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June 1979

Prepared for
the U.S. Nuclear Regulatory Commission
under a Related Services Agreement with the
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TOXICITY AND EFFECTS OF BROMOFORM ON FIVE MARINE SPECIES

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ABSTRACT

Bromoform has been identified as the single most abundant halogenated organic compound produced by the chlorination of marine waters. To determine the potential biological effects of its release into marine waters, short-term toxicity bioassays and 28-day uptake/28-day depuration studies were conducted with five marine species: Protothaca staminea, Mercenaria mercenaria, Crassostrea virginica, Penaeus aztecus and Brevoortia tyrannus. The bioassay studies indicate that 96-hr LC_{50}'s ranged from approximately 7 ppm for B. tyrannus to greater than 40 ppm for P. staminea. The behavior of P. aztecus and B. tyrannus was significantly altered by exposure to bromoform.

INTRODUCTION

Bromoform has been identified as the major organohalogen compound formed by the chlorination of sea water (Bean et al. 1978; Carpenter and Smith 1978). Coastal-sited steam electric stations chlorinate cooling water to prevent condensor fouling and slime buildup; consequently, these stations may have the potential to release considerable amounts of bromoform into the marine ecosystem. To test the relative toxicity of bromoform and the probability of bioaccumulation, a series of bioassays with bromoform were conducted using five marine species. The purpose of the bioassays was to obtain an estimate of the potential toxicity of bromoform and bioaccumulation of bromoform by organisms that may be exposed to cooling water discharges. This report discusses the toxicity of bromoform to five species of marine organisms: littleneck clam (Protothaca staminea), Eastern oyster (Crassostrea virginica), quahaug (Mercenaria mercenaria), shrimp (Penaeus aztecus), and menhaden (Brevoortia tyrannus).

METHODS AND MATERIALS

The bioassays with P. staminea were conducted at the Battelle Marine Research Laboratory, Sequim, Washington, and those with M. mercenaria, C. virginica, P. aztecus and B. tyrannus were conducted at the Battelle Florida Marine Research Facility, Daytona Beach, Florida.

Collection and Exposure--Sequim

Specimens of P. staminea were collected from Sequim Bay, Washington, and held in unfiltered, ambient running sea water for four days prior to testing. There was less than 1% mortality during the holding period. The exposure of P. staminea was conducted by bubbling air saturated with bromoform directly into the exposure tanks (Figure 1). The tanks were 30-liter glass aquaria layered with approximately 5 cm of coarse sand. Seventy-five clams were randomly selected and placed in each tank. Concentration of bromoform and mortality was monitored five days a week. Bromoform/air flows were adjusted to maintain nominal concentrations of 0, 1, 5, 10, and 20 mg/l.

Collection and Exposure--Daytona

With the exception of shrimp (P. aztecus) which were purchased from a local bait shrimp dealer, clams (M. mercenaria), oysters (C. virginica) and juvenile menhaden (B. tyrannus) were collected by Battelle staff members in the Halifax River within one mile of the Florida Marine Research facility. Shrimp and menhaden were held in 11,350-liter outdoor holding tanks with a continually circulating supply of Halifax River sea water, filtered through sand and activated carbon. Purina Trout Chow® was fed daily at a rate of 5% body weight.

