



NEW BEDFORD HARBOR PILOT STUDY
PRE-OPERATIONAL MONITORING - PROGRESS REPORT:

Results of Toxicity Tests Conducted on Receiving Water Samples
During July 1987.

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INTRODUCTION

The Environmental Research Laboratory, Narragansett, Rhode Island (ERL-N) has developed a suite of toxicity tests designed to assess the chronic toxicity of effluents entering marine or estuarine environments. Several of these tests were employed in a study to determine the toxicity of receiving waters collected from four sites in New Bedford Harbor, New Bedford, Massachusetts from July 9 through July 16, 1987 (a schedule of tests conducted on each day may be found in Table 1). The test methods used in this evaluation were the sea urchin (Arbacia punctulata) sperm cell test, the macroalgal (Champia parvula) reproductive test, the mysid (Mysidopsis bahia) 7-day survival, growth, and reproductive test, and the sheepshead minnow (Cyprinodon variegatus) 7-day larval survival and growth test.

METHODS

Two composite samples were collected daily by ERL-N staff over five hour periods on the ebb and flood tides throughout the test period. Simultaneous tests were conducted on control water from Narragansett Bay, RI (ERL-N control) and West Island, Buzzard's Bay (site control) for statistical comparison with exposure treatments.

The following method descriptions are a summary of the

toxicity test techniques employed during the New Bedford Harbor project. A detailed description of the various test protocols may be obtained, in draft form, from Bruce Reynolds, USEPA Environmental Research Laboratory, Narragansett, Rhode Island.

Arbacia punctulata

The sea urchin (Arbacia punctulata) sperm cell test was used to evaluate the ebb and flood tide waters from New Bedford Harbor on five days (Table 1). Each day's test included a test control (autoclaved Narragansett Bay water, ANSW), a site control (from West Island, Buzzard's Bay) and a Narragansett Bay control (NSW) used in other toxicity tests, against which the results of the receiving water tests were compared. The test involves the exposure of dilute sperm solutions to effluents or receiving waters for one hour. Eggs are added following this exposure period and the gametes are allowed to incubate for twenty minutes. Toxicity is expressed as a significant reduction in egg fertilization relative to the controls. Data are analyzed with Dunnett's test following arcsine transformations.

Champia parvula

The macroalgal (Champia parvula) reproductive test was used to evaluate the ebb and flood tide waters collected on three days (Table 1). Included in each test was a site control (West Island, Buzzard's Bay) and a Narragansett Bay control. The test consists of a simultaneous 2-day exposure

of male and female plants to an effluent or receiving water. After this exposure period, the female plants are placed in a control medium for a 5- to 7-day recovery period during which the cystocarps, evidence of sexual reproduction, develop. Toxicity is expressed as a significant reduction in the number of cystocarps developed relative to the controls. Data analysis is a one-way analysis of variance (ANOVA) followed by Dunnett's Test.

Mysidopsis bahia

The mysid shrimp (Mysidopsis bahia) was used to evaluate the flood and ebb tide waters from four sites in New Bedford Harbor. Included in the test was a site control (West Island, Buzzard's Bay) and a Narragansett Bay control. The mysid rapid-chronic test consists of exposing 7-day old Mysidopsis bahia juveniles to a complex effluent or receiving water for 7 days. This is a static test, with the water being replaced daily by a freshly collected (within 24-hours) sample. The females mature during this exposure period, usually producing and carrying eggs by the end of the test. The test endpoints are reproduction (measured as the percentage of females with eggs), growth (measured as dry weight), and survival. The percent survival and percent females with eggs data are subjected to an arcsine transformation before a one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test.

Cyprinodon variegatus

The sheepshead minnow (Cyprinodon variegatus)

rapid-chronic test was used to evaluate the ebb and flood tide waters at four sites in New Bedford Harbor. Included in the test were a site control (from West Island, Buzzard's Bay) and a Narragansett Bay control. The test consists of the exposure of newly-hatched (less than 24 hours) larvae to complex effluents and/or receiving waters for 7 days. This is a static test, with the exposure water being renewed daily. The test endpoints are growth, measured as dry weight per fish, and survival. Survival data are analyzed, after an arcsin transformation, by one-way analysis of variance (ANOVA) and Dunnett's test. No transformations are performed on growth data prior to analysis using ANOVA and Dunnett's test.

RESULTS

Arbacia punctulata

No samples yielded results significantly different from any of the controls on any of the test days (Table 2).

Champia parvula

Tests conducted on 9 and 15 July were unacceptable due to poor Narragansett Bay and site (West Island) control response (<10 cystocarps per plant). The test conducted on 13 July did have marginally acceptable laboratory control response, but the quality of this data is questionable and will not be considered in this study.

Mysidopsis bahia

No significant differences were detected in either female dry weight or total dry weight from this test (Table 3). There was, however, significant mortality in the receiving water from NBH-2 Flood relative to the Narragansett Bay controls, but not relative to the site (West Island) controls. Egg development in the controls was below the criterion for acceptability for this portion of the test.

