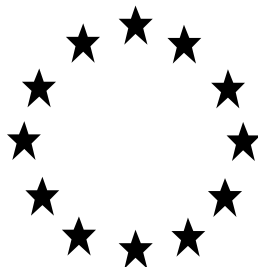


Competent Authority Report



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 **Activated Sludge Respiration Inhibition Test** submitted in September 2012 as part of the dossier for DDAC in PTs1-4. Since for DDAC in PT8 a data gap for micro-organisms in STP was concluded following the evaluation of the original dossier, upon EQC permission the eCA used this study also under PT8 and filled in the gap.

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

Section A7.4.1.4 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4**

			Official use only
1 REFERENCE			
1.1 Reference	OECD Guideline 209: Activated Sludge, Respiration Inhibition Test (2010)		X
1.2 Data protection	Yes		
1.2.1 Data owner	EUROPEAN QUATS CONSORTIUM P/A Akzo Nobel Chemicals B.V. Postbus 247 3800 AE Amersfoort The Netherlands		
1.2.2			
1.2.3 Criteria for data protection			
2 GUIDELINES AND QUALITY ASSURANCE			
2.1 Guideline study	Yes		
2.2 GLP	Yes		
2.3 Deviations	None		
3 MATERIALS AND METHODS			
3.1 Test material	[REDACTED]		
3.1.1 Lot/Batch number	[REDACTED]		
3.1.2 Specification	[REDACTED]		
3.1.3 Purity	[REDACTED]		
3.1.4 Composition of Product	-		
3.1.5 Further relevant properties	[REDACTED]		
3.1.6 Method of analysis			
3.2 Preparation of TS solution for poorly soluble or volatile test substances	-		
3.3 Reference substance	Yes [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]		

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Section A7.4.1.4 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4**

3.3.1 Method of analysis
for reference
substance

3.4 Testing procedure

3.4.1 Culture medium Synthetic waste water according to OECD 209

3.4.2 Inoculum /
test organism

Criteria	Details
Nature	Activated sludge
Species	N/A
Strain	N/A
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	████████████████████ ████████████████████
Laboratory culture	No
Method of cultivation	None
Preparation of inoculum for exposure	The sludge was washed twice with chlorine free tap water. The sludge was aerated and used within 24 h after sampling.
Pretreatment	See above
Initial cell concentration	████████████████████ ████████████████████ ████████████████████

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Section A7.4.1.4 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4**

3.4.3 Test system

Criteria	Details
Culturing apparatus	500 mL Erlenmeyer flasks
Number of culture flasks/concentration	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]
Aeration device	[REDACTED] [REDACTED] [REDACTED]
Measuring equipment	pH-meter, Multi 350 I, WTW Oximeter, Oxi 197-S, WTW Flat bed recorder, L250 E, LINSEIS
Test performed in closed vessels due to significant volatility of TS	No

3.4.4 Test conditions

Criteria	Details
Test temperature	[REDACTED]
pH of the activated sludge	[REDACTED]
pH of the synthetic waste water	[REDACTED]
Aeration of dilution water	[REDACTED]
Dry solid concentration [g/L] of sludge	[REDACTED]
Dry solid concentration [g/L] in test solution	[REDACTED]

3.4.5 Duration of the test 2012-08-16

3.4.6 Test parameter Respiration inhibition

3.4.7 Analytical parameter Oxygen depletion

3.4.8 Sampling

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

Section A7.4.1.4 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4**

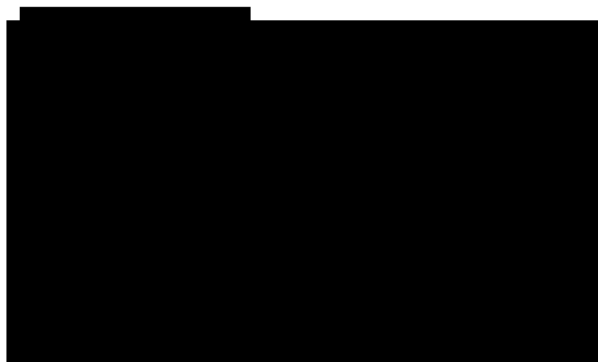
3.4.9	Monitoring of TS concentration	Yes, at test start	X
3.4.10	Controls		
3.4.11	Statistics	Evaluation: <div style="background-color: black; height: 15px; width: 100%;"></div> <div style="background-color: black; height: 15px; width: 95%;"></div> <div style="background-color: black; height: 15px; width: 85%;"></div> <div style="background-color: black; height: 15px; width: 100%;"></div> <div style="background-color: black; height: 15px; width: 65%;"></div> <div style="background-color: black; height: 15px; width: 25%;"></div>	
<p>The oxygen uptake rate (R) according to the raw data was calculated by formula (1).</p>			
$R = md \cdot f \cdot t \quad (1)$			
<p>R : oxygen uptake rate [mg O₂/L·h] md : measured distance [mm/3 min] f : 0.02 mg O₂/L·mm t : 20 [3 min/h]</p>			
<p>The inhibition of respiration rates was calculated by a standard method:</p>			
$\text{Inhibition [\%]} = \left[1 - \frac{R}{R_{c_{1-6}}(mv)} \right] \cdot 100 \quad (2)$			
<p>R : oxygen uptake rate of test concentration [mg O₂/L·h] R_{c₁₋₆}(mv) : mean oxygen uptake rate of controls 1-6 [mg O₂/L·h]</p>			
<p>Statistical calculation The NOEC was determined by calculation of statistical significance of the inhibition of respiration in comparison to the control. One Way Analysis of Variance (ANOVA) and DUNNETT's test was used for NOEC calculations. When running a One Way Analysis of Variance a Normality test and an Equal Variance test were done first. P-values for both Normality and Equal Variance test are 0.05. The α-value (acceptable probability of incorrectly concluding that there is a difference) is α=0.05. The EC-values of the test item were calculated by sigmoidal dose-response regression and the EC₅₀-value of the reference item by linear regression using software GraphPadPrism. Calculations of the confidence intervals for the EC-values were carried out using standard procedures.</p>			

