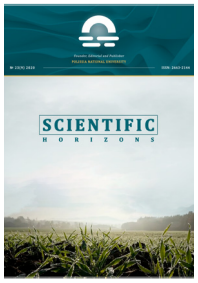

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Toxicity and Virucidal Activity of Chlorine Dioxide Disinfectant

Olha Chechet[1], Vyacheslav Kovalenko[2], Olha Haidei[1], Ivan Polupan[1*], Oleksii Rudoi[1]

[1]State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise
03151, 30 Donetska Str., Kyiv, Ukraine

[2]State Scientific Control Institute of Biotechnology and strains microorganisms
03151, 30 Donetska Str., Kyiv, Ukraine

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Abstract. The implementation of intensive technologies to produce livestock products requires the use of disinfectants at all stages. Analysis of the effectiveness of disinfectants begins with testing at the stage of creating or selecting substances, since different disinfectants have different activity against microorganisms, are toxic, immunosuppressive, and cause long-term effects on animals. This necessitates further development and research of preparations with optimal toxicity and virucidal action. The purpose of this study was to investigate the toxicity and virucidal effect of the new Diolide disinfectant, specifically on such samples as Aujeszky's disease virus, swine enzootic encephalomyelitis virus (Teschen disease) and rabies virus. The study was conducted according to national and international guidelines for the characterisation of virucidal properties of new disinfectants. The toxicity of Diolide disinfectant was investigated under protein load conditions in SPEV and BHK-21/C13 cell cultures. The virucidal activity of the Diolide disinfectant was determined under protein load conditions on models of shell viruses of Aujeszky's disease (Arsky strain) and rabies virus (CVS-11 strain) and using shell-free virus of enzootic encephalomyelitis of swine (Perechinsky-642 strain). The toxicity of Diolide disinfectant was determined for 0.16% (400 mg/l), 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l) and 0.004% (10 mg/l) concentrations of chlorine dioxide with an exposure time of 30 and 60 minutes. The virucidal effect of the preparation was determined for 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/L) and 0.004% (10 mg/l) concentrations relative to the working dilutions of viral suspensions: for the Aujeszky's disease virus – 5.3 CPE_{50}/ml – for swine enzootic encephalomyelitis virus – 5.5 CPE_{50}/ml, for rabies virus – 5.5 $TCID_{50}$/ml. The results of the study showed that Diolide disinfectant is non-toxic to transplanted SPEV and BHK-21/C13 cell cultures in 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l) and 0.004% (10 mg/l) concentrations of chlorine dioxide. The preparation is 100% virucidal against enveloped viruses such as Aujeszky's disease virus (Arsky strain) and rabies virus (CVS-11 strain) in concentrations from 0.1% (250 mg/l) to 0.004% (10 mg/l) when exposed for 30-60 minutes under protein load conditions. It has 100% virucidal activity against the shell-free enzootic encephalomyelitis virus of swine (Perechinsky-642 strain) in concentrations from 0.1% (250 mg/l) to 0.004% (10 mg/l) at an exposure of 60 minutes and in concentrations from 0.1% (250 mg/l) to 0.008% (20 mg/l) at an exposure of 30 minutes under protein load conditions. The coefficient of reduction of infectious activity of the enzootic encephalomyelitis virus of swine (Perechinsky-642 strain) established in experiments after 30 minutes of exposure with the Diolide disinfectant at a concentration of 0.004% (10 mg/l) under protein load conditions exceeded 4 lg (4.47 lg CPE_{50}/0.02 ml), which indicates a high virucidal activity of the Diolide disinfectant. Further research may be aimed at further increasing the virucidal activity of the disinfectant

Keywords: disinfection, Aujeszky's disease virus, swine enzootic encephalomyelitis virus, rabies virus, virus titre, cell culture

*Corresponding author

INTRODUCTION

Disinfection of pathogens is a necessary part of any modern commercial production, which is crucial both in obtaining high-quality products and in a complex of veterinary and sanitary measures for the prevention of the introduction of pathogenic microorganisms and the development of opportunistic microorganisms, the elimination of infectious diseases at veterinary supervision facilities (Paliy *et al.,* 2018; Wales *et al.,* 2021).

One of the components of the effectiveness and efficiency of any medical and preventive measures is the comprehensive use of disinfectants. The main purpose of disinfection is to create conditions for breaking the epizootic chain. To solve this problem, biocidal products are used, which are designed to destroy, neutralise, or inhibit the reproduction of bacteria, viruses, and fungi by chemical or biological means. The main factors affecting the effectiveness and efficiency of disinfectants are the spectrum of antimicrobial action (effectiveness against viruses and bacteria at different ambient temperatures and pH, no mutagenic effect on microorganisms), safety (no embryotoxic, teratogenic, carcinogenic, allergenic, and cumulative properties), corrosion activity, high permeability, environmental safety, etc. (Wales *et al.,* 2021; Chechet, 2022).

Currently, there are no ideal disinfectants, which encourages researchers to search for new compounds and study various combinations of known chemical compounds as disinfectants (Matsuzaki *et al.,* 2021; Cadnum *et al.,* 2021; Edmiston *et al.,* 2020).

