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Physiological Response of Rainbow Trout (*Salmo gairdneri*) to Acute Fenvalerate Intoxication[1]

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The physiological responses of rainbow trout (*Salmo gairdneri*) to fenvalerate intoxication during aqueous exposure were examined to provide information about the pyrethroid mode of action in fish. Trout ($n = 4$) were exposed to 412 ± 50 μg/liter fenvalerate and died in 10.9 ± 1.5 hr. Brain, liver, and carcass fenvalerate concentrations associated with mortality were 0.16 ± 0.05, 3.62 ± 0.57, and 0.25 ± 0.05 mg/kg, respectively. Visible signs of intoxication included elevated cough rate, tremors, and seizures. Histopathological examination of gill tissue showed damage consistent with irritation. An evaluation of respiratory–cardiovascular and blood chemistry responses indicated an elevated rate of metabolism associated with increasingly severe seizures. A cessation of ventilatory and cardiac activity, occurring with the seizures, was also observed. Finally, urine osmolality, Na^+ and K^+ concentrations, and Na^+ and K^+ excretion rates were elevated with intoxicated trout. The physiological responses of rainbow trout to fenvalerate intoxication suggest that besides effects on the nervous system, effects on respiratory surfaces and renal ion regulation may be associated with the mechanism of pyrethroid action in fish.

INTRODUCTION

The pyrethroid insecticides are extremely toxic to fish (1). Fenvalerate ([*R,S*]-α-cyano-3-phenoxybenzyl [*R,S*]-2-[4-chlorophenyl]-3-methylbutyrate) flow-through 96-hr LC_{50} values of 5.4 to 0.69 μg/liter have been reported for rainbow trout (*Salmo gairdneri*) and fathead minnows (*Pimephales promelas*) (2, 3). In contrast, mammals (4) and birds (5) are relatively insensitive to the pyrethroids. The extreme toxicity of these insecticides to fish could be a function of (i) efficient gill uptake, (ii) inefficient detoxification and elimination, and (iii) sensitivity at the site(s) of action. Results from a recent study with rainbow trout indicate that the gill uptake of fenvalerate is relatively inefficient and not a contributing factor in the compound's toxicity to fish (6). Low rates of fenvalerate elimination and metabolism do, however, seem to play a significant role in the piscicidal activity of this insecticide (6). Low rates of metabolism have also been implicated in the toxicity of permethrin (3-phenoxybenzyl [*R,S*]-*cis, trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) to fish (7, 8).

Although low rates of detoxification may be partly responsible for the susceptibility of fish to the pyrethroid insecticides, sensitivity at the site(s) of action may also be an important consideration. The pyrethroid insecticides are generally accepted to be neurotoxicants (4). Specific hypotheses regarding the effect of the pyrethroids in the nervous system include interactions with (i) sodium channels (9, 10), (ii) the γ-aminobutyric acid–receptor–ionophore complex (11–13), and (iii) ATPase-utilizing systems (14, 15). Glickman and Lech (16), using permethrin as a model compound, reported 3- to 10-fold lower brain concentrations at death in rainbow trout than in mice. These findings suggest either that there are differences in sensitivity at the

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site of action in the nervous system or that additional nonneural modes of action (e.g., gill damage) (17) are relevant in evaluating the toxicology of pyrethroids in fish.

Detailed observations of toxicological signs can reveal information about an insecticide's site of poisoning and mode of action (18), thereby providing a coherent framework for the planning and interpretation of mechanistic studies. At present, no detailed report of the response of fish to acute pyrethroid exposure is available in the literature. The study presented here describes the physiological responses of rainbow trout to lethal aqueous exposures of fenvalerate and provides baseline information for future mechanistic research.

MATERIALS AND METHODS

Fish preparation. The respirometer–metabolism chambers, exposure system, and surgical procedures used were as described previously for metabolism and gill uptake studies (6, 19–21), with some additions (22, 23).

Fish were initially anesthetized with 100 mg/liter of MS-222 (tricaine methanesulfonate, Ayerst Laboratories, New York) and immobilized by spinal transection. Transections were made at the eighth or ninth vertebrae, well behind the respiratory and cardiac control center located in the medulla oblongata (21). Twenty-four hours later, fish were reanesthetized, and a latex rubber-oral membrane was sutured around the mouth to separate inspired and expired water. Fish were also fitted with urinary catheters (PE-60, polyethylene tubing), indwelling dorsal aortic cannulas (24), and copper-wire heart electrodes (25). After surgery, fish were placed in the metabolism chambers, and physiological measurements began approximately 24 hr later. Four rainbow trout were exposed to fenvalerate. Three additional trout, handled in an identical manner but not exposed to the insecticide, were used as controls. The fish were maintained at the USEPA Environmental Research Laboratory, Duluth, Minnesota, for several months before use and acclimated to a temperature of 11 to 12°C. The fish weighed between 0.622 and 0.919 kg and were kept on a 12-hr photoperiod (incandescent lighting, 11.0 lx at water surface) during an experiment. Trout food (Glencoe Mills, Glencoe, MN) was withheld from fish 24 hr before their use.

Toxicant preparation and exposure. Technical fenvalerate (Lot No. 80115, 93% purity) was provided by the Shell Development Company, Modesto, California. A fresh solution of fenvalerate was prepared with distilled water in an 18-liter stock bottle for each exposure period. Required aliquots of the insecticide, dissolved in DMF,[2] were added to the stock bottle, followed by addition of water. The contents were agitated during an exposure with a magnetic stir bar.

