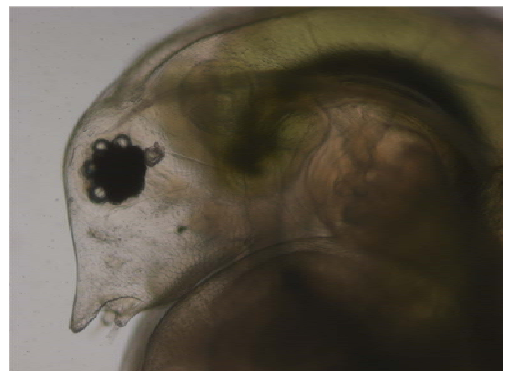


Ecotoxicity testing of an amine and amine waste water samples.



| | | | | |
|--|--|---|--|---|
| Main Office Gautstadalléen 21 NO-0349 Oslo, Norway Phone (47) 22 18 51 00 Telefax (47) 22 18 52 00 Internet: www.niva.no | Regional Office, Sørlandet Televeien 3 NO-4879 Grimstad, Norway Phone (47) 22 18 51 00 Telefax (47) 37 04 45 13 | Regional Office, Østlandet Sandvikaveien 41 NO-2312 Ottestad, Norway Phone (47) 22 18 51 00 Telefax (47) 62 57 66 53 | Regional Office, Vestlandet Nordnesboder 5 P.O.Box 2026 NO-5817 Bergen, Norway Phone (47) 22 18 51 00 Telefax (47) 55 23 24 95 | Regional Office Central Pirsenteret, Havnegata 9 P.O.Box 1266 NO-7462 Trondheim Phone (47) 22 18 51 00 Telefax (47) 73 54 63 87 |
|--|--|---|--|---|

| | | |
|---|---|---------------------|
| Title Ecotoxicity Testing of an amine and amine waste water samples | Serial No. | Date August 2009 |
| | Report No. Sub-No. 5832-2009 | Pages Price 12 |
| Author(s) Steven Brooks Adam Lillicrap Harald Hasle Heiaas | Topic group Ecotoxicity and Risk assessment | Distribution |
| | Geographical area Oslo | Printed NIVA |

| | |
|------------------------------------|-------------|
| Client(s) Jon Hovland - Tel-tek | Client ref. |
|------------------------------------|-------------|

| |
|--|
| <p>Abstract</p> <p>The following report investigates the toxicity of three water samples provided by Tel-Tek AS. The three samples include ethanolamine (Sigma) and two waste water effluent samples, one of which was treated prior to sample receipt. No information is provided on the treatment process. The toxicity of each of these three test solutions to the unicellular algae <i>Pseudokirchneriella subcapitata</i>, the freshwater crustacean <i>Daphnia magna</i>, and the embryo of the zebrafish <i>Danio rerio</i> was carried out in accordance to standard protocols. Of the three taxonomic groups tested, the algae were the most sensitive followed by <i>Daphnia</i> with the zebrafish embryo the least sensitive. Algal EC₅₀ concentrations were 127 mg/L MEA, and 0.014% and 12.1% untreated and treated waste respectively. The untreated waste was approximately 1000 fold more toxic to the algae than the treated waste. Due to the salinity of the waste effluents, daphnia toxicity data was only available for MEA and the untreated waste, with EC₅₀ concentrations of 284 mg/L and 0.091% respectively. The treated waste was not toxic to the zebrafish larvae at the concentrations tested (max 10% original concentration). Due to the salinity of the sample higher concentrations were not tested. Exposure to 1000 mg/L MEA had no significant effect on zebrafish embryo development.</p> |
|--|

| | |
|---|--|
| <p>4 keywords, Norwegian</p> <ol style="list-style-type: none"> 1. 2. 3. 4. | <p>4 keywords, English</p> <ol style="list-style-type: none"> 1. MEA 2. Algae 3. Daphnia 4. Zebrafish larvae |
|---|--|

