Bull. Environ. Contam. Toxicol. (1989) 42:359–366


Environmental Contamination and Toxicology

# Effects of Water Hardness and Salinity on the Acute Toxicity and Uptake of Fenvalerate by Bluegill (*Lepomis macrochirus*)

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Ample data exist documenting the acute toxicity of pyrethroid insecticides to fish. A few reports indicate that water hardness (Mauk et al. 1976) and salinity (McKenney and Hamaker 1984) alter the toxicity of pyrethroids to aquatic species. For fish, ionic homeostasis is largely regulated by the transepithelial potential of the gill and by active transport processes involving enzymes, such as ATPases. Passive movements of monovalent ions are influenced by the transepithelial potential (Eddy 1975). In addition, monovalent and divalent ion levels are actively regulated by Na-K ATPase (Eddy 1981) and Ca-ATPases (Hunn 1985), respectively. DDT has been shown to inhibit Na-K ATPase in gill epithelia (Leadem et al. 1974). Recently, squid axon Ca-ATPases were found to be inhibited by pyrethroids and DDT (Matsumura 1983). Fenvalerate intoxication has been shown to affect rates of excretion of cations in urine of rainbow trout (Bradbury et al. 1987). Therefore, it is postulated that pyrethroid insecticides, in addition to their neurotoxicity, may interfere with osmoregulatory processes to such an extent that osmotic stress contributes to the overall toxicity. The objectives of this research were to: 1) determine the role of osmoregulation in pyrethroid toxicity to fish, using osmoregulatory stressors such as hardness and salinity; and 2) evaluate the relationship between residue levels in fish and differential toxicity incurred by osmotic stressors. Fenvalerate was the model pyrethroid and bluegill (Lepomis macrochirus) the test species.

## MATERIALS AND METHODS

The influences of hardness and salinity on fenvalerate toxicity were examined separately, using a static system. Four grades of hardness were prepared (6, 36, 156, and 309 mg/L $CaCO_3$), referred to as W1, W2, W3, and W4, respectively, based on American Society for Testing Materials (ASTM 1980) recommendations. Four salinity levels (12.5, 25, 33, and 40% SW) were prepared by diluting full-strength reconsituted saltwater (34 o/oo, ASTM 1980). Test mixtures were aerated and aged 18–24 h before water characteristics were determined. Mean alkalinities for the

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hardness test were: 4, 28, 27, and 33 mg/L $CaCO_3$. Mean conductivity levels were: 49, 158, 431, and 745 umhos. Mean conductivity for 12.5% SW was 10,410 umhos, other salinities exceeded 19,990 umhos. pH's for both hardness and salinity tests ranged from 7.34 to 7.66. Tests were performed at 22°C and with a 12 h photoperiod.

Stock solutions of technical fenvalerate (courtesy of Shell Development Company, Modesto, California) were prepared in certified grade acetone. Appropriate portions of these solutions were pipetted into 4-L aquaria containing 3.5 L of water using a 10 µl syringe to minimize solvent addition. Three replicates of four fenvalerate concentrations, plus controls, were used for each hardness and salinity test.

The concentration of fenvalerate in water was monitored in each test by using methods adapted from Bradbury et al. (1985). Water was extracted with pesticide-grade hexane. Quantitative analysis of the hexane extracts was determined by GLC, using an electron capture detector.

Bluegill were obtained from Hickory Grove Lake (Story County, Iowa; hardness = 150 mg/L $CaCO_3$) and held at Iowa State University (164 mg/L $CaCO_3$) for at least 7 d before testing. At test's end, control fish from the hardness and salinity tests weighed 371 $\pm$ 114 mg (mean + S.E. for 15 control groups) and 608 $\pm$ 100 mg (mean + S.E. for 15 control groups), respectively. Four or five fish were randomly assigned to each aquarium. Fish were not fed 24 h before testing nor during a test. Eight observations were made during the exposure period. The time to death was recorded for each fish. Dead fish were removed, rinsed with acetone, placed in foil-lined jars, and stored at -40°C. At the completion of a test, live fish were sacrificed and handled as previously described. Survivors or dead fish in each treatment were pooled for residue analysis. Tissue analysis was based on methods described by Bradbury and Coats (1982). Whole carcasses were extracted with acetonitrile, which was subsequently extracted with hexane. Hexane extracts were run through cleanup columns filled with Florisil® and anhydrous sodium sulfate. Collected extracts were analyzed by GLC. Extraction and cleanup of spiked bluegill samples resulted in 95.8 + 7.3% (N = 12) recovery.

