THE UNIVERSITY OF MANITOBA

The Effects of an Organophosphorus Insecticide (Sumithion-Fenitrothion) on Swimming Performance and Respiration in Brook Trout, Salvelinus fontinalis (Mitchill)

by

LARRY ALEXANDER HUNTER

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THE EFFECTS OF AN ORGANOPHOSPHORUS INSECTICIDE (SUMITHION-FENITROTHION) ON SWIMMING PERFORMANCE AND RESPIRATION IN BROOK TROUT, *SALVELINUS FONTINALIS* (MITCHILL)

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

THIS THESIS IS DEDICATED TO MY WIFE,

HEATHER R. HUNTER

ABSTRACT

Brook trout (Salvelinus fontinalis Mitchill) were exposed to the organophosphorus insecticide Sumithion (fenitrothion) in two acute lethal bioassays in order to determine the median lethal concentrations. The 96-hour LC50 was calculated as 2.26 milligrams of fenitrothion per liter and 4.85 mg/liter for the 24-hour LC50. As an organophosphate, fenitrothion is a cholinesterase inhibitor which may become more toxic after biotransformation within the liver of fish. Brain, gill and heart tissues were sampled from dead and surviving fish in the bioassays for determination of acetylcholinesterase activity. The critical swimming speed, ventilation rate, oxygen uptake and certain behavioural parameters of brook trout pre-exposed to fenitrothion for 24 hours were monitored while swimming in incremental velocity tests. Immediately following fatigue in the swimming tunnel, the trout were killed, physical measurements recorded and tissue samples collected for enzyme analysis. These parameters were examined in an attempt to understand the toxic effects of fenitrothion in a non-target organism.

Acetylcholinesterase inhibition in the brain was not found to be correlated with the concentration of fenitrothion

which produced mortalities. Enzyme activities were severely depressed at nearly all of the concentrations tested. Gill acetylcholinesterase activities were consistently inhibited to well below 40 percent of the control levels at all concentrations tested in the acute lethal bioassays. Heart tissues demonstrated a more gradual but steady decrease in activity with increasing fenitrothion concentration. The tissues from the brook trout used in the swimming performance tests showed enzyme activities comparable to those from the 24-hour bioassay. A high correlation existed between decreased brain enzyme activity and critical swimming speeds in brook trout exposed to concentrations of fenitrothion up to 25.0 percent of the 24-hour LC50.

Swimming performance was significantly reduced at 0.3 mg/liter, which represented 6.25 percent of the 24-hour LC50. Oxygen uptake was predominantly dependent on activity, regardless of insecticide concentration, but at the higher levels of fenitrothion, oxygen uptake was substantially reduced. Ventilation rates were significantly elevated at the concentrations up to 50 percent of the 24-hour LC50, but were reduced at higher concentrations. Tailbeat frequencies increased curvilinearly with swimming speed, and were not influenced by insecticide concentration.

Brain weights, corrected for body weight, of trout exposed for 96 hours were significantly higher than control brain weights. The increase in weight was directly proportional to concentration of fenitrothion and length of exposure to the insecticide. It is uncertain whether swelling of the brain may have contributed to fish mortality.

The ecological implications of stream fish unable to attain their critical swimming speeds are readily imaginable. An impaired ability to catch food, to avoid predators and maintain social organization through territories is likely to jeopardize survival of the population and result in serious disruptions in the balance of the stream ecosystem.

ACKNOWLEDGMENTS

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INTRODUCTION

1. OBJECTIVES OF THIS STUDY

The objective of this study was to determine the effects of fenitrothion on the swimming performance and respiration in brook trout. This study will also investigate the toxicological effects of the insecticide on the inhibition of the enzyme acetylcholinesterase in the brain, gill and heart tissues after exposure to lethal and sublethal concentrations. Physiological target sites such as ventilation rates and cough frequency will also be monitored to help identify sites of action of organophosphorus insecticides. Theories will be proposed from information collected concomitantly during the exposures and in the swimming performance tests to explain the effects of fenitrothion on the swimming and on other physiological and behavioural parameters.

2. BACKGROUND INFORMATION

The potential use of organophosphorus compounds as insecticides was first realized during the Second World War by Gerhard Schrader in Germany while chemical-warfare agents were being developed (O'Brien 1967; Matsumura 1976). In 1959, fenitrothion was introduced as an experimental insecticide by Sumitomo Chemical Co. and by Farbenfabriken Bayer-AG (Nishizawa et al. 1961; NRCC 1975). Pressure to

develop 'low-toxicity' insecticides was strongly demanded after frequent accidental poisonings while using other highly toxic organophosphates such as parathion (Suzuki 1972) and methyl parathion. Organophosphorus insecticides account for over 30 percent of the registered synthetic insecticides and acaricides in the United States. All of these compounds are recognized for their anti-cholinesterase properties.

Fenitrothion is a broad-spectrum insecticide and has been used throughout Europe, Pakistan, East Africa, China, New Zealand, U.S.A., Brazil, Japan and Canada (Miyamoto 1973). It is used extensively to control insects of medical and economic importance. In Canada, fenitrothion has been used to combat the defoliating spruce budworm Choristoneura fumiferana (Clem.) predominantly in the Maritime Provinces. A chlorinated hydrocarbon insecticide, DDT, was used from 1952 into the late 1960's. By 1970, it was eventually phased out because the effects of DDT have been damaging to non-target biological systems in the spray areas. In New Brunswick, DDT sprayed at 0.5 pounds per acre resulted in heavy fish kills within three weeks and severe reductions in the movements of brook trout and white suckers in the forest streams (Kerswill 1967; Kerswill and Edwards 1967). Public concern over these undesirable

side effects led to useful spray modifications and the search for a more selective insecticide. Fenitrothion has fulfilled the basic requirements and has been widely used to date as a substitute for the chlorinated hydrocarbons. Fenitrothion was initially used in 1968, and the ban on DDT meant an increased dependence on organophosphorus compounds and carbamates (Goldberg 1976).

Biological and economic implications of spruce budworm infestations showed that chemical control was the only practical alternative to protect the four million acres of forest in New Brunswick, Quebec and Maine (USDA 1971; Wildish and Lister 1973). Between 1968 and 1972, 2,002 metric tons of fenitrothion had been sprayed over the forest and streams in New Brunswick alone (Symons 1977). Operational or experimental programs using fenitrothion against the spruce budworm and other forest pests have also been carried out in the provinces of Newfoundland, Ontario, British Columbia and Manitoba (Prebble 1975). The quantities of fenitrothion used and the vast areas sprayed have prompted this study of its effects on non-target organisms such as the trout, that use the streams as habitats and breeding grounds.

Following operationally-applied dosages of 4 ounces per acre, or 280 grams per hectare, maximum concentrations of fenitrothion in forest streams were approximately 0.08

mg/liter (NRCC 1975), with levels as high as 0.7 mg/liter in stagnant pools (Lockhart et al. 1977). Concentrations of the insecticide were found to be extremely variable among streams and years (Eidt and Sundaram 1975). Uptake by different fish species was also variable, therefore, generalized no-effect levels may not be applicable in all situations.

The toxic action of fenitrothion is believed to be the result of inhibition of cholinesterases, the hydrolytic enzymes for acetylcholine. The latter compound is a neurotransmitter in the presynaptic membranes of the parasympathetic nervous system. In vertebrates, acetylcholine is both excitatory and inhibitory depending on the tissue involved. Therefore, disruption of normal nervous function by inhibition of acetylcholinesterase can result in widespread deterioration of bodily functions and may possibly result in the death of the organism. Acetylcholinesterase inhibitors cause death in higher vertebrates by blocking neurotransmission in the respiratory center of the brain or in the neuromuscular junctions of the respiratory apparatus (Heath 1961), but this has not been confirmed in fish. Sublethal effects of fenitrothion on fish include decreased acetylcholinesterase activity in a variety of tissues (Lockhart et al. 1973; Wildish and Lister 1973; Duangsawasdi 1977; Symons 1977),

reduced locomotory behaviour (Peterson 1974) and reduced hierarchial behaviour (Wildish and Lister 1973). There is no unequivocal evidence that operational spraying has resulted in fish kills nor is any expected (Symons 1977), but the sublethal effects on the behaviour and physiology of fish may be detrimental to their survival under natural conditions. As behaviour is dependent upon the proper functioning of the biochemical and physiological processes in the intact organism, it is a sensitive indicator of sublethal effects produced by the toxicant (Welsh and Hanselka 1972; Scherer 1977). Delayed mortality is a possibility but is unlikely due to the low persistence of fenitrothion after four days in most parts of the aquatic ecosystem. Indirect mortalities have been shown to be significant (Hatfield and Anderson 1972) and may account for biomass changes of stream fishes after spraying (Symons and Harding 1974). These behavioural and physiological parameters are the focus of this study and will be used in the elucidation of the mechanisms of toxic action of fenitrothion on freshwater fish.

The relatively low persistence of fenitrothion in the environment is as a result of a number of degradation processes. Other routes of disappearance, from the water phase, include sorption by the sediments and

microbial degradation (Marshall and Roberts 1977). Low persistence of fenitrothion in natural waters also necessitated the shortening of experimental exposure periods to be more applicable and realistic.

Swimming performance, as measured by the capacity of a fish to maintain its position in a current, has been suggested as a criterion in the determination of the sublethal effects of toxicants on fish (Brett 1967; Sprague 1971). Swimming performance will be used as a tool to measure the degree of toxicity of fenitrothion on an observable and quantifiable behaviour. Measurements of swimming performance generally require acclimation periods of at least 24 hours and incremental velocity steps lasting 1 hour or more. Due to the relatively rapid recovery of some bodily functions after removal from fentrothion-treated water, time constraints impose limits on the test procedure for swimming performance.

Swimming performance and related behaviours represent the final integrated result of a diversity of bodily processes and therefore can be comprehensive and sensitive tests(Dodson and Mayfield 1979). Swimming performance has been used to indicate changes in fish behaviour (Lindahl and Schwanbom 1971; Greenland and Thomas 1972; Griffiths and Alderdice 1972), as a response to chemicals (Oseid and Smith 1972; Waiwood and Beamish 1978) and as a measure of

the effects of insecticides (Peterson 1974; Rand 1977). Swimming endurance is also a measure of survival since the social organization of stream-dwelling salmonids is heavily dependent upon swimming in order to maintain territories and optimize food utilization.

Increased activity in response to induced water velocities imposes increased demands on respiration (Dwyer and Kramer 1975). Respiratory impairment resulting from exposure to a toxicant will have a serious influence on swimming ability as the scope for activity will be substantially reduced (Fry 1957; Brett 1964). The relationship between swimming performance and respiration may provide some insight into the mechanics of fenitrothion toxicity in fish.

Acute lethality experiments supply the median lethal concentrations which are of great theoretical value (Lesel 1976). The results are fundamental for toxicity tests because they provide an initial toxicity assessment and form the point of reference for all subsequent research. In addition, the LC50's can be used for comparative purposes such as between species of fish or between toxicants. The tissue samples studied for enzyme inhibition include whole brains, gills and hearts. These samples will provide a baseline with which the sublethal effects can be related after measuring other parameters, including swimming performance.

METHODS AND MATERIALS

1. FISH STOCKS

Brook trout (Salvelinus fontinalis) were obtained from the Rockwood Experimental Fish Hatchery in October 1980. These trout were from a common, domestic stock (God's River, August 1980) and were reared at Rockwood. They were transported from the hatchery via tank truck supplied with oxygen. The fish were received in good condition with no mortalities directly attributable to transport.

The fish were held in the Animal Holding Facility (A.H.F.), Department of Zoology until required for experimentation. The fish were distributed equally among three 500 L fiberglas tanks. These holding tanks received a continuous flow of dechlorinated City of Winnipeg tap water (total hardness as $CaCO_3$ = 77 mg/L, specific conductance = 160 μmho/cm, pH = 7.5) and aeration. Dechlorination was accomplished by carbon filtration of the incoming city water. Chlorine levels were maintained below 0.05 parts per million.

The fish were fed daily on a diet of dry pellet trout food (Ewos No. 4P Trout Grower Pellet, Rundle Feed Mill Ltd., Palmerston, Ontario) at a rate of approximately 1 percent of body weight per day. This maintenance diet was sufficient for normal growth and health, and no significant mortality ($p < 0.05$) was recorded throughout the experimental period.

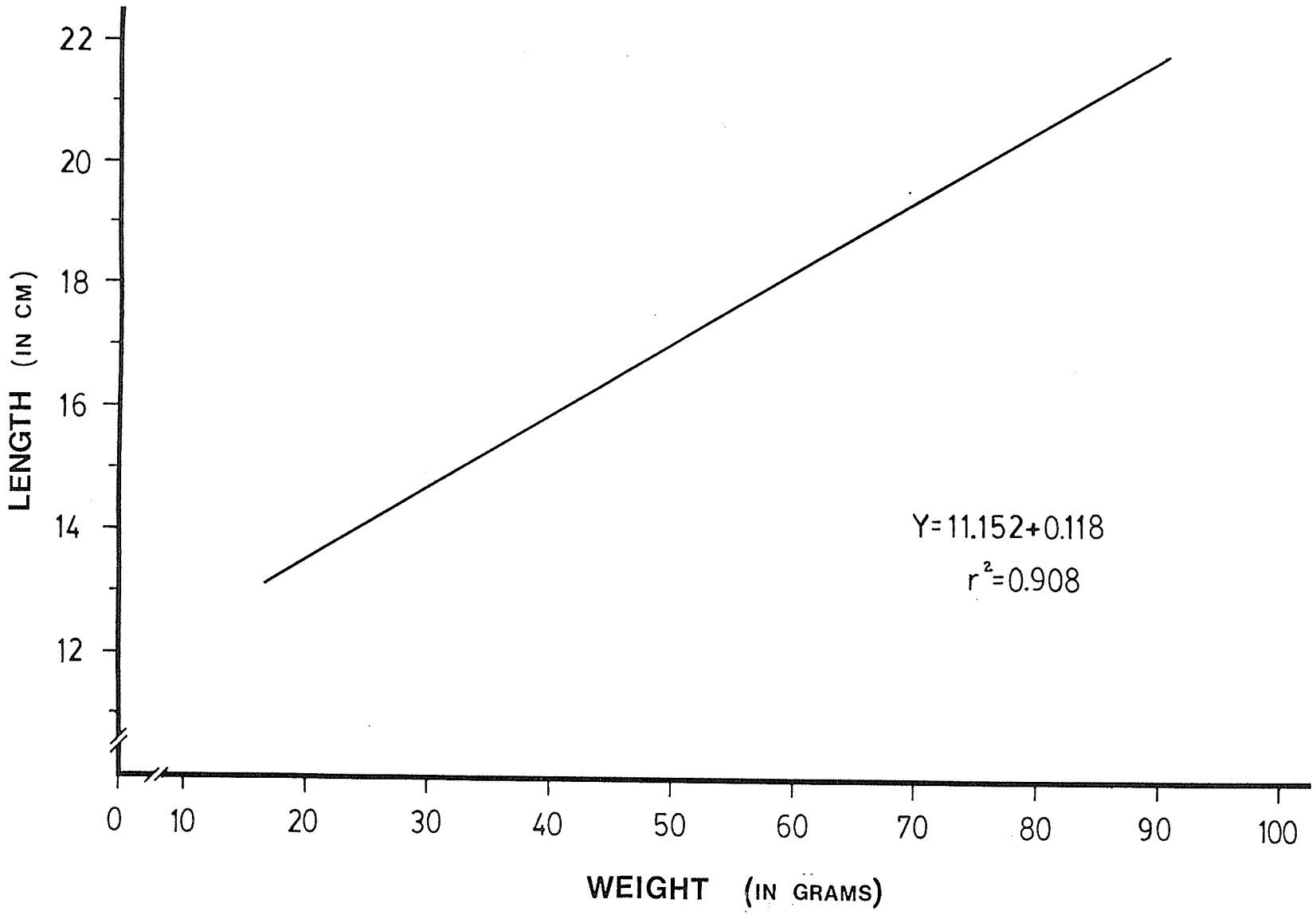
Water temperature in the tanks was maintained at 12°C, with a variation of less than ± 1°C. Aeration was achieved by air stones in each tank. The holding tanks were illuminated by electronically controlled fluorescent lights which provided a photoperiod of 12 hours light and 12 hours darkness. The brook trout were allowed to acclimate to the holding conditions for four weeks before commencement of any exposure experiments (Peterson and Anderson 1969; APHA 1981).

Brook trout were chosen regardless of sex and were used in all three stages of this study. The mean body weights and lengths for the fish used in each experiment as well as the overall mean body weight and length are given in Table 1. The average values will be used as baselines for further measurements. The length-weight relationship for these brook trout is best described by the equation, $Y = 11.152 + 0.118x$ and is illustrated in Figure 1. Sprague (1969) recommended that the largest fish should not be more than 1.5 times as long as the smallest fish within any single experiment to eliminate size induced variation in the bioassay results. The relationship between physical size and toxicity is inconsistent and there are conflicting reports in the literature between authors and compounds (Herbert and Merkens 1952; Norris and Miller 1974; Post

TABLE 1. Mean body lengths and weights and lines of best fit for each experimental section, as well as for all groups combined.

Experiment	Mean length ± 1 S.D.	Mean weight ± 1 S.D.	Line equation	r^2
96-hour LC50	15.02±1.383	33.48±10.77	Y = 10.922 + 0.1224X	0.908
24-hour LC50	15.67±1.448	38.16±11.110	Y = 10.926 + 0.1243X	0.910
Swimming Performance	17.19±1.481	50.69±13.011	Y = 11.930 + 0.1038X	0.834
All fish combined	15.98±1.702	40.91±13.744	Y = 11.152 + 0.1180X	0.908

Figure 1. Length-weight relationship for the brook trout used in this study.


LENGTH (IN CM)
22
20
18
16
14
12
0
10
20
30
40
50
60
70
80
90
100
WEIGHT (IN GRAMS)
Y=11.152+0.118
r²=0.908

and Schroeder 1971; Harrison 1975). The brook trout were within the size range recommended by Sprague (1969), except for a few individuals accounting for less than 5 percent of the total number of fish.

Fish were removed from the holding tanks only when required for research purposes. They were held in a 200 L fiberglas tank in a Controlled Environment (C.E.) Room and allowed to acclimate to these conditions for an additional 10 days prior to use (APHA 1981). The temperature and photoperiod were identical to those used previously. If any additional transfers of fish were required within the C.E. room, a period of 24-48 hours was allowed to enable the fish to recover from handling. These transfers were necessary to randomly sort the fish into the various aquaria and/or concentrations. Buckets were used in these transfers whenever feasible to avoid the removal of the fish from water. Feeding was discontinued 24 hours prior to initiation of any test or exposure experiments.

2. EXPERIMENTAL CONDITIONS

2.1. TEST AQUARIA

The test aquaria were used in the acute lethality tests and in the pre-exposures of brook trout to sublethal doses prior to the swimming performance tests. These test vessels were 48 L glass aquaria, each equipped with an

airstone and a continuous flow-through water delivery system as recommended by Sprague (1969). The rate of water flow to each tank was calibrated by a Gilmont Flowmeter (R. Gilmont Ind.) and was regulated by neoprene diaphragm valves (Grinnell Diaphragm Valve) and clamps. The volume of water entering each test aquaria was constant at 0.3 L/min and exceeded the minimum flow rates set by Sprague (1969) and APHA (1981). Harrison (1975) reported that under experimental conditions, flow rates down to 50 percent of the recommended values did not greatly alter the mean survival time. Exposure/test aquaria were located in the C.E. room. The temperature and photoperiod were controlled, which removed any possible inconsistencies these two variables may have introduced. Photoperiod has been shown to affect the outcome of a bioassay experiment (Harrison 1975). The mean survival time is consistently lower when the test photoperiod is different from the acclimation photoperiod. As a result of lowering the survival time, the lethal concentration will also be misleadingly low. The likelihood of accidental external disturbances was also reduced in the controlled environment room.

The glass aquaria were meticulously cleaned prior to the commencement of any exposure experiment to prevent

residual contamination of the aquaria between experiments. Test aquaria were also rinsed continually with dilution water for 48 hours before the introduction of fish. Dilution water for both the aquaria and the holding tanks was obtained from the same source. Green corrugated fiberglass covers prevented the fish from jumping out of the aquaria.

2.2. INSECTICIDE DELIVERY SYSTEM

The dosing apparatus consisted of a multiple head Masterflex Pump (model WZ1R03, Cole-Palmer Instrument Company) in combination with a Variable Speed Masterflex Controller (Cole-Palmer Instrument Company), the appropriate sized tubing for the pump heads and a stock bottle situated on a magnetic stirrer. The speed of the Masterflex pump was adjusted to deliver the precise amount of the concentrated stock solution to be diluted by the incoming flow of water. The toxicant pump was set at a rate of 4.8 ml/min. This incoming flow produced a Sprague ratio (Sprague 1969) of 2.74 and provided a 95 percent replacement time of 8.4 hours (99 percent replacement time of 13 hours). This relatively high turnover rate was adopted to eliminate the possibility of a significant drop in the level of the active ingredient in Sumithion due to metabolism, sorption or elimination through biological or other means. The high flow rate

also ensured that metabolic wastes were being efficiently removed from the exposure aquaria (Sprague 1973).

2.3 STOCK SOLUTIONS

Fresh stock solutions of fenitrothion (Appendix A) were prepared in the morning, prior to the beginning of each experiment. Stock solutions were prepared at the appropriate concentrations to give the desired final concentration in the exposure aquaria. The final concentrations of fenitrothion in the exposure tanks formed a logarithmic series. Preliminary tests used concentrations with intervals of at least one-order-of-magnitude to locate the critical lethal range for this species of fish under these conditions. The calculated final concentrations used in determining the 96-hour LC50 were 4.6, 3.5, 2.6, 2.0, 1.5, 1.1, and 0.8 mg/L fenitrothion and two controls. In addition to the normal control tank, with no insecticide, another tank received a dose of the emulsifier Atlox 3409, equal to the quantity used to solubilize 4.6 mg/L fenitrothion, approximately 1.15 mg/L. The theoretical final concentrations used in determining the 24-hour LC50 were 9.6, 8.0, 6.0, 4.5, 3.4, 2.5, and 1.9 mg/L fenitrothion and two controls. The Atlox control received approximately 2.3 mg/L Atlox for the 24-hour exposure period. The concentrations used in the swimming performance tests are expressed as percentages of

the 24-hour LC50, and the stock solutions were prepared accordingly. Final concentrations are referred to as calculated or theoretical concentrations since the actual levels of fenitrothion were never measured from water samples. Previous work and samples indicated that measured insecticide concentrations were within reasonable limits (± 5% of expected values).

A correction factor was employed in the amount of Technical grade Sumithion used. Its purity was given as 96.4% active ingredient being fenitrothion. Therefore, a correction factor of 1.037 was applied to each measurement of Sumithion weighed. It should be understood that all concentrations stated hereafter will be read as active ingredient of the insecticide.

