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Toxicity of pulse-exposed fenvalerate and esfenvalerate to larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*)

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Abstract

The effects of pulse exposure with two synthetic pyrethroids, fenvalerate and esfenvalerate, on survival of larval Australian crimson-spotted rainbow fish (*Melanotaenia fluviatilis*) were studied. Fenvalerate and esfenvalerate were both highly toxic to larval rainbow fish with 1-h esfenvalerate pulse-exposure concentrations as low as 0.32 $\mu g\ l^{-1}$, and 1-min fenvalerate pulse-exposure concentrations of 4.5 $\mu g\ l^{-1}$, sufficient to cause significant mortality. Technical grades were significantly more toxic than emulsified grades of the pesticides. The 1-h pulse-exposure 96-h LC_{50}'s were 12.75, 30.25, 1.18, and 1.99 $\mu g\ l^{-1}$ for technical fenvalerate, emulsified fenvalerate, technical esfenvalerate, and emulsified esfenvalerate, respectively. Emulsified fenvalerate and esfenvalerate were also extremely toxic to adult crimson-spotted rainbow fish. The 96-h continuous exposure LC_{50}'s for adult fish using 50% daily partial replacement were 14.58 and 6.24 $\mu g\ l^{-1}$, respectively, comparable with results for holarctic species. Fenvalerate is a racemic mixture containing four optical isomers (2R, aR; 2R, aS; 2S, aR, 2S, aS), while esfenvalerate contains mainly the 2S isomers. Esfenvalerate contains approximately twice the quantity of 2S isomers as does fenvalerate on a weight basis. Pulse-exposure LC_{50}'s for esfenvalerate were 10 to 15 times lower than for fenvalerate, a much higher toxicity of esfenvalerate than the roughly two fold difference which would have been predicted solely on a weight basis. Thus the 2R isomers, which make up approximately 50% of the fenvalerate formulation and which previously were regarded as non-active, appear to have reduced the expected toxicity of fenvalerate relative to measured esfenvalerate toxicity, perhaps via the inhibition of sodium channel activation. There was a complex relationship between pesticide concentration and time to mortality. At low concentrations of pesticide, most mortality occurred within the first 24 h, while at higher

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concentrations, mortality continued for 96 h after exposure. This suggests that mortality within the first 24 h was due to direct physiological effects of the pesticide on the larvae, while subsequent mortality was primarily due to starvation of larvae unable to recover from the initial insult. Duration of exposure (0 to 120 min) to emulsified fenvalerate and esfenvalerate significantly affected the acute toxicity to larval rainbow fish. For esfenvalerate, there was a linear relationship between duration of exposure and acute toxicity, while for fenvalerate, exposures of longer than 20 min did not significantly increase 96-h mortality.

Key words: Fenvalerate; Esfenvalerate; Pulse exposure; Rainbow fish; Toxicity

1. Introduction

Australia has a distinctive piscofauna characterised by high endemicity and low diversity; total of only 180–190 species distributed among 39 families have been described from the continent. In addition, there are at least 19 introduced fish species with self-maintaining populations (Lake, 1971). Surveys suggest that the range and abundance of many species of native fish is declining (e.g., Williams, 1967; Cadwallader, 1978; Pollard et al., 1980; Ride and Wilson, 1982). Cadwallader (1978) identified at least six possible causes of this decline including river regulation schemes, forestry and farming practices, de-snagging and channelisation of rivers, competition by introduced species, overfishing, and the effects of pollution.

A major source of water pollution in rural regions is deliberate or accidental release of effluent from farms. Fish kills resulting from application of agricultural compounds have frequently been recorded, and sublethal concentrations of pesticides have been noted for a number of species (Butcher, 1967). Little information, however, is available on the effects of xenobiotics on Australian native fish.

The crimson-spotted rainbow fish (*Melanotaenia fluviatilis*) is widespread in the temperate waters of eastern Australia, ranging from the Murray-Darling basin to just north of the Queensland border (Lake, 1978). Rainbow fish inhabit billabongs, streams and back-waters of large rivers, preferring localities where sub-surface vegetation is abundant (Backhouse and Frusher, 1978; Lloyd and Walker, 1986). Male crimson-spotted rainbow fish reach a maximum size of 90 mm and females 70 mm. The species is sexually dimorphic and is capable of reproduction at approximately 3 months of age.

The widespread distribution of the rainbow fish suggests it may be an ideal organism for monitoring the effects of xenobiotics on the aquatic environment, both in the field and in laboratory toxicity studies. Recent research has indicated that the newly-hatched larvae of the crimson-spotted rainbow fish may be the most vulnerable stage in the life cycle (Barry et al., unpublished results), a finding generally consistent with the literature (Woltering, 1984). Neonates were therefore used in all tests. Continuous exposure 96-h LC_{50}'s for adult rainbow fish were also determined to facilitate comparison of the sensitivity of rainbow fish with other species of fish.

The pesticide fenvalerate ([*R*,*S*]-α-cyano-3-phenoxybenzyl[*R*, *S*]-2-[4-chlorpheny]-3-methylbutyrate) is one of the most widely used pyrethroids. In 1980, 473 tonnes were used world wide of which 90% was used for cotton (WHO, 1990). Fenvalerate

and esfenvalerate are widely used in Australia for control of insect pests. Fenvalerate may enter the aquatic environment through agricultural run-off, drift or erosion (Loranzo et al., 1992). Unlike the organochloride and organophosphate pesticides, the pyrethroids have low persistence and minimal toxicity to higher mammals (Spehar et al., 1983). However, they are highly toxic to fish and invertebrates with LC_{50}'s ranging from 0.08 to 2 $\mu g\ l^{-1}$ (e.g., Anderson, 1982a,b; Day and Kaushik, 1987; Bradbury et al., 1987a,b), creating the potential for significant detrimental impact on sensitive aquatic environments.

