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Effect of Fenvalerate on Metabolic Ion Dynamics in the Fathead Minnow (*Pimephales promelas*) and Bluegill (*Lepomis macrochirus*)

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The recently developed synthetic pyrethroid insecticides, including fenvalerate, are extremely toxic to aquatic species with LC_{50} values generally <10 µg/L (Bradbury et al. 1987a). The primary mode of action of pyrethroids is generally accepted to be disruption of nerve transmission by altering ion permeability of nerve membranes (Gray and Soderlund 1985). Exposure to fenvalerate may also secondarily induce an osmotic imbalance that contributes to the extreme toxicity of this compound to fish. Acute fenvalerate toxicity in rainbow trout resulted in major losses of sodium in urine (Bradbury et al. 1987b). In addition to sodium, calcium and chloride ions have been shown to play essential roles in osmotic regulation by aquatic species (Foskett et al. 1983; Clark and Matsumura 1982; Pic 1978). The current study attempted to develop a relatively simple exposure test whereby secondary osmotic effects of neurotoxicants could be evaluated with whole-body ion levels in live fish by using radioactive ions. The uptake and depuration of sodium in bluegill (Lepomis macrochirus) exposed to salt stress, ouabain, or fenvalerate were monitored to determine if a measurable whole-body alteration in ionic concentration could be detected. The uptake and depuration of sodium, calcium, and chloride ions in both bluegill and fathead minnows (Pimephales promelas) exposed to fenvalerate were also monitored.

MATERIALS AND METHODS

Bluegill (7.2 ± 2.3 g; mean ± standard deviation (S.D.); N = 370) and fathead minnows (1.1 ± 0.5 g; mean ± S.D.; N = 845) were obtained from the Osage Catfishery (Osage Beach, MO). Fish were not fed 24 h before testing. Radioactive ^{22}Na, ^{45}Ca, and ^{36}Cl (NEN, DuPont Corp., Boston, MA) were the ions chosen for study. For ^{22}Na, no quenching was found in any of the tissue or water

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samples due to the high energy of the ^{22}Na γ-emission. Overall counting efficiencies, as determined on a solid scintillation counter, were 42% in both tissue and water samples. Quench curves were developed employing a liquid scintillation counter for both ^{45}Ca and ^{36}Cl, with quenching increasing linearly with the amount of fish tissue oxidized. Counting efficiencies in water were 61% and 69% for ^{45}Ca and ^{36}Cl, respectively. Background counts were taken throughout the counting runs, averaged, and subtracted from the sample counts for each run. Fish tissue was prepared for scintillation counting by first homogenizing each fish individually with a mortar and pestle. For the bluegill, triplicate tissue samples were taken from each individual and averaged to generate a value for that exposure chamber. Because there were five to six fathead minnows per exposure chamber, only one tared sample was taken from each individual; the five to six individual values were averaged for that exposure chamber. Samples containing ^{22}Na were counted directly while those containing ^{45}Ca or ^{36}Cl were chemically digested before counting. Approximately 250 mg of tissue was digested with two ml of Protosol® tissue solubilizer (DuPont, Inc.; NEN Research Products, Boston, MA). Vials were placed in a 50°C oven for six h, followed by addition of 0.3 ml of 30% hydrogen peroxide for four h, and finally ten ml of Ready-solv MP® scintillation cocktail (Beckman Instruments, Inc., Fullerton, CA). Samples were counted 24 h later to insure complete dissolution.

Triplicate static tests were run in 3.5-L glass aquaria, each containing either one randomly assigned bluegill or five fathead minnows. Fish were lightly anesthetized with MS-222 for transfer between storage and test aquariums. Tests were run at 22°C with a 12:12 light:dark photoperiod using either carbon-filtered Ames, IA, municipal water or reconstituted "fresh" (RF) water. Artificial salt water (ASTM 1980; used at a 0.1% concentration), $CaCl_2$, and $KHCO_3$ were used to reconstitute distilled water to make the RF water. Alkalinity, hardness, and conductivity were determined (APHA, 1985) at the initiation and completion of each test. Overall means ($\pm$ S.D.) for the Ames municipal water were: alkalinity and hardness 29 $\pm$ 2.5 and 142 $\pm$ 6.5 mg/L as $CaCO_3$ (N = 15), respectively; conductivity 447 $\pm$ 15 mho/cm (N = 15). Overall means for the RF water were: alkalinity and hardness 61.7 $\pm$ 7.0 and 150 $\pm$ 5.0 mg/L as $CaCO_3$ (N = 22); conductivity 840 $\pm$ 53 µmho/cm (N = 22). The overall mean dissolved oxygen concentration at test initiation was 8.5 $\pm$ 0.5 mg/L (N = 23) and 3.8 $\pm$ 1.0 mg/L (N = 9) after 48 h. Each individual aquarium pH was adjusted to 7.03 $\pm$ 0.1 (N = 88), except in the pH variability study, which monitored sodium uptake at pH 6.5, 7.4, and 8.3.

