Copper Toxicity in the Pacific Oyster *Crassostrea gigas*

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Copper (Cu) is considered to be one of the more toxic of the trace metals (BRYAN 1971). HARVEY (1960) states that Cu concentrations of 1.0 ppm are generally poisonous to marine organisms. Bivalves, especially oysters, are known to concentrate Cu. However, the toxicity levels of Cu have not been established for the Pacific oyster (Crassostrea gigas), an important shellfish grown commercially on the Pacific coast. Thus to fulfill this lack of information, a laboratory study was designed to determine the lethal concentrations of Cu to Crassostrea gigas.

MATERIALS AND METHODS

The oysters obtained from the Tomales Bay Oyster Company in Point Reyes Station, California, were 18 to 24 months old and sexually mature. Shell lengths ranged from 13 to 17 cm. All oysters were held in running unfiltered sea water (33 °/oo) at 12-15°C for one week prior to testing.

The oysters were exposed to Cu concentrations of 0.10, 0.25, 0.50, 0.75, and 1.00 ppm for three separate 96-hr experiments to determine the median tolerance limits (TL_m), the concentration at which 50% of an experimental population are dead after 96 hours for this study. Gaped oysters, displaying no muscular responses to probing, were considered dead. In addition to the above, one 336-hr (14 day) exposure to Cu concentrations of 0.010, 0.025, 0.050, 0.075, and 0.100 ppm was carried out to test sublethal effects.

The animals were exposed to Cu in the form of cupric sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) through a continuous-flow proportional diluter (MOUNT and BRUNGS 1967). Unfiltered sea water delivered to the diluter through polyvinylchloride pipes was used during the experiments to stimulate filtering and feeding responses by the oysters. Salinities, temperatures, and pH measured every 24 hours were 33 ± 1 °/oo, 13 ± 1°C, and 8.0 ± .1, respectively.

For each experiment, ten randomly selected oysters scrubbed of epifauna were placed with hinges downwards on plastic mesh screens in each of the six tanks. The number of survivors were recorded every 12 hours, at which time the feces were flushed from the tanks and dead oysters were removed.

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For the determination of Cu in the tanks, water samples of 200 ml were collected initially and every 24 hours and acidified with 2 ml redistilled 1:1 HCl. Control water samples were taken at the beginning and end of the 96-hr experiments. A third control sample was collected at 168-hr (7 day) for the 336-hr experiment. These samples were prepared according to the methyl isobutyl ketone and ammonium pyrrolidine dithiocarbamate extraction method described by PERKIN-ELMER CORP. (1971) and analyzed by atomic absorption spectrophotometry.

RESULTS

Table 1 shows the Cu concentrations in the test tanks. The values conformed closely to the concentrations selected for the experiments. Deviations are attributed to analytical and instrumental errors.

TABLE 1.

Cu concentrations (ppm) in the test tanks. Values are means ± one S.E.M. a=(n=2); b=(n=3); c=(n=5); d=(n=15).

96-hr Test	Control[a]	0.10[c]	0.25[c]	0.50[c]	0.75[c]	1.00[c]
#1	0.005± .0005	0.12± .03	0.24± .04	0.52± .06	0.70± .06	1.04± .07
#2	0.004± .0009	0.10± .03	0.26± .06	0.61± .08	0.80± .06	1.09± .09
#3	0.005± .0005	0.10± .03	0.26± .04	0.55± .07	0.73± .06	1.04± .06
336-hr Test	Control[b]	0.010[d]	0.025[d]	0.050[d]	0.075[d]	0.100[d]
	0.005± .0004	0.011± .002	0.026± .003	0.057± .004	0.075± .003	0.110± .020

The numbers of oysters and per cent survival for the three 96-hr tests are tabulated in Table 2. No deaths occurred during the first 72 hours; however, high mortalities were observed in the final 24-hour period. The per cent survival decreased proportionally to increasing exposures except at 1.00 ppm where 67% survival was observed.

TABLE 2

Numbers of oysters surviving 0.10-1.00 ppm Cu^{++} at 24-hr intervals in three 96-hr experiments (n=10).