Steven Brooks
Project manager

Kevin Thomas
Research manager

Jarle Nygard
Strategy Director

ISBN 978-82-577-5567-6

**Ecotoxicity testing of an amine and amine waste
water samples**

Contents

| | |
|--|-----------|
| Summary | 5 |
| 1. Scope of Work | 6 |
| 2. Objectives | 6 |
| 3. Method | 6 |
| 3.1. Unicellular algae | 6 |
| 3.2. Daphnia 48h immobilisation test | 7 |
| 3.3. Zebrafish early life stage test | 7 |
| 4. Results | 9 |
| 4.1. Effects on algal growth | 9 |
| 4.2. Effects of Daphnia immobilisation | 10 |
| 4.3. Effects of zebrafish larvae | 11 |
| 5. Conclusions | 12 |
| 6. References | 12 |

Summary

The following report investigates the toxicity of three water samples provided by Tel-Tek AS. The three samples include ethanolamine (MEA, Sigma) and two waste water effluent samples, one of which was treated prior to sample receipt. No information was provided on the treatment process. The toxicity of each of these three test solutions to the unicellular algae *Pseudokirchneriella subcapitata*, the freshwater crustacean *Daphnia magna*, and the embryo of the zebrafish *Danio rerio* was carried out in accordance to standard protocols. Of the three taxonomic groups tested, the algae were the most sensitive followed by *Daphnia*, with the zebrafish embryo the least sensitive. Algal EC₅₀ concentrations were 127 mg/L MEA, and 0.014% and 12.1% untreated and treated waste respectively. The untreated waste was approximately 1000 fold more toxic to the algae than the treated waste. Due to the salinity of the waste effluents, daphnia toxicity data was only available for MEA and the untreated waste, with EC₅₀ concentrations of 284 mg/L and 0.091% respectively. The treated waste was not toxic to the zebrafish larvae at the concentrations tested (max 10% original concentration). Due to the salinity of the sample higher concentrations were not tested. Exposure to 1000 mg/L MEA had no significant effect on zebrafish embryo development.

1. Scope of Work

The following describes the results of three toxicity tests carried out on three test solutions. The test solutions were supplied by Tel-Tek AS. The organisms chosen represent three major aquatic phyla and include algae, crustacea and fish.

2. Objectives

The main objectives of the work were as follows:

1. To conduct three toxicity tests on environmental samples provided by Tel-Tek AS.
2. To determine the ecotoxicity endpoints including NOEC, LOEC and EC/LC50 of the environmental samples for the unicellular freshwater algae *Pseudokirchneriella subcapitata*, the freshwater crustacean *Daphnia magna*, and the larvae of the zebrafish *Danio rerio*.

3. Method

The test solutions were supplied by Tel-tek and transported on ice to the NIVA Oslo laboratory where they were stored until analysis.

3.1. Unicellular algae

The tests were carried out in accordance with the guidelines set out in the ISO 8692, OECD 201: Algal growth inhibition test. The effect of 72 h exposure of the test solutions on the growth rate of the freshwater algae was observed. The test parameters have been included in the table below (Table 1).

Table 1. Specific parameters for the algal 72h growth inhibition test.

| | |
|-------------------------------|--|
| Test method: | ISO 8692, OECD 201: Algal growth inhibition test |
| Organism: | <i>Pseudokirchneriella subcapitata</i> NIVA CHL1 |
| Test parameter: | Growth rate 72 hours |
| Stem culture: | Semi-static in 10% Z8 growth medium (Staub 1961) |
| Start date: | 6/7/09 |
| Pretreatment of sample | pH adjusted with 10M HCl, 0.45µm filtered |
| Test concentrations: | MEA: 3.2, 10, 32, 100, 320, 1000 mg/L Untreated waste: 0.0056, 0.01, 0.018, 0.032, 0.056, 0.01 % of original concentration Treated waste: 0.32, 1, 3.2, 10, and 32% of original concentration. |
| Samples prepared: | 23/6/09 |
| Test medium: | ISO 8692 |
| Replicates: | 3 for each test concentration, 6 for control |
| Test vessels: | 30 ml glass vials with 12 ml sample |
| Light conditions: | 70 µmol m ⁻² s ⁻¹ , continuous from daylight fluorescent tubes. |
| Temperature: | 20.4 – 21.1°C |

| | |
|---|---|
| pH | 7.91 -8.08 |
| Algal density | 5×10 ⁶ cells/L |
| Estimation of cell density | Particle count with Coulter Multisizer after 72 hours (±2h) |
| Estimation of growth rate | Logarithmic increase in cell density from start to 72 hours. |
| Calculation of EC _x ¹ | Non-linear regression (Hill 1910, Vinidiman <i>et al.</i> 1983) |
| Calculation of NOEC ² | Dunnett's test/ t test for non-homogenous variance. |

¹EC_x: The estimated concentration where x % effect is observed.