Fenvalerate has two chiral centres, in the acid and alcohol moieties, resulting in four optical-isomers: 2R, αR; 2R, αS; 2S, αR; 2S, αS (Fig. 1). Research suggests that the 2S, aS isomer may be the most biologically active followed by the 2S, aR (Bradbury et al., 1987a,b). Fenvalerate is a racemic mixture of the four isomers in equal proportions; esfenvalerate predominantly consists of the more toxic 2S isomers. With the exception of three recent papers on the ecosystem level effects of esfenvaler-

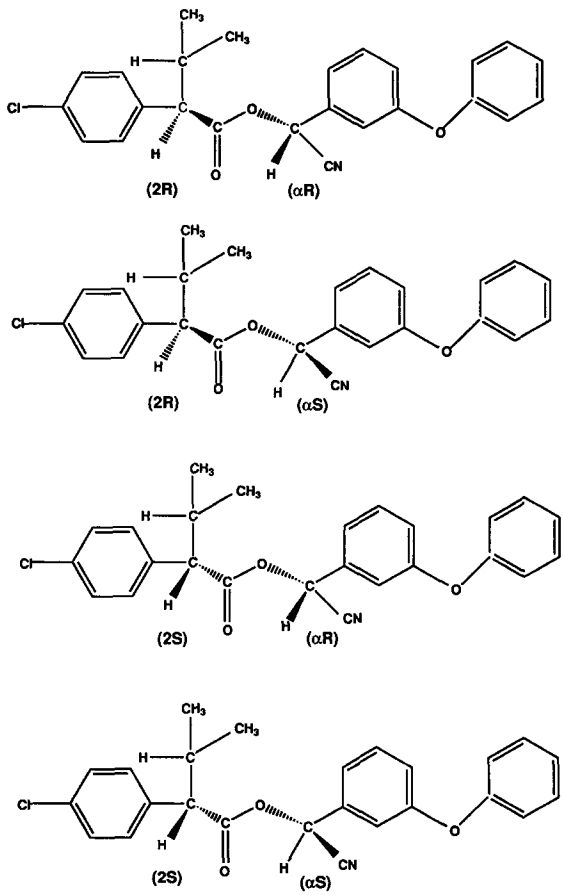


Fig. 1. Chemical structure of the four isomers of fenvalerate.

ate (Fairchild et al., 1992; Loranzo et al., 1992; Webber et al., 1992), little information is available on this pesticide.

Pyrethroids do not normally persist in the water column for long periods, and thus pulse exposure would provide the most realistic method of modelling the effects of these pesticides on fish. Recent studies have also suggested that the presence of an emulsifier may enhance the toxicity of pyrethroids to fishes (Coates and O'Donnel-Jeffery, 1979). Other studies, however, have shown this effect to be an artifact of experimental design (Bradbury et al., 1985, 1987a,b).

This research was undertaken: (1) to test the null hypotheses that neither pesticide concentration, pesticide formulation, duration of exposure nor feeding significantly affected the acute toxicity of pulse-exposed fenvalerate and esfenvalerate to larval rainbow fish; (2) to determine the relative sensitivity of crimson-spotted rainbow fish to pyrethroid pesticide exposure; and (3) to assess the relative risk that brief exposures to fenvalerate and esfenvalerate represent to Australian freshwater fish.

2. Materials and Methods

Culture methods

The original rainbow fish stock was collected from the Murray River. Fish populations were maintained and bred in 36 or 60 l all-glass flow-through aquaria. Third generation, laboratory bred specimens were used for all experiments. Tanks were bare except for plastic air-driven filters containing nylon wool which provided a substrate for nitrogenous bacteria. Photoperiod was 16 h light/8 h dark, with 30 min of simulated dawn/dusk.

A continuous flow of filtered water (FW) was maintained to all tanks at a rate sufficient to ensure 90% molecular turnover in 12 h or 99% in 24 h (Sprague, 1973). Water was drawn directly from the mains using PVC piping and pumped via a sand and carbon filter to a 1000 l storage tank above the laboratory. Water in the storage tank was aerated and heated to 25°C ± 1° before entering the laboratory. The main chemical features of the FW are summarised in Table 1. The main water chemistry parameters (dissolved O_2, pH, conductivity, and temperature) were measured daily when experiments were being conducted. If any parameter varied by more than 10% during the course of an experiment, the test was discontinued. Total hardness, Mg hardness and Ca hardness were measured weekly. Water quality was stable and proved to be ideal for culture of rainbow fish.

Adult rainbow fish were fed six times a day: each morning fish were offered newly-hatched live brine shrimp and a standard commercial flake food. One hour later fish were fed frozen adult brine shrimp which had been thawed and rinsed. The same feeding regime was repeated in the late afternoon. The amount of food added per tank was sufficient for the fish to consume within 5 min. The bottom of each tank was siphoned daily to remove uneaten food and faeces. Rainbow fish larvae have low lipid reserves and commence feeding within hours of birth. They were fed a mixture consisting of 50% finely ground adult flake food and 50% Hatchfry Encapsulon Grade 1 (Argent®), a micro-encapsulated larval food for aquatic organisms.

A ratio of three males to seven females in 36 l tanks or three males to ten females in 60 l tanks proved the optimal combination for spawning. Two spawning mops were placed in each breeding tank to provide a substrate for egg laying. Each mop consisted of 30 strands of nylon wool tied together and weighted at one end. Spawning females would attach groups of eggs to the substrate by fine filamentous threads. Between 30 and 500 eggs could be harvested from each breeding tank per day. Wild populations of the rainbow fish normally reproduce in spring and summer. Under the controlled laboratory conditions provided, no seasonal trends in the breeding cycle were observed and spawning continued throughout the year.

