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Final Report

Acute Effect of Free Chlorine on Selected Estuarine Invertebrates and Vertebrates

by

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Introduction

The objective of this project was to determine acute toxic effects of chlorine on selected estuarine organisms found adjacent to the projected outfall of a sewage treatment plant in the lower York River. The test species specified under contract were oyster (*Crassostrea virginica*) and clam (*Mercenaria mercenaria*) larvae, *Acartia tonsa* (a dominant copepod), and the fishes, menhaden (*Brevoortia tyranus*), pipefish (*Syngnathus fuscus*), blennies (*Hypsoblennius hentzi*) and sheepshead minnow (*Cyprinodon variegatus*). The parameter of interest in the tests was mortality when the animals were exposed to a constant level of chlorine for a 48 or 96 hr period.

Difficulties in acquisition of some species lead to use of alternate species; naked gobies (*Gobiosoma bosci*) for blennies, silversides (*Menidia menidia*) for menhaden, and mummichogs (*Fundulus heteroclitus*) for the sheepshead minnow. In addition to the prescribed tests, assays were also conducted with glass shrimp (*Palaemonetes pugio*) and juvenile oysters (shell deposition rate measured rather than mortality).

Chlorine was tested as free residual chlorine. In a sewage effluent, much of the chlorine would be present as mono- or dichloramine because of the high concentration of ammonia in sewage waste. Studies of freshwater organisms suggest that chlorine and chloramines have similar toxicity values (Brungs, 1974). If this holds for marine species,

the toxicity of chlorine should approximate that of chloramines and chlorine-chloramine mixtures. The present studies considered only chlorine toxicity, to avoid possible problems in evaluating toxicity of both chlorine and chloramines.

The assumption was made that the addition of chlorine to the River system would be continuous, producing an essentially constant concentration about the outfall. Therefore in these tests, chlorine addition was continuous, resulting in essentially constant concentrations in the test chambers.

Materials and Methods

Test animals with the exception of oyster and clam larvae were obtained by field collection. Field collected animals were held in static aquaria in the laboratory for a minimum of ten days prior to testing with the exception of Acartia tonsa which were held four days. During this preliminary holding period, external parasites were removed when observed. All species were fed an appropriate food until 48 hr. prior to testing at which time feeding was discontinued. Mortality for all animals used in tests was less than 5% during the two days prior to testing, and usually less than 1%.

Oyster and clam larvae, no more than 6 hr. post fertilization, were provided by the Culture Department with the exception of one batch of oyster larvae obtained from the Eastern Shore Laboratory. Larvae were used immediately for experiments.

Two test systems were used for three experiments, both designed to provide as constant a concentration of chlorine as possible. The system used for oyster and clam larvae and copepods (Acartia tonsa) was a constant addition system. A series of stock solutions was continuously introduced into the 3 liter test aquaria at a rate empirically determined to maintain desired concentrations for 48 hr. Aeration was provided as large bubbles in later experiments to prevent establishment of concentration gradients in the aquaria.

All other species were tested in a flow-through system in which both toxicant and diluent was pumped into a mixing chamber and apportioned between two test aquaria. Five concentrations and a control were included in each assay. Experience with the system led to modifications to alleviate the formation of gradients; aeration, drain siphons extending to the bottom of each tank, and relocation of the input tube.

Stock solutions were prepared by dissolving calcium hypochlorite in deionized water buffered to ca. pH 8. Concentrations of stock solutions were essentially constant for periods of 24 to 48 hr.

The ambient chlorine concentration in each test chamber was assayed at least daily during each experiment. During the first half of the contract period analyses were performed by amperometric titration. In several cases, the analysis was performed to distinguish monochloramine from free chlorine. In all cases, no monochloramines was detected, and the

assumption has been made that only free chlorine was present in all experiments. During the latter half of the contract period, the prototype of an instrument to measure chlorine with columetric calibration and amperometric detection was available. This instrument was sensitive to as little as 0.002 ppm free chlorine (readable to 0.001 ppm). The total sample size required is only 25-30 ml vs. 200 ml for the amperometric method, a significant advantage.

Temperature, salinity and dissolved oxygen in the tests were those ambient in the incoming diluent water. Temperature ranged from 17C in later experiments to 28C in experiments conducted in the middle of the summer. During any single experiment, temperature varied by $\pm$ 2C at most. Salinities during the contract period ranged from 18.2 to 20.4. Dissolved oxygen concentrations were always near saturation.

Mortality data (as %) for 24, 48, and 96 hr were plotted against log concentration. The concentration was taken to be the mean measured concentration over the test period. The TL50 for each time interval was determined from the graph. In the case of juvenile oysters in which the rate of shell deposition was measured, the response at each toxicant concentration was expressed as a percent of control animal deposition. The EC50 for 96 hr was then determined in a manner analogous to that used to determine TL50 values.

Results

The results are summarized in Table 1. The 48 or 96 hour TL50 values ranged from 0.28 ppm for Syngnathus fuscus and 0.22 ppm for Palaemonetes pugio to less than 0.005 for oyster and clam larvae.

Several tests were attempted with oyster larvae with varying degrees of success. In an early experiment in which no aeration was used to mix the chlorine and chlorine was added manually at 6 to 8 hour intervals, the apparent 48 hr TL50 of 0.10 ppm was obtained. With constant addition of chlorine stock but no aeration to mix the chlorine, the test results did not permit calculation of a TL50 value, but high mortality was observed at 0.05 ppm, the lowest concentration tested. The new instrument for chlorine analysis allowed sampling at surface and bottom revealing a concentration gradient. Aeration of the test chamber alleviated the gradient. In a test with aeration applied, the TL50 was below the lowest concentration tested, 0.005 ppm.

