Bull. Environ. Contam. Toxicol. (1993) 51:303–308

Environmental Contamination and Toxicology

Toxicity of Cadmium and Lead to Juvenile Red Swamp Crayfish, *Procambarus clarkii*, and Effects on Fecundity of Adults

Syed M. Naqvi and Rosie D. Howell

Department of Biological Sciences, Southern University, Baton Rouge, Louisiana 70813, USA

Heavy metals such as Cd and Pb may affect aquatic organisms adversely if they are sublethally exposed for a long time. Acute toxicities of a number of heavy metals to different species of crayfish have been reported by several investigators (Mirenda 1986), Thorp and Gloss (1986), Diaz-Mayans et al. (1986), Del Ramo et al. (1987) and Naqvi et al. (1987). However, the chronic toxicities of different heavy metals have not been reported as frequently.

P. clarkii is of considerable commercial importance to the economy of Louisiana, which produces 98% of the total harvest of the U.S., worth approximately 143 million dollars annually (Huner 1989). This prompted us to use this crayfish for: (1) determining the 96-hr LC_{50} values for Cd and Pb using juvenile P. clarkii and (2) assessing the chronic effects of Cd and Pb on the fecundity of adults and hatching success of metal-exposed eggs.

MATERIALS AND METHODS

Adult crayfish (P. clarkii) were obtained from Ben Hur Experiment Station, Louisiana State University, Baton Rouge, LA. These organisms are grown in specially constructed crayfish ponds. They were brought to our laboratory and acclimatized in glass aquaria (70 cm X 125 cm X 20cm), containing aged tap water (aerated continuously for 2 weeks prior to use). Aged tap water was used throughout the study to minimize mortality in controls. The juveniles (1-1.5 cm length), which were raised in our laboratory were used in static bioassays. Range-finding tests were conducted to achieve 0 and 100% mortalities prior to definitive tests.

A total of 50 juveniles per concentration of $CdCl_2$ and

Send reprint requests to Dr. Syed Naqvi at the above address.

Pb $(NO_3)_2$ were exposed for a period of 96 hrs. For this purpose, all-glass aquaria (70 cm x 125 cm x 20 cm) were used. Each all-glass aquarium contained 10 juveniles, and 5 aquaria/concentration were used. Thus a total of 50 juveniles/concentration of Pb and 50 juveniles for Cd were exposed, similarly. Aged tap water was used for making test solutions and to maintain controls. Aged tap water was prepared by aerating the tap water continuously for 2 wks before use. We have noticed that aged tap water usage minimizes the mortality of crayfish in controls. Test solutions of $CdCl_2$ were 0.5, 1.0, 2.0, 3.0 and 4.0 mg/L Cd; and $Pb(NO_3)_2$ solutions were 100, 300, 600, 700, 800, 900 and 1000 mg/L Pb. Water parameters measured included: temperature, dissolved oxygen, pH and total hardness. Juveniles were fed before, but not during testing. The LC_{50} and LC_{99} values and 95% confidence intervals were computed by probit analysis on an IBM computer with the MICROPROBIT 3.0 software package (Sparks and Sparks, 1987).

For chronic toxicity testing, 48 males and 48 females were allowed to mate during the months of May and June when mating activity reaches its peak. In the month of July, males were removed to avoid cannibalism. From July to November, 24 females were exposed to 0.5 mg/L $CdCl_2$ and 24 to 100 mg/L Pb $(NO_3)_2$, individually; and 24 females were kept in aged tap water only as control. During this exposure period (July to November), 7 control, 8 Cd-exposed and 8 Pb-exposed females died. The remaining (17 control, 16 Cd-exposed and 16 Pb-exposed) were maintained in metal solutions or aged tap water, till egg-laying occurred in November. Test solutions were replaced on a fortnightly basis and the actual amount of Cd and Pb present in solution was determined once a month using flame AAS technique.

When eggs were laid they remained attached to females till hatching occurred. Eggs were counted individually and dead eggs were removed, which were discerned by their yellow coloration. Dead eggs which were found loose or attached to the female were also counted. After hatching, the number of hatchlings from treated and controls were recorded. Data for hatching success were analyzed statistically by Student's t test.

Test solutions were analyzed to determine the exact amount of heavy metals present in solution. Due to the fact that no organic matter was expected in aged tap water (which was used for preparation of test solutions), no digestion was done. A 100-ml sample was evaporated to 20 ml, which was then used to detect the amount of metal by a flame atomic absorption (Perkin-Elmer, Model 3030) equipped with a CRT display. Standards for lead and cadmium were purchased from

Table 1. Mortalities of juvenile Louisiana swamp crayfish, *Procambarus clarkii*, exposed to various concentrations of cadmium as the chloride and lead as the nitrate (mg/L) for 96 hr (N=50/conc.)