Eggs were harvested by removing the spawning mops and carefully picking the eggs from the substrate with well-washed fingers. Eggs were placed in 600 ml polyethylene beakers to which buoyancy rings had been attached. Beakers were modified by drilling holes in the bottom and sides to permit free exchange of water. The holes were covered with 240 μm nylon mesh. To increase water exchange a direct flow of water was provided to each beaker from a manifold system suspended above the tank. To reduce the occurrence and spread of fungus the beakers containing eggs were dipped in malachite green (4 mg l^{-1}) for 5 min each day, for the first 5 days post-fertilization (Holdway et al., 1988). Larvae hatched in 7 to 9 days at 25°C, with hatching success generally in the range of 40–70%.

Experimental design

For each experiment, six nominal pesticide concentrations plus a control were tested as follows: 0.1, 0.32, 1.0, 3.2, 10.0, 31.6 μg l^{-1} active ingredient. An acetone

Table 1
Summary of main physical and chemical parameters of FW

Parameter	Mean (± SE)	Metal	Concentration (mg l^{-1})
Temperature	25°C (± 1)	[F]	0.84
pH	7.0 (± 0.2)	[Al]	0.038
Dissolved O_2	9.0 mg l^{-1} (± 1.0)	[Cd]	< 0.0002
Conductivity	90 μS cm^{-1} (± 10)	[Cr]	< 0.002
Total hardness	20 mg l^{-1} as $CaCO_3$ (± 4)	[Cu]	0.003
Ca hardness	12 mg l^{-1} as $CaCO_3$ (± 2)	[Ni]	< 0.001
Mg hardness	8 mg l^{-1} as $CaCO_3$ (± 2)	[Pb]	< 0.002
		[Hg]	< 0.00005
		[As]	< 0.001
		[Zn]	< 0.006
Nitrite and Nitrate as N		0.22 mg l^{-1}	
Ammonia as N		0.1 mg l^{-1}	
Chlorinated pesticides (screen)		Not detected	

Temperature, pH, oxygen, conductivity, hardness, calcium, and magnesium results are based on regular readings. All other data are based on results of analysis of three water samples performed by State Water Laboratory of the Rural Water Commission of Victoria.

control was also tested for each pesticide in the technical form and an acetone/emulsifier control for the emulsified form. Acetone controls were equivalent to the highest concentrations of pesticide. Test solutions were made-up in two litres of FW in eight small all-glass aquaria. The technical form of each pesticide was a viscous yellow paste. As pyrethroids have low solubility in water, acetone was used as a carrier in all experiments. To minimise loss of pesticides through adsorption onto the walls of mixing containers and experimental vessels, solutions were prepared immediately prior to the commencement of an experiment.

Between 15 and 20 larvae, less than 48 h old, were collected using a small plastic ladle and transferred to sixteen 100-ml polyethylene beakers floating in an aquarium. The beakers were fitted with flotation rings and had large holes drilled in the sides and bottom. The holes were covered with 240 μm nylon mesh. Two beakers containing larvae were then added to each of the eight aquaria and exposed to the toxicant for 1 h. To minimise stress on the larvae during transfer, a glass beaker was placed under the test beaker ensuring that the larvae remained in water almost the whole time. The aquaria were placed in a large water bath maintained at 25°C. Every 15 min the beakers were stirred to ensure complete exchange of the water with the surrounding medium.

After 1 h, the beakers were removed from the toxicant and placed in a 36 l flow-through aquarium in the laboratory. Controls were placed directly in the aquarium. Acetone controls were first dipped in the control water to remove any carrier. Commencing with the lowest concentration, test solutions were dipped first in the acetone control and then the control water to remove traces of pesticide and carrier before being placed in the aquaria.

Larvae were fed three times each day. Mortality in each container was measured at 2, 4, 8, 24, 32, 48, 72 and 96 h. Corpses were removed from the beakers using a Pasteur pipette and examined under a stereo-binocular microscope. Mortality was defined as the absence of heart-beat or circulation in a larva. At least four replicates of each experiment were performed.

The effects of length of exposure to emulsified fenvalerate and esfenvalerate were measured by placing eight beakers, containing 15 larval rainbow fish, in an aquarium filled with 8 l of FW and pesticide at a nominal concentration of 5 $\mu g\ l^{-1}$. A control group was placed directly in a flow-through aquarium containing FW only. A single beaker was removed from the pesticide at each of 1, 2, 5, 10, 20, 40, 60 and 120 min after addition. Beakers were dipped twice in fresh FW before being placed in the aquarium containing the controls. Following placement in the flow-through aquarium, larvae were treated exactly as for the LC_{50} experiments above. The experiment with emulsified esfenvalerate was repeated twice and with emulsified fenvalerate five times.

Continuous-exposure, acute-toxicity experiments were performed by placing ten adult rainbow fish (five males and five females) in each of seven aquaria containing 4 l of FW. Aquaria were aerated by a gentle stream of air directed from a Pasteur pipette placed in each tank. For fenvalerate, pesticide was added at nominal concentrations of 20, 30, 35, 40, 45, and 50 $\mu g\ l^{-1}$. For esfenvalerate, nominal concentrations were 1, 4, 5, 6, 8 and 10 $\mu g\ l^{-1}$. At 24 h intervals 50% of the water was replaced with

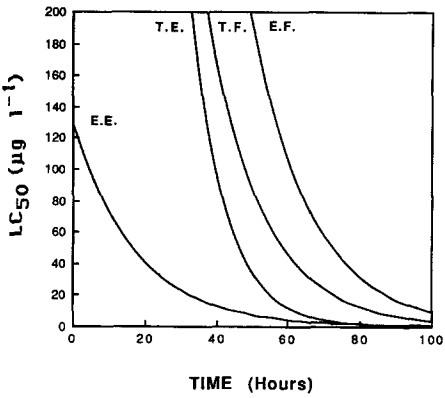


Fig. 2. Comparison of change in the LC_{50} for crimson-spotted rainbow fish larvae with time following 1 h pulse exposure. The LC_{50}'s were calculated at 8, 24, 32, 48, 72 and 96 h after exposure on four replicates for each pesticide (five replicates for technical fenvalerate). Lines of best fit were calculated using a negative exponential. Key: T.F., technical fenvalerate; E.F., emulsified fenvalerate; T.E., technical esfenvalerate; E.E., emulsified esfenvalerate.

fresh FW containing the appropriate amount of pesticide. Fish were not fed for the duration of the experiment.

