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Arsenic Uptake and Depuration by Red Crayfish, *Procambarus clarkii*, Exposed to Various Concentrations of Monosodium Methanearsonate (MSMA) Herbicide

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Like many other heavy metals, arsenic is known to accumulate in the tissues of aquatic organisms including crayfish. One of the earliest reports on red crayfish, Procambarus clarkii, was published by Woolson et al. (1976) who determined the bioaccumulation factor (BF) ratios for radioactive sodium methanearsonate which ranged from 80-480. Abdelghani et al (1976) found P. clarkii accumulated 3.7 times more As than available in water after 56 days of exposure. Other heavy metals, i.e., Cr, Cd, Pb and Hg have also been reported to accumulate experimentally in P. clarkii tissues (Hernandez et al. 1986; Dickson et al. 1982; Pastor et al. 1988 and Del Ramo et al. 1988, respectively). Hernandez et al. (1987) found Cd, Pb, Cu and Zn residues in P. clarkii collected from Donana National Park in Spain.

This study was conducted to evaluate in the laboratory the bio-accumulative potential of As by the American red crayfish, Procambarus clarkii, which is abundant in Louisiana; and also to assess the level of arsenic present in the tissues of fieldcollected individuals. Total revenues from the sales of this crayfish exceeds $143 million annually (Huner 1983). MSMA is an organo-arsenical selective herbicide for the post-emergence control of crabgrass, dallis grass and other weedy grasses found alongside highways (Herbicide Handbook 1983). It has been used in Louisiana for the past 16 years at the application rate of 272 g/A.

MATERIALS AND METHODS

Adult crayfish (10-12 cm length) were obtained from Ben Hur Experiment Station, Louisiana State University, where these animals are grown in experimental ponds. Since no pesticide use is allowed in the vicinity of the Experimental Station, contamination of the growing ponds is expected to be minimal. The animals were

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acclimatized to laboratory conditions (temperature 22± 2°C, dissolved oxygen 4.3-5.6 ppm, pH 7.1-7.8 and total water hardness 32 ppm).

In order to assess the As-uptake by P. clarkii (wholebody), a total of 96 crayfish were exposed to 3 different concentrations (0.5, 5 and 50 ppm) of MSMA herbicide for a period of 8 wks. Thirty-two crayfish were exposed to each concentration of the herbicide in 16 all-glass aquaria (24 x 15.5 x 17 cm) each containing 1 male and 1 female. The amount of test solution was 1 L/aquarium, which was replaced by a new solution twice a week. These tests were conducted in static bioassay conditions. Thirty-two crayfish were similarly kept in aged tap-water as controls. A total of 128 crayfish were used in this part of the experiment. Biweekly, 8 crayfish (4 males and 4 females) were removed from the treated and control groups, wrapped in aluminum foil and frozen. Each crayfish pair was offered 1 g crayfish food/wk.

The depuration of As by crayfish was assessed with another group of 96 crayfish which were exposed to 0.5, 5 and 50 ppm MSMA for 8 wks. At the end of 8 wks, they were transferred to As-free, aged tap-water. Similarly, 4 males and 4 females from each exposed and control group were removed and frozen. To assess the amount of As taken up by crayfish tissues from test solutions during the 8 wks of 'uptake' and 8 wks of 'depuration' period, samples of test solutions and aged tap-water were collected at the beginning and end of each sampling period (2 wks).

Arsenic levels were determined by a slightly modified method of Standard Methods, 16th Ed (1985). Samples were digested in 400 ml beakers containing a 3:1 mixture of nitric and perchloric acid at 130°C on a pre-heated hot plate until dense fumes appeared. The sample was then transferred to a 100 ml volumetric flask where the final volume (100 ml) was achieved with deionized water. A sample aliquot (40 ml) was transferred to a 125 ml Erlenmeyer flask, and 5 ml HCl was added. Two ml KI and 7 drops of stannous chloride were also added and the mixture was allowed to stand for 15 min. Both ends of the arsenic apparatus were plugged with glass wool and the lower plug (rubber stoppered) was saturated with 4 drops of acetate solution. Silver diethyldithiocarbamate-pyridine (4 ml) was pipetted into a 15-ml spectrophotometer tube. A gas dispersing tube was attached to the opposite end of the lead acetate apparatus tube which was dispensed in the pyridine solution. Zinc metal (30 mesh) was added to catalyze the reaction and immediately connected to the delivery tube apparatus. The mixture

was allowed to react for 30 min and the optical density was read at 522 nm on a Bausch & Lomb spectrophotometer (Spectronic 20). A standard curve was prepared using an arsenic trioxide reference solution. The final volume of the working standard (2 UG AS/L) was made up to 40 ml with deionized water. Standards were also read at 522 nm and the optical density was plotted against ug of arsenic.

To assess levels of arsenic in field-collected crayfish, animals were transported back to the laboratory in ice coolers and frozen immediately upon arrival. Crayfish were collected from bayous and ditches alongside major highways of Louisiana from 24 different sites representing the entire state.

RESULTS AND DISCUSSION

Arsenic uptake by crayfish (whole-body) during 8 wks exposure by both male and female crayfish was dose-dependent but not time dependent, since the amount of As present in the tissues varied during the exposure period (Table 1). There was no statistically significant difference between male and female residues during uptake or depuration (Table 3).

