- Cover sheet / Short test report -

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This summary report is based on the test:

- Examination of the virucidal activity of the disinfectant product *TSS40-06AD* against *Vacciniavirus (Elstree)* using the suspension test method following EN 14476:2019 - Screening test S4 at a temperature of T = 20 °C from 24.06.2020

The present summary report consists of the following parts:

1. Cover sheet [1 page]. . . . . ☒
2. Summary of the test protocol(s) [2 pages]. . . . . ☒

Co-applicable documents:

1. DIN EN 14476:2019
2. Finalised test protocol of the screening test S4 dated 14.07.2020

This summary report has been finalised and released:

Date/Signature: 13.09.2020 _____

Dr. Ch. Jursch, Laboratory Manager

Information about the testing

Principal:	EcoWays Umwelt Innovationen GmbH	*Test run:*	S4 / Vacciniavirus
Product(s):	TSS40-06AD	*Test date:*	24.06.2020
Test system:	Vacciniavirus *(Elstree)* + Vero-76 cells	*Analysis:*	08.07.2020 *(14 p.i.)*

Test methodology and test parameters

Test method: Screening test using the methodology of the EN 14476 (quantitative virucidal suspensions test)
Test mixture: 1 VT protein load + 1 VT virus suspension + 8 VT neat product
Protein load: „clean conditions“ *(low protein load)*
Test parameter: T = 20 °C / t = 60 min.

Tested product sample(s)

1st product: *TSS40-06AD* *[Product sample tested as received; Arrival: 29.04.2020; Storage at RT; the product under test was introduced into the experimental test with a preliminary product designation]*

Tab. 1: Weight of content

Set	Product (s)	Product conc.	Product conc. in test	Dosage	pH [1] of Working Sol.
#1	*TSS40-06AD*	undiluted *(neat product)*	80%	n.a.	*not reliably measurable*

[1] = pH was constantly drifting. The composition of the product under test was not communicated but this effect is typical for e.g. quarternary ammonium compounts which covers the pores of the electrode.

Test system:

- Vacciniavirus (strain: Elstree)
  (Origin: Institute of virology and antiviral therapy of the University of Jena, Germany)
- Vero-76 cells
  (Origin: Institute of virology and antiviral therapy of the University of Jena, Germany)

Test results

1. Annotations:

- No changes were made to the test plan
- No abnormalities were observed.

2. Virus titration:

Tab. 2.1: Virus control *(Virus titration: by limiting dilution)*

Samples	VK-1a	VK-1b	Ø
	Virus control		
Titer/test vol. ($lg\ ID_{50}$) [1]	5,95 ± 0,49	5,95 ± 0,57	**5,95**
Average ± CI (95%) [1]	**6,95 ± 0,39 / mL**		

[1] = calculation of virus titer and its 95% confidential interval was performed according to EN 14476:2019

Tab. 2.2: Cytotoxicity control *(Virus titration: by limiting dilution)*

Samples	Tox Test	T-1
	data of cytotoxicity test *(T1; 18.06.2020)*	Cytotoxicity *(actual test run)*
Titer/test vol. ($lg\ ID_{50}$) [1]	3,45 / mL	3,45 / mL

[1] = calculation of virus titer and its 95% confidential interval was performed according to EN 14476:2019.

***Tab. 2.3*: Virus inactivation** (Virus titration: by limiting dilution)*

Samples	In-1a	In-1b	**Ø**
	Inactivation: neat / 60 min. / CC		
Titer/test vol. ($lg\ ID_{50}$)	≤ 2,45	≤ 2,45	**≤ 2,45**
Average ± CI (95%) [1]	≤ 3,45 / mL		
Reduction [2] ($lg\ ID_{50}$ ± CI [95%])	**≥ 3,50 ± 0,39**		

[1] = virus titer and its 95% confidental interval were calculated according to EN 14476:2019
[2] = virus reduction = titer of virus control ($lg\ ID_{50}$) minus titer of sample ($lg\ ID_{50}$)

***Tab. 2.4*: Virus inactivation** (Virus titration: by Large Volume Plating [LVP])*

Samples	In-1
	Inactivation: neat / 60 min. / CC
Dilution factor (VF)	1.000
Sample vol. analysed	10 µL + 10 µL = 20 µL
Cell cultures inoculated	96
Virus positive cells	0
Virus input [mL]	6,95 ± 0,39
Titer/test vol. ($lg\ ID_{50}$)	≤ 2,33 / mL
Reduction [2] ($lg\ ID_{50}$ ± CI [95%])	**≥ 4,62 ± 0,39**

[1] = virus titer and its 95% confidential interval were calculated according to EN 14476:2019
[2] = virus reduction = titer of virus control ($lg\ ID_{50}$) minus titer of sample ($lg\ ID_{50}$)

Conclusions:

- When the product ***TSS40-06AD*** (neat product) was introduced into test under „clean conditions“ no residual test virus was detected above the cytotoxicity level ($lg\ TD_{50}$ = 3,45/mL; cf. Tab. 2.3).
- Using the LVP-titration method also no residual virus was detected with the 1.000-fold diluted test sample. With the Poisson-formula applied the (virtual) virus titer amounted to $lg\ ID_{50} \leq 2,33$/mL, corresponding to a virus reduction of RF ≥ 4,62 ± 0,34 (cf. Tab. 2.4).
- Based on the present data as obtained in the corresponding screening test and to my opinion a complete validation test according to EN 14476 vs. Vacciniavirus (virucidal claim: "virucidal active against enveloped viruses") can be striven with the tested product. With this virucidal claim a virucidal activity against all enveloped virus is assumed (e.g. HIV, HBV, HCV, the influenza viruses, the corona viruses [incl. SARS-CoV-2] and others).
- From the data obtained it cannot be concluded whether or not a virucidal activity against the non-enveloped viruses is also given (virus claim: "limited virucidal PLUS" or "virucidal").