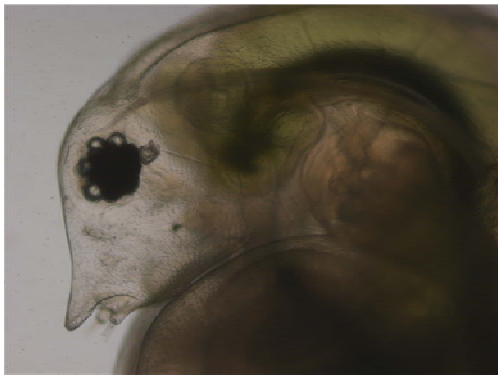
Norwegian Institute for Water Research

REPORT NO: 5832-2009

Ecotoxicity testing of an amine and amine waste water samples.



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REPORT

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Title	Serial No.	Date
Ecotoxicity Testing of an amine and amine waste water samples		August 2009
	Report No. Sub-No. 5832-2009	Pages Price 12
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	Geographical area Oslo	Printed NIVA

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

Abstract
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 *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 EC_{50} 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 EC_{50} 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.

4 keywords, Norwegian	4 keywords, English
1.	1. MEA
2.	2. Algae
3.	3. Daphnia
4.	4. Zebrafish larvae

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ISBN 978-82-577-5567-6

Ecotoxicity testing of an amine and amine waste water samples

Contents

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 EC_{50} 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 EC_{50} 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:	*Pseudokirchneriella subcapitata* 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^{o}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 *et al*. 1983)
Calculation of NOEC[2]	Dunnett's test/ t test for non-homogenous variance.

[1] ECx: 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 *Daphnia magna*" The method is in accordance with the OECD Guideline 202; "Daphnia sp. acute immobilization test"
Test organism	*Daphnia magna*, clone A. Maintained in Elendt M7 and fed *Pseudokirchneriella subcapitata* 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
O_2 saturation, 48 t	8.0 – 8.92 mg/L
Calculation of EC_{50} *	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 *'Zebrafish Embryo Toxicity Test'*
Test organism	Zebrafish (*Danio rerio*) 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
O_2 saturation, 48 t	6.25 – 7.4 mg/L