Uptake and depuration assays were run separately. Uptake results were obtained by transferring fish directly into 3.5-L aquaria containing toxicant and labelled ion and monitoring at four postexposure time intervals through 48 h. Fish in the depuration study were all kept in a single 56-L aquarium containing labelled ion and no toxicant for 48 h, then transferred into individual aquaria containing toxicant but no labelled ion for the actual

assay. Uptake was monitored for 48 h because preliminary studies indicated no difference between uptake rate values at 48 and 96 h, and virtually all mortality occurred before 30 h. Ion uptake amounts were expressed on a µg ion/g fish weight/h exposure basis. Specific activity (dpm/g), determined from the aquaria water, was used to convert dpms to mass of ion. Each exposure aquarium was considered an experimental unit with three replicates at each of four sample times (postexposure) and each of three exposure levels (control plus two toxicant levels). The mean ± S.D. of the three replicates were used to generate an uptake rate for each exposure level at each sample time. A standard two-tailed t-test was used to determine significant differences between control and exposed fish. Preliminary sodium studies utilized reconstituted sea water (ASTM, 1980) to evaluate salt stress effects on sodium uptake, ouabain (Sigma Chemical Co., Milwaukee, WI) because of its known effect on sodium regulation, and the synthetic pyrethroid fenvalerate (Shell Development Co., Modesto, CA). Fenvalerate exposure levels were determined by methods of Bradbury et al. (1987a), with a spike recovery of 101% ± 11% (N = 6).

RESULTS AND DISCUSSION

Studies were undertaken in an attempt to isolate and reduce the large variability noted in individual fish sodium uptake rates in preliminary assays. Water type (RF vs. filtered tap), level of anesthetization with MS-222, water purity (single or double distilled), pH, fish weight, and aeration levels were all examined, but no clear definition of the variability (as indicated by the S.D.) could be gained. All subsequent assays were run using RF water since all ion levels could then be quantified nominally.

Initial studies employed exposures to several different salinities and produced significantly different rates of sodium uptake (Table 1). Sodium uptake rate increased at higher salinities. Dange (1985) found that succesively greater aqueous salinities increased the activity of the Na-K-ATPase enzyme, which is primarily responsible for the active transport of sodium. Under conditions of massive osmoregulatory insult, the test system, as designed, was sensitive to differences in sodium uptake.

Ouabain has been used previously in gill perfusion and intraperitoneal injection studies (Silva et al. 1977) and in stripped eel (Anguilla japonica) intestine studies (Ando 1981). Silva et al. (1977) stated that inhibition of gill Na-K-ATPase (i.e., Na-K adenosine triphosphatase) by ouabain resulted in almost complete inhibition of the efflux of both sodium and chloride. This study monitored both uptake and depuration or sodium at a nominal aqueous exposure level of 10 mg/L ouabain, with no significant differences between exposed and control fish noted. Ten mg/L was the highest no-effect concentration found in rangefinding studies. The Na-K-ATPase of the gill chloride cell is considered to be facing inward (Silva et al. 1977); indicating

Table 1. Sodium uptake rates by bluegill exposed to various salinities

Exposure[a]	Sodium Uptake Rate (µg/g fish/h)		
	9 h[b]	26 h	48 h
0.04% Salt Water	1.6 ± 0.2[c]	1.9 ± 0.2	1.4 ± 0.3
0.14% Salt Water	2.2 ± 0.8	2.5 ± 0.7	2.5 ± 0.3
0.71% Salt Water	3.7 ± 0.7	3.0 ± 0.3	2.6 ± 0.3
2.90% Salt Water	5.0 ± 1.0	3.3 ± 1.1	3.2 ± 1.3

[a]All exposures contain 1.1 x 10^6 dpm ^{22}Na per aquarium. Percentages are ASTM salt water in double-distilled water.

[b]Time postexposure that individual was sacrificed.

[c]Mean ± S.D.; N = 3.

that the ouabain must be absorbed internally by the organism before it can effectively inhibit ion transfer. No statistically significant differences were determined in the present study, probably because of poor uptake of the ouabain into the fish.