Calculated	Actual	Dead Crayfish in Hours				%
$CdCl_2$ conc.	conc.	24	48	72	96	Mort.
0.0		0	0	0	0	0
0.5	0.10	0	0	2	4	8
1.0	0.21	0	0	10	22	44
1.5	0.29	0	9	24	48	92
2.0	0.33	0	5	23	47	94
3.0	0.58	5	12	22	50	100
3.5	0.74	24	26	0	0	100
$Pb(NO_3)_2$						
0.0		0	0	0	0	0
100	16.84	0	0	0	0	0
300	50.58	0	0	0	0	0
600	87.60	0	0	0	0	0
700	215.41	1	5	12	22	44
800	254.66	4	11	18	26	52
900	287.88	0	16	25	38	76
1000	331.20	14	24	39	46	92

Table 2. LC_{50} and LC_{99} values for cadmium- and lead-exposed juvenile Louisiana swamp crayfish, *Procambarus clarkii*.

Conc.	Lethal Con. Values		95% Fiducial Limits		Chi-Sq.
mg/L	LC50	LC99	Lower	Upper	
$CdCl_2$	1.04		0.91	1.18	
		3.24	2.52	4.84	0.348223*
$Pb(NO_3)_2$	751.57		698.82	788.18	
		1289.27	1148.37	1613.55	0.927089*

*Chi-square values are not significant.

Mallinckrodt Co., and Fisher Scientific Co., and the levels of detection for these metals were 0.5 and 0.025 ppm, respectively. No graphite furnace was used for this purpose since the amounts of metals present in test solutions were greater than the detection limits. The actual concentrations of Cd and Pb present in test solutions are given in Table 1. However, the LC_{50} and LC_{99} values are based on the serial dilution of freshly prepared 1% aqueous stock solution (Table 2).

RESULTS AND DISCUSSION

The water parameters measured during acute bioassays were: temperature 22±2°C, dissolved oxygen 5.5-6.5 ppm, pH 7-7.8 and total hardness 30.32 ppm. Percent mortalities of juvenile crayfish in cadmium and lead are given in Table 1, and the LC_{50} and LC_{99} values are given in Table 2. It is evident from the data that mortalities due to cadmium and lead were time and dose-dependent.

Table 3. Mean (S.D.) number of eggs laid (fecundity) and eggs hatched (hatching success) by control and treated crayfish, Procambarus clarkii

No. of crayfish (N)	Treatment	Mean No. of eggs laid	Mean No. of eggs hatched	Mean % hatch
17	Control	203 S.D.(145.57)	193 (145.35)	95.07 (5.28)
16	0.5 mg/L Cd	48 S.D. (22.97)	9 (9.68)	16.66 (18.76)
16	100 mg/L Pb	180 S.D. (98.59)	112 (63.03)	62.22 (10.55)

Table 4. Student's t test for the number of eggs laid by crayfish exposed to 0.5 mg/L cadmium chloride and 100 mg/L lead nitrate for a period of 148 days

Source	DF	SS	F-value	PR F	P
Pb 100 mg/L	1	135304.06	6.63	0.0031	**
Cd 0.5 mg/L	1	180110.47	11.10	0.0001	***

**P<0.05 ***P<.01

The mean actual amount of Cd in test solutions was 5.18 times less than the calculated amount, and the Pb was 4.46 times less. However, comparing the LC_{50} values for Cd and Pb (based on calculated concentrations in test solutions), it was evident that cadmium was 722.66 times more toxic than lead to juvenile crayfish. Another noticeable fact was that no crayfish died up to 600 mg/L Pb exposure while cadmium caused 8% mortality in 0.5 mg/L concentration.

Table 3 gives the number of adult crayfish, mean number of eggs laid, mean number of eggs hatched, mean percent hatched, and standard deviation.

Student's t test (Table 4) indicated that the number of eggs laid and hatched by Pb- and Cd-treated crayfish was reduced significantly as well as the hatching success in comparison to controls.

This was further exemplified by the average of 203 eggs (S.D. 145.57) produced by control vs 48 (S.D. 22.97) by Cd-treated, and 180 (S.D. 98.59) by Pb-treated crayfish. Similarly, 95% of the control eggs hatched in comparison to 17% for the Cd-treated and 62% for the Pb-treated. Based on the above data, we conclude that both heavy metals caused a statistically highly significant decrease in egg-production as well as hatching ($P = <0.05$ for Pb and $P = <0.01$ for Cd).

