

**Section A7.4.1.4 Inhibition to microbial activity (aquatic)****Annex Point IIA VII.7.4**

			Official use only
<b>1 REFERENCE</b>			
<b>1.1 Reference</b>		G. Mueller, 2001, Preventol A 4-S Toxicity to Bacteria, Bayer AG, Institute for Environmental Analysis and Evaluation, Leverkusen, Germany, Study number 1135 A/01 B (unpublished), 2001-10-01	
<b>1.2 Data protection</b>		Yes	
1.2.1 Data owner		Bayer Chemicals AG	
1.2.2 Companies with letter of access		-	
1.2.3 Criteria for data protection		Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
<b>2 GUIDELINES AND QUALITY ASSURANCE</b>			
<b>2.1 Guideline study</b>		Yes  Commission Directive 88/302/EEC, Part C. This test method is in most parts identical with OECD guideline No. 209	
<b>2.2 GLP</b>		Yes	
<b>2.3 Deviations</b>		No,  the study is comparable to OECD guideline No. 209	
<b>3 MATERIALS AND METHODS</b>			
<b>3.1 Test material</b>		As given in Section 2 of dossier	
3.1.1 Lot/Batch number		Batch number: [REDACTED]	
3.1.2 Specification		As given in Section 2 of dossier	
3.1.3 Purity		[REDACTED]	X
3.1.4 Composition of Product		-	
3.1.5 Further relevant properties		-	
3.1.6 Method of analysis		Test substance concentrations are not confirmed by analytical method	X
<b>3.2 Preparation of TS solution for poorly soluble or volatile test substances</b>		Type of application of the test substance: direct weighing.  The test substance has been added to about 130 ml deionised water and stirred overnight before testing (equilibration phase).	
<b>3.3 Reference substance</b>		Yes,  3,5-Dichlorophenol	
3.3.1 Method of analysis for reference substance		Reference substance concentrations are not confirmed by analytical method	X

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3.4.1	Culture medium	Synthetic medium	X
3.4.2	Inoculum / test organism	see table A7_4_1_4-1	
3.4.3	Test system	No data	X
3.4.4	Test conditions	see table A7_4_1_4-2	
3.4.5	Duration of the test	3 hours with permanent aeration	
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Oxygen measurement	
3.4.8	Sampling	The oxygen concentration was measured in the controls and in every concentration of the test and reference substance at the beginning and at the end of the test period.  pH and temperature were determined in the controls and in every test concentration of test and reference substance during the test period.	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Two controls without test substance are included in the test design, one at the start and the other at the end of the test series.  A physico - chemical oxygen consumption control with a test substance concentration of 10000 mg/l was carried out, since some substances can also consume oxygen by chemical reactivity.	
3.4.11	Statistics	An EC <sub>50</sub> value is calculated from determinations at different concentrations using statistical methods (probit analysis).	

**4      RESULTS**

<b>4.1</b>	<b>Preliminary test</b>	Not performed	
4.1.1	Concentration	-	
4.1.2	Effect data	-	
<b>4.2</b>	<b>Results test substance</b>		
4.2.1	Initial concentration of test substance	Nominal concentrations: 5.6, 10, 18, 32 and 56 mg/l	
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical methods	
4.2.3	Growth curves	No graph available	
4.2.4	Cell concentration data	Not reported	

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4.2.5	Concentration/ response curve	Concentration/response curves (inhibition vs. concentration) are given in the report on page 17 (test substance) and on page 20 (reference substance)
4.2.6	Effect data	EC <sub>50</sub> = 19 mg/l
4.2.7	Other observed effects	-
<b>4.3</b>	<b>Results of controls</b>	No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration.
<b>4.4</b>	<b>Test with reference substance</b>	Performed with 3,5-Dichlorophenol
4.4.1	Concentrations	2.5, 5, 10, 20 and 40 mg/l
4.4.2	Results	EC <sub>50</sub> = 12 mg/l

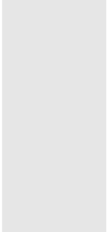
**5      APPLICANT'S SUMMARY AND CONCLUSION**

