The toxicity of MEA and amine waste water samples using standardised freshwater bioassays
The toxicity of MEA and amine waste water samples using standardised freshwater bioassays

Water samples provided by Tel-Tek AS were assessed for their toxicity to freshwater organisms from three trophic groups. The water samples included pure monoethanolamine (MEA) and two amine waste water mixtures described as Amine Reactor Waste (ARW) and treated amine waste water (TW). The toxicity of these three test solutions to the unicellular algae *Pseudokirchneriella subcapitata*, the freshwater crustacean *Daphnia magna*, and the embryos of the zebra fish *Danio rerio* were performed in accordance with standard protocols. Of the three taxonomic groups tested, the algae were found to be the most sensitive to both MEA and ARW followed by daphnids, with the zebra fish embryo the least sensitive. For the algae, EC50 concentrations of 151 mg/L MEA, 0.019% ARW and 2.4% TW were similar to that recorded in previous tests performed at NIVA in 2009. The pattern in toxicity for TW was different with the lowest EC50 concentration calculated in the zebra fish followed by the algae and daphnids. However, the EC50 concentrations for TW were very close ranging from 1.91% TW in the zebra fish to 3.4% TW in daphnids.
The toxicity of MEA and amine waste water samples using standardised freshwater bioassays
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Summary

Three water samples provided by Tel-Tek AS were assessed for their toxicity to freshwater organisms from three trophic groups. The water samples included two mixtures of amine waste water, which were by-products of the CO$_2$ capturing methodology using amines. The mixtures included an untreated waste water sample described as *Amine Reactor Waste* (ARW) and a treated waste water sample (TW). In addition, pure monoethanolamine (MEA) an important amine used in the CO$_2$ capturing process was provided for the toxicity assessment.

The toxicity test performed on the three test solutions included: 1) the unicellular freshwater algae *Pseudokirchneriella subcapitata*; 2) the freshwater crustacean *Daphnia magna*; and 3) the embryos of the freshwater zebra fish *Danio rerio*. All tests were performed in accordance with their standard protocols.

For both MEA and ARW, the unicellular algae were the most sensitive followed by daphnids and then the zebra fish. However for TW, the zebra fish was the most sensitive closely followed by the unicellular algae and the daphnids. The effect concentration (EC) endpoints are summarised in the table below.

<table>
<thead>
<tr>
<th>Test chemical</th>
<th>Trophic group</th>
<th>EC$_{10}$</th>
<th>EC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA (mg/L)</td>
<td>Unicellular algae</td>
<td>30</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Daphnids</td>
<td>128</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>Zebra fish</td>
<td>164.6</td>
<td>617.5-</td>
</tr>
<tr>
<td>ARW (%)</td>
<td>Unicellular algae</td>
<td>0.0089</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Daphnids</td>
<td>0.060</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>Zebra fish</td>
<td>0.034</td>
<td>0.194</td>
</tr>
<tr>
<td>TW (%)</td>
<td>Unicellular algae</td>
<td>0.74</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Daphnids</td>
<td>2.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Zebra fish</td>
<td>-</td>
<td>1.91</td>
</tr>
</tbody>
</table>
1. Scope of the work

The following report describes the results of three freshwater toxicity tests performed on three test solutions. The test solutions were supplied by Tel-Tek AS and represent pure monoethanolamine (MEA); and mixtures of treated and the untreated amine waste water from a CO$_2$ capturing facility.

The organisms used within the standardised bioassays represent three major freshwater phyla and include the freshwater algae, (*Pseudokirchneriella subcapitata*) the crustacea (*Daphnia magna*) and zebra fish (*Danio rerio*) embryos.

2. Objective

The main objective of the work was to conduct standard freshwater toxicity tests on the three environmental samples provided by Tel-Tek AS, which included: 1) Pure MEA; untreated amine waste water (amine reclaimed waste, ARW); and treated waste water (TW).

3. Materials and Methods

The three test solutions were supplied by Tel-Tek AS and delivered on ice to the NIVA Oslo laboratory where they were stored until analysis. The MEA and ARW were stored in the dark at 4°C, whilst the TW arrived frozen in approximately fifteen 200 ml plastic containers with screw lids that were stored at -20°C until required for testing.