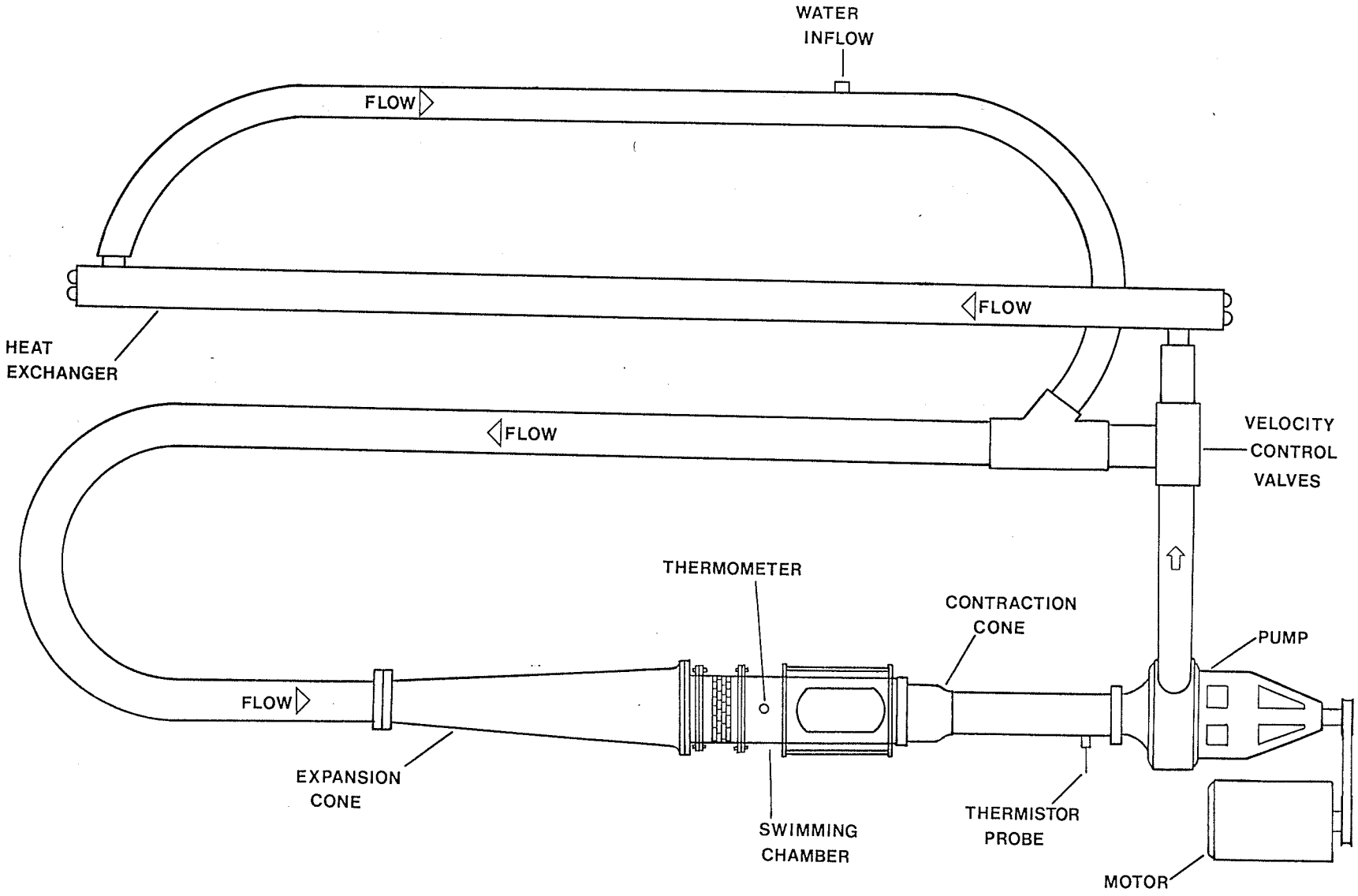
Technical grade Sumithion (fenitrothion, 96.4% emulsifiable concentrate, Sumitomo Chemical Company) was mixed with an emulsifier, Atlox 3409 (Atlas Chemical Industry Ltd.), in the amounts of 25 percent by weight of insecticide, before it was added to water to readily permit mixing of stock solution in test aquaria. Atlox 3409 is an anionic-nonionic blend of dodecylbenzene sulphonate and polyoxyethylene ethers and is a regular additive in New Brunswick spray program (Eidt 1978). Problems with separation of the emulsions such as that experienced by Symons and

Harding (1974) were not apparent. The precise pharmacodynamics and toxicological properties of Atlox 3409 are not clear with respect to the aquatic ecosystem (Safe et al. 1977), therefore caution must be used when interpreting the results of combinations of chemicals. Stock solutions were all prepared from the same source of technical grade insecticide and the same method of preparation was used in each case. Stock solution bottles were covered at all times with aluminum foil and kept stirred with magnetic stirrers to ensure homogeneous solutions. At time zero of each exposure experiment, the appropriate volume of stock solution was added to each aquaria to immediately increase the concentration of the toxicant to the desired level.

2.4. SWIMMING TUNNEL DESIGN

The swimming tunnel (Fig. 2) used in this study was a modification of Brett's (1964) respirometer. For the type of parameters measured, this swimming tunnel was the most suitable of the available designs (Blazka et al. 1960; Thomas et al. 1964; MacLeod 1967). This swimming tunnel design is characterized by high horsepower, high head and low volume (Smith and Newcomb 1970). These favorable traits enable the swimming tunnel to act as an apparatus which not only measures swimming performance but is also capable

Figure 2. Swimming tunnel design showing the major components of the structure.


WATER
INFLOW
FLOW
FLOW
HEAT
EXCHANGER
FLOW
VELOCITY
CONTROL
VALVES
THERMOMETER
CONTRACTION
CONE
PUMP
FLOW
EXPANSION
CONE
SWIMMING
CHAMBER
THERMISTOR
PROBE
MOTOR

of producing accurate oxygen consumption data. Under forced activity, oxygen consumption may be useful in explaining the mechanism(s) of action of fenitrothion. The Blazka-type tunnel was designed solely as a respirometer and as such had a minimal volume, minimal head and minimal power. The latter two characteristics are not desirable in a swimming tunnel because the result is a poor flow profile. Measurement of swimming ability or respiration is best performed in a tunnel of the appropriate design (Bell and Terhune 1970; Beamish 1978).

The swimming tunnel was built as a recirculating water tunnel and was supplied with a continuous flow-through volume of water at 1.15 ± 0.1 L/min. The total volume of circulated water was approximately equal to 32 liters. The 90 percent replacement time was 60 minutes. The swimming tunnel consisted of a clear plexiglass fish chamber connected to a pump through polyvinyl chloride (PVC) pipe, a contraction cone and a fiberglass expansion cone. The water was driven by a Century Type SC motor (Gould Manufacturing of Canada) and the Hayward-Gordon centrifugal pump (Model AB 316 SS) mounted on a separate, large concrete block in order to reduce the amount of vibration transmitted through to the swimming chamber. This pump/motor combination had a maximum output of 760 L/min and was capable of forcing

water through the fish chamber at a maximum speed of 84 cm/sec.

Temperature was controlled by a primary heat exchange system (Bell and Terhune 1970) incorporated in the swimming tunnel design. The heat exchanger (Fig. 2) consisted of a stainless steel outer shell 195 cm long, with six lengths of cooling coils inside. The 2 ton compression unit was regulated by a Thermistemp Temperature Controller (Model 63RB, Yellow Springs Instrument Company) and a Y.S.I. temperature probe situated in the swimming tunnel behind the fish chamber. Heating the water was unnecessary, since fluid friction within the swimming tunnel and the heat produced by the pump was sufficient to offset any temperature irregularities created by the heat exchanger.

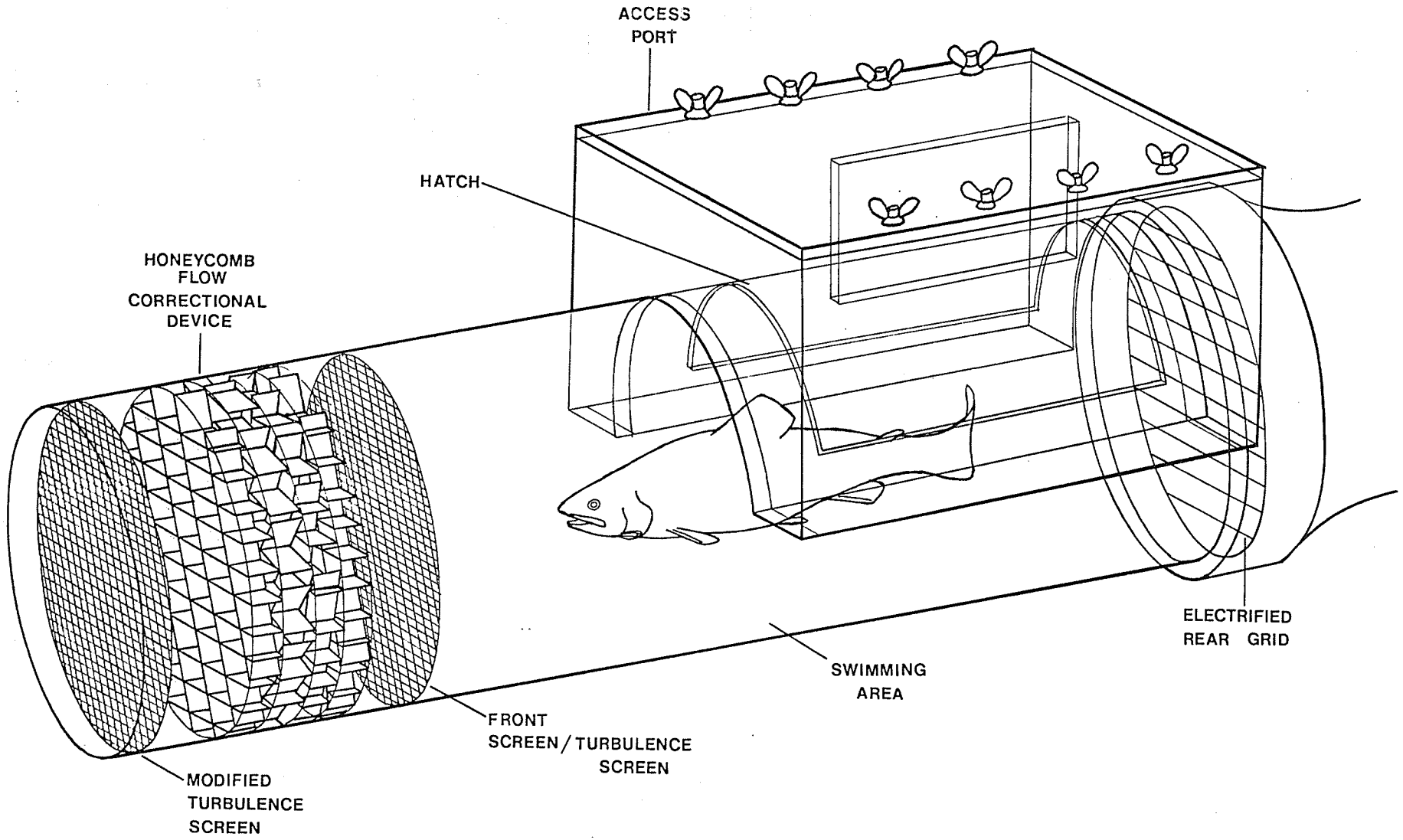
The expansion cone was an important component of the swimming chamber in maintaining a relatively flat, consistent velocity flow profile. The diffuser converted the kinetic energy of the flow to static pressure energy as efficiently as possible without converting the energy to heat through vortices and viscosity (Bell and Terhune 1970). The build up of a boundary layer was a contributing factor to the development of laminar flow. However, the series of turbulence grids and honeycombs retarded this process. Laminar flow was not desirable because the velocity front

is curved. A rectilinear flow profile, associated with uniform turbulent flow, was best suited for the swimming performance tests.

Interference effects occurring within a closed swimming tunnel can be important contributors to the outcome of any test. In swimming tests with fish, the solid blocking effect is of greatest concern (Bell and Terhune 1970). Here, the fish body was blocking the flow of water through part of the tunnel, which increases the velocity of the water around the body to satisfy continuity requirements (Bell and Terhune 1970). As a result, the dynamic pressure, Reynolds number and, most important, the effective test section velocity, were increased. Bell and Terhune (1970) recommend that to avoid excessive velocity changes due to this type of interference effect, the maximum cross-sectional area of the object to be tested should not occupy more than 10 percent of the available test section area. The maximum cross-sectional area encountered did not exceed the 8 percent of the swimming chamber cross-section. Therefore, no corrections for this source of error were applied.

The fish swimming chamber (Fig. 3) was made of clear acrylic plastic with an inside diameter of 10 cm, which was equal to the maximum diameter at the outflow of the

Figure 3. Close-up view of the major components comprising the swimming chamber of the swimming tunnel.


ACCESS PORT
HATCH
HONEYCOMB FLOW CORRECTIONAL DEVICE
ELECTRIFIED REAR GRID
SWIMMING AREA
FRONT SCREEN / TURBULENCE SCREEN
MODIFIED TURBULENCE SCREEN

expansion cone. The chamber had a length of 50 cm, which was approximately three times the average body length of the fish. A hatch or port, allowing access into the fish chamber, was cut from the top half of the chamber. This access port provided a means by which fish could be loaded into the chamber with a minimum of excitement. The inside curvature of the hatch was identical to the fish chamber which permitted uninterrupted water flow within the testing chamber. An acrylic box was built around the hatch cover which served to collect the overflow from the swimming chamber and supplied a means by which the hatch could be secured. On one side and at the bottom of the fish chamber, a series of alternating black and white vertical stripes gave the fish a point of reference. The optomotor response has been well documented (Harden Jones 1963; Pavlov 1969) and has been used frequently in toxicity studies (Dodson and Mayfield 1979; Scherer and Harrison 1979).

To confine the fish's movements within the swimming chamber a stainless steel wire mesh was placed inside the swimming tunnel. This steel mesh at the upstream end of the chamber also served to reduce the level of turbulence in the flow and perturbations of velocity about the mean flow rate at each particular setting (Bell and Terhune 1970). The mesh was made of wire 0.074 cm in diameter with 0.25 cm between the grid wires (3.2 meshes per

centimeter). Upstream from this wire mesh was a series of honeycomb structures which served to reduce the transverse component of velocity fluctuations. Successive honeycombs were "crossed" at 30^{o} from each other to further dissipate strong turbulence. The restricted amount of space in which the swimming tunnel was set up, required bends that were too severe. These restrictions necessitated the flow correctional devices. In front of the honeycombs, another stainless steel wire grid was inserted in the swimming tunnel.

A suitable flow profile (Fig. 4) is one in which the velocity is uniform (i.e. turbulent flow) and not laminar flow. Depending on pipe roughness (low Rn), the profile will progress into a laminar-type flow and the resulting parabolic flow profile (Fig. 5) will arise. Turbulence has a characteristic rate of decay depending on the mesh size and water velocity (Brett 1964). Therefore, this decay is inevitable. The initial flow profile was unacceptable and required an adjustment in the form of a modified grid (Fig. 6). The modification consisted of the addition of 52 machine screws with round heads, 0.5 centimeters in diameter, in strategic locations in the grid. The final smoothed velocity profile (Fig. 7) in the swimming chamber was acceptable. As demonstrated in Fig. 7, the velocities (v) measured at the various locations within the

Figure 4. Simplified diagram of the uniform, turbulent velocity flow profile which is desirable for a swimming tunnel.

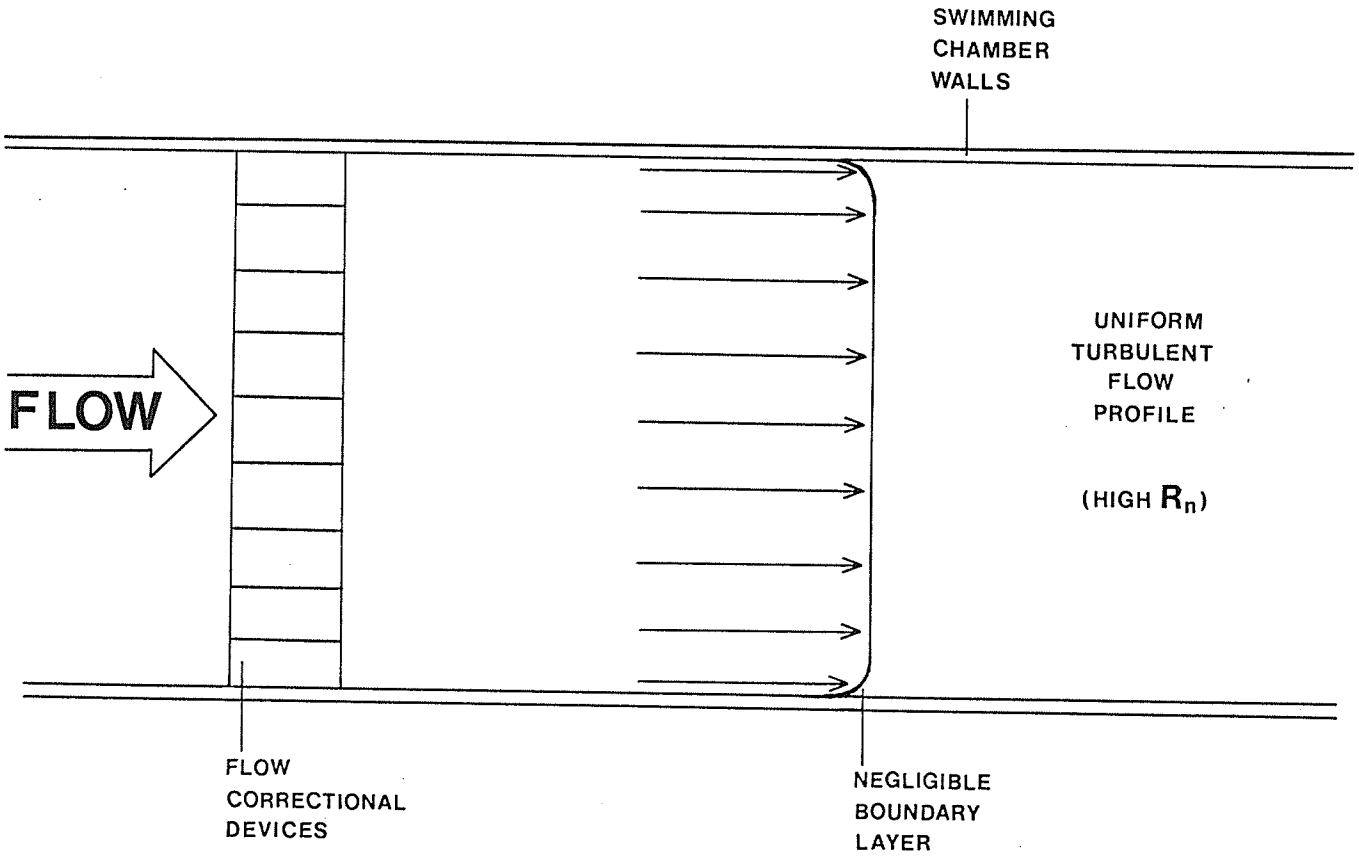

SWIMMING
CHAMBER
WALLS
FLOW
UNIFORM
TURBULENT
FLOW
PROFILE
(HIGH R_n)
FLOW
CORRECTIONAL
DEVICES
NEGLIGIBLE
BOUNDARY
LAYER

Figure 5. Simplified laminar type flow profile, the result of a low Rn and a significant boundary layer effect within the swimming tunnel.

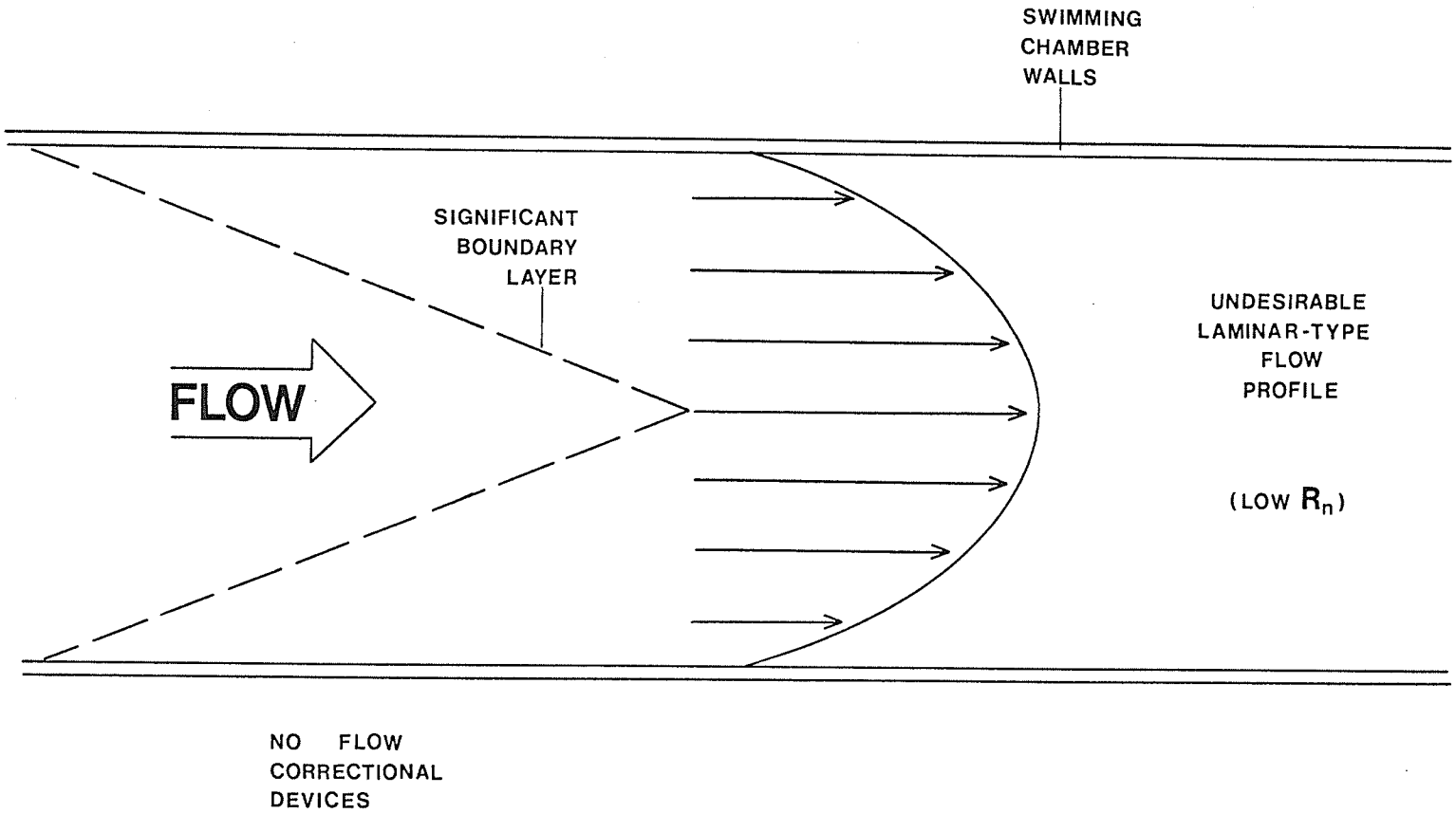

SWIMMING
CHAMBER
WALLS
SIGNIFICANT
BOUNDARY
LAYER
FLOW
UNDESIRABLE
LAMINAR-TYPE
FLOW
PROFILE
(LOW R_n)
NO FLOW
CORRECTIONAL
DEVICES

Figure 6. Modified screen used in the swimming tunnel to correct for major hydrodynamic distortions in the velocity flow profile.
⊠ - machine screw locations in the screen.

