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BIOASSAYS ON MARINE ORGANISMS: ACUTE TOXICITY TEST OF MERCURY, CADMIUM AND COPPER TO ARKSHELL, *Anadara broughtonii*, FROM JIN-DONG BAY, AND TO OYSTER, *Crassostrea gigas*, FROM KWANG-DO BAY, SOUTH COAST OF KOREA

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ABSTRACT

Short-term acute toxicity of mercury, cadmium and copper to arkshell, *Anadara broughtonii*, and to oyster, *Crassostrea gigas*, was determined by static bioassays from 20 May to 27 June in 1977. During the observations of the opening rate of the shell mercury was the most sensitive toxicant of the three toxic substances to the test animals and caused them to close their shellvalves together after being exposed to a mercury solution for an hour during the test. Opening rate to cadmium and copper increased gradually at the higher concentration. The 96hr-LC50 values for the test animals are 4.84mg/*l* for mercury and 1.86mg/*l* for cadmium, while the 72hr-LC50 value for copper is 0.31mg/*l*.

The death rate of oysters for cadmium showed lower than that of the mercury and copper test solutions. The 96hr-LC50 values of mercury, copper and cadmium were 1.1mg/*l*, 2.54mg/*l* and 19.5mg/*l*, respectively.

For oysters mercury was the most toxic substance, and cadmium was the least toxic one. The medium lethal time (LT 50) value decreased gradually at higher concentration of heavy metals. The LT 50 of 2mg/*l* was found within 96 hours for copper, 104 hours for mercury and 121 hours for cadmium. The lethal threshold concentrations for 7 days were found to be about 1mg/*l* for mercury and copper, and 2mg/*l* for cadmium.

INTRODUCTION

The increase of water pollution problems is leading us toward official "Water Quality Criteria" for aquatic organisms. It is known that these pollutants from the discharge of industrial wastes, domestic sewage and other municipal wastes have resulted in degradation of the receiving waters. In particular heavy metals are regarded as serious pollutants of the marine ecosystem because of their environmental persistence, their toxicity at low concentration and their ability to be incorporated into food chains and concentrated by aquatic organisms.

To know how organisms respond to toxic chemical materials and various environmental factors bioassays have been conducted and advanced in the fields of pharmacology, toxicology, microbiology any fisheries, such tests provide some meaningful results. Reproducible and relevant kinds of toxicity tests were recommended in assessing the acute toxicity of substances by several authors; e.g., Doudroff(1951), Gaddum (1953), Connor(1962), Sprague(1951), and Bliss and Finney(1964) since the need for information for protection of aquatic life has arisen. For oysters, the toxicity of heavy metals has been studied by Calabrese *et al.*(1973), Wisely and Blick(1967), and soon.

There is no information about acute and sublethal effects of heavy metals on shellfish and marine fish in the south coast of Korea, except a toxicity test of phenol on the clams (Wui *et al.* 1974). The investigation was initiated to provide basic information on the toxicity of heavy metals to protect living resources in Jin Dong Bay. Arkshell was chosen as the test animal because of their commercial importance in aquaculture and their availability and abundance in the bay. Oysters from Kwang-do Bay were also collected for the same purpose.

MATERIALS AND METHODS

Collection of test animals

A thousand arkshells, *Anadara broughtonii*, from Jin-Dong Bay and oyester samples(2.63cm in length) from Kwang-do Bay and were collected and were washed in fresh sea water and were transported in plastic containers with covering wet patchs to the laboratory where they were placed in a holding tank, The mean length and width were 2.86 and 2.1cm respectively.

Acclimation of test animals and diluted water

Test animals were acclimated for a week(22 May to 29 May) to reduce unexpected impacts of temperature and metabolism on natural and laboratory conditions before use. The salinity, temperature and *p*H of the seawater that flowed into the holding tank were 34.1‰, 19±2°C and 7.9 units. The water quality conditions of test diluents is shown in Table 1.