3.1 Physicochemical parameters of test mixtures

Some important physicochemical parameters of the two test mixtures ARW and the TW were assessed prior to analysis. In addition a third test mixture, identified as ‘Feed to bioreactor’ was measured for the same parameters. These parameters included chemical oxygen demand (COD), ammonium (NH$_4$), sulphate (SO$_4$), chloride (Cl), potassium (K) and sodium (Na).
3.2 Freshwater algal growth test

Growth inhibition of the algae *Pseudokirchneriella subcapitata* (NIVA-CHL 1) was performed in accordance with OECD guideline 201 (OECD, 2011). The specifics of the test are found in Table 1.

**Table 1.** Information for the algal growth inhibition test (OECD 201), which was performed on the three test solutions.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Organism:</td>
<td><em>Pseudokirchneriella subcapitata</em> (NIVA-CHL1)</td>
</tr>
<tr>
<td>Test parameter:</td>
<td>Growth rate 72 hours</td>
</tr>
<tr>
<td>Stock culture:</td>
<td>Semi-continuous in 10 % Z8 growth medium (Staub, 1961)</td>
</tr>
<tr>
<td>Test date:</td>
<td>03.12.2012 - 06.12.2012 (MEA, TW, ARW)</td>
</tr>
<tr>
<td>Pretreatment of sample:</td>
<td>MEA: 1M HCl was added to the test concentrations to adjust pH to 8.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>TW: Test compound was filtered with GF/F filter followed by 0.45 µm filter, and added ISO 8692 stock solutions.</td>
</tr>
<tr>
<td></td>
<td>ARW: Test compound was filtered with 0.45µm syringe filter, and 0.2M HCl were added to test concentrations to adjust pH to 8.0 ± 0.2</td>
</tr>
<tr>
<td>Test concentrations:</td>
<td>MEA: 10, 18, 32, 56, 100, 180 mg/L</td>
</tr>
<tr>
<td></td>
<td>TW: 1st test: 3.2, 5.6, 10, 18, 32 % of original concentration; 2nd test: 0.18, 0.32, 0.56, 1.0, 1.8, 3.2 % of original concentration</td>
</tr>
<tr>
<td></td>
<td>ARW: 0.0018, 0.0032, 0.0056, 0.010, 0.018, 0.032, 0.056, 0.1 % of original concentration</td>
</tr>
<tr>
<td>Test medium:</td>
<td>ISO 8692</td>
</tr>
<tr>
<td>Replicates:</td>
<td>3 in each test concentration, 6 in controls</td>
</tr>
<tr>
<td>Incubation:</td>
<td>Incubator with orbital shaking</td>
</tr>
<tr>
<td>Test containers:</td>
<td>30 ml glass vials with ca. 12 ml sample</td>
</tr>
<tr>
<td>Light:</td>
<td>87 µmol m⁻² s⁻¹, continuous from daylight type fluorescent tubes</td>
</tr>
<tr>
<td>Inoculum:</td>
<td>5×10⁶ cells/ L (nominal concentration) of an exponentially growing culture.</td>
</tr>
<tr>
<td>Registration of growth:</td>
<td>Particle count with Beckman Coulter Multisizer 3 after 24, 48 and 72 ± 2 hours.</td>
</tr>
<tr>
<td>Calculation of growth rate:</td>
<td>Logarithmic increase in density from start to 72 hours.</td>
</tr>
<tr>
<td>Calculation of ECX¹</td>
<td>Non linear regression with Excel macro Regtox 7.0.5 (Hill 1910, Vinidiman et al 1983)</td>
</tr>
</tbody>
</table>

¹ ECX: The concentration which results in x % reduction in growth rate compared to the control
3.3 *Daphnia magna* immobilisation test

Acute immobilisation of *Daphnia magna* was performed in accordance with OECD guideline 202 (OECD, 2004). Test vessels were examined under microscope once daily for the duration of the test, and immobilised or dead animals were recorded. The specifics of the test are found in Table 2.

Table 2. Information for the *D. magna* immobilisation test (OECD 202), which was performed on the three test solutions.