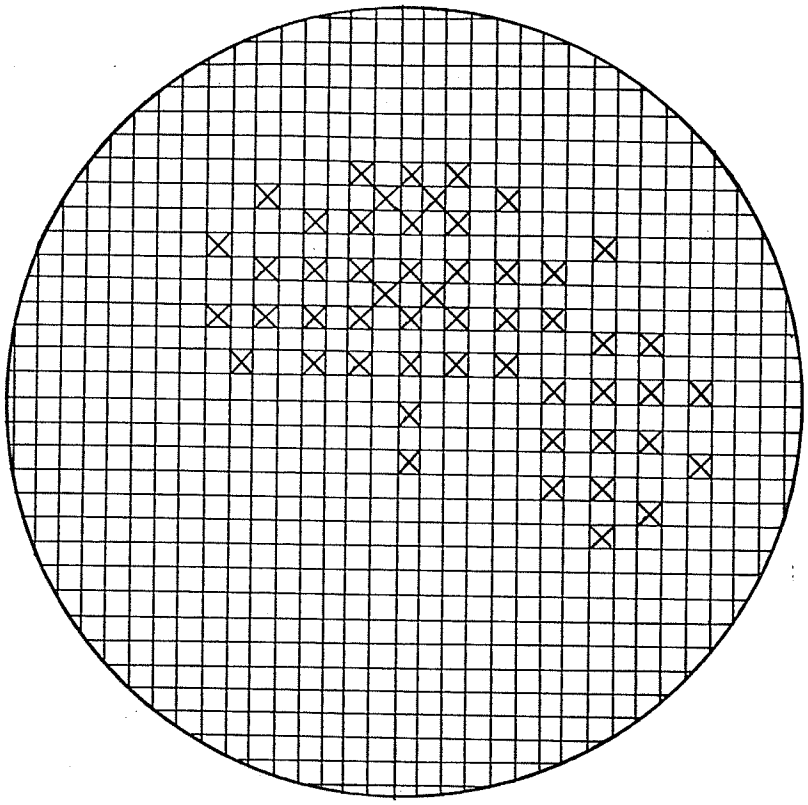
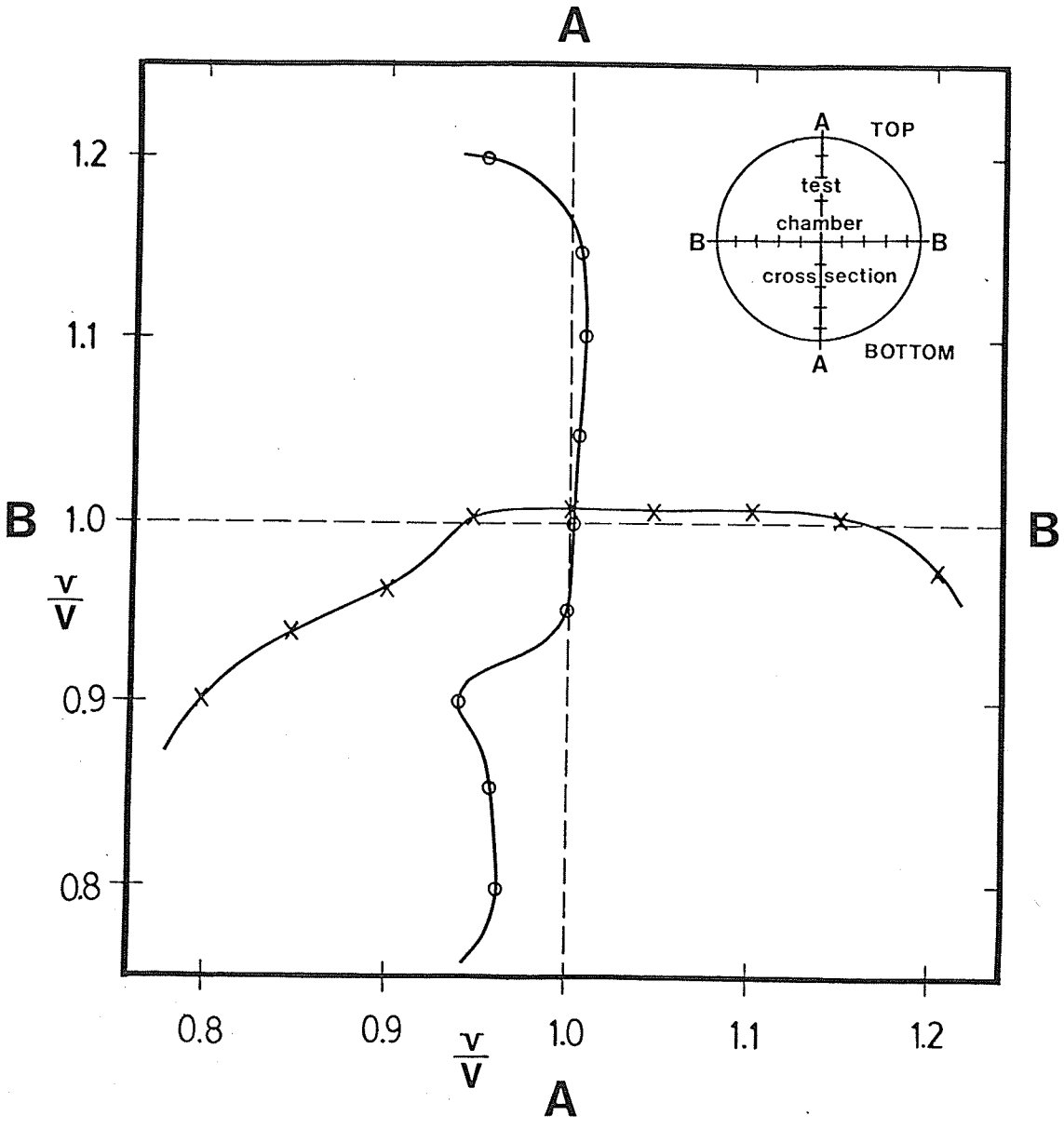


Figure 7. Final velocity profile in the test section of the swimming tunnel.

INSET- cross-section of swimming chamber

v - measured velocity at point indicated on inset

V - average water velocity

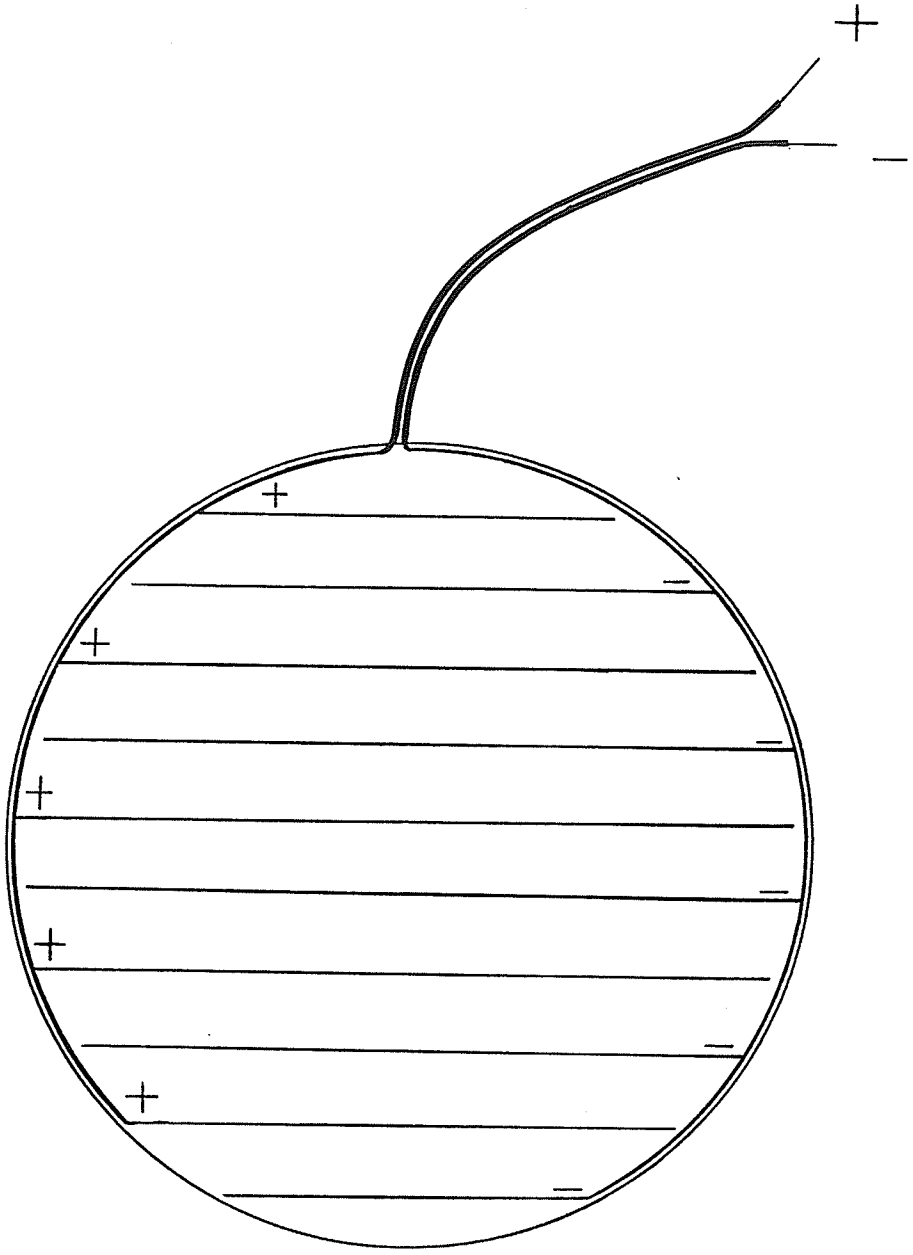

A
1.2
1.1
B
1.0
B
v/V
0.9
0.8
0.8
0.9
v/V
1.0
1.1
1.2
A
A
TOP
test
chamber
B
B
cross section
A
BOTTOM

swimming chamber (see inset) deviated little from the desired average velocity (V).

The acrylic swimming chamber was extremely smooth, therefore, it had a relatively high Rn value and the development of laminar flow from the desirable turbulent flow profile was negligible. This fact has been supported by velocity measurements taken in the swimming chamber. An exception to this favorable profile occurred to some extent at the upper end of the velocity regime, but its effect was minimal since few fish attained these velocities.

At the downstream end of the swimming chamber another stainless steel wire grid prevented the escape of the fish. This wire grid alone was insufficient to make the fish swim at all velocities. The grid was improved with the addition of an electrified grid of geometrically parallel, horizontal electrodes (Fig. 8) located on the swimming chamber side of the stainless steel grid. A Grass S6 Stimulator (Grass Instrument) supplied the necessary current to stimulate the swimming response. The minimal amount of current required to produce the swimming response was used in all cases. Weak electric fields created a fright reaction which was the useful stage of behaviour of fish in an electric field (Bell and Terhune 1970). The voltage gradient varied with fish size, but voltages over 7 volts were rarely used.

Figure 8. Details of the electrified grid, located at the rear of the swimming chamber for stimulation of non-swimming fish.



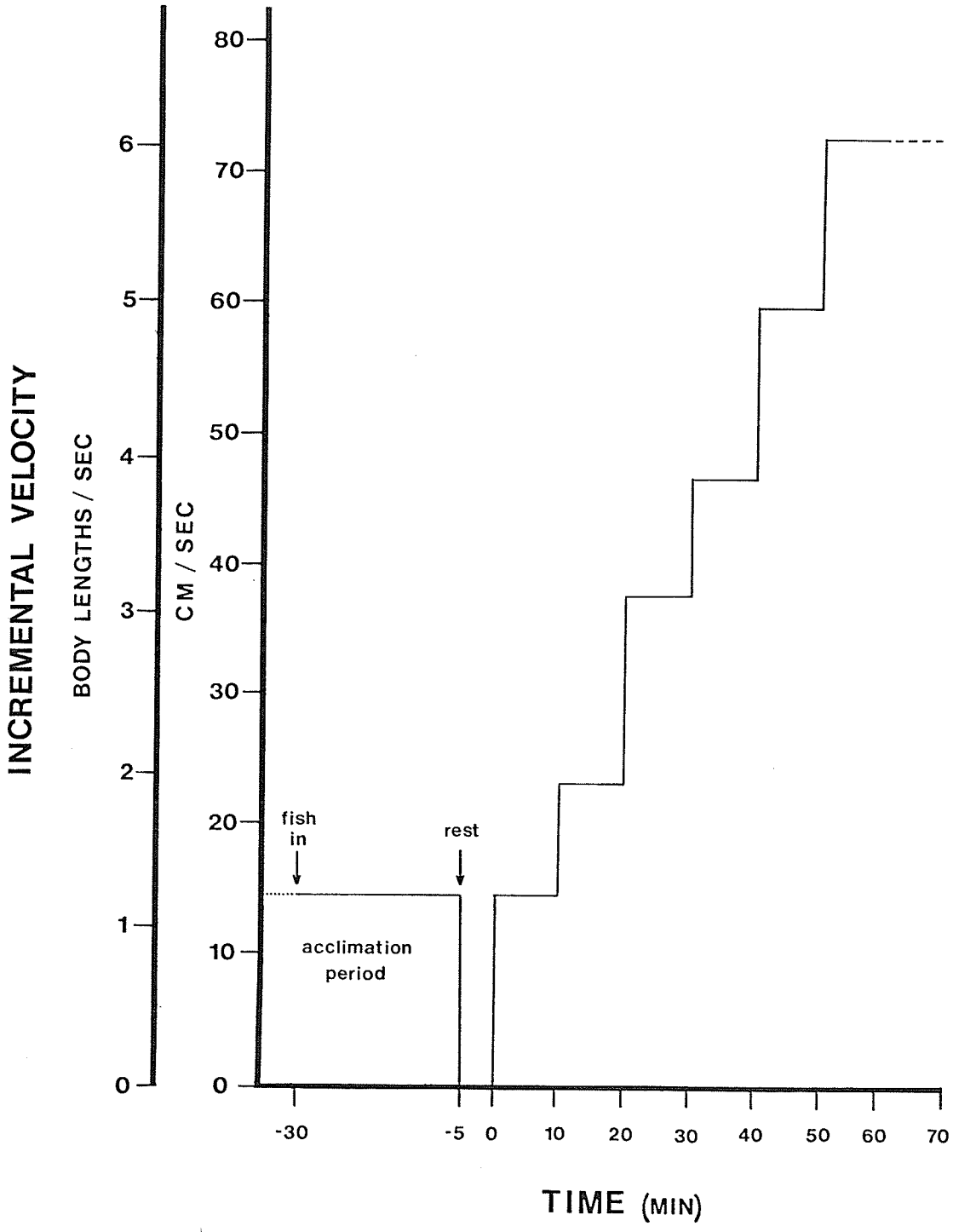
POSITIVE

NEGATIVE

Step-wise velocity increments were achieved by the manipulation of two 1½ inch PVC Y-pattern globe valves (Chemline Plastics). Each increment approximated a velocity increase of 1 body length per second (10-15 cm per sec) and was calibrated by an indwelling Ott Meter (Type C1, A. Ott Meters). These measurements were repeated and refined by a Kent Miniflo meter (Type 265, Kent Industries) in combination with a Type 265-3 low speed probe. The final incremental velocity test sequence was as illustrated (Fig. 9). The Ott meter was subsequently removed for the swimming performance test. Velocities were monitored throughout the experiment to ensure that constant and repeatable speeds had been achieved.

The entire swimming tunnel was built on a plywood table with the supports lined with neoprene foam pads to absorb some of the vibrations produced by the motor/pump assembly. Water temperature was continually checked during all runs by the use of an indwelling thermometer located at the upstream end of the fish chamber. Oxygen concentration in the outflow was also monitored and recorded throughout all swimming performance tests by a YSI Oxygen meter (Model 54, Yellow Springe Instrument) and a YSI temperature-compensating, membrane oxygen electrode (Model 5450). The oxygen meter was calibrated and checked regularly in water saturated with air. Similar oxygen monitoring equipment

Figure 9. Incremental velocity test sequence used in the swimming performance tests.


INCREMENTAL VELOCITY
BODY LENGTHS / SEC
CM / SEC
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
fish in
rest
acclimation period
-30
-5
0
10
20
30
40
50
60
70
TIME (MIN)

has been used for measuring critical oxygen levels by Klyashtorin (1975).

3. EXPERIMENTAL PROCEDURES

3.1 ACUTE LETHALITY EXPERIMENTS

3.1.1. ACUTE BIOASSAYS

To determine the acute lethality of fenitrothion, 96-hour and 24-hour exposure periods were used to obtain the respective LC50's, specific to this population of brook trout under the described conditions. The bioassay experiments were designed on the basis of recommendations made by Sprague (1969, 1973).

Ten fish of either sex, chosen at random, were introduced into each of nine exposure tanks containing dilution water, and allowed to acclimate to the tanks for 48 hours. The 96-hour exposure experiments began on a Monday morning and ran continually until Friday morning. The fish were not fed after the transfer to the exposure aquaria nor during the experiment. The 24-hour exposure tests usually began mid-morning on day one and were terminated the next day.

Exposure tanks were monitored closely for any mortalities. Aquaria were checked at half hour intervals for the first six hours, and hourly up to twelve hours, and at 14, 16, 18 and 24 hours for the 24-hour tests.

Additional checks at 30, 36, 48, 60, 72, 84 and 96 hours were made for the 96-hour exposure. The criterion for death was cessation of breathing motions and lack of response to tactile stimuli (Sprague 1973). Dead fish were removed and the time of death recorded. Each fish was immediately weighed and measured, and tissue samples were removed. The sex, girth, height and fork length of each fish were recorded. Two blood samples for measurement of hematocrits were collected from the caudal blood vessels by severing the tail at the caudal peduncle and drawing the blood directly into heparinized micro-hematocrit capillary tubes. After centrifugation at 10,000 x g for five minutes in a micro-capillary centrifuge (International Equipment Company, model MB), the hematocrit as a percent of blood cell volume in whole blood was recorded.

3.1.2. TISSUE SAMPLES

Brain, gill, heart and liver samples were taken from the brook trout used in all sections of this experiment (96-hour and 24-hour bioassays, and the swimming performance tests). The liver samples were weighed and discarded. The brain, gill and heart samples were weighed and stored in individual glass jars at -15^{o}C until analyzed for protein and cholinesterase. Tests have shown that freezing does not significantly affect cholinesterase activity compared to

fresh samples given that the analyses were performed within two months (Klaverkamp et al. 1977). Comparisons of similar tissues during preliminary tests performed in the course of this experiment tend to support the findings of Klaverkamp et al. (1977).

Tissue samples were excised from the mortalities in the acute lethality studies or from the sacrificed fish, immediately after completion of the acute lethality studies and swimming performance tests. Whole brain samples were obtained by shearing off the top of the skull and cutting the brain loose at the base of the medulla, the optic nerves and the olfactory tract. A check was made after removal of the brain to ensure an intact specimen, including the pituitary. Gill tissues were obtained by a method modified from that used by Zaugg and McLain (1971). Gill samples were collected by cutting off the first, second, third and fourth gills arches, complete with filaments, arches, and some associated musculature at the dorsal side of the arches. This complex of tissues will hereafter be referred to as simply, gills. The gills were rinsed with distilled water and stored in individual glass vials. No attempt was made to flush the resident blood from the gill arches and filaments. Heart samples were taken by opening the pericardial sac, cutting the ventral aorta, anterior to the

bulbus anteriosus and lifting out the ventricle and bulbus. The whole liver was sampled by cutting the blood vessels and mesentary free from the liver and removing the organ from the visceral cavity. The variability in the size and fullness of the gall bladder necessitated its removal before weighing the liver sample.

3.1.3. DETERMINATION OF LC50's

The median lethal concentration (LC50) was the concentration that produced 50 percent mortality at the exposure time specified. The 96-hour and 24-hour LC50's and the 95 percent confidence intervals were calculated using the probit analysis method by Finney (1971). The mathematical solution to the linear regression equation is dependent upon the transformation of the observed percent mortality to probits and the concentrations to logs. The sigmoid nature of the biological response can be linearized by using logarithmic scales. The sigmoid curve results from variable individual responses causing the curve to resemble a cumulative normal distribution (Bliss 1957). The log-probit transformation is an acceptable manipulation of bioassay data (Bliss 1937; Finney 1964).

3.2. SWIMMING PERFORMANCE TESTS

3.2.1. PROCEDURES

The concentrations of fenitrothion used when exposing the brook trout prior to testing their swimming performance

are stated as percentages of the 24-hour LC50. These sublethal concentrations are presented in this way to permit comparisons and to indicate the proximity of the concentration to a lethal dose of fenitrothion.

Brook trout of either sex were chosen at random from the holding tank in the controlled environment room and placed individually in 48 liter aquaria. A 24-hour acclimation period was allowed to enable the fish to recover from the transfer and handling. Exposure conditions for these experiments were identical to the conditions described for the bioassays. Feeding was discontinued 24 hours before commencement of the experiment and was withheld for the duration of the tests. Gastric evacuation was usually completed in this time period, ensuring a post-absorptive state (Davis et al. 1963; Windell et al. 1969).

A total of 100 brook trout with a mean weight of 50.69 grams (range, 20.5 - 90.3; standard deviation, 13.01) and a mean length of 17.19 centimeters (range, 13.3 - 20.0; standard deviation, 1.48) were used in the swimming performance tests. Twenty brook trout were treated as controls, being subjected to the same conditions and handling procedures, but without being exposed to a dose of the insecticide or emulsifier. Of the remaining 80 fish, 10 brook trout were exposed to each of the following concentrations of fenitrothion: 0.15, 0.30, 0.61, 1.21,

1.82, 2.43, 3.03 and 3.64 mg/L, hereafter referred to as 3.12, 6.25, 12.5, 25.0, 37.5, 50.0, 62.5 and 75.0 percent respectively of the 24-hour LC50. Each fish was exposed for 24 hours then immediately transferred to the swimming tunnel where its swimming performance was measured. This transfer was exceptionally critical because if the fish were overly exerted, their performance in the swimming chamber could be jeopardized. Transfers were carefully executed by means of a bucket and the fish were literally poured into the fish chamber of the swimming tunnel using a minimal amount of water. This method proved to be the most efficient way to transfer the fish with a minimum of excitement.

The procedures for preparing the swimming tunnel and running a swimming performance test were identical for all runs. Fresh, dechlorinated water at 12^{o}C was continually running through the system at about 1.2 L/min to ensure that fresh, uncontaminated water was available for each run. Twenty minutes prior to the introduction of a fish into the fish chamber, the water pump system, the refrigeration unit and the temperature control unit circuits were energized to allow for temperature stabilization. During this interval, the electric grid was wired to the stimulator and the oxygen probe and meter were calibrated. Oxygen concentrations were monitored to make certain that the initial levels of

oxygen were between 95-100 percent of saturation. The velocity control valves were 'cracked' to clear any accumulated debris, and were reset to the introductory acclimation velocity of 14.5 cm/sec.

Once the temperature had stabilized at 11.5 $\pm$ 0.5^{o}C and the oxygen concentration was adequate, a brook trout, having just completed 24 hours of exposure to fenitrothion was placed in the fish chamber and was allowed to acclimate for 25 minutes. This acclimation period also served as a training session, in which the fish learned to avoid the rear electric wire grid due to low frequency, low voltage shocks passing through the grid. At precisely 25 minutes, the pump and the electrified grid were shut off for a 5 minute rest period. At the end of the rest period, the water pump was restarted, indicating the beginning of the swimming performance test and t = 0 minutes. The grid was used only when the fish appeared to drift back onto the grid. The shocks were delivered singly until swimming resumed. The velocity increments were applied for 10 minutes, with each increment occurring instantaneously with the appropriate adjustment of the valves. The longest period that any one fish was able to swim was approximately 62 minutes, and 6 velocity increments. The step-wise increases in velocity were imposed upon the brook trout until the fish

were unable to prevent a collapse against the electrified grid. This was the criterion which constituted fatigue.

3.2.2. DETERMINATION OF CRITICAL SWIMMING SPEEDS

The time of fatigue, within the particular increment, was recorded and the critical swimming speed was calculated by a method proposed by Brett (1964). The critical swimming speed is defined as the maximum attainable velocity by the brook trout. Collapse usually occurred before the end of a prescribed time interval, and therefore, the critical speed was calculated by adding that fraction of the velocity increment (in cm/sec) to the last recorded speed according to the fraction of the time interval that swimming was maintained. Swimming speeds were also recorded in units relative to fish size, in body lengths per second.

3.2.3. RESPIRATION

The concentrations of oxygen were recorded at the end of each increment and at the end of the run. Corrections for lag and damping effects caused by fluctuations in the oxygen content in the swimming tunnel were made using the formula developed by Evans (1972). This correction factor was made for a continuous flow swimming tunnel/respirometer, into which water must be flowing at a constant rate (Fry 1971). Prior to transferring the fish from the exposure

tanks to the swimming tunnel, a baseline ventilation rate was recorded and the cough frequency was noted by the visual method (Heath 1972). Ventilation rates were also recorded at the mid-way point of each increment. In addition, tailbeat frequencies were monitored throughout the swimming performance tests.

3.2.4. TISSUE SAMPLES

At the completion of each swimming performance test, the exhausted fish were sacrificed by spinal transection. The physical parameters were measured as described previously and the brain, gill, heart and liver tissue samples were excised, weighed and stored for acetycholinesterase analysis.

3.3. ACETYLCHOLINESTERASE ANALYSIS

Tissues to be analyzed for cholinesterase activity were homogenized in a 0.1 M phosphate buffer pH 7.2 (Fisher Scientific) using a Tri-R Stir-R homogenizer (Tri-R Instruments) with a teflon pestle and glass mortar. Brain, heart and gill tissues were homogenized with the phosphate buffer in the ratio of 20,200 and 1000 milligrams of tissue per milliliter of buffer, respectively. Tissues were homogenized for 1 minute in an ice bath to prevent warming. They were capped and allowed to sit for ninety minutes in an ice bath. The homogenates were vortexed briefly and centrifuged at 10,000 x g for 10 minutes which was sufficient

time to clear the supernatant. The supernatant was transferred to clean tubes, and kept in an ice bath until analyzed. Both protein and enzyme analyses were performed using the same supernatant from each sample tissue. The gill supernatant required further dilution to stay within the range of the protein assay.

