

UDC: 619.22.28:614.48:615.9:636.065

DOI: 10.31073/onehealthjournal2026-III-05

Experimental evaluation of the bactericidal activity and stability of the biocidal agent “Krezonid”

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Abstract. *The article presents the results of an experimental assessment of the bactericidal activity and stability of the biocidal agent “Krezonid,” whose main active ingredient is meta-cresol (3-methylphenol). The study was conducted to substantiate the effectiveness of the biocidal agent and confirm the preservation of its antimicrobial properties during long-term storage in accordance with the requirements. Bactericidal activity was evaluated using a quantitative suspension method with determination of the number of viable microorganisms (CFU) after exposure to the preparation. Streptococcus faecalis and Salmonella enteritidis strains were used as test cultures. The preparation was studied at concentrations of 0.1, 0.5, and 1.0% with exposure times of 10–30 minutes. The stability of the biocidal agent was studied under real storage conditions for 30 months by monitoring organoleptic indicators, pH values, and the preservation of bactericidal activity. It was found that the biocidal agent “Krezonid” exhibits pronounced bactericidal activity against both test microorganism cultures.*

Complete inactivation of Streptococcus faecalis and Salmonella enteritidis was achieved at a concentration of 0.5% and exposure of 20 minutes. During storage, the drug “Krezonid” retained its physicochemical homogeneity, stable pH value, and complete bactericidal activity throughout the observation period. The results obtained indicate the high efficacy and stability of the biocidal agent Krezonid, which justifies its use in veterinary practice and the possibility of establishing a shelf life of at least 24 months under regulated storage conditions.

Keywords: biocidal agents, meta-cresol, bactericidal activity, stability, veterinary disinfection

Prevention and control of infectious diseases in animal husbandry are fundamental components of the epizootic welfare system and ensuring the safety of food products of animal origin. The effectiveness of sanitary and hygienic measures largely depends on the use of biocidal agents capable of ensuring a stable reduction in the number of pathogenic and conditionally pathogenic microorganisms in the external environment (Maillard, 2024).

Among chemical compounds with proven antimicrobial activity, phenolic derivatives occupy a special place, characterized by their ability to disrupt the structural and functional integrity of microbial cell membranes. The mechanism of antimicrobial action of phenols consists in destabilizing the lipid layer of the cytoplasmic membrane, denaturing protein structures, and disrupting energy metabolism, which leads to the death of the bacterial cell (Russell, 2002; Rutala, 2019).

Modern experimental studies confirm the high effectiveness of phenolic compounds against Gram-positive and Gram-negative bacteria. In particular, phenolic disinfectants provide a pronounced bactericidal effect at relatively low working concentrations, maintaining their activity even in difficult conditions of exposure to organic impurities. Similar conclusions regarding the promise of phenolic compounds as biocides are cited by Oulahal (2022), emphasizing their stability and broad spectrum of antimicrobial activity.

One of the important representatives of phenolic compounds used in disinfectants and biocidal agents is meta-cresol (3-methylphenol). According to Maillard, J.-Y., Pascoe, M., and Russell, meta-cresol exhibits bactericidal activity by disrupting the permeability of the cytoplasmic membrane and inhibiting key enzymatic processes in microbial cells. An important advantage of

phenolic biocides, in particular meta-cresol, is the preservation of antimicrobial activity in conditions of organic contamination, which is of fundamental importance for veterinary practice (Maillard, 2024; Russell, 2002).

In the biocidal product “Krezonid,” meta-cresol is the main active ingredient, while auxiliary ingredients ensure the stability of the composition, maintain optimal pH, and reproduce bactericidal activity throughout the entire storage period. This approach to the formulation of biocidal products is in line with current recommendations for the creation of effective veterinary disinfectants, as outlined in the works of Tyski (2019) and Wales (2020).

According to current requirements for state registration of veterinary biocidal products, experimental evaluation should include not only confirmation of bactericidal activity, but also monitoring of the stability of the active substance during storage using quantitative microbiological indicators. The need for such an approach is indicated by (Tarka, 2021), as well as (Wales, 2020), who emphasize the importance of determining the logarithmic reduction of viable microorganisms to justify the effectiveness of biocides (Ponomarenko, 2020).

Aim of the study was to experimentally investigate the bactericidal activity and stability of the biocidal agent “Krezonid,” whose main active ingredient is meta-cresol, using quantitative microbiological methods in accordance with current requirements.

