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EVALUATION OF THE EFFECTIVENESS OF DISINFECTANTS FOR DRINKING WATER DECONTAMINATION UNDER EXTREME CONDITIONS

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ABSTRACT

Objective. To test methods for determining the specific activity of disinfectants used for drinking water decontamination under extreme conditions and to develop a phased evaluation algorithm aligned with Ukrainian and European requirements.

Methods. Laboratory studies were conducted using quantitative suspension tests and methods simulating practical water-treatment conditions. Six disinfectants with different chemical compositions were evaluated: Eco Chlor, Novochlor-extra, Blanidas-C Hypochlorite, Sanika-Anolyte, Bioluft CL, and SterilOx. Reference strains of Gram-positive and Gram-negative bacteria, bacterial spores, yeasts, and moulds were used to assess bactericidal, sporicidal, yeasticidal, and fungicidal activity. Tests were performed under clean conditions at 20.0 ± 1.0 °C using the concentrations and contact times specified in the study protocols.

Results. Eco Chlor was effective against bacteria and fungi at 0.5% for 15 minutes. Novochlor-extra showed bactericidal and yeasticidal activity at 0.5% for 30 minutes, fungicidal activity at 0.5% for 60 minutes, and sporicidal activity at 2.0% for 60 minutes. Blanidas-C Hypochlorite was effective against bacteria and fungi at active-chlorine concentrations of 0.03% and 0.2% for 15 minutes. Sanika-Anolyte, Bioluft CL, and SterilOx were effective in the tested practical-use regimes; both SterilOx regimes achieved complete decontamination from bacteria and yeast-like fungi. The findings supported a phased algorithm for evaluating disinfectants intended for emergency drinking-water treatment.

Conclusions. The investigated disinfectants demonstrated efficacy in European-standard suspension tests and in conditions simulating practical use. A phased assessment combining basic activity testing, quantitative suspension testing, and practical-use evaluation can support the selection of agents for drinking water decontamination when centralized water supply is unavailable.

Keywords: disinfectants, microorganisms, specific activity, drinking water

Introduction

Ukraine lacks approved approaches for the hygienic evaluation of reagents used in drinking water treatment, including disinfectants (or biocides), as stipulated by European legislation—Directive (EU) 2020/2184 on water intended for human consumption and Regulation (EU) No 528/2012. This Regulation establishes rules for: creating a Union-level list of active substances that can be used in biocidal products; and the mutual recognition of authorizations within the Union.

The use of disinfectants must be carried out following comprehensive studies by the manufacturer or supplier of disinfectants based in specialized accredited laboratories and drinking water supply enterprises. These must follow the order and criteria established by legislative requirements,

and in EU countries, take into account the requirements of Directive (EU) 2020/2184 on the quality of water intended for human consumption [1] and Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products [2].

With the beginning of the full-scale invasion by the Russian Federation, population access to centralized drinking water supply and centralized wastewater disposal significantly deteriorated in various regions.

The aim is to test methods for determining the specific activity of disinfectants for drinking water decontamination.

Materials and methods

Drinking water must meet the requirements of DSanPiN 2.2.4-171-10 "Hygienic requirements for drinking water

intended for human consumption" [3]. To ensure the epidemic safety of drinking water under conditions of its contamination in extreme situations (military actions, natural and man-made disasters, epidemics, etc.), studies were conducted in the Laboratory of Sanitary Microbiology and Disinfectology using European methods for determining the effectiveness of disinfectants and the requirements of the guidelines "Test methods for disinfectants to assess their safety and effectiveness", 1998 [4]. The work was carried out on an approved scientific topic, disinfectants are registered in Ukraine.

Test strains of microorganisms were used to determine bactericidal, sporicidal, and fungicidal activity: *Staphylococcus aureus* ATCC 6538 (*S. aureus*), *Pseudomonas aeruginosa* ATCC 15442 (*P. aeruginosa*), *Escherichia coli* ATCC 10536 (*E. coli*), *Enterococcus hirae* ATCC 10541 (*E. hirae*), *Salmonella enterica* subsp. *enterica* sv Abony NCTC 6017 (*S. enterica*), *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231 (*C. albicans*), *Aspergillus brasiliensis* ATCC 16404 (*A. brasiliensis*).