$LC_{50}$ values were calculated by the Trimmed Spearman-Karber method (Hamilton et al. 1977). Total numbers of dead fish from triplicate treatments were combined for statistical analysis. An average of the fenvalerate concentrations in water at 0 and 24 h (after fish addition) was used for the hardness tests. In salinity tests, the mean of the 0-, 24-, and 48-h values were used. For $LC_{50}$ comparisons, points with non-overlapping 95% confidence limits (CL) were deemed significantly different. One way analysis of variance of the least squared means for the hardness and salinity tests was employed to test for treatment effects on dead fish residues. In addition, this procedure was used to test for differences between live and dead fish residue

levels. Slopes from log-log regressions of 48-h $LC_{50}$ values vs. $Ca^{2+}$ and $CaCO_3$ levels were calculated. Slopes of fenvalerate concentrations in fish vs. water concentrations were also determined. ANOVA was used to determine linear fitness and differences between slopes. $P<0.05$ was deemed significant.

## RESULTS AND DISCUSSION

Median lethal fenvalerate concentrations for all water hardnesses decreased over time (Table 1). The 36- and 48-h $LC_{50}$'s for W1 were significantly greater than for W4. Throughout the test period, W2, W3, and W4 $LC_{50}$ values were not significantly different.

Table 1. The effects of hardness on fenvalerate median lethal concentrations ($LC_{50}$ for bluegill over time (with 95% confidence limits). Concentrations expressed in ug/L.

| Water | 27 h | 31 h | 36 h | 48 h |
|---|---|---|---|---|
| Very Soft (W1) (6 mg/L)[a] | | 2.03 (1.74-2.37) | 2.03 (1.74-2.37) | 1.89 (1.65-2.17) |
| Soft (W2) (36 mg/L) | 1.71 (0.93-3.15) | 1.43 (1.15-1.77) | 1.14 (0.93-1.39) | 1.09 (0.53-2.26) |
| Hard (W3) (156 mg/L) | 1.60 (1.34-1.94) | 1.46 (1.30-1.64) | 1.21 (1.05-1.39) | 0.95 (0.45-2.04) |
| Very Hard (W4) (309 mg/L) | 1.74 (1.66-1.83) | 1.51 (0.84-2.69) | 1.16 (0.98-1.39) | 0.90 (0.75-1.08) |

[a]Hardness concentration (expressed as mg/L $CaCO_3$).

Average $uMCa^{2+}$ levels were 44, 172, 698, and 1395. Regression analysis of log $LC_{50}$ and log $CaCO_3$ indicated that hardness was significantly correlated with toxicity ($P$ 0.05, $r^2 = 0.91$; log $LC_{50} = 0.39 - 0.19$ (log $CaCO_3$). In addition, a high degree of correlation ($r^2 = 0.87$; log $LC_{50} = 0.57 - 0.21$ (log $uMCa^{2+}$)) was also found with log $LC_{50}$ and log $uMCa^{2+}$.

Residues for dead fish at comparable fenvalerate exposure concentrations were similar among the hardnesses, with mean values ranging from 192 to 398 ng/g (Table 2) based on wet weight. One-way ANOVA of the least squared means of the residues for each hardness indicated that water hardness did not significantly affect residue levels. Net uptake rates (residue concentration/mean time to mortality) and concentration factors (residue concentration/mean water concentration) were also not affected by hardness. Fenvalerate residue values for dead and surviving fish from hardness tests were pooled separately; means ($\pm$ standard

error of the least-squared mean) were calculated and tested for differences. Dead fish (268 ± 24 ng/g) did not contain significantly (P=0.06) more fenvalerate than live fish (196 ± 28 ng/g).

