TOXICITY TEST REPORT

For:
SRK (Clean Stream Scientific Services)
Screening toxicity testing of samples B-eff and C-eff

Survey:
March 2010
1. Analyses requested

<table>
<thead>
<tr>
<th>Analyses</th>
<th>Laboratory sample reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 and 30 minute <em>Vibrio fischeri</em> bioluminescent screening test</td>
<td>B-eff &amp; C-eff</td>
</tr>
<tr>
<td>15 and 30 minute <em>Vibrio fischeri</em> bioluminescent definitive test</td>
<td></td>
</tr>
<tr>
<td>72h <em>Selenastrum capricornutum</em> growth inhibition screening test</td>
<td>B-eff &amp; C-eff</td>
</tr>
<tr>
<td>72h <em>Selenastrum capricornutum</em> growth inhibition definitive test</td>
<td></td>
</tr>
<tr>
<td>24 and 48h <em>Daphnia magna</em> acute toxicity screening test</td>
<td>B-eff &amp; C-eff</td>
</tr>
<tr>
<td>24 and 48h <em>Daphnia magna</em> acute toxicity definitive test</td>
<td></td>
</tr>
<tr>
<td>96h <em>Poecilia reticulata</em> acute toxicity screening test</td>
<td>B-eff &amp; C-eff</td>
</tr>
<tr>
<td>96h <em>Poecilia reticulata</em> acute toxicity definitive test</td>
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</tr>
</tbody>
</table>

2. Methodology

2.1 Test conditions

All tests were conducted in environmental controlled rooms using the following internationally standardized methods:

2.1.1 *Vibrio fischeri* bioluminescent test

- Deviation from standard method: None
- Test species: *Vibrio fischeri* (NRRL B-11177)
- Vibrio fischeri batch no: VF4408
- Reagent diluent’s batch no: RD4708
- Sample diluent’s batch no: SD4508
- Exposure period: 15 and 30 minutes
- Test sample volume: 500 ul
- Measurement equipment: Luminoscan TL, Hygiene Monitoring System
- Test endpoint: Screening test - % growth inhibition or stimulation relative to control
  Definitive test - EC20 and EC50 values
- Statistical method used: BioOrbit software
2.1.2 Selenastrum capricornutum growth inhibition test

Standard method: OECD Guideline 201, 1984
Deviation from standard method: None
Kit number: SC118
Expiry date: 30.9.2008
Test species: Selenastrum capricornutum, Printz (CCAP 278/4 Cambridge, UK)
Algal beads batch no: SC060809
Matrix dissolving media batch no: SC250609
Exposure period: 72h
Test sample volume: 25 ml
Test chamber type: 10 cm long cell
Test temperature: 21-25°C
Measurement equipment: Jenway 6300 spectrophotometer
Test endpoint: Screening test - % growth inhibition or stimulation relative to control.
Definitive test - EC20 and EC50 values
Statistical method used: EXCEL spreadsheet

2.1.3 Daphnia magna acute toxicity test

Deviation from standard method: None
Test species: Daphnia magna
Test species age: Less than 24h old
Batch number of ephippia: DM161209
Exposure period: 24 and 48h
Test sample volume: 25 ml
Number of test organisms per beaker: 5
Replicate number beakers per sample: 4
Test temperature: 21±2°C
Test endpoint: Screening test - %mortality. Definitive test - LC10 and LC50 values
Statistical method used: Probit software

2.1.4 Poecilia reticulata acute toxicity test

Deviation from standard method: None
Test species: Poecilia reticulata
Test species age: Less than 21 days
Exposure period: 96h
Test sample volume: 200 ml
Number of test organisms per beaker: 5
Replicate number beakers per sample: 2
Test temperature: 21±2°C  
Test endpoint: Screening test - %mortality  
Definitive test - LC10 and LC50  
values  
Statistical method used: Probit software  

3. Quality assurance  
The following quality assurance information would be made available on request:  
- In-house reference toxicant test data and control charts.  
- Additional lot and batch numbers and raw test data.  