Table 2 shows the amount of As present in test solutions at the beginning and end of each sampling period. Invariably, the levels were higher at the beginning of each sampling period indicating that As was removed by crayfish and incorporated in their tissues. Since a small amount of As was present in aged tap-water also, control water samples contained 0.1-0.3 ppm As. In comparing the As levels in the 0.5, 5 and 50 ppm test solutions, it is evident that the amount of metal actually present in the test solutions was significantly lower than the intended concentration, i.e., the maximum amount of As in the 50 ppm test solution at the beginning of 6th week sampling period was only 10.93 ppm. This difference was quite obvious in all test solutions, inspite of the fact that MSMA is a water soluble compound. A possible explanation is that since MSMA is an organoarsenical herbicide (sodium salt of methanearsenic acid), the actual amount of As in the whole molecule is considerably less than inorganic As compunds.

Arsenic in the test solutions ranged between 50-74% (in 0.5 ppm MSMA), 24-35% (5 ppm) and 16-23% (50 ppm), indicating that the amount of As in these solutions was inversely proportional to the increase in test solution concentration. The greater amount of As in solutions quantitated at the beginning of each sampling period indicated that it was being removed by crayfish

tissues. The difference in As between beginning and end test solutions was greater in 50 ppm MSMA (1-8 times), followed by 0.5 ppm (1-6 times) and 5 ppm (1-2 times).

During depuration, the amount of arsenic present in the test solutions was also generally greater at the beginning of each sampling period. These amounts were fairly consistent over an 8-week depuration period, and were dose dependent. There was, however, no statistically significant difference between males and females (Table 3). A noticeable fact was that most of the metal accumulated during the uptake period was rapidly lost (depurated) within the first 2 weeks of depuration and continued to be depurated thereafter.

Table 1. The amount of arsenic (ppm) present in the whole-body of male and female crayfish, Procambarus clarkii during 'uptake' and 'depuration. (Samples are the average of 4 males or 4 females)

Sampling Period (Wks)	Male (M) Female	Exposure Concentrations (PPM) Control	0.5	5.0	50
		U p t a k e*			
2	M	0.19	0.23	1.42	2.81
	F	0.18	0.67	1.28	7.39
4	M	0.15	1.36	2.33	4.61
	F	0.44	1.17	2.23	3.80
6	M	0.00	0.42	2.47	9.02
	F	0.27	0.31	2.26	4.81
8	M	0.36	0.86	2.86	5.79
	F	0.11	0.93	4.29	4.60
		D e p u r a t i o n*			
2	M	0.25	0.43	1.07	5.64
	F	0.19	0.43	1.10	1.19
4	M	0.24	0.45	0.64	2.57
	F	0.15	0.51	0.81	1.99
6	M	0.27	0.45	0.83	2.63
	F	0.00	0.20	0.75	2.65
8	M	0.19	0.32	0.89	4.45
	F	0.00	0.30	0.63	2.10

Crayfish collected from 24 different locations, which included bayous and shallow ditches alongside major highways of Louisiana, had As residues (whole-body) which ranged from 0.38-2.65 ppm. This suggests a moderate environmental contamination by As since the levels of residue were also moderate in concentration.

Table 2. The amount of arsenic (ppm) present in tested solutions and aged tap-water, quantitated at the beginning and end of each 2 wk sampling period.

Sampling Period (Wks)	Beginning(B) End (E)	Exposure Concentrations (PPM Control	0.5	5.0	50
		Uptake			
2	B	0.27	0.25	1.72	7.92
	E	0.04	0.24	2.00	1.03
4	B	0.02	0.15	1.68	9.89
	E	0.00	0.08	1.10	1.44
6	B	0.01	0.18	1.73	10.93
	E	0.00	0.03	0.91	5.73
8	B	0.01	0.37	1.19	8.46
	E	0.00	0.11	0.69	5.85
		Depuration			
2	B	0.11	0.02	0.13	0.21
	E	0.11	0.09	0.17	0.04
4	B	0.00	0.03	0.01	0.20
	E	0.00	0.01	0.06	0.02
6	B	0.30	0.03	0.02	0.25
	E	0.00	0.10	0.07	0.21
8	B	0.00	0.03	0.01	0.23
	E	0.00	0.09	0.06	0.01

Table 3. Analysis of variance for the amount of arsenic present in crayfish (Procambarus clarkii) tissues (MSMA treated and control), field-collected crayfish and test solutions.

Source	Degrees of Freedom	Sum of Squares	F-value	PR F
Uptake				
Male vs Female	1	0.0107	0.05	0.8311N.S.
Treated vs Control	1	6.5472	27.52	0.0002
Depuration				
Male vs Female	1	22.8126	0.96	0.3473N.S.
Field Crayfish				
Regions	4	0.5942	1.57	0.2235N.S.
Locations	4	0.4731	1.46	0.4357N.S.
Test Solutions				
Control vs Treated	1	0.6951	4.55	0.0500*

*Level of Significance

The ANOVA did not reveal any significant differences between different regions of the state (NE, NW, SE, SW and Central), as well as between different locations

where they were collected. ANOVA and Student's t-test were done on an IBM computer using the SAS program.

A linear relation to exposure concentrations of As in crayfish tissues was obtained, as expected. Similar findings were reported by Diaz-Mayans et al. (1986) who reported that 3.2-10 ug Cd/L 96 h exposure to P. clarkii resulted in a dose-dependent concentration of Cd in various tissues. Mirenda (1986) found that O. virilis exposed to 0.7-6.1 mg Cd/L for 14 days also had dose-dependent response. Earlier, Thorp et al. (1979) found a positive correlation between Cd uptake by Cambarus latimus exposed to 0.2, 5 and 10 ug/L Cd for 5 months. Alikhan and Zia (1989) exposed C. bartoni to 0.2-0.8 mg/L Ni for 4 wks, but the uptake of this metal was neither dose nor time dependent. Zia and Alikhan (1989) reported similar results for Cu uptake by C. bartoni. However, Anderson and Brower (1978) found that Pb accumulation in the gills and exoskeleton of O. virilis was both dose and time dependent.

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