Department of Biocidal Products Implementation
Ekolabos sp. z o.o. (Ltd)
Duńska Street 9
54-427 Wrocław

Report No.: **RB/5173/03/21**

Date issued: 9 April 2021

Biocidal efficacy assessment report

for the product

Oxine Pro 500ppm

according to PN-EN 13697+A1:2019-08 standard

made for the company

EUROPEAN HYGIENE TECH AS

Skjergardsvegen 333

5353 Straume, Norway

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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, and *Escherichia coli* ATCC 10536, *Enterococcus hirae* ATCC 10541, *Candida albicans* ATCC 10231, and *Aspergillus brasilensis* ATCC 16404 strains.

3. FORMAL BASIS

The assessment of biocidal efficacy was carried out on the basis of the agreement/order dated 26 November 2020 (Agreement No.: AFC/022361/02/21/WRO) concluded between the Contracting Party and the Contractor.

Contracting Party:

EUROPEAN HYGIENE TECH AS

Skjergardsvegen 333

5353 Straume, Norway

Contractor:

EKOLABOS sp. z o. o.

Environmental Research Laboratory

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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 13697+A1:2019-08 Chemical disinfectants and antiseptics – quantitative method for the determination of the antibacterial and antifungal effect of chemical disinfectants used in the food sector, industrial and domestic conditions, and in public utility facilities in relation to non-porous surfaces. Test method and requirements (phase 2, stage 2). According to the standard, the disinfectant has a biocidal effect on the strain used if the logarithm for bacterial cell reduction obtained in the test is ≥4 and for yeast and mould cells ≥3.

5. SAMPLE IDENTIFICATION [1]

The tested sample was the biocidal product in the form of a ready-to-use liquid. The preparation was accepted for testing on 10 February 2021. Sample code assigned by the laboratory: 087/10/02/21.

Product name: Oxine Pro 500ppm

Batch No.: 25012021

Product reference number: 25012021

Manufacturer: Biocide International – European Hygiene Tech AS.

Date of manufacture: 25.01.2021

Expiry date: 12 months from production date

Product appearance: liquid, light green, light smell

Recommended product solvent: N/A

Storage conditions: Store in cool, dark place. Room temperature or below. Avoid sunlight.

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

- according to the SDS of the product

[1] Declaration by the Principal

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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: 01 04 2021 – 08 04 2021

Identification of the microbial strain:

Staphylococcus aureus ATCC 6538,

Pseudomonas aeruginosa ATCC 15442,

Escherichia coli ATCC 10536,

Enterococcus hirae ATCC 10541,

Candida albicans ATCC 10231,

Aspergillus brasilensis ATCC 16404.

Incubation for 24 h at 37 °C ± 1 °C for bacteria, 48 h at 30 °C ± 1 °C for yeast, 5 days at 30 °C ± 1 °C for mould.

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

Test temperature: 20 °C ± 1 °C

Required duration of the product contacting the bacterial suspension: 2 min± 10 sec

Required duration of the product contacting the suspension of yeast or mould: 2 min± 10 sec

Aggravating substances: beef albumin 3g/l

Solvent used during the test:

Demineralised water

Stability of product mixture with solvent:

No precipitation formed during the test.

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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) for bacteria and Malt-extract Agar (MEA) for yeast and moul

7.TESTS RESULTS

The results of product testing are presented in tables 1-4.

Table 1. Results of bacterial validation tests

Tested organism	Bacterial suspension for testing	Neutraliser toxicity Test	Validation test	Test using water
	N	**NT**	**NC**	**Nc**
Staphylococcus aureus ATCC 6538	10^{-6}: >330 10^{-7}: 44 **N: 7,04**	**NT: 6,96**	**NC: 6,95**	**Nc: 6,98**
Pseudomonas aeruginosa ATCC 15442	10^{-6}: >330 10^{-7}: 38 **N: 6,98**	**NT: 6,87**	**NC: 6,89**	**Nc: 6,92**
Escherichia coli ATCC 10536	10^{-6}: >330 10^{-7}: 36 **N: 6,95**	**NT: 6,82**	**NC: 6,87**	**Nc: 6,87**
Enterococcus hirae ATCC 10541	10^{-6}: >330 10^{-7}: 41 **N: 7,01**	**NT: 6,89**	**NC: 6,90**	**Nc: 6,93**

N – $\log_{10}$ of the number of CFU/ml applied to the test surface

NT – $\log_{10}$ of the number of CFU/ml on the test surface for the neutraliser's toxicity

NT – $\log_{10}$ of the number of CFU /ml on the test surface for validation test

Nc – $\log_{10}$ of the number of CFU /ml on the test surface to be tested in control with water

Nts – the of CFU remaining on the surface after the test

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Table 2. Results of the bacterial test

