Mar. Res. Indonesia Vol.33, No.1, 2008: 49-53

PARTIAL CHRONIC TOXICITY TEST OF COPPER (Cu^{2+}), ZINC (Zn^{2+}) AND CYANIDE (CN^-) FOR YOUNG COBIA FISH (*RACHYCENTRON CANADUM*)

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ABSTRACT

The partial toxicity tests of copper (Cu^{2+}), zinc (Zn^{2+}) and cyanide (CN^-) for young cobia (*Rachycentron canadum*) fishes 45 days-old were conducted in Doson station during ten days (yr 2005). These three toxic substances affected strongly the growth rate of the Cobia fish (length and weight of the body), even at the lowest treated concentration. The LC_{50} values of copper, zinc and cyanide were 0.32 mg l^{-1}; over mg l^{-1} and 0,049 mg l^{-1}, respectively. Based on the statistical analysis of growth rate of cobia by determining IC25, IC50, NOEC and LOEC values, we have addressed the safe concentrations of these two heavy metals and cyanide in the environmental aquaculture which must be lower than 0.025 mg Cu l^{-1}, 0.5 mg Zn l^{-1} and 0.005 mg CN l^{-1}, respectively. The result also showed that cyanide was the most toxic to Cobia, followed by copper and zinc.

Keywords: Chronic toxicity test, Cobia, Heavy metals and cyanide

INTRODUCTION

Vietnam is developing rapidly and the priority polices are the development of industrialization and modernization. Industries are being promoted in the urban areas along Vietnamese coast and the current environmental problems have been disregarded, especially zones along Red river delta. The rapid development of industries plus the weakness of the environmental management are not only decreasing people's quality of life but also affecting the environment in general as well as marine environment in particular. In addition, run-offs from coal mining in Quang Ninh, off Red river delta, has brought pollutants to surrounding marine areas during rainy seasons. This has also been revealed by the increased levels of contaminants in samples collected by Marine Environment Monitoring system. According to the data of Vietnam Environment Protection Agency (VEPA, 2005 and 2006), pollutant levels have been increasing rapidly in the Haiphong-Quangninh area in recent years, especially copper, zinc and cyanide. Besides, this area is facing potential pollution for the environment caused by human activities, such as water-deep seaports, marine transportation, coal mining, overfishing, etc. These pollutants will produce negative impacts on the mariculture area along the coast.

Cobia is one of the most important commercial fish commonly cultured in the floating cages in Quangninh - Haiphong area. The production of this fish plays an important role in export strategy of the Ministry of Fishery. When Cobia are 45 days old they are transported from the hatchery to the floating cages in aquaculture areas, where they are being commercially cultured. The fish is known to be very sensitive regarding the water quality, both in the hatchery (Do Van Minh, 2002), as well as in the cultivating area in the marine environments, especially when exposed to copper, zinc and cyanide (Le Quang Dung, 2005).

The concentrations of copper, zinc and cyanide in the water column are too high to protect this species (Le Quang Dung, 2005). It seems that the Vietnam Marine Water Quality Standard is too high for these compounds and therefore Cobia species is threatened. Therefore, the purpose of this paper is to contribute a reference for policy-makers to

DOI: 10.14203/mri.v33i1.505

consider the threshold for the safe concentrations of copper, zinc and cyanide for Cobia juvenile by partial chronic test.

MATERIALS AND METHODS

Test animals were 45 days old Cobia (*Rachicentron canadum*). The average length and weight of the fish were 10.8 cm and 5.12 gram, respectively. Cobia fishes were purchased at Ngochai hatchery. The fishes selected for each test were healthy and similar in size and physiological condition.

Stock solutions were prepared by dissolving 3.9063 g CuSO4.5H2O and 2.0923 g ZnCl2 in 100 ml of 2% sulfuric acid solution and filled to 1000 ml with distilled water, respectively. The cyanide stock solution was prepared by dissolving 2.51 g KCN and 1.6 g NaOH pellets in distilled water in a 1-L volumetric flask. All stock solutions were preserved at 4^{0}C to keep them in good condition. Before commencing the experiments, the stock solutions were diluted to the desired concentrations.