The cholinesterase activity in the brain, gill and heart supernatants was determined with a Cholinesterase Test-Combination kit (Boehringer Mannheim). This test was based on the colorimetric method by Ellman et al. (1961) and used acetylthiocholine iodide as the substrate. This substrate and acetylcholine react with a group of enzymes that hydrolyze cholinesters. As a result, all measured activity, under the conditions of the assay, was related to acetyl- and other arylcholinesterases (Boehringer Mannheim). Pharmacological investigations on trout brains have revealed that the cholinesterase activity reported represents that of acetylcholinesterase (Hobden and Klaverkamp 1977). The reagents were prepared as directed in the test combination kit and the procedure was followed as described except that the change in absorbance was monitored for 4 minutes rather than 90 seconds. The absorbance was read at 405 nm against air every 30 seconds on a Spectronic 20 spectrophotometer (Bausch and Lomb).

To correct for spontaneous substrate hydrolysis, a reaction blank was monitored, using water as the sample. Activity was calculated using the formula provided in the test kit.

Protein content in the tissue samples was determined by using the Bio-Rad Protein assay (Bio-Rad Laboratories), a dye-binding technique based on the differential color change of a dye in response to various concentrations of protein (Bradford 1976). This method yields comparable results to those of the Lowry method (Lowry et al. 1951), yet the Bio-Rad protein assay is quicker, less complex, more stable and free of many interferences limiting the application of the Lowry method (Bio-Rad 1979). The standard assay procedure was followed, reading the optical densities at 595 nm. The readings were corrected using a phosphate buffer blank. A bovine plasma albumin protein standard was used to prepare a standard curve.

Acetylcholinesterase activity was expressed in micromoles of acetylcholine hydrolyzed per milligram of protein per hour at 12°C (μ moles ACh hydrolyzed/mg protein/hr) and also as a percentage of control value. All activities at each concentration of fenitrothion were pooled for statistical analysis.

RESULTS

1. ACUTE LETHALITY STUDIES

1.1 LC50 DETERMINATION

The probit analysis results and graphs are presented (Table 2, Figs. 10 and 11). The median survival times were calculated according to Litchfield (1949) (Fig. 12) and the median concentrations were recalculated using the method of Litchfield and Wilcoxon (1949) for comparative purposes. A mortality curve (Fig. 13) was constructed for each bioassay, plotting log median survival time against log concentration. The slope and the y-intercept were determined using regression analysis.

The median lethal concentration (LC50) values are expressed as milligrams of active ingredient of fenitrothion per liter (mg/liter). The 96-hour LC50 was 2.26 mg/L with a standard deviation of 0.18. The 24-hour LC50 was 4.85 mg/liter with a standard deviation of 0.42. These LC50 values were determined using Figures 11 and 12. The corresponding value on the x-axis is obtained by extrapolating across from the 5 on the y-axis, giving the log value of the LC50.

No mortalities were recorded in the control tanks in either bioassay. The Atlox-control tanks used in the acute lethality experiments demonstrated that the emulsifier did not contribute to the mortalities recorded in the

TABLE 2. Result of the probit regression analyses by maximum likelihood estimation for the 96-hour and the 24-hour acute lethality studies.

	96-hour bioassay	24-hour bioassay
b	7.6817	6.9906
Y	Y = 2.276 + 7.682X	Y = 0.20518 + 6.991X
Max. Like. Est.	0.3546	0.6859
LC50	2.26	4.85
X^2	0.12, df = 1	0.57, df = 1
Heterogeneous deviation	none	none
S.E.	$\pm$0.18	$\pm$0.42

Figure 10. Probit line and 95% confidence limits for the relation between the probit of kill of brook trout and the concentration of fenitrothion from the 96-hour bioassay.

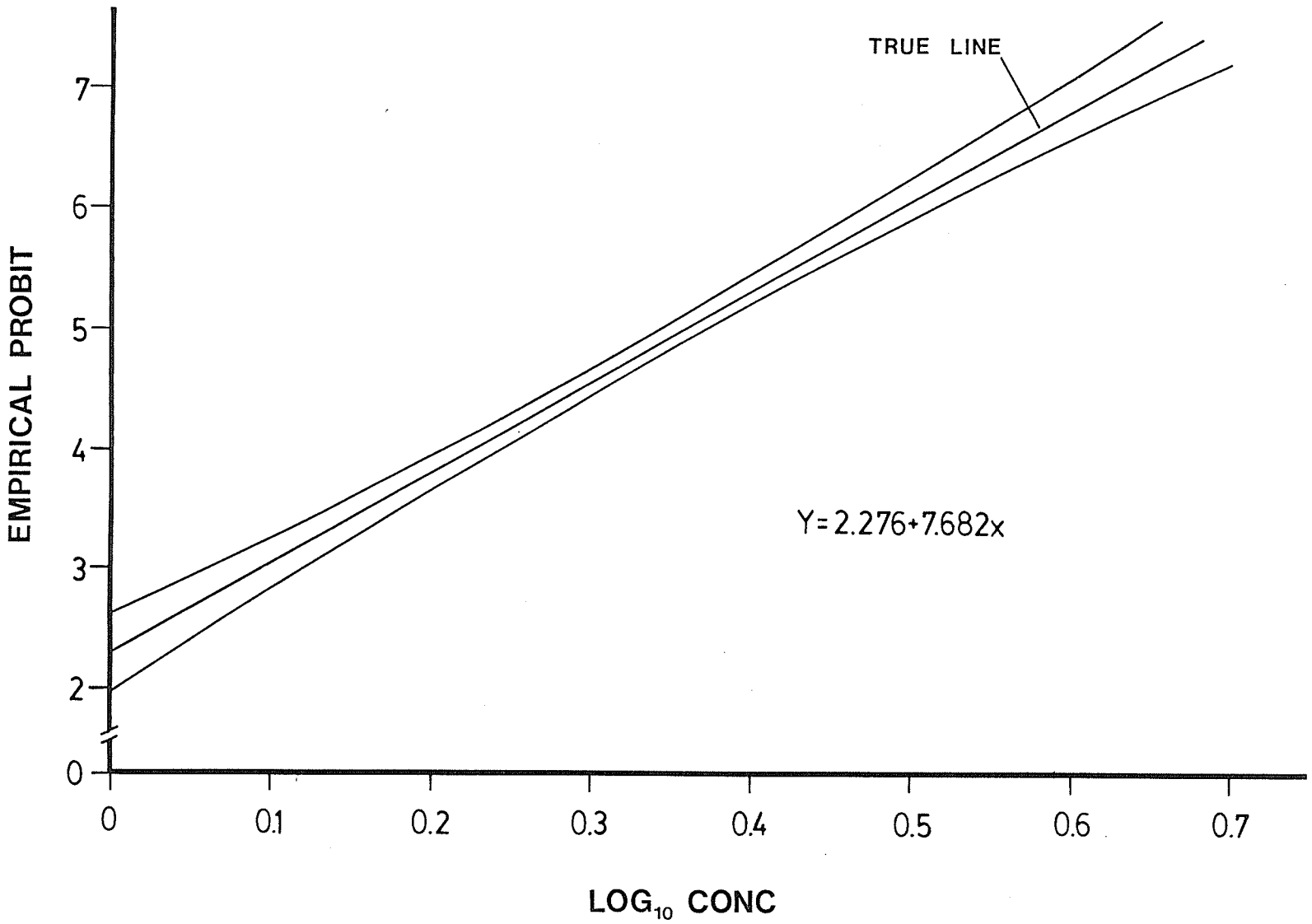

TRUE LINE
Y=2.276+7.682x
EMPIRICAL PROBIT
7
6
5
4
3
2
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
LOG10 CONC

Figure 11. Probit line and 95% confidence limits for the relation between the probit of kill of brook trout and the concentration of fenitrothion from the 24-hour bioassay.

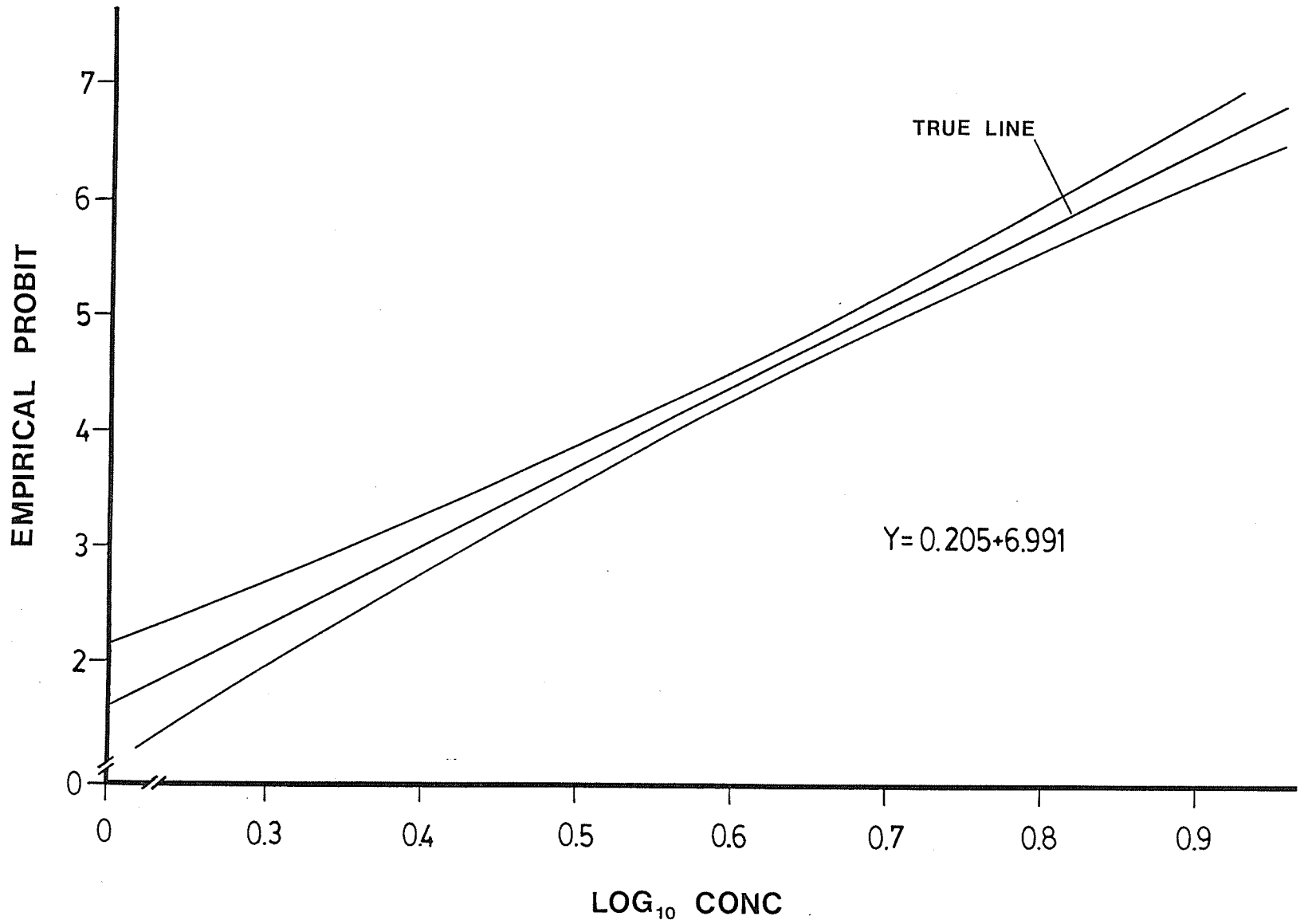

EMPIRICAL PROBIT
7
6
5
4
3
2
0
TRUE LINE
Y= 0.205+6.991
0
0.3
0.4
0.5
0.6
0.7
0.8
0.9
LOG10 CONC

Figure 12. Mortality curves for each concentration of fenitrothion resulting in mortalities during the course of the two bioassays.

9.6_{24} - represents the mortality curve from those exposed to 9.6 mg fenitrothion/liter in the 24-hour bioassay.

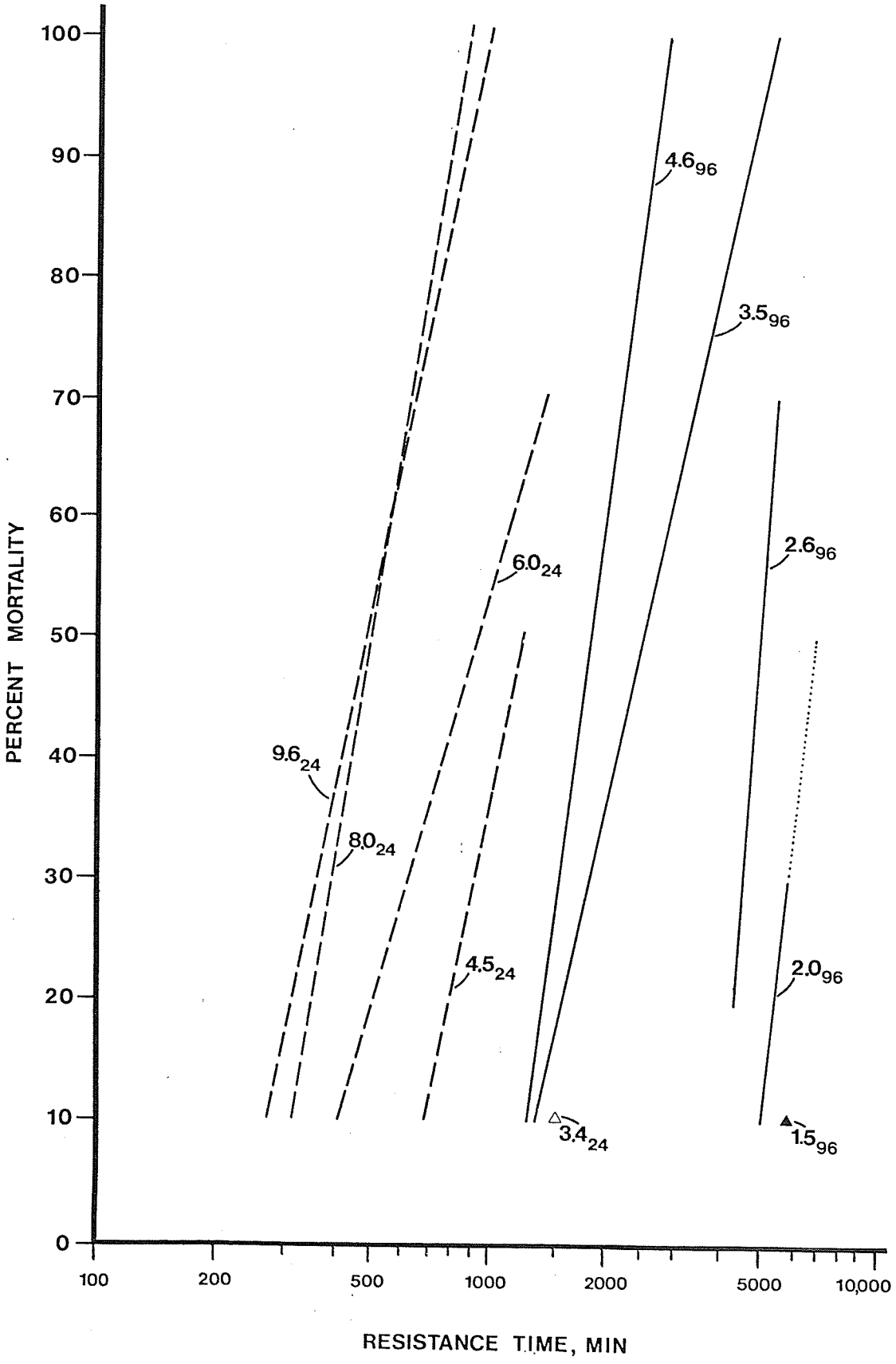
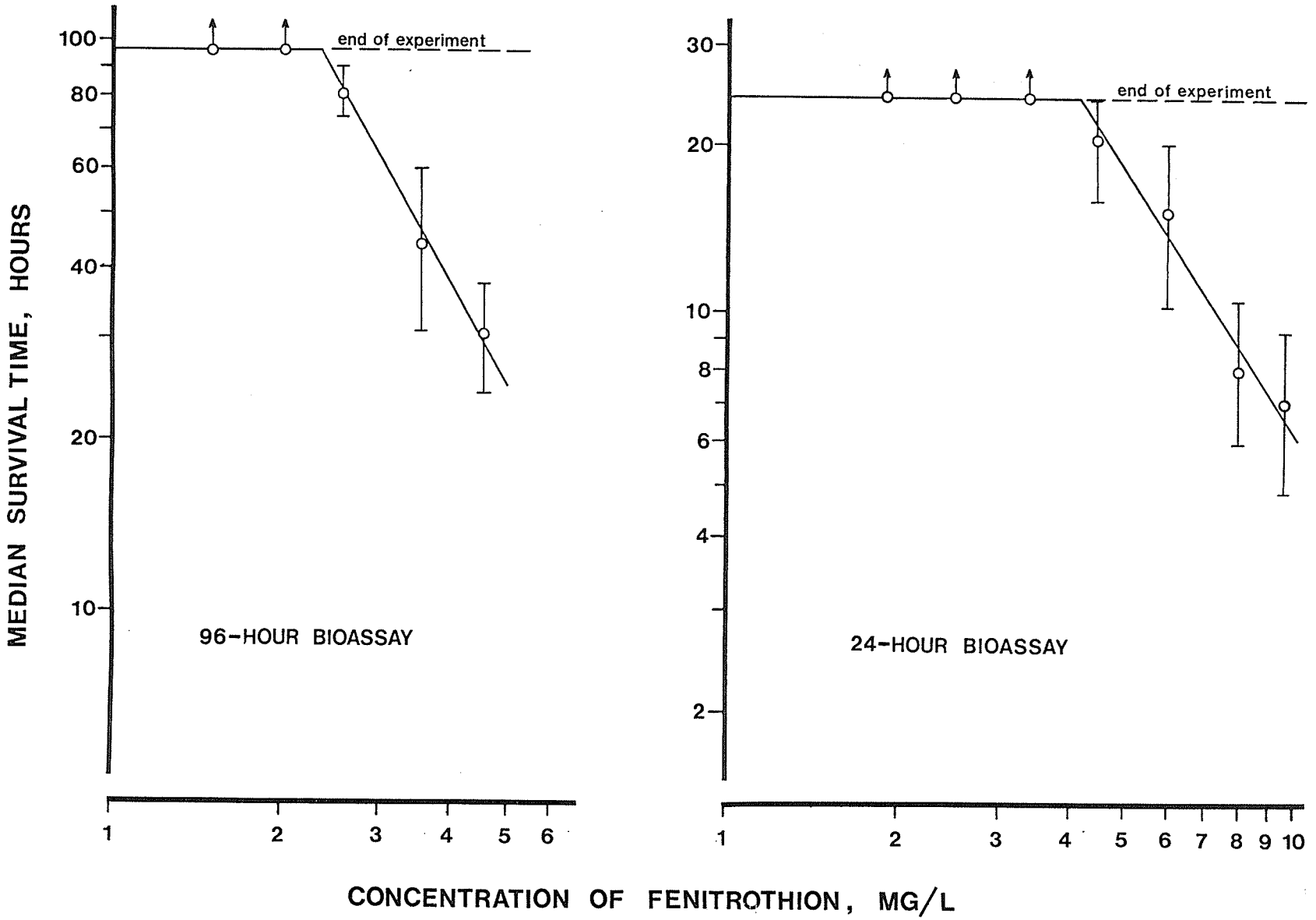

PERCENT MORTALITY
100
90
80
70
60
50
40
30
20
10
0
100
200
500
1000
2000
5000
10,000
RESISTANCE TIME, MIN
9.6$_{24}$
8.0$_{24}$
6.0$_{24}$
4.5$_{24}$
△3.4$_{24}$
4.6$_{96}$
3.5$_{96}$
2.6$_{96}$
2.0$_{96}$
▲1.5$_{96}$

Figure 13. Mortality curves for the 24- and 96-hour bioassays. Each point represents the median survival time in hours as determined from the individual mortality curves. The vertical bars represent the 95 percent confidence intervals.


MEDIAN SURVIVAL TIME, HOURS
100
80
60
40
20
10
end of experiment
96-HOUR BIOASSAY
30
20
10
8
6
4
2
end of experiment
24-HOUR BIOASSAY
1
2
3
4
5
6
1
2
3
4
5
6
7
8
9
10
CONCENTRATION OF FENITROTHION, MG/L

fenitrothion-treated tanks. In addition, the Atlox-control fish were similar, both behaviourally and, in most cases, physiologically to the control trout. Therefore, the differences recorded between the controls and the fenitrothion-treated trout cannot be attributed to the use of Atlox 3409 as the emulsifier.

The slopes of the lines in Figure 12 are statistically similar, suggesting that the mortality pressures acting in both the 96-hour and 24-hour bioassays are similar. As indicated in Figure 12, mortalities occurred in the 96-hour bioassay at concentrations of 4.6, 3.5, 2.6, 2.0 and 1.5 mg of fenitrothion/liter and in the 24-hour bioassay at concentrations of 9.6, 8.0, 6.0, 4.5 and 3.4 mg/liter. Resistance time is the time in minutes from the beginning of the test until the trout are pronounced dead as a result of fenitrothion exposure. Mortality curves for the 96-hour and 24-hour exposure periods (Fig. 13) confirmed the LC50 values above, determined by probit analysis (Finney 1971). The reciprocals of the median survival time, otherwise known as the rates of mortality, are presented in Table 3. These rates increased with increasing fenitrothion concentrations.

At concentrations where the number of mortalities was significant, there was a tendency for the larger fish to be among the early fatalities. Intermediate sized fish were most resistant to the acutely lethal effects of fenitrothion.

TABLE 3. Rates of mortality in the 96-hour and 24-hour acute lethal bioassays. Rate of mortality is equivalent to 1/median survival time (in hours).

96-hour bioassay		24-hour bioassay	
Conc. (mg/L.)	Rate of Mortality	Conc. (mg/L.)	Rate of Mortality
4.6	0.0333	9.6	0.1389
3.5	0.0247	8.0	0.1250
2.6	0.0119	6.0	0.0667
		4.5	0.0500

1.2. BEHAVIOURAL OBSERVATIONS

Brook trout exposed to levels of fenitrothion resulting in few or no mortalities displayed some behavioural anomalities attributable to insecticide poisoning. At all concentrations tested, the initial addition of fenitrothion produced mild to severe periods of coughing. Coughing subsided at sublethal levels only, but persisted at concentrations that resulted in significant mortalities. Trout that were exposed to fenitrothion in both bioassays exhibited an obvious loss of color compared to the controls (Figs. 14 and 15). At higher concentrations, the loss of color was apparent earlier in the bioassays. Most spontaneous locomotory behaviour appeared depressed, even at the lowest concentrations, compared to the controls. Untreated trout would frequently engage in exploratory behaviour, freely moving about the aquaria.

At concentrations approaching lethal levels, several behavioural changes were apparent. These behaviours have been recorded as respiratory distress, loss of equilibrium and the occurrence of the tail flex action (Figs. 14 and 15). Respiratory distress included laboured breathing, irregular ventilation rates and gulping motions at the surface. At the highest concentrations, respiratory distress was widespread, affecting all of the fish for the entire exposure

Figure 14. Onset and duration of five behavioural abnormalities during the 96-hour bioassay.