Fenvalerate stock solutions were delivered to the toxicant-mixing cell at a rate of 0.2 ml/min with an FMI (Fluid Metering, Inc., Oster Bay, NY) chemical-metering pump. The stock solutions were then diluted with Lake Superior water flowing at a rate of 1000 ml/min. The fenvalerate solutions flowed into two replicate metabolism chambers at a rate of 500 ml/min. Mean fenvalerate aqueous concentrations ($\pm$SD) were maintained at 412 $\pm$ 50 μg/liter (n = 4 chambers). The fenvalerate concentration was selected to cause mortality within 12 hr. The nominal concentration of DMF was 200 mg/liter. Previous studies indicated that a DMF concentration of 200 mg/liter does not have an effect on the physiological (see Physiological Monitoring) or histopathological parameters examined (unpublished data). Filtered Lake Superior water was

[2] Abbreviations used: AChE, acetylcholinesterase; CR, cough rate; DF, discriminant function; DFA, discriminant function analysis; DMF, dimethylformamide; Hb, hemoglobin; Hct, hematocrit; HR, heart rate; O_2E, oxygen uptake efficiency; PC, principal component; PCA, principal component analysis; pHa, arterial pH; $TaCO_2$, total arterial CO_2; TaO_2, total arterial O_2; V_G, ventilation volume; VO_2, oxygen consumption; VR, ventilation rate.

maintained at 11.0 to 11.5°C. Overall means and standard deviations (n = 8) for hardness and alkalinity (26) were 42.59 ± 0.62 and 44.62 ± 1.20 mg/liter as $CaCO_3$, respectively. Dissolved oxygen, measured with an oxygen electrode (Beckman Instruments, Inc., Arlington Heights, IL), ranged from 10.5 to 11.0 mg/liter (n = 42). Mean pH (n = 34) was 7.92 ± 0.18.

Physiological monitoring. During each experiment, a variety of physiological variables were monitored (Table 1). Measurements were made approximately every 2 hr, except for blood gases and blood pH, which were monitored every 2 to 4 hr throughout the exposure period. Preceding exposure, two to three sets of all variables were measured on each fish to provide predose (control) values.

VR, V_G, O_2E, VO_2, and urine flow rate were measured as previously described (6, 19–21). CR was monitored via the free-standing electrodes used to measure VR, and HR was monitored by the copper electrodes.

Arterial blood samples, obtained from the aortic cannulas, were analyzed for pHa, $TaCO_2$, TaO_2, Hb, and Hct. Not more than 10% of the total blood volume was taken from a fish; the blood volume removed was replaced with Cortland saline for freshwater teleosts (27). A Radiometer BGA 3 Blood Micro system (Westlake, OH) was used to determine pHa, $TaCO_2$, and TaO_2. The pH meter was thermostated to 11°C. The methods of Cameron (28) and Tucker (29) were used to determine $TaCO_2$ and TaO_2, with the electrodes maintained at 37°C (30). Hct was measured on a Becton Dickinson & Company 0575 centrifuge (Rutherford, NJ). Hb measurements were made using the cyanomethemoglobin pro-

TABLE 1

Physiological Measurements Completed on Fenvalerate-Exposed[a] and Control Rainbow Trout

Physiological variables	Absolute predose value		Postdose percentage change[b]	
	Control trout	Fenvalerate-exposed trout	Control trout	Fenvalerate-exposed trout
Ventilation rate, VR (No./min)	73.9 ± 3.3[c]	69.3 ± 4.7	−6.1 ± 11.7	3.7 ± 13.3
Ventilation volume, V_G (ml/min)	120.8 ± 27.1	152.6 ± 64.7	4.3 ± 2.9	40.7 ± 61.3
Cough rate, CR (No./min)	0.8 ± 0.3	1 ± 1	0 ± 0	205 ± 167
Heart rate, HR (No./min)	51.6 ± 4.6	42 ± 7.4	−2.8 ± 10.9	−3.3 ± 21.4
Oxygen consumption, VO_2 (mg/kg/h)	48.6 ± 4.0	76.5 ± 16.2	12.1 ± 5.1	4.1 ± 28.7
Oxygen uptake efficiency, O_2E (%)	52.7 ± 12.2	54.8 ± 7.0	2.3 ± 4.1	−16.4 ± 18.6
Arterial blood oxygen, TaO_2 (g/100 ml)	12.2 ± 1.3	5.7 ± 2.9	−21.9 ± 10.8	11.0 ± 36.7
Arterial blood carbon dioxide, $TaCO_2$ (mmol/liter)	6.4 ± 0.4	9.1 ± 3.0	27.6 ± 24.8	−19.9 ± 15.6
Arterial blood pH, pHa	7.83 ± 0.09	7.99 ± 0.10	0.44 ± 0.74	−0.60 ± 3.6
Hematocrit, Hct (%)	31.1 ± 2.0	22.4 ± 1.8	−9.1 ± 6.4	9.1 ± 16.5
Hemoglobin, Hb (g/100 ml)	8.8 ± 0.6	7.7 ± 1.2	−9.4 ± 3.9	6.0 ± 10.3
Urine flow rate (ml/hr/kg)	3.31 ± 0.67	5.82 ± 2.45	1.8 ± 24.4	15.2 ± 16.0
Urine Na^+ concentration (mmol/liter)	5.07 ± 3.03	8.92 ± 9.27	−38.3 ± 14.6	23.9 ± 59
Urine Na^+ excretion rate (mmol/hr/kg)	0.0165 ± 0.008	0.0332 ± 0.0206	−57.4 ± 27.9	74.0 ± 103
Urine K^+ concentration (mmol/liter)	1.25 ± 0.63	3.53 ± 2.34	−7.5 ± 5.1	14.3 ± 14.8
Urine K^+ excretion rate (mmol/hr/kg)	0.00509 ± 0.00424	0.0205 ± 0.0227	5.2 ± 18.5	53.4 ± 63.9
Urine osmolality (mosmol/kg H_2O)	25.7 ± 5.8	32.2 ± 23.5	−5.0 ± 8.6	14.6 ± 15.9

[a] Fish exposed to 412 ± 50 μg/liter fenvalerate.