Testing procedures

A logarithmic-series of concentrations of solutions were made by diluting standard solutions of cadmium hloride ($CaCl_2 \cdot 2\frac{1}{2}H_2O$), cupric sulfate ($CuSO_4 \cdot 5H_2O$) and mercury bichloride ($HgCl_2$). Healthy and parasite-free animals were selected and then distributed to test containers made of glass for preliminary tests. According to the results of preliminary estimation of half of the animals respond to toxicity, five series of test solutions were employed with ten animals per each test container.

Table 1. Average Water Quality Conditions for Test Solution

Conc. mg/*l*		Temp. °C	Salinity ‰	*p*H	Field (Sampling Station)
Hg	2.0	15.9	34.4	7.97	Temp.: 19.3°C
	3.0	15.8	34.3	7.94	Sal.: 34.2‰
	4.0	15.7	34.5	7.96	*p*H: 7.92
	6.0	16.1	34.4	7.90	
	8.0	16.2	34.6	7.94	
	10.0	16.0	34.2	7.96	
Cd	2.0	16.1	34.2	7.95	
	6.0	16.4	34.6	7.92	
	8.0	16.4	34.3	7.98	
	16.0	17.0	34.3	7.94	
	32.0	16.5	34.4	8.00	
Cu	1.0	15.7	33.9	7.79	
	1.5	15.8	34.1	7.69	
	2.0	16.4	34.3	7.88	
	4.0	17.1	33.7	7.38	
	6.0	16.1	33.8	7.77	
	8.0	17.4	34.2	7.67	
	10.0	16.7	33.7	7.69	
Control		16.1	34.2	7.94	

During the test, the test solution was aerated for the oxygen supply and analysed for heavy metal once a day, and then the newly-made test solution was replaced every 24 hours for the stability of the lowered fixed metal concentration owing to the uptake by animals, chemical and biological degradation and adsorption to the container wall, which made it difficult to maintain a constant concentration of poison. Control tests were also made for comparison. Water diluent was taken so that the weight of all shellfish in a test container should not exceed 1g per liter of the liquid medium tested. According to the results of preliminary estimation of half of the oysters response to toxicity, five series of test solutions were employed with ten or

more healthy animals per each regular test tank (22W×37L×28cmH). During the test no test animals were fed.

Observation

Shell opening rate was checked at 10 minutes intervals for one hour after the start. For observation of the test, exposure time is part of the experimental stimulus (Gaddum 1953), so responses are according to the time observed since concentration is fixed within a given test tank. When apparently dead animals were observed, according to the criteria for death they were removed and both the dead and surviving animals counted. These observation were continued to meet 100 percent dead rate.

Criteria for death

Arkshells which remained opened after the shellvalves had been slightly opened were considered to be dead after being stimulated by prodding, and animals with shells pressed together were transfered to natural seawater to judge whether they were capable of recovering. Only animals that failed to recover were considered as dead.

Shell opening rate

Most of shellfish pressed their shellvalves together, when experimental conditions were not suited for them. Test animals which naturally opened their shells in seawater were distributed into test containers of five series of test solution, and opening animals thereafter were counted every 10 minutes for one hour. The opening rate of test animals might be informative for study of toxic tests as outlined above.

Data presentation

Mortalities in several concentrations of the heavy metals under tests were recorded to look for the concentration at which a certain percentage of the test specimens responed in a previously set time span at fixed intervals, usually 24, 48 and 96 hours, and the lethal concentration for half of the animals (LC50) is extrapolated from these data. The mortalities were modified by the Tattersfield and Morris Formula (1924). On the toxicity response curve, the cumulative percentage is plotted against concen-

Table 2. Opening rate of *Anadara broughtonii* for each concentration every 10 minute for an hour

