Competent Authority Report

ADDENDUM to Document IIIA, Section 7

Study Summaries Active Substance

DDAC
(CAS no.7173-51-5)

Product-types 3&4
(Veterinary hygiene; Food and feed)

eCA: Italy

September 2018

This Addendum supplements Doc. IIIA Section 7 of the Draft Competent Authority Report (CAR) which was prepared by the eCA (Italy) according to Regulation (EU) No 528/2012 for the purpose of the review of the existing biocidal active substance **didecyldimethylammonium** chloride (**DDAC**, CAS no. 7173-51-5) as Product Types 3 and 4 (Veterinary hygiene; Food and feed area).

This Addendum presents the **growth inhibition test on algae** submitted by EQC in April 2012 under PT8 in order to fill the data gap which had been remarked by the eCA-IT following the evaluation of the original Dossier.

The eCA-IT conclusions, resulting from the evaluation of the new documentation (already peer-reviewed under PT8), are available under the relevant evaluation box.

Section A7.4.1.3		Growth inhibition test on algae	
		1 REFERENCE	**Official use only**
1.1	**Reference**	[redacted]): [redacted] *Alga, Growth Inhibition Test with Pseudokirchneriella subcapitata, 72 h.* [redacted] *(IIIA7-4-1-3 - DDAC - final report - acute aglae inhibition test - SPO140621 - 8sep2011.pdf)*	
1.2	**Data protection**	*Yes*	
1.2.1	Data owner	*European Quats Consortium*	
1.2.2			
1.2.3	Criteria for data protection	*Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]*	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	*Yes, OECD 201*	
2.2	**GLP**	*Yes*	
2.3	**Deviations**	*No*	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	*As given in section 2*	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	*Only if investigation with biocidal product, give percentage of any ingredients*	
3.1.5	Further relevant properties	*Appearance:* [redacted] *Water solubility:* [redacted]	
3.1.6	Method of analysis	*Analysis of DDAC, lead compound of* [redacted]*, was performed to verify the various concentrations of the test item. Analysis was carried out via Ultra Performance LC-MS/MS on a reversed-phase column in gradient mode using external standard calibration. Detection was carried out by electrospray ionisation in positive mode*	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	*Not applicable*	
3.3	**Reference substance**	[redacted]	

<table>
<tr><td colspan="2">Section A7.4.1.3</td><td>Growth inhibition test on algae</td><td></td></tr>
<tr><td>3.3.1</td><td>Method of analysis for reference substance</td><td>[redacted]</td><td></td></tr>
<tr><td>3.4</td><td>Testing procedure</td><td>Non-entry field</td><td></td></tr>
<tr><td>3.4.1</td><td>Culture medium</td><td>
<table>
<tr><th>Component</th><th>Concentration [mg/L]</th></tr>
<tr><td>NH_4Cl</td><td>15</td></tr>
<tr><td>$MgCl_2 \cdot 6\ H_2O$</td><td>12</td></tr>
<tr><td>$CaCl_2 \cdot 2\ H_2O$</td><td>18</td></tr>
<tr><td>$MgSO_4 \cdot 7\ H_2O$</td><td>15</td></tr>
<tr><td>KH_2PO_4</td><td>1.6</td></tr>
<tr><td>$FeCl_3 \cdot 6\ H_2O$</td><td>0.064</td></tr>
<tr><td>$Na_2EDTA \cdot 2\ H_2O$</td><td>0.1</td></tr>
<tr><td>H_3BO_3</td><td>0.185</td></tr>
<tr><td>$MnCl_2 \cdot 4\ H_2O$</td><td>0.415</td></tr>
<tr><td>$ZnCl_2$</td><td>3×10^{-3}</td></tr>
<tr><td>$Na_2MoO_4 \cdot 2\ H_2O$</td><td>7×10^{-3}</td></tr>
<tr><td>$CoCl_2 \cdot 6\ H_2O$</td><td>1.5×10^{-3}</td></tr>
<tr><td>$CuCl_2 \cdot 2\ H_2O$</td><td>1×10^{-5}</td></tr>
<tr><td>$NaHCO_3$</td><td>50</td></tr>
<tr><td>pH-value</td><td>8.1 ± 0.2</td></tr>
</table>
This medium had a nominal hardness of 0.24 mmol Ca+Mg/L.</td><td></td></tr>
<tr><td>3.4.2</td><td>Test organisms</td><td>[redacted]</td><td></td></tr>
<tr><td>3.4.3</td><td>Test system</td><td>See table A7_4_1_3-3</td><td>X</td></tr>
<tr><td>3.4.4</td><td>Test conditions</td><td>See table A7_4_1_3-4</td><td></td></tr>
<tr><td>3.4.5</td><td>Duration of the test</td><td>72 hours</td><td></td></tr>
<tr><td>3.4.6</td><td>Test parameter</td><td>Cell density, inhibition of growth rate and yield</td><td></td></tr>
<tr><td>3.4.7</td><td>Sampling</td><td>From the series of concentrations the lowest, middle and the highest test concentration and the control were analytically verified at the beginning of the test. Separate replicates of the lowest, middle and highest test concentration were prepared without algae for analysis after 0 and 72 hours. Additionally two replicates of the middle concentration with algae (incubated under test conditions) were analysed after 72 hours. Sorption to the walls of the glass container was checked too.</td><td></td></tr>
<tr><td>3.4.8</td><td>Monitoring of TS concentration</td><td>Yes (see 3.4.7)</td><td></td></tr>
</table>

