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

Report No.: **RB/4948/02/21**

Date issued: 24 March 2021

Biocidal efficacy assessment report

for the product

Oxine Pro 500ppm

according to PN-EN 13624:2013-12 standard

made for the company

EUROPEAN HYGIENE TECH AS

Skjergardsvegen 333

5353 Straume, Norway

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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 has progressed 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

The aim of the study was to assess the biocidal efficacy of the product in relation to *Candida albicans* ATCC 10231 and *Aspergillus brasilensis* ATCC 16404 strain.

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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4. LEGAL BASIS

The legal basis for the conducted tests is:

The Act of 9 October 2015 on biocidal products

PN-EN 13624:2013-12 Chemical disinfectants and antiseptics – quantitative suspension method for the evaluation of fungicidal or biocidal effect on yeast-like fungi in the medical area. Test method and requirements (phase 2, stage 1). According to the standard, the disinfectant has a biocidal effect on the strain used if the logarithm for yeast or mould cell reduction obtained in the test is ≥4.

Guidance on the Biocidal Products Regulation - Volume II Efficacy - Assessment and Evaluation (Parts B+C), Version 3.0, April 2018.

Regulation of the European Parliament and of the Council (EU) No. 528/2012 of 22 May 2012 concerning the making available on the market and use of biocidal products.

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 February2021. Sample code assigned by the laboratory: 086/10/02/21.

Product name: Oxine Pro 500ppm

Batch No.: 25012021

Product reference number: 25012021

Manufacturer: Biocide International - EUROPEAN HYGIENE TECH AS.

Date of manufacture: 25012021

Expiry date: 12 mnd. 25.01.2022

Product appearance: liquid, light green, light smell

Recommended product solvent: N/A

Storage conditions: cold and dark

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

- information in SDS

[1] Declaration by the Principal

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6. SCOPE OF TASKS PERFORMED

Phase 2, stage 1 assessment consists in using the dilution and neutralisation method in which the test organism is exposed to the preparation at different concentrations, time 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

Analysis period: 15 March 2021 – 22 March 2021

Identification of the microbial strains:

Candida albicans ATCC 10231,

Aspergillus brasilensis ATCC 16404.

Incubation for 48h at 22 °C ± 1 °C

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

Required test temperature: 20 °C ± 1 °C

Required duration of the product contacting the microbial suspension:

60 sec ± 5 sec, 120 sec ± 5 sec

interfering substances: beef albumin 0.3 g/l, beef albumin 3 g/l plus 3.0 ml/l erythrocytes

Solvent used during the test:

Demineralised water

Stability of product mixture with solvent:

no precipitation formed during the test.

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 - 15 g/l

Lecithin - 3 g/l

The neutralizer used allowed the method to be validated. Substrate used: Malt Extract Agar (MEA)

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7. TESTS RESULTS

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

Table 1. Results of validation tests (clean conditions)

Test organism	Test microbial suspension	Validation suspension		Control of conditions	Control of neutraliser	Method validation
	N	**Nv_0**	**Nv_B**	**A**	**B**	**C**
Candida albicans ATCC 10231	10^{-5}: >330 10^{-6}: 40 **log N: 7,60**	**Nv_0= 81**	**Nv_B=8,8*10^4**	**A: 77**	**B: 82**	**C: 80**
Aspergillus brasilensis ATCC 16404	10^{-5}: >165 10^{-6}: 22 **log N: 7,34**	**Nv_0= 68**	**Nv_B= 7,2*10^4**	**A: 65**	**B: 70**	**C: 62**

N – number of CFU /ml in test suspension
Nv0 - number of CFU/ml in validation suspension
Nv – 10x Nv0
A – number of CFU in the control of test conditions
B – number of CFU in the control of neutraliser
C – number of CFU in method validation

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Table 2. Test results (clean conditions)

