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Coolhands BV.

Subject

Screening test

Please find enclosed the results of the disinfection screening tests TNO performed with your product on two bacteria, *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442. The product was received from Coolhands BV on 12th of April 2016. The product was tested in three concentrations (0.5%, 1.5% and 3%) and during three different contact times (1 min., 5 min. and 10 min).

The screening test was performed applying the exposure condition described in NEN-EN 1276 "Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)" without the validation tests. The requirements for a disinfectant is showing a reduction > 5 Log units for the bacteria in the test. The results of the screening test are shown in Table 1.

From the results we conclude:

1. The product shows bactericidal activity on *Staphylococcus aureus* at a concentration of 0.5%, 1.5% and 3% (v/v) in 1 minute exposure time in distilled water at 20°C.
2. The product shows bactericidal activity on *Pseudomonas aeruginosa* at a concentration of 1.5% (v/v) and 3% (v/v) in distilled water in 1 minute exposure time at 20°C and in 5 minutes exposure time at a concentration of 0.5% (v/v) in distilled water at 20°C.

The test results are valid for this test subject only.

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Our reference

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Project number

060.20946/01.39.01

The General Terms and Conditions for commissions to TNO, as filed with the Registry of the District Court in the Hague and with the Chamber of Commerce and Industry in The Hague, shall apply to all commissions to TNO.

Our General Terms and Conditions are also available on our website www.tno.nl. A copy will be sent upon request.

Trade register number 27376655

Since the product has achieved the target minimum reduction of 5 log units, this is encouraging for further work which is necessary toward registration of the product as a disinfectant for specific applications.

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Yours faithfully,



M. Heerikhuisen
Project manager Microbiology

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Table 1. Test results (bactericidal suspension test)

Test organism: Staphylococcus aureus ATCC 6538
Incubation temperature: 37°C

Test suspension (N and No):	N	Vc1	Vc1	Xg(wm) = 5,7x10 ⁸ logN = 8.76 No=N/10 logNo = 7.76 7,17 ≤ logNo ≤ 7,70? yes <input type="checkbox"/> no <input checked="" type="checkbox"/> V
	10 ⁶	>300	>300	
	10 ⁷	65	49	

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
0,5%	10 ⁰	<14	<14	<14	1.15	6.61	1	10 ⁰	<14	<14	<14	1.15	6.61	5	10 ⁰	<14	<14	<14	1.15	6.61	10
	10 ⁻¹							10 ⁻¹							10 ⁻¹						
	10 ⁻²							10 ⁻²							10 ⁻²						
	10 ⁻³							10 ⁻³							10 ⁻³						

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
1,5%	10 ⁰	<14	<14	<14	1.15	6.61	1	10 ⁰	<14	<14	<14	1.15	6.61	5	10 ⁰	<14	<14	<14	1.15	6.61	10
	10 ⁻¹							10 ⁻¹							10 ⁻¹						
	10 ⁻²							10 ⁻²							10 ⁻²						
	10 ⁻³							10 ⁻³							10 ⁻³						

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
3%	10 ⁰	<14	<14	<14	1.15	6.61	1	10 ⁰	<14	<14	<14	1.15	6.61	5	10 ⁰	<14	<14	<14	1.15	6.61	10
	10 ⁻¹							10 ⁻¹							10 ⁻¹						
	10 ⁻²							10 ⁻²							10 ⁻²						
	10 ⁻³							10 ⁻³							10 ⁻³						

Legend

- N = number of cfu/ml of the bacterial test suspension
- No = number of cfu/ml in the test suspension at the begin of the exposure time
- R = reduction of viability
- Vc = viable count
- Na = number of cfu/ml in the test mixture at the end of the exposure time

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Table 1. Test results (bactericidal suspension test)

Test organism: *Pseudomonas aeruginosa* ATCC 15442
Incubation temperature: 37°C

Test suspension (N and No):	N	Vc1	Vc1	$Xg(wm) = 7,5 \times 10^8$ $\log N = 8.87$ $No = N/10$ $\log No = 7.87$ $7,17 \leq \log No \leq 7,70?$ yes <input type="checkbox"/> no <input type="checkbox"/> V
	10^{-6}	>300	>300	
	10^{-7}	69	81	

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
0,5%	10^0	>330	>330	169	3.23	4.64	1
	10^{-1}	169	168				
	10^{-2}	17	18				
	10^{-3}						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10^0	110	116	115	2.06	5.81	5
10^{-1}	14	12				
10^{-2}						
10^{-3}						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10^0	35	39	37	1.57	6.3	10
10^{-1}						
10^{-2}						
10^{-3}						

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
1,5%	10^0	<14	<14	<14	1.15	6.72	1
	10^{-1}						
	10^{-2}						
	10^{-3}						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10^0	<14	<14	<14	1.15	6.72	5
10^{-1}						
10^{-2}						
10^{-3}						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10^0	<14	<14	<14	1.15	6.72	10
10^{-1}						
10^{-2}						
10^{-3}						

Concentration of the product	Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
3%	10^0	<14	<14	<14	1.15	6.72	1
	10^{-1}						
	10^{-2}						
	10^{-3}						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10^0	<14	<14	<14	1.15	6.72	5
10^{-1}						
10^{-2}						
10^{-3}						

Dilution step	Vc1	Vc2	Na = (Xg(wm))	lg Na	lg R (lg No - lg Na)	Contact time (min)
10^0	<14	<14	<14	1.15	6.72	10
10^{-1}						
10^{-2}						
10^{-3}						

Legend

- N = number of cfu/ml of the bacterial test suspension
- No = number of cfu/ml in the test suspension at the begin of the exposure time
- R = reduction of viability
- Vc = viable count
- Na = number of cfu/ml in the test mixture at the end of the exposure time