Cyprinodon variegatus

Initial mortality of seven to twenty-seven percent was observed in all but two of the treatment and control exposure chambers (Table 5). The survival in the Narragansett controls was below the criterion for acceptability, but survival in the site (West Island) controls did remain high enough for them to be used for statistical comparisons and maintain the acceptability of the test. Results of data analysis show that neither survival nor growth was significantly affected by any of the receiving water samples tested.

SUMMARY

Toxicity tests were conducted on ebb and flood tide receiving water samples from four sites located in New Bedford Harbor, Massachusetts. These samples were composites of five hourly samples taken daily on each tide. The toxicity tests used were the sea urchin (Arbacia punctulata) sperm cell test,

the macroalgal (Champia parvula) reproductive test, the mysid shrimp (Mysidopsis bahia) 7-day survival, growth, and reproductive test, and the sheepshead minnow (Cyprinodon variegatus) 7-day larval survival and growth test.

No sample evaluated with the Arbacia punctulata sperm cell test or the Cyprinodon variegatus indicated any signs of toxicity. The Cyprinodon test did, however, show initial mortality in most test chambers. The larvae used in this test were the last of their cohort to hatch, perhaps indicating that they were weaker or less healthy than their older siblings. We believe that this may be the cause of this mortality.

None of the Champia parvula tests conducted were considered acceptable due to poor control response. The low control response is believed to be due to the poor condition of stock cultures maintained in Narragansett Bay seawater, which is often of poor quality during the month of July.

The Mysidopsis bahia test exhibited no effect on total or female weight, but there was significant mortality in receiving water NBH-2 Flood. Egg development in the controls in the mysid test was too low to permit consideration of this portion of the mysid test.

In conclusion, we feel that despite some problems with test acceptability, this preliminary study of New Bedford Harbor water was successful. The receiving water quality was found to be generally acceptable to the organisms tested. Where effects were observed, they were marginal in nature and are not believed to be significant. These preliminary test

results should make a firm basis for comparison for future New Bedford Harbor toxicity testing.

REFERENCES

- Duncan, D.B. 1955. Multiple range and multiple F tests.
Biometrics 11:1-42
- Dunnett, C.W. 1955. A multiple comparisons procedure for
comparing several treatments with a control.
JASA 50:1096-1101.

Table 1. The following is a list of sampling dates and tests performed on the New Bedford Harbor samples. Each sample was a hourly composite of bottom, mid and surface water collected on an ebb and flood tide at each station respectively.

The Cyprinodon variegatus and Mysidopsis bahia tests are seven day exposures with the water replaced daily. These two tests reflect the integrated water quality over that time period. The Champia parvula, Laminaria saccharina, and Arbacia punctulata tests are non-water replacement procedures and thus are an indication of the quality of a specific sample.

COLLECTION DATE	<u>C. variegatus</u>	<u>M. bahia</u>	<u>C. parvula</u>	<u>A. punctulata</u>
7/08/87	x	x	x	x
7/09/87	x	x	-	x
7/10/87	x	x	-	-
7/11/87	x	x	-	-
7/12/87	x	x	x	x
7/13/87	x	x	-	x
7/14/87	x	x	x	x

Table 2. Summary table of the results of New Bedford Harbor receiving water evaluation using the sea urchin, Arbacia punctulata. Results are presented as percent fertilized on each day of testing. Controls included are Narragansett Bay water (NSW), autoclaved Narragansett Bay water (ANSW), and West Island control water (site control).

SITE/ TIDE	EFFECT, PERCENT FERTILIZED				
	07/09/87	07/10/87	07/13/87	07/14/87	07/15/87
CONTROL (NSW)	92.0 \pm 2.8	93.5 \pm 0.7	94.5 \pm 2.1	96.5 \pm 0.7	95.0 \pm 2.8
CONTROL (ANSW)	91.5 \pm 2.1	92.5 \pm 4.9	94.0 \pm 2.8	96.5 \pm 3.5	94.0 \pm 1.4
CONTROL (SITE)	91.0 \pm 4.2	94.0 \pm 1.4	96.0 \pm 1.4	96.0 \pm 2.8	95.5 \pm 0.7
NBH-1 EBB	91.5 \pm 0.7	94.5 \pm 3.5	96.5 \pm 2.1	95.0 \pm 2.8	96.5 \pm 0.7
NBH-1 FLOOD	91.0 \pm 2.8	93.0 \pm 2.8	95.0 \pm 2.8	96.0 \pm 1.4	95.0 \pm 4.3
NBH-2 EBB	89.5 \pm 0.7	93.6 \pm 0.9	95.5 \pm 2.1	94.0 \pm 2.8	96.0 \pm 1.4
NBH-2 FLOOD	89.5 \pm 3.5	94.5 \pm 0.7	93.5 \pm 3.6	96.0 \pm 2.8	95.0 \pm 1.4
NBH-3 EBB	90.1 \pm 1.6	97.0 \pm 1.4	93.5 \pm 2.1	94.0 \pm 1.4	97.5 \pm 0.6
NBH-3 FLOOD	89.0 \pm 1.4	96.0 \pm 1.4	95.5 \pm 3.5	97.0 \pm 1.4	94.0 \pm 1.4
NBH-4 EBB	91.0 \pm 2.8	92.0 \pm 2.8	96.0 \pm 1.4	95.5 \pm 2.1	91.5 \pm 0.7
NBH-4 FLOOD	91.5 \pm 2.1	93.5 \pm 2.1	94.5 \pm 0.7	96.5 \pm 2.1	94.0 \pm 1.4

Table 3. The effects of New Bedford Harbor receiving waters on reproduction, growth, and survival of *Mysidopsis bahia*. The test includes a Narragansett Bay control (lab control) and a West Island control (site control). Test conditions: daily static renewal, 8 replicates of 5 animals per treatment.