[b] Percentage change from predose mean; postdose absolute value is derived from the mean of the measurements between 25 and 75% survival time.

[c] Mean ± SD, for control (n = 3) and fenvalerate-exposed trout (n = 4), with two to five measurements per fish.

cedure (31); blood was collected and diluted by using the Unopette microcollection system (Becton Dickinson & Co.). Absorbance was measured on a Beckman Instruments, Inc. DU7 spectrophotometer.

Urine samples (approximately 1 ml) were collected and analyzed for Na^+ and K^+ concentration by atomic absorption spectroscopy (32) on a Perkin–Elmer 5000 spectrophotometer (Norwalk, CT). Urine osmolality was determined on a Precision Instruments, Inc., Micro Osmette 5004 system (Utica, MI).

Water and tissue analysis. Fenvalerate concentrations in the inspired water of each chamber were monitored every 2 to 3 hr ($n = 4$ samples per exposure period per chamber). Water was collected and analyzed for fenvalerate as previously described (2, 6). Recovery of fenvalerate from spiked water samples ($n = 4$) was 99 ± 5%. At death the liver, brain, and two to three gill arches were removed and weighed. The remaining carcass was weighed and homogenized. Fenvalerate concentrations in brain, liver, and remaining carcass were determined by previously published methods (2, 5). Fenvalerate recoveries from spiked brain, liver, and carcass samples ($n = 4$ for each matrix) were 96 ± 4, 85 ± 9, and 94 ± 3%, respectively. A histopathological examination was performed on the gill tissue.

Statistical analysis. Each of the seven fish was treated as an experimental unit. The mean percentage change of physiological parameters from their predose means was determined for the 25 to 75% survival time interval for each fish. Measurements only within the 25 to 75% survival time interval were used in the analysis to reduce responses due to initial stress and death itself. Before analysis, all variables were examined for normality and log-transformed when necessary.

PCA was used to compare the toxic response of the fenvalerate-exposed fish with that of the controls. This multivariate statistical technique has been typically employed in ecological studies (33) and more recently has been used in toxicological investigations as well (34). Using PCA new and orthogonal variables (PCs) are calculated on the basis of correlation or covariance between the original variables. Through this approach, the variation among the experimental units can be explained by fewer dimensions. This technique was used as an exploratory/descriptive procedure; no hypotheses were tested with this analysis. All analyses were performed using BMDP statistical software (35). Interpretation of the principal component axes were based on those variables with correlations of greater than 0.70 ($P < 0.05$) with the PCs.

RESULTS

Rainbow trout were exposed to a mean concentration of 412 ± 50 μg/liter fenvalerate and died in 10.9 ± 1.5 hr ($n = 4$ fish). Visible signs of intoxication were evident anterior to the site of spinal transection. Shortly after exposure to fenvalerate (10% survival time), CR seemed to increase, which was confirmed through evaluation of ventilatory traces (Table 1 and Fig. 1). Visual examination of expired gill water indicated that elevated secretion of mucus was associated with increased CR. Except for these effects on CR and mucus secretion, fenvalerate-exposed fish seemed normal, compared with control fish and predose behavior, through approximately 30% survival time, at which point fine tremors were observed. Tremors progressed to periods of violent head shaking and twisting that culminated in episodes in which the head was held at a 30° angle from the body for periods of up to 8 sec. During these seizures, the opercula were flared and in a state of tetany. The intensity and frequency of seizures increased through approximately 70% survival time, after which they subsided, and the fish became inactive and expired.

Fenvalerate residues associated with mortality were determined for brain, liver,

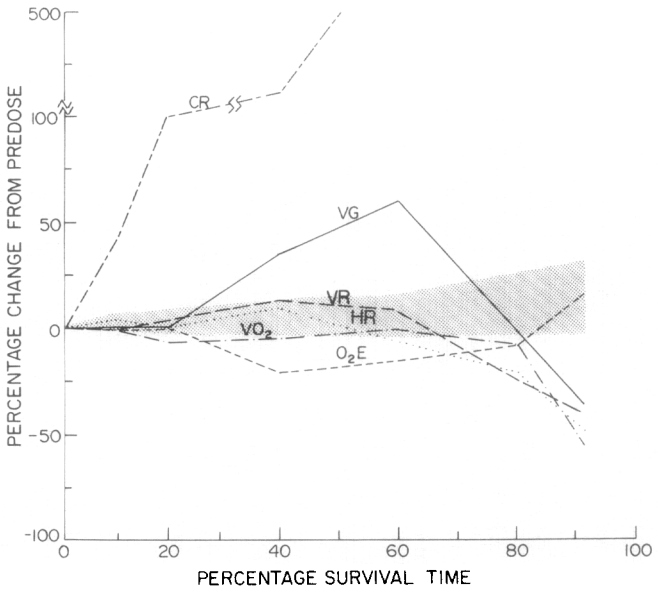

FIG. 1. *Mean respiratory–cardiovascular responses (CR, – · –; V_G, —; VR, – –; HR, . . .; O_2E, ---; VO_2, – · –) of rainbow trout (n = 4) exposed to a lethal aqueous concentration of fenvalerate (412 μg/liter). Stippled area represents range of the mean response for control fish (n = 3).*

and remaining carcass tissue (Table 2). Liver contained the highest concentrations (about 3.5 mg/kg), followed by remaining carcass (about 0.25 mg/kg) and brain (about 0.15 mg/kg). Fenvalerate was not detected in control fish (<0.01 μg/kg).