² NOEC: The highest tested concentration without significant effect on the actual parameter.

3.2. Daphnia 48h immobilisation test

The toxicity of the water samples were carried out in accordance with ISO 6341 -Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea) - Acute toxicity test (ISO 1996). The test parameters are described below in Table 2.

Table 2. Test parameters for the Daphnia 24h immobilisation test.

| | |
|---|---|
| Test method | ISO 6341, "Water Quality - Determination of the inhibition of the motility of <i>Daphnia magna</i> " The method is in accordance with the OECD Guideline 202; "Daphnia sp. acute immobilization test" |
| Test organism | <i>Daphnia magna</i> , clone A. Maintained in Elendt M7 and fed <i>Pseudokirchneriella subcapitata</i> grown in 10% Z8 nutrient salt solution. Age at start of test < 24 timer. |
| Test period | 30.06.09 |
| Pretreatment of sample: | pH adjusted with 10M HCl 2 ml Untreated waste diluted to 200 ml, pH adjusted with ca. 700µl 10M HCl |
| Dilution medium: | ISO 6341 (made 30.6) |
| Test concentrations | MEA: 56, 100, 180, 320, 560, 1000 mg/L Untreated waste: 0.0032, 0.01, 0.032, 0.1, 0.32% |
| Replicates | 4 vessels for each concentration, with 5-7 animals per vessel |
| Test containers | 50 ml polystyrene cups with ca. 40 ml medium |
| Temperature | 20.2 – 20.6 |
| pH | 7.62 - 8.52 |
| O₂ saturation, 48 t | 8.0 – 8.92 mg/L |
| Calculation of EC₅₀ * | Probit (Statens Naturvårdsverk, 1989) |

Daphnia immobilisation is effected by salinities above 1 ppt. The highest concentration of untreated waste was approximately 3ppt and may have had some influence on the animals. Due to high salinities the treated waste was not tested with Daphnia.

3.3. Zebrafish early life stage test

Zebrafish (*Danio rerio*) embryos, obtained from the Norwegian Veterinary Institute, Oslo, were individually exposed in 24-well microtiter plates to a series of test concentrations. The test concentrations were chosen based on preliminary results from the algae and daphnia toxicity tests. The

test method was based on using five test concentrations as well as an appropriate negative control. For the main parameters see table 3.

The test was initiated immediately after fertilization and continued for 48 hours in duration. Lethal effects were recorded at 48 hours and were based on four apical observations (coagulation of the embryo, non-detachment of the tail, non-formation of somites, and non-detection of the heartbeat). Observations of any of these 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. Test parameters for the Zebrafish embryo toxicity test

| | |
|---------------------------------------|---|
| Test method | OECD draft guidelines ' <i>Zebrafish Embryo Toxicity Test</i> ' |
| Test organism | Zebrafish (<i>Danio rerio</i>) embryos, obtained from the Norwegian Veterinary Institute, Oslo. First brood hatch |
| Test period | 29.07.09 – 31.07.09 (48 h) |
| Pretreatment of sample: | pH adjusted with 10M HCl |
| Dilution medium: | Reconstituted freshwater from the Norwegian Veterinary Institute |
| Test concentrations | MEA: 10, 32, 100, 320 and 1000 mg/L Untreated waste: 0.001, 0.01, 0.1, 1.0 and 10% v/v of original concentration Treated waste: 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 | 25 ± 1°C |
| pH | 7.51 - 8.79 |
| O₂ saturation, 48 t | 6.25 – 7.4 mg/L |