Metal	Elapsed Time / Conc. mg/*l*	No. of shell opening (10 animals each)						opening numbers	opening rate (%)
		10 min.	20 min.	30 min.	40 min.	50 min.	60 min.		
Hg	2.50	0	0	0	0	0	0	0	0
	3.15	0	0	0	0	0	0	0	0
	4.00	0	0	0	0	0	0	0	0
	5.00	0	0	0	0	0	0	0	0
	6.31	0	0	0	0	0	0	0	0
Cd	1.25	8	7	10	10	5	4	44	73.3
	1.60	8	7	9	9	4	4	41	68.3
	2.00	10	10	8	9	2	3	42	70.0
	2.50	10	9	8	8	2	3	40	66.6
	31.5	8	8	7	5	1	0	29	48.3
Cu	0.20	8	6	6	5	7	8	40	66.6
	0.25	7	5	5	5	7	7	36	60.0
	0.32	10	3	3	2	7	7	32	53.3
	0.40	7	3	2	3	9	8	32	53.3
	0.50	6	3	1	2	7	7	26	43.3
Control		6	7	7	7	5	5	37	61.6

tration and lethal concentration for 50% of the test organisms was described.

RESULTS

Opening rate of the shell

Most of test animals, which had been normally opening their shells in the natural seawater, closed their shellvalves within 10 minutes and no opened animals were observed one hour after placement in the mercury solution. Arkshells seemed to be very sensitive to the toxicity of mercury(Table 2).

For cadmium generally speaking, the more concentration and the more time, the less the opening rate showed. They seemed to move with their foot at the bottom of the container immediately after being distributed in the cadmium solution of the test container. It is remarkable that the opening rate of specimens for the test container is larger than that of the control container. The more time elasped, the opening range of shell is smaller than the initial range (Table 2).

The opening rate of the shell in the copper solution varies gradually according to concentration and elasped time since introduction of the poison. The number of opening animals decreased at a higher concentration of the test solution (Table 2).

Table 3. Modified death rate (%) of *Anadara broughtonii* for every concentration in the experiments with heavy metals

(10 specimen used for each tank)

Metal	Time / Conc. mg/l	Time elapsed 24 hrs	48 hrs	72 hrs	96 hrs
Hg	1.00	0	0	0	20.0
	1.26	0	0	0	28.6
	2.00	0	0	0	28.6
	3.16	0	0	0	41.4
	4.00	0	0	0	40.0
	5.00	0	0	0	41.5
	6.31	0	0	20	57.1
	7.94	0	0	0	60.0
	10.00	20	100	100	100.0
Cd	1.25	0	0	0	20.0
	1.60	0	0	0	59.0
	2.00	0	0	20	80.0
	2.50	0	20	30	70.0
	3.15	0	5	30	82.8
	5.01	0	0	42.9	100.0
Cu	0.20	0	0	0	5
	0.25	0	0	40	100.0
	0.32	0	0	53	100.0
	0.40	0	0	70	100.0
	0.50	0	0	55.5	100.0
	1.00	0	0	87.5	100.0

Modified death rate of arkshells

The results of the death rate from each concentration in the test containers modified by the Tattersfield and Morris Formula (1924) are shown in Table 3. A hundred percent death rate appeared at 10mg/*l* after 48 hours for mercury, and both mercury and cadmium showed zero death rate at 0.1mg/*l* concentration of the test solution for 96 hours, and a hundred percent death rate of cadmium occurred at 5.01 mg/*l* concentration for 96 hours. In view of selected series of each concentration, a higher death rate appeared at the lowest series for copper.

Mortality of osyters

In the effects of heavy metals, no dead animals were found at all heavy metal concentration of 10mg/*l* within 24 hours, and at 3mg/*l* for mercury, 16mg/*l* for cadmium and 2mg/*l* for copper within 48 hours. On the other hand, a mortality of 100 percent of the test animals was found at 2mg/*l* for mercury and 32mg/*l* for cadmium within 144 hours and at 4mg/*l* for copper within 120 hours (Table 4). It was remarkable that the cadium solution caused effects of mortality of the animals at higher concentration than the other two metals.