The tests were carried out under static renewed conditions of the partial life-cycle toxicity test (USEPA, 2002) during 10 days. Before carrying out the experiment, Cobia fishes were acclimatized in seawater of 15 ppm salinity (the same salinity as that at the hatchery) for few days at room temperature (28-30^{0}C). The clean seawater used for the acclimation and experiments was transported from Ngochai hatchery. Cobia fishes were treated in 30 L containers with 20 L test solution. All containers used for the experiments were the same size *i.e.*, 40 cm x 25 cm x 30 cm.

The concentrations of three substances used in the experiments were as follows, 1/ copper : 0.025; 0,05; 0,1; 0,2, 0,4 mg l^{-1} and control; 2/ zinc: 0.025; 0.05; 0,1; 0,5, 1,0 mg l^{-1} and control; and 3/ cyanide: 0.001; 0.005; 0.01; 0.025; 0.05, 0.1 mg l^{-1} and control. The test was considered to be valid and acceptable if mean control survival was ≥90% and water quality parameters were maintained within acceptable limits

The experiment was conducted with two replicates at each treatment and control. Test solutions of heavy metals were renewed twice a day, and of cyanide, three times a day. Cobia fishes were fed with small live shrimps at the rate of 5% body weight twice a day (morning and evening) 45 minutes before renewing water.

The numbers of survivors of each replicate were counted and recorded every 12 hours. Dead animals were removed immediately. The mortality of fish was used for determining the lethal concentration of 50% (LC_{50}). Growth rate of cobia was measured as the change of body weight and length over the growth period of 10 days. The growth rate was used as a criteria of the effect of sub-lethal treatments on fish i.e., the inhibition concentrations of 25% (IC25), 50% (IC50), no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC)

Water quality parameters (temperature, dissolved oxygen, pH and salinity) were measured routinely. Mortality data was used for calculating lethal concentration (LC) by assistance of Probit and Spearman Karber software. The growth rate was analyzed for statistical significance using Toxstat and Dunnet test to determined IC25, IC50, NOEC and LOEC.

RESULTS AND DISCUSSION

The proportion of survivors in the controls always exceeded 90% in all tests, ensuring the accuracy of the data (USEPA, 2002). During toxicity testing, the experimental conditions were kept stable to suit cobia growth and cultivation conditions were maintained corresponding to biological and ecological characteristics of cobia.

In all three treatments, the mortality of fish increased in corresponding to the elevated toxic concentrations. However, each treatment had a different mortality (Table 1, 2 and 3).For copper and zinc, the mortality of fish was only found in the highest concentrations (0.4 mg l^{-1} for Cu and 1.0 mg l^{-1} for Zn). The mortality after 12 h exposure to Cu was 20% and after 24 h was 50%. There were no mortality within the first 12 h exposure to Zn, and after 24 h, mortality was only 20%. In contrast, for cyanide exposure of 0.05 mg l^{-1} and 0.1 mg l^{-1}, the mortality was detected within the first 30 minutes with the mortality was 20% and 70% respectively. A 100% mortality of fish was found after 12 h exposure to 0.1 mg l^{-1} CN. It is interesting to note that the highest mortality for Cu occurred after 120 h exposure (Table 3), For Zn, the mortality after 24 h was 20%, and for CN

Table 1. Mortality of fish after 10-days exposure to various copper concentrations

No	Cu (mg l^{-1})	Fish	Mortality of fish at different time (%)											
			12h	24h	36h	48h	72h	96h	120h	144h	168h	192h	216h	240h
1	0	10	0	0	0	0	0	0	0	0	0	0	0	0
2	0,025	10	0	0	0	0	0	0	0	0	0	0	0	0
3	0,05	10	0	0	0	0	0	0	0	0	0	0	0	0
4	0,1	10	0	0	0	0	0	0	0	0	0	0	0	0
5	0,2	10	0	0	0	0	0	0	0	0	0	0	0	0
6	0,4	10	30	50	60	60	60	60	70	70	70	70	70	70