Section A7.4.1.3	Growth inhibition test on algae	
3.4.9 Statistics	*Growth rate* $\mu = (\ln(Nn) - \ln(N0)) / (tn - t0)$ μ = growth rate of the cell density (1/day) Nn = biomass after tn d in cells/mL $N0$ = biomass at t0 in cells/mL $t0$ = time of beginning of test tn = time of nth measurement after beginning of test *Rate related inhibition* $I\mu t = ((\mu c - \mu t) / \mu c) \cdot 100\ \%$ $I\mu t$ = rate related inhibition % μc = growth rate of the control after n days μt = growth rate of the test concentration after n days *Yield* $Y = Nn - N0$ Y = yield $N0$ = nominal number of cells/mL at the beginning of the test Nn = measured number of cells/mL at time n *Yield inhibition* $Iy = (YC - YT) / YC \cdot 100\ \%$ I = percent inhibition YC = mean value for yield in the control group YT = value for yield for the treatment replicate *EC_{10}-, EC_{20}- and EC_{50}-values of the growth rate and yield inhibition after 72 hours were calculated by maximum likelihood regression using the probit transform.* *NOEC/LOEC were determined by calculation of statistical significance of growth rate and yield. Dunnett's multiple comparison test was used. The Shapiro-Wilk-Test was used to test for normally distributed populations. Variances were tested by the Bartlett's test.*	
	4 RESULTS	
Limit Test	Not performed *(If performed, fill in 4.1.1, 4.1.2 and 4.1.3)*	
4.1.1 Concentration	*N.A.*	
4.1.2 Number/ percentage of animals showing adverse effects	*N.A.*	
Results test substance	*Non-entry field*	

Section A7.4.1.3		Growth inhibition test on algae	
4.1.3	Initial concentrations of test substance	[redacted]	
4.1.4 4.1.5	Actual concentrations of test substance	[redacted]	

Section A7.4.1.3	Growth inhibition test on algae	
4.1.6 Growth curves	[redacted]	

Section A7.4.1.3		Growth inhibition test on algae	
4.1.7	Concentration / response curve	[redacted]	
4.1.8	Cell concentration data	*See table A7_4_1_3-5*	

Section A7.4.1.3	Growth inhibition test on algae	
4.1.9 Effect data (cell multiplication inhibition)	[redacted]	
4.1.10 Other observed effects	[redacted]	
Results of controls	*See table A7_4_1_3-5*	
Test with reference substance	*Performed*	
4.1.11 Concentrations	[redacted]	
4.1.12 Results	[redacted]	
	5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	[redacted]	

