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

Report No.: **RB/5296/04/21**

Date issued: **28 April 2021**

Tuberculocidal efficacy assessment report for the product

Oxine Pro 500ppm

according to PN-EN 14563:2012 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

The study aims to assess the antibacterial efficacy of the product in relation to *Mycobacterium avium* ATCC 15769 and *Mycobacterium terrae* ATCC 15755 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

ul. Duńska 9, 54-427 Wrocław

Poland

4. LEGAL BASIS

The legal basis for the conducted tests is:

The Act of 9 October 2015 on biocidal products

PN-EN 14563:2012 Chemical disinfectants and antiseptics – quantitative suspension method for the evaluation of tuberculocidal activity of chemical disinfectants used in the medical area including instrument disinfectants – Test method and requirements (phase 2, stage 2). According to the standard, the disinfectant has an antibacterial effect on the strain used if the logarithm for bacterial cell reduction obtained in the test is ≥4.

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: 090/10/02/21.

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 2 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

Tests performed on: 16 04 2021 –23 04 2021

Identification of the bacterial strains: *Mycobacterium avium* ATCC 15769,
Mycobacterium terrae ATCC 15755.

Incubation for 24h at 37 °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 bacterial suspension: 60 min ± 10sek

interfering substances beef albumin 3g/ l and sheep erythrocytes 3ml /l

Solvent used during the test:

Distilled 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 – 10 g/l
Lecithin – 3 g/l

The neutralizer used allowed the method to be validated.

Substrate used: Middlebrook and Cohn's 7H10 supplemented with 10% OADC (MCA)

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7. TESTS RESULTS

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

Table 1. Results of validation tests for contact time 30 s

Test organism	Test bacterial suspension	Validation bacterial suspension	Validation test	Control of neutraliser toxicity	Test using water
	N	**Nv_0**	**A**	**B**	**C**
Mycobacterium avium ATCC 15769	**N: 9,59**	**Nv_0: 78**	**A: 74**	**B: 69**	**C: 77**
Mycobacterium terrae ATCC 15755	**N: 9,64**	**Nv_0: 83**	**A: 80**	**B: 75**	**C: 76**

N – log from the number of CFU/ml put in the test suspension
Nv0 – 1/10 of CFU/ml in the validation suspension
A – number of CFU/ml in the mixture intended for the validation test
B – number of CFU/ml in the mixture for neutraliser toxicity test
C - number of CFU/ml in the mixture to be controlled using water and the highest concentration of the active substance

Table 2. Test results for contact time 30 s

Test organism	N_w	Results for individual concentrations in% by volume (test conditions: contact time: 30 s, temperature: 20 ° C ± 1 ° C)		
		100 %	**1 %**	**0,1 %**
Mycobacterium avium ATCC 15769	**7,18**	<14, <14	>330, >330	>330, >330
		Na: <140 Na: <2,15	**Na: >3300 Na: <3,52**	**Na: >3300 Na: <3,52**
R (N_w–Na)		**R:>5,03**	**R:<3,66**	**R:<3,66**
Mycobacterium terrae ATCC 15755	**7,24**	<14, <14	>330, >330	>330, >330
		Na: <140 Na: <2,15	**Na: >3300 Na: <3,52**	**Na: >3300 Na: <3,52**
R (N_w – Na)		**R:>5,10**	**R:<3,72**	**R:<3,72**

N_w – number of cells per ml of mixture to be tested at the end of contact time before neutralization;
Na – number of CFU / ml in the test mixture after treatment with the preparation;
R – logarithm bacterial cell reduction obtained during the test (Nw-Log Na)

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Table 3. Results of validation tests for contact time 60 s

Test organism	Test bacterial suspension	Validation bacterial suspension	Validation test	Control of neutraliser toxicity	Test using water
	N	**Nv_0**	**A**	**B**	**C**
Mycobacterium avium ATCC 15769	**N: 9,60**	**Nv_0: 92**	**A: 86**	**B: 80**	**C: 85**
Mycobacterium terrae ATCC 15755	**N: 9,66**	**Nv_0: 86**	**A: 82**	**B: 77**	**C: 84**

N – log from the number of CFU/ml put in the test suspension
Nv0 – 1/10 of CFU/ml in the validation suspension
A – number of CFU/ml in the mixture intended for the validation test
B – number of CFU/ml in the mixture for neutraliser toxicity test
C - number of CFU/ml in the mixture to be controlled using water and the highest concentration of the active substance

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Table 4. Test results for contact time 60 s

Test organism	N_w	Results for individual concentrations in% by volume (test conditions: contact time: 60 s, temperature: 20 ° C ± 1 ° C)		
		100 %	**1 %**	**0,1 %**
Mycobacterium avium ATCC 15769	**7,18**	<14, <14	>330, >330	>330, >330
		Na: <140 Na: <2,15	**Na: >3300 Na: <3,52**	**Na: >3300 Na: <3,52**
R (N_w–Na)		**R:>5,03**	**R:<3,66**	**R:<3,66**
Mycobacterium terrae ATCC 15755	**7,30**	<14, <14	>330, >330	>330, >330
		Na: <140 Na: <2,15	**Na: >3300 Na: <3,52**	**Na: >3300 Na: <3,52**
R (N_w – Na)		**R:>5,15**	**R:<3,78**	**R:<3,78**

N_w – number of cells per ml of mixture to be tested at the end of contact time before neutralization;
Na – number of CFU / ml in the test mixture after treatment with the preparation;
R – logarithm bacterial cell reduction obtained during the test (Nw-Log Na)

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Specific comments:

Verification of methodology – requirements and limits:

- N is between $1.5x10^9$ CFU/ml and $5.0x10^9$ CFU/ml ($9.17 \leq \log N \leq 8.70$),
- N_{V0} is between 30 CFU/ml and 160 CFU/ml
- N_w is not less than $1.5x10^6$ CFU / ml (6.15 log) and not greater than 0.05N (logN-1.3)
- Control of the weighted average of the successive dilutions used for calculations is from 5.0 to 15.0
- the average number of bacteria on each plate used for the calculation and obtained from the active concentration test is between 14 and 330
- A, B and C are equal to or greater than $0.5xN_{V0}$
- 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 in order to demonstrate the biocidal effect of the product.

8. CONCLUSIONS

The product tested in accordance with PN-EN 14563:2012 standard, after the contact time 30 s and 60 s, in temperature of 20°C, with the presence of aggravating substance, shows tuberculocidal efficacy antiviral effect (reduction ≥4 log) against:

Mycobacterium avium	ATCC 15769	at 100% concentration
Mycobacterium terrae	ATCC 15755	at 100% concentration

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

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Date issued: 28 April 2021

Report prepared by: Agnieszka Pawelec, M.Sc

The results were authorised by: Mateusz Latosiński, Eng

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