Histopathological examination of gill tissue, collected at death, from fenvalerate-exposed trout indicated lamellar aneurysms on the distal third of many filaments (Fig. 2). Lamellae adjacent to aneurysmal lamellae often had necrotic pillar cells. The filamental tissue between adjacent lamellae was commonly separated from its anchorage on the connective tissue that forms the central sinus of the filament. Numerous necrotic cells were found within these separated interlamellar zones (Fig. 2).

TABLE 2
Fenvalerate Residues in Rainbow Trout Tissues Associated with Mortality[a]

Tissue	Fenvalerate concentration (mg/kg)
Brain	0.16 ± 0.05[b]
Liver	3.62 ± 0.57
Remaining carcass	0.25 ± 0.05

[a] Fish were exposed to 412 ± 50 μg/liter fenvalerate (n = 4 chambers). Mortality occurred after 10.9 ± 1.5 hr (n = 4 fish) of exposure.

[b] Mean ± SD for n = 4 fish.

Predose values for the physiological variables monitored in the study are listed in Table 1. The respiratory–cardiovascular values (VR, V_G, CR, HR, VO_2, and O_2E) were similar between control and fenvalerate-exposed fish and comparable to previously reported values for transected rainbow trout (6, 19–21); however, VO_2 in the fenvalerate-exposed fish was elevated. The predose blood chemistry values (TaO_2, $TaCO_2$, pHa, Hct, and Hb) from the trout in this study were also comparable to previously published data (36, 37). Blood chemistry measurements were similar between treatment groups, except that TaO_2 and $TaCO_2$ were depressed and elevated, respectively, in the fenvalerate group as compared with the controls. Predose urinary values were similar between treatment groups and comparable to values previously reported for rainbow trout (38–40).