Comparative data on the toxicities of Cd and Pb to juvenile P. clarkii are not available. However, the 96hr LC_{50} value for adults have been reported to be 18.4 mg/L Cd at 28°C (Del Ramo et al. 1987). Diaz-Mayans et al. (1986) reported the 96hr LC_{50} value for Cd at 24°C to be 10mg/L for adult P. clarkii. We have determined the 96hr LC_{50} value for our juvenile crayfish at 24°C to be only 1.04 mg/L, which was expected since juveniles are generally more susceptible to xenobiotics than adults (Leung et al. 1980; Rice 1983). We also found considerable difference in the susceptibility of juveniles for Cd and Pb toxicity. The 96 hr LC_{50} values for these metals were 1.04 and 751.57 mg/L Cd and Pb, respectively. Juvenile crayfish were 723 times more susceptible to Cd than Pb.

No comparative data exist concerning the effects of Cd and Pb on fecundity and hatching success of P. clarkii. Therefore, this work might serve as a baseline for further study. Control crayfish produced 80.9% more eggs (on an average basis) than 0.5 mg/L Cd-treated crayfish, and 53.0% more than those treated with 100 mg/L Pb. Similarly, the hatching of control eggs was 95.5 and 63.3% more than Cd-treated and Pb-treated eggs, respectively. Thus, both heavy metals caused a considerable reduction in egg-laying capability of adult crayfish and the hatching of eggs produced by females sublethally exposed for approximately 7 months prior to egg-laying.

Long-term sublethal exposure to heavy metals seems to be critical for the fecundity and hatching success of crayfish. Rice (1983) noticed that P. clarkii (developing) eggs exposed to 250 ug/L Cu for 600 hr had 17% hatching success while those exposed to 2840 ug/L Cu had 100% hatching success. Similarly, Chaisemartin (1975) reported for another crayfish (Orconectes limosus), that 0.2 mg/L Cu treatment of adults for 20 days resulted in degeneration of 50% eggs. We reported earlier (Naqvi and Flagge 1990) that adult P. clarkii exposed to 100 mg/L MSMA (an organoarsenical herbicide) caused 19.1% reduction in fecundity and 82.4% reduction in hatching as compared to controls. We extrapolate from these results that certain heavy metals (e.g., Pb and Cd) have a potential to produce detrimental effects on Louisiana's crayfish farming if the ambient water of bayous in which the wild populations of crayfish exist contains very high amounts of these metals.

Acknowledgments. We thank Dr. Robert Romaire of Fisheries & Wildlife Department, Louisiana State University, for supplying us the adult crayfish. This study was supported by a grant from NIGMS (No. 8175) through the MBRS Program.

REFERENCES

Chaisemartin C (1975) Comparative experimental analysis of the toxicity of copper in water to Astacidae. CR Congr Nat Soc Savantes Sci 98: 145-155

Del Ramo J, Diaz-Mayans J, Torreblance A, Nunez A (1987) Effects of temperature on the acute toxicity of heavy metals (Cr, Cd, Hg) to the freshwater crayfish, Procambarus clarkii. Bull Environ Contam Toxicol 38: 736-741

Diaz-Mayans J, Torreblance A, Del Ramo J, Nunez A (1986) Oxygen uptake by excised gills of Procambarus clarkii from Albufera Lake of Valencia, Spain under heavy metal treatments. Bull Environ Contam Toxicol 36: 912-917

Huner JV (1989) Overview of international and domestic freshwater crawfish production. J Shellfish Res 8: 259-265

Leung TS, Naqvi SM, Naqvi NZ (1980) Paraquat toxicity to Louisiana crayfish (Procambarus clarkii). Bull Environ Contam Toxicol 25: 465-469

Mirenda RJ (1986) Toxicity and accumulation of cadmium in the crayfish, Orconectes virilis. Arch Environ Contam Toxicol 15: 401-407

Naqvi SM, Hawkins R, Naqvi NH (1987) Mortality response and LC_{50} values for juvenile and adult crayfish, Procambarus clarkii exposed to Thiodan and Cutrine-plus. Environ Pollut 48: 275-283

Naqvi SM, Flagge CT (1990) Chronic effects of arsenic on American red crayfish, Procambarus clarkii exposed to monosodium methanearsonate (MSMA) herbicide. Bull Environ Contam Toxicol 45: 101-106

Rice DW Jr (1983) Sensitivity of adult, embryonic and larval crayfish, Procambarus clarkii to copper. Govt Repts Announc & Index 23, Rept UCRL-53048, US NTIS 83: 5580 Washington DC USA

Romaire RP, Lutz CH (1989) Population dynamics of red swamp crawfish (Procambarus clarkii) and the white river crawfish (Procambarus acutus acutus), in commercial ponds. Aquaculture 81: 253-274

Sparks TC, Sparks A (1987) Microprobit Analysis for the IBM PC and Compatibles. Department of Entomology, Louisiana State University, Baton Rouge, Louisiana

Thorp JH, Gloss SP (1986) Field and laboratory tests on acute toxicity to freshwater crayfish. Bull Environ Contam Toxicol 37: 355-361

Received June 10, 1992; accepted February 3, 1993