The first sodium uptake test with fenvalerate was run using bluegills. Significant differences were found after 48 h, with means indicating a trend towards increased sodium uptake with exposure to fenvalerate (Table 2). A clear dose response was not obtained, however, probably because of the occurrence of mortality in the high exposure level. As death approaches, fish exhibit seizures and lose the ability to maintain an upright position. Thrashing and loss of body control probably increases the urinary loss of sodium because renal excretion rates of sodium increase significantly during swimming activity (Wood and Randall 1973). This increased loss could account for the lower sodium uptake value at the higher exposure level at 48 h.

The uptake study was repeated using fathead minnows. Although results at 3 h indicated a lower sodium uptake rate, mean uptake rates of the exposed fish at 24 and 48 h were greater than those of controls, which was consistent with the bluegill results. Deviation from control rates may be due to either increased uptake of sodium with exposure to fenvalerate or an increase in the time required for the exposed fish to come to equilibrium with the labelled ion.

No statistical differences were found in the depuration rate (Table 2) for bluegill. Fathead minnows did exhibit a higher depuration rate at 8 h ($P<0.05$), postexposure, when exposed to fenvalerate (Table 2). The depuration value we generated indicates the amount of ion clearing the body in a given time span. Whole-body effects may be minimized, however, because of

Table 2. Dynamics of sodium in bluegill and fathead minnows (FHM) exposed to fenvalerate

Species	Process	Exposure[a]	µg sodium/g fish weight/h			
			3 h[b]	8 h	24 h	48 h
Bluegill	Uptake[c]	Control	N.A.[d]	4.4 ± 2.0	3.3 ± 0.4	2.6 ± 0.8
Bluegill	Uptake	Fenval (1.4 ppb)	N.A.	4.6 ± 1.7	3.6 ± 0.5	4.3 ± 0.2*
Bluegill	Uptake	Fenval (8.9 ppb)	N.A.	6.2 ± 1.6	4.6 ± 1.4	3.6 ± 0.1+
Bluegill	Depuration[e]	Control	24.1 + 5.6	9.1 ± 0.5	3.6 ± 0.3	1.6 ± 0.2
Bluegill	Depuration	Fenval (1.2 ppb)	30.5 + 6.4	10.2 ± 1.0	3.0 ± 0.5	N.A.
Bluegill	Depuration	Fenval (3.5 ppb)	N.A.	10.3 ± 1.6	3.5 ± 0.5	1.4 ± 0.1
FHM	Uptake	Control	14.9 + 1.4	10.7 ± 1.1	6.8 ± 0.3	4.6 ± 0.2
FHM	Uptake	Fenval (2.0 ppb)	12.4 + 1.1*	9.4 ± 1.4	7.6 ± 0.4**	5.5 ± 1.5
FHM	Depuration	Control	50.3 + 3.8	17.5 ± 1.0	4.0 ± 0.2	1.0 ± 0.2
FHM	Depuration	Fenval (1.6 ppb)	47.0 + 2.9	18.6 ± 0.4*	4.0 ± 0.4	1.1 ± 0.4

[a]Mean, N = 9; samples taken at 0, 24, and 48 h postexposure. Controls <0.05 ppb.

[b]Time, postexposure.

[c]460 dpm ^{22}Na per ml in each exposure aquarium.

[d]N.A. = data not available; other values are mean ± SD (N = 3 for FHM and for bluegill). +indicates difference from controls at $P<0.2$; *indicates difference at P 0.05.

[e]All fish exposed for 48 h to 460 dpm ^{22}Na per ml in a 56-L tank, then transferred into individual nonradioactive aquaria for the assay. FHM not exposed to fenvalerate contained 159 ± 38 µg Na/g fish tissue at 0 h, whereas bluegill contained 93 ± 21 µg Na/g fish.

ionic shifts between body compartments, an important aspect of ion-osmotic adaptation (Kostecki 1984).

Fathead minnows seemed to accumulate more sodium per g of wet weight than the larger bluegill. This may be due to relatively greater respiration rates generally present in smaller fish. Differences in sodium accumulation in the control fish exposed to ^{22}Na for 48 h in the uptake study and the fish preadapted to ^{22}Na for 48 h for the depuration study may be due to the different exposure conditions. There were more fish present per aquaria in the uptake period of the depuration studies than in the uptake study. The variability between individual uptakes, however, remains very high for both fathead minnows and bluegill. When only the means are considered, there is an increase in the uptake rate of sodium with exposure to fenvalerate, but the trend is not highly statistically significant.