Table 2. Mortality of fish after 10-days exposure to various zinc concentrations

No	Zn (mg l^{-1})	Fish	Mortality of fish at different time (%)											
			12h	24h	36h	48h	72h	96h	120h	144h	168h	192h	216h	240h
1	0	10	0	0	0	0	0	0	0	0	0	0	0	0
2	0,025	10	0	0	0	0	0	0	0	0	0	0	0	0
3	0,05	10	0	0	0	0	0	0	0	0	0	0	0	0
4	0,1	10	0	0	0	0	0	0	0	0	0	0	0	0
5	0,5	10	0	0	0	0	0	0	0	0	0	0	0	0
6	1,0	10	0	20	20	20	20	20	20	20	20	20	20	20

Table 3. Mortality of fish after 10-days exposure to various cyanide concentrations

No	CN (mg l^{-1})	Fish	Mortality of fish at different time (%)											
			30 min	12h	24h	48h	72h	96h	120h	144h	168h	192h	216h	240h
1	0	10	0	0	0	0	0	0	0	0	0	0	0	0
2	0,001	10	0	0	0	0	0	0	0	0	0	0	0	0
3	0,005	10	0	0	0	0	0	0	0	0	0	0	0	0
4	0,01	10	0	0	0	0	0	0	0	0	0	0	0	0
5	0,025	10	0	10	10	10	10	10	10	10	10	10	10	10
6	0,05	10	20	40	40	40	40	40	40	40	40	40	40	40
7	0,1	10	70	100	100	100	100	100	100	100	100	100	100	100

the highest mortality occurred after 12 h and was the same until the end of the experiment.

The lethal concentrations of 50 % of the three substances in 10 days are listed in Table 4. LC_{50} is 0.049 mg l^{-1} for CN at 240 h, with the confidence limit ranges from 0.036 to 0.066 mg l^{-1}. Thus, cobia seems to be very sensitive to cyanide. The 240 h LC_{50} of current tests are a little bit higher than the 96h LC_{50} of the previous acute tests at the same age of cobia (Le Quang Dung *et al.*, 2005), however this difference is not significant.

The 240-h LC_{50} of copper for cobia is 0.32 mg l^{-1} (confidence limit 0.25 to 0.4 mg l^{-1}). Noorzah *et al.* (1997) found that the 96-h LC_{50} of Cu for 8 week-old seabass (*Lates calcarifer*) and PL-14 prawn (*Penaeus monodon*) are 3.2 mg Cu l^{-1} (2.7 – 3.8 mg Cu l^{-1}) and 1.2 mg Cu l^{-1} (0.6 – 2.4 mg Cu l^{-1}), respectively. Comparing to sea bass and prawn, cobia is 10 and 3 times more sensitive to copper than that of sea bass and prawn. Somtawin *et al.* (1997) reported that the 96h-LC_{50} values of acute tests on juvenile tiger shrimp (*Penaeus monodon*, PL 20-26) and Seabass (*Lates calcarifer*, 15-20 days old) were 0.58 ± 0.05 mg Cu l^{-1} and over 1.82 mg Cu l^{-1}, respectively. These values are also relatively higher that that for cobia.

The value of Zn 240h-LC_{50} for cobia was over 1 mg Zn/l without data of confidence limit, because

Table 4. Lethal concentrations and confidence limit of the three substances

Value / Toxicants	LC_{50}	Confidence limit	
Cu	0,32	0,25	0,40
Zn	> 1	NC	NC
CN	0,049	0,036	0,066

Note: NC is non-countable

the mortality in this test was below 50%. Hence, it is difficult to determine the exact value of 240h-LC_{50} of Zn, however, this result might be accepted as accurate data because it was not significantly different from the result of previous study of the acute test on cobia (Le Quang Dung *et al.*, 2005). The 96h LC_{50} of zinc was 0.82 mg Zn l^{-1} and confidence limit ranges from 0.48 – 1.19 mg Zn l^{-1}. Comparing to the 96h-LC_{50} of acute tests on zinc for juvenile tiger shrimp (*Penaeus monodon*, PL 20-26) and seabass (*Lates calcarifer*, 15-20 days old) which was 0.91 ± 0.35 mg l^{-1} and over 7.87 mg l^{-1}, respectively (Somtawin *et al.* (1997). The results of the present study showed that the 240 h LC_{50} value has similar value as that for tiger shrimp, but much lower compared to that for seabass. Based on the results of previous and the current tests, the effects of zinc on cobia in marine water seems to be weaker than of copper and cyanide.