It can be considered that one of the universal means of disinfection is sodium hypochloride and glutaraldehyde. However, they are corrosive, and their vapours adversely affect humans and animals (Rutala & Weber, 2019). That is why in developing disinfectants, the top priority is to create a best broad-spectrum disinfectant, which is non-toxic, non-irritating, non-corrosive, safe for humans, animals, and the environment (Rabenau *et al.,* 2020; Wales *et al.,* 2021).

In the last decade, chlorine dioxide (ClO_2) has been increasingly used as an effective biocide, both in soluble and gaseous forms. Chlorine dioxide is effective against bacteria, viruses, protozoa, mould and yeast fungi, mycobacteria, and bacterial spores. This chemical compound has powerful oxidising properties, thanks to which it destroys the protein structures of the cell wall or viral envelope. The chlorine dioxide molecule is oxidised with high efficiency, and therefore considerably affects viruses even at low concentrations (Miura & Shibata, 2010). Given that chlorine dioxide is a strong oxidising agent, disinfectants based on it are widely used for sterilisation, disinfection, and wastewater treatment. Chlorine dioxide is used for disinfection of drinking water and the environment, for disinfection of vegetables and fruits (strawberries, lettuce, cabbage, cucumbers) in gaseous form (Yu *et al.,* 2014). Furthermore, chlorine dioxide-based disinfectants can be used in medical institutions and public places where bioaerosols are significant (Du *et al.,* 2017).

Notably, chlorine dioxide does not belong to chlorine-type disinfectants, it does not enter chlorination reactions, does not produce carcinogens and is a safe compound for human body cells. At the same time, its biocidal effect on pathogens such as bacteria, viruses, fungi, and other pathogens is more powerful than that of chlorine due to active oxygen (Hsu *et al.,* 2015).

The most dangerous group of pathogenic microorganisms are viruses because the vast majority of nosological units of particularly dangerous infectious diseases of animals and humans are caused by viruses. The introduction of such pathogens into livestock or poultry farms causes mass deaths and the need for strict anti-epizootic measures, where forced disinfection is a part (Mummert & Weiss, 2017; Kindermann *et al.,* 2020; Wales & Davies, 2021).

It is known that animal viruses are classified into six groups and, depending on the structure, type of viral genome and size, differ in their resistance to disinfectants (Paquette *et al.,* 2020; Tarka & Nitsch-Osuch, 2021).

For a broad scientific assessment of new disinfectants for their virucidal activity, it is necessary to use various viruses, including shell-free ones, which are resistant to acidic pH values (International Committee..., 2022). Given that viruses must multiply in cell cultures, the virucidal activity should also be determined in culture systems, which will vary depending on the test virus used (Rabenau *et al.,* 2020).

Recommendations for the practical use of disinfectants that can be obtained from the results of tests in cell cultures are quite limited, since the conditions modelled in homogeneous suspensions are not factually found in practice. However, conclusions about the overall activity of the disinfectant under study, including under simulated protein load conditions, can be drawn from the results of tests in cell culture.

The purpose of this study was to investigate the toxicity and virucidal activity of the Diolide disinfectant preparation in cell culture on models of Aujeszky's disease virus, porcine enzootic encephalomyelitis virus (Teschen disease) and rabies virus.

MATERIALS AND METHODS

The toxicity and virucidal activity of Diolide disinfectant were studied according to national and international guidelines (Rabenau *et al.,* 2020; Kovalenko & Nedosekov, 2011).

The object of the study was the Diolide disinfectant, which is a two-component powdered product. Active substances of component 1: sodium chlorite – 42%, sodium chloride – 46%, functional additives – 12%. Active substances of component 2 are citric acid – 95%, adipic acid – 3%, functional additives – 2%.

To obtain the necessary working concentrations of the Diolide disinfectant, water of standard hardness was used, which was obtained according to the method (Rabenau *et al.,* 2020).

The toxicity study of the Diolide disinfectant was performed in re-grafted cell cultures of SPEV and BHK-21/C13 (ATCC CCL-10).

The virucidal effect of the Diolide disinfectant was determined under protein load conditions (adding 10% FBS to the DMEM medium) on models of Aujeszky's disease virus (Arsky strain); swine enzootic encephalomyelitis virus (Perechinsky-642 strain) and rabies virus (CVS-11 strain, ATCC VR 959). The infectious titre of virus-containing suspensions used in the studies was 7.31±0.20 lg CPE 50/ ml for the Aujeszky's disease virus (Arsky strain); swine enzootic encephalomyelitis virus (Perechinsky-642 strain) – 9.52±0.25 lg CPE_{50}/ ml; rabies virus (CVS-11 strain) with infectious activity – 7.53±0.11 lg TCID50/ml.

The following reagents were used for the research: DMEM (Dulbecco's Modified Eagle Medium), Sigma (GB); Fetal Bovine Serum (FBS), Gibco (Brazil); Dulbecco's Phosphate Buffered Saline (DPBS), Sigma (GB); Trypsin-EDTA (0.5%), no phenol red, Gibco (GB); Plasmocin, InvivoGen (France); Antibiotic-Antimycotic, Sigma (Israel); culture microplates (96 well), Sarstedt (Germany); Tissue Culture Flask (75 cm^2), Sarstedt (Germany); AR grade acetone, 80%, (Ukraine); FITC Anti-Rabies Globulin Kit, Fujirebio (USA).