Concentration (ppm)	Experiment 1 24 hrs.	48 hrs.	72 hrs.	96 hrs.
Control (0.005)	10	10	10	10
0.10	10	10	10	10
0.25	10	10	10	7
0.50	10	10	10	5
0.75	10	10	10	3
1.00	10	10	10	5

Concentration (ppm)	Experiment 2 24 hrs.	48 hrs.	72 hrs.	96 hrs.
Control (0.005)	10	10	10	10
0.10	10	10	10	10
0.25	10	10	10	6
0.50	10	10	10	5
0.75	10	10	10	5
1.00	10	10	10	8

Concentration (ppm)	Experiment 3 24 hrs.	48 hrs.	72 hrs.	96 hrs.
Control (0.005)	10	10	10	10
0.10	10	10	10	10
0.25	10	10	10	7
0.50	10	10	10	5
0.75	10	10	10	4
1.00	10	10	10	7

Total % Survival

Concentration (ppm)	%
Control (0.005)	100
0.10	100
0.25	67
0.50	50
0.75	40
1.00	67

Figure 1 presents the combined data for the three 96-hr test, excluding the results for the 1.00 ppm exposure. The regression coefficient (-26.6) calculated by the method of least squares (WOOLF 1968) is highly significant ($P<0.01$; $F=44.1$; 3 d.f.). Extrapolation shows that the 96-hr TL_m is estimated to be 0.56 ± .15 ppm (95% confidence limits).

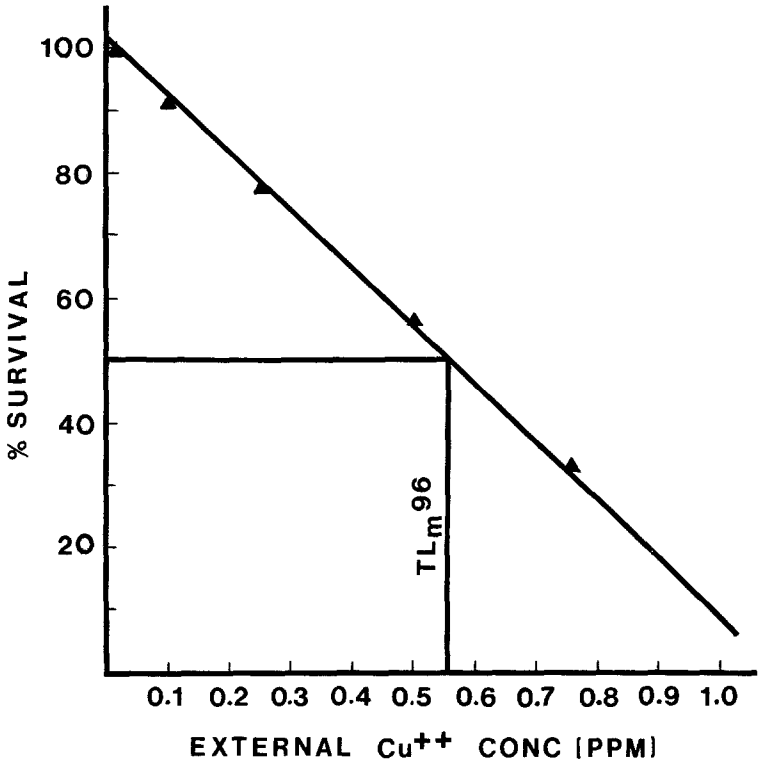

Figure 1. Per cent survival of oysters exposed to 0.005 (control), 0.10, 0.25, 0.50, and 0.75 ppm Cu^{++} and 96-hr TL_m.

The numbers of oysters and per cent survival for the 336-hr test are compiled in Table 3. The oysters were able to survive these concentrations without eliciting a 50% mortality response. Mortalities were first observed in the 144-hour period, and survival ranged from 60-80%.

TABLE 3

Numbers of oysters surviving 0.010-0.100 ppm Cu^{++} at 24-hr intervals in a 336-hr experiment (n=10).

