- 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 products *TSS40-06AD* against the *Transmissible Gastroenteritis Virus of Swine (TGEV; strain: Toyama 36)* 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) [3 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 _____

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Information about the testing

Principal:	EcoWays Umwelt Innovationen GmbH	*Test run:*	S4 / TGEV-Coronavirus
Product(s):	TSS40-06AD	*Test date:*	24.06.2020
Test system:	TGEV *(Toyama 36)* + ST75/2 cells	*Analysis:*	01.07.2020 *(7 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 compounds which covers the pores of the electrode.

Test system:

- Transmissible Gastroenteritis Virus of Swine (TGEV); strain: Toyama 36
  (Origin: Virusbank der BFA f. Viruskrankheiten der Tiere; Friedrich Löffler-Institut, Insel Riems, Germany)
- ST75/2 cells (foetal testis cells of swine)
  (Origin: Robert Koch-Institute, Berlin, 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]	5,43 ± 0,35	5,43 ± 0,35	**5,43**
Average ± CI (95%) [1]	**6,43 ± 0,25 / 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	2,75 / 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**
Parameter	Inactivation		
Titer/Test vol. ($lg\ ID_{50}$)	≤ 1,75	≤ 1,75	**≤ 1,75**
Average ± K (95%) [1]	≤ 2,75/mL		
Reduction [2] ($lg\ ID_{50}$ ± CI [95%])	**≥ 3,68 ± 0,25**		

[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 ($lg\ ID_{50}$)

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

Samples	In-1
	Inactivation
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]	6,43 ± 0,25
Titer/test vol. ($lg\ ID_{50}$)	≤ 2,33 / mL
Reduction [2] ($lg\ ID_{50}$ ± CI [95%])	**≥ 4,10 ± 0,25**

[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 ($lg\ TD_{50}$ = 2,75/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 ≥ 4,10 ± 0,25 (cf. Tab. 2.4).
- With the present screening test the TGEV-Coronavirus (cf. Test system) was used as the test virus. This virus is a member of the virus family *coronaviridae* to which SARS-CoV and SARS-CoV-2 also belong. Based on the data as obtained a basic virucidal activity against the coronaviruses was demonstrated.
- With respect to product validation a complete validation test according to EN 14476 vs. coronavirus (virus claim: specifically virucidal active against coronaviruses) can be striven.
- In my opinion, however, a complete validation test according to EN 14476 vs. Vacciniavirus is more advantageous. With a successful testing vs. Vacciniavirus a virucidal activity against all enveloped virus can be claimed. This virucidal claim includes (for example) HIV, HBV, HCV, the influenza viruses, the corona viruses [incl. SARS-CoV-2] and others.