Report No.: **1430/11/2022**

Date issued: 24th of November 2022

Biocidal efficacy assessment report for the product

PROOXINE

according to PN-EN 14349:2013-05 standard

made for the company

Kemin Europa NV

Toekomstlaan 42

2200 Herental, Belgium

Ekolabos sp. z o.o. (Ltd)
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Signed by: Mateusz Latosiński Eng.
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1. INTRODUCTION

The properties of biocidal preparations, before they are authorised for use, are assessed based on tests carried out in accordance with European standards or other methods accepted by designated national authorities.

The standardisation of testing methods in recent years, through the development of successive European standards on the efficacy of disinfectants and antiseptics, allows a uniform, objective assessment of the antimicrobial effect of these agents and guarantees that the products offered in the market have adequate efficacy.

2. PURPOSE OF THE STUDY

This study aims to assess the biocidal efficacy of the product in relation to *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Proteus vulgaris* ATCC 13315, *Enterococcus hirae* ATCC 10541.

3. FORMAL BASIS

The assessment of biocidal efficacy was carried out on the basis of the agreement/order dated 25th of October 2022 (Agreement No.: 44/11/2022) concluded between the Contracting Party and the Contractor.

Contracting Party:

Kemin Europa NV

Toekomstlaan 42

2200 Herental, Belgium

Contractor:

EKOLABOS sp. z o. o.

Environmental Research Laboratory

ul. Duńska 9 54-427 Wrocław

Poland

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4. LEGAL BASIS

The legal basis for the conducted tests is:

The Act of 9 October 2015 on biocidal products

PN-EN 14349:2013-05 Chemical disinfectants and antiseptics -- Quantitative surface method for determining the bactericidal effect of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action -- Test method and requirements (phase 2, step 2) According to the standard, a disinfectant has a biocidal effect against the strain used if the logarithm of bacterial cell reduction obtained during the test is ≥4.

5. SAMPLE IDENTIFICATION [1]

The tested sample was the biocidal product in the form of a product for dilution. The preparation was accepted for testing on 3th of November 2022. Sample code assigned by the laboratory: 19/03/11/22. Product was delivered by the Contracting Party. After the time it was accepted for testing, and before the start of analysys the product was kept according to the storage conditions. Container was not breached until product testing started. Contractor does not bear responsibility for product stability after opening.

Product name: PROOXINE

Lot No.: no data

Product reference number: no data

Manufacturer:

Kemin Europa NV

Toekomstlaan 42

2200 Herental, Belgium

Date of manufacture: no data

Expiry date: no data

Product appearance: two-component product - liquid + white powder (activator) - yellow liquid after mixing

Recommended product solvent: no data

[1] Declaration by the Principal

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Storage conditions: no data

Active substances present in the product provided by the Contracting Party and their concentrations:

- no data

6.SCOPE OF TASKS PERFORMED

Phase 2, stage 2 assessment consists in using the dilution and neutralisation method in which the test organism is exposed to the preparation at different concentrations, times and temperatures with the addition of aggravating substances. These methods are to confirm the efficacy of the product in laboratory conditions, similar to the intended use.

6.1 CONDITIONS OF THE TEST PERFORMED

Tests performed on: 15th of November 2022– 21th of November 2022

Identification of the microbial strain:

Staphylococcus aureus ATCC 6538,

Pseudomonas aeruginosa ATCC 15442,

Proteus vulgaris ATCC 13315,

Enterococcus hirae ATCC 10541,

Incubation for 24 h at 37 °C ± 1 °C

Number of times the test is repeated on the microbe: 1

Test temperature: 10 °C ± 1 °C

Required duration of the product contacting the bacterial suspension:

5 min± 10 sec (product at 100% concentration);

30 min± 10 sec (determination of effective concentration);

Aggravating substances: beef albumin 3g/l

Solvent used during the test:

Hard Water according to PN-EN 14349:2013-05 standard

Stability of the product during the test:

Product stable during the study.

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6.2 TESTING METHOD AND VALIDATION

Method used: neutralisation of solutions

Counting method: deep inoculation on plates

Neutraliser used, composition:

- Polysorbate 80 – 30 g/l
- Sodium thiosulphate – 10 g/l
- Lecithin – 3 g/l

The neutralizer used allowed the method to be validated.