Test organism	N_0	Results for individual volumetric concentrations of the product (test conditions: contact time – 60 seconds, temperature – 20°C ± 1°C, clean conditions)		
		80%	**1%**	**0,1%**
Candida albicans ATCC 10231	**log N_0: 6,60**	<14, <14	>330, >330	>330, >330
		Na: <140 log Na: <2,15	**Na: >3300 log Na:>3,52**	**Na: >3300 log Na:>3,52**
log R (log N_0 – log Na)		**log R: >4,46**	**log R: <3,08**	**log R: <3,08**
Aspergillus brasilensis ATCC 16404	**log N_0: 6,34**	<14, <14	>165, >165	>165, >165
		Na: <140 log Na: <2,15	**Na: >1650 log Na:>3,22**	**Na: >1650 log Na:>3,22**
log R (log N_0 – log Na)		**log R: >4,20**	**log R: <3,12**	**log R: <3,12**

N0 – N/10
Na – number of CFU/ml after treatment
log R – logarithm obtained during the test of bacterial cell reduction

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Table 3. Results of validation tests (dirty conditions)

Test organism	Test microbial suspension	Validation suspension		Control of conditions	Control of neutraliser	Method validation
	N	**Nv_0**	**Nv_B**	**A**	**B**	**C**
Candida albicans ATCC 10231	10^{-5}: >330 10^{-6}: 42 **log N: 7,62**	**Nv_0= 85**	**Nv_B= 8,0*10^4**	**A: 78**	**B: 77**	**C: 84**
Aspergillus brasilensis ATCC 16404	10^{-5}: >165 10^{-6}: 21 **log N: 7,32**	**Nv_0= 71**	**Nv_B= 7,9*10^4**	**A: 70**	**B: 72**	**C: 66**

N – number of CFU /ml in test suspension
Nv0 - number of CFU/ml in validation suspension
Nv – 10x Nv0
A – number of CFU in the control of test conditions
B – number of CFU in the control of neutraliser
C – number of CFU in method validation

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Table 4. Test results (dirty conditions)

Test organism	N_0	Results for individual volumetric concentrations of the product (test conditions: contact time –120 seconds, temperature – 20°C ± 1°C, dirty conditions)		
		80%	**1%**	**0,1%**
Candida albicans ATCC 10231	**log N_0: 6,62**	<14, <14	>330, >330	>330, >330
		Na: <140 log Na: <2,15	**Na: >3300 log Na:>3,52**	**Na: >3300 log Na:>3,52**
log R (log N_0 – log Na)		**log R: >4,48**	**log R: <3,10**	**log R: <3,10**
Aspergillus brasilensis ATCC 16404	**log N_0: 6,32**	<14, <14	>165, >165	>165, >165
		Na: <140 log Na: <2,15	**Na: >1650 log Na:>3,22**	**Na: >1650 log Na:>3,22**
log R (log N_0 – log Na)		**log R: >4,18**	**log R: <3,10**	**log R: <3,10**

N0 – N/10
Na – number of CFU/ml after treatment
log R – logarithm obtained during the test of bacterial cell reduction

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Specific comments:

Verification of methodology – requirements and limits:

- N is between $1.5x10^7$CFU/ml and $5x10^7$ CFU/ml (7,17≤logN≤7,70),
- N_0 is between $1.5x10^6$ CFU/ml and $5x10^6$ CFU/ml (6,17≤logN0≤6,70),
- N_{V0} is between 30 CFU/ml and 160 CFU/ml
- N_V is between $3.0 x10^2$ CFU/ml and $1.6 x10^3$ CFU/ml
- N_{VB} is between $3.0 x10^4$ CFU/ml and $1.6 x10^5$ CFU/ml
- Control of the weighted average of the successive dilutions for N is between 5.0 and 15.0
- A and C are equal to or greater than $0.5xN_{V0}$ B is equal to or greater than $0.0005xN_{VB}$
- At least one test concentration of the product must show a reduction log ≥4
  and at least one test concentration of the product must show a reduction log <4.

8. CONCLUSIONS

The product, tested in accordance with PN-EN 13624:2013-12 standard, during 60 and 120 seconds at 20°C, in the presence of an aggravating substance, shows biocidal effect (reduction ≥4 log) in relation to:

Candida albicans	ATCC 10231	at 80% concentration
Aspergillus brasilensis	ATCC 16404	at 80% concentration

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

Date issued: 24 March 2021

Report prepared by: Agnieszka Pawelec, M.Sc

The results were authorised by: Mateusz Latosiński, Eng

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