Research was conducted using the following equipment: Esco CelCulture and Jouan 150 CO_2 incubators; microscope C.Zeizz – Aviovert 40CFL; fluorescent inverted microscope Zeiss AXIOVERT 25CA; Eppendorf and Biohit variable volume dispensers for 20-200 and 100-1,000 µl; biological safety cabinets Jokan MSC9; Holten SAFE-2010 and Hereus HS-18; Neubauer chamber for cell counting.

Study of the toxicity of the Diolide disinfectant. SPEV and BHK-21/C13 cell cultures were prepared in 96-well microplates (seeding concentration 1–1.2x105 cells per well). After 24 hours, the medium was removed from the 96-well microplates (provided that 80-90% of the monolayer is present) and corresponding dilutions of the disinfectant were made at 0.05 ml/well. Experimental dilutions of the Diolide disinfectant in the final concentration according to chlorine dioxide 0.16% (400 mg/l), 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l) and 0.004% (10 mg/l) were previously prepared from 20% disinfectant and 80% DMEM medium (with 10% FBS).

Contact of SPEV and BHK-21/C13 cells with corresponding disinfectant dilutions was carried out in an incubator at 37°C (for BHK-21/C13 cell culture, also 5% CO_2) for 30 and 60 minutes. For one concentration of Diolide disinfectant, 32 wells were used.

To control SPEV and BHK-21/C13 cells, a mixture of 80% DMEM medium (with 10% FBS) and 20% sterile water of standard hardness was used, which was introduced into 32 wells of a 96-well microplate of 0.05 ml/well for the same period of contact of cells with the disinfectant.

At the end of the contact period, disinfectant solutions were removed from 96-well microplates, DPBS was washed three times, and 0.20 ml of a supporting medium containing 10% FBS was introduced into the wells. Incubation of 96-well micro-panels with SPEV and BHK-21/C13 cell cultures was performed for 72 hours using daily monolayer microscopy of cells in wells to detect the cytopathic effect (CPE). The presence of a cytopathic effect was evaluated visually, and the presence of a monolayer of cells was expressed as a percentage.

Study of the virucidal effect of the Diolide disinfectant. The specific effect of the Diolide disinfectant was determined for concentrations of 0.16% (400 mg/l), 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l) and 0.004% (10 mg/l). Test objects: Aujeszky's disease virus (Arsky strain), swine enzootic encephalomyelitis virus (Perechinsky strain-642) and rabies virus (CVS-11 strain, ATCC VR 959). SPEV and BHK-21/C13 cell cultures were pre-inoculated in 96-well microplates (inoculation concentration for both cell lines was 1-1.2x105 cells per well). The selection of viruses was based on several factors, namely: 1) viruses that do not cause (rabies virus) and cause CPE (Aujeszky's disease virus and swine enzootic encephalomyelitis virus); 2) shell-containing viruses (rabies virus and Aujeszky's disease virus) and shell-free viruses (swine enzootic encephalomyelitis virus).

The necessary amount of virus was added to the determined experimental dilutions of the Diolide disinfectant to obtain its working dilution. In each experiment, the working dilution of viral suspensions was obtained based on virus activity titres: for Aujeszky's disease virus – 5.3 lg CPE_{50}/0.2 ml, for swine enzootic encephalomyelitis virus – 5.5 lg CPE_{50}/0.2 ml, and for rabies virus – 5.5 lg TCID50/0.2 ml.

Contact (exposure) of viral suspensions with corresponding dilutions of the disinfectant was carried out at room temperature (recommended for disinfection) for 30 and 60 minutes. 32 wells with SPEV and BHK-21/C13 cell cultures were used for each concentration of Diolide disinfectant. Later, corresponding dilutions of the disinfectant with viruses were made as follows:

- disinfectant dilution + working dilution of Aujeszky's disease virus (Arsky strain) on a daily monolayer of SPEV cell cultures;
- disinfectant dilution + working dilution of swine enzootic encephalomyelitis virus (Perechinsky-642 strain) on a daily monolayer of SPEV cell cultures;
- dilution of disinfectant + working dilution of rabies virus (CVS-11 strain) on a daily monolayer of BHK-21/C13 cell culture.

In all experiments, the mixture of the working dilution of the virus and experimental dilutions of the Diolide disinfectant in cell cultures was adsorbed within 60 minutes.

Then, to neutralise the action of the disinfectant, the dilution of the disinfectant with viruses was removed from 96-well micro-panels, DPBS was washed three times and 0.20 ml of the supporting medium with a content of 10% FBS was introduced into the wells.

96-well micropanels with SPEV cell culture, in which various concentrations of the Diolide disinfectant and working dilutions of the Aujeszky's disease virus (Arsky strain) and swine enzootic encephalomyelitis virus (Perechinskyi-642 strain) were added, were incubated for 72 hours with daily microscopy of the monolayer of cells in the wells for the detection of cytopathic effect. In each experiment, to control the titre of infectious activity of the applied viruses, titration of the working dose of viral suspensions was performed.