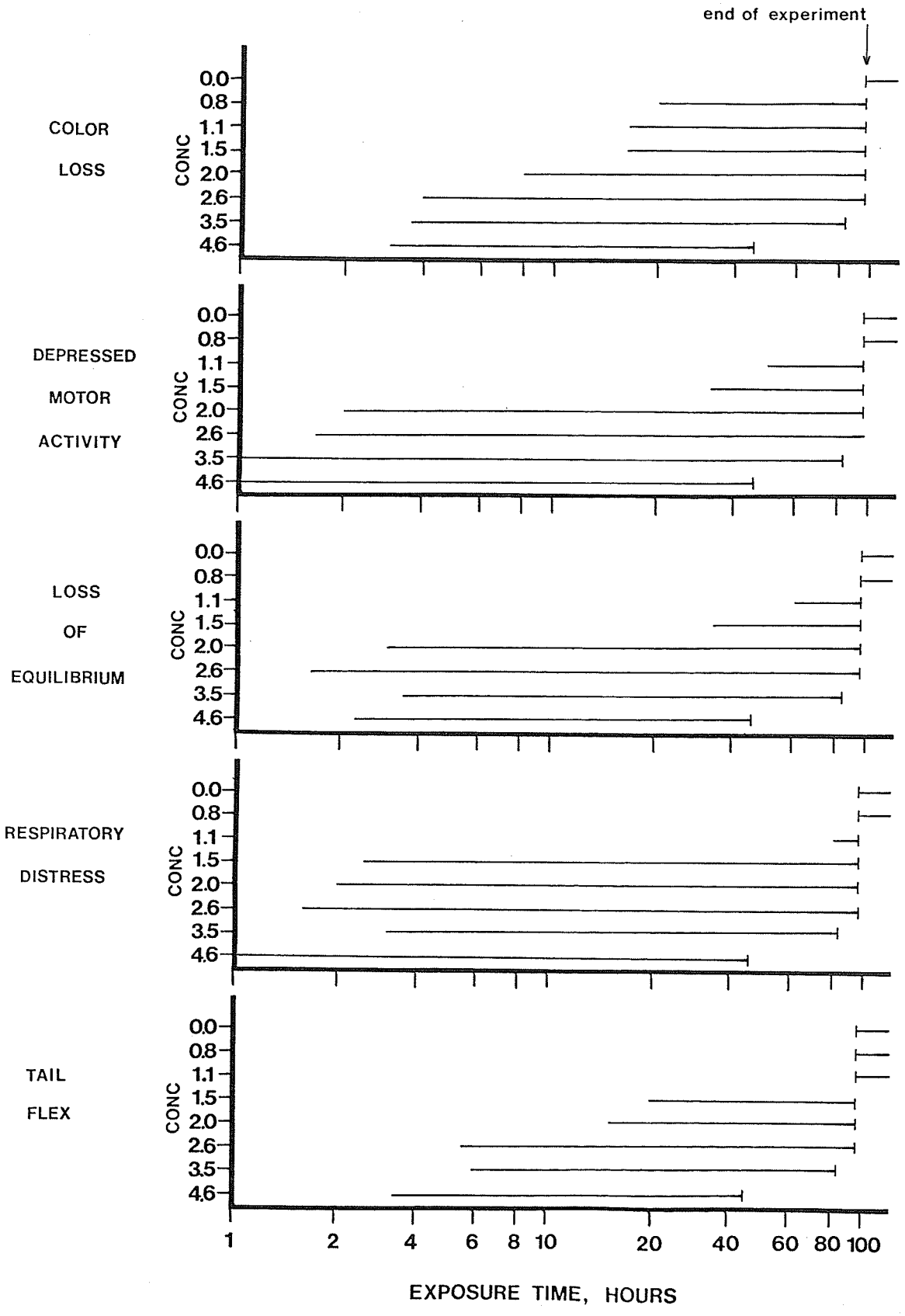
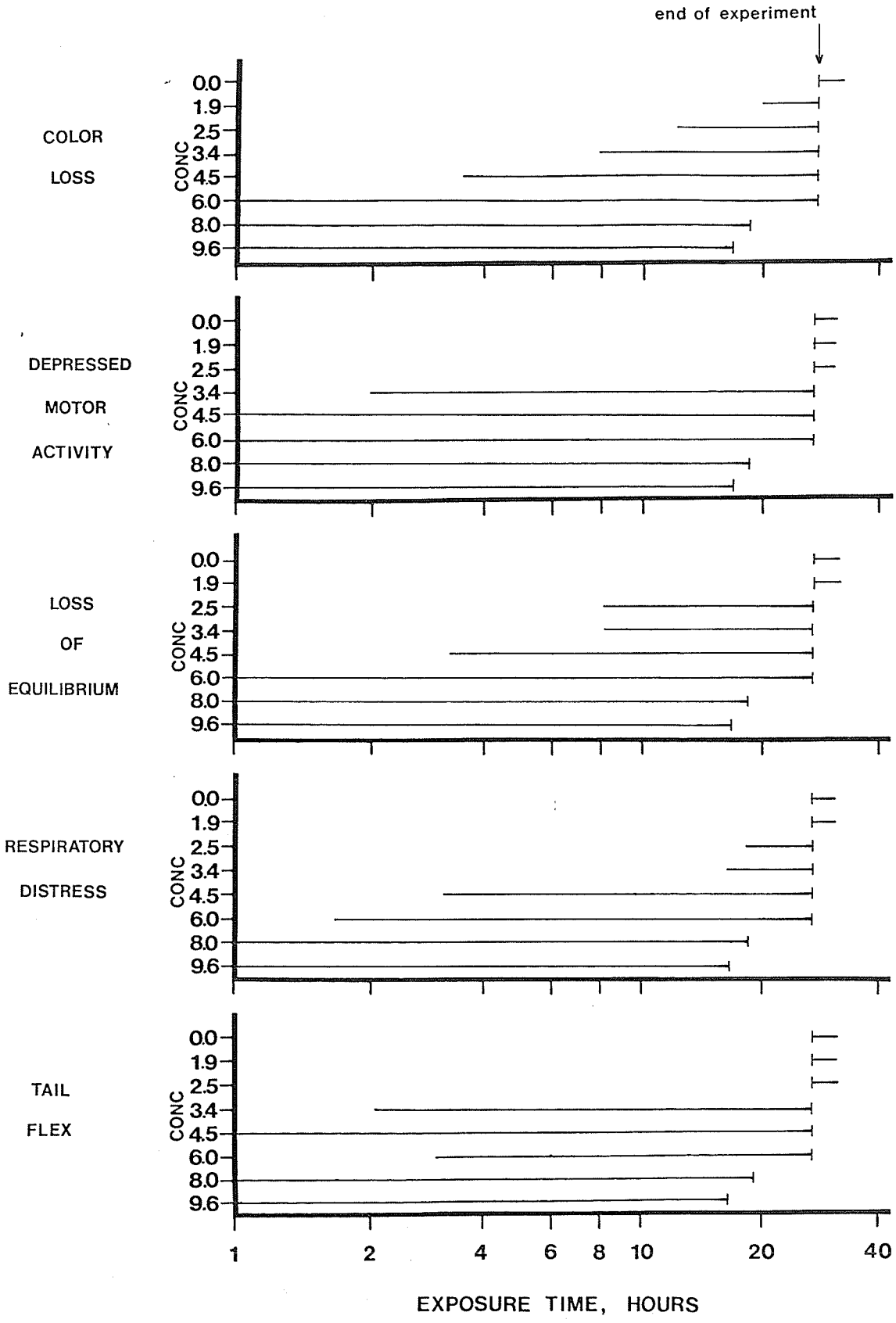

end of experiment
COLOR LOSS
DEPRESSED MOTOR ACTIVITY
LOSS OF EQUILIBRIUM
RESPIRATORY DISTRESS
TAIL FLEX
CONC
0.0
0.8
1.1
1.5
2.0
2.6
3.5
4.6
1
2
4
6
8
10
20
40
60
80
100
EXPOSURE TIME, HOURS

Figure 15. Onset and duration of five behavioural abnormalities during the 24-hour bioassay.


end of experiment
COLOR LOSS
DEPRESSED MOTOR ACTIVITY
LOSS OF EQUILIBRIUM
RESPIRATORY DISTRESS
TAIL FLEX
CONC
0.0
1.9
2.5
3.4
4.5
6.0
8.0
9.6
1
2
4
6
8
10
20
40
EXPOSURE TIME, HOURS

period (Fig. 15). Dissection of these fish, and others which were noticeably bloated, revealed a clear, mucus-like liquid in the stomach and upper intestine, often with large air bubbles present. Gall bladders in the majority of these fish also appeared exceptionally large. Brook trout that were recorded gulping at the surface, displayed bloated swim bladders in addition to the other observations above. Occasionally, symptoms similar to these were observed in the pre-exposed trout used in the swimming performance tests. Loss of equilibrium included body rolling to the sides and the occurrence of the head-up, tail-down posture usually at the surface. Loss of equilibrium was common at levels of fenitrothion approaching the median lethal concentrations and above. At most sublethal concentrations severe losses of equilibrium were infrequent and occurred only towards the end of the bioassay (Fig. 14). The tail flex phenomenon is a spastic body movement where the tail is curled back against the body and will often quickly flip over to the other side of the body. Tail flexes were accompanied with fin twitching, body spasms and paralytic type postures. At lethal levels, fish were observed to develop a body spasm which terminated with temporary paralysis of the entire body, usually with fins erect. At lower levels, body spasms and tail flexes were

less frequent and were initiated later in the exposure period (Fig. 14). Prior to death, opercles were open and the gills were flared. Body rolling and twitching fins dominated any movement.

2. SWIMMING PERFORMANCE

2.1 CRITICAL SWIMMING SPEEDS

The critical swimming speed attained by the control group of brook trout (Fig. 16) averaged 62.61 $\pm$ 5.97 centimeters per second, or an average of 3.98 body lengths per second (BL/s). The critical velocities of brook trout after a 24-hour exposure to sublethal concentrations of fenitrothion were lower than those of the controls (Table 4, Fig. 17). The decrease in critical swimming speeds was a function of the concentration of the insecticide used.

Trout exposed to the lowest concentration tested, 0.15 mg/liter, had a mean critical swimming velocity of 3.58 BL/s. This represented a non-significant decrease in velocity at $\alpha = 0.05$, using an extension of Duncan's New Multiple Range Test. All of the other sublethal concentrations tested produced critical swimming speeds significantly lower than the controls. At 3.64 mg/liter, which represented 75 percent of the 24-hour LC50, the trout were unable to maintain equilibrium and were incapable of swimming at the lowest speed tested ($<$ 1 BL/s).

Figure 16. Relationship between critical swimming speed and length for the control group of brook trout.

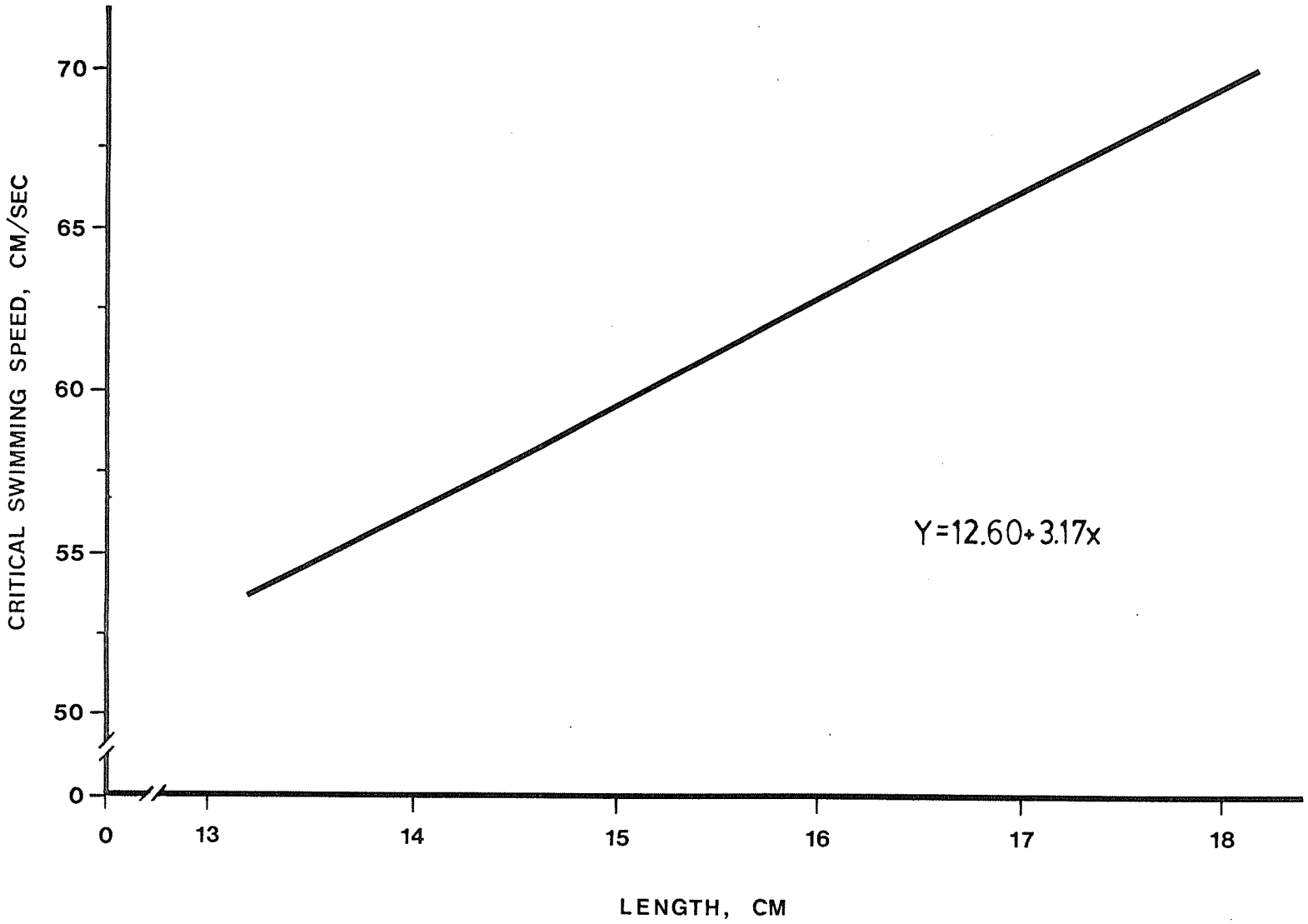

CRITICAL SWIMMING SPEED, CM/SEC
70
65
60
55
50
0
0
13
14
15
16
17
18
LENGTH, CM
Y=12.60+3.17x

TABLE 4. The average critical swimming speeds for all groups of trout tested in the swimming performance tests. Critical velocities are presented and then are corrected for the length of the individual fish and presented as body lengths per second.

Treatment mg/l	% of LC50	N	Critical velocity cm/sec $\bar{X}$	±	S.D.	Body length/second $\bar{X}$	±	S.D.
Control	0.0	10	62.61		5.97	3.98		0.22
0.15	3.13	10	63.58		7.57	3.58		0.46
0.30	6.25	10	56.56		6.05	3.23		0.35
0.61	12.5	10	47.67		5.88	2.83		0.25
1.21	25.0	10	47.22		5.60	2.74		0.29
1.82	37.5	6	24.40		3.58	1.38		0.20
		4	47.76*		4.92*	2.73*		0.19*
2.42	50.0	0	-		-	-		-
		10	56.31*		5.17*	3.36*		0.41*
3.03	62.5	3	19.32		4.91	1.19		0.31
		7	49.59*		6.51*	2.79*		0.28*
3.64	75.0	10	17.01		8.30	0.95		0.42

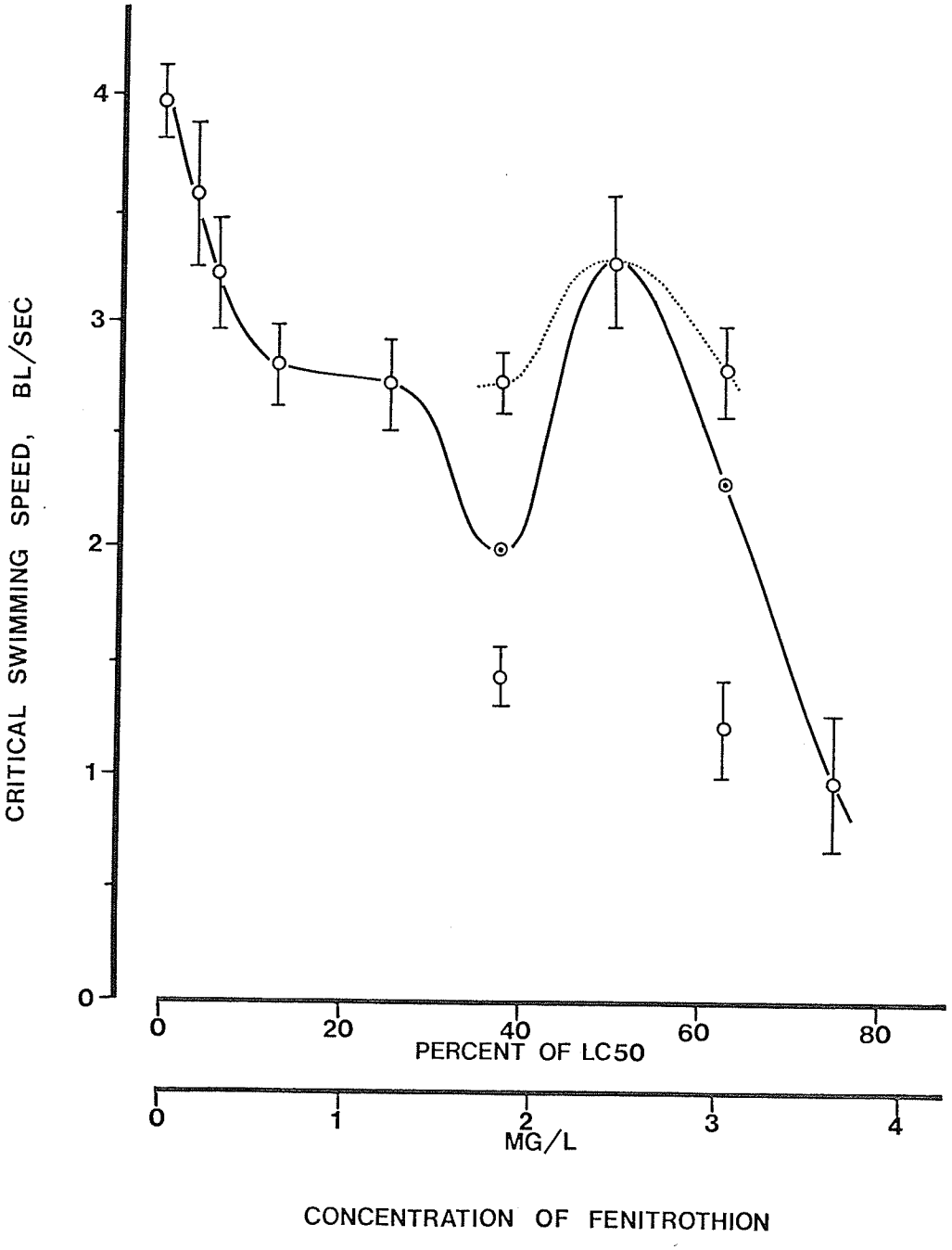
* Brook trout displaying mechanical-like swimming and exceptionally high critical swimming speeds.

Figure 17. Effect of fenitrothion on the critical swimming of brook trout exposed to sublethal concentrations of the insecticide. The vertical bars represent the 95 percent confidence intervals.

Symbols:

····· - line connecting those trout displaying exceptionally high swimming speeds

⊙ - average value of the two groups exposed to the same concentration


CRITICAL SWIMMING SPEED, BL/SEC
0
1
2
3
4
0
20
40
60
80
PERCENT OF LC50
0
1
2
3
4
MG/L
CONCENTRATION OF FENITROTHION

At 37.5 percent of the LC50, the population of fish showed a biphasic response to that dose. Forty percent of the fish swam with mechanical-like efficiency and constancy to the current imposed upon them. The two groups were easily segregated by both behavioural observations and swimming performance results. At 50 percent of the LC50, all ten of the fish tested displayed the mechanical-type swimming and performed extraordinarily well compared to fish exposed to lower concentrations. At 62.5 percent of the LC50, there was a return to a definite biphasic response with two behaviourally distinct groups. Seven of the 10 fish exposed at 62.5 percent swam significantly better than the other three. Those individuals with the lower critical velocities, appeared more 'normal' behaviourally, displaying typical deviations in swimming such as inconsistent swimming, periodic rest stops on the bottom of the swimming tunnel and some exploratory behaviour. The mechanical behaviour of the other brook trout consisted of continuous swimming with no deviations until they became fatigued. As a result, these fish generally performed better than the 'normal' fish. A line of best fit represented by the equation $Y = 3.564 - 0.027x$, does not adequately describe the data. Therefore, an undulating curve must be employed to demonstrate the relationship of the decrease

in the critical swimming speed of brook trout exposed to a variety of concentrations of fenitrothion (Fig. 17).

The initial decrease in the critical swimming speeds of those brook trout exposed to 3.125 to 12.5 percent of the 24-hour LC50 is highly correlated ($\alpha = 0.05$) with the depression of brain acetylcholinesterase activity over the same range of fenitrothion concentrations. At 25 percent, the two curves (Figs. 17 and 27) become curvilinear with concentration. Beyond 25 percent of the LC50, critical swimming speeds are not correlated with brain enzyme activity.

All brook trout exposed to fenitrothion initiated swimming behaviours earlier than control fish (Fig. 18). In the first three velocity increments, a higher proportion of treated fish were swimming compared to the controls. At all times, control trout were better able to maintain position in the swimming chamber at the lower water velocities without actively swimming.

2.2. BEHAVIOURAL OBSERVATIONS

Several behavioural parameters were monitored while the swimming performance tests were conducted and many were observed to change with increasing insecticide concentration. The control fish displayed spurts of swimming at the slower speeds with some irregular swimming throughout

Figure 18. Initiation and percent of fish swimming at each velocity increment according to the sublethal concentrations of fenitrothion used in the swimming performance tests.

Symbols:

● - control brook trout

○ - 3.125 percent of 24-hour LC50

◑ - 6.25 percent

◒ - 12.5 percent

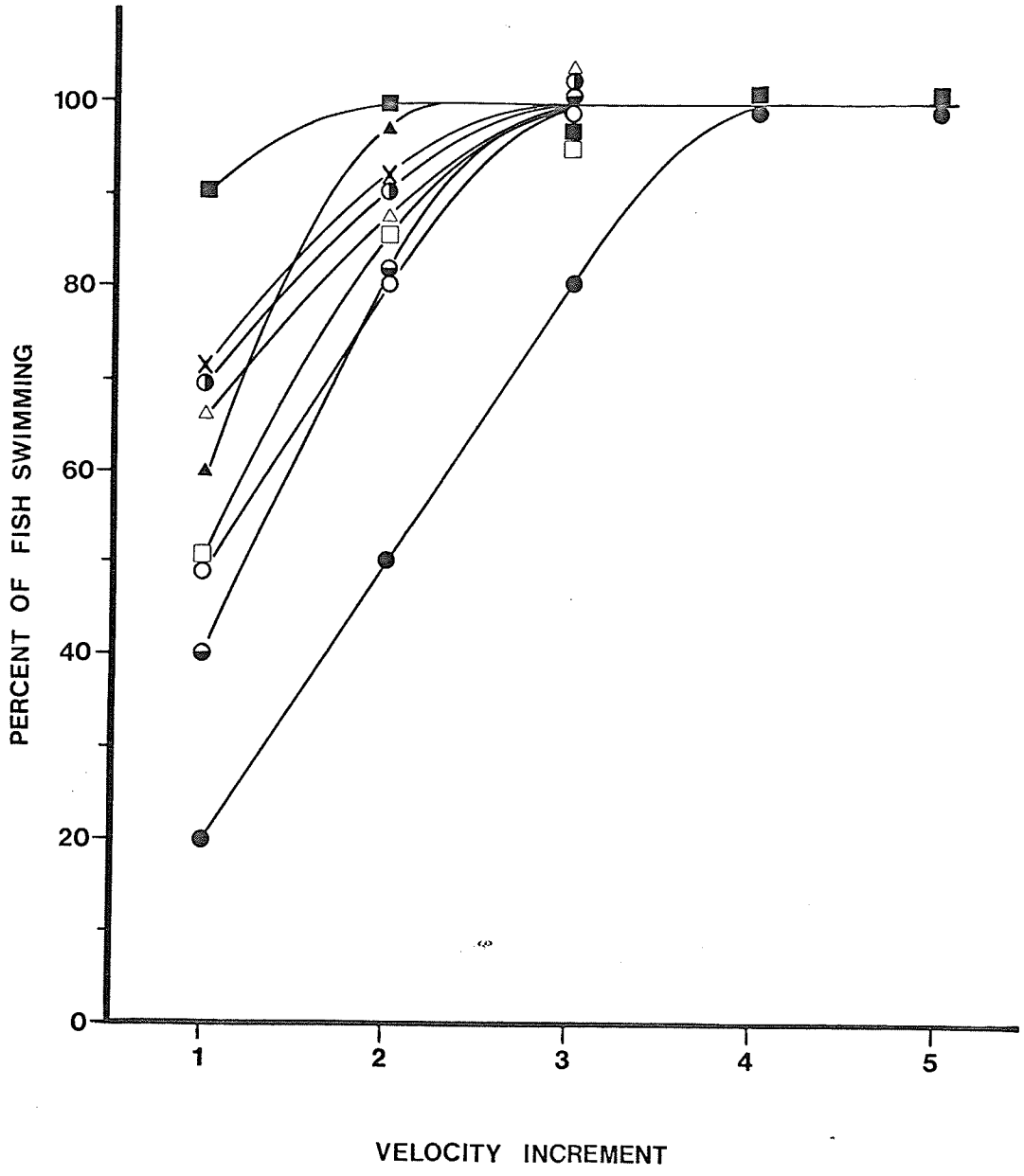
X - 25.0 percent

□ - 37.5 percent

■ - 50.0 percent

△ - 62.5 percent

▲ - 75.0 percent


PERCENT OF FISH SWIMMING
100
80
60
40
20
0
1
2
3
4
5
VELOCITY INCREMENT

the test sequence. This involved swimming back and forth in the swimming chamber, often nudging the front screen for varying periods and sitting on the bottom of the chamber with the paired fins outstretched. In some cases the larger fish were not inclined to swim until the second increment was imposed upon them. The controls, as a whole, were moderately excitable, but would adapt to the swimming chamber quickly. The instantaneous velocity increments did not alarm the control fish and they were able to adjust easily. Ventilation, which consisted of deep, wide-open mouthed cycles at the lower water velocities, became quick, short bursts with large amplitudes at the higher water velocities.

