Bull. Environ. Contam. Toxicol. (1990) 45:101–106

Environmental Contamination and Toxicology

Chronic Effects of Arsenic on American Red Crayfish, *Procambarus clarkii*, Exposed to Monosodium Methanearsonate (MSMA) Herbicide

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Bioaccumulative and biomagnifying effects of arsenic on crayfish have been reported by Abdelghani et al. (1976) and Woolson et al. (1976). However, no work has been done on the chronic effects of this heavy metal on crayfish populations. There is a great concern for MSMA (Monosodium Methanearsonate) herbicide in the vicinity of natural waters due to its high water solubility and bioaccumulative potential (Anderson et al. 1975). MSMA has been used in Louisiana for the past 16 years to control a number of non-crop weeds alongside highways is 76 L/A (pers. commun. with Mr. Bennie St. Romain, Highway Forman II for Louisiana State Hy. Dept. 1987).

American red crayfish (Procambarus clarkii) account for 98% of the annual crayfish harvest in North America (Huner 1984). Up to 60 million pounds have been harvested commercially each year from swamps and marshes of Atchafalaya Basin and thousands of acres of culture ponds. The total revenues from the sales of crayfish exceeds $143,000,000/yr. About 80% of this harvest is consumed alone by the people of Louisiana State (USA). These crayfish also serve as an important link between the primary producers and consumers in lentic habitats and freshwater aquatic ecosystems. Those pesticides which have greater water solubility (i.e. MSMA) than other less soluble compounds may cause higher mortalities of aquatic organisms (Mulla and Mian 1981), or cause adverse chronic effects if the non-target animals are sublethally exposed. This work was conducted in the laboratory to assess the possible chronic effects of arsenic (which is the main constituent of MSMA herbicide) on crayfish.

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MATERIALS AND METHODS

Chronic effects of arsenic on adult P. clarkii in this study included fecundity, hatchability and growth-rate of juveniles. Adult crayfish were obtained from a relatively pesticide-free environment (Ben Hur Experiment Station, Louisiana State University, Baton Rouge, LA) and were acclimatized in the laboratory prior to the initiation of experiments. To assess the sublethal effects of arsenic eighteen adult male and 18 females were exposed to 100 ppm MSMA for a period of 12 weeks. The males were removed after mating, but the females were exposed to MSMA for an additional period of 12 weeks, totalling 24 weeks before they laid eggs. The exposure was done in all-glass aquaria using one male and one female crayfish per acquarian.

MSMA solutions were prepared by diluting a freshly prepared 1% stock solution to the desired concentration. The exposure concentration (100 ppm) was based on the 96 h LC_{50} (1019 ppm) reported for MSMA by Naqvi et al (1987). Aged tap-water was used throughout the study to insure least mortalities. Tap-water was aerated continuously in 60 L polyethylene carboys for a period of 1 wk before use and 20 drops of saturated sodium thiosulfate were added for the complete removal of chlorine.

Both male and female crayfish were provided with 1 g pet food (Gaines Top Choice^R) once a week. The oxygen concentration and water temperature were measured by an electrode type oxygen meter (YSI Model 51A), and pH was measured by a Digi-Sense^R digital pH meter (Cole-palmer Instrument Co.). Total water hardness was measured using a water hardness test kit (La Motte Chemical Products). These parameters were measured daily from 3 aquaria containing control crayfish. The water hardness was measured at the beginning and end of each week both for treated and control water. In order to assess if the initial habitat of crayfish contained arsenic, Ben Hur Experiment Station pond water and soil were analyzed. Crayfish food (Gaines Top Choice^R) was also analyzed by a colorimetric technique (Standard Methods 1985).

As soon as the females began egg laying, males were removed to prevent cannibalism. The total number of eggs attached to the swimmerets of each female were recorded and loose or dead eggs were counted simultaneously during the egg-laying period. The number of eggs produced by a female varies from 100 to 500 depending upon the total body length and size of the ovary. All eggs are laid in a single batch during each reproductive cycle. Loose eggs were those which

became detached from the female's body but had the same color as those which were still attached. They were considered dead when they acquired an orange color, which generallly occurred 24 hrs after detachment.