The uptake and depuration values obtained for ^{36}Cl are presented in Table 3. As noted in the sodium studies, the fathead minnows

Table 3. Dynamics of chloride in bluegill and fathead minnows (FHM) exposed to fenvalerate

Species	Process	Exposure[a]	µg chloride/g fish weight/h			
			3 h[b]	8 h	24 h	32 h
Bluegill	Uptake[c]	Control	36.3 ± 16.0[d]	14.2 ± 1.5	4.1 ± 1.3	3.4 ± 0.8
Bluegill	Uptake	Fenval (1.8 ppb)	29.8 ± 11.0	12.3 ± 3.6	2.9 ± 0.5	3.8 ± 1.4
Bluegill	Uptake	Fenval (8.2 ppb)	17.3 ± 7.6	10.7 ± 3.0	3.3 ± 0.1	2.9 ± 0.7
Bluegill	Depuration[e]	Control	49.3 ± 12.0	22.5 ± 6.6	6.6 ± 0.6	5.8 ± 1.1
Bluegill	Depuration	Fenval (1.9 ppb)	38.0 ± 14.6	17.4 ± 2.6	N.A.	3.1 ± 0.8*
Bluegill	Depuration	Fenval (3.5 ppb)	23.9 ± 3.1*	10.7 ± 1.6*	3.1 ± 0.6**	2+6 ± 1.3*
			3 h[b]	8 h	24 h	48 h
FHM	Uptake	Control	94.2 ± 17.0	58.0 ± 16.0	27.6 ± 0.3	15.8 ± 1.1
FHM	Uptake	Fenval (1.3 ppb)	126 ± 27.0+	52.7 ± 19.0	24.3 ± 4.4	17.8 ± 4.3
FHM	Uptake	Fenval (5.5 ppb)	128 ± 29.0+	56.7 ± 2.1	18.3 ± 4.8**	13.3 ± 5.1
FHM	Depuration	Control	146 ± 6.6	52.8 ± 15.5	16.3 ± 2.4	7.7 ± 1.4
FHM	Depuration	Fenval (1.2 ppb)	105 ± 11.0**	40.8 ± 6.7	13.3 ± 3.7	5.3 ± 1.3*
FHM	Depuration	Fenval (3.4 ppb)	117 ± 25.0*	37.3 ± 12.0	12.5 ± 2.6*	4.8 ± 1.3**

[a]Mean, N = 6; samples taken at 0, and 24 h postexposure. Controls <0.05 ppb.

[b]Time, postexposure.

[c]305 dpm ^{36}Cl per ml in each exposure aquarium.

[d]Values are mean ± SD (N = 3 for FHM and for bluegill); N.A. = data not available. +indicates difference from controls at $P<0.1$; *indicates difference at $P<0.05$; **indicates difference at a $P<0.01$.

[e]All fish exposed for 48 h to 305 dpm ^{36}Cl per ml in a 15 gal tank, then transferred into individual non-radioactive aquaria for the assay. FHM not exposed to fenvalerate contained 460 ± 104 µg Ca/g fish tissue at 0 h, whereas bluegill contained 400 ± 104 µg Cl/g fish.

seemed to accumulate more ^{36}Cl than the bluegill on a per gram of body weight basis. The bluegill test lasted only 32 h because of mortality in the higher exposures. There were no statistical differences in uptake between control and exposed fish for bluegill. Fathead minnows indicated a significant increase in uptake at 3 h, postesposure ($P<0.1$) and then a significant reduction in uptake in the highest exposure after 24 h ($P<0.01$). Means for both bluegills and fathead minnows would seem to indicate a reduction in uptake with increasing exposure to fenvalerate. There were statistically significant differences in depuration of chloride ion between exposed and control fish for both bluegill and fathead minnows (Table 3). Mean values followed

a dose response, reflecting greater depuration with increasing fenvalerate exposure. Pic (1978) reported that the effects on sodium and chloride excretion caused by stress were virtually instantaneous, followed by a relatively slow recovery, and that nervous and/or hormonal factors were responsible for the noted effects. These observations are consistent with our results, showing differences even at the earliest sample time. Several studies have reported that chloride permeability is increased in stressed animals, resulting in an increased efflux rate (Pic 1978; Ando 1981). Ando (1981) also found that membrane permeability to chloride increases almost instantaneously with the introduction of ouabain as an osmotic stressor. It has been shown that sodium and chloride transport are interdependent and that water transport is linked to the coupled Na^+/Cl^- transport (Ando 1980).