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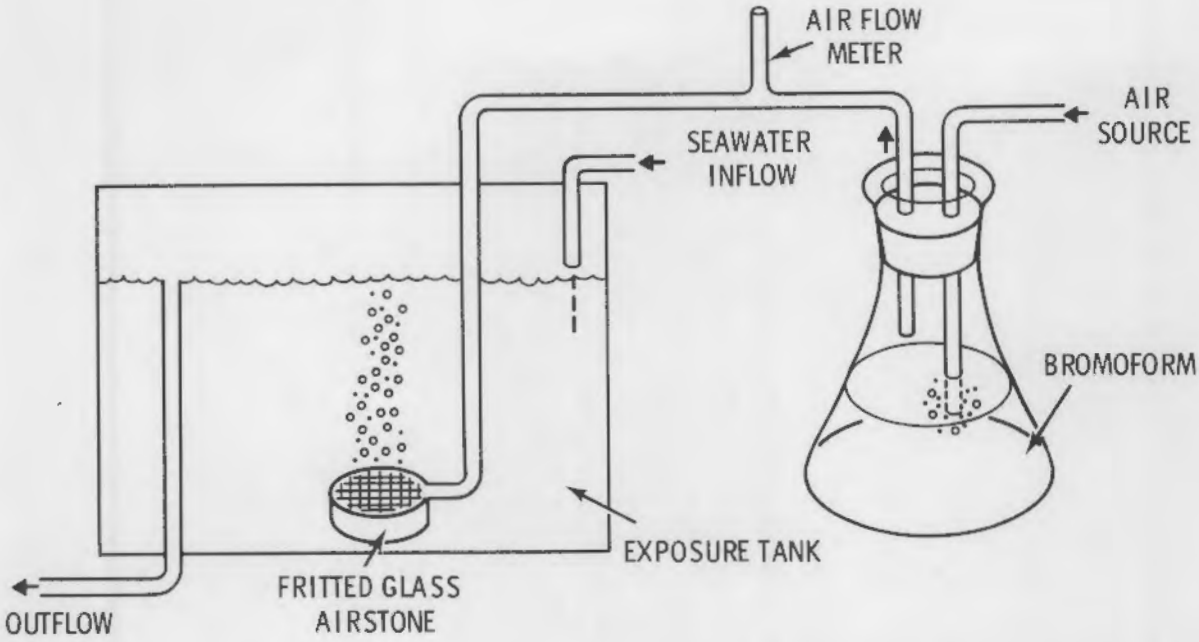

FIGURE 1. Toxicant Delivery System for Seawater/Bromoform Bioassays Conducted at Sequim

Clams and oysters, held in 265-liter water tables, were supplied with unfiltered water as a food source. Clams were placed in 5 cm of sand. All organisms were held for at least one week prior to exposure and showed less than 1% mortality. LC_{50} values were calculated by the method of Litchfield and Wilcoxon (1948).

Bromoform exposures were conducted with a modified Chadwick® diluter (Figure 2). Exposure chambers were 40-liter glass aquaria. Sea water used for the experiments was pumped throgh activated carbon filters before being returned to the Halifax River.

Bromoform was introduced into the sea water by bubbling air through liquid bromoform and then into sea water in a mixing chamber via silicone tubing and a glass airstone. Air flow was regulated with glass air valves and rates were measured with Gilmont® flow meters. The concentration of bromoform in the sea water was controlled by regulating the air and seawater flow rate to the mixing chamber.

Sampling and Monitoring Procedures

During all exposures at Daytona, daily water quality measurements of incoming diluter water were taken. Water quality parameters had the following ranges: Salinity, 25 to 35 ppt; temperature, 25° to 30°C, pH, 8.0 to 8.5; D.O., 5.5 to 7.5 mg/l. Water samples were taken for bromoform analysis from selected