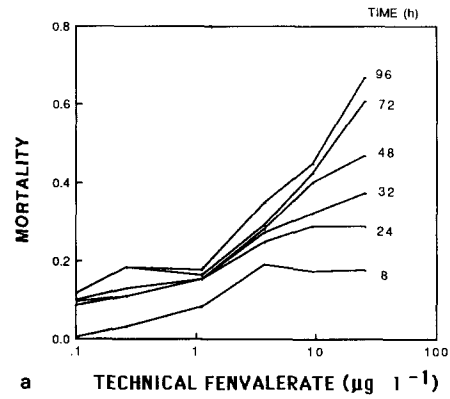
Chemical analyses

It was not possible to measure actual pesticide values at the time of the experiments because the GC was not yet operational. However, conversion of nominal pesticide concentrations to actual values was made on a parallel series of tanks using identical treatment and conditions but without test organisms. Newly-hatched larvae were used in most experiments and thus loss of fenvalerate to the test organisms was generally negligible. However, in the adult rainbow fish continuous-exposure experiments using 50% partial replacement per day, significant quantities of pesticide were absorbed by the fish. The concentration of emulsified fenvalerate was thus measured in tanks containing fish. Measurement of concentrations of esfenvalerate, however, were made on blank (fish free) aquaria and thus the LC_{50} values calculated for these experiments were higher than actual values.

Pesticide samples were extracted by concentrating 10 ml of water in 1 ml of iso-octane. The extracted samples were chromatographed on a SE-54 (film thickness 0.25 μm) fused silica column (30 mm × 0.25 mm i.d., Alltech USA®) mounted in a Shimadzu 14A gas chromatograph with electron capture detection. Two 2 μl splitless injections were made with a Shimadzu AOC-14 autosampler. Helium was used as the carrier gas. The temperature program was 80°C to 280°C at 16°C min^{-1}; the injection port was maintained at 280°C and the detector at 300°C. Retention times were 23.0 min (RS and SR) and 23.6 min (SS and RR).

Statistical analyses

Acute mortality data was analysed using probit analysis (Finney, 1971), and 96-h



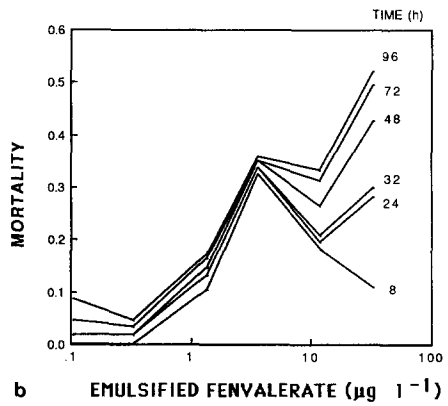
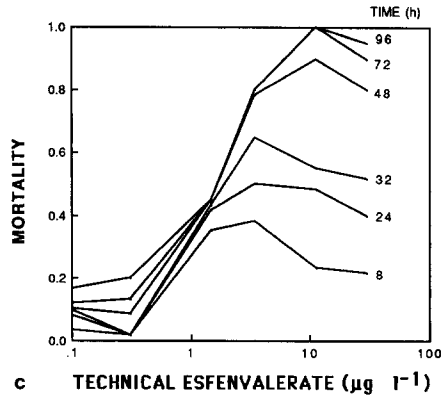


Fig. 3. Mortality of crimson-spotted rainbow fish in relation to pesticide exposure concentration at six times following 1 h pulse exposure: (a) technical fenvalerate; (b) emulsified fenvalerate; (c) technical esfenvalerate; (d) emulsified esfenvalerate.

LC_{50}'s and LT_{50}'s were calculated. To test for the statistical significance between the pulse-exposure acute toxicity results, data was analysed using a two-way ANOVA, with pesticide and formulation as the factors. Data was log-transformed prior to analyses to ensure homoscendacity of the means (Zar, 1984). Duration of exposure data was analysed using a randomised complete block design ANOVA to determine if length of exposure to fenvalerate had a significant effect on 96-h mortality. The independent variable was exposure time, the dependent variable was percentage mortality after 96 h, and the blocking factor was replicate. Data was square root arcsine-transformed before analysis to meet the assumption of normality implicit in analysis of variance (Zar, 1984). Fisher's Least Significant Difference test ($P \leq 0.05$) was used



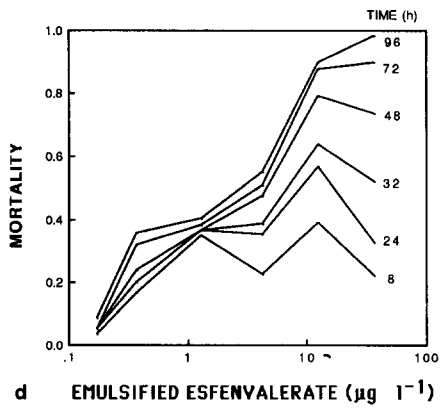


Fig. 3. Continued.