The lethal threshold concentration of heavy

Table 4. Mortality of *Crassostrea gigas* for every concentration in the experiments with heavy metals (unit : %)

Metal	Conc. mg/*l*	Time elapsed 24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
Hg	2.0	0	0	19.9	52.3	86.2	100
	3.0	0	0	23.5	58.8	100	100
	4.0	0	9.1	42.2	69.7	80	100
	6.0	0	3.6	25.0	54.0	72.2	100
	8.0	0	10.7	39.3	67.9	82.8	100
	10.0	0	13.7	46.3	71.6	87.9	100
Cd	2.0	0	0	0	12.5	37.5	87.5
	6.0	0	0	0	20.0	55.0	80.0
	8.0	0	0	8.8	24.2	79.2	81.8
	16.0	0	0	35.8	50.9	80.0	93.3
	32.0	0	40	60.0	70.0	—	100
Cu	1.0	0	0	0	27.3	63.6	72.7
	1.5	0	0	14.3	42.9	78.6	85.7
	2.0	0	0	18.6	42.6	84.2	100
	4.0	0	27.5	41.1	69.1	100	100
	6.0	0	25.4	88.6	92.3	100	100
	8.0	0	34.5	100	73.6	100	100
	10.0	0	6.3	87.5	100	100	100
Control	0	0	0	0	0	0	0

metals for 7 days was found to be in the vicinity of 1mg/*l* for copper and mercury, and 2mg/*l* for cadmium (Fig. 1).

DISCUSSION

Response/Concentration relation in toxicity test

The studies on the effects of heavy metal on marine shellfish, especially their embryonic and larval stage of oyster were very few. Mercury concentrations of 2mg/*l* produced a mortality of 100 percent after 6 days exposure. Wisely and Blick (1967) exposed larvae of the oyster, *Crassostrea commercialis* to mercury. Fifty percent of the oyster larvae died in 2 hours at mercury concentration of 180.5ppm and Mytilus at 13.0ppm mercury. The 96hr-LC506 value of the persent oyster for mercury was 1.1mg/*l*

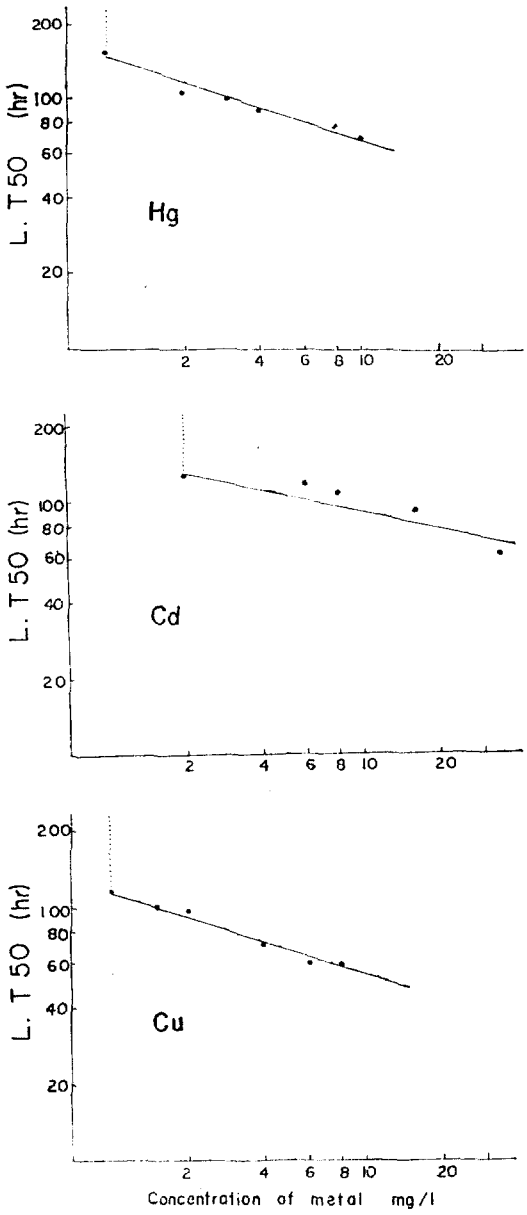

Fig. 1. The lethal threshold concentration of mercury, cadmium and copper

which was a much higher concentration compared with the values of 0.0056ppm within 48 hours (Calabrese et al 1973).