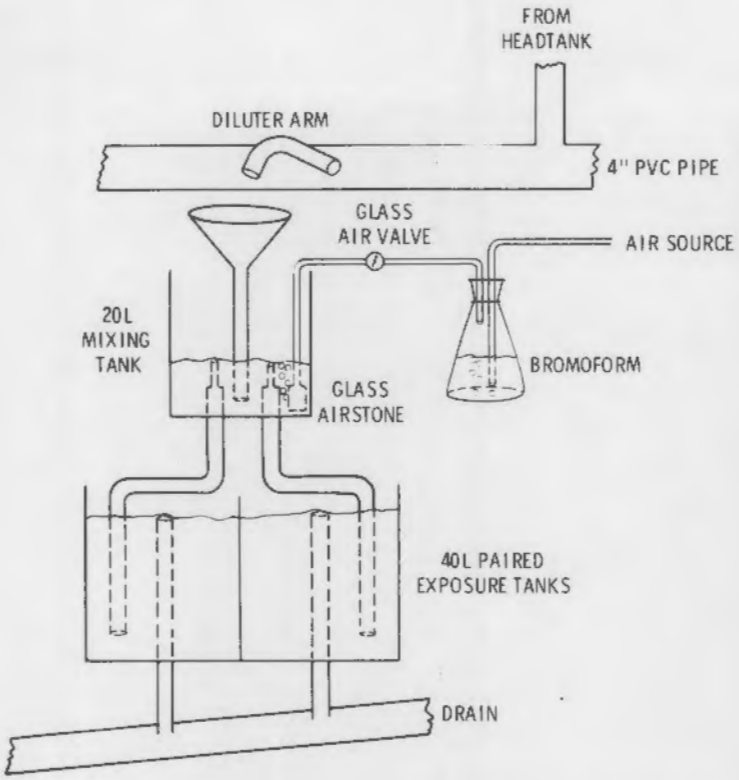

FIGURE 2. A Section of the Bromoform Exposure System Used at the Beach Laboratory

exposure tanks at each concentration, and were collected daily in amber glass bottles and immediately refrigerated for shipment to Sequim. Water and airflow rates were monitored daily.

At Sequim, water quality was not monitored. Previous studies have shown the water quality to be stable with only slight seasonal variation. Salinity ranges from 29 to 31 ppt and temperature from 7° to 13°C. Oxygen is normally at 100% saturation (9.4 mg/l) except in late August and September when concentrations down to 80% saturation have been observed. Water samples for bromoform analysis were collected and stored under refrigeration.

Bromoform Analysis

Water samples collected from the bioassay tanks were stored in tightly capped and compeltely filled 60-ml bottles at 4°C prior to analysis. Subsamples (5 to 10 ml) were removed from the bottles and transferred to 25 ml screw-cap vials containing 10 ml hexane. The vials were hand-shaken for 90 seconds and allowed to stand until phase separation occurred. One ml of the hexane phase was transferred to a 2 ml Hewlett Packard® autosampler vial with septum cap. An internal standard was then added to the vial by syringe (3 µl of 152 µg/ml, 3 dibromopropane). The samples were then analyzed by electron capture gas chromatography, utilizing a Hewlett Packard model 5840® with autosampler.

The analysis conditions were as follows: Column--30 meter SP2100 glass capillary with 15 to 1 split ratio; carrier gas--helium; oven temperature--85°C, detector--$^{63}N_1$ electron capture. Calculation of sample concentration was conducted by the internal standard calibration method.

RESULTS

Protothaca staminea

Preliminary testing indicated that obtaining a 96-hr LC_{50} would be difficult because the clams closed up and did not pump water when exposed to high levels of bromoform. At concentrations in the 300 to 400 mg/l range, P. staminea retracted their siphons and closed. They remained closed and died in that position in one test where the concentration was approximately 800 mg/l. To obtain an estimate of toxicity and potential for bioaccumulations, exposures to 1, 5, 10 and 20 mg/l were conducted for 28 days. Considerable difficulty was experienced in maintaining the desired bromoform concentrations. At the highest level (nominal 20 mg/l), the average of 20 values for the 28-day exposure was 27 mg/l with a range from 9 mg/l to 76 mg/l. A plot of the concentrations measured are given in Figure 3. The other exposure tanks had similar variation (Figures 4, 5, and 6) and averaged 2 mg/l, 7 mg/l, and 19/l for the target concentrations of 1, 5, and 10 mg/l, respectively.