4. Results

4.1. Effects on algal growth

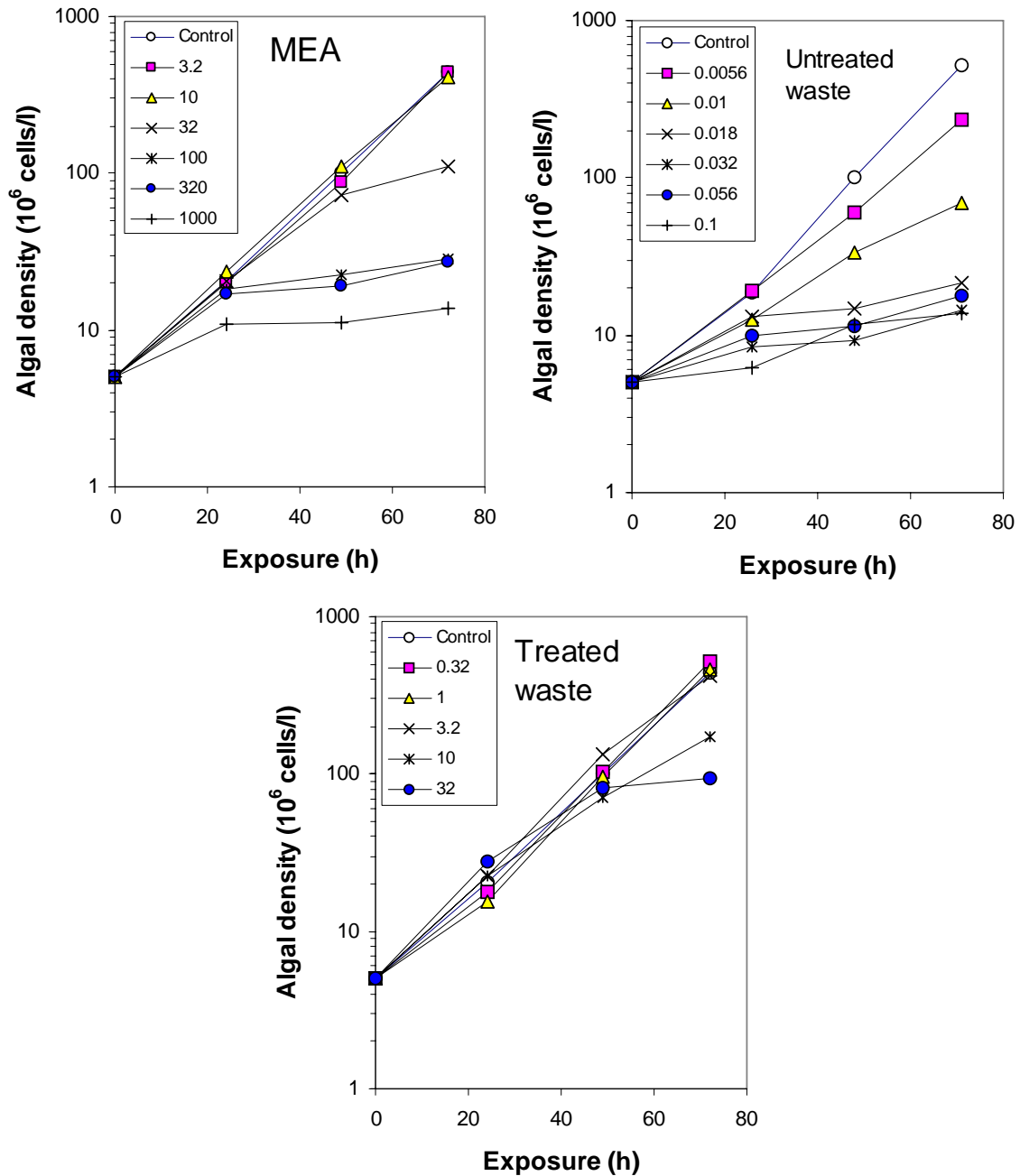


Figure 1. The effects on algal growth rate over time for ethanolamine (MEA mg/L) and the treated and untreated waste (%) exposures.

For the treated waste, the effects of salinity on the reduction in algal growth at the two highest concentrations (10 and 32%) can not be ruled out. At 10% and 32% treated effluent the salinity of the test solution was approximately 2ppt and 6.4ppt respectively.

