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Toxicity Evaluation of the Proposed Secondary and the Primary Effluents Discharged to Massachusetts Bay

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The discharge of industrial and municipal effluents into the nation's surface waters is regulated by the National Pollutant Discharge Elimination System (NPDES). The U.S. Environmental Protection Agency (EPA) released its "Policy for the Development of Water Quality-Based Permit Limitations for Toxic Pollutants" (EPA 1984) in which EPA addresses the control of toxic pollutants beyond technology-based requirements in order to meet water quality standards. To implement this policy, guidance was provided to the respective state and regional permit personnel in the EPA's "Technical Support Document for Water Quality-Based Toxics Control" (EPA, 1985). Both documents recommended that, where appropriate, permit limits based on effluent toxicity should be developed. An important aspect of EPA's policy is the use of effluent toxicity tests to determine if a discharge will likely have any adverse effect on representative species in aquatic environment. In 1986 EPA Region I issued a State Permit for the toxicity evaluation of effluents discharged from Publicly Owned Treatment Works (POTW) and Combined Sewer Overflow (CSO) outfalls to Massachusetts Bay.

MATERIALS AND METHODS

The experimental pilot wastewater treatment pilot unit consisted of a bench-scale sequencing batch reactor in which both biological treatment and solid-liquid separation occured was constructed and operated in laboratory. The secondary effluent produced during the pilot plant operation was used for testing. Chlorination of the secondary effluent was completed by adding sufficient amounts of sodium hypochlorite 1000 parts per million (ppm) solution to obtain 1 mg/L total residual chlorine (TRC) concentration after the 40-minute contact period. Dechlorination of the secondary effluent was completed by adding sufficient amounts of metabisulfite solution that reduced TRC concentration to bellow detection limit.

The effluent samples were collected from the Deer Island and the Nut Island wastewater treatment facilities. Two sets of 7-day samples were collected placed on ice and delivered to the Battelle Ocean Sciences laboratory in September 1987 (first set of effluent samples) and in October 1987 (second set of effluent samples). Each effluent sample was a 24-h composite, collected each day for 7 days either prior to or after chlorination at each treatment plant. A combined sample was prepared each day by combining a

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70 percent Deer Island chlorinated effluent with 30 percent Nut Island chlorinated effluent.

Natural seawater from Duxbury Bay, a high-salinity inlet of Massachusetts Bay, was used in culturing and holding animals and as test dilution water. The chemical analysis of selected metals in dilution water conducted in October 1987 revealed dissolved and particulate concentrations of 0.08 and 0.06 μg/L for copper, 0.03 and 0.004 μg/L for cadmium, 0.39 and 0.04 μg/L for nickel, 0.12 and 0.08 μg/L for lead, and 0.00 and 0.04 μg/L for chromium (unpublished data, 1987, C.Hunt, Battelle Ocean Sciences, Duxbury, MA).

Mature sea urchins *Arbacia punctulata* were held in continuously flowing seawater at a temperature of 15 ± 3°C. The sea urchins were fed field-collected kelp, *Laminaria sp.* Urchins were induced to spawn using electrical stimulation according to the procedure of Nacci et al. (1986). Eggs and sperm were collected from the gonophore by means of a disposable syringe fitted with a large-gauge blunt-tipped needle. The concentration was adjusted to 2000 eggs/ml of seawater. The density of sperm was estimated spectrophotometrically.

Haploid male and female gametophytes of the red macroalga *Champia parvula* were maintained in separate unialgal cultures. Unialgal stock cultures of males and females were maintained in 2000-ml aerated Erlenmeyer flasks containing natural seawater and GP2 culture medium (Thursby et al., 1985). Culture flasks were illuminated with cool white fluorescent light (500 fc) on a 16-h light, 8-h dark cycle. The short-term toxicity tests followed standard procedures with two exceptions: the number of cystocarps per female branch tip was determined microscopically at the end of a 9-day recovery period. The culture medium was renewed once after 3 days during the recovery period.