Those fish exposed to the lower concentrations of fenitrothion (up to 12.5 percent of the LC50) demonstrated a transition phase in the swimming behaviour. The majority of these trout were easily excitable and were often hyperactive in the exposure tanks. Swimming continued to be irregular at low water speeds with all fish swimming before the end of the first increment. The frequency of fish sitting on the bottom was reduced and there was a clear trend for the fish to swim in midwater. Exploratory behaviour was almost negligible. Burst swimming was predominant as the critical swimming velocity was approached. The frequency of external

stimulation required, in the form of electrical shocks, increased slightly compared to the controls. In addition, the number of fish requiring stimulation to initiate swimming also increased. Tail flexes and body spasms became apparent in some fish at the moderate concentrations. Immediately after an incremental increase in water velocity, a few fish exposed to 12.5 percent of the LC50 were observed to burp up air bubbles. Respiration was not affected but body undulations appeared to be less efficient compared to the controls. These fish also failed to capitalize on the opportunity to rest on the bottom or to use those areas favored by the control fish, where the water turbulence presumably made swimming easier.

Trout exposed to the mid-range of the concentrations began to display the biphasic response as previously described. Starting at the 25 percent level of the LC50, the fish were noticeably more calm and adjusted quickly to the swimming chamber. Fish spent the majority of the time at the top of the chamber, and often nudged at the top in an attempt to reach the surface. The head-up, tail-down posture also became more common which contributed to a less efficient mode of swimming. Body undulations required the recruitment of the entire body as opposed to mostly tail- and caudal peduncle-oriented locomotion. Cycles of respiration

were occasionally interrupted with a phenomenon best described as lock-jaw. In the middle of the mouth-opening phase of respiration, the jaw of these trout would appear to lock open. In an attempt to close the mouth, the thrashing would force the fish against the back grid, or the severe head shaking would produce a mild trauma. These tetanic muscle contractions may be the result of repeated stimuli of the muscles by excessive amounts of endogenous acetylcholine.

With progressively higher concentrations of fenitrothion, the tail flex spasms were common, breathing became labored or almost forced, the lock-jaw phenomenon became a regular occurrence and swimming became awkward and unproductive. As a complication, some of the fish at these moderate concentrations, and all the fish at 50 percent of the 24-hour LC50, actually performed better than expected. These fish are generally docile, non-excitable and were usually sitting up in the current or at the top of the swimming chamber. Swimming was initiated immediately after the pump was started and remained continuous. Deviations from swimming were negligible and the fish proceeded with a mechanical-type of consistency. Most of these fish were somewhat tipsy, and tended to roll from one side to the other which may explain why the dorsal fin and the paired fins were fully extended. Breathing was shallow with a

dominance of stiff-mouthed breathing and short quick opercle flaps. The response to electrical stimulation at the back grid was weak and occasionally delayed. At 62.5 percent of the LC50, swimming was more variable and there was a return to the biphasic response (Table 4). Respiration amplitudes were greatly reduced with mouths slightly agape and opercular flaps weak. Lock-jaw appeared during respiratory distress and occurred frequently during periods of brief stress or excitement. Breathing was discontinuous with the trout possibly adopting ram-jet ventilation as an alternative to active respiration. Head-up, tail-down posture was common and twitching fins became a regular event.

At the highest concentration tested, swimming activity became almost non-existent (Fig. 17). Equilibrium was lost in all cases with only minor recoveries to the head-up, tail down posture. Severe coughing occurred and was interspersed with weak, shallow breathing. Only two fish were able to complete the first increment and they subsequently failed early in the second increment. In all cases, the fish were positioned at the top of the swimming chamber with their caudal fins in close proximity to the electric grid. The voltage required to elicit the fright reaction at the grid was three times higher with this group of fish as compared to the controls.

2.3. RESPIRATION DATA

2.3.1. BASELINE RATES

The sublethal concentrations used in the 24-hour pre-exposure period did not result in any mortalities, even at the highest dose of 3.64 mg/liter or 75 percent of the 24-hour LC50. Prior to transferring the fish to the swimming tunnel, the frequency of coughing was recorded and a baseline ventilation rate was noted. The ventilation rate was monitored throughout the swimming performance tests, but was initially used after the transfer to the swimming chamber as a measure of excitement/calmness and as an indicator to commence the test.

2.3.2. COUGH FREQUENCY

The cough frequency data is presented (Fig. 19). The increase in cough frequency was directly proportional to the increase in fenitrothion concentration.

2.3.3. VENTILATION RATES

Throughout the swimming performance tests, ventilation rates, oxygen consumption and tailbeat frequency were monitored. The ventilation rates, recorded in the middle of each increment and while the fish were actively swimming, are presented (Table 5). These data were converted to a percentage of the resting ventilation rate and were

Figure 19. Effects of fenitrothion on the frequency of coughing in brook trout used in the swimming performance tests. The vertical bars represent the 95 percent confidence intervals.

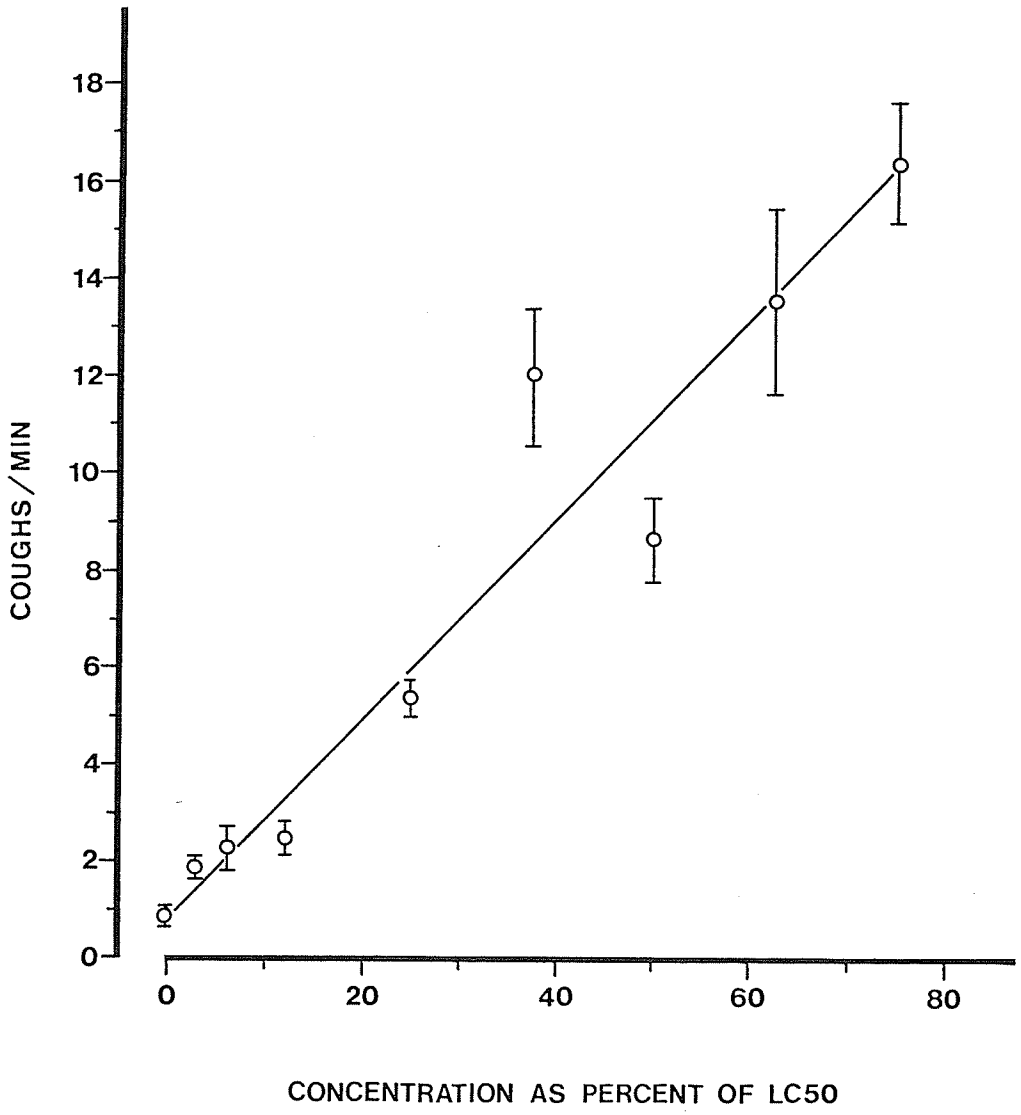

COUGHS/MIN
0
2
4
6
8
10
12
14
16
18
0
20
40
60
80
CONCENTRATION AS PERCENT OF LC50

TABLE 5. Summary of ventilation rates of brook trout in the swimming performance tests after pre-exposure to sublethal concentrations of fenitrothion.

Conc. % of LC50		Resting rate	Increment				
			1	2	3	4	5
Control	n	10	10	10	10	10	3
	mean ± S.D.	65.6±4.95	69.2±7.50	70.5±2.79	78.2±6.49	75.8±10.56	82.7±8.33
	% of resting rate	100	105.5	107.5	119.2	115.6	126.0
	range	60-72	60-84	64-72	68-88	52-90	76-92
3.13	n	10	10	10	10	10	4
	mean ± S.D.	76.3±6.68	84.6±6.75	91.3±3.20	96.3±3.30	99.7±4.35	102.8±3.77
	% of resting rate	100	110.9	119.7	126.21	130.7	134.7
	range	63-86	72-93	87-96	90-102	96-108	99-108
6.25	n	10	10	10	10	7	
	mean ± S.D.	69.2±10.22	90.6±8.46	94.5±9.30	100±3.16	100.3±4.54	
	% of resting rate	100	130.9	136.6	144.5	144.9	
	range	57-90	75-99	72-102	96-105	96-108	
12.5	n	10	10	10	8	3	
	mean ± S.D.	74.6±8.34	93.6±5.44	101.6±5.56	102.6±5.68	103.0±6.24	
	% of resting rate	100	125.5	136.2	137.6	138.1	
	range	66-84	87-105	93-114	90-108	96-108	
25.0	n	10	10	10	10	1	
	mean ± S.D.	69.6±4.06	88.5±6.82	93.0±8.00	94.5±8.36	96.0± -	
	% of resting rate	100	127.2	133.6	135.8	137.9	
	range	66-75	72-96	75-99	85-105	-	
37.5	n	10	10	4			
	mean ± S.D.	71.0±13.39	91.2±8.85	93.0±5.48			
	% of resting rate	100	128.5	131.0			
	range	45-92	78-105	87-96			
50.0	n	10	10	10	10	7	
	mean ± S.D.	70.0±4.11	83.3±10.20	86.0±7.47	89.3±10.79	87.4±8.68	
	% of resting rate	100	119.0	122.9	127.6	124.9	
	range	63-75	72-96	73-99	70-108	71-96	
62.5	n	10	9	8	7	3	
	mean ± S.D.	66.1±7.88	73.3±5.22	73.9±7.16	72.1±11.39	57.7±15.04	
	% of resting rate	100	110.9	111.8	109.1	87.2	
	range	54-81	63-81	66-87	50-87	48-75	
75.0	n	10	3	1			
	mean ± S.D.	82.0±10.28	101.0±6.24	99.0± -			
	% of resting rate	100	123.2	120.9			
	range	69-96	96-108	-			

plotted versus swimming speed (Fig. 20). Only the trout exposed to the lowest concentration tested had ventilation rates similar to the controls through the entire range of velocities. In the other concentrations tested, the ventilation rate differed from the controls by at least 5 percent during one or more of the velocity increments. At low to moderate concentrations, the ventilation rates were markedly higher than the controls at all increments. Fish exposed to the high concentrations (50 percent of the LC50 or greater) exhibited ventilation rates initially similar to the controls, but dropping off as the critical velocity was approached. A major difference between the exposed fish and the controls, was the fact that as the critical velocity was approached, ventilation rates tended to level off or decrease, whereas with the controls, these rates continued to increase. The adoption of ram ventilation by the fenitrothion-treated trout at higher water velocities may explain, in part the leveling of ventilation rates. The lower levels of the insecticide produced elevated ventilation rates and the higher concentrations inhibited ventilation rates above 40 cm/second.

Within any one velocity increment, the highest recorded increase in ventilation rate usually occurred in those fish exposed to 6.25 percent of the 24-hour LC50.

Figure 20. Ventilation rates in brook trout exposed to sublethal concentrations of fenitrothion while in the swimming tunnel, compared to a control group of trout.

Symbols:

● - control brook trout

○ - 3.125 percent of the 24-hour LC50

◑ - 6.25 percent

⊖ - 12.5 percent

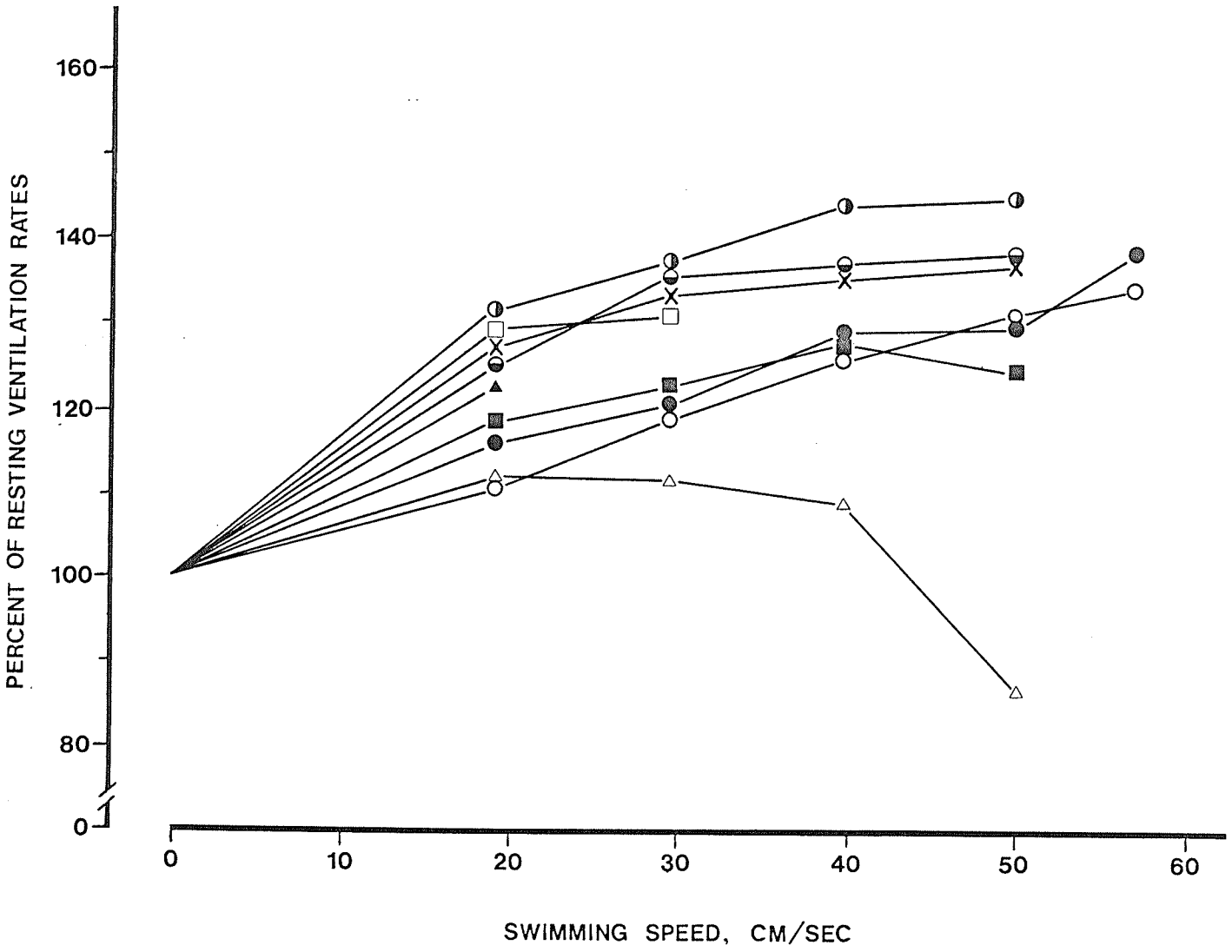
X - 25.0 percent

□ - 37.5 percent

■ - 50.0 percent

△ - 62.5 percent

▲ - 75.0 percent


160
140
120
100
80
0
PERCENT OF RESTING VENTILATION RATES
0
10
20
30
40
50
60
SWIMMING SPEED, CM/SEC

At higher concentrations, ventilation rates were significantly lower, regardless of the water velocity.

2.3.4. OXYGEN CONSUMPTION

Oxygen concentration was monitored as an indicator of the oxygen consumption by the fish under forced exercise. The logarithm of oxygen consumption was linearly related to swimming speed, but with increasing concentrations of insecticide the relationship became weaker. Compared to the controls, oxygen consumption in the treated groups of fish was significantly reduced (Fig. 21). The rate of oxygen consumption was lower in all treated groups with the exception of those fish exposed to 50 percent of the LC50. This group performed better as a whole than anticipated and their higher activity was reflected in the oxygen consumption data. This relationship suggested that oxygen consumption may be more closely related to swimming activity, rather than to the direct influence of fenitrothion.

2.4 TAILBEAT FREQUENCY

Tailbeat frequencies were recorded at the mid-way mark of each increment. Using the average frequency during acclimation as the baseline, subsequent tailbeat rates were recorded as percentages of this baseline and were plotted against swimming speed (Fig. 22). A curvilinear

Figure 21. Effect of swimming activity on the uptake of oxygen by fenitrothion-treated brook trout compared to a control group of trout.

Symbols:

● - control brook trout

○ - 3.125 percent of 24-hour LC50

◑ - 6.25 percent

◒ - 12.5 percent

X - 25.0 percent

□ - 37.5 percent

■ - 50.0 percent

△ - 62.5 percent

▲ - 75.0 percent

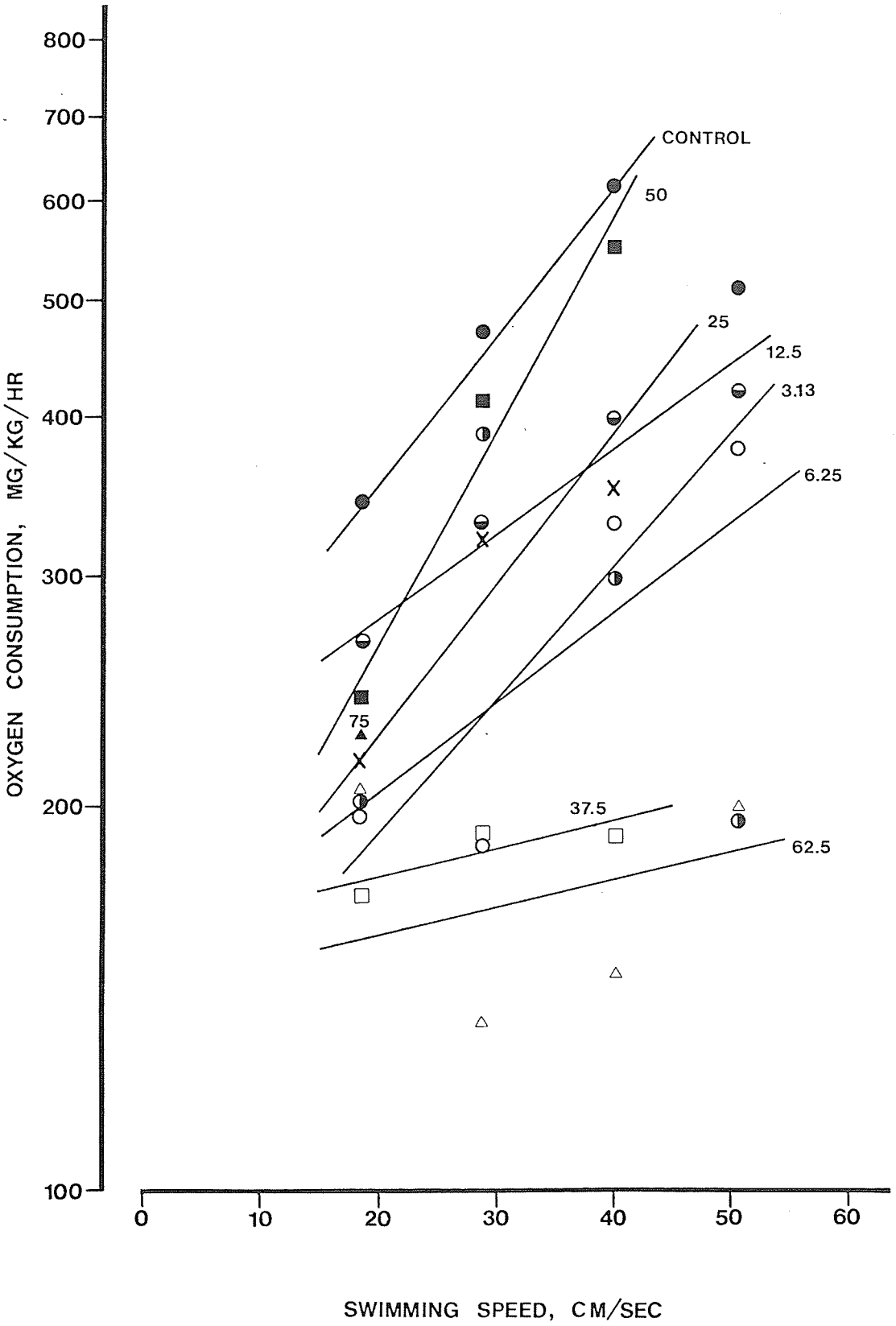

800
700
600
500
400
300
200
100
OXYGEN CONSUMPTION, MG/KG/HR
CONTROL
50
25
12.5
3.13
6.25
75
37.5
62.5
0
10
20
30
40
50
60
SWIMMING SPEED, CM/SEC

Figure 22. Tailbeat frequency of the brook trout used in the swimming performance tests. Symbols:

● - control brook trout

○ - 3.125 percent of the 24-hour LC50

◑ - 6.25 percent

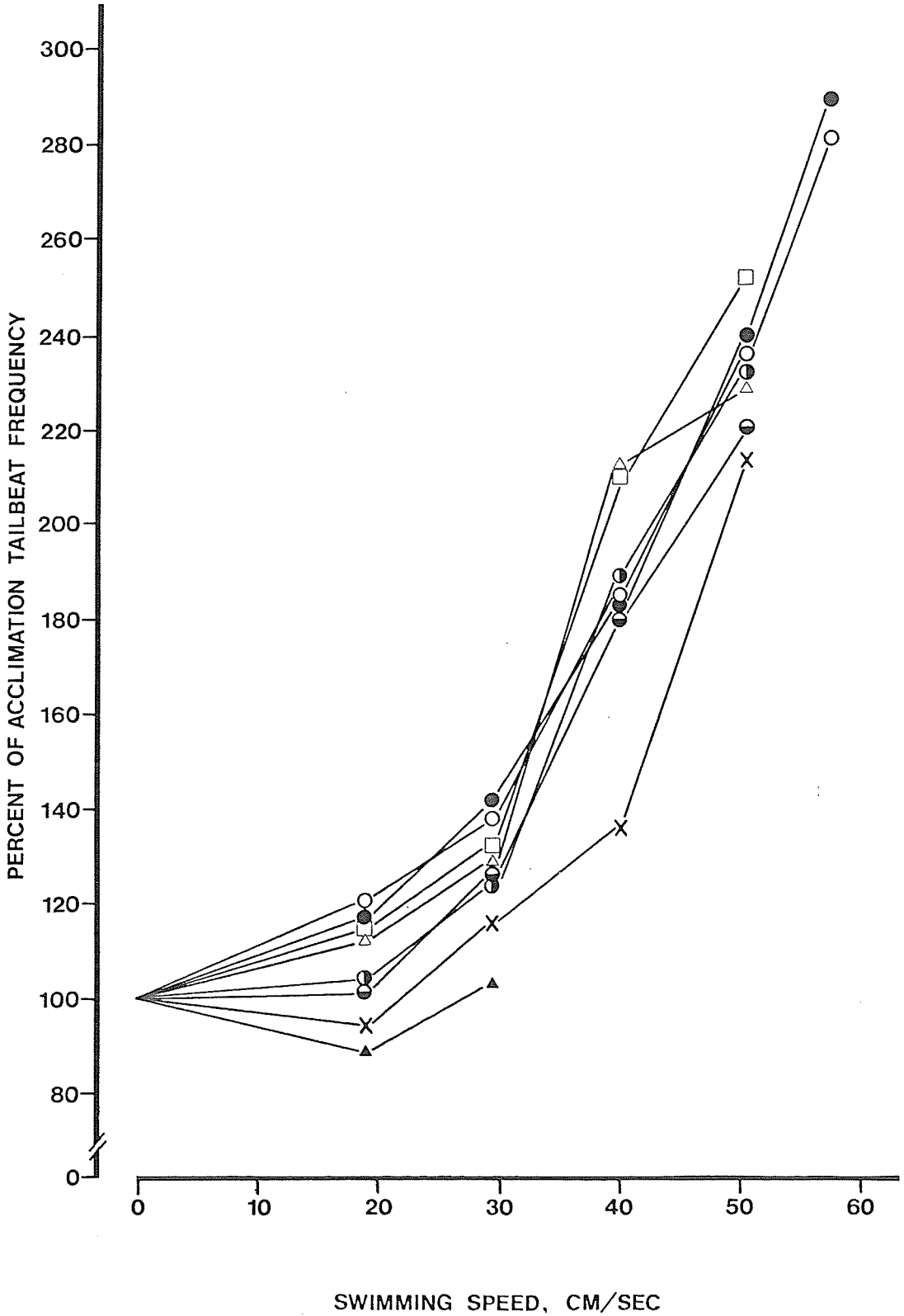
⊖ - 12.5 percent

X - 25.0 percent

□ - 37.5 percent

△ - 62.5 percent

▲ - 75.0 percent


300
280
260
240
220
200
180
160
140
120
100
80
0
PERCENT OF ACCLIMATION TAILBEAT FREQUENCY
0
10
20
30
40
50
60
SWIMMING SPEED, CM/SEC

relationship exists between tailbeat frequency and swimming speed, similar to observations by Feldmeth and Jenkins (1973). With increasing fenitrothion concentrations, the deviations from the relationship increased. At the highest concentrations, the general trend was still discernible but the relationship was weaker and more variable.

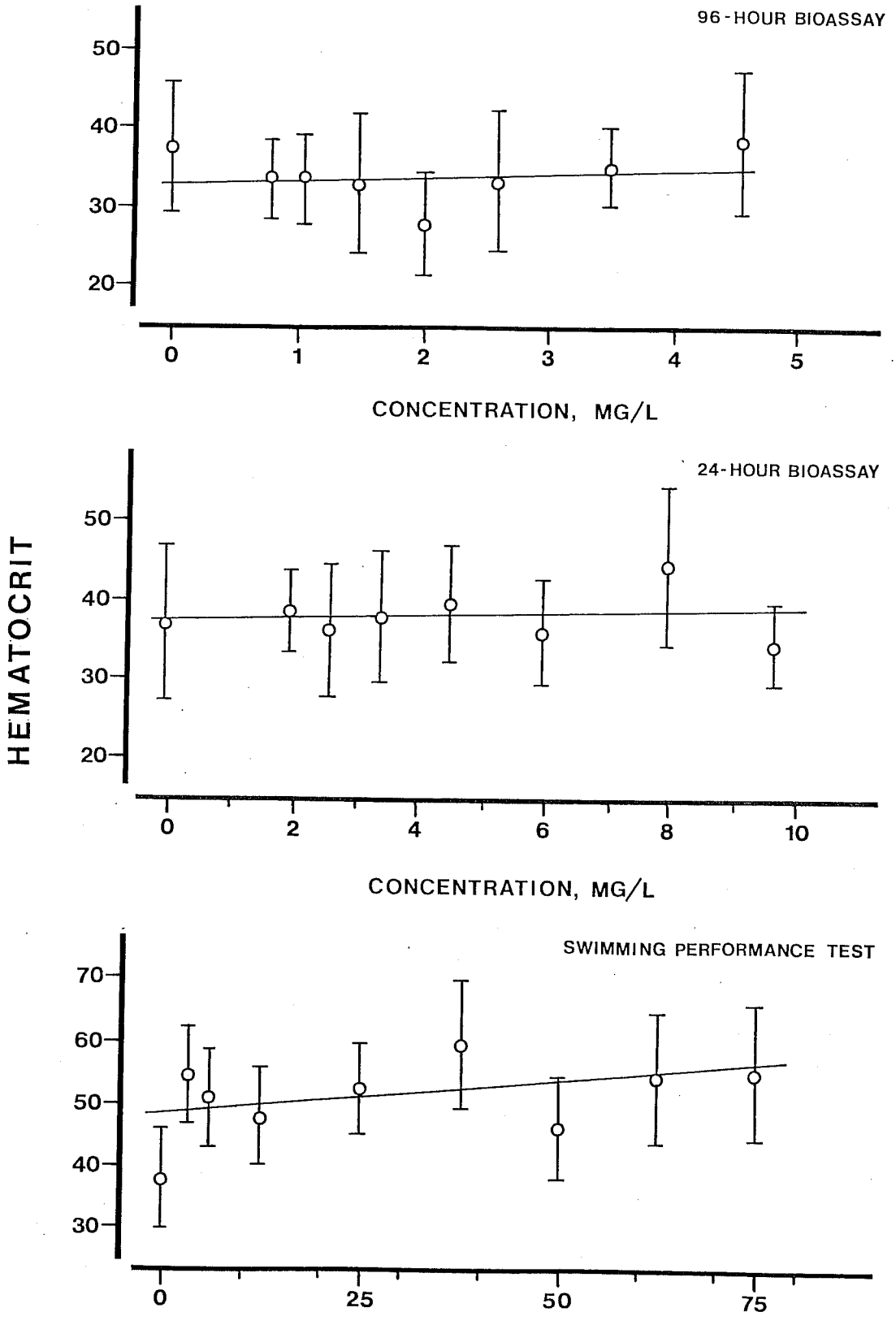
3. HEMATOCRIT

Hematocrit results from the two bioassays and from those fish used in the swimming performance tests are presented (Fig. 23). The slope of the line of best fit for the data from the 96-hour bioassay is significantly different from 0 at $\alpha = 0.05$. This longer exposure time was responsible for the difference, since exposure to comparable concentrations for 24 hours did not change the slope of the line significantly (Fig. 23). Large variations between groups and between individuals within the groups made any further inferences difficult. Those brook trout used in the swimming performance tests displayed average hematocrit values that were consistently higher than the control values.

4. ORGAN WEIGHTS

Brain, heart and liver weights were recorded immediately after removal of the trout from the exposure

Figure 23. Hematocrit values for the brook trout in the 96-hour and 24-hour bioassays and swimming performance tests plotted against concentration of fenitrothion. The vertical bars represent the 95 percent confidence intervals.


96-HOUR BIOASSAY
50
40
30
20
0
1
2
3
4
5
CONCENTRATION, MG/L
24-HOUR BIOASSAY
HEMATOCRIT
50
40
30
20
0
2
4
6
8
10
CONCENTRATION, MG/L
SWIMMING PERFORMANCE TEST
70
60
50
40
30
0
25
50
75
CONCENTRATION AS PERCENT OF LC50

tanks. Comparison of the organ weights as recorded was inconclusive. A correction factor was applied to the weights by dividing the organ weights by the body weight of the individual fish. The corrected brain weights of the brook trout used in the acute lethality tests (Fig. 24) tended to increase with fenitrothion concentration compared to control brain weight values. The slope of the line of best fit for the 96-hour corrected brain weight data differed significantly from 0 at $\alpha = 0.05$. In Figure 24, two curves are presented for each bioassay, one for those trout dying before the completion of the bioassay and the other curve for those fish surviving the exposure period. The largest brain weight to body weight ratios were obtained by those fish surviving the 96-hour exposure to fenitrothion. The smallest increases in the brain weight ratio were recorded by those trout dying before the end of the 24-hour exposure tests. This rate of increase was insignificantly different from the trout surviving the 24-hour exposure period. The length of time that the fish were exposed to the insecticide seemed to influence the rate of increase of the weight ratio. Similar analyses of the corrected heart weight and liver weight ratios did not reveal any significant changes when compared to control organ weight ratios.

Figure 24. Effect of exposure of brook trout to lethal and sublethal concentrations of fenitrothion on brain weight, corrected by the body weight of the individual trout.

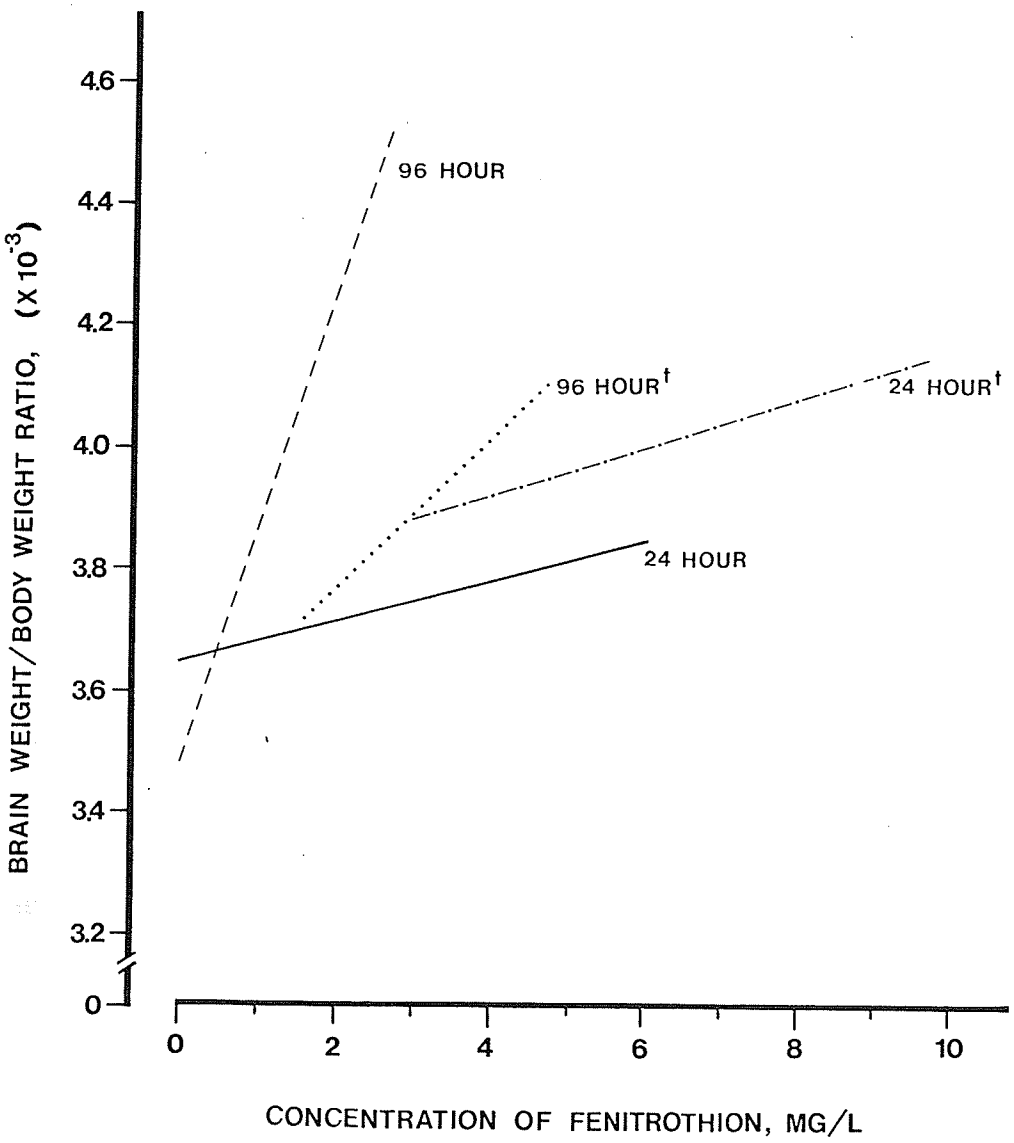
Symbols:

– – – – – – Surviving trout from the 96-hour bioassay

..... Dead trout† from the 96-hour bioassay

——— Surviving trout from the 24-hour bioassay

–·–·–·– Dead trout† from the 24-hour bioassay


BRAIN WEIGHT/BODY WEIGHT RATIO, (X 10^{-3})
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
0
96 HOUR
96 HOUR^{t}
24 HOUR^{t}
24 HOUR
0
2
4
6
8
10
CONCENTRATION OF FENITROTHION, MG/L

5. ACETYLCHOLINESTERASE ACTIVITY

Acetylcholinesterase activity in the brain, gill and heart tissues from brook trout surviving the 96-hour exposure to fenitrothion are presented in Table 6, and the activity of those tissues from dead fish are presented in Table 7. The acetylcholinesterase activity in the brain, gill and heart tissues from surviving and dead brook trout used in the 24-hour acute lethality study are presented in Tables 8 and 9, respectively. Enzyme activity in the brain, gill and heart tissues from the fish used in the swimming performance tests are presented in Table 10. The control brook trout had average tissue enzyme activity values of 16.93 ± 3.28 for whole brain, 1.76 ± 0.55 for gill and 10.46 ± 2.28 for heart samples. The units of activity are expressed as micromoles of acetylcholine hydrolyzed per milligram of protein per hour at $12^{o}C$.

Brook trout which survived exposure to fenitrothion for 96 hours had brain, gill and heart acetylcholinesterase activities compared to controls of 39.94 to 46.53 percent, 22.40 to 35.18 percent and 54.40 to 70.00 percent respectively. Moribund and dead fish in the 96-hour exposure had brain, gill and heart enzyme activities of 34.01 to 42.97 percent, 15.88 to 40.46 percent and 40.25 to 55.26 percent of the controls, respectively. Brook trout surviving 24 hours exposure to fenitrothion had brain, gill and heart

TABLE 6. Acetylcholinesterase activity (in micromoles of acetylcholine hydrolyzed per milligram of protein per hour at 12°C) in brain, gill and heart tissues of brook trout surviving after 96 hours of exposure to fenitrothion.

Conc.		Brain	Gill	Heart
0.0	n	10	10	10
(control)	mean ± S.D.	16.35±3.06	1.89±0.69	10.62±2.59
	% control	100.00	100.00	100.00
	range	11.17-20.93	0.92-3.35	8.22-16.67
atlox	n	10	10	10
control	mean ± S.D.	14.72±1.59	1.07±0.17	11.60±2.00
	% control	90.02	56.52	109.25
	range	11.43-17.50	0.75-1.23	8.97-14.78
0.8	n	10	10	10
	mean ± S.D.	7.12±0.92	0.56±0.07	6.47±0.88
	% control	43.55	29.58	60.94
	range	6.11-8.69	0.46-0.63	5.09-7.73
1.1	n	10	10	10
	mean ± S.D.	6.53±0.63	0.42±0.10	5.78±1.10
	% control	39.94	22.40	54.40
	range	5.51-7.33	0.29-0.58	3.05-6.92
1.5	n	9	9	9
	mean ± S.D.	7.25±0.83	0.58±0.07	6.94±1.67
	% control	44.34	30.69	65.40
	range	6.06-8.39	0.50-0.71	4.38-8.72
2.0	n	7	7	7
	mean ± S.D.	7.00±1.65	0.67±0.27	5.81±0.95
	% control	42.82	35.18	54.76
	range	4.96-9.42	0.37-1.12	4.72-7.17
2.6	n	3	3	3
	mean ± S.D.	7.61±1.38	0.61±0.04	7.43±0.26
	% control	46.53	32.38	70.00
	range	6.45-9.14	0.57-0.65	7.15-7.65

TABLE 7. Acetylcholinesterase activity (in micromoles of acetylcholine hydrolyzed per milligram of protein per hour at 12°C) in brain, gill and heart tissues of dead brook trout after 96 hours of exposure to fenitrothion.

Conc.		Brain	Gill	Heart
1.5	n	1	1	1
	mean ± S.D.	5.56± -	0.62± -	4.92± -
	% control	34.01	32.54	46.34
	range	-	-	-
2.0	n	3	3	3
	mean ± S.D.	6.13±0.30	0.30±0.07	5.67±0.76
	% control	37.48	15.58	55.26
	range	5.90-6.47	0.24-0.37	5.05-6.56
2.6	n	7	7	7
	mean ± S.D.	6.95±2.68	0.38±0.18	5.38±1.08
	% control	42.53	20.18	50.70
	range	3.66-9.75	0.08-0.59	4.35-7.14
3.5	n	10	10	10
	mean ± S.D.	5.65±1.69	0.41±0.07	5.04±1.25
	% control	34.56	21.45	47.46
	range	4.06-8.55	0.30-0.54	3.65-6.72
4.6	n	10	10	10
	mean ± S.D.	7.02±1.43	0.77±0.20	4.27±1.17
	% control	42.97	40.46	40.25
	range	5.19-9.37	0.52-1.18	2.27-5.87

TABLE 8. Acetylcholinesterase activity (in micromoles of acetylcholine hydrolyzed per milligram of protein per hour at 12°C) in brain, gill and heart tissues of brook trout surviving after 24 hours of exposure to fenitrothion.

Conc.		Brain	Gill	Heart
0.0	n	10	10	10
(control)	mean ± S.D.	16.35±3.06	1.89±0.69	10.62±2.59
	% control	100.00	100.00	100.00
	range	11.17-20.93	0.92-3.35	8.22-16.67
atlox	n	10	10	10
control	mean ± S.D.	13.68±1.39	1.22±0.15	9.76±3.42
	% control	83.68	64.55	91.87
	range	11.46-16.02	1.04-1.50	7.01-17.07
1.9	n	10	10	10
	mean ± S.D.	9.20±1.61	0.73±0.15	7.60±2.16
	% control	56.25	38.51	71.57
	range	6.19-11.21	0.54-1.00	5.41-12.83
2.5	n	10	10	10
	mean ± S.D.	7.60±1.63	0.78±0.14	6.69±0.90
	% control	46.46	40.94	63.03
	range	5.09-10.47	0.56-0.99	4.88-8.26
3.4	n	9	9	9
	mean ± S.D.	7.44±0.79	0.60±0.26	6.42±1.03
	% control	45.50	31.64	60.46
	range	6.24-8.69	0.35-1.10	5.38-7.74
4.5	n	5	5	5
	mean ± S.D.	9.63±1.62	0.58±0.10	8.81±2.14
	% control	58.88	31.06	82.93
	range	8.09-11.51	0.50-0.72	5.83-11.14
6.0	n	3	3	3
	mean ± S.D.	9.52±2.21	0.52±0.08	6.34±1.00
	% control	58.22	27.58	59.68
	range	8.03-12.05	0.43-0.59	5.50-7.45

TABLE 9. Acetylcholinesterase activity (in micromoles of acetylcholine hydrolyzed per milligram of protein per hour at 12°C) in brain, gill and heart tissue of dead brook trout after 24 hours of exposure to fenitrothion.

Conc.		Brain	Gill	Heart
3.4	n	1	1	1
	mean ± S.D.	4.22± -	0.64± -	6.91± -
	% control	25.81	33.81	65.08
	range	-	-	-
4.5	n	5	5	5
	mean ± S.D.	6.87±1.49	0.66±0.22	5.92±0.37
	% control	42.01	35.18	55.75
	range	5.47-8.72	0.40-0.88	5.31-6.29
6.0	n	7	7	7
	mean ± S.D.	7.27±2.75	0.54±0.11	6.26±0.84
	% control	44.49	28.42	58.95
	range	3.81-10.64	0.39-0.66	5.19-7.37
8.0	n	10	10	10
	mean ± S.D.	4.15±1.43	0.49±0.20	5.75±1.24
	% control	25.35	25.62	54.15
	range	1.52-6.33	0.24-0.71	3.73-7.51
9.6	n	10	10	10
	mean ± S.D.	5.59±1.44	0.82±0.26	6.18±1.33
	% control	34.20	43.48	58.22
	range	4.35-8.13	0.50-1.19	4.08-8.55

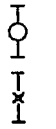
TABLE 10. Acetylcholinesterase activity (in micromoles of acetylcholine hydrolyzed per milligram of protein per hour at 12°C) in the brain, gill and heart tissue of the brook trout used in the swimming performance tests (pre-exposed to fenitrothion for 24 hours).

Conc. mg/L	Conc. % 24-hr LC50		Brain	Gill	Heart
0.0	0.0	n	10	10	10
Control	control	mean ± S.D.	18.09±3.73	1.51±0.27	10.15±1.65
		% control	100.00	100.00	100.00
		range	10.46-23.15	1.01-1.98	8.45-14.08
0.15	3.13	n	10	10	10
		mean ± S.D.	13.36±1.98	0.57±0.05	7.18±1.58
		% control	73.84	38.09	70.76
		range	10.56-15.88	0.47-0.64	5.22-9.82
0.30	6.25	n	10	10	10
		mean ± S.D.	13.50±1.73	0.66±0.14	7.88±1.68
		% control	74.65	43.93	77.60
		range	10.56-16.13	0.55-1.01	5.69-11.18
0.61	12.5	n	10	10	10
		mean ± S.D.	10.92±2.30	0.56±0.08	6.96±0.80
		% control	60.38	36.96	68.52
		range	8.05-14.20	0.43-0.69	5.42-7.94
1.21	25.0	n	10	10	10
		mean ± S.D.	8.63±1.75	0.66±0.11	6.30±0.81
		% control	47.68	43.73	62.05
		range	5.63-10.75	0.50-0.82	5.30-7.64
1.82	37.5	n	10	10	10
		mean ± S.D.	7.58±1.13	0.51±0.15	5.18±1.54
		% control	41.91	33.97	50.97
		range	5.76-9.47	0.27-0.68	2.55-6.96
2.43	50.0	n	10	10	10
		mean ± S.D.	7.63±1.03	0.71±0.12	5.32±1.77
		% control	42.16	47.25	52.39
		range	4.89-8.65	0.50-0.86	3.08-8.03
3.03	62.5	n	10	10	10
		mean ± S.D.	4.18±0.58	0.34±0.06	3.26±1.15
		% control	23.13	22.56	32.06
		range	3.08-4.99	0.23-0.43	1.88-5.14
3.64	75.0	n	10	10	10
		mean ± S.D.	5.90±1.08	0.36±0.07	4.10±1.05
		% control	32.62	24.02	40.42
		range	4.38-7.64	0.26-0.47	2.39-5.82

acetylcholinesterase activities of 45.54 to 58.88 percent, 27.58 to 40.94 percent and 59.68 to 82.93 percent of control values, respectively. Those fish found either moribund or dead in the 24-hour lethality study displayed brain, gill and heart enzyme activities of 25.33 to 44.49 percent, 25.62 to 43.48 percent and 54.15 to 65.08 percent of the control enzyme activities, respectively. Brook trout pre-exposed for 24 hours in fenitrothion and used in the swimming performance tests, had brain, gill and heart acetylcholinesterase activities of 23.13 to 74.65 percent, 22.56 to 47.25 percent and 32.06 to 77.60 percent of the controls, respectively. In general, gill enzyme activity was the most severely depressed of the three tissues examined. Heart tissues were the least affected, displaying enzyme activities closest to the control levels. Average enzyme levels of dead fish tissues were generally lower than those in surviving brook trout, however, this difference was not statistically significant. The level of enzyme activity tended to decrease with increasing insecticide concentrations, with some deviations from this trend at the extremely high levels of fenitrothion (Figs. 25 and 26). At these extreme concentrations, the number of mortalities was high with the majority of the fish succumbing early in the exposure tests. Their enzyme

Figure 25. Acetylcholinesterase activities as percent of control values in the brain, gill and heart tissues in the surviving and dead brook trout following exposure to fenitrothion at various concentrations during the 96-hour bioassay. The vertical bars represent the 95 percent confidence intervals.