A departure from predose respiratory–cardiovascular status was noted in the fen-

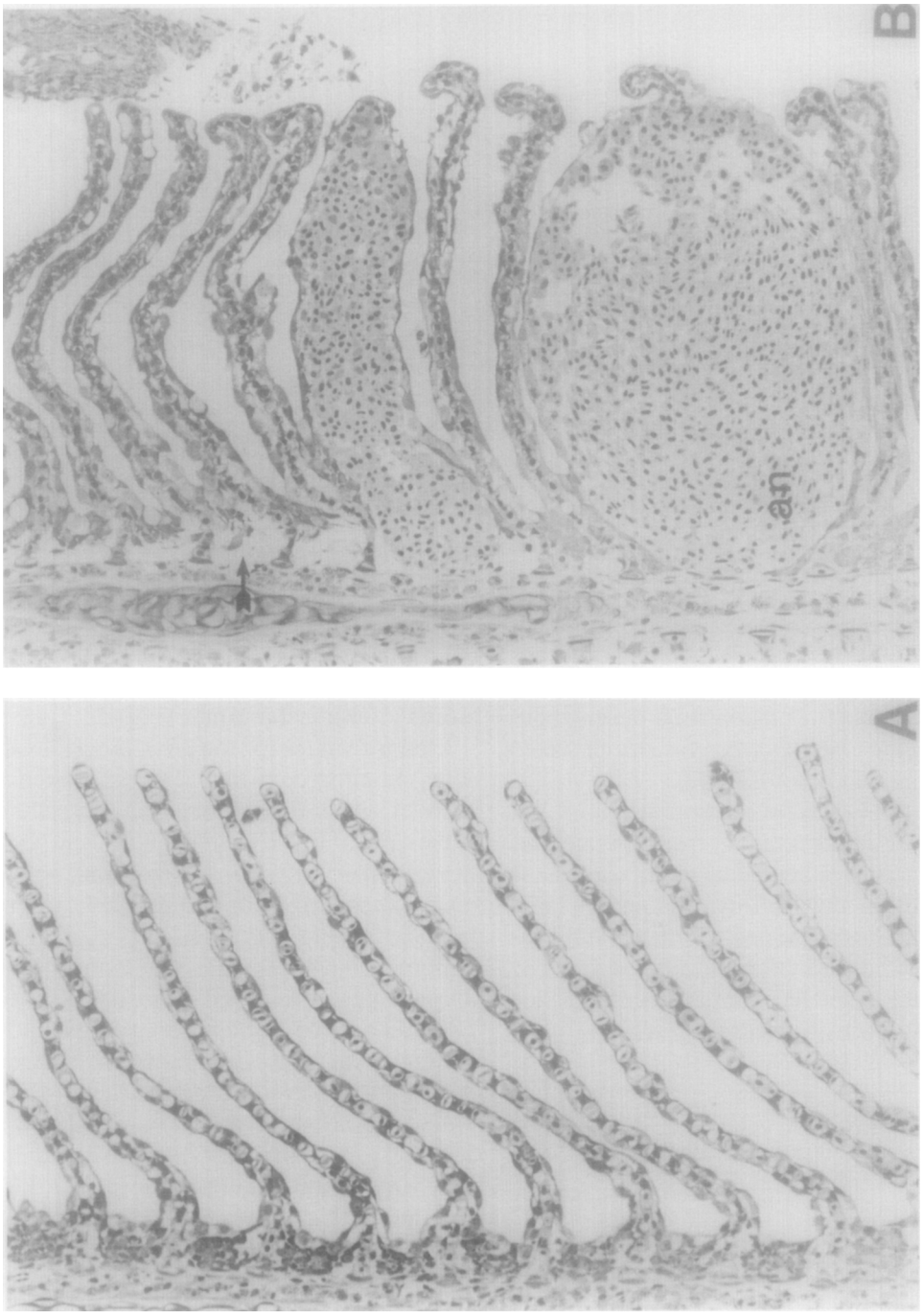

FIG. 2. *Representative photomicrographs of gill sections from control (A) and fenvalerate-intoxicated (B) rainbow trout. Arrow, interlamellar separation and necrotic cells; an, lamellar aneurysms* × *195.*

valerate-intoxicated fish (Table 1 and Fig. 1) while the response of control fish was generally consistent with the "predose" condition. Most notable in the intoxicated fish was a dramatic increase in CR (500%) over predose levels at 50% survival time. The ventilatory pattern of intoxicated fish was sufficiently distorted beyond this point that an accurate determination of cough response was no longer possible. An increase in V_G was observed through about 50% survival time; V_G then dropped below predose levels near death (overall mean increase of 40.7% over predose values). Associated with the rising V_G was a mean drop in O_2E of 16.4%. Due to the inverse relationship between O_2E and V_G, VO_2 remained fairly constant until the fish were near death, when rates dropped substantially with falling VR and V_G. HR in intoxicated fish showed a somewhat variable response. Initially HR was elevated; however, at about 50% survival time periods of heart inactivity occurred. These periods of inactivity, coincident with seizures, were seemingly responsible for the falling HR during the later half of the exposure periods. HR between periods of inactivity was generally similar to predose values. VR showed a similar trend to that of HR, with periods of inactivity associated with seizures. To quantify seizures, periods of coupled HR and VR inactivity on physiograph traces (Fig. 3) were noted. During predose periods seizures were not observed in the control or fenvalerate-exposed trout. During the 25 to 75% survival periods, 0 ± 0 and 1.3 ± 0.4 seizures/hr were recorded in the control and fenvalerate groups, respectively. The seizure values were used in subsequent PCA. These seizure values should be viewed as only a relative, not absolute, measure since the physiograph was not continuously monitoring responses throughout an exposure.

Marked changes were also noted in the blood chemistry parameters of the fenvalerate-exposed trout (Table 1 and Fig. 4). TaO_2 showed an initial increase, followed

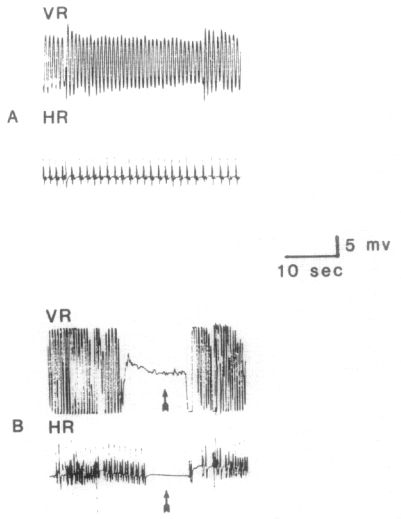

FIG. 3. *Representative simultaneous recordings of VR and HR from an individual fish prior to (A) and during (B) fenvalerate exposure (66% survival time). Periods of coupled VR and HR inactivity (arrows) were associated with seizures.*

by a dramatic drop midway through the exposures (overall mean increase of approximately 11% over predose values). $TaCO_2$ and pHa remained fairly constant until dropping at about 40 to 50% survival time. Fenvalerate-intoxicated trout also had rapidly rising Hct midway through the exposures. This increase in Hct (mean of 9.1% over predose values) was likely caused by red blood cell swelling, since Hb remained constant, and may have been caused by the dropping pHa (41) or associated with an osmoregulatory response to Na^+ and K^+ loss (see paragraph below). The decreases in TaO_2, $TaCO_2$, and pHa and the increase in Hct occurred at similar times during exposure and were associated with increasingly severe seizures and dropping V_G, VR, and HR. In control fish, $TaCO_2$ and pHa showed a steady increase throughout the exposure period. These changes in $TaCO_2$ and pHa might have been a function of serial blood sampling and/or elevated CO_2 and pH in the replacement saline. Control fish also had gradually falling Hb and Hct during exposures, an expected result caused by serial blood sampling.

Urinary changes in the fenvalerate-ex-

posed trout were substantially different from those in the control fish (Table 1 and Fig. 5). Except for urinary Na^+ alterations, no substantial changes between "predose" and "exposure" values were noted in the control trout. Initially, control trout showed a 30 to 50% drop in Na^+ concentration from "predose" values; however, by 10 to 20% "survival time," Na^+ excretion reached a steady state. This initial drop in Na^+ excretion might reflect a final recovery stage from surgery. In contrast to the control trout, the intoxicated fish had mean elevations of Na^+ concentration (23.9%), Na^+ excretion rate (74.0%), K^+ concentration (14.3%), K^+ excretion rate (53.4%), and osmolality (14.6%). In fenvalerate-exposed fish, urine flow rates remained constant until near death, when rates dropped.