It was observed that hatching occurred generally within a period of 2 weeks after the egg-laying, and that the hatchlings clung to each other when isolated from the mother or when disturbed. After 30 days, hatchlings no longer cling to the female.

Twenty hatchlings (length 1.4-1.6 cm, weight 0.06-1.0 g) which were produced by treated females (exposed to 100 ppm MSMA) were acclimatized to laboratory conditions in 15 ppm MSMA solution in a 30 L aquarium for a period of 1 week. They were then individually placed in finger bowls (11 cm diameter, 4 cm height) containing 15 ppm MSMA solution. This sublethal concentration was chosen on the basis of the 96 h LC_{50} value for juveniles as 101 ppm MSMA (Naqvi et al. 1987). The same number of hatchlings from control females were maintained in aged tap-water as control for juvenile studies. Temperature, pH and dissolved oxygen were measured from 3 control finger-bowls daily for the entire period of an additional 36 weeks in which the growth-rate of hatchlings was monitored. The total water hardness was measured at the beginning and end of each week. The weight of hatchlings was measured using a Mettler weighing scale capable of measuring to 0.0001 g, lengths were recorded monthly, and molting frequency and behavioral activities of hatchlings recorded daily. Analysis of variance and Student's t-test were done on an IBM computer using the SAS Program for data on growth, fecundity and hatchability.

RESULTS AND DISCUSSION

Water parameters measured throughout the study period (average of 59 readings) were: temperature 17.7-19.6°C, total water hardness 25.6-29.3 ppm, dissolved oxygen 4.2-5.3 ppm and pH 7.8-8.3. The effect of 100 ppm MSMA exposure of P. clarkii for a period of 168 days is shown in Table 1.

MSMA-exposed crayfish produced 1149 eggs, whereas controls produced 1419. The difference was not statistically significant. Hatching success of control crayfish was 78.08% vs only 16.97% for treated crayfish. MSMA reduced egg hatching drastically. Rice (1983) pointed out that the length of exposure to a toxicant is more important than its concentration. This author exposed P. clarkii embryos to 250 ug Cu/L for 600 hrs and hatching was 17%. In comparison, 100%

Table 1. Effects of MSMA herbicide (100 ppm) on fecundity and hatchability of crayfish, Procambarus clarkii for a period of 168 days.

Crayfish No.	No. of eggs laid		No. of eggs hatched	
	Control	Treated	Control	Treated
1	91	0	26	0
2	33	146	0	0
3	290	32	283	0
4	0	0	0	0
5	305	124	305	124
6	0	139	0	54
7	316	378	110	0
8	384	330	384	17
9	0	0	0	0
Total	1419*	1149*	1108**	195**

* Not significantly different $P < 0.5131$
** Statistically significant $P < 0.0707$

of the eggs exposed to 2840 ug Cu/L for 250 hours hatched.

The initial average weight of 20 newly hatched control crayfish was 0.0642 g and the final weight (after 254 days) was 0.2941 g, representing an increase of 4.6 times the original weight. The initial average weight of MSMA-exposed hatchlings was 0.1088 g and the final weight 0.2223 g, which was only 2.05 times their initial weight. However, this difference was not statistically significant (Table 2). Perhaps a greater concentration of MSMA herbicide (more than 15 ppm MSMA) would have reduced the weight-gain more effectively.

The average initial length of 20 control hatchlings was 1.456 cm and after 254 days they grew to be 2.151 cm (1.47 times than the original). In comparison, the treated hatchlings increased in length from 1.642 to 21.88 cm (1.33 times). However, the difference between control and MSMA-exposed crayfish was not statistically significant (TAble 2). Since there are no comparative studies reported in the literature, for MSMA herbicide our findings stand alone as they are presented here. Oladimeji et al (1984) reported that exposure of rainbow trout, Salmo gairdneri, to 30 mg/kg arsenic inhibited growth due to a decrease in hemoglobin content of RBCs. Although this comparison might not be relevant, one can surmise that arsenic might have a similar inhibitory effect on hemocyanin of crayfish. This hypothesis, however, needs confirmation.