Mercenaria mercenaria larvae were tested in the constant addition system with aeration. The 48 hr EC50 based on corrected percent straight hinge larvae was 0.0056 ppm. The 48 hr. TL50 was again less than 0.005 ppm, probably on the order of 0.001 ppm.

Acartia tonsa were tested in the constant addition system without aeration. The minimum concentration tested was 0.05 ppm since that was the minimum detectable level

TL50 (or EC50) values for all species tested in ppm of free chlorine.

Species	2 hr.	24 hr.	48 hr.	96 hr.
Invertebrates				
Crassostrea virginica	0.75	0.27	0.11	------
larvae (intermittent Cl_2 addition)				
larvae (aerated)	----	----	0.005	------
juvenile (shell deposition)	----	----	-----	0.023*
Mercenaria mercenaria	----	----		------
larvae EC50+	----	----	0.0056	
TL50	----	----	[0.001]	
Acartia tonsa	----	<0.05	<0.05	------
Palaemonetes pugio	----	0.38	----	0.22
Vertebrates				
Syngnathus fuscus	----	0.28	0.27	0.27
Gobiosoma bosci	0.64	0.08	0.08	0.08
Menidia menidia	----	0.095	0.038	0.037

* extrapolated; lowest concentration tested 0.04 ppm
\+ based on corrected % straight hinge after 48 hr.
[] extrapolated value since suitable low concentrations could not be tested.

with the amperometric technique. The 48 hr. TL50 was less than 0.05 ppm, perhaps on the order of 0.005 ppm (based on extrapolation of the data).

The oyster shell deposition test was conducted in the flow-through system. The deep aquaria used in this system are not ideal for this test since water flow is not directed over the oysters. Food availability was also probably less than ideal. Nevertheless, good shell deposition was observed for control and some test animals. The shell deposition at each test concentration was expressed as a percentage of shell deposition by controls. The percent shell deposition was then plotted against concentration. Since no test animals exhibited a relative shell deposition greater than 30%, an EC50 cannot be derived. If one extrapolates from the data, an estimate of 0.023 ppm is obtained.

Palaemonetes pugio was tested in the flow-through system. After 24 hr, the TL50 was 0.38 ppm, after 96 hr, 0.22 ppm. Inaccuracy in counts at 48 hr. precluded an estimate of the TL50 for that interval.

Of the three fish species successfully tested, Syngnathus fuscus was the most tolerant with a 96 hr. TL50 of 0.28 ppm and Menidia menidia was most sensitive with a TL50 of 0.037 ppm. A test with Fundulus heteroclitus was attempted, but the concentrations delivered to the aquaria were not consistent. Attempts to obtain additional fish to repeat this test were unsuccessful. The available data suggests that the TL50 for 48 hr. is between 0.5 and 1.0 ppm, for 96 hr, between 0.07 and 0.2 ppm.

Discussion

It is clear from these results that various marine animals are extremely sensitive to the continuous presence of free chlorine in sea water. The most sensitive forms among the invertebrates tested were molluscan larvae and the copepod Acartia tonsa. The most sensitive fish species was Menidia menidia, the silverside, which is an important food source for many commercial species in estuaries.

Juvenile oysters are markedly less sensitive than larval stages. Only a few juvenile oysters actually died during the test, but these mortalities cannot be clearly attributed to chlorine exposure. The TL50 for juvenile oysters would have been greater than the EC50 for shell deposition.

Oyster larvae are markedly more tolerant of chlorine when the exposure is intermittent rather than continuous, with a 48 hr TL50 two orders of magnitude higher in the former case than the latter. Even when exposed intermittently, however, the maximum exposure concentration is less than ambient concentrations adjacent of many sewage outfalls.

Acartia tonsa is the dominant copepod in many estuaries, including the York River, Va., and serves as a major food source for various invertebrates and vertebrates. Continuous exposure to 0.05 ppm chlorine permits only 14% survival over 48 hr. Dressel (1971) showed that the 96 hr. TL50 was on the order of 1 ppm for a single addition of

chlorine at 20°C, and slightly less at 25°C. Dressel's study was addressed to problems of chlorination of power plant cooling water when the biocide is introduced intermittently. In the case of sewage treatment, chlorine addition is more nearly continuous such that the receiving water adjacent to the outfall reaches a steady state concentration of chlorine.

The more tolerant species, Palaemonetes pugio and Syngnathus fuscus, feed in part on zooplankton including Acartia. Even though themselves relatively tolerant, decreased food supplies could reduce their populations.

The data should be interpreted with caution because of the extreme lability of chlorine in aquatic systems, and the possibility at these low levels of significant uptake by larger species tested. Measurement of ambient concentrations in test aquaria may be quite misleading. Stock solutions were as much as two orders of magnitude above the concentration calculated stoichiometrically to be necessary to produce any desired concentration assuming no loss to the atmosphere. Further, in the test with Menidia, the two lowest concentrations tested were nearly identical in ambient concentration (0.034 ppm vs. 0.040 ppm). The apparatus was set up to produce ca. 0.05 and 0.1 ppm respectively. At the nominal 0.1 ppm level, 95% mortality was observed after 96 hr, whereas at the nominal 0.05 ppm level, only 5% mortality was observed. Analysis of the inflowing water indicated concentrations of 0.16

and 0.23 ppm for nominal 0.05 and 0.1 ppm levels respectively. The ambient levels in the tank, used to calculate the TL50, are not true estimates of the exposure level, but neither are the concentrations of the inflowing water appropriate, since some fraction of this chlorine was lost by volitalization. Presumably the high mortality at the nominal 0.1 ppm level reflects uptake by the fish themselves at a greater rate than was occurring at the nominal 0.05 ppm level.