The response of trout to fenvalerate intoxication was monitored by measuring 18 variables. This response set could, theoretically, be explained in an 18-dimensional space; however, interpretation of the data would obviously be difficult. Through PCA, the dimensionality of a complex data set is reduced by creating a new set of orthogonal variables, PCs, that are derived from linear combinations of the original data (33). In the present study, the fenvalerate-exposed fish were analyzed with the control fish in a two-dimensional plot (PC1 vs PC2) that incorporated 71% of the variation for the 18 variables (Fig. 6). PC1 accounted for 41% of the overall variance, whereas PC2 accounted for 30%. Seizures ($r = 0.985$), urinary K^+ concentration ($r = 0.962$), urinary Na^+ concentration ($r = 0.876$), urinary Na^+ excretion rate ($r = 0.871$), urine osmolality ($r = 0.813$), TaO_2 ($r = 0.777$), and urinary K^+ excretion rate ($r = 0.704$) were positively correlated with PC1. HR and V_G were positively correlated with PC2 ($r = 0.927$ and 0.886, respectively), whereas pHa was negatively correlated with PC2 ($r = -0.820$). The third PC, which accounted for an additional 13% of the overall variance, was most strongly correlated with VR ($r = 0.700$). The pattern of the plot indicated that the response of the fenvalerate-intoxicated fish was clearly different from that of the controls along PC1; i.e., seizures, increasing renal

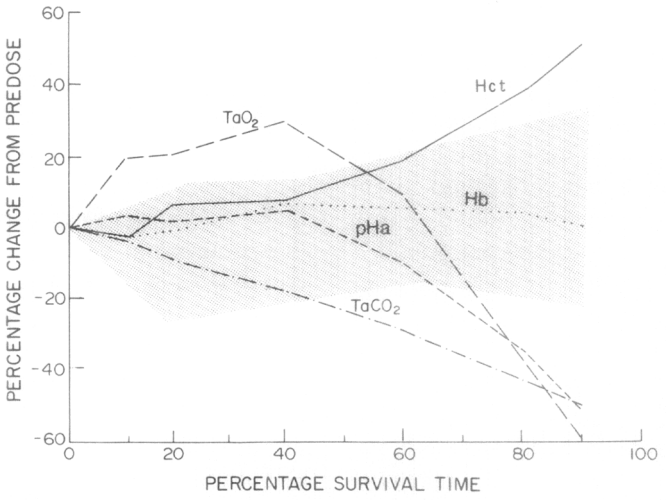

FIG. 4. *Mean blood chemistry responses (TaO_2, – –; $TaCO_2$, – · –; pHa, ---; Hct, —; Hb, . . .) from rainbow trout ($n = 4$) exposed to a lethal aqueous concentration of fenvalerate (412 μg/liter). The pHa response has been expanded 10-fold to normalize this parameter with the other data. Stippled area represents range of the mean response for control fish ($n = 3$).*

ion loss, and elevated TaO_2 in the intoxicated fish were predominately responsible for separating the two groups of experimental animals. The spread in PC scores among fenvalerate-exposed fish was greater than that in the control group and reflected the larger variability among the intoxicated fish (Table 1). Although CR was elevated about 200% in fenvalerate-exposed trout, this toxic response did not strongly correlate ($r < 0.700$) with the other physiological parameters. Instead, CR was moderately correlated with both PC1 ($r = 0.595$) and PC2 ($r = 0.492$; $r = 0.0$ indicating no correlation).

DISCUSSION

Results of the current study indicate that rainbow trout are extremely sensitive to fenvalerate. Mean brain residues, associated with 100% mortality after approximately 10 hr of exposure, were about 0.15 mg/kg fenvalerate. Brain concentrations of permethrin (16) and cypermethrin ([*R,S*]-α-cyano-3-phenoxybenzyl [*R,S*]-*cis, trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) (42) in rainbow trout at death are also similar to those reported here for fenvalerate. Liver fenvalerate concentrations associated with mortality were about 10-fold higher than those measured in brain and remaining carcass. This degree of fenvalerate accumulation in the liver was not observed in rainbow trout during sublethal exposures (6) and may reflect changes in kinetics with dose rate. The fenvalerate body burden associated with mortality in rainbow trout was approximately 0.25 mg/kg. The fenvalerate body burden in fathead minnows at death has been reported to be about 1.0 mg/kg (2), which is consistent with the somewhat lower sensitivity of this species compared with that of rainbow trout (3). Lethal intraperitoneal