There was very little difference in molting frequencies of MSMA-exposed and control hatchlings, which was a total of 53 and 56, respectively. Considering that the

Table 2. Analysis of variance for differences between weight-gain, length-gain and molting frequency of newly hatched control and MSMA exposed (15 ppm) crayfish, Procambarus clarkii.

Source	Degrees of Freedom	Sum of Squares	Mean S Square	F Value	PR F
Weight	2	0.0031	0.0015	0.02	0.0978*
Total	39	2.6136			
Length	2	0.3719	0.1857	0.60	0.5562*
Total	39	11.9168			
Molting Frequency	2	2.1434	1.0717	0.35	0.7081*

*Not statistically significant

96 h LC_{50} values for adult and juvenile P. clarkii (1019 and 101 ppm MSMA, respectively) as reported by Naqvi et al (1987), the concentration used in the present study for newly hatched crayfish might have been low, and, thus did not produce a significant inhibitory effect on growth-rate. The recommended rate of MSMA application is 272 g/A (= 3 ppm). If this application rate is strictly adhered to, MSMA might produce no ill effects on growth rate of natural populations of crayfish in Louisiana. Water from a crayfish pond contained 0.057 ppm As, the aged tap-water had 0.045 ppm As, pond soil had 0.217 ppm As and crayfish food contained 0.053 ppm As.

Acknowledgments. We thank Dr. Dudley Culley of Louisiana State University for supplying adult crayfish for this study. This work was supported by National Institutes of Health (NIH) through the MBRS Program

REFERENCES

Abdelghani AA, Mason JW, Anderson AC, Englande AJ, Diem JE (1976) Bioconcentration of MSMA in crayfish (Procambarus clarkii). Trace Subst Environ Health 10: 235-245

Anderson AC, Abdelghani AA, Smith PM, Mason JW, Englande AJ (1975) The acute toxicity of MSMA to black bass Micropterus dolomiu, crayfish Procambarus clarkii and channel catfish, Ictalurus lacustris. Bull Environ Contam Toxicol 14:330-333

Huner JV, Barr JE (1984) Red Swamp Crayfish. Biology and Exploitation. Louisiana Sea Grant Program, Center for Wetland Resources, LSU. pp. 135

Mulla MS, Mian LS (1981) Biological and environmental impact of the insecticide malathion and parathion on non-target biota in aquatic ecosystems. Res Reviews 78: 101-135

Naqvi SM, Hawkins R, Naqvi NH (1987) Mortality

response and LC_{50} values for juvenile and adult crayfish, Procambarus clarkii, exposed to Thiodan (insecticides) and Cutrine-plus (algicide). Environ Pollut 48: 275-283

Oladimeji AA, Qudri SU, De Freitas ASW (1984) Long-term effects of arsenic accumulation in rainbow trout, Salmo gairdneri. Bull Environ Contam Toxicol 32: 732-742

Rice DW Jr (1983) Sensitivity of adult, embryonic and larval crayfish, Procambarus clarkii to copper. Govt. Repts. Announ. & Index No. 23, Rept. UCRL-53048, US NTIS 83: 5580, Washington, D.C.

Standard Methods (1985) For the Examination of Water Wastewater. 16th Ed. Amer Publ Health Assoc., Washington, D.C., pp. 1268

Woodson EA, Isenee AR, Kearney PC (1976) Distribution and isolation of radioactivity from ^{74}As-arsenate and ^{14}C-methanearsonic acid in an aquatic system. Pest Biochem Physiol 6:261-2690

Received July 31, 1989; accepted September 16, 1989.