96-well micropanels with BHK-21/C13 cell culture, in which different concentrations of disinfectant and working dilution of rabies virus (CVS-11 strain, ATCC VR 959) were added, were incubated for 72 hours. After the end of the incubation period, the cells in the wells were fixed with 80% acetone and after drying they were stained with FITC Anti-Rabies Globulin Kit. After washing the DPBS cells, the presence of a specific glow of the rabies virus was evaluated under a luminescent microscope.

To control the cells, a mixture of 80% DMEM medium (with 10% FBS) and 20% sterile water of standard hardness was used, which was introduced into 32 wells of a 96-well microplate for the same period of adsorption of the virus mixture with Diolide disinfectant. Virus suspensions in working dilution were used as positive controls (virus of Aujeszky's disease – 5.3 lg CPE_{50}/0.2 ml, virus of enzootic encephalomyelitis of swine – 5.5 lg CPE_{50}/0.2 ml, rabies virus – 5.5 lg TCID50/0.2 ml).

The specific effect of Diolide disinfectant on experimental viruses was expressed in the absence of virus expression in cell cultures, namely: the absence of CPE in SPEV cell culture for Aujeszky's disease viruses and enzootic encephalomyelitis of swine, as well as in the absence of a specific glow of rabies virus in BHK-21/C13 cell culture with visual detection of characteristic changes in positive controls.

RESULTS AND DISCUSSION

The study of the Diolide disinfectant was performed in two stages. The first stage involved the detection of cytotoxic effects in transplanted SPEV and BHK-21/C13 cell culture lines, and the second – directly virucidal activity against three different viral strains.

Study of the toxicity of the Diolide disinfectant. The manifestation of the cytotoxic effect was determined for 0.16% (400 mg/l), 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l) and 0.004% (10 mg/l) concentrations of Diolide disinfectant in SPEV and BHK-21/C13 cell cultures at 30 and 60 minutes of exposure (Table 1).

Table 1. *Cytotoxic effect of various concentrations of Diolide disinfectant in SPEV and BHK-21/C13 cell cultures, n=3*

Final concentration of Diolide disinfectant for chlorine dioxide	Exposition	Presence of a monolayer of cells in 96-well micro-panels, %					
		SPEV cell culture			cell culture BHK-21/C13		
		24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.16% (400 mg/l)	30 min	10	10	20	0	0	0
0.1% (250 mg/l)		80	80	90	80	80	80
0.06% (150 mg/l)		80	100	100	80	80	90
0.02% (50 mg/l)		90	100	100	90	90	90
0.008% (20 mg/l)		90	100	100	80	100	100
0.004% (10 mg/l)		90	100	100	90	100	100
control		90	100	100	80	100	100
0.16% (400 mg/l)	60 min	0	0	0	0	0	0
0.1% (250 mg/l)		80	90	90	70	80	80
0.06% (150 mg/l)		80	100	100	80	80	100
0.02% (50 mg/l)		90	100	100	80	90	100
0.008% (20 mg/l)		90	100	100	80	90	100
0.004% (10 mg/l)		90	100	100	90	100	100
control		90	100	100	80	100	100

The digital material presented in Table 1 shows that the use of Diolide disinfectant in different concentrations caused different manifestations of cytotoxic effects on SPEV and BHK-21/C13 cells.

A substantial cytotoxic effect (90-100% cell death in the central part of the wells for 24 hours of cultivation, changes in the morphostructure and lack of active proliferation of living cell residues) of the Diolide disinfectant was established in SPEV and BHK-21/C13 cell cultures at a concentration of 0.16% (400 mg/l) at an exposure of 30 and 60 minutes. In a separate experiment, after exposure as an inactivator of the action of the active components of the Diolide disinfectant, a 50% FBS solution was added to SPEV cells for 30 minutes, but it was not possible to get rid of the cytotoxic effect (20-40% of cells remained alive and no proliferation was observed for 72 hours cultivation).

The use of Diolide disinfectant at a concentration of 0.1% (250 mg/l) did not cause cell death or other cytotoxic manifestations that can be identified visually. However, during the entire follow-up period (72 hours), cell proliferation was insignificant compared to the control cells SPEV and BHK-21/C13 at 30 and 60 minutes of exposure.

In SPEV and BHK-21/C13 cells treated with Diolide disinfectant at concentrations of 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l) and 0.004% (10 mg/l) at exposure for both 30 and 60 minutes, no cell death was detected during the entire follow-up period (72 hours). Cell proliferation and visual filling of the monolayer were comparable to similar cells in the control group.

Study of the virucidal effect of Diolide disinfectant. Considering the results obtained to investigate the cytotoxic manifestation of various working dilutions of Diolide disinfectant in SPEV and BHK-21/C13 cell cultures, concentrations of 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l), and 0.004% (10 mg/l) were selected to study its direct virucidal effect.

To investigate the virucidal activity of the Diolide disinfectant, DNA- and RNA-containing viruses were used, namely Aujeszky's disease virus (DNA-containing, family Herpesviridae, Arsky strain); swine enzootic encephalomyelitis virus (RNA-containing, family Teschovirus, Perechinsky-642 strain); rabies virus (RNA-containing, family *Rhabdoviridae*, CVS-11 strain).

