## Section A7.4.1.4 Inhibition to microbial activity (aquatic)

**Annex Point IIA7.4**

Official use only

### 1 REFERENCE

| | | |
|---|---|---|
| **1.1** | **Reference** | L.M. Bouman, Activated sludge respiration inhibition test with PURAC HS 88, NOTOX project no. 483211, unpublished report, February 2007 |
| **1.2** | **Data protection** | Yes |
| 1.2.1 | Data owner | Purac Biochem B.V.<br>Arkelsedijk 46<br>4206 AC Gorinchem , The Netherlands |
| 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:<br>OECD Guidelines 209, ISO 8192 |
| **2.2** | **GLP** | Yes |
| **2.3** | **Deviations** | Yes, a limited test with one concentration was carried out. |

### 3 MATERIALS AND METHODS

| | | |
|---|---|---|
| **3.1** | **Test material** | As given in section 2 |
| 3.1.1 | Lot/Batch number | 0602001247 |
| 3.1.2 | Specification | As given in section 2. |
| 3.1.3 | Purity | 88.2% activated substance in solution and 100% calculated on the dried basis. |
| 3.1.4 | Composition of Product | |
| 3.1.5 | Further relevant properties | |
| 3.1.6 | Method of analysis | Not relevant |
| **3.2** | **Preparation of TS solution for poorly soluble or volatile test substances** | Not applicable |
| **3.3** | **Reference substance** | Yes: 3,5-Dichlorophenol |
| 3.3.1 | Method of analysis for reference substance | Not relevant |
| **3.4** | **Testing procedure** | |
| 3.4.1 | Culture medium | Synthetic sewage |
| 3.4.2 | Inoculum / test organism | Activated sludge from municipal sewage treatment plant that predominantly treats domestic sewage. See table A7_4_1_4-2. |
| 3.4.3 | Test system | see table A7_4_1_4-3. |
| 3.4.4 | Test conditions | see table A7_4_1_4-4 |

## Section A7.4.1.4 Inhibition to microbial activity (aquatic)

**Annex Point IIA7.4**

| | | | |
|---|---|---|---|
| 3.4.5 | Duration of the test | 3h | |
| 3.4.6 | Test parameter | Respiration inhibition | |
| 3.4.7 | Analytical parameter | Oxygen measurement | |
| 3.4.8 | Sampling | The oxygen concentration was measured continuously for 10 minutes. | |
| 3.4.9 | Monitoring of TS concentration | No | |
| 3.4.10 | Controls | One control without test substance was tested at the start and one at the end of each test series (test substance and reference test), four activity control flasks with reference substance. | |
| 3.4.11 | Statistics | % inhibition= $(1-\frac{2*Rt}{Rc(start\ test\ series)+Rc\ (end\ series)})*100\%$<br>Rc = respiration rate of the control<br>Rt= respiration rate of the test/ reference substance (mg $O_2$/l/hr) | |
| | | **4 RESULTS** | |
| **4.1** | **Preliminary test** | Not performed | |
| 4.1.1 | Concentration | Not applicable | |
| 4.1.2 | Effect data | Not applicable | |
| **4.2** | **Results test substance** | | |
| 4.2.1 | Initial concentrations of test substance | 100 mg/l (duplicate test flasks) | x |
| 4.2.2 | Actual concentrations of test substance | Not determined. | |
| 4.2.3 | Growth curves | Not determined | |
| 4.2.4 | Cell concentration data | Only given as 4.3 g/l MLSS | |
| 4.2.5 | Concentration/ response curve | Not relevant | |
| 4.2.6 | Effect data | EC50>100 mg/l. No toxic effects were found at the tested concentrations. | x |
| 4.2.7 | Other observed effects | None observed | |
| **4.3** | **Results of controls** | Oxygen consumption in blank control: 44 mg $O_2$/l/hr | |
| **4.4** | **Test with reference substance** | Performed | |
| 4.4.1 | Concentrations | 1.0, 3.2, 10, 32 | |
| 4.4.2 | Results | EC50=5.9 mg/l | x |