<table>
<thead>
<tr>
<th>Test method</th>
<th>The method is in accordance with the OECD Guideline 202; &quot;Daphnia sp. acute immobilization test&quot;</th>
</tr>
</thead>
</table>
| Pretreatment of sample: | MEA: Stock solution (10 g/L) was adjusted to pH 7.9 with 18.5 % HCl  
TW: Filtered with 0.45µm membrane filter  
ARW: Filtered (0.45µm membrane capsule filter) and adjusted pH with 1M HCl in each test concentration |
| Test concentrations  | MEA: 18, 31, 55, 98, 176, 312 mg/L  
TW: 0.56, 1.0, 1.8, 3.2, 5.6 % v/v  
ARW: 0.009, 0.016, 0.030, 0.056, 0.1 % v/v |
| Replicates           | 4 vessels for each concentration, with 5-7 animals per vessel |
| Test containers      | 50 ml polystyrene cups with ca. 40 ml medium |
| Observations:        | Every 24 ± 2 hours with a microscope |
| Temperature          | Max: 20.7  
Min: 19.3 |
| pH in control (Start – End) | MEA: 7.96 – 7.87  
TW: 8.20 – 8.05  
ARW: 8.14 – 7.97 |
| pH in highest conc. (Start – End) | MEA: 7.87 – 7.97  
TW: 8.34 – 8.48  
ARW: 8.10 – 7.96 |
| O₂saturation, 48 h Highest conc. | MEA: 9.77 mg/L  
TW: 9.43 mg/L  
ARW: 9.02 mg/L |
| Control | MEA: 9.76 mg/L  
TW: 9.49 mg/L  
ARW: 8.43 mg/L |
| Calculation of EC₅₀* | Post hoc logarithmic regression between values of highest concentration without immobilized animals and lowest concentration with 100 % immobilized animals. Performed using Microsoft Office 2010 Excel. |

3.4 Zebra fish embryo test

Embryos of the zebra fish, *Danio rerio* were obtained from the Norwegian Veterinary Institute, Oslo. The test method was based on the OECD draft guideline ‘Zebra fish Embryo Toxicity Test’. The specifics of the tests are provided in Table 3.

The test was initiated immediately after fertilization and continued for 96 hours in duration. Lethal effects were recorded every 24 hours and were based on four apical observations (coagulation of the

* EC₅₀ = The concentration which gives x % immobilization of the test animals.

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*EC₅₀ = The concentration which gives x % immobilization of the test animals.*
embryo, non-detachment of the tail, non-formation of somites, and non-detection of the heartbeat). Observations of any one of these four malformations were indicative of lethality. This was compared to the occurrence in the dilution water control to provide sufficient information to calculate lethal concentration (LC) toxicity endpoints.

Table 3. Information on the zebra fish embryo toxicity test, which was performed on the three test solutions.

<table>
<thead>
<tr>
<th>Test method</th>
<th>OECD draft guidelines ‘Zebra fish Embryo Toxicity Test’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test organism</td>
<td>Zebra fish (<em>Danio rerio</em>) embryos, obtained from the Norwegian Veterinary Institute, Oslo.</td>
</tr>
<tr>
<td>Test period</td>
<td>12.12.12 – 15.12.12 (96 h)</td>
</tr>
<tr>
<td>Pretreatment of sample:</td>
<td>pH adjusted with 10M HCl</td>
</tr>
<tr>
<td>Dilution medium:</td>
<td>Reconstituted freshwater from the Norwegian Veterinary Institute</td>
</tr>
</tbody>
</table>
| Test concentrations  | MEA: 10, 32, 100, 320 and 1000 mg/L  
                         | ARW: 0.001, 0.01, 0.1, 1.0 and 10% v/v of original concentration  
                         | TW: 0.1, 0.32, 1.0, 3.2 and 10% v/v of original concentration |
| Replicates           | 20 embryos per test concentration |
| Test containers      | 24-well plate |
| Temperature          | 27 ± 1°C (incubator controlled temperature) |
| pH                   | 8.27 – 8.70 |

4. Results

4.1 Physicochemical parameters of the test mixtures

Due to the high chemical oxygen demand (COD), the waste water mixtures required dilution to 10,000 times its original concentration before analysis. This dilution resulted in some of the parameters falling below the detection limit and in these cases data was not reported.