IC_{25}, IC_{50}, NOEC and LOEC values for copper, zinc and cyanide were analyzed statistically and showed in Table 5. Based on the hypothesis of the statistical analysis, the endpoint values obtained from the test of the three toxic substances on cobia are quite low. In cyanide test, the obtained values of IC_{25}, IC_{50}, NOEC and LOEC were 0.015 mg l^{-1}, 0.049 mg l^{-1} CN, 0.005 mg l^{-1} CN and 0.01 mg l^{-1} CN, respectively. This indicated that the cobia is very sensitive to cyanide and even a small amount of cyanide in marine water will be able to severely affect cobia juveniles. This can partly explain the reason why the reduction of the species richness has occurred in natural environments recently. Meanwhile, the values of zinc seem to be highest of the three compounds, with values of IC_{25} = 0.773 mg l^{-1} , IC_{50} > 1 mg l^{-1} , NOEC = 0.50 mg l^{-1} and LOEC = 1.00 mg l^{-1}. For copper, the LOEC value (0.05 mg l^{-1}) was two times higher than that the NOEC value (0.025 mg l^{-1}). On the other hand, IC_{50} (0.289 mg l^{-1} Cu) was 3 times higher than that of IC_{25} value (0.076 mg l^{-1}).

The results of the present study showed that juvenile cobia was sensitive to copper, zinc and cyanide in the experimental conditions. The tested substances certainly influence on the growth of cobia juveniles even at low concentrations in marine waters. Therefore, this information is useful for fishermen to know the risks of environmental degradation that can affect the quality of fish

Table 5. IC25, IC50, NOEC and LOEC values of chronic toxicity test on copper, zinc and cyanide for cobia (*Rachycentron canadum*)

GT / Substances	IC25	IC50	NOEC	LOEC
Cu	0,076 (0.021–0.141)	0,289 (0.200–0.406)	0,025	0,05
Zn	0,773	> 1	0,50	1,00
CN	0,015 (0.009-0.018)	0,049	0,005	0,01

Note: GT – growth rate test

production, and also for policy makers in deciding the regulation for the protection of marine life.

Vietnam marine water quality standard (VNMQS) for copper, zinc and cyanide for the protection of aquatic life in coastal areas are 0.01 mg l^{-1} for all three substances (MoNRE, 1995). Comparing with VNMQS, the toxic values of copper and cyanide for cobia were lower than those of the current VNMQS, whereas the toxic value of zinc was higher than that of the standard. Therefore, the current national criteria may not be protective of this species for copper and cyanide in marine environment and to be safe for toxic values of zinc. Hence, it is time to consider a new water quality criterion for protection of the aquatic life. Based on the present study, it is suggested that to protect cobia, the safe concentrations of copper and cyanide in marine environment must be lower than 0.005 mg CN l^{-1} and 0.025 mg Cu l^{-1}, respectively.

CONCLUSION

The results of these experiments suggest that cyanide is the most toxic substance to 45 day-old cobia, followed by copper and zinc. The cyanide exposure had immediate effects on the test biota. By contrast, the effects of copper and zinc exposure often occurred later, within the first 12–24 hour period.

All three toxic substances affect adversely on the growth rate of cobia (size and weight of body) at the low toxic concentrations. The safe concentration for copper and cyanide to protect cobia in the marine environment must be lower than 0.005 mgCN l^{-1} and 0.025 mg Cu l^{-1}.

Acknowledgment. This study was supported by Vietnam Academic Science and Technology (VAST) and authors would like to thank staffs at Doson Marine Station for their assistance.

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