The disinfectants studied according to European methods [5–9] included:

"Eco Chlor" – active substance (as): sodium hypochlorite (4% active chlorine).

"Novochlor-extra" – as: sodium hypochlorite, 7.0–9.0% active chlorine.

"Blanidas-C Hypochlorite" – as: sodium hypochlorite – 10.0% (active chlorine).

Disinfectants studied according to [1]:

"Sanika-Anolyte" – as: hypochlorous acid, peroxide compounds <0.01%.

"SterilOx" – as: active chlorine 0.04%.

"Bioluft CL" – as: sodium chlorite – 42%, sodium chloride – 47%, citric acid – 95%, adipic acid – 3%.

A solution with the following composition was used as a neutralizer: sodium thiosulfate 20.0 g with the addition of polysorbate-80 30.0 cm³; lecithin 3.0 g; water up to 1000 cm³. The test temperature was (20.0 ± 1.0) °C, interfering substance: 0.03% BSA (clean conditions). Working solutions of the agents were used for research according to the

manufacturer's documentation, which belonged to toxicity class 4 based on toxicological characteristics.

Results and discussion

Studies conducted using European methods to determine the effectiveness of disinfectants [5–9] in working solutions and contact times showed that:

"Eco Chlor" (0.5% for 15 min and 2.0% for 60 min) and **"Blanidas-C Hypochlorite"** (0.001% for 30 min, 0.5% for 60 min, and 2.0% for 60 min) are effective for water decontamination against bacteria and fungi.

"Novochlor-extra" (in regimes of 0.5% for 30 min; 0.5% for 60 min and 2.0% for 60 min) is effective against bacteria, fungi, and spores.

The research results are presented in Tables 1–7.

Experimental studies using methods [5–9] demonstrate that "Eco Chlor" at a solution concentration of 0.5% (product-based) and an exposure time of 15 min is an effective disinfectant for water decontamination against bacteria and fungi.

The disinfecting effect of "Novochlor-extra" was studied against test strains of bacteria and yeast-like fungi in a suspension test [5–9] under "clean" conditions at 20°C: at a concentration of 0.5% and an exposure of 30 min; against *A. brasiliensis* at a concentration of 0.5% for 60 min; and sporicidal activity on *B. subtilis* spores at a concentration of 2.0% for 60 min. The results are presented in Table 2.

In the suspension test, a 0.5% solution of "Novochlor-extra" (product-based) exhibited bactericidal and yeasticidal activity against Gram-negative and Gram-positive bacteria and yeast-like fungi under "clean" conditions at 20°C with an exposure of 30 minutes. Fungicidal activity was confirmed for the *A. brasiliensis* test strain under "clean" conditions at 20°C with an exposure of 60 min for the 0.5% disinfectant solution. It was established that a 2.0% disinfectant solution with an exposure of 60 min under "clean" conditions exhibited sporicidal activity.

To determine the specific activity of the disinfectant "Blanidas-C Hypochlorite" in a suspension test, microorganism

Table 1. Microbiological research results to determine the effectiveness of the disinfectant "Eco Chlor" 0.5% for 15 min and 2.0% for 60 min for water decontamination*

Test strain	Disinfection regime	Lg R	lg Na	lg N0
<i>S. aureus</i>	0.5% (product-based), 15 min**	>5.24	<2.15	7.39
<i>P. aeruginosa</i>	0.5% (product-based), 15 min**	>5.13	<2.15	7.28
<i>E. hirae</i>	0.5% (product-based), 15 min**	>5.20	<2.15	7.35
<i>S. enterica</i>	0.5% (product-based), 15 min**	>5.23	<2.15	7.38
<i>C. albicans</i>	2.0% (product-based), 60 min**	>4.23	<2.15	6.38
<i>A. brasiliensis</i>	2.0% (product-based), 60 min**	>4.19	<2.15	6.34

*Note: Determination was performed in a suspension test under clean conditions.