4. Toxicity test results classification systems  
   Individual tests results  
Criteria, as suggested by the Direct Estimation of the Ecological Effect Potential (DEEEP) approach (DWAF, 2003), for the ecological hazard assessment for discharges, has been based on criteria provided for the TEM method by RIZA in the Netherlands. These criteria classifies individual toxicity test units obtained from acute toxicity tests as follows:  

<table>
<thead>
<tr>
<th>Toxicity Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>Not acutely toxic</td>
</tr>
<tr>
<td>1-2</td>
<td>Negligibly acutely toxic</td>
</tr>
<tr>
<td>2-10</td>
<td>Mildly acutely toxic</td>
</tr>
<tr>
<td>10-100</td>
<td>Acutely toxic</td>
</tr>
<tr>
<td>&gt;100</td>
<td>Highly acutely toxic</td>
</tr>
</tbody>
</table>

Battery of test results  
Hazard classification system for natural waters (screening tests)  
After the determination of the percentage effect (EP), obtained with each of the battery of toxicity screening tests performed, the sample is ranked into one of the following five classes:  

<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>No acute hazard - none of the tests shows a toxic effect.</td>
</tr>
<tr>
<td>Class II</td>
<td>Slight acute hazard – a statistically significant percentage effect is reached in at least one test, but the effect level is below 50%.</td>
</tr>
<tr>
<td>Class III</td>
<td>Acute hazard – the percentage effect level is reached or exceeded in at least one test, but the effect level is below 100%.</td>
</tr>
</tbody>
</table>
Toxicity classification system for waste water discharges in the aquatic environment (definitive tests)

The samples are classified into one of the following five classes on the basis of the highest toxicity unit (TUa) found in the battery of toxicity definitive tests performed:

- **Class I**  
  *No acute hazard* - none of the tests shows a toxic effect.

- **Class II**  
  *Slight acute hazard* – the percentage effect observed in at least one toxicity test is significantly higher than in the control, but the effect level is blow 50% (TUa is <1).

- **Class III**  
  *Acute hazard* – the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is below 50% (TUa is between 1 and 10).

- **Class IV**  
  *High acute hazard* – the L(E)C50 is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution (TUa is between 10 and 100).

- **Class V**  
  *Very high acute hazard* – the L(E)C50 is reached in the 100 fold dilution for at least one test (TUa is >100).
## 5. Results

<table>
<thead>
<tr>
<th></th>
<th>B-eff</th>
<th>C-eff</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project</strong></td>
<td>SRK 2010-03</td>
<td></td>
</tr>
<tr>
<td><strong>Samples taken</strong></td>
<td>2010-03-15</td>
<td></td>
</tr>
<tr>
<td><strong>Tests performed</strong></td>
<td>2010-03-19 to 29</td>
<td></td>
</tr>
<tr>
<td><strong>Test operator</strong></td>
<td>Juan Potgieter / Brenton Niehaus</td>
<td></td>
</tr>
</tbody>
</table>

Tests performed

**Screening**

- *V. fischeri* (bacteria) B-eff, C-eff
- *S. capricornutum* (algae) B-eff, C-eff
- *D. magna* (waterflea) B-eff, C-eff
- *P. reticulata* (guppy) B-eff, C-eff

**Definitive**

- *V. fischeri* (bacteria) none
- *S. capricornutum* (algae) none
- *D. magna* (waterflea) none
- *P. reticulata* (guppy) none

<table>
<thead>
<tr>
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<th>C-eff</th>
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<tbody>
<tr>
<td><strong>Results</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water quality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.31</td>
<td>7.27</td>
</tr>
<tr>
<td>EC (Electrical conductivity) (mS/m)</td>
<td>58.1</td>
<td>37.3</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/l)</td>
<td>4.35</td>
<td>3.75</td>
</tr>
<tr>
<td><strong>30min inhibition (-) / stimulation (+) (%)</strong></td>
<td>-21</td>
<td>45</td>
</tr>
<tr>
<td>Individual toxicity test classification</td>
<td>some degree of acute toxicity, refer to overall hazard class</td>
<td>not acutely toxic</td>
</tr>
<tr>
<td><strong>72hour inhibition (-) / stimulation (+) (%)</strong></td>
<td>37</td>
<td>-9</td>
</tr>
<tr>
<td>Individual toxicity test classification</td>
<td>not acutely toxic</td>
<td>not acutely toxic</td>
</tr>
<tr>
<td><strong>48hour mortality rate (-%)</strong></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Individual toxicity test classification</td>
<td>not acutely toxic</td>
<td>not acutely toxic</td>
</tr>
<tr>
<td><strong>96hour mortality rate (-%)</strong></td>
<td>-30</td>
<td>-20</td>
</tr>
<tr>
<td>Individual toxicity test classification</td>
<td>some degree of acute toxicity, refer to overall hazard class</td>
<td>some degree of acute toxicity, refer to overall hazard class</td>
</tr>
</tbody>
</table>

**Overall classification - Hazard class**

<table>
<thead>
<tr>
<th></th>
<th>B-eff</th>
<th>C-eff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (%)</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

**Class II - Slight acute hazard**
6. Literature references