Sheepshead minnows were cultured, held, and tested at a temperature of 25 ± 2°C. Ten days prior to the scheduled start of the test, two spawning trays were placed in a brood stock holding tank. Each tray was 20 x 45 x 5 cm with a 0.5-mm Nitex screen-meshed bottom covered with a 2-cm layer of dolomite. After 3 days of spawning, trays were removed and the dolomite was transferred in seawater to a plastic tub. The dolomite was swirled in the tub to free the eggs, and the seawater containing the eggs was decanted over a 0.5-mm-mesh screen. Approximately 600 embryos were pipetted into 1-L glass jars containing 800 ml of continuously aerated seawater. Twenty-four hours prior to the start of the toxicity test, all hatched fish larvae were removed from incubation jars and transferred to the 1-L holding jars. The larvae were fed ≤24-h-old brine shrimp *Artemia salina* nauplii.

The percent mortality of mysids and sheepshead minnows after the specified exposure period was used to calculate median lethal concentration (LC50) values. The median effective concentration (EC50) values for the sea urchin test were calculated based on a percentage of unfertilized eggs. The Trimmed Spearman-Karber (Hamilton et al., 1977) method with Abbott's correction and Probit Analysis (SAS 1985) were applied to the data to

calculate LC/EC50 values with respective 95 percent confidence intervals. An analysis of variance followed by Duncan's multiple range test (SAS 1985) was used to determine the no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) from rapid-chronic test data. The maximum acceptable toxicant concentration (MATC) was calculated as the geometric mean of the NOEC and LOEC. Statistical differences between LC50 values were determined by the method suggested by Sprague (1977).

RESULTS AND DISCUSSION

Standard physicochemical parameters such as pH, salinity, and alkalinity remained at a similar level in each effluent sample. Salinity ranged from 0.5 to 3.0 ppt, pH from 6.63 to 7.22, and alkalinity from 89 to 117 mg $CaCO_3$. Hardness varied from 120 to 330 mg $CaCO_3$. Dissolved oxygen concentrations were higher in chlorinated samples which were collected before aeration. The average concentrations of dissolved oxygen in unchlorinated and chlorinated samples were 2.8 and 6.5 mg/L, respectively. Total residual chlorine (TRC) concentrations were higher in samples collected from the Nut Island treatment. The average concentration of TRC from the Deer Island sampling site was 1.6 mg TRC/L, whereas the average TRC concentration from the Nut Island sampling site was 2.5 mg TRC/L. Chlorine concentrations were reduced to 0.7 mg/L (Nut Island primary effluent) and to 0.05 mg/L (Deer Island primary effluent) 3 hours after the last composite sample was collected and prior to salinity adjustment, and to 0.5 mg/L (Nut Island effluent) and bellow detection limit (Deer Island effluent) 1 hour later, after the salinity of effluents was adjusted. In most effluent samples, chlorine was not detected 3 h after initiation of the toxicity tests (Fig 1). TRC concentrations in secondary effluents were similar for both samples and ranged between 1.0 and 1.12 mg TRC/L. All toxicity tests were initiated within 30 minutes after salinity of the samples was adjusted.

The results of the 7-day static-renewal toxicity test indicated that the survival of sheepshead minnows in unchlorinated effluent samples collected from both plants in September and October was significantly reduced when compared with survival in chlorinated effluents (Table 1). The toxicity of the combined chlorinated effluent was expected to be similar to the observed in Deer Island chlorinated effluent because the combined effluent contained the Deer Island chlorinated effluent at a ratio of 70% to 30% (v/v) of the Nut Island chlorinated effluent. The fish mortality in the combined chlorinated effluents in September was similar to that observed in the Deer Island chlorinated samples. The 7-day LC50 values for the chlorinated Deer Island and combined effluents were also similar, 62.5 and 70.7 percent, respectively. In contrast, the fish mortality of the combined chlorinated effluents in October was similar to fish mortality recorded in Nut Island chlorinated effluents, reflected by similar LC50 values of 74.6 and 69.6 percent, respectively. The fish survival in the seawater controls was 95.6 percent in September, and 96.9 percent in October at test termination, indicating the fish used for testing were healthy. Analysis of the fish survival data, using Duncan's multiple range test, indicated that in all undiluted effluent samples tested, survival was significantly reduced ($P>0.05$) when compared with the survival in the controls. Fish survival was also significantly reduced after 7