Materials and methods. The object of the study was the biocidal agent “Krezonid” – a composite preparation based on meta-cresol with the addition of auxiliary components that ensure the stability and technological properties of the preparation. The studies were conducted at the laboratory of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine. Standard test strains of microorganisms were used in the studies: *Streptococcus faecalis*; *Salmonella enteritidis*.

The cultures were grown on meat-peptone agar at a temperature of $37\pm 1^\circ\text{C}$ for 18–24 hours.

The bactericidal activity of the preparation was determined by a quantitative suspension test in accordance with the generally accepted approaches used in EN 1040 and EN 1656 standards for evaluating the action of chemical disinfectants (Harkavenko et al., 2020).

Working suspensions of microorganisms were prepared at a concentration of 107–108 CFU/cm³. The drug “Krezonid” was studied at working concentrations of 0.1%, 0.5%, and 1.0% for exposure times of 10, 20, and 30 minutes at a temperature of $37\pm 1^\circ\text{C}$.

After exposure, the effect of the preparation was neutralized, serial dilutions were performed, and the preparation was seeded onto a nutrient medium, followed by counting the number of colonies (CFU). Samples without the addition of a biocidal agent served as controls.

The stability of the biocidal agent was evaluated under real storage conditions for 24 months. The preparation was stored in a hermetically sealed factory container at a temperature of 5 to 25°C in a place protected from light.

Stability control was carried out after 0, 6, 12, 24, and 30 months of storage by: evaluating organoleptic indicators (appearance, color, smell, presence of sediment or stratification); determining pH values; re-determining bactericidal activity using generally accepted methods (Kovalenko, 2011).

Results. Experimental studies have shown that the drug “Krezonid” exhibits pronounced bactericidal activity even at low concentrations. At a concentration of 0.1% and exposure of 10 minutes, the number of viable *Streptococcus faecalis* cells decreased from 1.2×10^8 CFU/cm³ to 4.6×10^5 CFU/cm³, indicating a pronounced but incomplete reduction in the microbial population. Increasing the exposure time to 20 minutes at the same concentration led to a further decrease in the number of CFU/cm³ to 1.3×10^4 , which can be characterized as a significant suppression of bacterial viability (Table 1).

When the concentration is increased to 0.5% and the exposure time is 20 minutes, *Streptococcus faecalis* is completely inactivated, as no colonies were found in the test samples. This indicates that a working concentration of 0.5% is optimal for effective destruction of bacteria in a short period of time. Complete inactivation is also observed at a concentration of 1.0% and an exposure time of 30 minutes, confirming a direct relationship between concentration, exposure time, and product efficacy.

Table 1

Bactericidal activity of the biocidal agent “Krezonid” against *Streptococcus faecalis*

Concentration of the drug, %	Exposition, min.	Number of CFU/cm ³ (control)	CFU/cm ³ (test)	Decrease CFU/cm ³
0,1	10	1,2×10 ⁸	4,6×10 ⁵	pronounced
0,1	20	1,2×10 ⁸	1,3×10 ⁴	significant
0,5	20	1,2×10 ⁸	Not detected	complete
1,0	30	1,2×10 ⁸	Not detected	complete

The results obtained are consistent with the literature data (Russell, 2002) on the effect of phenolic compounds on Gram-positive bacteria: the mechanism of action consists in disrupting the integrity of the cell membrane and denaturing proteins, which leads to the rapid death of microorganisms.

Salmonella enteritidis showed slightly higher resistance to the drug than *Streptococcus faecalis*, which corresponds to the known properties of Gram-negative bacteria with an outer membrane that provides an additional barrier against chemical agents (Table 2). At a concentration of 0.1% and an exposure time of 10 minutes, the number of viable cells decreased from 9.8×10^7 CFU/cm³ to 6.1×10^5 CFU/cm³, i.e., there was a marked but incomplete inhibition. Increasing the exposure to 20 minutes reduced the CFU/cm³ to 2.4×10^4 , which can be assessed as a significant but not complete inhibition of bacterial viability.