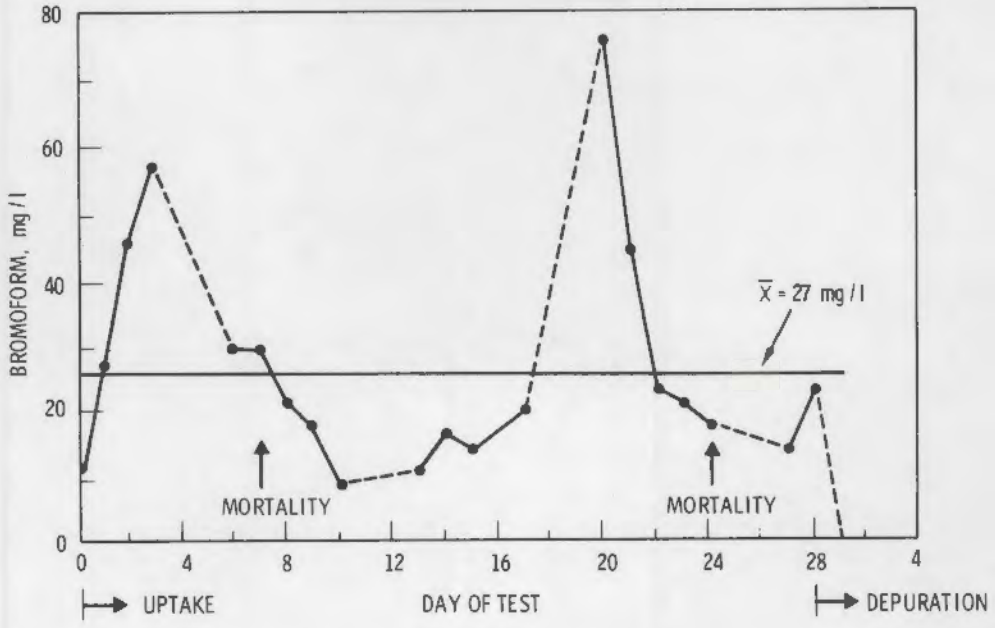

FIGURE 3. Measured Concentrations in 20 mg/l Exposure Tank

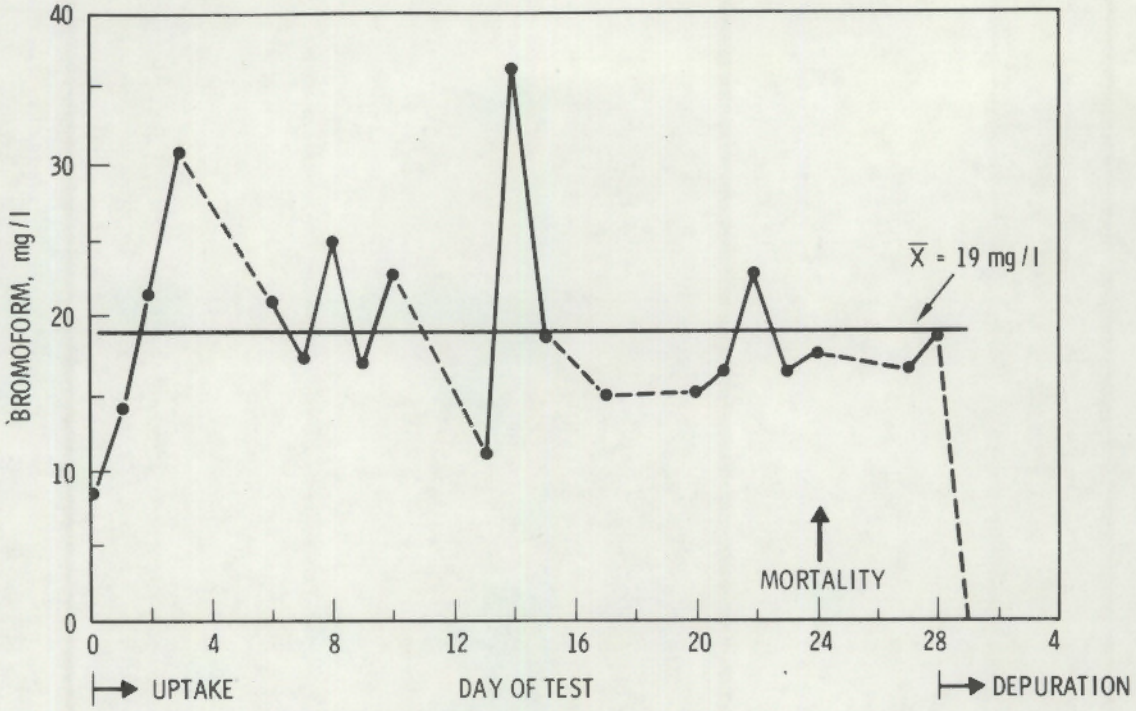

FIGURE 4. Measured Concentrations in 10 mg/l Exposure Tank

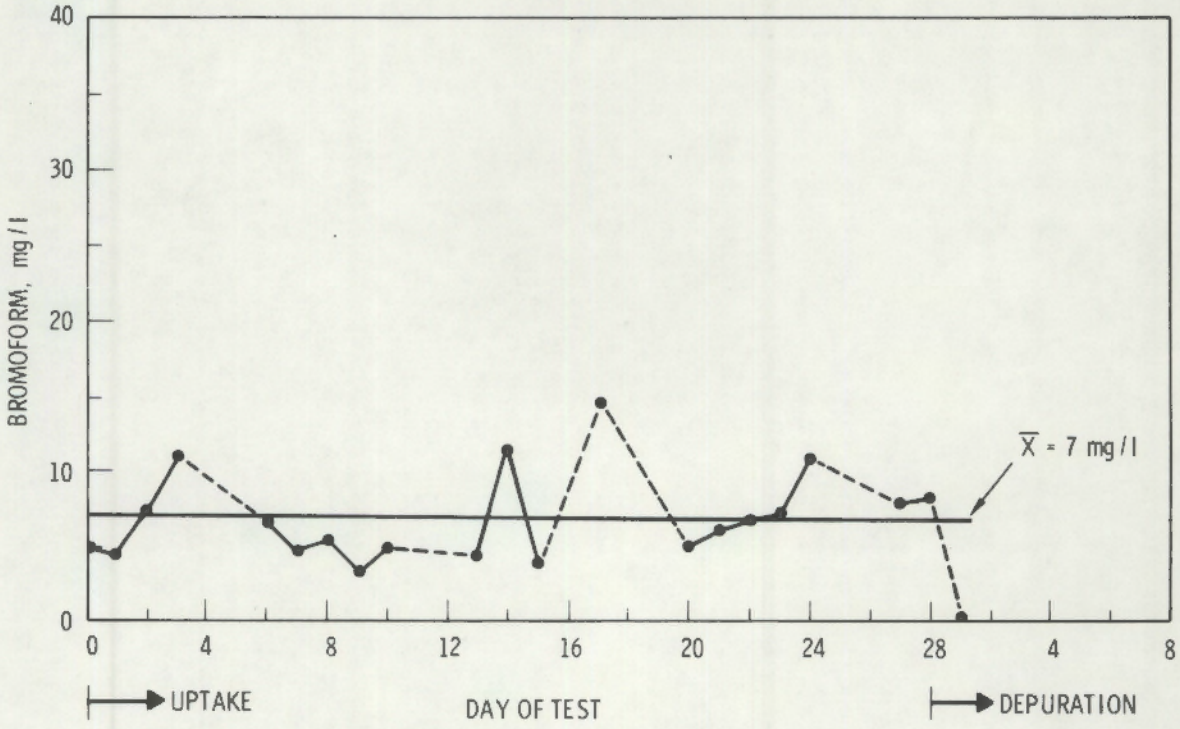

FIGURE 5. Measured Concentrations in 5 mg/l Exposure Tank

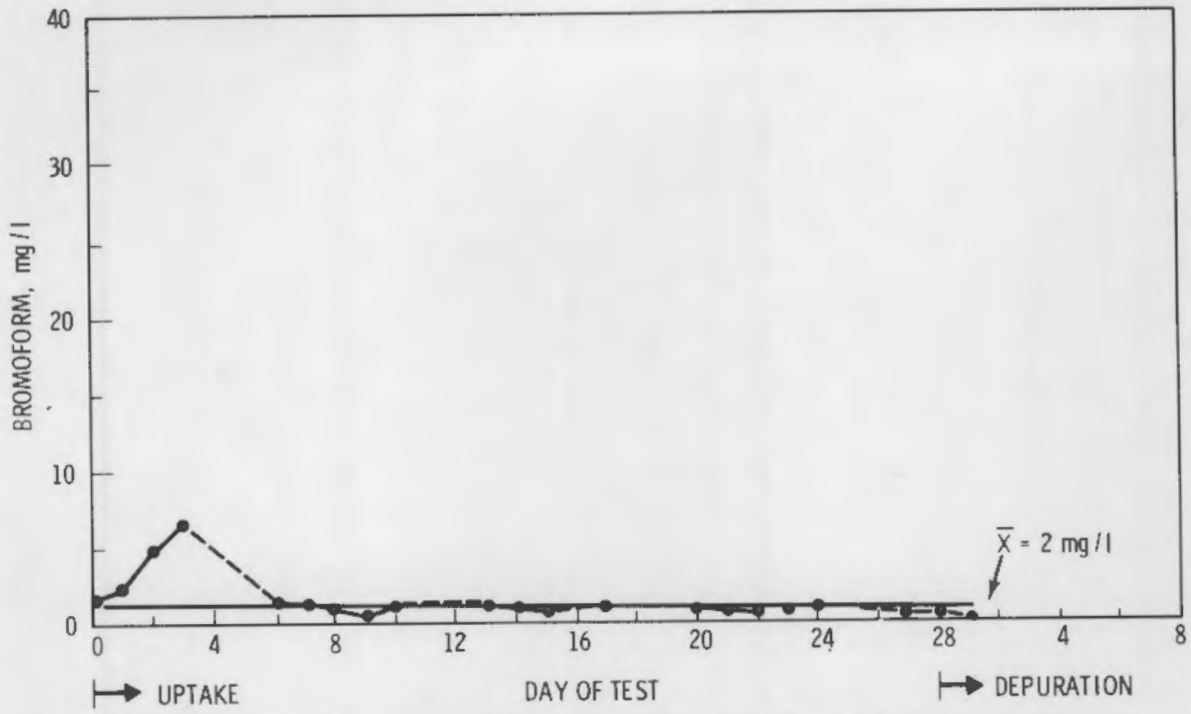

FIGURE 6. Measured Concentrations in 1 mg/l Exposure Tank

Mortality was observed only in the two higher concentrations. At the highest concentrations (average 27 mg/l), 21 of 60 clams were found dead on day seven of the exposure, and then 10 of 24 clams were found dead on day 25. In the 10 mg/l exposure (average concentration of 19 mg/l), 9 of 45 clams were found dead on day-25. During the depuration cycle of the test, two more mortalities occurred at the highest concentrations, one recorded on day four, and one recorded on day seven. The exact