The study of the virucidal effect of the Diolide disinfectant on the model of the DNA-containing virus of Aujeszky's disease (Arsky strain) in the SPEV transplantable culture system showed that 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l), and 0.004% (10 mg/l) concentrations of the Diolide disinfectant both during 30 minutes of exposure and during 60 minutes of exposure, provided absolute (100%) virucidal effect (Table 2).

***Table 2**. Virucidal effect of Diolide disinfectant on Aujeszky's disease virus (Arsky strain) in SPEV cell culture, n=3*

Final concentration of Diolide disinfectant for chlorine dioxide	Exposure, min	Presence of a virus	Cell control	Virus control (CPE presence)
0.1% (250 mg/l)	30	—	#	+
	60	—	#	+
0.06% (150 mg/l)	30	—	#	+
	60	—	#	+
0.02% (50 mg/l)	30	—	#	+
	60	—	#	+
0.008% (20 mg/l)	30	—	#	+
	60	—	#	+
0.004% (10 mg/l)	30	—	#	+
	60	—	#	+

***Note**: "—" is the absence of CPE in cell culture; "+" is the presence of CPE in cell culture; "#" is the presence of 100% monolayer at 72 hours of cultivation in all control wells of a 96-well plate*

In all wells with SPEV cells, in which mixtures of different concentrations of Diolide disinfectant and working dilution (5.3 lg CPE_{50}/0.2 ml) of the Aujeszky's disease virus (Arsky strain), no CPE was detected, which would indicate virus reproduction (Figure 1a – concentration of 0.06% (150 mg/l) at 60 minutes of exposure; Figure 1b – concentration of 0.004% (10 mg/l) at 60 minutes of exposure).

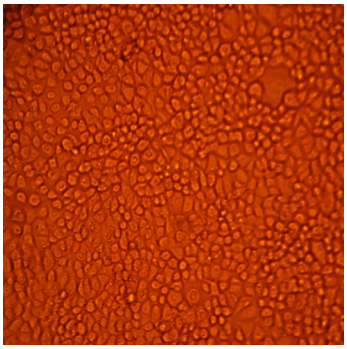

***Figure 1a**. SPEV cell culture Aujeszky's disease virus (Arsky strain) + Diolide at a concentration of 0.06% (150 mg/l) at an exposure of 60 minutes, 24 hours of cultivation*

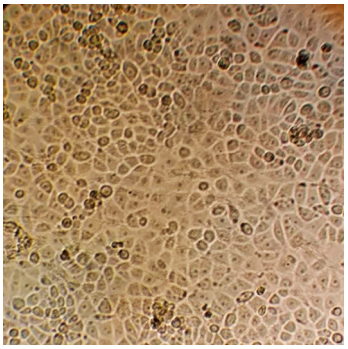

***Figure 1b**. SPEV cell culture Aujeszky's disease virus (Arsky strain) + Diolide at a concentration of 0.004% (10 mg/l) at an exposure of 60 minutes, 72 hours of cultivation*

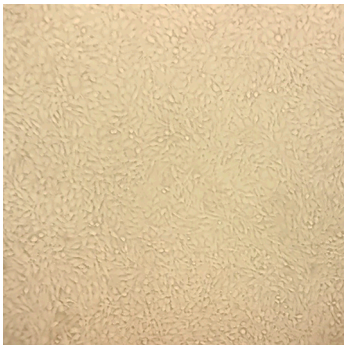

***Figure 1c**. SPEV cell culture Cell control, 48 hours of cultivation*

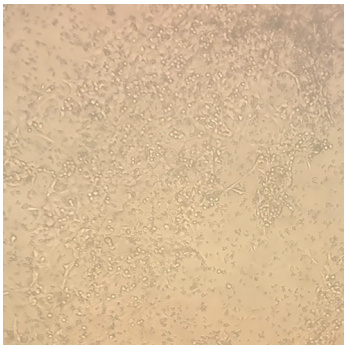

***Figure 1d**. SPEV cell culture Control of Aujeszky's disease virus (Arsky strain), CPE in cell culture for 24 hours of cultivation*

SPEV cells in the control wells remained intact for the entire follow-up period, forming a 100% monolayer after 72 hours of incubation (Figure 1c). In wells with virus control (Figure 1d), 100% CPE was noted as early as 24 h after infection. The infectious titre of the working dose of the Aujeszky's disease virus (Arsky strain) used in the experiments was 5.22±0.15 lg CPE_{50}/0.02 ml.

Experiments on the characterisation of the virucidal effect of the Diolide disinfectant on a model of the RNA-containing virus of enzootic encephalomyelitis of swine (Perechinsky-642 strain) in the re-grafted culture system SPEV showed slightly different results (Table 3).

***Table 3**. Virucidal effect of Diolide disinfectant on swine enzootic encephalomyelitis virus (Perechinsky-642 strain) in SPEV cell culture, n=4*

Final concentration of Diolide disinfectant for chlorine dioxide	Exposure, min	Presence of a virus	Cell control	Virus control (CPE presence)
0.1% (250 mg/l)	30	—	#	+
	60	—	#	+
0.06% (150 mg/l)	30	—	#	+
	60	—	#	+
0.02% (50 mg/l)	30	—	#	+
	60	—	#	+
0.008% (20 mg/l)	30	—	#	+
	60	—	#	+
0.004% (10 mg/l)	30	+	#	+
	60	—	#	+

***Note**: "—" is the absence of CPE in cell culture; "+" is the presence of CPE in cell culture; "#" is the presence of 100% monolayer at 72 hours of cultivation in all control wells of a 96-well plate*

It was found that 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l), and 0.004% (10 mg/l) concentrations of Diolide disinfectant during 60 minutes of exposure provided a 100% virucidal effect relative to the working dose of enzootic encephalomyelitis virus used in SPEV cells (Figure 2a – concentration of 0.1% (250 mg/l) at an exposure of 60 minutes; Figure 2b – concentration of 0.004% (10 mg/l) at an exposure of 60 minutes).