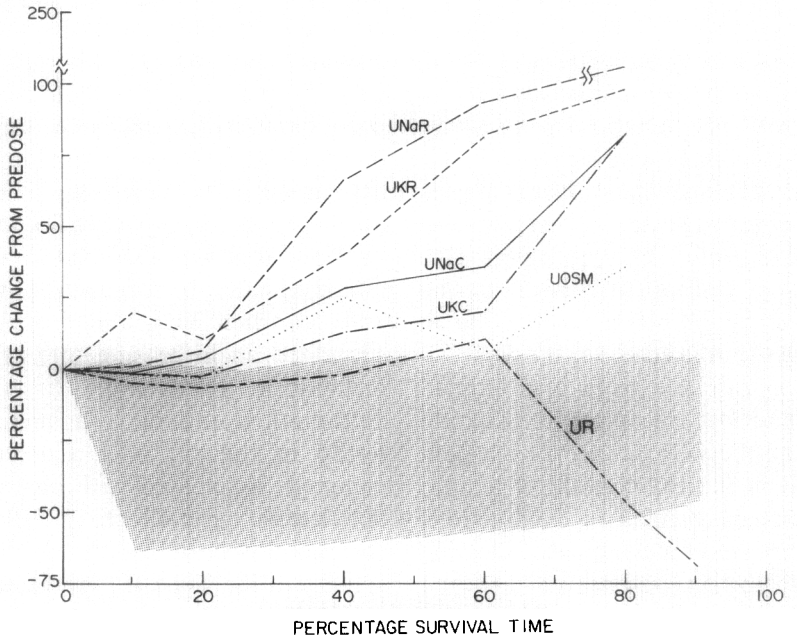

FIG. 5. *Mean urinary responses (UR, – – –; UOSM, . . .; UNaC, —; UNaR, – –; UKC, – · –; UKR, ---) from rainbow trout (n = 4) exposed to a lethal aqueous concentration of fenvalerate (412 μg/liter). Stippled area represents range of the mean response for control fish (n = 3). Abbreviations used: UR, urine excretion rate; UOSM, urine osmolality; UNaC, urinary Na^+ concentration; UNaR, urinary Na^+ excretion rate; UKC, urinary K^+ concentration; UKR, urinary K^+ excretion rate, respectively.*

doses of about 0.5 mg/kg fenvalerate in bluegill (*Lepomis macrochirus*) (43) are similar to rainbow trout and fathead minnow body burdens associated with mortality.

Pyrethroid doses and brain residues associated with mortality in mammals and birds are substantially higher than those reported for fish. Oral LD_{50} values of >4000 and 450 mg/kg fenvalerate have been reported for bobwhite quail (*Colinus virginianus*) (5) and rat (44), respectively. Intravenous LD_{50} values (probably the best comparisons to fish body burdens at death) of 50 to 100 mg/kg fenvalerate in the rat (45) further substantiate significant species differences in susceptibility. Fenvalerate brain residues in immature bobwhite quail of 1.26 mg/kg are associated with 70% mortality and an oral dose of 4000 mg/kg (5). Approximately 10-fold differences in lethal brain concentrations between trout and mice and Japanese quail (*Coturnix coturnix*) have also been reported with permethrin (16) and cypermethrin (42). The lower doses (body burdens) associated with mortality in rainbow trout is partly a function of less efficient pyrethroid metabolism than that observed in mammals and birds (6–8, 42, 46); however, the striking between-species differences in fenvalerate, permethrin, and cypermethrin brain concentrations also suggest that a difference related to the mechanism of pyrethroid action may be an important factor in species sensitivity.

Seizures in rainbow trout and fathead minnows (2) during fenvalerate intoxication indicate that effects on the nervous system are involved in the toxic mode of action. Toxic signs characterized by convulsions have been reported for mammals following exposure to Type II pyrethroids (4). During fenvalerate intoxication in rainbow trout, changes in a variety of respiratory–cardiovascular and blood chemistry variables were also similar to those changes observed in the rat during deltamethrin ([*S*]-α-cyano-3-phenoxybenzyl [1*R*,3*R*]-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) intoxication (34, 47). During advanced stages of choreoathetosis and convulsions in rats, increased blood glucose, blood lactate, plasma adrenaline, and plasma noradrenaline were observed and associated with decreasing pHa. Presumably, the dropping pHa in intoxicated rainbow trout was also related to rising lactate levels and, in conjunction with the initially elevated TaO_2 and dropping $TaCO_2$, suggested an increasing rate of anaerobic metabolism. Bradycardia, associated with deltamethrin-induced spasms, was also noted in the rat (47). Convulsions in mammals associated with increased metabolic activity and effects on HR have been correlated with the central nervous system activity of the Type II pyrethroids (47, 48).

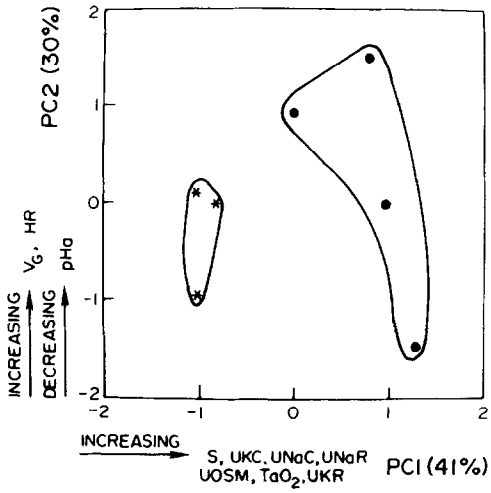

FIG. 6. *Scores for the first and second PCs, calculated by the PCA multivariate statistical technique, for rainbow trout exposed to 412 μg/liter fenvalerate* (●) *and control fish* (*). *Abbreviations used: S, seizures; UKC, urinary K^+ concentration; UNaC; urinary Na^+ concentration; UNaR, urinary Na^+ excretion rate; UOSM, urine osmolality; UKR, urinary K^+ excretion rate.*

In addition to nervous system involvement, the findings of the current study also suggest that fenvalerate may have adverse effects on gill structure. Histopathological examination of gill tissue indicated changes generally associated with a response to irri-

tation (49, 50), which is consistent with the observed hypersecretion of mucus (49). Similar changes in gill tissue have been reported in rainbow trout after aqueous and oral exposure to permethrin (17) at subacute levels. These changes in gill structure could cause disruptions in oxygen uptake and ion balance and may have contributed to the increase in V_G and decrease in O_2E observed in fenvalerate-exposed rainbow trout. Increased CR may have been a function of the morphological changes in the gills and/or may have been due to interactions of fenvalerate with receptors on the walls of the pharnyx or gill arches (49). Fenvalerate has been reported to cause skin paresthesias in humans, presumably due to effects on sensory nerves (51–53).