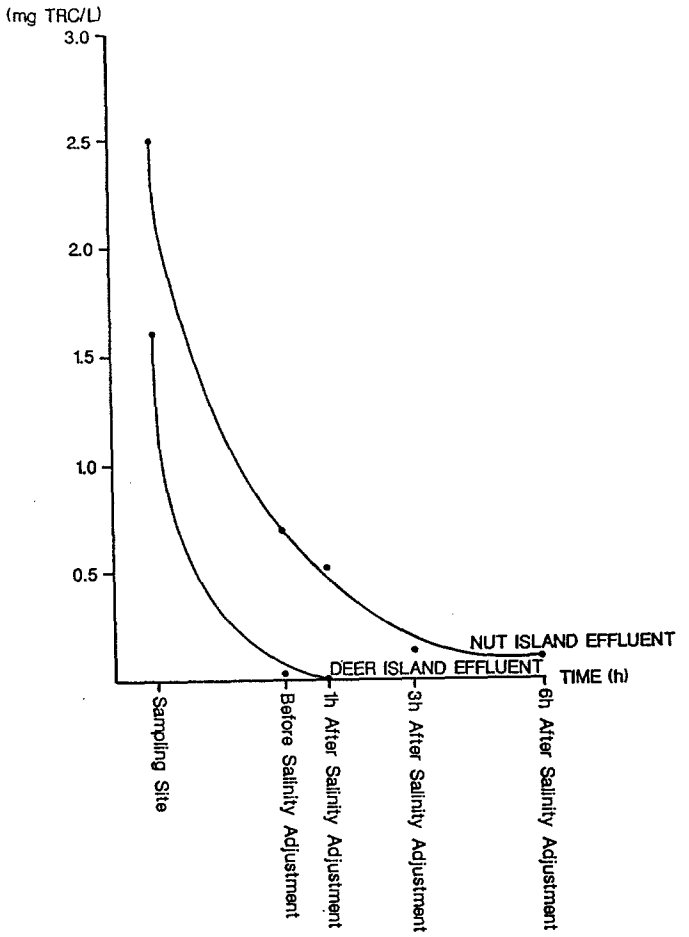

Figure 1. Total residual chlorine concentration in primary effluents.

days in the 50 percent effluent dilution from Deer and Nut Island unchlorinated effluents tested in September.

The mysid survival during 96-h in the seawater controls averaged 99 and 97 percent in tests conducted in September and in October, respectively. The results of the 96-h static acute toxicity tests indicated that survival of mysids in the chlorinated effluent samples collected from both plants in September and in October was reduced when compared with survival in the unchlorinated effluents; however, mysid response to chlorine was not substantially different (Table 2). The LC50 values for Deer Island effluents collected and tested in September were similar (37.8 and 32.2 percent), whereas the LC50 values for the October samples were similar (67.0 and 51.6 percent) for Nut Island effluents (Table 2).

The average number of cystocarps produced (9.6) for all seawater controls in September was below a minimum required for acceptance. Only two tests conducted with effluents from Deer Island produced more than 10 cystocarps in the controls. At the same time, the average production of cystocarps in the remaining tests ranged between 0 and 1.5.

Although the production of cystocarps was poor in the controls from these three tests, these tests were included as valid in this report because the number of cystocarps produced in low effluent concentrations was sufficient and produced well-defined concentration-response curves. In contrast the October cystocarp production in the seawater controls was high and averaged 30.6. The production of cystocarps in the seawater controls in December averaged 30.1 during the first week of testing, while one week later cystocarp production dropped and averaged 7.2. The results of reproduction toxicity tests with macroalga showed that Nut Island effluent samples were generally more toxic than Deer Island samples (Table 3). However, differences in macroalga response to the effluents from both treatment plants were more pronounced in September than in October. At the same time the response of the red macroalga to the secondary effluent was inconsistent.