**Controls: *A, B, C* met the requirements.

Table 2. Microbiological research results to determine the effectiveness of the disinfectant "Novochlor-extra" 0.5% for 30 min, 0.5% for 60 min, and 2.0% for 60 min for water decontamination*

Test strain	Disinfection regime	Lg R	lg Na	lg N0
S. aureus	0.5% (product-based), 30 min**	>5.11	<2.15	7.26
P. aeruginosa	0.5% (product-based), 30 min**	>5.12	<2.15	7.27
E. hirae	0.5% (product-based), 30 min*	>5.20	<2.15	7.35
C. albicans	0.5% (product-based), 30 min**	>4.13	<2.15	6.28
A. brasiliensis	0.5% (product-based), 60 min**	>4.24	<2.15	6.39
B. subtilis (spores)	2.0% (product-based), 60 min	>5.13	<2.15	7.28

*Note: Determination was performed in a suspension test under clean conditions.

**Controls: A, B, C met the requirements.

Table 3. Microbiological research results to determine the effectiveness of the disinfectant "Blanidas-C Hypochlorite" 0.001% for 30 min, 0.5% for 60 min, and 2.0% for 60 min for water decontamination*

Test strain	Disinfection regime	Lg R	lg Na	lg N0
S. aureus	0.03% and 0.2% (active chlorine), 15 min**	>5.09	<2.15	7.24
P. aeruginosa	0.03% and 0.2% (active chlorine), 15 min**	>5.07	<2.15	7.22
E. hirae	0.03% and 0.2% (active chlorine), 15 min**	>5.07	<2.15	7.22
C. albicans	0.03% and 0.2% (active chlorine), 15 min**	>4.09	<2.15	6.24
A. brasiliensis	0.03% and 0.2% (active chlorine), 15 min**	>4.07	<2.15	6.22

*Note: Determination was performed in a suspension test under clean conditions.

**Controls: A, B, C met the requirements.

Table 4. Microbiological research results to determine the effectiveness of "Sanika-Anolyte" for water decontamination

Test strain	Water with "Sanika-Anolyte" added, 50 ml/l	Water with "Sanika-Anolyte" added, 100 ml/l	Control, CFU/100 cm ³ (water without agent)	Culture control, CFU/cm ³
E. coli	No growth	No growth	Confluent growth	$\backslash(3.9 \times 10^8\backslash)$
S. aureus	No growth	No growth	Confluent growth	$\backslash(4.2 \times 10^8\backslash)$
B. subtilis	No growth	No growth	Confluent growth	$\backslash(4.5 \times 10^8\backslash)$
C. albicans	No growth	No growth	Confluent growth	$\backslash(3.1 \times 10^8\backslash)$

Table 5. Microbiological research results to determine the effectiveness of the drinking water disinfectant "Bioluft CL"

Test strain	Water with "Bioluft CL" added, CFU/200 cm ³	Control, CFU/100 cm ³ (water without agent)	Initial culture control, CFU/cm ³
E. coli	Absence of growth	Confluent growth	$\backslash(3.2 \times 10^8\backslash)$
P. aeruginosa	Absence of growth	Confluent growth	$\backslash(4.6 \times 10^8\backslash)$
B. subtilis	Absence of growth	Confluent growth	$\backslash(4.5 \times 10^8\backslash)$

Table 6. Results of determining the drinking water disinfection effect of the disinfectant "SterilOx" – Regime 1

Test cultures of microorganisms	Decontamination regime: 10 ml of disinfectant per 100 ml of contaminated water – exposure 10 min	Culture control, CFU/l
<i>E. coli</i>	absence of growth	$\backslash(2.2 \times 10^6\backslash)$
<i>E. hirae</i>	absence of growth	$\backslash(5.3 \times 10^6\backslash)$
<i>S. aureus</i>	absence of growth	$\backslash(8.7 \times 10^6\backslash)$
<i>P. aeruginosa</i>	absence of growth	$\backslash(6.9 \times 10^6\backslash)$
<i>C. albicans</i>	absence of growth	$\backslash(8.3 \times 10^5\backslash)$

Table 7. Results of determining the drinking water disinfection effect of the disinfectant "SterilOx" – Regime 2