The 96hr-LD50 value of copper for the test animals was 2.54mg/*l*. Fujiya (1960) and Calabrese (1973) had reported a 96hr-LC50 value of 1.9mg/*l* for Japanese oyster, *Crassostrea*

gigas and 48hr-LC50 value of 0.103ppm for oyster embryos, *Crassostrea virginica*, but a 48hr-LC50 value of 22.5mg/*l* for copper had been reported for *Mytilus* by Wisely and Blick (1967).

On the other hand, the 96hr-LC50 value of cadmium for the oyster was 19.5mg/*l*. Using *Crassostrea virginica*, Calabrese (1973) reported that the 48hr-LC50 value for cadmium was 3.8mg/*l*, while Eisler (1971) found that the 96hr-LC50 value for cadmium was 25mg/*l* to *Mytilus edulis*, and 2.2mg/*l* to *Mya arenaria*.

In the test, mercury was the most toxic to oyster and copper was the next toxic metal of the test metals. These relative toxicity effects in that order were similar to those reported by Calabrese (1973), who used the embryos of oyster.

Median lethal time for the mercury test tank was longer than copper at the same levels of relative toxicity. This comparative resistance was due to the ability of these organisms to withdraw their bodies into their shells, thereby reducing the penetration of the toxic matter into the parts of the body(Calabrese 1973). Therefore, the LC50 values obtained would be influenced by the length of time that a particular species could remain closed.

As there is more than one abiotic factor in test solutions and they act simultaneously, so the responess of the test animals did not evenly appear, which make it difficult to understand the status condition of animal with our present knowledge.

To find out the lethal concentration at which half of the test animals respond (LC 50), a toxicity curve was established at a previously fixed time span. Both mortalities in ordinate and concentration in abscissa were plotted in logarithmic scales., The toxicity curve for mercury and copper showed linear relation as log $Y=1.3158+0.5597\ \log X$ and $\log Y=1.9413+0.4759\ \log X$ (Fig. 2). However the curve for cadmium appeared to be curved (Fig. 2). Median lethal concentration of heavy metals(LC 50) to arkshell was determined from the regression line from the data of mortalities and concentration in a logarithmic scales. The 96hr LC 50 values for mercury and cadmium were