Site/ Tide	Survival (%)	Females with eggs (%)	Females Mean Dry/wt (mg)	Total Mean Dry/wt (mg)	Mean Temperature (°C)	Mean Salinity (ppt)	Mean D.O. (mg/l)
Lab Control	100.5	20 a	0.320 ± 0.06	0.287 ± 0.03	25.8 ± 0.1	31.0 ± 1.4	5.3 ± 0.1
Site Control	95.0	16 a	0.317 ± 0.04	0.290 ± 0.02	25.7 ± 0.1	32.0 ± 1.9	5.2 ± 0.1
NBH-1 Ebb	97.5	56	0.364 ± 0.13	0.308 ± 0.11	25.6 ± 0.1	28.0 ± 0.3	5.0 ± 0.2
NBH-1 Flood	97.5	13	0.283 ± 0.04	0.284 ± 0.02	25.6 ± 0.1	28.0 ± 1.4	5.1 ± 0.1
NBH-2 Ebb	100.0	40	0.321 ± 0.03	0.319 ± 0.03	25.6 ± 0.1	29.0 ± 1.7	5.4 ± 0.1
NBH-2 Flood	90.0 b	75	0.341 ± 0.04	0.308 ± 0.03	25.7 ± 0.1	30.0 ± 0.3	5.0 ± 0.0
NBH-3 Ebb	92.5	74	0.279 ± 0.04	0.262 ± 0.03	25.6 ± 0.1	30.0 ± 1.4	5.3 ± 0.1
NBH-3 Flood	92.5	64	0.301 ± 0.06	0.279 ± 0.03	25.6 ± 0.3	30.0 ± 0.6	5.3 ± 0.1
NBH-4 Ebb	100.0	37	0.306 ± 0.04	0.285 ± 0.02	25.6 ± 0.1	30.0 ± 1.7	5.4 ± 0.1
NBH-4 Flood	92.5	13	0.316 ± 0.09	0.287 ± 0.05	25.6 ± 0.1	30.0 ± 1.4	5.4 ± 0.1

a) Below criterion for acceptability.

b) Significantly different from Narragansett Bay controls.

Table 4. Effects on sheepshead minnow (*Cyprinodon variegatus*) larvae during 7-day laboratory exposure to receiving water samples from sites in New Bedford Harbor, New Bedford, Massachusetts. Effects measured are growth (weight) and survival. Included in each test are a Narragansett Bay control (lab control) and a West Island control (site control). Test conditions: daily static renewal, 3 replicates of 15 fish used in each treatment. All treatments are statistically compared to site controls.

STATION/ TIDE/	SURVIVAL (%)	FINAL MEAN DRY WT/ INDIVIDUAL (mg)	MEAN TEMPERATURE (°C)	MEAN SALINITY (ppt)	MEAN D.O. (mg/l)
LAB CONTROL	71.1 a	1.15 ± 0.06	25.6 ± 0.4	30.6 ± 0.7	5.6 ± 0.3
SITE CONTROL	88.9	1.14 ± 0.07	25.6 ± 0.3	30.8 ± 0.4	5.4 ± 0.6
NBH-1 EBB	75.6	1.28 ± 0.06	25.5 ± 0.4	27.3 ± 1.0	5.5 ± 0.5
NBH-1 FLOOD	68.9	1.25 ± 0.12	25.7 ± 0.4	28.5 ± 0.7	5.5 ± 0.5
NBH-2 EBB	84.4	1.24 ± 0.01	25.5 ± 0.4	29.8 ± 0.5	5.5 ± 0.5
NBH-2 FLOOD	80.0	1.25 ± 0.04	25.5 ± 0.3	30.0 ± 0.2	5.5 ± 0.5
NBH-3 EBB	88.9	1.26 ± 0.06	25.6 ± 0.3	30.1 ± 0.4	5.5 ± 0.5
NBH-3 FLOOD	88.9	1.19 ± 0.19	25.6 ± 0.3	30.3 ± 0.4	5.5 ± 0.4
NBH-4 EBB	73.3	1.31 ± 0.10	25.4 ± 0.4	31.0 ± 0.0	5.6 ± 0.5
NBH-4 FLOOD	77.8	1.31 ± 0.06	25.6 ± 0.3	30.9 ± 0.3	5.6 ± 0.4

a) Below criterion for acceptability.