Department of Biocidal Products Implementation
Ekolabos sp. z o.o. (Ltd)
Duńska Street 9
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Report No.: **RB/3854/10/20**

Date issued: **19.10.2020**

Virucidal efficacy assessment report

for the product

Oxine 50ppm for surface desinfektion

according to PN-EN 14476+A2:2019-08 standard,

made for the company

EUROPEAN HYGIENE TECH AS

Skjergardsvegen 333

5353 Straume, Norway

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Signed by: Mateusz Latosiński Eng.
Signed by QES

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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 aim of the study was to assess the biocidal efficacy of the product in relation to *Poliovirus type 1,* LSc 2ab (NIBSC 10/164), *Murine norovirus* (S99), *Adenovirus* type 5 (ATCC VR-5).

3. FORMAL BASIS

The assessment of antibacterial efficacy was carried out on the basis of the agreement/order dated 21 August 2020 (agreement number: AFC/019208/08/20/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 14476+A2:2019-08 Chemical disinfectants and antiseptics – quantitative suspension method for the evaluation of virucidal effect in the medical area – test method and requirements (Phase 2 / Stage 1)

5. SAMPLE IDENTIFICATION

The tested sample was the biocidal product in the form of a ready-to-use liquid. The preparation was accepted for testing on 21 August 2020. Sample code assigned by the laboratory: 060/21/08/20.

Product name: Oxine 50ppm for surface desinfektion

Lot No.: 202003002

Product reference number: N/A

Manufacturer:

Bio Cide International, Inc.

2845 Broco Drive, Norman, OK 73072 USA

Date of manufacture: 02.Mars 2020

Expiry date: 12 months from production date

Product appearance: Clear liquid. Nearly odourless.

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:

- Sodium (CAS: 7440-23-5); <0,2 % weight
- Chlorine Dioxide (CAS: 10049-04-4); <0,01 % weight
- Citric Acid (CAS: 77-92-9); <0,01 % weight

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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, 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: 15.10.2020 – 19.10.2020

Identification of the microbial strains and cell lines:

Virus strain	Collection catalogue number	Cell line	Collection catalogue number
Poliovirus type 1 LSc 2ab	NIBSC 10/164	HeLa	ATCC CCL-2
Murine norovirus	S99 Berlin	RAW 264.7	ATCC TIB-71
Adenovirus type 5	ATCC VR-5	HeLa	ATCC CCL-2

Incubation for 72h at 37 °C ± 1 °C + 5%CO2

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

Test temperature: 20°C ± 1°C

Duration of the product contacting the microbial suspension: 60 s ± 10 s

Interfering substances: beef albumin 0.3g/l

Solvent used during the test:

Hard water according to PN-EN 14476+A2:2019

Stability of product mixture with solvent:

No precipitation formed during the test.

Reference biocidal product: 0.7% (w/v) glutaraldehyde

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6.2 TESTING METHOD AND VALIDATION

Method used: neutralisation of solutions

Counting method: observation of cytopathic effect

Neutraliser used, composition:

- Eagle minimal medium (MEM)

Substrates used:

- Eagle minimal medium (MEM) + 10% FCS - medium used to multiply cell lines,

- Eagle minimal medium (MEM) + 2% FCS - medium used to determine the product's activity, cytotoxicity, susceptibility of tested strains to glutaraldehyde, and to determine the concentration of viral strains (the medium was prepared with 4% FCS concentration, for the final concentration of FCS to be at 2%).

7. TESTS RESULTS

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

Table 1. Results of validation tests

Organizm testowy	***a***	**A**	**B**	**C**	**D**
Poliovirus type 1 LSc 2ab	**N: 8,55**	<10%	**8,34**	**1,8**	**3,7**
Murine norovirus	**N: 8,24**	<10%	**8,12**	**1,8**	**2,8**
Adenovirus type 5	**N: 8,31**	10%	**8,33**	**4,0**	**4,9**

***a*-** the titre of the virus used for testing expressed in logarithmic form.
A- cytotoxic concentration of the tested product (w/v).
B- control of the virus titre using PBS instead of the tested product.
C- Reduction of the virus titre after 30 minutes testing with the reference biocidal product.
D- Reduction of the virus titre after 60 minutes testing with the reference biocidal product.

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Table 2. Audit results

Organizm testowy	*a*	Wyniki dla poszczególnych stężeń w % objętościowych produktu (warunki badania: czas kontaktu: 60 s, temperatura: 20°C ± 1°C)					
		80%		1%		0,1%	
Poliovirus type 1 LSc 2ab	**N: 8,55**	***b:* 4,09**	**R*:* 4,46**	***b:* 6,35**	**R*:* 2,20**	***b:* 8,14**	**R*:* 0,41**
Murine norovirus	**N: 8,24**	***b:* 2,53**	**R*:* 5,71**	***b:* 7,12**	**R*:* 1,12**	***b:* 8,05**	**R*:* 0,19**
Adenovirus type 5	**N: 8,31**	***b:* 1,87**	**R*:* 6,44**	***b:* 6,78**	**R*:* 1,53**	***b:* 7,86**	**R*:* 0,45**

R- *a-b achieved* reduction of the virus titre.
b- remaining virus titre after time of contact t

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Specific comments:

Verification of methodology – requirements and limits:

- The titre of virus *a* used in the test is at least 10^8 (≥8 lg), or is at least high enough to demonstrate R above 4lg,
- Cytotoxicity allows to show the reduction of virus titre >4 lg,
- B is less than *a* by not more than 1,
- C is between 0.5 and 2.5; 1.0 and 3.0; 3.0 and 5.0 for respectively *Poliovirus* type 1 LSc 2ab, *Murine norovirus, Adenovirus* type 5.
- D is between 2.0 and 4.5; 2.0 and 4.0; 3.5 and 5.5 for respectively *Poliovirus* type 1 LSc 2ab, *Murine norovirus, Adenovirus* type 5.

8. CONCLUSIONS

The product tested in accordance with PN-EN 14476:2013+A2:2019 standard, during 60 s, in temperature of 20°C, diluted in hard water, with the presence of interfering substance, shows Virucidal efficacy antiviral effect (reduction ≥4 lg) in relation to:

Poliovirus type 1 LSc 2ab	NIBSC 10/164	at 80% concentration
Murine norovirus	S99 Berlin	at 80% concentration
Adenovirus type 5	ATCC VR-5	at 80% concentration

Date issued: 19-10-2020

Report prepared by: Jakub Jałówko, M.Sc

The results were authorised by: Mateusz Latosiński, Eng

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