Section A7.4.1.3		Growth inhibition test on algae	
5.2	**Results and discussion**	*Summarize relevant results; discuss relevant test material-specific properties (e.g. solubility, stability, adsorption behaviour, volatility)*	
5.2.1	NOE_rC	*Rate-related inhibition: 0.0340 mg/L* *Inhibition of yield: 0.0150 mg/L*	X
5.2.2	E_{r50}	*Rate-related inhibition: 0.156 (0.0981 – 0.249) mg/L*	X
5.2.3	E_bC_{50}	*Inhibition of yield: 0.0435 (0.0252 – 0.0723) mg/L*	X
5.3	**Conclusion**	[redacted] *was found to inhibit the growth of the freshwater green alga Pseudokirchneriella subcapitata after 72 hours with the following effect values: The EC50-values with 95 % confidence intervals for inhibition of specific growth rate (ErC50) and yield (EyC50) after 72 hours were 0.156 (0.0981 – 0.249) mg/L and 0.0435 (0.0252 – 0.0723) mg/L, respectively. The EC10-values with 95 % confidence intervals for inhibition of specific growth rate (ErC10) and yield (EyC10) after 72 hours were 0.0514 (0.00848 – 0.0862) mg/L and 0.0128 (0.00137 – 0.0230) mg/L, respectively. The NOEC-values for inhibition of specific growth rate and yield after 72 hours were 0.034 mg/L and 0.015 mg/L, respectively. After 72 hours algae were transferred from the nominal test item concentrations 0.0750 – 0.800 mg/L and the control to fresh untreated medium and allowed to grow for further 4 – 7 days under test conditions. The test item effect was observed to be reversible at these concentrations. Therefore, there is potential for recovery following exposure up to 0.800 mg/L (highest test concentration). All effect values are given based on nominal test item concentrations* [redacted]	
5.3.1	Reliability	*1 (reliable without restriction)*	X
5.3.2	Deficiencies	*No*	X

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	Acceptable. NOEC values should be interpreted with caution. EC_{10} values might be underestimated.

Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Tables for Applicant's Summary and Conclusion

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	No
Concentration of vehicle	*n.a.*
Vehicle control performed	n.a.
Other procedures	n.a.

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Pseudokirchneriella subcapitata HINDÁK*
Strain	*SAG 61.81*
Source	*Sammlung von Algenkulturen (SAG)* *Pflanzenphysiologisches Institut der Universität Göttingen, Nikolausberger Weg 18, D-37073 Göttingen*
Laboratory culture	*Yes*
Method of cultivation	*Fresh stocks were prepared every month on Z-Agar. Light intensity amounted 35-70 µE ·m^{-2} ·s^{-1} for 24 hours per day.*
Pretreatment	*A three day old exponential growing preculture was used as inoculum. Incubation was performed in 500 mL Erlenmeyer flasks with dilution water. The preculture was exposed to the same environmental conditions relative to the definitive test.*
Initial cell concentration	*9846 cells/mL*

Table A7_4_1_3-3: Test system

Criteria	Details
Volume of culture flasks	[redacted]
Culturing apparatus	[redacted]
Light quality	[redacted]
Procedure for suspending algae	[redacted]
Number of vessels/ concentration	[redacted]
Test performed in closed vessels due to significant volatility of TS	[redacted]

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	[redacted]
pH	[redacted]
Aeration of dilution water	[redacted]
Light intensity	[redacted]
Photoperiod	[redacted]

Table A7_4_1_3-5: Cell concentration data

Test-Substance Concentration (nominal[1] [mg/l]	Cell concentrations (mean values) [cells/ml]							
	Measured				Percent of control			
	0 h	24 h	48 h	72 h	0 h	24 h	48 h	72 h
0.800	9846	< 9846	< 9846	< 9846	[redacted]			
0.364	9846	< 9846	< 9846	< 16860				
0.165	9846	< 9846	33030	244796				
0.0750	9846	15995	67409	482631				
0.0340	9846	26979	179767	1208609				
0.0150	9846	34026	264867	1661689				
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]				
Temperature [°C]	See above							
pH	See above							

[1] specify, if TS concentrations were nominal or measured