Organism tested	Nc	Results for individual concentrations in volumetric % of the product (test conditions: contact time: 2 minutes, temperature: 20°C ± 1°C)		
		100%	**1%**	**0,1%**
Staphylococcus aureus ATCC 6538	6,98	10^{-0}:<14 10^{-1}:<14 10^{-2}:<14	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330
		Nd:<2,15 Nts:0	**Nd:>5,52 Nts:>330**	**Nd:>5,52 Nts:>330**
R=(Nc – Nd)		**R: >4,48**	**R: <1,46**	**R: <1,46**
Pseudomonas aeruginosa ATCC 15442	6,92	10^{-0}:<14 10^{-1}:<14 10^{-2}:<14	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330
		Nd:<2,15 Nts:0	**Nd:>5,52 Nts:>330**	**Nd:>5,52 Nts:>330**
R=(Nc – Nd)		**R: >4,77**	**R: <1,40**	**R: <1,40**
Escherichia coli ATCC 10536	6,87	10^{-0}:<14 10^{-1}:<14 10^{-2}:<14	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330
		Nd:<2,15 Nts:0	**Nd:>5,52 Nts:>330**	**Nd:>5,52 Nts:>330**
R=(Nc – Nd)		**R: >4,72**	**R: <1,35**	**R: <1,35**
Enterococcus. hirae ATCC 10541	6,93	10^{-0}:<14 10^{-1}:<14 10^{-2}:<14	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330	10^{-0}:>330 10^{-1}:>330 10^{-2}:>330
		Nd:<2,15 Nts:0	**Nd:>5,52 Nts:>330**	**Nd:>5,52 Nts:>330**
R=(Nc – Nd)		**R: >4,78**	**R: <1,41**	**R: <1,41**

Nd – $\log_{10}$ of the number of CFU/ml on the surface for testing disinfectant efficacy
R – reduction of microbial count during the test
Nts – the of CFU remaining on the surface after the test

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Table 3. Results of the validation tests for yeast and mould

Test organism	Fungi suspension for testing	Toxicity control for the neutraliser	Validation test	Test using water
	N	**NT**	**NC**	**Nc**
Aspergillus brasilensis ATCC 16404	10^{-5}: >165 10^{-6}: 32 **N: 5,90**	**NT: 5,81**	**NC: 5,76**	**Nc: 5,82**
Candida albicans ATCC 10231	10^{-5}: >330 10^{-6}: 41 **N: 6,01**	**NT: 5,90**	**NC: 5,91**	**Nc: 5,94**

N – log10 of the number of CFU/ml applied to the test surface
NT – log10 of the number of CFU /ml on the surface for testing the neutraliser's toxicity
NT - log10 of the number of CFU /ml on the test surface for validation test
Nc - log10 of the number of CFU /ml on the test surface to be tested in control with water
Nts – the of CFU remaining on the surface after the test

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Table 4. Results of yeast and mould tests

Organism tested	Nc	Results for individual concentrations in volumetric % (test conditions: contact time: 2 min, temperature: 20°C ± 1°C)		
		100%	**1%**	**0,1%**
Aspergillus brasilensis ATCC 16404	5,82	10^{-0}:<14 10^{-1}:<14 10^{-2}:<14	10^{-0}:>165 10^{-1}: >165 10^{-2}: >165	10^{-0}:>165 10^{-1}: >165 10^{-2}: >165
		Nd:<2,15 Nts:0	**Nd:>5,22 Nts:>165**	**Nd:>5,22 Nts:>165**
R=(Nc – Nd)		**R: >3,67**	**R:<0,60**	**R:<0,60**
Candida albicans ATCC 10231	5,94	10^{-0}:<14 10^{-1}:<14 10^{-2}:<14	10^{-0}:>165 10^{-1}: >165 10^{-2}: >165	10^{-0}:>165 10^{-1}: >165 10^{-2}: >165
		Nd:<2,15 Nts:0	**Nd:>5,22 Nts:>165**	**Nd:>5,22 Nts:>165**
R=(Nc – Nd)		**R: >3,80**	**R:<0,43**	**R:<0,43**

Nd – $\log_{10}$ of the number of CFU /ml on the surface for testing disinfectant efficacy
R – reduction of microbial count during the test
Nts – the of CFU remaining on the surface after the test

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Specific comments:

Verification of methodology – requirements and limits:

- N is between 6.57 and 7.10 for bacteria and between 5.57 and 6.10 for yeast and mould,
- Nc is high enough to determine the reduction factor (R) above 4lg for bacteria and 3lg for yeast and mould
- NC - Nc is at a maximum of ±0.3lg,
- NT - Nc is at a maximum of ±0.3lg,
- The average number of bacteria and yeasts, on each plate used for the calculation and obtained from the active concentration test, is between 14 and 330, and the number of moulds between 14 and 165,
- Nts on each plate remaining from the active concentrations and used in the calculation is less than 100,
- The control of the weighted average quotient of the successive dilutions when determining N is between 5.0 and 15.0.

8. CONCLUSIONS

The product tested in accordance with PN-EN 13697+A1:2019-08 standard, during 2 min, in temperature of 20°C, with the presence of aggravating substance, shows antibacterial effect on surfaces (reduction ≥4log) in relation to:

Pseudomonas aeruginosa	ATCC 15442	at 100% concentration
Escherichia coli	ATCC 10536	at 100% concentration
Staphylococcus aureus	ATCC 6538	at 100% concentration
Enterococcus hirae	ATCC 10541	at 100% concentration

The product tested in accordance with PN-EN 13697+A1:2019-08 standard, during 2 min, in temperature of 20°C, with the presence of aggravating substance, shows antifungal effect on surfaces (reduction ≥3 log) in relation to:

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Aspergillus brasilensis	ATCC 16404	at 100% concentration
Candida albicans	ATCC 10231	at 100% concentration

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

Date issued: 9 April 2021

Report prepared by: Agnieszka Pawelec, M.Sc

The results were authorised by: Mateusz Latosiński, Eng

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