Table 2
Equations describing the change with time after pesticide exposure in acute toxicity of crimson-spotted rainbow fish, measured as 1-h pulse exposure LC_{50}'s for technical and emulsified fenvalerate and esfenvalerate

Emulsified Fenvalerate	$LC_{50} = 4073.2 \cdot 10^{(-0.026T)}$ $R^2 = 0.81$
Technical Fenvalerate	$LC_{50} = 91.7 \cdot 10^{(-0.013T)}$ $R^2 = 0.91$
Emulsified Esfenvalerate	$LC_{50} = 6478.0 \cdot 10^{(-0.045T)}$ $R^2 = 0.78$
Technical Esfenvalerate	$LC_{50} = 129.0 \cdot 10^{(-0.025T)}$ $R^2 = 0.80$

Equations were calculated using LC_{50}'s calculated at 8, 24, 32, 48 and 96 h after pesticide exposure, where T is the time after exposure in hours, and LC_{50} is given in $\mu g\ l^{-1}$ pesticide.

to determine the levels at which time was significant. A t-test ($P \leq 0.05$) was used to assess the affect of feeding on pulse-exposure acute toxicity.

3. Results

One-hour pulse exposures to fenvalerate and esfenvalerate were highly toxic to rainbow fish larvae with significant mortality occurring at concentrations as low as 0.32 $\mu g\ l^{-1}$ esfenvalerate. Larvae showed symptoms that included whole body tremors, spasmodic movements, and occasional bursts of hyper-activity, typical of Type II pyrethroid toxicity. Mortality of larvae was first observed within 2 h of pesticide exposure. Death, however, continued throughout the 96-h observation period. There was a negative exponential relationship between the time after exposure and the estimated value of the pulse-exposure (PE) LC_{50} (Fig. 2; Table 2). At 96 h post-exposure, the LC_{50} had reached an asymptote indicating that acute mortality had ceased.

There was a complex relationship between concentration of pesticide, formulation, larval mortality and time (Fig. 3a, b, c, d). Thus, at technical and emulsified fenvalerate concentrations of 3.2 $\mu g\ l^{-1}$ (Fig. 3a, b), and technical and emulsified esfenvalerate concentrations of 1.0 $\mu g\ l^{-1}$ (Fig. 3c, d), most mortality occurred within the first 24 h. However, at the higher concentrations mortality continued over the 96 h period. Mortality during the first 24 h was correlated with pesticide concentration above a threshold level, but large changes in concentration produced only small changes in toxicity. After 24 h, there was a strong response to pesticide concentration at levels of 3.2 $\mu g\ l^{-1}$ and above for technical fenvalerate (Fig. 3a), 10.0 $\mu g\ l^{-1}$ and above for emulsified fenvalerate (Fig. 3b), and 3.2 $\mu g\ l^{-1}$ and above for both technical and emulsified esfenvalerate (Fig. 3c, d).

There was a highly significant difference between pesticides and between formulations ($P < 0.01$). The interaction term, however, was not significant ($P > 0.05$), indicating significant differences in toxicity between all four test compounds. The 96-h LC_{50} (± standard error) values for 1-h pulse-exposed, newly-hatched rainbow fish larvae, were: 30.3 (± 6.1) $\mu g\ l^{-1}$ for emulsified fenvalerate; 12.8 (± 3.1) $\mu g\ l^{-1}$ for technical fenvalerate; 2.0 (± 0.4) $\mu g\ l^{-1}$ for emulsified esfenvalerate; and 1.2 (± 0.2) $\mu g\ l^{-1}$ for technical esfenvalerate (Table 3). The technical forms of fenvalerate and esfenvalerate were 2.3 and 1.7 times as toxic to rainbow fish larvae as the respective emulsified forms, while the LC_{50}'s for emulsified and technical esfenvalerate were 15.2 and 25.6 times greater than for emulsified and technical fenvalerate, respectively.

There was significant mortality in unfed control larvae 96 h after esfenvalerate pulse exposure. Therefore, the effect of feeding on sensitivity to esfenvalerate was measured at 48 h after pulse exposure. The 48-h P.E. LC_{50} (± SE) for unfed larvae was 3.8 $\mu g \cdot l^{-1}$ (± 0.8), not significantly different from 4.4 $\mu g\ l^{-1}$ (± 1.5) for fed larvae ($P > 0.05$).

The results of experiments to test the effects of exposure time on toxicity to rainbow fish larvae (resistance) indicated that the highest mortality of rainbow fish larvae pulse-exposed to 4.5 $\mu g\ l^{-1}$ fenvalerate occurred at an exposure time of 20 min and

Table 3
Summary of 1-h pulse exposure (PE) LC_{50}'s from larval rainbow fish experiments as calculated by probit analysis, including 95% upper and lower fiducial limits and the slope of the probit line

Pesticide	*N*	PE 96 h LC_{50} ($\mu g\ l^{-1}$)	95% Fiducial limits		Slope
			Lower	Upper	
Emulsified					
Fenvalerate	4	38.8	8.5	–	0.53
		12.3	6.3	35	1.08
		33.5	18	–	2.03
		36.5	10	–	0.64
Mean (SE)		30.3 (6.1)			
Technical					
Fenvalerate	5	7.5	3.0	17	1.20
		3.9	0.6	480	0.34
		19.2	9.0	110	1.01
		14.3	6.4	56	1.33
		18.8	7.9	130	0.90
Mean (SE)		12.8 (3.1)			
Emulsified					
Esfenvalerate	4	2.9	0.0	1.0	1.30
		1.8	0.9	3.3	1.27
		0.9	–	–	1.30
		2.4	0.7	5.2	1.19
Mean (SE)		2.0 (0.4)			
Technical					
Esfenvalerate	4	1.3	0.0	16	1.03
		1.7	0.5	3.5	1.10
		0.9	0.6	1.3	3.51
		0.8	0.0	6.2	1.62
Mean (SE)		1.2 (0.2)			

was lower at 40, 60 and 120 min (Fig. 4a). Exposure time had a highly significant effect on mortality ($P < 0.001$), but even a 1-min exposure to fenvalerate at 4.5 $\mu g\ l^{-1}$ was sufficient to cause significant mortality to larval rainbow fish. Toxicity was lower at 1 min of exposure time than at 20 or 40 min (the times with the highest mortality), but not significantly different than at 60 or 120 min. No other consistent differences could be detected for fenvalerate.