## 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** | Test performed according to OECD 209, with as deviation that a limit test with one concentration was carried out. | |
| **5.2** | **Results and discussion** | No inhibition of the respiration rate was observed for the test substance. The EC50 for the reference substance was 5.9 mg/l, which is within the accepted range. The variation within the controls was acceptable (<15%). | x |
| 5.2.1 | $EC_{20}$ | | |
| 5.2.2 | $EC_{50}$ | >100 mg/l | |
| 5.2.3 | $EC_{80}$ | | |
| **5.3** | **Conclusion** | The test was valid, because the reference test resulted in an acceptable EC50 and the blank controls showed limited variation (<15%) | |
| 5.3.1 | Reliability | 1 | |
| 5.3.2 | Deficiencies | No | |

| **Evaluation by Competent Authorities** | |
|---|---|
| | Use separate "evaluation boxes" to provide transparency as to the comments and views submitted |
| | **EVALUATION BY RAPPORTEUR MEMBER STATE** |
| **Date** | 2009/04/29 |
| **Materials and Methods** | Applicant's version is acceptable. |
| **Results and discussion** | Applicant's version is adopted with the following amendments:<br>4.2.1: A limit test was carried out with only one test substance concentration.<br>4.2.6: Test substance; Effect data: $EC_{50}$ > 100 mg/L<br>4.4.2: Reference substance; Results: $EC_{50}$ = 5.9 mg/L. This result is within the range of 5-30 mg/L, therefore validity criterion is fulfilled.<br>5.2: The difference between the respiration rates of the blanks is 5%. The value is less than 15 %, therefore validity criterion is fulfilled. |
| **Conclusion** | The applicant's version is adopted. |
| **Reliability** | 1 |
| **Acceptability** | Acceptable |
| **Remarks** | Deviation from Guideline OECD 209 – a limit test with one concentration was carried out. |
| | **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.*<br>*Discuss if deviating from view of rapporteur member state* |
| **Results and discussion** | *Discuss if deviating from view of rapporteur member state* |

## Section A7.4.1.4 Inhibition to microbial activity (aquatic)

**Annex Point IIA7.4**

| | |
|---|---|
| **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** | |

**Table A7_4_1_4-2: Inoculum / Test organism**

| Criteria | Details |
|---|---|
| Nature | Activated sludge |
| Species | Unknown |
| Strain | Unknown |
| Source | Sewage treatment plant treating predominantly domestic sewage |
| Sampling site | Waterschap de Maaskant, 's Hertogenbosch, The Netherlands |
| Laboratory culture | No |
| Method of cultivation | Not relevant |
| Preparation of inoculum for exposure | The sludge was coarsely sieved, washed and diluted with ISO medium. Total suspended solids content was set at 4.3 $g.l^{-1}$, pH 7.5. Sludge was kept aerated at test temperature until use. |
| Pretreatment | Before use 50 ml synthetic sewage was added to each litre of sludge at the end of the collection day. |
| Initial cell concentration | Cell concentration only given as 4.3 g/l mixed liquor suspended solids. |

**Table A7_4_1_4-3: Test system**

| Criteria | Details |
|---|---|
| Culturing apparatus | 1 litre glass test bottles for the incubation and glass 300 ml oxygen bottles for the oxygen measurements. |
| Number of culture flasks/concentration | Two test flasks for the test substance, 1 litre flask per concentration for the reference substance, four (2x2) blank control flasks. |
| Aeration device | Pipette |
| Measuring equipment | $O_2$-electrode (WTW inolab Oxi 730 & WTW Cellox 325 oxygen electrode) |
| Test performed in closed vessels due to significant volatility of TS | No |

**Table A7_4_1_4-4: Test conditions**

| **Criteria** | **Details** |
|---|---|
| Test temperature | 18.0-18.6 (measured continuously) |
| pH | 7.9 |
| Aeration of dilution water | No |
| Suspended solids concentration | 4.3 g/l |