Test cultures of microorganisms	Decontamination regime: 5 ml of disinfectant per 100 ml of contaminated water – exposure 15 min	Culture control, CFU/l
<i>E. coli</i>	absence of growth	$\backslash(2.2 \times 10^6\backslash)$
<i>E. hirae</i>	absence of growth	$\backslash(5.3 \times 10^6\backslash)$
<i>S. aureus</i>	absence of growth	$\backslash(8.7 \times 10^6\backslash)$
<i>P. aeruginosa</i>	absence of growth	$\backslash(6.9 \times 10^6\backslash)$
<i>C. albicans</i>	absence of growth	$\backslash(8.3 \times 10^5\backslash)$

test strains were used under "clean" conditions at 20°C with an exposure of 15 min at active chlorine concentrations of 0.001%, 0.03%, and 0.2%. The results are presented in Table 3.

Experimental studies according to methods [3–7] demonstrate that "Blanidas-C Hypochlorite" under conditions of using solutions with concentrations of 0.03% and 0.2% (active chlorine) and an exposure of 15 min is an effective disinfectant for decontamination against bacteria and fungi.

Thus, studies conducted using European methods to determine the effectiveness of disinfectants in working solutions showed that "Eco Chlor" (0.5% 15 min and 2.0% - 60 min) and "Blanidas-C Hypochlorite" (0.03% and 0.2% - 15 min) are effective disinfectants for water decontamination against bacteria and fungi, while "Novochlor-extra" (0.5% 30 min, 0.5% 60 min, and 2.0% - 60 min) is effective against bacteria, fungi, and spores.

The study of the disinfecting activity of "Sanika-Anolyte" was conducted according to [1].

A test sample (concentration of active substances <0.01%) was added in an amount of 50 cm³ per 1 dm³ of water (1st regime) and 100 cm³ per 1 dm³ of water (2nd regime) with an exposure of 30 minutes. Water samples contaminated with cultures of the corresponding test microorganisms without the addition of a decontaminating agent were used as controls. The research results are shown in Table 4.

The "Sanika-Anolyte" sample possesses sufficient antimicrobial activity against *E. coli*, *S. aureus*, *C. albicans*,

and *B. subtilis* in both regimes (50 ml and 100 ml of product per 1 liter of water) with an exposure of 30 minutes. Based on the test results, it was established that the disinfectant sample "Sanika-Anolyte" possesses sufficient disinfecting activity and is effective for water decontamination in emergency conditions.

The evaluation of the effectiveness of the drinking water disinfectant "Biolut CL" was performed using test microorganisms *E. coli*, *P. aeruginosa*, and *B. subtilis* according to method [4]. The research results are shown in Table 5.

As seen in Table 5, the sample of the drinking water disinfectant "Biolut CL" possesses antimicrobial activity against *E. coli*, *P. aeruginosa*, and *B. subtilis* in the regime of 2 ml of native solution per 1 liter of water (which corresponds to a chlorine dioxide concentration of 0.8 mg/l) with an exposure of 30 min. It possesses disinfecting activity and is effective for water decontamination under emergency conditions.

The study of the disinfecting properties of "SterilOx" was conducted in the following regime: a disinfectant concentration of 50 ml per 1000 ml of water artificially contaminated with test strains with an exposure of 30 min.

Based on the results of experimental studies on drinking water decontamination with the disinfectant "SterilOx", it was established that the 2 studied regimes ensure 100% decontamination of water from bacteria and yeast-like fungi at a temperature of 20°C. The studied water decontamination regimes can be recommended for obtaining epidemiologically safe drinking water in emergency conditions, including

wartime.

The effectiveness (bactericidal, fungicidal, and sporicidal action) of the studied disinfectants was confirmed in a quantitative suspension test according to [5–9]. The studied disinfectants, whose specific activity was evaluated for drinking water decontamination, showed their effectiveness both in studies according to European requirements (quantitative suspension test) [5–9] and in practical specific testing [4] of disinfectants for drinking water.

Developed Algorithm for Researching Bactericidal, Fungicidal, and Sporocidal Activity of Agents Used for Drinking Water Disinfection in Emergency Conditions (in the absence of access to centralized water supply):

Stage 1. Determine the basic bactericidal and fungicidal activity of the disinfectant according to DSTU EN 1040 [9] (strains *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442) and DSTU EN 1275 [5] (*Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* ATCC 16404).