Mortality of larval rainbow fish pulse-exposed to esfenvalerate at 96 h was directly proportional to the duration of the exposure (Fig. 4b). The mean 96-h LT_{50} for emulsified esfenvalerate based on two replicates was 26.5 min at a measured pesticide concentration of 3.9 $\mu g\ l^{-1}$. The pattern of time-to-mortality following pulse exposure of larval rainbow fish to 3.9 $\mu g\ l^{-1}$ of emulsified esfenvalerate shows that all mortality occurred within the first 24 h for exposure times below 10 min (Fig. 4b). For longer

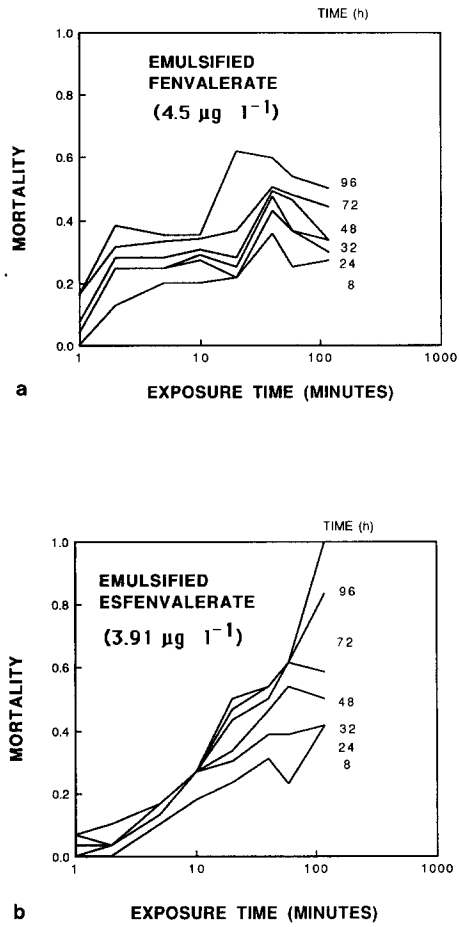


Fig. 4. Mortality of crimson-spotted rainbow fish in relation to exposure time at six times following pulse exposure to a single concentration of pesticide: (a) emulsified fenvalerate; (b) emulsified esfenvalerate.

exposure times, mortality continued over the next 72 h. The time-to-mortality response of the larval rainbow fish pulse-exposed to emulsified fenvalerate was quite different. In this case, mortality continued steadily over 96 h at all exposure times in a roughly proportional manner (Fig. 4a).

The continuous-exposure 96-h LC_{50} for adult rainbow fish exposed to emulsified fenvalerate, with 50% partial replacement was 14.6 $\mu g\ l^{-1}$ (95% C.I. 12.7–16.7; slope 17.2). The continuous-exposure 96-h LC_{50} for emulsified esfenvalerate was 6.2 $\mu g\ l^{-1}$ (95% C.I. 5.7–6.9; slope 11.2). The actual value for emulsified esfenvalerate was probably lower because LC_{50}'s were calculated using measured chemical values of test solutions taken from tanks without fish.

4. Discussion

Bradbury et al., (1987a,b) found that most of the toxicity of fenvalerate to fish is due to the 2S isomer. Thus, it is not surprising that rainbow fish larvae showed greater sensitivity to esfenvalerate than to the racemic mixture. On a weight basis, esfenvalerate contains twice the amount of the 2S isomers as does fenvalerate and would thus be expected to be twice as toxic as fenvalerate. This, however, was not the case in this study. Acute pulse-exposure esfenvalerate toxicity to larval rainbow fish was some 10 to 15 times higher than for fenvalerate-exposed fish. The acute toxicity of esfenvalerate observed in this study was therefore much greater than would have been predicted solely on the basis of the findings of Bradbury et al. (1987a,b).

The principal target for pyrethroid insecticides is believed to be the voltage-sensitive sodium channels of nerve membranes (Sattelle and Yamamoto, 1988; Soderlund and Bloomquist, 1989; Rubin and Soderlund, 1992). Studies using mammals and terrestrial insects have led to the classification of Type I and Type II responses to pyrethroids (Vershoyle and Aldridge, 1980; Gammon et al., 1981). Type I pyrethroids act to prolong opening of the sodium channels, resulting in enhancement of depolarisation of after-potentials to the threshold level for production of action potentials in the nerve. This results in repetitive discharges analogous to the effects of DDT. The Type I syndrome is normally elicited by non-alpha cyano pyrethroids such as permethrin, and signs of toxicity include restlessness, poor coordination and paralysis. The Type II pyrethroids act by maintaining the sodium channels open for a much greater length of time (Clarke and Brooks 1989). This syndrome is normally elicited in response to pyrethroids containing an S-α-cyano-3-phenoxybenzyl alcohol moiety such as fenvalerate. Toxicity signs include poor coordination, convulsions and hyperactivity.