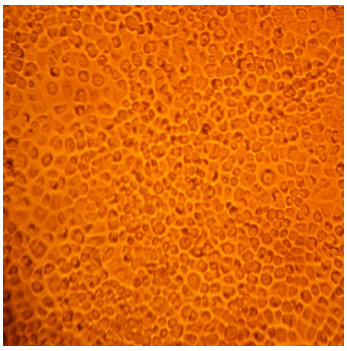

***Figure 2a**. SPEV cell culture Swine enzootic encephalomyelitis virus (Perechinsky-642 strain) + Diolide preparation in a concentration of 0.1% (250 mg/l) at an exposure of 60 minutes, 48 hours of cultivation*

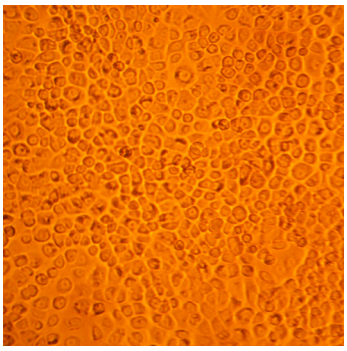

***Figure 2b**. SPEV cell culture Swine enzootic encephalomyelitis virus (Perechinsky-642 strain) + Diolide preparation in a concentration of 0.004% (10 mg/l) at an exposure of 60 minutes, 24 hours of cultivation*

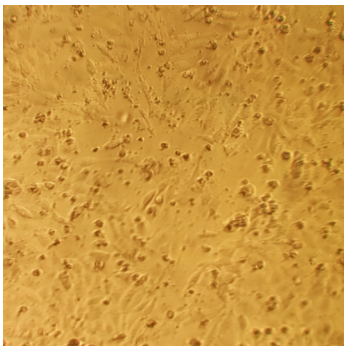

***Figure 2c**. SPEV cell culture Swine enzootic encephalomyelitis virus (Perechinsky-642 strain) + Diolide preparation in a concentration of 0.004% (10 mg/l) at an exposure of 30 minutes, 48 hours of cultivation*

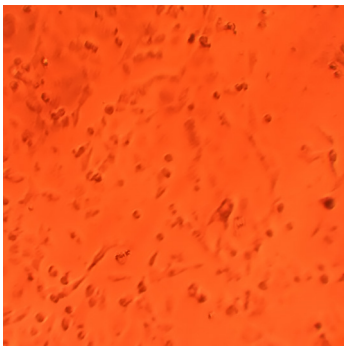

***Figure 2d**. SPEV cell culture Control of swine enzootic encephalomyelitis virus (Perechinsky-642 strain), CPE in cell culture for 24 hours of cultivation*

However, after 30 minutes of exposure and a concentration of 0.004% (10 mg/l), residual infectious activity of the swine enzootic encephalomyelitis virus was detected (Figure 2c), which was manifested as CPE after 48 hours of cell culture. Determination of the infectious activity of swine enzootic encephalomyelitis virus (Perechinsky-642) after 30 minutes of exposure with Diolide disinfectant at a concentration of 0.004% (10 mg/l) showed a titre of 1.25±0.19 lg CPE_{50}/0.02 ml.

The virus control (Fig. 2d) showed 100% CPE as early as 24 hours after infection, and the SPEV cells in the control wells remained intact for the entire follow-up period.

Determination of the infectious titre of the working dose of swine enzootic encephalomyelitis virus (Perechinsky-642), which was used in experiments, showed a titre of 5.72±0.12 lg CPE_{50}/0.02 ml. That is, the decrease in infectious activity (reduction coefficient – RF) of the pig enzootic encephalomyelitis virus (Perechinsky-642 strain) after 30 minutes of exposure with Diolide disinfectant at a concentration of 0.004% (10 mg/l) was 4.47 lg CPE_{50}/0.02 ml.

The disinfectant is considered to have caused a sufficient reduction in the titre if the average RF is at least 4 lg. That is, a decrease in the infectious activity of the enzootic encephalomyelitis virus of pigs when using the Diolide disinfectant at a concentration of 0.004% (10 mg/l) for more than 4 lg is an acceptable virucidal effect (especially for shell-free viruses and in studies under protein load conditions).

In another series of experiments, the virucidal activity of the Diolide disinfectant was investigated on a model of a shell RNA-containing rabies virus (CVS-11 strain) in a transplanted BHK-21/C13 culture system. Studies have shown that 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l), and 0.004% (10 mg/l) concentrations of Diolide provided 100% virucidal action during 30 and 60 minutes of exposure (Table 4).