Table 2. Fenvalerate residue data for dead bluegill from hardness tests.

| Water | Fenvalerate Water Conc. (ug/L) | Fenvalerate Residue Conc. (ng/g) | Uptake Rate (ng/g/h)[a] | Concentration Factor |
|---|---|---|---|---|
| Very Soft (W1) | 1.32±0.17[b] | 362.1±138.3 | 9.17±3.50 | 274.3±104.8 |
| | ----------[c] | 261.6± 25.8 | 8.47±0.84 | ---------- |
| | 2.67±0.32 | 212.7± 80.2 | 7.18±2.66 | 79.0± 29.3 |
| Soft (W2) | 1.15±0.20 | 191.6± 3.2 | 5.86±0.09 | 166.6± 2.8 |
| | 2.02±0.09 | 344.2±134.6 | 11.63±4.54 | 170.4± 66.6 |
| | 2.12±0.25 | 248.8± 54.4 | 10.28±2.25 | 117.4± 25.6 |
| Hard (W3) | 1.24±0.15 | 397.6± 66.8 | 10.09±1.69 | 320.7± 53.9 |
| | 1.62±0.39 | 319.8± 88.9 | 10.12±2.80 | 197.4± 54.9 |
| | 2.30 | 217.1± 23.5 | 9.52±1.03 | 94.4± 10.2 |
| Very Hard (W4) | 0.86±0.35 | 317.9± 36.7 | 7.64±0.87 | 369.7± 42.0 |
| | 1.61±0.17 | 347.8± 32.0 | 10.38±0.96 | 215.9± 19.9 |
| | 1.89±0.09 | 222.4± 42.8 | 8.42±1.62 | 117.7± 22.6 |

[a]Uptake rate = $\frac{\text{Residue concentration}}{\text{Time to mortality}}$ based on wet weight

[b]Mean ± standard deviation (N = 3, except for hard 2.30 ug/L water conc. where N = 1.

[c]No water concentrations available.

In general, toxicity increased with salinity (Table 3). The 37-h $LC_{50}$ for 33% SW exposed fish was significantly less than 12.5 and 25% SW. By 48 h, the $LC_{50}$ for fish exposed to fenvalerate in 33% SW was significantly lower than those in 25, 40, and 12.5% SW. There were no significant differences between 25, 40, and 12.5% SW during the test.

Residues of fenvalerate in dead fish for 12.5% SW exposed fish were significantly greater than those of the other three salinities ($P<0.01$). Fenvalerate residues in fish from 25, 33, and 40% SW were not significantly different from one another (Table 4). Regression analysis of residue concentration plotted against water concentration for all salinities indicated that the slopes for 25, 33, and 40% SW were similar and had overlapping 95% CL. ANOVA confirmed that the slopes of the three curves were not significantly different, thus they were pooled and characterized by a common slope: residue concentration = 141 + 10 (water concentration). For 12.5% SW, however, a pronounced negative

Table 3. The effects of salinity on fenvalerate median lethal concentrations ($LC_{50}$) for bluegill over time (with 95% confidence limits). Concentrations expressed in ug/L.

| Salt Water (SW) | 24 h | 27 h | 31 h | 37 h | 48 h |
|---|---|---|---|---|---|
| 12.5% (4.25%) | | 1.92 (1.70+2.16) | 1.64 (1.46-1.85) | 1.64 (1.46-1.85) | 1.54 (1.35-1.76) |
| 25% (8.5%) | 2.49 (1.89-3.28) | 2.07 (1.55-2.77 | 1.83 (1.56-2.14) | 1.55 (1.35-1.78) | 1.22 (1.09-1.37) |
| 33% (11.2%) | 2.10 (1.64-2.70) | 1.55 (1.36-1.78) | 1.23 (1.04-1.47) | 1.11 (0.95-1.31) | 0.89 (0.84-0.94) |
| 40% (13/6%) | | | 2.14[a] | 1.77 (1.18-2.64) | 1.19 (0.99-1.43) |

[a]Confidence limits not reliable.

Table 4. Fenvalerate residue data for dead bluegill from salinity tests.