The pyrethroid pesticides by themselves do not directly stimulate sodium uptake by nerve preparations in vitro. However, they do enhance uptake in the presence of sodium channel activators (Jaques et al., 1980; Vijverberg et al., 1982). Recently, Rubin et al. (1993) found that the 2S stereo-isomers of fenvalerate allosterically enhanced binding of [^{3}H]batrachotoxin A-20-a-benzoate to mouse brain voltage-sensitive sodium channels. The non-toxic 2R isomers, however, were either inhibitory or without significant effect, suggesting possible antagonism between the 2S and the 2R stereo-isomers of fenvalerate.

If the results of the in vitro study by Rubin et al. (1993) are applicable at the whole organism level, the inhibitory effect of the 2R stereo-isomers of fenvalerate may explain the high potency of esfenvalerate observed in this study. Molecular binding at nerve endings by the 2S isomers may prolong potentials through the voltage sensitive sodium channels, while competitive or antagonist binding by the 2R isomers may mitigate these effects. The toxicity of fenvalerate, which is a racemic mixture containing equal proportions of the four isomers, would be reduced by interactions of the 2S and 2R isomers. Such interactions would be minimal in esfenvalerate, which predominantly consists of the 2S isomers.

The technical grade pesticides used in this study were significantly more toxic to larval rainbow fish than the respective emulsified formulations. These results are in

agreement with the data of Bradbury et al. (1985) who found that the 96-h LC_{50} for continuous exposure of fathead minnow (*Pimephales promelas*) was higher for technical grade fenvalerate than for the emulsified form. At 168 h, they found no significant difference between the two forms of the pesticide, indicating that uptake of the emulsified fenvalerate was slower than for the technical material. In contrast, Coats and O'Donnell-Jeffery (1979) and Zitko et al. (1979) both found emulsified pesticides to be more toxic. However, both of these studies relied on nominal pesticide concentrations and did not measure the levels of the toxins directly. Bradbury et al. (1985) suggested that the results of the earlier studies might have been an artifact of the techniques used. Since use of an emulsifier may increase the half-life of fenvalerate in the water column compared with technical material, the use of nominal concentrations may have over-estimated the concentration of the technical form.

Bradbury et al. (1985) indicated that the uptake of emulsified fenvalerate by fish is slower than the uptake of technical material, and suggested that the emulsifier may coat the gills, making absorption of the toxin more difficult. This may explain the higher toxicity of technical fenvalerate to larval rainbow fish found in this study. Rainbow fish were pulse-exposed to the pesticides only briefly (1 h or less), and thus the total dose of technical fenvalerate received by the larvae was probably higher compared to emulsified fenvalerate. Unfortunately, owing to the small size of the larvae (< 0.1 mg), it was not possible to measure uptake of pesticide directly in these experiments.

In continuous-exposure studies using adult fish (Bradbury et al., 1985) mortality of fish exposed to the emulsified form of fenvalerate continued for 168 h. In contrast, in response to the 1-h pulse exposures of technical and emulsified fenvalerate in this study, acute larval fish mortality had ceased by 96 h. This difference in mortality may simply be a consequence of the methodological differences between the two studies.

Mortality of pulse-exposed larval rainbow fish occurred in two distinct phases. The first phase lasted from time of exposure up to 24 h, depending on the pesticide used. During this period, mortality was relatively independent of pesticide concentration above a threshold dose. The second phase lasted for the following 72 h, during which time mortality was dependent on pesticide concentration. Observations on larvae following exposure to the pesticides suggest the initial mortality was due to the direct effects of the pesticide insult on the fish. Larvae which were exposed to the higher concentrations of fenvalerate and survived the initial phase often appeared damaged and unable to feed. Newly-hatched rainbow fish larvae do not have high levels of endogenous lipids and normally commence feeding within 12 h of hatching. Thus, starvation may be a likely cause of death in the period following initial mortality.

This hypothesis is supported by the data of Haya and Waiwood (1983) who found a significant interaction between the toxicity of fenvalerate and feeding regime in juvenile Atlantic salmon. Starved fish showed much greater sensitivity to the pesticide. They found that both fenvalerate and starvation caused depletion of glycogen stores in liver and muscle. In contrast, adenylates and creatine phosphates remained constant, suggesting that glycogenolysis was induced to maintain availability of immediate sources of energy. Bradbury et al. (1987a,b) also speculated that detrimental

effects of fenvalerate on renal and respiratory systems may be associated with the cause of death in addition to the impact on the nervous system.

No significant interaction was found between the toxicity of esfenvalerate and feeding over 48 h. However, the fact that starved control fish showed mortality due to lack of food within 96 h is evidence of the precarious nutritional status of larval rainbow fish. Rainbow fish larvae, however, undergo many rapid changes during the first 96 h of life, and thus other explanations cannot be eliminated. In particular, metabolic detoxication processes within the liver of larval rainbow fish commence between days 1 and 3 post-hatch (Reid, 1992; Reid et al., 1992). Stresses involved with the activation of this important detoxication organ may thus play an additional role in mortality.

Larvae showed significant mortality following exposures as short as 1 min to emulsified fenvalerate and esfenvalerate. This highlights the extremely high toxicity of these compounds to larval rainbow fish. The response of the larvae to emulsified fenvalerate was particularly interesting, where mortality reached a plateau or possibly declined with increasing exposure time. One possible explanation for this may be the metabolism and elimination of the toxic components of fenvalerate. However, fish are believed to have low rates of elimination of fenvalerate, and half-lives in trout are in excess of 48 h (Glickman et al., 1981; Bradbury et al., 1986).