Table 4. Summary of the algal toxicity data for the three test compounds.

| | 72 h exposure | | | | |
|---------------------|---------------|--------|------------------|------------------|-------------------------|
| | NOEC | LOEC | EC ₁₀ | EC ₅₀ | 95% confidence interval |
| MEA (mg/L) | 10 | 32 | 7.13 | 127 | 107 – 190 |
| Untreated waste (%) | <0.0056 | 0.0056 | 0.0016 | 0.014 | 0.011 – 0.017 |
| Treated waste (%) | 3.2 | 10 | 8.9 | 12.1 | 11.6 – 12.4 |

4.2. Effects of *Daphnia* immobilisation

It was not possible to carry out a daphnia test on the treated waste due to the unexpectedly high salinity of this sample water. Salinities greater than 1ppt can lead to elevated background mortalities in daphnia. A 25% concentration of the treated waste was found to have a salinity of 5ppt. Based on the algae toxicity data, a minimum of 32% treated water exposure would be required to obtain EC values. Therefore, an EC₅₀ for the treated waste water would not be obtained without the influence of salinity on daphnia survival.

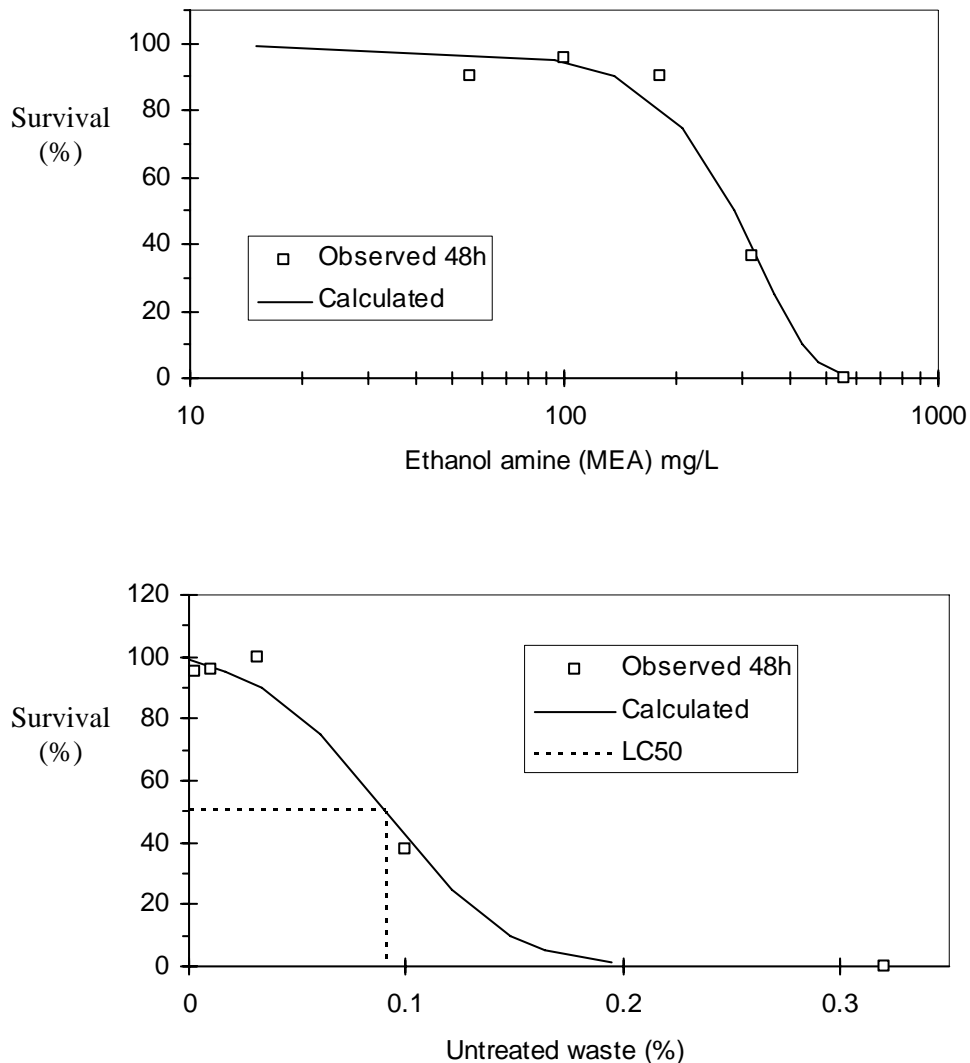


Figure 2. Effects on *Daphnia magna* immobilisation after 48 hour exposure. Two samples: MEA and Untreated waste.