***Table 4**. Virucidal effect of Diolide disinfectant on rabies virus (CVS-11 strain) in BHK-21/C13 cell culture, n=3*

Final concentration of Diolide disinfectant for chlorine dioxide	Exposure, min	Presence of a virus	Cell control	Virus control (presence of TCID)
0.1% (250 mg/l)	30	—	#	+
	60	—	#	+
0.06% (150 mg/l)	30	—	#	+
	60	—	#	+
0.02% (50 mg/l)	30	—	#	+
	60	—	#	+
0.008% (20 mg/l)	30	—	#	+
	60	—	#	+
0.004% (10 mg/l)	30	—	#	+
	60	—	#	+

***Note**: "—" is the absence of specific fluorescence in cell culture; "+" is the presence of a specific glow in the cell culture; "#" is the presence of 100% monolayer at 72 hours of cultivation in all control wells of a 96-well plate*

In all wells with BHK-21/C13 cells, in which mixtures of different concentrations of Diolide disinfectant and working dilution (5.5 lg $TCID_{50}$/0.2 ml) of rabies virus (CVS-11 strain), no specific glow was detected at 72 hours of incubation, which indicated no reproduction of the virus. In wells with virus control after 72 hours, a specific glow of the rabies virus was detected (Figure 3a – transplanted culture of VNA-21/C13 cells for 48 hours of incubation after applying the rabies virus and Diolide at a concentration of 0.1% (250 mg/l) at an exposure of 60 minutes; Figure 1b – control of the virus in the VNA-21/C13 culture for 72 hours of incubation). Titration revealed that the infectious activity of the working dose of rabies virus (CVS-11 strain) was 5.82±0.07 lg $TCID_{50}$/0.2 ml.

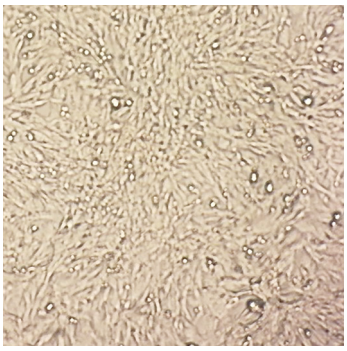

***Figure 3a**. Culture of BHK-21/C13 cells. Rabies virus (CVS-11 strain) + Diolide preparation at a concentration of 0.1% (250 mg/l) at an exposure of 60 minutes, 48 hours of cultivation*

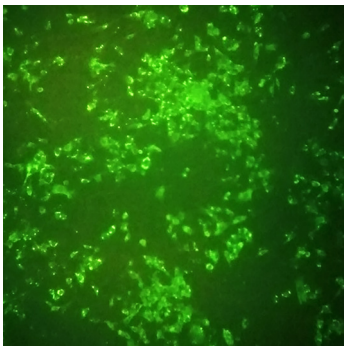

***Figure 3b**. Luminescence microscopy of rabies virus (CVS-11 strain) in BHK-21/C13 cell culture after 72 hours of incubation*

According to J. Ma et al. (2017), the antiviral activity of chlorine dioxide is detected after 2 minutes for H1N1 virus and influenza Type B virus. T. Sanekata et al. (2010) evaluated the antiviral activity of chlorine dioxide solution against feline calicivirus, influenza virus, measles virus, canine distemper virus, human herpes virus, human adenovirus, canine adenovirus, and canine parvovirus. Chlorine dioxide in low concentrations was found to exhibit strong antiviral activity, inactivating 99.9% of viruses at 15-second exposure (Yu *et al.,* 2010; Ogata & Shibata, 2008).

In this study, the high virucidal activity of various concentrations of the Diolide disinfectant, the main active substance of which is chlorine dioxide, was similarly established. The studies used other animal viruses as models, namely Aujeszky's disease virus, swine enzootic encephalomyelitis virus (Teschen disease) and rabies virus. A prerequisite for the use of these viruses was the toxicity study of the Diolide disinfectant, which was tested on two re-grafted cultural systems: SPEV and BHK-21/C13.

Thus, the results of laboratory tests presented in this paper indicate the safety and high efficiency of 0.1-0.004% concentration of the Diolide disinfectant based on chlorine dioxide, which opens up prospects for its wide application in production in the implementation of preventive and forced disinfection treatment of surfaces and liquids.

CONCLUSIONS

1. Diolide disinfectant is non-toxic to re-grafted SPEV and BHK-21/C13 cell cultures in 0.1% (250 mg/l), 0.06% (150 mg/l), 0.02% (50 mg/l), 0.008% (20 mg/l),

and 0.004% (10 mg/l) concentrations of chlorine dioxide.

2. Diolide disinfectant has 100% virucidal activity against envelope viruses, such as Aujeszky's disease virus (Arsky strain) and rabies virus (CVS-11 strain) in concentrations from 0.1% (250 mg/l) to 0.004% (10 mg/l) when exposed for 30-60 minutes under protein load conditions.

3. Diolide disinfectant has 100% virucidal activity against the shell-free virus of enzootic encephalomyelitis of swine (Perechinsky-642) in concentrations from 0.1% (250 mg/l) to 0.004% (10 mg/l) at an exposure of 60 minutes and in concentrations from 0.1% (250 mg/l) to 0.008% (20 mg/l) at an exposure of 30 minutes under conditions of protein load.