4. Results

4.1. Effects on algal growth

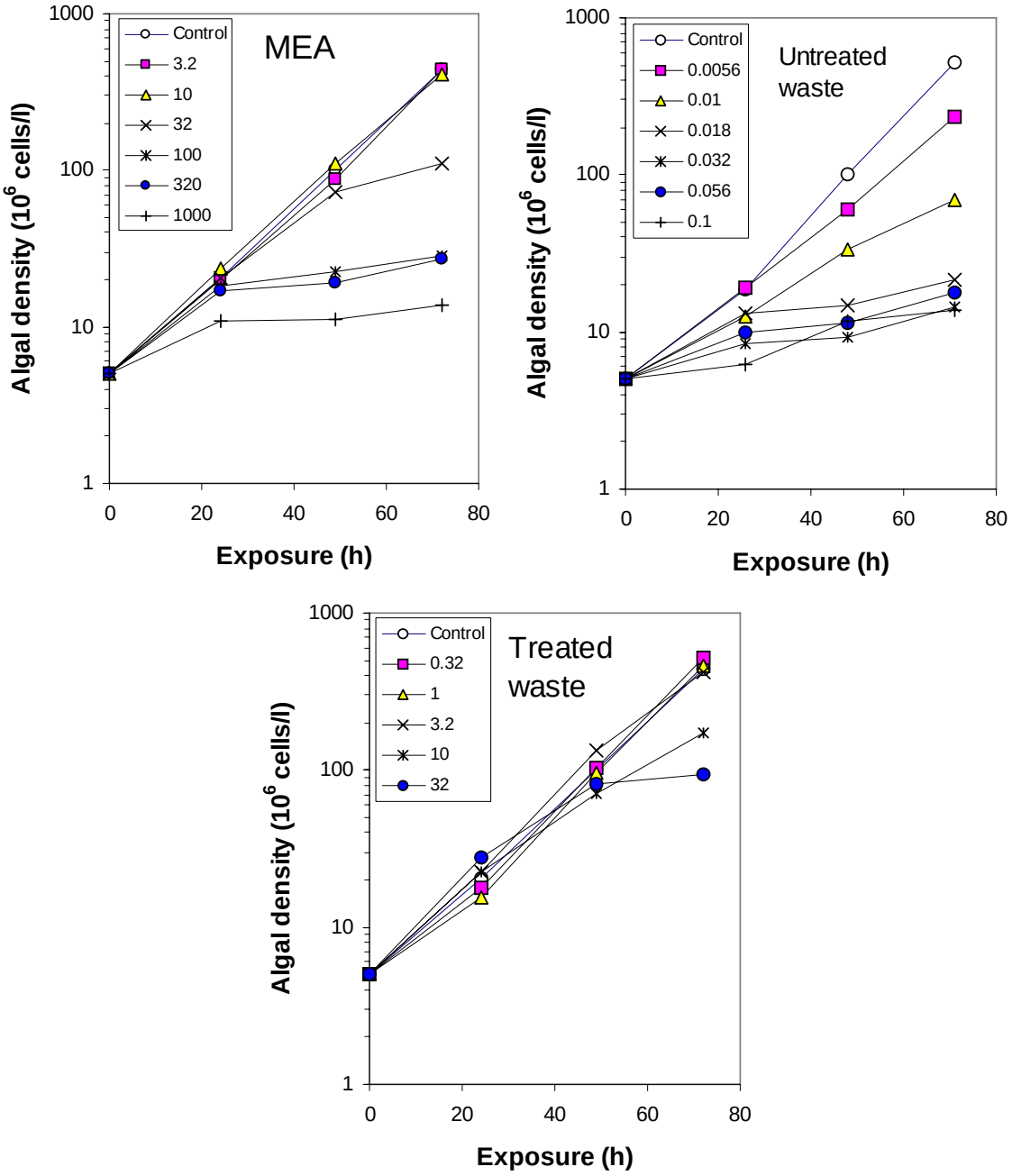


Figure 1. The effects on algal growth rate over time for ethanolamine (MEA mg/L) and the treated and untreated waste (%) exposures.

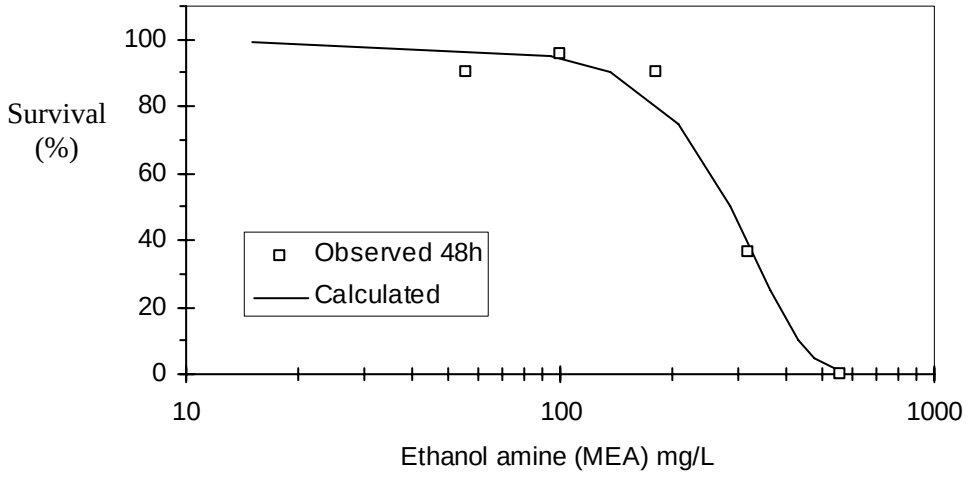
For the treated waste, the effects of salinity on the reduction in algal growth at the two highest concentrations (10 and 32%) can not be ruled out. At 10% and 32% treated effluent the salinity of the test solution was approximately 2ppt and 6.4ppt respectively.

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 EC_{50} for the treated waste water would not be obtained without the influence of salinity on daphnia survival.



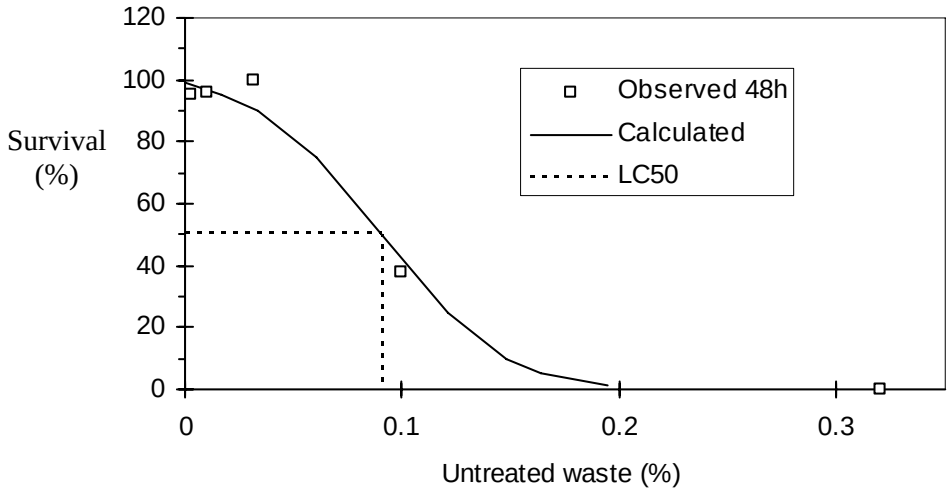


Figure 2. Effects on *Daphnia magna* immobilisation after 48 hour exposure. Two samples: MEA and Untreated waste.

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 EC_{50} 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 EC_{50} 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.

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