Concentration (ppm)	Hours														
	24	48	72	96	120	144	168	192	216	240	264	288	312	336	% Survival
Control (0.005)	10	10	10	10	10	10	10	10	10	10	10	10	10	10	100
0.010	10	10	10	10	10	10	10	10	10	10	10	10	9	8	80
0.025	10	10	10	10	10	10	8	8	8	8	8	8	8	8	80
0.050	10	10	10	10	10	8	7	6	6	6	6	6	6	6	60
0.075	10	10	10	10	10	10	7	7	7	7	7	7	7	7	70
0.100	10	10	10	10	10	9	7	7	7	7	7	7	7	7	70

DISCUSSION

In this study the 96-hr TL_m was estimated to be 0.56 ppm (see Figure 1). This value is much higher than the concentrations varying from 0.001-0.010 ppm found in the natural environment (SPECTOR 1956). FUJIYA (1960), however, calculated a 96-hr TL_m of 1.9 ppm for the Japanese oyster (presumably Crassostrea gigas). These conflicting values may reflect differences in experimental conditions and/or exposure levels of Cu^{++}. Fujiya experimented under static conditions, while this study used a continuous-flow system. ADEMA et al. (1972) favored a continuous-flow system for testing the mussel Mytilus edulis after Cu^{++} concentrations decreased from 0.1 ppm to natural levels within a few hours under static conditions. In addition, the accumulation of Cu^{++} and its effects may vary with genetically different populations, activity (feeding vs. nonfeeding), physiological state, salinity, temperature, and pH.

In this study concentrations greater than the TL_m value of 0.56 ppm might be presumed to be lethal. However, at 1.00 ppm exposure, oyster mortality averaged 67% for the three 96-hr tests. Thus, the TL_m may represent the upper limit of response to lower but lethal concentrations of Cu^{++}. Exposures to concentrations somewhat greater than this value may not affect the oyster which may show varying responses to high Cu^{++} exposures. For example, Cu^{++} at high concentrations may not be chemically similar to that existing at lower concentrations. The metal may be precipitating out at a faster rate at 1.00 ppm, and the oysters may be able to sense this form and cease feeding by closing their valves. My preliminary experiments have shown 100% survival of oysters exposed to Cu^{++} levels of 5.6 and 7.5 ppm for 96 hours. YAGER and HARRY (1966) also observed decreased uptake of Cd^{++} and Zn^{++} in the snail Taphius glabratus at higher exposures. Further studies are needed to investigate the possibilities of chemical changes of the metal occurring at high concentrations and the effects and availability of different forms of Cu.

BRYAN (1971) has suggested that regulation of metals may be less efficient in the larval than in the adult stages. Because adult oysters were used in this study, concentrations lower than 0.56 ppm may be presumed to be lethal to the more susceptible egg and larval stages. Cu^{++} concentrations of 0.10 and 0.13 ppm were found to affect egg development in Crassostrea gigas (OKUBO and OKUBO 1962) and to induce 100% mortality in the embryos of the American oyster Crassostrea virginica (CALABRESE et al. 1973), respectively. However, PRYTHERCH (1934) stated that Cu concentrations >0.5 ppm were toxic to larvae of Crassostrea virginica.

In the 96- and 336-hr experiments, deaths occurred at the 72- and 144-hr period, respectively. This indicates that the length of exposure becomes critical since Cu^{++} uptake is a cumulative process which may eventually overwhelm or slowly damage regulatory mechanisms and sites of accumulation in the tissues.

In summary, the TL_m appears to be a relative value with defined parameters of salinity, temperature, and pH and experimental conditions, such as continuous-flow versus static and duration of the experiments. In this study, salinity (33 °/oo), temperature (13°C), and pH (8.0) were kept constant. Oysters usually found in estuaries are more likely to experience lower salinities, warmer temperatures, and fluctuating pH values. Metal toxicity or uptake has been found to be influenced by these three parameters (PRINGLE et al. 1968; BRYAN and HUMMERSTONE 1971; TSAI et al. 1975). Thus, further studies using lower salinities and pH and higher temperatures may have elicited a lower TL_m value than 0.56 ppm and resulted in mortalities at earlier time exposures.

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