Table 1. Chronic values from sheepshead minnow static-renewal short-term chronic tests. All values are expressed as percent of effluent.

		Growth		Survival		
Sample		NOEC	LOEC	LC50	NOEC	LOEC
Deer Island Unchlorinated	S	50	>50	36.8	25	50
	O	50	>50	70.7	50	100
Deer Island Chlorinated	S	25	50	62.5	50	100
	O	25	50	>100	50	100
Nut Island Unchlorinated	S	50	>50	39.4	25	50
	O	50	>50	69.6	50	100
Nut Island Chlorinated	S	<3.1	3.1	>50	50	>50
	O	25	50	69.6	50	100
Combined	S	25	50	70.7	50	100
	O	25	50	74.6	50	100

S - September samples
O - October samples

Thirty-one sea urchin sperm cell toxicity tests were conducted during the testing program. Twenty-five tests were performed on primary effluents collected on five different days, and six tests were conducted with secondary effluents. The results of sea urchin tests showed that the toxicity of each effluent varied throughout the program. The most consistent response was observed with the most toxic Nut Island chlorinated effluent, and the most variable response was observed with the least toxic Deer Island unchlorinated effluent.

Generally, the chlorinated primary effluents were more toxic than the unchlorinated, and Nut Island effluents decreased sperm viability more than Deer Island effluents. The results of five sea urchin rapid-chronic tests

Table 2. Acute values from mysid static toxicity tests. All values are expressed as percent of effluent.

Sample	LC50		NOAEL	
	September	Ocotber	September	October
Deer Island Unchlorinated	37.8	92.7	12.5	25
Deer Island Chlorinated	32.2	53.7	12.5	25
Nut Island Unchlorinated	31.2	67.0	6.2	25
Nut Island Chlorinated	15.0	51.6	6.2	25
Combined	30.5	>50	12.5	50

Table 3. Chronic values from red macroalga short-term chronic tests. All values are expressed as percent effluent.

Sample	September		October		December	
	NOEC	LOEC	NOEC	LOEC	NOEC	LOEC
Primary Effluents						
Deer Island Unchlorinated	12.5	25	3.1	6.2		
Deer Island Chlorinated	12.5	25	ND	3.1		
Nut Island Unchlorinated	3.1	6.2	3.1	6.2		
Nut Island Chlorinated	3.1	6.2	1.6	3.1		
Combined	12.5	25	ND	3.1		
Secondary Effluents						
Unchlorinated 12/09/87					10	20
12/16/87						510
Chlorinated 12/09/87					5	10
12/16/87					10	20
Dechlorinated 12/09/87					5	10
12/16/87					1	5

showed that toxicity in the combined chlorinated effluents was similar to that which was observed in the chlorinated Deer Island samples. Based on measured chlorine concentration in samples, the threshold level for total residual chlorine was empirically estimated at 0.003 mg TRC/L. The unchlorinated and the chlorinated/dechlorinated secondary effluents were practically not toxic to sea urchin.

The percent of the unfertilized eggs in those two effluents at the highest test concentrations (100 percent effluent) ranged from 37.3 to 79.7. The EC50 values obtained from those tests ranged from 73.4 to 96.2 percent for the unchlorinated effluents and >100 percent for the chlorinated/dechlorinated

effluents (Table 4).

The results of this study showed that the wastewaters discharged currently to Boston Harbor are toxic, however the source of toxicity is unknown. The chlorination treatment currently used at both treatment facilities increases TRC concentration in the primary effluents and exaggerates toxicity of the wastewaters. The dosing of chlorine is more consistent at the Nut Island plant what was reflected by less variable and higher concentrations of TRC in the effluent during the course of this study. The introduction of chlorine to marine receiving waters provokes its decay from a diatomic gas to hypochlorous acid, hypochlorite ions, and sodium hypochlorite (Lewis 1966). This reaction occurs rapidly and is usually completed within seconds after the addition of chlorine. Subsequently, hypochlorite ions form chloramines and bromoamines, which is a function of temperature, pH, ammonia, and bromine concentrations. The activity of oxidizing agents like chlorine in an aquatic environment is affected by the initial dose concentration, the quality of the receiving water (especially relative concentrations of potential reactants), and time of reactions. At the same time chlorine activity is reduced by organic matter, hardness, volatilization, and other reducing agents present in wastewaters (Hoss et al. 1975).