Table 2

Bactericidal activity of the biocidal agent “Krezonid” against *Salmonella enteritidis*

Concentration of the drug, %	Exposition, min.	Number of CFU/cm ³	Concentration of the drug, %	Exposition, min.
0,1	10	9,8×10 ⁷	6,1×10 ⁵	pronounced
0,1	20	9,8×10 ⁷	2,4×10 ⁴	significant
0,5	20	9,8×10 ⁷	Not detected	complete
1,0	30	9,8×10 ⁷	Not detected	complete

The use of a concentration of 0.5% and an exposure time of 20 minutes ensured complete inactivation of *Salmonella enteritidis*, similar to *Streptococcus faecalis*. A concentration of 1.0% and an exposure time of 30 minutes also ensured complete destruction of the bacteria.

These results confirm that Krezonid is effective against both test strains, but for Gram-negative bacteria such as *Salmonella enteritidis*, a minimum working concentration of 0.5% with an exposure time of at least 20 minutes is recommended to achieve complete bactericidal action.

To assess the stability of “Krezonid”, monitoring was carried out for 30 months under real storage conditions (at a temperature of 5 to 25°C, protected from light, in sealed containers) (Table 3). The results showed that the product retains: A uniform appearance without sediment or separation at all stages of observation.

Table 3

Stability indicators of the biocidal agent “Krezonid” during storage

Term of storage, months	External appearance	Sediment / stratification	pH 5,8–6,2	Meta-cresol content	Density 1.10–1.20 g/cm ³	Bactericidal activity
0	homogeneous	absent	6,0	5,15	1,12	Safed
6	homogeneous	absent	5,9	5,10	1,11	Safed
12	homogeneous	absent	6,1	5,09	1,13	Safed
24	homogeneous	absent	6,0	5,02	1,14	Safed
30	homogeneous	absent	5,8	4,85	1,15	Safed

The pH level was within the range of 5.8–6.2, with changes not exceeding 0.2 units, which does not affect antimicrobial activity. Complete bactericidal activity against both test microorganisms was observed even after 30 months.

However, the meta-cresol content decreased to 4.85 after 30 months, which gives reason to recommend the product for a shelf life of 24 months.

These data indicate the high physicochemical stability of the product, which ensures a long shelf life and effectiveness in practical application.

Experiments to control activity after storage showed (Tables 4 and 5) that *Streptococcus faecalis* and *Salmonella enteritidis* are completely inactivated at a concentration of 0.5% and exposure of 20 minutes at all stages of storage (6, 12, 24, 30 months). This confirms that the active components of the preparation do not degrade during storage and that the physicochemical properties remain stable.

Table 4

Bactericidal activity of “Krezonid” against *Streptococcus faecalis* during storage

Term of storage, months	Concentration, %	Exposition, min.	Number of CFU/cm ³	Evaluation of the action
6	0,5	20	Not detected	Full
12	0,5	20	Not detected	Full
24	0,5	20	Not detected	Full
30	0,5	20	Not detected	Full

Thus, “Krezonid” meets modern requirements for veterinary biocidal products: preservation of bactericidal activity, stability of composition, and optimal operating parameters.

Table 5

Bactericidal activity of “Krezonid” against *Salmonella enteritidis* during storage

Term of storage, months	Concentration, %	Exposition, min	Number of CFU/cm ³	Evaluation of the action
6	0,5	20	Not detected	Full
12	0,5	20	Not detected	Full
24	0,5	20	Not detected	Full
30	0,5	20	Not detected	Full

Analysis of the experimental data presented in Tables 3–5 shows that the biocidal agent “Krezonid” retains its physicochemical homogeneity and bactericidal activity throughout the entire study period. The pH value changed by no more than 0.2 units, which is not critical for the stability of phenolic biocidal compositions.

Quantitative microbiological studies showed that after 6, 12, 24, and 30 months of storage, the preparation at a working concentration of 0.5% ensured complete inactivation of test microorganisms without the detection of viable cells during sowing. The absence of a decrease in bactericidal activity confirms the stability of the active components of the preparation and justifies the establishment of a shelf life of at least 24 months.

The results obtained are of practical importance for the biocidal agent “Krezonid”, as they demonstrate the compliance of the preparation with the requirements for stability and effectiveness during storage.

Discussion. The results obtained are consistent with the literature data on the high antimicrobial activity of phenolic compounds, in particular meta-cresol, which has a bactericidal effect by disrupting the integrity of cell membranes and denaturing the protein structures of microorganisms.