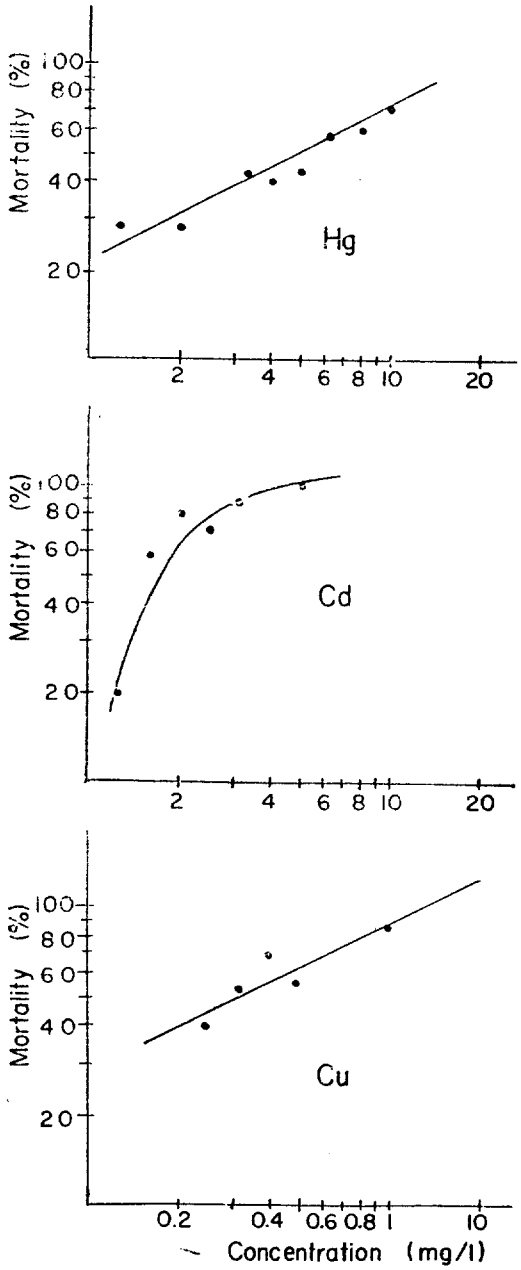

Fig. 2. Toxicity curve of mercury and cadmium for 96hrs and copper for 72hrs

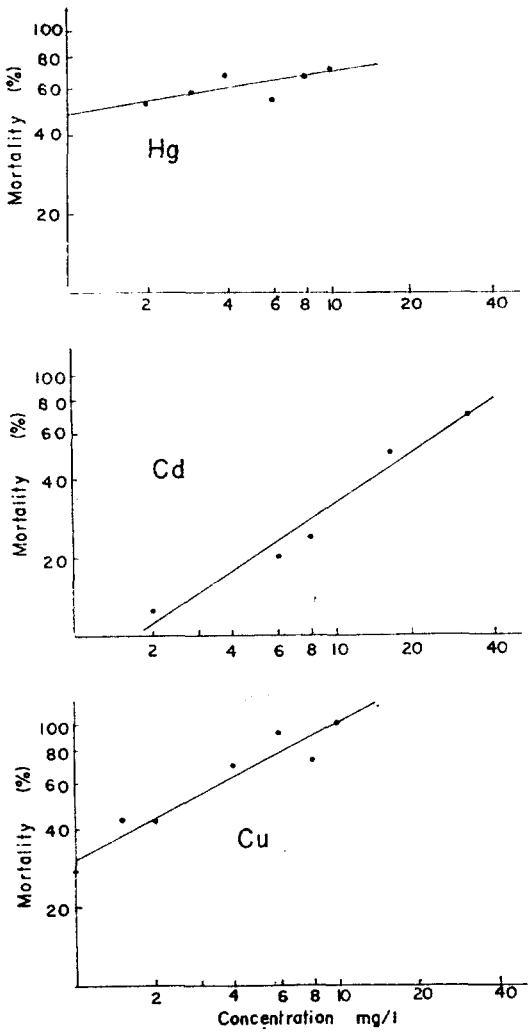

Fig. 3. Toxicity curve of mercury, cadmium and copper for 96hours

4.84mg/*l* and 1.86mg/*l* respectively. The 72hr -LC 50 value for copper was 0.31 mg/*l*.

For oysters the toxicity curve for 96 hours had been shown in linear relation when plotted logarithmically as shown in Fig. 3. In this test, the toxicity curve of mortality in ordinate (Y) and concentration in abscissa (X) showed linear relation as $\log Y=0.1458 \log X+1.6950$ ($r=0.6587$) for mercury, $\log Y=0.5149 \log X+1.4906$ ($r=0.9587$) for copper and $\log Y=0.6588 \log X+0.8486$ ($r=0.9765$) for cadium.

The 96hr-LC50 value of each test metal from the linear relation was 1.1mg/*l* for mercury, 2.54/*l* for copper and 19.5mg/*l* for cadmium, The most toxic metal was mercury, and copper was the next. However, cadmium was the least toxic substance as the LC50 value of 19.5mg/*l*.

Table 5. Time for 50% mortality at each test tank
unit : hour

Hg		Cd		Cu	
Conc. mg/*l*	LT50	Conc. mg/*l*	LT50	Conc. mg/*l*	LT50
1.0	151	2.0	121	1.0	114
2.0	104	6.0	117	1.5	100
3.0	100	117	106	2.0	96
4.0	88	16.0	89	4.0	71
8.0	79	32.0	60	6.0	60
10.0	69			8.0	61

The lethal threshold concentration

Median lethal time at each test tank was extended when a little toxic metal was intaken. At 2 mg/*l* test solutions, the LT50 values derived from this study were 96 hours for copper, 104 hours for mercury and 121 hours for cadmium (Table 5).