Substrates used: Trypticasein Soy LAB-Agar (TSA)

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7.TESTS RESULTS

The results of product testing are presented in tables 1-2

Table 1. Results of bacterial validation tests

Tested organism	Bacterial suspension for testing	Neutraliser toxicity Test	Validation test	Test using water
	N	**B**	**C**	**Nw**
Staphylococcus aureus ATCC 6538	10^{-7}: >330 10^{-8}: 45 **N: 8,05**	**NT: 7,66**	**NC: 7,55**	**Nc: 7,85**
Pseudomonas aeruginosa ATCC 15442	10^{-7}: >330 10^{-8}: 38 **N: 7,98**	**NT: 7,67**	**NC: 7,50**	**Nc: 7,76**
Proteus vulgaris ATCC 13315	10^{-7}: >330 10^{-8}: 40 **N: 8,00**	**NT: 7,67**	**NC: 7,67**	**Nc: 7,86**
Enterococcus hirae ATCC 10541	10^{-7}: >330 10^{-8}: 44 **N: 8,04**	**NT: 7,78**	**NC: 7,75**	**Nc: 7,98**

N - $\log_{10}$ number of cfu/ml applied on the test surface
B - $\log_{10}$ number of cfu /ml on the surface intended for the neutralizer toxicity test
C - $\log_{10}$ number of cfu /ml on the surface intended for the validation test
Nw - $\log_{10}$ number of cfu /ml on the surface intended for the control test with water

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Table 2. Test results

Organism tested	Nw	Results for individual concentrations in volumetric % of the product (test conditions: temperature: 10°C ± 1°C)					
		100% contact time: 5 min	**1,6% contact time: 30 min**	**0,8% contact time: 30 min**	**0,4% contact time: 30 min**	**0,2% contact time: 30 min**	**0,0001% contact time: 30 min**
Staphylococcus aureus ATCC 6538	7,85	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:>330 10^{-2}:>330
		Na:<2,15 Nts:0	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:>5,52 Nts:>330**
R=(Nw – Nd)		**R: >5,70**	**R: >5,70**	**R: >5,70**	**R: >5,70**	**R: >5,70**	**R: <2,33**
Pseudomonas aeruginosa ATCC 15442	7,76	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:>330 10^{-2}:>330
		Na:<2,15 Nts:0	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:>5,52 Nts:>330**
R=(Nw – Nd)		**R: >5,61**	**R: >5,61**	**R: >5,61**	**R: >5,61**	**R: >5,61**	**R: <2,24**
Proteus vulgaris ATCC 13315	7,86	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:>330 10^{-2}:>330
		Na:<2,15 Nts:0	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:>5,52 Nts:>330**
R=(Nw – Nd)		**R: >5,71**	**R: >5,71**	**R: >5,71**	**R: >5,71**	**R: >5,71**	**R: <2,34**
Enterococcus hirae ATCC 10541	7,98	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:<14 10^{-1}:<14	10^{-0}:>330 10^{-2}:>330
		Na:<2,15 Nts:0	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:<2,15 Nts:0**	**Na:>5,52 Nts:>330**
R=(Nw – Nd)		**R: >5,83**	**R: >5,83**	**R: >5,83**	**R: >5,83**	**R: >5,83**	**R: <2,46**

Nd - $\log_{10}$ of the number of cfu /ml on the surface to be tested for disinfectant effectiveness
R - reduction in the number of microorganisms during the test
Nts - number of cfu remaining on the surface after the test is performed

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Specific comments:

Verification of methodology – requirements and limits:

- N is between 7.57 and 8.10
- Nw is ≥6,2
- B and C are ≥0,5xNw
- The average number of bacteria on each plate used for the calculation and obtained from the active concentration test, is between 14 and 330,
- Nts on each plate remaining from the active concentrations and used in the calculation is less than 100,
- The quotient control of the weighted averages from successive dilutions used in the calculations ranges from 5.0 to 15.0.

8. CONCLUSIONS

The product tested in accordance with PN-EN 14349:2013-05 standard, in temperature of 10°C, diluted in hard water, with the presence of aggravating substance, shows antibacterial effect on surfaces (reduction ≥4log) in relation to:

Staphylococcus aureus	ATCC 6538	in concentrations of 0.2%, 0.4%, 0.8%, 1.6% after 30 min contact time in 100% concentration after 5 min contact time
Pseudomonas aeruginosa	ATCC 15442	in concentrations of 0.2%, 0.4%, 0.8%, 1.6% after 30 min contact time in 100% concentration after 5 min contact time
Proteus vulgaris	ATCC 13315	in concentrations of 0.2%, 0.4%, 0.8%, 1.6% after 30 min contact time in 100% concentration after 5 min contact time
Enterococcus hirae	ATCC 10541	in concentrations of 0.2%, 0.4%, 0.8%, 1.6% after 30 min contact time in 100% concentration after 5 min contact time

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The results obtained during all controls and tests met all the requirements of the methodology and were within the limits set.

Date issued: 24th of November 2022

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The results were authorised by: Jakub Jałówko, Eng, M.Sc

The report was approved by: Mateusz Latosiński, Eng

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