Table 4. Measured parameters of the amine waste water mixtures

<table>
<thead>
<tr>
<th>Description (g/L)</th>
<th>ARW (dark liquid)</th>
<th>Feed to bioreactor</th>
<th>Treated waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>74.6</td>
<td>5.4</td>
<td>2.39</td>
</tr>
<tr>
<td>NH₄</td>
<td>8.87</td>
<td>1.01</td>
<td>0.19</td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>68.8</td>
<td>1.1</td>
<td>0.94</td>
</tr>
<tr>
<td>pH</td>
<td>11.1</td>
<td>9.82</td>
<td>7.86</td>
</tr>
<tr>
<td>Conductivity</td>
<td>11.26</td>
<td>6.22</td>
<td>15.22</td>
</tr>
<tr>
<td>Salinity</td>
<td>4.8</td>
<td>2.8</td>
<td>8.5</td>
</tr>
</tbody>
</table>
4.2 Toxicity to freshwater algae

The increase in cell numbers of the control group during the test was almost exponential. The variation between the control replicates were within the acceptable test criteria (Table 5).

MEA:
There was no observed difference in algal growth after 24 h of exposure to MEA up to a concentration of 177 mg/L (Figure 1A). However, after 48 h, toxic effects of the MEA on algal growth were evident. There were no differences in the effects seen from exposure to the two highest MEA concentrations with a plateau in the observed toxicity. Effect concentrations were calculated using a nonlinear regression with adjustable minimum effect and are shown in Figure 2A.

ARW:
There was no apparent effect of the lower concentrations of ARW (<0.01%). As with MEA, the two top concentrations had higher growth rates compared with the third highest test concentration (Figure 1B). Effect concentrations were calculated by nonlinear regression with adjustable minimum effect and are shown in (Figure 2B).

TW:
Since inhibition of algal growth was found in the lowest concentration of TW (3.2%) (Figure 1C), the test was repeated with a weaker concentration series (Figure 1D). However, the second test exhibited less inhibition at 3.2% TW as shown in the first test. Differences in age and sample storage are likely factors, since the first test was performed on a recently thawed sample that was stored at -20°C, whilst the second test was performed on the same sample that was stored in the dark at 4°C for 8 days. Effect concentrations were calculated by non-linear regression (Figure 2C&D).

Table 5. Validity criteria for the algal growth inhibition test (OECD, 201).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of variation in control less than 7 %</td>
<td>1.9 %</td>
<td>3.1 %</td>
</tr>
<tr>
<td>Coefficient of variation in section by section growth rate less than 35 %</td>
<td>16.1 %</td>
<td>34.6 %</td>
</tr>
<tr>
<td>More than 16 times increase in cell concentration from the start to the end of experiment</td>
<td>49 times</td>
<td>53 times</td>
</tr>
<tr>
<td>pH increase in the control less than 1 unit</td>
<td>-0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>
Figure 1. Average cell density over time when exposed to concentrations of the test media: A) MEA (mg/L); B) ARW (%); C) TW (%) 1st test; D) TW (%) 2nd test.
Figure 2. Calculation of effect concentrations of A) MEA, B) ARW C) TW 1st test and D) TW 2nd test.

All test substances were found to inhibit the growth of the algae *P. subcapitata*, although MEA and ARW did not show full inhibition at the highest concentrations tested, but instead the effect appeared to reach a plateau. This was also observed in a previous test with MEA, where test concentrations were even higher (Brooks et al., 2009).

The inhibiting effect of TW on algal growth was lower in the aged (8 days) sample than a freshly thawed sample. The toxic substances in the aged sample might have evaporated or degraded during that time, resulting in lower toxicity. The effect concentrations are summarized in Table 6.

Table 6. Effect concentrations of the tested substances on the growth of *P. subcapitata*. For TW the EC$_{50}$ is calculated from the first test whilst the EC$_{10}$ is calculated from the repeated test.