In the present study, the 96hr-LC50 value of mercury for *Anadara broughtonii* is 4.84mg/*l* which is much higher in concentration compared with the values of 6.5×10^{-5}M and 9.0×10^{-4}M for *Mytilus edulis* and *Crassostrea commercialis* (Wisely and Blick 1967, Table 3).

The 96hr-LC50 value of cadmium for the test species is 1.86mg/*l*. The 48hr-LC 50 for *Crassostrea virginica* is 3.8mg/*l* (Calabrease et al 1973). Ahsanullah (1971) has reported a 96hr-LC 50 value 1.62mg/*l* for *Mytilus edulis*. However, Eisler (1971) gave a 96hr-LC 50 value of 25mg/*l* for *Mytilus edulis*, which is much higher compared with the above values and a 19hr-LC 50 value of 2.2mg/*l* for *Mya arenaria* (Table 6).

The 72hr-LC 50 value of copper for the species is 0.31mg/*l*. Fujiya (1960) has reported a 96hr-LC 50 value of 1.9mg/*l* for the Japanese oyster, *Crassostrea gigas*, but a 48hr-LC 50

Table 6. Literature LC 50 values of mercury, cadmium and copper for marine organisms

Metal	Species	Time (hr)	LC 50	Reference
Hg	*Cyprinus carpio*	96	0.30mg/*l*	Malacea 1966
	Daphnia magna	24	26 ppm	Ballard & Oliff 1969
	Artemia salina	75	5 mg/*l*	Corner & Sparrow 1956
	Bivalve larva	not given	0.027mg/*l*	Weelke 1961
	Mytilus edulis	96	6.5×10^{-5}M	Wisely & Blick 1967
	Crassostrea virginica	48	0.0056ppm	Calabrese 1973
	Crassostrea Commercialis (embryo of)	96	9.0×10^{-4}M	Wisely & Blick 1967
Cd	*Pimephales promelas*	96	5 ppm	Henderson & Tarzwell 1960
	Salmo gairdneri	24	30 mg/*l*	Ball 1967
	Mytilus edulis	96	1.62 mg/*l*	Ahsanullah 1976
	Mytilus edulis	96	25 mg/*l*	Eisler 1971
	Cardium edule	96	2.0 mg/*l*	Portman & Wilson 1971
	Mya arenaria	96	2.2 mg/*l*	Eisler 1971
	Crassostrea virginica (embryo of)	48	3.80 ppm	Calabrese 1973
Cu	*Salmo gairdneri*	48	0.5-0.4 ppm	Brown 1968
	Pimephales promelas	96	1.4 ppm	Tarzwell & Henderson 1960
	Carassius auratus	24	90 ppm	Floch et al 1963
	Mytilus	48	22.5 mg/*l*	Wisely & Blick 1967
	Cardium edule	48	1 mg/*l*	Portman 1968
	Crassostrea gigas	96	1.9 mg/*l*	Fujiya 1960

value of 22.5 mg/*l* for copper has been given for *Mytilus* by Wisely and Blick (1967).

As metioned above, for *Anadara broughtonii* in the present study, mercury is more toxic than cadmium and copper. The differentiation of the LC 50values depend on the experimental conditions, size of test fish, kind of species and physiological conditions of the animals used. Methodology of acute toxicity research should be considered in uniformity.

During the test, some difficulties arose as followings:

—During the test period, because no specimens opened their shell for mercury tests, mercury exposure time to toxic material seemed to be shorter than cadmium and copper, which make it difficult to compare the toxicity of mercury with of copper and cadmium.

—It was hard to know the toxicity of substances dissolved in the test water due to their interaction by chemical and hydrochemical reaction in the water, e.g., synergism and antagonism of mercury and chlorine when they hydrolized in mercury test solution.

—The key to death assessment is very poor for observation.

—It is difficult to apply the test results to field situation to field situation since they are misleading, such as the results of simple numbers based on quantal responses.

—Having static test, lethal concentration of toxic material does not remain constant as to the uptake by animals, adsorption to the container walls and biological and chemical degradation. Exchange of diluting water every 24 hours does not fully protect the loss of toxic substance in the test.

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