| Water | Fenvalerate Water Conc. (ug/L) | Fenvalerate Residue Conc. (ng/g) | Uptake Rate (ng/g/h) | Concentration Factor |
|---|---|---|---|---|
| 12.5% SW | 1.16 ± 0.15 | 413.8 ± 8.8 | 11.50 ± 0.26 | 57.1 ± 8.2 |
| 12.5% SW | 1.48 ± 0.33 | 306.9 ± 105.8 | 9.71 ± 3.35 | 207.4 ± 71.5 |
| 12.5% SW | 2.49 ± 0.46 | 212.3 ± 22.2 | 8.04 ± 0.84 | 85.3 ± 8.9 |
| 25% SW | 1.33 ± 0.44 | 171.3 ± 65.2 | 4.31 ± 1.64 | 128.8 ± 48.9 |
| 25% SW | 1.68 ± 0.36 | 144.0 ± 6.8 | 3.84 ± 0.18 | 85.7 ± 4.0 |
| 25% SW | 2.75 ± 0.59 | 161.0 ± 16.1 | 6.17 ± 0.62 | 58.5 ± 5.9 |
| 33% SW | 1.09 ± 0.12 | 124.5 ± 5.0 | 3.17 ± 0.13 | 114.2 ± 4.6 |
| 33% SW | 1.59 ± 0.24 | 137.2 ± 26.3 | 4.39 ± 0.84 | 86.3 ± 16.5 |
| 33% SW | 2.31 ± 0.23 | 178.8 ± 17.4 | 6.74 ± 0.67 | 76.1 ± 7.6 |
| 40% SW | 1.12 ± 0.17 | 108.9 ± 46.7 | 2.36 ± 1.01 | 97.3 ± 41.7 |
| 40% SW | 1.78 ± 0.33 | 207.6 ± 132.2 | 5.65 ± 3.59 | 116.6 ± 74.3 |
| 40% SW | 2.14 ± 0.26 | 161.2 ± 52.2 | 4.72 ± 1.54 | 75.3 ± 24.4 |

[a]Uptake rate = $\frac{\text{Residue concentration}}{\text{Time to mortality}}$ based on dry weight

[b]Mean ± standard deviation (N = 3).

slope was found: residue = 542 - 135 (water concentration). ANOVA showed that the common and 12.5% SW slopes were significantly different ($P<0.05$). Uptake rates of fish exposed in 12.5% SW were significantly greater than rates found in the other salinities. ANOVA for net uptake rates and concentration factors vs. water concentration indicated that the slopes for 25, 33, and 40% SW were not significantly different, thus they were pooled and characterized by common slopes: net uptake rate (ng/g/h) = 0.9 + 2.2 (water concentration) and concentration factor = 148 - 32 (water concentration). Regression curves for 12.5% SW were significantly different ($P<0.01$) from the salinities and were characterized by the following slopes: net uptake rate = 13.8 - 2.4 (water concentration) and concentration factor = 531 - 184 (water concentration). Residues in pooled dead fish (190 $\pm$ 14 ng/g) were significantly greater ($P<0.01$) than residues in pooled live fish (80 $\pm$ 18 ng/g).

The $LC_{50}$ values of the hardness and salinity tests compare well with previously published values for technical fenvalerate. Bradbury et al. (1985) reported an $LC_{50}$ value of 0.69 µg/L for fathead minnows (Pimephales promelas). In addition, Mauk et al. (1976) reported that 96-h $LC_{50}$ values for bluegill exposed to natural pyrethrum decreased significantly from 62.0 µg/L in very soft water to 46.5 µg/L in very hard water. In this study, fenvalerate toxicity increased with increased $CaCO_3$ and $uMCa^{2+}$ levels. One explanation could be that fish preadapted to hard water and then transferred to W1 (low calcium) underwent an ion-regulatory adaptation (increased gill permeability) antagonistic to the action of fenvalerate, whereas fish transferred to W3 or W4 did not undergo as great an adaptation, thus greater toxicity resulted. Eddy (1975) reported that low calcium water concentrations caused increased gill permeability to $Na^+$ and $Cl^-$ (increased influx and efflux rates). Hence, if permeability to $Na^+$ and/or $Cl^-$ is crucial to the toxicity of fenvalerate, the lower $Ca^{2+}$ levels may increase $Na^+$ and $Cl^-$ influx and thereby minimize toxicity. A second possible explanation concerns active ion-regulatory processes (ATPases). Flik et al. (1984) reported that exposure of eels to low calcium freshwater (0.1 mg $Ca^{2+}$/L) resulted in a 161% increase in gill epithelial Ca+Mg-ATPase activity. Thus, more fenvalerate would be required to sufficiently inhibit the osmoregulatory enzymes needed for death to occur in W1 as compared to W2-W4. However, it must be noted that induction of enzymes is a longer term adaptation to osmoregulatory stress than the previous epithelial explanation.

The salinity study indicated that 33% SW was the most toxic condition. Leadem et al. (1974) and Eddy (1975) have stated that 33% SW approximates an isotonic solution for fish such as rainbow trout and goldfish. Theoretically, this solution should be ideal in regard to osmoregulation, where water and ion fluxes occur with no expenditure of energy, causing minimal osmoregulatory stress. However, the least (theoretically) stressful water was the most toxic. The inducible enzyme activity for fish 33% SW may have been reduced to such an extent that less fenvalerate was required

to sufficiently inhibit the osmoregulatory ATPases and cause death.