<b>5.1</b>	<b>Materials and methods</b>	<p>To assess the toxicity of dichlofluanid to bacteria a test was investigated according to the Commission Directive 88/302/EEC, Part C. This method is in most parts identical with OECD guideline No. 209. Activated sludge was exposed to dichlofluanid at different concentrations. The respiration rate of each mixture was determined after aeration periods of 3 hours.</p> <p>The test shows no significant deviations from the OECD guideline No. 209.</p>
<b>5.2</b>	<b>Results and discussion</b>	<p>50% inhibition of respiration was determined at EC<sub>50</sub> = 19 mg/l dichlofluanid.</p> <p>No physico – chemical oxygen consumption has been determined at 10000 mg/l test substance concentration. Therefore lower concentrations of the test substance cause no physico – chemical oxygen consumption (deduced values).</p> <p>At nominal test concentrations of 5.6 – 56 mg/l, inhibition of respiration in activated sludge was observed between 23.4% and 76.4% (see table A7_4_1_4-3)</p>
5.2.1	EC <sub>20</sub>	-
5.2.2	EC <sub>50</sub>	19 mg/l
5.2.3	EC <sub>80</sub>	-
<b>5.3</b>	<b>Conclusion</b>	<p>All validity criteria of the test method were met:</p> <ul style="list-style-type: none"> <li>* respiratory rate of the two controls differs less than 15%</li> <li>* respiratory rate of the controls is &lt; 60 mg O<sub>2</sub>/l-h</li> <li>* EC<sub>50</sub> of the reference substance 3.5-Dichlorophenol is in the range 5–30 mg/l</li> </ul> <p>A dose – response relationship can be seen from the test.</p>

X

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5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes, Some reporting deficiencies: No data about the test system. Information incomplete about culture medium and test organism.	

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<b>Evaluation by Competent Authorities</b>	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	<b>EVALUATION BY RAPPORTEUR MEMBER STATE</b>
<b>Date</b>	13/07/06
<b>Materials and Methods</b>	Accept applicant's version noting the following minor deviations: <b>3.1.3</b> The purity was only [REDACTED]. <b>3.1.6</b> Test substance concentrations are not confirmed by analytical method, as stated by the applicant in 3.4.9. <b>3.3.1</b> Reference substance concentrations are not confirmed by analytical method. <b>3.4.1</b> Culture medium is described only as synthetic. This is highlighted as a deficiency by the applicant (5.3.2). <b>3.4.3</b> No data is given on the test system. This is highlighted as a deficiency by the applicant (5.3.2).
<b>Results and discussion</b>	Accept applicant's version
<b>Conclusion</b>	Accept applicant's version noting the following minor deviations: <b>5.2.2</b> The endpoint is in excess of the solubility of the a.s. (1.58 mg/l at 20°C)
<b>Reliability</b>	Reliability = 2
<b>Acceptability</b>	Acceptable  The lack of measured concentrations is considered a minor deficiency as the guideline does not specify measured concentrations and significant degradation of dichlofluanid would not be expected during the 3 hour test.
<b>Remarks</b>	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	<b>COMMENTS FROM ...</b>
<b>Date</b>	<i>Give date of comments submitted</i>
<b>Materials and Methods</b>	<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>
<b>Results and discussion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Conclusion</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Reliability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Acceptability</b>	<i>Discuss if deviating from view of rapporteur member state</i>
<b>Remarks</b>	



**Table A7\_4\_1\_4-1: Inoculum/Test organism**

<b>Criteria</b>	<b>Details</b>
Nature	activated sludge (mixed population of aquatic micro-organisms)
Species	-
Strain	-
Source	Waste water treatment plant treating predominantly domestic sewage
Sampling site	Aeration tank of the waste water treatment plant (Wupper area water authority)
Laboratory culture	Yes
Method of cultivation	-
Preparation of inoculum for exposure	No data
Pretreatment	Aeration of the activated sludge; daily feed with synthetic medium
Initial cell concentration	Only 280 mg suspended solids/l were used because of strong respiration of the activated sludge

**Table A7\_4\_1\_4-2: Test conditions**

<b>Criteria</b>	<b>Details</b>
Test temperature	18.6 – 19.3 °C
pH	8.1 – 8.3 7.6 (physico chemical oxygen consumption control)
Aeration of dilution water	No data
Suspended solids concentration	280 mg/l

**Table A7\_4\_1\_4-3: Test results of test substance (based on nominal concentrations) and controls**

<b>Test Compound [mg/l]</b>	<b>Respiratory Rate [mg O<sub>2</sub>/l h]</b>	<b>Inhibition [%]</b>
5.6	24.8	23.9
10	24.0	26.4
18	15.4	52.8
32	12.0	63.2
56	7.7	76.4

<b>Control</b>	<b>Respiratory rate [mg O<sub>2</sub>/l·h]</b>
Control 1	33.6
Control 2	31.5