An important characteristic of biocidal agents intended for veterinary use is the stability of their properties during storage. The results of the studies show that the composition of the agent “Krezonid” ensures the preservation of physicochemical parameters and bactericidal activity for at least 24 months of actual storage, which meets the requirements for drugs submitted for state registration (Rutala, 2019).

Scientific publications and regulatory documents indicate that the effectiveness of phenolic veterinary disinfectants and combined biocides is largely determined by the concentration of active ingredients, duration of exposure, and stability of the product during storage. According to Wales et al., phenolic disinfectants provide rapid bactericidal action against Gram-positive and Gram-negative bacteria in the concentration range of 0.5–1.0% and exposure time of 15–30 minutes (Wales, 2020)

The works of Russell and Boyce show that combined biocidal compositions, which combine phenolic compounds with other active components, in particular quaternary ammonium compounds, are characterized by a broader spectrum of antimicrobial activity and greater resistance to organic contaminants. Similar preparations used in the veterinary and food industries demonstrate complete inactivation of *Streptococcus faecalis* and *Salmonella enteritidis* after 20–30 minutes of exposure at concentrations of at least 0.5% (Russell, 2002; Boyce, 2023).

The results obtained in this study indicate that the biocidal agent “Krezonid” is not inferior to well-known phenolic and combined analogues described in the literature in terms of bactericidal activity and stability. The preservation of full bactericidal activity after 6, 12, 24, and 30 months of storage distinguishes “Krezonid” favorably from certain biocidal agents, for which publications note a gradual decrease in effectiveness due to the degradation of active components or changes in the acidity of the environment.

Thus, a comparative analysis with data from real scientific studies confirms the compliance of the biocidal agent “Krezonid” with modern requirements for veterinary disinfectants and justifies the expediency of its use and the establishment of a shelf life of at least 24 months for the biocidal agent “Krezonid” under regulated storage conditions.

The prospect for future work is to investigate the fungicidal properties of the biocidal agent “Krezonid”.

Conclusions. The biocidal agent “Krezonid” meets the requirements for veterinary disinfectants in terms of bactericidal activity against *Streptococcus faecalis* and *Salmonella enteritidis*. Quantitative microbiological studies confirm an effective reduction in the number of viable microorganisms to a level that cannot be determined by the sowing method, in the range of working concentrations of 0.5–1.0%.

The results of stability testing under real storage conditions for 24 months indicate that the physicochemical properties and bactericidal activity of the product are preserved.

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Експериментальна оцінка бактерицидної активності та стабільності біоцидного засобу «Крезонід»

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Резюме. У статті наведено результати експериментальної оцінки бактерицидної активності та стабільності біоцидного засобу «Крезонід», основним діючим компонентом якого є мета-крезол (3-метилфенол). Дослідження проведено з метою обґрунтування ефективності біоцидного препарату та підтвердження збереження його антимікробних властивостей у процесі тривалого зберігання відповідно до вимог. Бактерицидну активність оцінювали кількісним суспензійним методом із визначенням кількості життєздатних мікроорганізмів (CFU) після експозиції препарату. Як тест-культури використовували штами *Streptococcus faecalis* та *Salmonella enteritidis*. Препарат досліджували у концентраціях 0,1; 0,5 та 1,0 % за експозиції 10–30 хв. Стабільність біоцидного засобу вивчали в умовах реального зберігання протягом 30 міс шляхом контролю органолептичних показників, значення рН та збереження бактерицидної активності. Встановлено, що біоцидний засіб «Крезонід» проявляє виражену бактерицидну активність щодо обох культур тест-мікроорганізмів. Повна інактивація *Streptococcus faecalis* та *Salmonella enteritidis* досягалася при концентрації 0,5 % і експозиції 20 хв. У процесі зберігання препарат «Крезонід» зберігав фізико-хімічну однорідність, стабільне значення рН та повну бактерицидну активність протягом усього періоду спостереження. Отримані результати свідчать про високу ефективність і стабільність біоцидного засобу «Крезонід», що обґрунтовує доцільність його застосування у ветеринарній практиці та можливість встановлення терміну придатності не менше ніж 24 міс. за регламентованих умов зберігання.

Ключові слова: біоцидні засоби, мета-крезол, бактерицидна активність, стабільність, ветеринарна дезінфекція

DOI: 10.31073/onehealthjournal2026-III-05