day of death for these individuals could not be determined. Of interest is the fact that no mortalities occurred at the lower two test levels through the 28-day uptake or 28-day depuration period.

Penaeus aztecus

The calculated 96-hr LC_{50} for P. aztecus was 26 mg/l with a 95% confidence interval between 33 mg/l and 20 mg/l. Of interest was the behavior exhibited by the shrimp at two levels of concentration. At bromoform/airflow rates delivering 19 mg/l and above, an avoidance response to the bromoform source occurred within 60 seconds of exposure. At flow rates delivering 31 mg/l and above, a narcotic-like effect, where the shrimp were observed lying on their sides on the bottom of the tank with their abdominal appendages undulating, occurred within 120 minutes and continued throughout the experiment or until death.

Brevoortia tyrannus

The calculated 96-hr LC_{50} for B. tyrannus was 12 mg/l with a 95% confidence interval between 15 mg/l and 9 mg/l. As the menhaden approached death they began to lose equilibrium and lay on their sides at the bottom of the tank. Opercular movement gradually decreased until all movement stopped.

Crassostrea virginica and Mercenaria mercenaria

A 96-hr exposure period appears to be inadequate to generate meaningful LC_{50} data on clams and oysters. At concentrations above 10 mg/l, filtering ceases and the bivalves close and remain closed for much of the exposure period. At the end of 96 hours there were no mortalities with either M. mercenaria or C. virginica. However, mortalities occurred during the 3-day period immediately following the 96 hours of exposure to bromoform. Based on this latent mortality data, the 50% mortality concentration for C. virginica and M. mercenaria was in the range of 40 mg/l and 140 mg/l, respectively.