<table>
<thead>
<tr>
<th>Substance</th>
<th>EC$_{50}$ (95% confidence interval)</th>
<th>EC$_{10}$ (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA (mg/L)</td>
<td>151 (95%)</td>
<td>30 (95%)</td>
</tr>
<tr>
<td>TW (%)</td>
<td>2.4 (1.9 – 2.9)</td>
<td>0.74 (0.58 – 0.98)</td>
</tr>
<tr>
<td>ARW (%)</td>
<td>0.019 (95%)</td>
<td>0.0089 (95%)</td>
</tr>
</tbody>
</table>
4.3 Toxicity to Daphnia magna

The test achieved the validity criteria described in OECD 202 “Daphnia sp. acute immobilization test” (Table 1). In all tests a monotone concentration-response was observed (Table 2). Due to only one partial response in each test EC50 at 48 h was determined by logarithmic regression (figure 1).

Table 7. Validity criteria for the Daphnia sp. acute immobilisation test

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 10% immobilization in control</td>
<td>MEA: 0 %</td>
</tr>
<tr>
<td></td>
<td>TW: 0 %</td>
</tr>
<tr>
<td></td>
<td>ARW: 3.1 %</td>
</tr>
<tr>
<td>≥3 mg/L dissolved oxygen by the end of test in highest test concentration</td>
<td>MEA: 9.77 mg/L</td>
</tr>
<tr>
<td></td>
<td>TW: 9.43 mg/L</td>
</tr>
<tr>
<td></td>
<td>ARW: 9.02 mg/L</td>
</tr>
</tbody>
</table>

Table 8. Observed immobilised D. magna after 24 and 48 hours in control and test media

<table>
<thead>
<tr>
<th>Test substance and concentration</th>
<th># Daphnia</th>
<th>Immobilized 24 h</th>
<th>Immobilized 48 h</th>
<th>pH start</th>
<th>pH 48 h</th>
<th>O2 48 h (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>7.96</td>
<td>7.87</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>7.87</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>7.95</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>7.82</td>
<td>7.95</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>7.70</td>
<td>7.94</td>
</tr>
<tr>
<td></td>
<td>176</td>
<td>24</td>
<td>3</td>
<td>4</td>
<td>7.87</td>
<td>7.96</td>
</tr>
<tr>
<td></td>
<td>312</td>
<td>21</td>
<td>4</td>
<td>20</td>
<td>7.87</td>
<td>7.97</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>8.20</td>
<td>8.05</td>
</tr>
<tr>
<td></td>
<td>0.56</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>8.29</td>
<td>8.07</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>8.28</td>
<td>8.19</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>8.29</td>
<td>8.29</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>21</td>
<td>0</td>
<td>7</td>
<td>8.34</td>
<td>8.46</td>
</tr>
<tr>
<td></td>
<td>5.6</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>8.34</td>
<td>8.48</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>32</td>
<td>0</td>
<td>1</td>
<td>8.14</td>
<td>7.97</td>
</tr>
<tr>
<td></td>
<td>0.009</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>7.89</td>
<td>8.23</td>
</tr>
<tr>
<td></td>
<td>0.016</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>7.98</td>
<td>8.03</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>8.01</td>
<td>8.01</td>
</tr>
<tr>
<td></td>
<td>0.056</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>8.03</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>22</td>
<td>3</td>
<td>17</td>
<td>8.10</td>
<td>7.96</td>
</tr>
</tbody>
</table>
Figure 3. Calculated concentration response curves for A) MEA, B) TW and C) ARW with respect to 24 h and 48 h survival in *D. magna.*
All test substances had a toxic effect on *Daphnia magna* (Table 9). ARW was almost 50 times more toxic than TW. EC$_{50}$ above 100 mg/L is not considered toxic to the aquatic environment.