4. The coefficient of reduction of infectious activity of the enzootic encephalomyelitis virus of swine (Perechinsky-642 strain) after 30 minutes of exposure with Diolide disinfectant at a concentration of 0.004% (10 mg/l) under protein load conditions exceeded 4 lg (4.47 lg CPE_{50}/0.02 ml).

5. The results of laboratory tests indicate a high virucidal activity of the Diolide disinfectant and give grounds for its widespread introduction into production.

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Токсичність та віруліцидна активність дезінфекційного засобу на основі діоксиду хлору

Ольга Миколаївна Чечет[1], Вячеслав Леонідович Коваленко[2], Ольга Сергіївна Гайдей[1], Іван Миколайович Полупан[1], Олексій Васильович Рудой[1]

[1]Державний науково-дослідний інститут з лабораторної діагностики та ветеринарно-санітарної експертизи
03151, вул. Донецька, 30, м. Київ, Україна

[2]Державний науково-контрольний інститут біотехнології і штамів мікроорганізмів
03151, вул. Донецька, 30, м. Київ, Україна

Анотація. Впровадження інтенсивних технологій виробництва продукції тваринництва вимагає використання дезінфікуючих засобів на всіх етапах. Аналіз ефективності дезінфектантів починається з випробування на етапі створення або відбору речовин, оскільки різні дезінфікуючі засоби мають різну активність проти мікроорганізмів, є токсичними, імуносупресивними та спричиняють довготривалий вплив на тварин. Це зумовлює необхідність подальшої розробки та досліджень засобів із оптимальними показниками токсичності та віруліцидної дії. Метою статті є дослідити токсичність та віруліцидну дію нового дезінфікуючого засобу «Діолайд», зокрема на таких зразках як вірус хвороби Ауєскі, вірус ензоотичного енцефаломіеліту свиней (хвороби Тешена) та вірус сказу. Дослідження проводили відповідно до національних і міжнародних керівництв щодо характеристики віруліцидних властивостей нових дезінфікуючих засобів. Вивчення токсичності дезінфікуючого засобу «Діолайд» проводили за умов білкового навантаження в культурах клітин SPEV та BHK-21/C13. Визначення віруліцидної активності дезінфікуючого засобу «Діолайд» проводили за умов білкового навантаження на моделях оболонкових вірусів хвороби Ауєскі (штам «Арський») і вірусу сказу (штам CVS-11) та використовуючи безоболонковий вірус ензоотичного енцефаломіеліту свиней (штам «Перечинський-642»). Токсичність дезінфікуючого засобу «Діолайд» визначали для 0,16 % (400 мг/л), 0,1 % (250 мг/л), 0,06 % (150 мг/л), 0,02 % (50 мг/л), 0,008 % (20 мг/л) та 0,004 % (10 мг/л) концентрацій за двоокисом хлору з тривалістю експозиції 30 та 60 хвилин. Віруліцидну дію засобу визначали для 0,1 % (250 мг/л), 0,06% (150 мг/л), 0,02% (50 мг/л), 0,008% (20 мг/л) та 0,004 % (10 мг/л) концентрацій відносно робочих розведень вірусних суспензій: для вірусу хвороби Ауєскі – 5,3 CPE_{50}/ml, для вірусу ензоотичного енцефаломієліту свиней – 5,5 CPE_{50}/ml, для вірусу сказу – 5,5 $TCID_{50}$/ml. Результати дослідження показали, що дезінфікуючий засіб «Діолайд» не токсичний для перещеплюваних культур клітин SPEV та BHK-21/C13 в 0,1 % (250 мг/л), 0,06 % (150 мг/л), 0,02 % (50 мг/л), 0,008 % (20 мг/л) та 0,004 % (10 мг/л) концентраціях за діоксидом хлору. Препарат 100% віруліцидно діє щодо оболонкових вірусів, таких як вірус хвороби Ауєскі (штам «Арський») та вірус сказу (штам CVS-11) в концентраціях від 0,1 % (250 мг/л) до 0,004 % (10 мг/л) за експозиції 30–60 хвилин в умовах білкового навантаження. Має 100 % віруліцидну активність щодо безоболонкового вірусу ензоотичного енцефаломіеліту свиней (штам «Перечинський-642») в концентраціях від 0,1 % (250 мг/л) до 0,004 % (10 мг/л) за експозиції 60 хвилин та в концентраціях від 0,1 % (250 мг/л) до 0,008 % (20 мг/л) за експозиції 30 хвилин в умовах білкового навантаження. Встановлений в дослідах коефіцієнт зниження інфекційної активності вірусу ензоотичного енцефаломіеліту свиней (штам «Перечинський-642») після 30 хвилин експозиції з дезінфікуючим засобом «Діолайд» в концентрації 0,004 % (10 мг/л) в умовах білкового навантаження становив більше 4 lg (4,47 lg CPE_{50}/0,02 ml), що свідчить про високу віруліцидну активність дезінфекційного засобу «Діолайд». Подальші дослідження можуть бути спрямовані на подальше підвищення віруліцидної активності дезінфікуючого засобу

Ключові слова: дезінфекція, вірус хвороби Ауєскі, вірус ензоотичного енцефаломіеліту свиней, вірус сказу, титр вірусу, культура клітин