The effluents tested in this program are essentially identical in chemical characteristics, all carrying large amounts of highly biodegradable organic material and metals. The concentration of metals in primary effluents ranged from 12 to 28 ug/L for chromium, 95 to 108 µg/L for copper, 13 to 18 µg/L for lead, and 150 to 258 µg/L for zinc. The organic chemicals detected in wastewaters are phenol, 1,2-dichlorobenzene, naphthalene, 2-methylnaphthalene, 2,4,5-trichlorophenol, methylene chloride, acetone, chloroform, trichloroethene, benzene, toluene, and chlorobenzene in concentrations ranging from 5 to 120 µg/L (CDM Report, 1987). The toxicity of chlorinated wastewaters depends on a variety of factors (i.e., exposure

Table 4. Chronic EC50 values from sea urchin short-term chronic tests. All values are expressed as percent effluent.

Sample	September			October		December	
Primary Effluents							
Deer Island Unchlorinated	35.4	27.2	16.7	21.6	3.0		
Deer Island Chlorinated	18.5	5.1	5.1	17.5	3.5		
Nut Island Unchlorinated	21.1	17.7	18.1	19.3	3.6		
Nut Island Chlorinated	9.7	3.0	3.7	5.5	2.7		
Combined	18.0	4.3	9.3	14.8	3.9		
Secondary Effluents							
Unchlorinated						96.2	73.4
Chlorinated						3.1	2.2
Dechlorinated						>100	>100

time, concentration of heavy metals, sensitivities of the species tested). Therefore, interpolation of the individual species response to chlorine in wastewaters is difficult, even knowing the toxicity of the effluent prior to chlorination.

Biological monitoring conducted in the future on effluent discharged from the new secondary treatment plant should include assessment of the short- and long-term biological effects of the discharged wastewaters. The relative sensitivity of a test or method used for detecting impacts associated with the wastewater disposal is very important. The results of this study showed that all test methods used in this program are useful for the short-term biomonitoring purposes, although each test method utilized different biological end points. The sea urchin gametes were extremely sensitive to chlorinated wastewaters. Dinnel et al. (1981) reported similar responses of sea urchin and sand dollar to chlorinated wastewaters. They found chlorine to be a potent spermicide. The sea urchin and sand dollar sperm cells were very sensitive to chlorinated seawater at concentrations from 0.002 to 0.02 mg TRC/L. Their results indicated that fertilization tests were approximately 5 to 10 times more sensitive than fish 96-h tests. The fertilization test should be included in future biomonitoring program of since secondary effluent will be chlorinated.

Each of these methods may be ranked depending of its biological relevance, degree of difficulty, sensitivity and associated costs. The sheepshead growth test for example requires relatively large volumes of samples that must be renewed each day once involves an elaborate and expensive sampling schedule especially when 24-hours composite samples are required. This test is generally less sensitive than the other short-term chronic tests, however, is biologically relevant because of the economical and ecological importance of fish. The mysid acute test uses mortality as a end point, is comparally insensitive and the relevance of this species to the Boston Harbor ecosystem could be questioned since mysids does not represent invertebrates potentially affected in the receiving water environment. The sexual reproduction test with red macroalga utilizes a biologically important stage of its life-cycle, however variability in cystocarp production in control treatments are difficult to define (Jop 1989). Although these test methods are valuable in a range of aquatic biomonitoring programs, it has become evident that the sea urchin fertilization test was particularly relevant to this program. This test is unique because it includes the evaluation of reproduction potential, is conducted in minutes therefore can follow the transient nature of same pollutants in the wastewaters, is very sensitive to variety of pollutants, and relatively simple to conduct.

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