The increased excretion of renal Na^+ and K^+ in fenvalerate-exposed rainbow trout (correlated with increasing urine osmolality) indicates that the insecticide may also interfere with ion regulation. An interaction between fenvalerate intoxication and osmoregulation has been implicated with the estuarine grass shrimp, *Palaemonetes pugio* (54), and the bluegill (unpublished data, S. Dyer, Iowa State University, Ames). Numerous studies have been published indicating that pyrethroid insecticides are capable of inhibiting a variety of ATPase-utilizing systems, including Ca^{2+}- and Ca^{2+} + Mg^{2+}-ATPase (15), mitochondrial Mg^{2+}-ATPase (55, 56), and Na^+ + K^+-ATPase (56). Inhibition of mitochondrial ATPase and/or the Na^+/K^+ pump in the kidneys could be responsible for the observed increase in ion loss from intoxicated rainbow trout. Dropping internal ion concentrations, especially Na^+, could accentuate the neurological perturbations associated with the insecticide. The PCA indicated that seizures and renal Na^+ loss were highly correlated.

McKim *et al.* (22, 23) have classified the responses of trout to intoxication by gill irritants (acrolein and benzaldehyde), oxidative phosphorylation uncouplers (2,4-dinitrophenol and pentachlorophenol), narcotics (MS-222 and 1-octanol), and AChE inhibitors (malathion and carbaryl) into fish acute toxicity syndromes using DFA, a multivariate statistical technique (57). The four syndromes defined thus far include response sets associated with a respiratory uncoupler syndrome, a narcosis syndrome, an AChE inhibitor syndrome, and a respiratory irritant syndrome. The toxic responses of rainbow trout to fenvalerate were significantly different from response sets obtained after exposure to chemicals from these previously identified syndromes. A DFA of these previous data sets (22, 23) (n = 32 trout exposed to 8 chemicals) combined with the data from fenvalerate-exposed trout resulted in correct classification of individual fish into the syndromes previously defined and the delineation of a new response set that consisted solely of those fish exposed to the pyrethroid. The results of the analysis are depicted graphically in a three-dimensional plot (Fig. 7). Seizures had the highest univariate F value (112.4; $P < 0.0001$) and was, therefore, selected first in the DFA. Seizures primarily separated the fenvalerate-exposed fish from the fish exposed to the other chemicals because those toxicants did not elicit such a response. This trend is shown in Fig. 7 where the fenvalerate-exposed fish are located in the extreme right of DF1 in the three-dimensional space. CR, which had the second highest F value (53.4; $P < 0.0001$), was also correlated with DF1 and contributed to the separation of the fenvalerate and gill-irritant groups from the other response sets. The remaining fish acute toxicity syndromes were correctly predicted by DF2 and DF3, which were related with CR, VO_2, O_2E, TaO_2, and pHa generally as discussed previously (23). Because a limited number of animals in the other chemical treatment groups were monitored for urinary data, inclusion of these responses in the DFA was not possible. However, individual PCA of

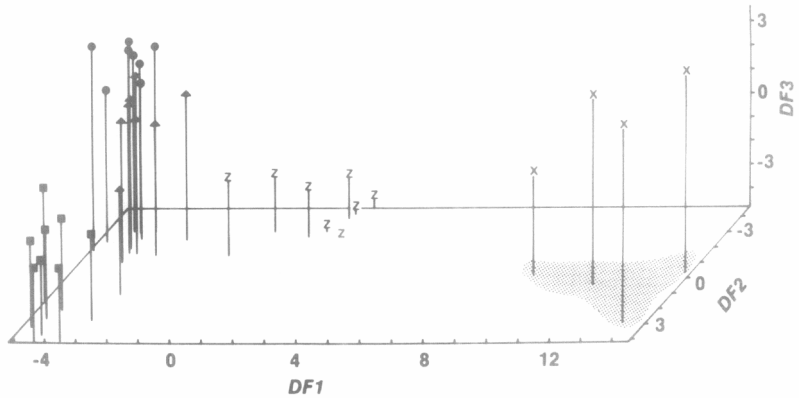

FIG. 7. *Three-dimensional plot of the first three DFs that separate five fish acute toxicity syndromes. Each point represents an individual fish exposed to one of the following nine chemicals as represented by the following symbols: acrolein and benzaldehyde (Z), carbaryl and malathion (▲), 2,4-dinitrophenol and pentachlorophenol (●), fenvalerate (X), MS-222 and 1-octanol (■). Stippled area denotes the syndrome associated with fenvalerate intoxication. The respiratory uncoupler syndrome (●), the narcosis syndrome (■), the AChE inhibitor syndrome (▲), and the respiratory irritant syndrome (Z) are correctly resolved as well (23); however, because of the scaling required to include the fenvalerate response on the plot the separation of the respiratory uncoupler and AChE inhibitor syndromes is not readily discerned.*

responses from control and chemical exposure groups from the other fish acute toxicity syndromes indicated no consistent changes between control and exposure values for urinary parameters. This also contrasts well with the response of fenvalerate-exposed trout. Further testing is planned to determine what other neurotoxicants are associated with the fish acute toxicity syndrome developed from fenvalerate.

In conclusion, the results of the current study further establish that fish are extremely sensitive to the pyrethroid insecticides. Interpretation of the physiological responses of rainbow trout to fenvalerate intoxication suggest that effects on respiratory structures and renal ion regulation, in addition to effects on the nervous system, may be important factors in evaluating the aquatic toxicology of the pyrethroid insecticides. A complete assessment of the contribution of these potential mechanisms will require further investigation.

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