Stage 2. Divided into 2 steps:

Step 2.1* – research of bactericidal activity in a quantitative suspension method according to DSTU EN 13727 [6] (on strains *Staphylococcus aureus* ATCC 6538; *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541; *Escherichia coli* ATCC 10536). Research of fungicidal activity according to DSTU EN 13624 [7] (*Candida albicans* ATCC 10231 and *Aspergillus brasiliensis* (A. niger) ATCC 16404). Research of sporocidal activity according to DSTU EN 13704 [8] (spores of *Bacillus subtilis* ATCC 6633). Research of virucidal activity on the reference F-RNA coliphage MS2, as it is a recognized useful model/surrogate for human enteric viruses. The reference test strain *E. coli* K12 Hfr is used as

bacterial hosts. The *Escherichia coli* bacteriophage MS2 is used in the form of a broth culture freed from bacterial debris and unlysed bacteria, having a titer of not less than 1010 plaque-forming units (PFU).

Step 2.2 – Evaluation of the disinfectant under conditions simulating practical use, described in the Methodological Guidelines "Test Methods for Disinfectants to Assess Their Safety and Efficacy", 1998.

Conclusions

A comprehensive analysis of the main approaches to assessing the effective and safe use of disinfectants for drinking water treatment in Ukraine and EU countries was conducted.

The experimental results on drinking water disinfection with various disinfectants confirmed the feasibility of a phased evaluation of their efficacy.

Experimental studies on the specific effects of disinfectants with different chemical compositions ("SANIKA-ANOLIT", "Novochlor-extra", "Eco Chlor", "BIOLUFT CL", "Blanidas-C Hypochlorite", and "SterilOx") confirmed their efficacy in drinking water disinfection ($\lg R > 5$) for bacteria and ($\lg R > 4$) for fungi).

According to the research results, the disinfectants approved for drinking water disinfection in Ukraine demonstrated their efficacy both in studies compliant with European standards and in tests simulating practical conditions.

A developed algorithm for investigating the bactericidal, fungicidal, and sporicidal activity of agents used for drinking water disinfection in emergency situations (in the absence of access to centralized water supply) is also presented.

Bibliography

1. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (recast) <http://data.europa.eu/eli/dir/2020/2184/oj>
2. Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products (consolidated text) <http://data.europa.eu/eli/reg/2012/528/oj>
3. Ministry of Health of Ukraine. Hygienic requirements for drinking water intended for human consumption (State Sanitary Rules and Norms DSanPiN 2.2.4-171-10). 2010.
4. Methodological Guidelines. Test methods for disinfectants to assess their safety and efficacy. Ministry of Health of Ukraine. 1998.
5. State Standard of Ukraine. Chemical disinfectants and antiseptics. Primary fungicidal activity. Test method and requirements (Stage 1) (DSTU EN 1275:2004). Ukrainian Agency for Standardization. 2004.
6. State Standard of Ukraine. Chemical disinfectants and antiseptics. Quantitative suspension test method for the evaluation of bactericidal activity in healthcare. Test method and requirements (Stage 2, Step 1) (DSTU EN 13727:2019). Ukrainian Agency for Standardization. 2019.
7. State Standard of Ukraine. Chemical disinfectants and antiseptics. Quantitative suspension test method for the evaluation of fungicidal or pseudofungicidal activity in the medical sector. Test method and requirements (phase 2, step 1) (DSTU EN 13624:2019). Ukrainian Agency for Standardization. 2019.
8. State Standard of Ukraine. Chemical disinfectants. Quantitative suspension test method for determining the sporicidal activity of chemical disinfectants used in food service establishments, industry, households, and public facilities. Test method and requirements (stage 2, step 1) (DSTU EN 13704:2019). Ukrainian Agency for Standardization. 2019.
9. State Standard of Ukraine. Chemical disinfectants and antiseptics. General bactericidal activity. Test method and requirements (stage 1) (DSTU EN 1040:2004). Ukrainian Agency for Standardization. 2004.

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