Hardness had no significant effect on fenvalerate residue concentrations in bluegill. The fact that very soft water (W1) was the least toxic condition yet had similar residue concentrations compared to the other hardnesses may indicate that osmotic adaptation for fish exposed to W1 exposed fish minimized fenvalerate toxicity.

The salinity test revealed that residues of 12.5% SW exposed fish were significantly greater than those in 25, 33, 40% SW. Residues of 12.5% SW exposed fish decreased at higher fenvalerate water concentrations. This pattern is similar to results found with fathead minnows (Bradbury et al. 1985). Spehar et al. (1982), however, reported a positive correlation between fish residue concentration and water concentration, which was also found in the present study for 25, 33, and 40% SW. The higher residue levels did not translate to greater toxicity. The three highest SW concentrations probably inhibited normal ventilation and respiration, while 12.5% SW allowed more normal functioning of the bluegill ventilation. In all salinities and hardnesses, the highest concentration factors resulted from the lowest fenvalerate exposure levels.

In summary, fenvalerate was the least toxic in the softest water tested (6 mg/L $CaCO_3$). The effect was not the result of lower uptake rates, since comparable rates were observed at all hardnesses. In salinity trials, fenvalerate was most toxic at the approximate isotonic concentration (33% SW), possibly due to lower levels of osmoregulatory enzymes present and their inhibition by fenvalerate.

Acknowledgments. The authors thank D. Symonik and S. Nuutero for their technical assistance during this project. Although the research described in this article has been funded by the U.S. Environmental Protection Agency under grant No. 810659 to J. R. Coats, it has not been submitted to the Agency's peer and administrative review and, therefore, may not necessarily reflect the views of the Agency and no official endorsement should be inferred. This is Journal paper No. J-12530 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa; Project 2306.

REFERENCES

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

Bradbury SP, Coats JR (1982) Toxicity of fenvalerate to bobwhite quail (Colinus virginianus), including brain and liver residues associated with mortality. J Toxicol Environ Health 10:307-319

Bradbury SP, Coats JR, McKim JM (1985) Differential toxicity and uptake of two fenvalerate formulations in fathead minnows (Pimephales promelas). Environ Toxicol Chem 4:533-541

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

Eddy, FB (1981) Effects of stress on osmotic and ionic regulation in fish. In: Pickering AD (ed) Stress and fish. Academic Press, New York, New York, pp 77-102

Flik B, Bonga SEW, Fenwick JC (1984) $Ca^{2+}$-dependent phosphatase and $Ca^{2+}$-dependent ATPase activities in plasma membranes of eel epithelium- II. Evidence for transport of high-affinity $Ca^{2+}$-ATPase. Comp Biochem Physiol 79B:9-16

Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714-719. Correction 12:417 (1978)

Hunn JB (1985) Role of calcium in gill function in freshwater fishes. Comp Biochem Physiol 82:543-547

Leadem TP, Campbell RD, Johnson DW (1974) Osmoregulatory responses to DDT and varying salinities in Salmo gairdneri- I. Gill Na-K-ATPase. Comp Biochem Physiol 49A:197-205

Matsumura F (1983) Influence of chlorinated hydrocarbons and pyrethroid insecticides on cellular calcium regulatory mechanisms. In: Miyamoto J, Kearney JC (eds) Mode of action, metabolism and toxicology, vol 3. Pesticide chemistry: Human welfare and the environment. Pergamon Press, NY, pp 3-13

Mauk WL, Olson LE, Marking LL (1976) Toxicity of natural pyrethrins and five pyrethroids to fish. Arch Environ Contam Toxicol 4:18-19

McKenney CL, Hamaker DB (1984) Effects of fenvalerate on larval development of Palaemonetes pugio (Holthuis) and on larval metabolism during osmotic stress. Aquat Toxicol 5:343-355

Spehar RL, Tanner DK, Gibson JH (1982) Effects of kelthane and pydrin on early life stages of fathead minnows (Pimphales promelas) and amphipods (Hyalella azteca). In: Peron JG, Foster R, Bishop WE (eds) Aquatic Toxicology and Hazard Assessment: Fifth Conference. American Society for Testing and Materials STP 766. Philadelphia, Pennsylvania, pp 234-244

Received February 17, 1988; accepted July 6, 1988.