The continuous-exposure 96-h LC_{50} for adult rainbow fish exposed to fenvalerate under conditions of 50% daily partial replacement was 14.6 $\mu g\ l^{-1}$. The most sensitive species to fenvalerate acute toxicity reported in the literature is the fathead minnow (*Pimephales promelas*), with a 96-h LC_{50} of 0.69 $\mu g\ l^{-1}$ (Bradbury et al., 1985). The least sensitive species reported in the literature is the tilapia (*Tilapia mossambica*), with a 48-h LC_{50} of 200 $\mu g\ l^{-1}$ (Mulla et al., 1978). Within this wide range, a number of values for other fish species have been reported. The sensitivity of the crimson-spotted rainbow fish to fenvalerate acute toxicity is therefore comparable with the holarctic species which have been tested.

There is little information available in the literature on the toxicity of esfenvalerate to fish. Bradbury et al. (1987b) found a 48-h LC_{50} of 0.34 $\mu g\ l^{-1}$ for a mixture containing the 2S R,S isomers of fenvalerate using fathead minnow as the test organism, while Fairchild et al. (1992) found a 96-h LC_{50} of 0.27 $\mu g\ l^{-1}$ for esfenvalerate-exposed bluegill sunfish (*Lepomis macrochiris*). The acute toxicity of esfenvalerate to adult rainbow fish in the current study was somewhat higher, with a 96-h LC_{50} of 6.2 $\mu g\ l^{-1}$. However, the LC_{50} values for adult rainbow fish to both fenvalerate and esfenvalerate fall within the range that may be encountered in the 'natural' environment. This again highlights the extreme potency of these pesticides to native aquatic fauna.

With the exception of the work of Holdway and Dixon on methoxychlor (1985, 1987), very few papers have studied the effects of pulse exposure of pesticides on fish. Continuous-exposure techniques provide a conservative estimate of pesticide impact. However, pulse exposure may offer a more realistic model of environmental impact for hydrophobic and/or rapidly degraded compounds such as fenvalerate which are quickly adsorbed onto sediments and particulate organic matter.

The use of pulse exposure may also provide a powerful tool for elucidation of the effect of environmental stresses on aquatic fauna. Underwood (1989) classified envi-

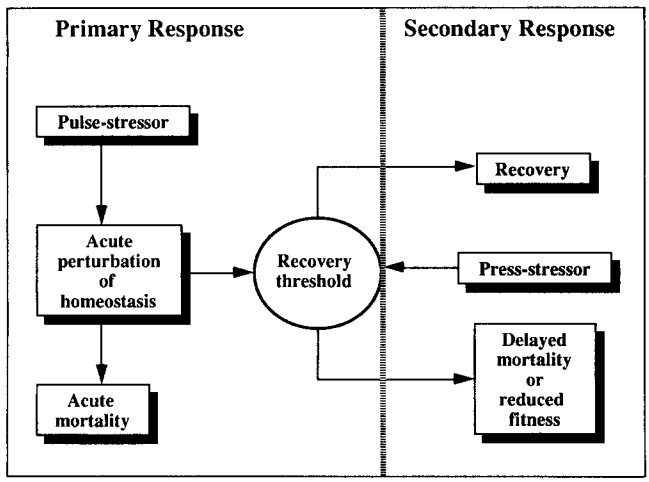


Fig. 5. A model summarising xenobiotic pulse exposure effects on fish.

ronmental stress with two categories: press and pulse. Studies which expose fish to pesticides or environmental toxins continuously over a period of time may be classified as press experiments. Such methods may be appropriate for work on metals, organochloride based pesticides and other xenobiotics with remain in the water for long periods of time. However, the use of rapidly biodegradable pesticides such as the pyrethroids is becoming increasingly widespread. The mode of action and time-frame with which such pesticides impact on the natural environment may be completely different from that of the longer lasting xenobiotics. It is therefore important to select the right time scale when framing hypotheses and developing experimental protocols with which to study such chemicals. The use of brief exposure times, which expose an organism to a pulse stress may be an important tool in such situations.

A generalised model and nomenclature for describing of the effects of pulse exposure of xenobiotics on aquatic organisms is thus proposed (Fig. 5). The pesticide is described as a pulse-stressor which disturbs physiological homeostasis of the fish. Disturbance above a certain threshold causes acute mortality. If the effect of the pulse-stressor is below the mortality threshold this leads to a secondary response: either a complete recovery or a delayed deleterious response. The nature of the secondary response may be strongly dependent on exogenous factors such as environmental conditions or the availability of food. Such factors may act over a long period of time and as such are referred to as press-stressors after the nomenclature of Underwood (1989). We suggest that the term 'primary response' be reserved for the direct effects of a toxin on the system of the target organism. The term 'secondary response' may be used to describe cascading effects which occur over time. Thus the threshold level is an important parameter to determine, if one indeed exists, beyond which an organism is not able to fully return to homeostasis and will experience delayed deleterious effects.

The pyrethroids have come to be regarded as 'environmentally benign', because of their relatively short half-lives within ecosystems and within animal tissues. However, the extreme toxicity of fenvalerate and esfenvalerate to larval rainbow fish illustrates the potential of these pesticides for causing large scale destruction of native fish populations. Exposures in this study as low as 0.32 $\mu g\,l^{-1}$ or as short as 1 min duration caused significant mortality to larval rainbow fish. McDowell et al. (1987) reported that up to 38% of fenvalerate could be washed off following rainfall events, resulting in aquatic ecosystems receiving contaminated run-off containing relatively large quantities of the pesticide.

This situation is of particular concern given that fenvalerate is used most heavily on cotton crops in Australia during the summer months. Summer is also the breeding season for the crimson-spotted rainbow fish as well as for many other species of Australian freshwater fish (Merrick and Schmida, 1984). Therefore fish larvae, which are among the most sensitive of life-stages to xenobiotic exposure (Woltering, 1984), may be exposed to the highest concentrations of pyrethroids. These results suggest an urgent need for stronger regulation and control of the use of these pesticides in agriculture, and for greater education of the end-users of pesticides concerning the potential hazards to native aquatic fauna of their use.

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