DISCUSSION

The results of the bioassays indicate that bromoform does not cause acute effects to the species tested at concentrations below 1 mg/l. Menhaden were the most sensitive, with a 96-hr LC_{50} of 10 mg/l. Shrimp were next in sensitivity with a 96-hr LC_{50} of 26 mg/l. The bivalves tested had 96-hr LC_{50}'s that were apparently above 40 mg/l. These bromoform concentrations are well above those one would expect in a power plant discharge, based on the findings of Carpenter and Smith (1978) and Bean et al. (1978). They reported bromoform concentrations of 30 to 350 ppb in sea water that had been chlorinated at a rate of 1 to 4 ppm. This is a conversion rate of about 0.02 to 0.08 parts bromoform for each part chlorine added; for this conversion rate, chlorine would have to be added at a rate of 500 mg/l to form sufficient bromoform to cause acute effects. At this rate of chlorination (unless there is an extremely heavy chlorine demand), the residual oxidant will have a much more pronounced effect than bromoform. The literature reports that total residual oxidant causes acute effects to the tested species in the 1.5 mg/l to 0.005 mg/l range (Roberts et al. 1975; Thatcher 1978; Scott et al. 1978).

The mortalities noted in the P. staminea 27 mg/l uptake/depuration exposure are curious in that they appear to have occurred at two single points in time. Both occurrences were four days after peak exposure concentrations were experienced (Figure 3). The first mortality occurred after a peak of 56 mg/l bromoform, and the second occurred after a peak of 76 mg/l bromoform. Thus, in this exposure tank it appears that there may be threshold concentration above which mortality begins.

The mortality that occurred in the 19 mg/l exposure did not follow the pattern found at the higher level. The concentration in this system was not as variable as in the 27 mg/l exposure, and the mortality did not occur until ten days after a peak concentration occurred.

The delayed mortalities noted in the oyster tests and the above clam mortalities indicate that the action of the bromoform at high concentrations can cause severe enough damage to prevent recovery. This action can result from short-term exposure to high concentrations (probably greater than 50 mg/l) or longer term exposure to lower concentrations in the 20 to 30 mg/l range. In regard to concern about the release of bromoform from steam electric stations, the exact concentrations required for either short-term or long-term mortality is academic, since these levels are approximately 1000 times those expected to be found.

At sublethal concentrations, the menhaden and shrimp exhibited some qualitative behavioral changes. After 48 hours, juvenile (under 7 cm T.L.) menhaden exposed to 6 mg/l and 9 mg/l bromoform exhibited extreme excitation to external stimuli such as loud noises, quick movements or sudden light changes. These stimuli would cause the fish to swim rapidly in random directions and frequently collide with the tank walls. In control tanks and at the higher concentrations, this response did not occur. This excitability continued for up to 20 days after the exposure to bromoform had been terminated.

The shrimp responded similarly at bromoform concentrations between 0.4 mg/l and 6 mg/l. However, at concentrations below 3 mg/l the response was no longer evident within one hour after bromoform addition was stopped. At concentrations between 3 mg/l and 6 mg/l, the response continued for at least one day.

These observations are qualitative but in complete opposition to the response noted for those organisms that died. At the higher levels the bromoform appeared to act as a narcotic. Both shrimp and menhaden gradually slowed down, lost orientation and eventually stopped pleopod or opercular movement. This condition was reversible for the shrimp, which recovered within a few hours if the bromoform input was stopped before pleopod motion ceased.

Based on the 96-hr LC_{50} studies and mortality data from the uptake and depuration studies, the potential for acute environmental effects (to the studied species) from bromoform created through chlorination of steam electric station cooling waters is minimal. The behavioral responses noted should be considered subjective observations that may or may not be related to bromoform exposures. To determine if the behaviorial responses noted are, in fact, real changes and caused by bromoform, further research will be necessary.

Bioaccumulation data not available at this writing will provide insights into the potential for long-term effects on food web transfer of bromoform via those species that spend significant time in discharge streams.

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