**Table 9.** Summary of the ecotoxicity data for MEA, TW and ARW on the immobilisation of *D. magna*.

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Unit</th>
<th>EC$_{50}$</th>
<th>95% conf. int.</th>
<th>EC$_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA</td>
<td>mg/L</td>
<td>209</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>TW</td>
<td>%</td>
<td>3.4</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>ARW</td>
<td>%</td>
<td>0.081</td>
<td>0.060</td>
<td></td>
</tr>
</tbody>
</table>

**4.4 Toxicity to the embryo-larvae of the zebra fish**

**Table 10.** The numbers of successfully hatched and surviving embryos of the zebra fish (*Danio rerio*) following 96 h exposure to the test media indicated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Hatched survival after 96h (n=20)</th>
<th>% hatched survival after 96h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated waste (ARW)</td>
<td>control</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>0.001%</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>0.01%</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>0.10%</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treated waste (TW)</td>
<td>control</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>0.10%</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>0.32%</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>3.20%</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MEA</td>
<td>control</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>10 mg/L</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>32 mg/L</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>100 mg/L</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>320 mg/L</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1000 mg/L</td>
<td>6</td>
<td>30</td>
</tr>
</tbody>
</table>

All test substances were found to be toxic to the developing larvae of the zebra fish. Of the two mixtures, ARW was the most toxic with a calculated EC$_{50}$ of 0.194 % of its original concentration compared to 1.91% for the TW (Table 11). Pure MEA had an EC$_{50}$ of 617.5 mg/L.
Figure 4. The percentage of surviving hatched larvae following 96 h exposure to MEA and to amine waste water mixtures, ARW and Treated amine waste.

Table 11. Summary of the ecotoxicity data for the zebra fish larvae after 96 h exposure. Calculated with ToxCalc scientific.

<table>
<thead>
<tr>
<th></th>
<th>96 h exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NOEC</td>
</tr>
<tr>
<td>MEA (mg/L)</td>
<td>320</td>
</tr>
<tr>
<td>ARW (%)</td>
<td>0.1</td>
</tr>
<tr>
<td>TW (%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>
5. Conclusion

Overall, of the three taxonomic groups tested, the algae was found to be the most sensitive to both MEA and ARW followed by daphnids, with the zebra fish embryo the least sensitive. For the algae, EC$_{50}$ concentrations of 151 mg/L MEA, 0.019% ARW and 2.4% TW, where similar to that recorded in previous tests performed at NIVA in 2009 (i.e. 127 mg/L MEA, 0.014% Untreated waste, 12.1% TW, Brooks et al., 2009).

For the TW a different pattern in toxicity was found with the lowest EC$_{50}$ concentration calculated in the zebra fish followed by the algae and daphnids. However, the EC$_{50}$ concentrations for TW were very close ranging from 1.91% TW in the zebra fish to 3.4% TW in daphnids. When compared to previous testing, the TW toxicity was found to be approximately 10 fold lower to the algae than that previously reported (Brooks et al., 2009).

In the previous study (Brooks et al. 2009) the TW was not toxic to the zebra fish after 48 h at the maximum concentration tested 10% TW. This was also the case in the present study, although toxicity was observed after the 96 h exposure duration.

Table 12. Summary of ecotoxicity endpoints for the three test chemicals for the three trophic groups

<table>
<thead>
<tr>
<th>Test chemical</th>
<th>Trophic group</th>
<th>EC$_{10}$</th>
<th>EC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEA (mg/L)</td>
<td>Algae</td>
<td>30</td>
<td>151</td>
</tr>
<tr>
<td></td>
<td>Daphnids</td>
<td>128</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>Zebra fish</td>
<td>164.6</td>
<td>617.5-</td>
</tr>
<tr>
<td>ARW (%)</td>
<td>FW algae</td>
<td>0.0089</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Daphnids</td>
<td>0.060</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>Zebra fish</td>
<td>0.034</td>
<td>0.194</td>
</tr>
<tr>
<td>TW (%)</td>
<td>FW algae</td>
<td>0.74</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>Daphnids</td>
<td>2.2</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Zebra fish</td>
<td>-</td>
<td>1.91</td>
</tr>
</tbody>
</table>
6. References

Hill, A.V. 1910: The possible effects of aggregation of the molecules of hemoglobin on its dissociation curves. *J. Physiol. (Lond.)* 40, IV-VII.
OECD 2011: Guidelines for testing of chemicals. no. 201; Alga. growth inhibition test. OECD. Paris
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