There was no statistical difference between control and fenvalerate-exposed fathead minnows when both uptake and depuration for ^{45}Ca are compared. Although mean uptake of calcium was approximately 10 percent higher in fathead minnows exposed to fenvalerate, this was not a significant difference due to the large variability in results. Both fathead minnows and bluegill seemed to take up and depurate the same amount of calcium on a per-gram basis. The uptake and depuration rates decreased in a linear manner similar to the sodium and chloride tests. A fenvalerate exposure of 0.7 µg/L caused a significant ($P<0.05$) increase in calcium uptake in bluegill at 3 h (25.9 µg/g/h vs 15.2 µg Ca/g/h for controls) and at 48 h (3.4 µg Ca/g/h vs 2.9 µg Ca/g/h) postexposure. Bluegill also exhibited a significantly ($P<0.01$) lower depuration rate at 3 h (34.9 µg Ca/g/L vs 49.2 µg Ca/g/L for control), postexposure, when exposed to 1.2 µg/L. These results are consistant with the findings of Rashatwar and Matsumura (1986), which showed that DDT affected the Na/Ca exchange system in the American lobster (Homerus americanus).

Fish osmoregulation depends on the integrated transport activities of gill, gut, and renal systems, making it difficult to identify any one source of an ion imbalance. In addition, in vivo ion concentrations are interdependent. The large variability in our results make trends difficult to characterize. The ion imbalance shown by the sodium, chloride and calcium results do suggest a probable osmoregulatory imbalance in fenvalerate-exposed fish. The stress introduced by this osmoregulatory imbalance may be a contributing factor in the extreme toxicity of fenvalerate to fish, but the whole-body ion methodology employed in this study was not adequate to document the effects quantitatively.

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REFERENCES

American Public Health Association and Water Pollution Control Federation (1985) Standard methods for the examination of water and wastewaters, 16th ed. American Public Health Association, Washington, DC

American Society for Testing and Materials (1980) Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. American Society for Testing and Materials, Philadelphia, PA

Ando M (1980) Chloride-dependent sodium and water transport in the seawater eel intestine. J Comp Physiol 138:87-91

Ando M (1981) Effects of ouabain on chloride movements across the seawater eel intestine. J Comp Physiol 145:73-79

Bradbury SP, Symonik DM, Coats JR, Atchison GJ (1987a) Toxicity of fenvalerate and its constituent isomers to the fathead minnow (Pimephales promelas) and bluegill (Lepomis macrochirus). Bull Environ Contam Toxicol 38:378-385

Bradbury SP, McKim JM, Coats JR (1987b) Physiological response of rainbow trout (Salmo gairdneri) to acute fenvalerate intoxication. Pestic Biochem Physiol 27:275-288

Clark JM, Matsumura F (1982) Two different types of inhibitory effects of pyrethroids of nerve Ca and Ca-Mg-ATPase activity in the squid (Logligo paelei). Pestic Biochem Physiol 18:180-190

Dange AD (1985) Branchial Na-K-ATPase activity during osmotic adjustments in two freshwater euryhaline teleosts, tilapia (Sarotherodon mossambicus) and orange chromid (Etropis maculatus). Mar Biol 87:101-107

Foskett JK, Bern HA, Machen TE, Conner M (1983) Chloride cells and the hormonal control of teleost fish osmoregulation. J Exp Biol 106:255-281

Gray AJ, Soderlund DM (1985) Mammalian toxicology of pyrethroids. In: Huston DH, Robert TR (eds) Insecticides, John Wiley and Sons, Ltd, New York, pp 193-242

Kostecki PT (1984) The effect of osmotic and non-osmotic stresses on the blood and urine composition and urine flow of rainbow trout (Salmo gairdneri). Comp Biochem Physiol 79(A)2:215-224

Pic P. (1978) A comparative study of the mechanism of Na^+ and Cl^- excretion by the gill of Mugil capito and Fundulus lieteroclitus: Effect of stress. J Comp Physiol 123:155-162

Rashatwar SS, Matsumura F (1986) Influence of calomodulin and ATP on DDT inhibition of Na/Ca exchange in the axolemma-rich nerve preparation from the American lobster. Pest Biochem Pysiol 26: 90-99

Silva P, Solomon R, Spikes K, Epstein FH (1977) Ouabain inhibition of gill Na-K-ATPase: Relationship to active chloride transport. J Exp Zool 199:419-426

Wood CM, Randall DJ (1973) The influence of swimming activity on water balance in the rainbow trout (Salmo gairdneri). J Comp Physiol 82:257-276

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