Table 5. Summary of toxicity data for Daphnia after 48 h exposure.

| | 48 h exposure | | | | |
|-----------------|---|------|------------------|------------------|-------------------------|
| | NOEC | LOEC | EC ₁₀ | EC ₅₀ | 95% confidence interval |
| MEA (mg/L) | 180 | 320 | 136 | 284 | 245 - 338 |
| Untreated waste | 0.032 | 0.01 | 0.033 | 0.091 | 0.073-0.12 |
| Treated waste | Not tested due to high salinity of the sample | | | | |

4.3. Effects of zebrafish larvae

Table 6. The effects of the three test solutions on the survival of zebra fish larvae. 48h exposure duration.

| Test solution | Concentration (mg/L) | Alive | Dead | Mortality (%) |
|-----------------|----------------------|-------|------|---------------|
| MEA | Control | 14 | 6 | 30 |
| | 10 | 14 | 6 | 30 |
| | 32 | 12 | 8 | 40 |
| | 100 | 13 | 7 | 35 |
| | 320 | 13 | 7 | 35 |
| | 1000 | 19 | 1 | 5 |
| | % of original conc. | | | |
| Untreated waste | Control | 14 | 6 | 30 |
| | 0.001 | 14 | 6 | 30 |
| | 0.01 | 16 | 4 | 20 |
| | 0.1 | 14 | 6 | 30 |
| | 1 | 12 | 8 | 40 |
| | 10 | 0 | 20 | 100 |
| Treated waste | Control | 14 | 6 | 30 |
| | 0.1 | 15 | 5 | 25 |
| | 0.32 | 18 | 2 | 10 |
| | 1 | 12 | 8 | 40 |
| | 3.2 | 16 | 4 | 20 |
| | 10 | 14 | 6 | 30 |

Table 7. Summary of the ecotoxicity data for the zebra fish larvae after 48h exposure.

| | 48 h exposure | | | |
|---------------------|---------------|-------|------------------|------------------|
| | NOEC | LOEC | EC ₁₀ | EC ₅₀ |
| MEA (mg/L) | 1000 | >1000 | - | - |
| Untreated waste (%) | 1 | 10 | 0.73 | 4.75 |
| Treated waste (%) | | | | |

Slightly high mortalities were found in the control group, although this was within the acceptability criteria for the test. MEA was not found to be toxic to the fish embryos up to the highest concentration tested of 1000mg/L. The treated waste was also not found to be toxic at the highest exposure concentration of 10%. Due to the salinity of the sample higher exposure concentrations were not tested. The Untreated waste was toxic at the highest concentration only (10%).

5. Conclusions

Of the three taxonomic groups tested, the algae were the most sensitive followed by daphnia with the zebrafish embryo the least sensitive. Algal EC₅₀ concentrations were 127 mg/L MEA, and 0.014% and 12.1% untreated and treated waste respectively. The untreated waste was approximately 1000 fold more toxic to the algae than the treated waste. Due to the salinity of the waste effluents, daphnia toxicity data was only available for MEA and the untreated waste, with EC₅₀ concentrations of 284 mg/L and 0.091% respectively. The treated waste was not toxic to the zebrafish larvae at the concentrations tested (max 10% original concentration). Due to the salinity of the sample higher concentrations were not tested. Exposure to 1000 mg/L MEA had no significant effect on zebrafish embryo development.

6. References

- Hill. A.V. 1910: The possible effects of aggregation of the molecules of haemoglobin on its dissociation curves. *J. Physiol. (Lond.)* 40. IV-VII.
- ISO/DIS 8692 : Water quality - Algal growth inhibition test
- OECD 1984: Guidelines for testing of chemicals. no. 201; Alga. growth inhibition test. OECD. Paris
- Staub. R. 1961: Ernährungsphysiologische Untersuchungen an der planktonischen Blaualge *Oscillatoria rubescens*. D.C. Schweiz. *Z. Hydrol.* 23:82-198.
- Vindimian. E. Robaut. R. and Fillion. G. 1983: A method for co-operative and non-cooperative binding studies using non-linear regression analysis on a microcomputer. *J. Appl. Biochem.* 5: 261-268