Section A7.4.1.4 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4****4 RESULTS****Preliminary test**

Performed

4.1.1 Concentration

4.1.2 Effect data

**Results test substance***Non-entry field*4.1.3 Initial
 concentrations of
 test substance4.1.4 Actual
 concentrations of
 test substance

N/A

4.1.5 Growth curves

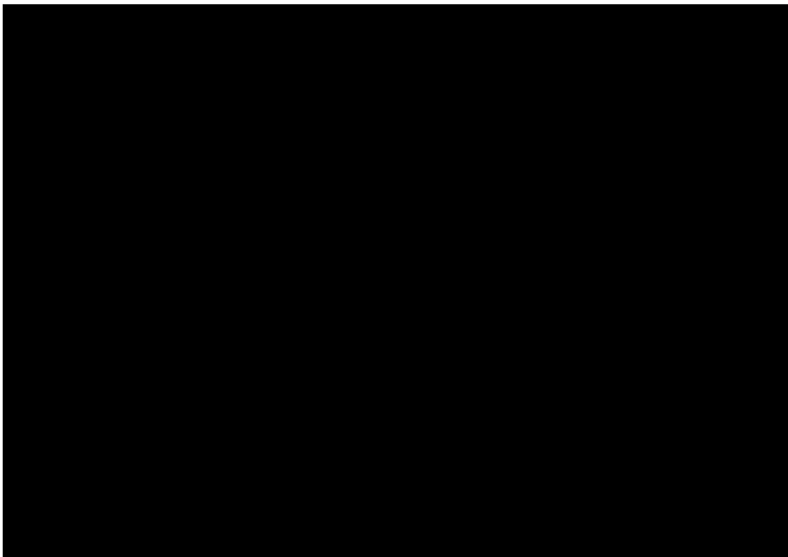
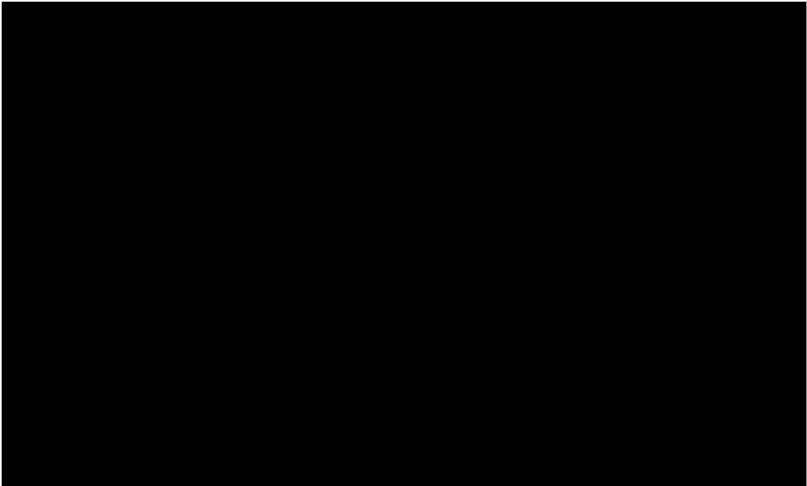


N/A

4.1.6 Cell concentration
 data

N/A

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.1.7 Concentration/ response curve	
4.1.8 Effect data	
4.1.9 Other observed effects	-
Results of controls	
Test with reference substance	Performed
4.1.10 Concentrations	
4.1.11 Results	EC ₅₀ = 115 mg/L 95 % confidence interval = 108 - 124 mg/L

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	Materials and methods	Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines
5.2	Results and discussion	Summarize relevant results; discuss relevant test material-specific properties (e.g. solubility, stability, adsorption behaviour, volatility) as well as any observations affecting test results
5.2.1	EC ₁₀	5.95 (5.22 - 6.73)
5.2.2	EC ₂₀	8.90 (8.06 - 9.89)
5.2.3	EC ₅₀	17.9 (16.4 - 19.5)
5.3	Conclusion	The validity criteria were fulfilled: [Redacted] [Redacted] [Redacted] [Redacted] [Redacted] [Redacted] [Redacted] [Redacted] [Redacted] [Redacted] [Redacted]
5.3.1	Reliability	[Redacted] [Redacted]
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[Redacted]
Materials and Methods	[Redacted] [Redacted] [Redacted] [Redacted]
Reference	[Redacted]. DDAC - Respiration Inhibition Test with Activated Sludge [Redacted]
Results and discussion	No comment
Conclusion	No comment
Reliability	1

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Section A7.4.1.4 Inhibition to microbial activity (aquatic)**Annex Point IIA7.4**

Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	<i>Give date of comments submitted</i>
Materials and Methods	<i>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</i>
Results and discussion	<i>Discuss if deviating from view of rapporteur member state</i>
Conclusion	<i>Discuss if deviating from view of rapporteur member state</i>
Reliability	<i>Discuss if deviating from view of rapporteur member state</i>
Acceptability	<i>Discuss if deviating from view of rapporteur member state</i>
Remarks	