Symbols:



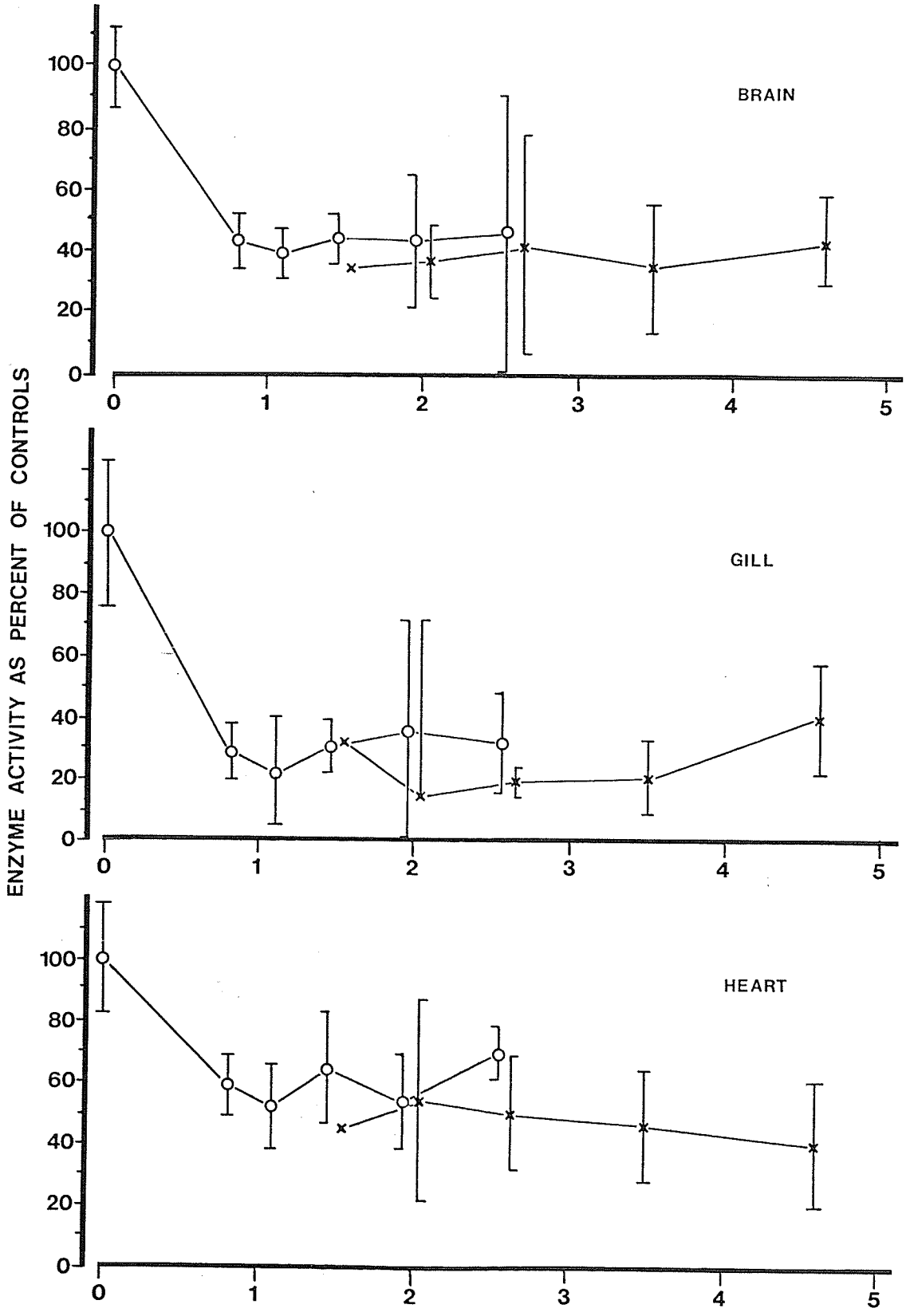
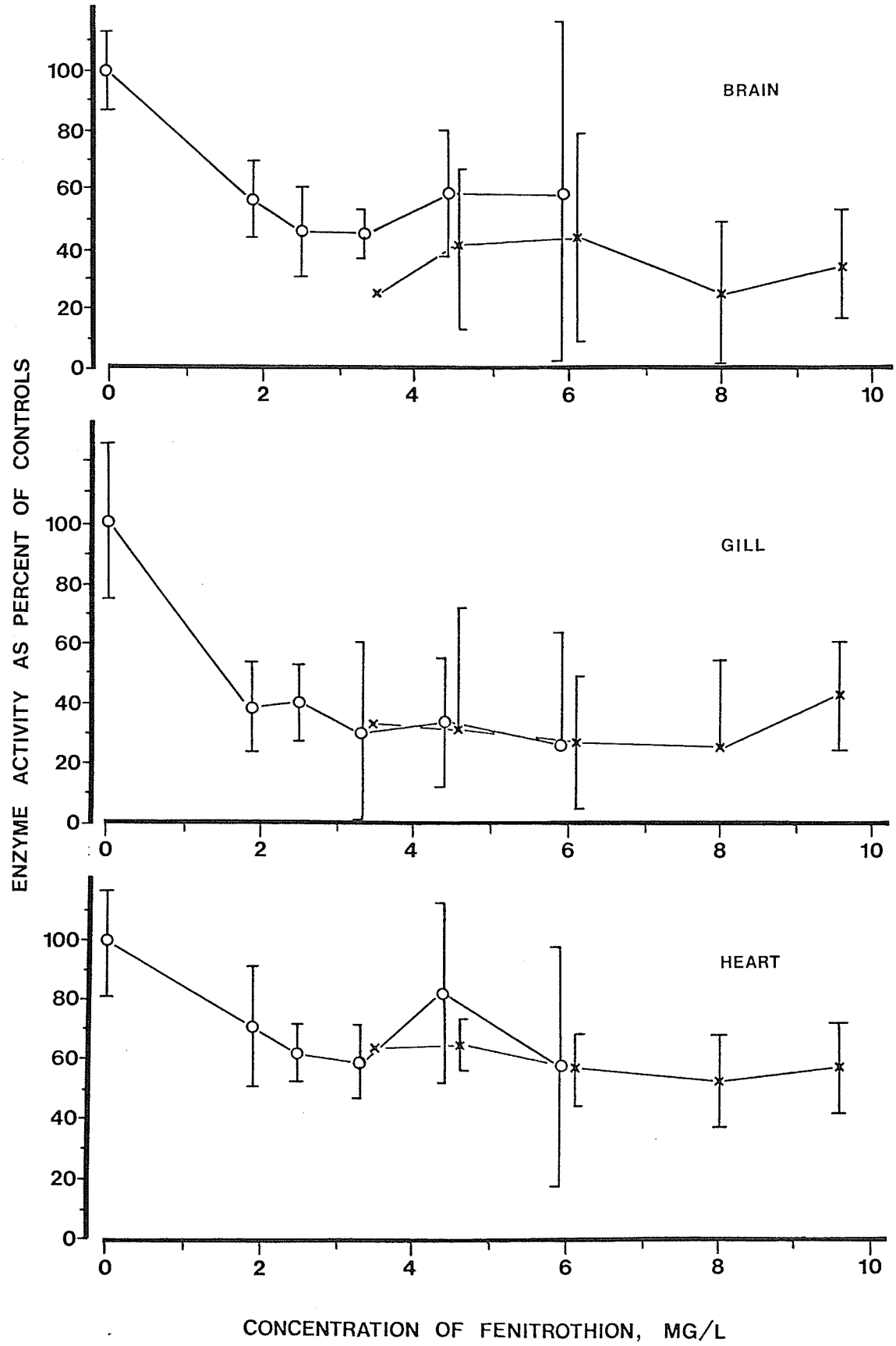

BRAIN
GILL
HEART
ENZYME ACTIVITY AS PERCENT OF CONTROLS
CONCENTRATION OF FENITROTHION, MG/L
0
20
40
60
80
100
0
1
2
3
4
5

Figure 26. Acetylcholinesterase activities as percent of control values in the brain, gill and heart tissues in the surviving and dead brook trout following exposure to fenitrothion at various concentrations during the 24-hour bioassay. The vertical bars represent the 95 percent confidence intervals.

Symbols:

 - surviving brook trout

 - dead brook trout


BRAIN
GILL
HEART
ENZYME ACTIVITY AS PERCENT OF CONTROLS
CONCENTRATION OF FENITROTHION, MG/L
0
20
40
60
80
100
0
2
4
6
8
10

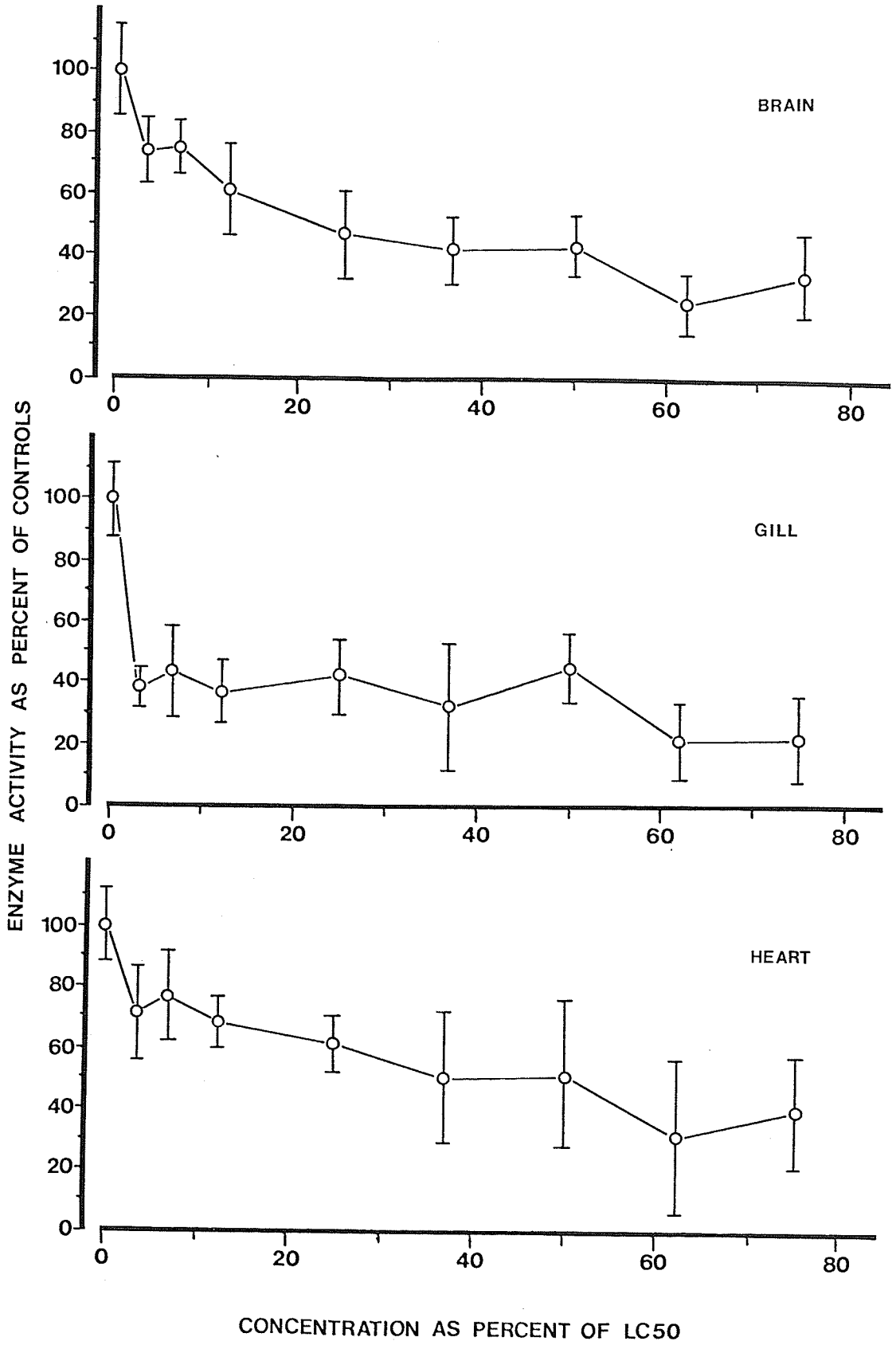
activities were frequently higher than the enzyme activities in fish at lower concentrations. This evidence suggested that the rate of enzyme inhibition was a factor in the mortality rate at these concentrations of fenitrothion. In several cases, the average brain acetylcholinesterase activity in surviving fish was lower than in brain samples taken from dead fish (Tables 6 through 9).

Figures 25, 26 and 27 show the acetylcholinesterase activity as a percentage of the controls in the three tissues of dead and surviving brook trout after exposure to fenitrothion under the specified conditions. The three tissues displayed a similar pattern of inhibition over the range of concentrations in each test, but the degree of inhibition between the tissues was significantly different. The inhibition of enzyme activity in all three tissues studied does not have a simple dose-response relationship. In the majority of cases, enzyme inhibition appears to have an all-or-nothing type of association with the concentration of fenitrothion. The brain enzyme activity in the fish that were used in the swimming performance tests had a temporarily linear relationship with insecticide at the lower levels only. This initial decrease in activity may be a significant factor in the decreased critical swimming speeds observed over the same range of concentrations.

Figure 27. Acetylcholinesterase activities as percent of control values in the brain, gill and heart tissues in the brook trout following pre-exposure to fenitrothion at sublethal levels and after testing for their critical swimming speeds. The vertical bars represent the 96 percent confidence intervals.

Symbol:

 - average value of 10 fish


BRAIN
GILL
HEART
ENZYME ACTIVITY AS PERCENT OF CONTROLS
CONCENTRATION AS PERCENT OF LC50
0
20
40
60
80
100

Beyond 25.0 percent of the 24-hour LC50, the brain enzyme activity levels off at approximately 40 percent of the control values. Similar plateaus are recognizable in the curves in the other tissues studied.

In order to determine how the mean enzyme activities differ as a result of exposure to the various concentrations of the insecticide, an extension of Duncan's New Multiple Range Test for group means with unequal numbers was used (Tables 11 through 15). This test was used to determine which of the differences between group means were significant and which were not at the 0.05 level of confidence. The results of the multiple comparison test are reported by the 'underlining' method. An examination of the Tables revealed that the mean enzyme activity of the tissues sampled from the treated groups of brook trout were significantly lower than the control groups. In some instances, the Atlox control group differed significantly from the control group. Given the variability in the values for enzyme activity, this difference was not unexpected. In general, the groups treated with fenitrothion did not differ significantly. The mean enzyme activity levels in surviving fish did not differ significantly from enzyme activities in dead fish for any of the tissues tested. The decreasing sample size as the LC50 concentrations were approached in both of the acute lethality studies contributed to the misleading high

TABLE 11. Comparisons of the mean acetylcholinesterase activity in the brain, gill and heart tissues from brook trout surviving a 96-hour exposure to fenitrothion, using an extension of Duncan's New Multiple Range Test. Insignificantly different means are indicated by the underlining method.

Tissue		Means in Ascending Order						
Brain	conc.	1.1	2.0	0.8	1.5	2.6	Atlox	0.0
	mean AChE activity	6.53	7.00	7.12	7.25	7.61	14.71	16.35
Gill	conc.	1.1	0.8	1.5	2.6	2.0	Atlox	0.0
	mean AChE activity	0.42	0.56	0.58	0.61	0.67	1.07	1.89
Heart	Conc.	1.1	2.0	0.8	1.5	2.6	Atlox	0.0
	mean AChE activity	5.78	5.81	6.47	6.94	7.43	10.62	11.60

TABLE 12. Comparisons of the mean acetylcholinesterase activity in the brain, gill and heart tissues from dead brook trout after 96 hours of exposure to fenitrothion, using an extension of Duncan's New Multiple Range Test. Insignificantly different means are indicated by the underlining method.

Tissue		Means in Ascending Order				
Brain	conc.	1.5	3.5	2.0	2.6	4.6
	mean AChE activity	5.56	5.65	6.13	6.95	7.02
Gill	conc.	2.0	2.6	3.5	1.5	4.6
	mean AChE activity	0.30	0.38	0.41	0.62	0.77
Heart	conc.	4.6	1.5	3.5	2.6	2.0
	mean AChE activity	4.27	4.92	5.04	5.38	5.87

TABLE 13. Comparisons of the mean acetylcholinesterase activity in the brain, gill and heart tissues from brook trout surviving a 24-hour exposure to fenitrothion, using an extension of Duncan's New Multiple Range Test. Insignificantly different means are indicated by the underlining method.

Tissue		Means in Ascending Order						
Brain	conc.	3.4	2.5	1.9	6.0	4.5	Atlox	0.0
	mean AChE activity	7.44	7.60	9.20	9.52	9.63	13.68	16.35
Gill	conc.	6.0	4.5	3.4	1.9	2.5	Atlox	0.0
	mean AChE activity	0.52	0.58	0.60	0.73	0.78	1.22	1.89
Heart	conc.	6.0	3.4	2.5	1.9	4.5	Atlox	0.0
	mean AChE activity	6.34	6.42	6.69	7.60	8.81	9.76	10.62

TABLE 14. Comparisons of mean acetylcholinesterase activity in the brain, gill and heart tissue from dead brook trout after 24-hours of exposure to fenitrothion, using an extension of Duncan's New Multiple Range Test. Insignificantly different means are indicated by the underlining method.

Tissue		Means in Ascending Order				
Brain	conc.	8.0	3.4	9.6	4.5	6.0
	mean AChE activity	4.15	4.22	5.59	6.87	7.27
Gill	conc.	8.0	6.0	3.4	4.5	9.6
	mean AChE activity	0.49	0.54	0.64	0.66	0.82
Heart	conc.	8.0	4.5	9.6	6.0	3.4
	mean AChE activity	5.75	5.92	6.18	6.26	6.91

TABLE 15. Comparisons of mean acetylcholinesterase activity in the brain, gill, and heart tissues of the brook trout used in the swimming performance tests, using an extension of Duncan's New Multiple Range Test. Insignificantly different means are indicated by the underlining method.

Tissue		Means in Ascending Order								
Brain	conc.	3.03	3.64	1.82	2.43	1.21	0.61	0.15	0.30	0.0
	% of LC50	62.5	75.0	37.5	50.0	25.0	12.5	3.12	6.25	0.0
	mean AChE activity	4.18	5.90	7.58	7.63	8.63	10.92	13.36	13.50	18.09
Gill	conc.	3.03	3.64	1.82	0.61	0.15	1.21	0.30	2.43	0.0
	% of LC50	62.5	75.0	37.5	12.5	3.12	25.0	6.25	50.0	0.0
	mean AChE activity	0.34	0.36	0.51	0.56	0.57	0.659	0.662	0.71	1.51
Heart	conc.	3.03	3.64	1.82	2.43	1.21	0.61	0.15	0.30	0.0
	% of LC50	62.5	75.0	37.5	50.0	25.0	12.5	3.12	6.25	0.0
	mean AChE activity	3.26	4.10	5.18	5.32	6.30	6.96	7.18	7.88	10.15

average enzyme activity levels at those concentrations (Tables 6 and 8). In the summary of the comparisons of mean enzyme activities for the swimming performance tests (Table 15), the control tissues had significantly higher enzyme activity levels than the treated groups of fish. The fish exposed to the highest sublethal concentrations of fenitrothion had significantly lower enzyme activities. The overlap of insignificantly different means is indicative of the variability in enzyme activities. The situation was further complicated by the small increments between the insecticide concentrations tested.

DISCUSSION

1. INTRODUCTION

Each section of this research project will be dealt with separately in this discussion. In each part of the discussion, interpretation of the literature pertinent to the results will be presented and the possible implications of the results will be discussed. A summary will follow the discussion, which will present a condensed analysis of the major findings of this thesis.

2. BIOASSAY

The 24-hour and 96-hour LC50's for brook trout exposed to fenitrothion were 4.85 and 2.26 mg/liter respectively, which agrees favourably with the results of similar studies using other salmonids. No data on median lethal concentrations with respect to brook trout could be found. Wildish et al. (1971), using static lethal tests, reported that the 24-hour LC50 values for Atlantic salmon fry and parr were 4.2 and 5.0 mg/liter respectively. Rainbow trout fingerlings were found to have a 24-hour LC50 of 3.4 mg/liter and a 96-hour LC50 of 2.0 mg/liter (Klaverkamp et al. 1977). Bull (1971) reported the 48-hour LC50 for juvenile coho salmon (Oncorhynchus kisutch) to be 1.4 mg/liter. Pickering et al. (1962) found that the 96-hour LC50's for some warmwater species exposed to

organophosphorus insecticides ranged from 0.01 to 680 mg/liter. The apparent selective toxicity of some organophosphorus insecticides may enable fisheries biologists to control undesirable fish species.

The slopes of the mortality curves for the individual concentrations tested were statistically similar and ranged from 0.023 to 0.065. This implies that the mechanism, resulting in mortalities, may be the same at all concentrations and for both exposure periods. There was no evidence suggesting that the mortality pressures in the 24-hour exposure were different from those occurring in the 96-hour exposure. The mortality rates, or the rates of toxicity (Hodson and Sprague 1975), increased with insecticide concentration.

The bioassay method chosen is best described as being acute lethal. It measured a quantal response (death) usually brought about by the primary lethal effects of the toxicants. Sprague (1969) reviewed the literature and concluded that 4-day exposures were best for comparative purposes. The acute lethal effects apparently ceased within four days for many toxicants. The data were mostly from static tests which may not always agree with empirical data obtained from continuous flow bioassays. The objectionable features of the static test, as described

by Burke and Fergusson (1968) include a decline in the concentration of the toxicant during the exposure period by adsorption onto the container or other surfaces, uptake by the experimental organism and by chemical alteration. In addition, accumulation of waste products, reduction of oxygen and the growth of microbial populations may produce undesirable test conditions. This situation complicates the interpretation of the results since the fish are under additional stress. This stress extends the adaptive responses beyond the normal range to the extent that the chances of survival are significantly reduced. In a continuous flow-through type bioassay, the test solution is constantly being replaced which reduces the inherent problems in static systems. Under these conditions, the only stress-producing element is the toxicant. Brown (1968) stated that exposure to a toxicant, results in a degree of shock with subsequent non-specific effects. Errors in interpretation of bioassay data may arise if trout are assumed to display constant sensitivity to a given poison. Physiological changes throughout the year are reflected in variations in resistance (LC50's) to toxicants, fluctuating by a factor as high as 2.5 (Brown 1968).

Fish size measured as wet body weight may be a factor in insecticide toxicity. There was an apparent

trend for the larger brook trout to die earlier than the smaller fish. The most probable site of uptake by the fish is at the gills. Muir (1969) found the total respiratory surface area in a variety of fish species increased with fish weight to the power of 0.8 to 0.9. Thus, as fish increase in size, the ratio of gill area to body weight decreases (Murphy and Murphy 1971). With the ratio decreasing in larger fish, gill impairment may become a critical factor sooner compared to smaller fish (Harrison 1975). The observation that larger trout were dying earlier in the acute bioassays, reinforces this possibility. Gill impairment may be in the form of increased mucus production to protect the gill epithelia from irritants in the water (Carpenter 1930), or a disrupted gill sieve due to prolonged or severe bouts of coughing. There was evidence for both increased mucous production by the gills and observations of disrupted gill filaments in this study.

No simple relationship exists between size and toxicity. Carpenter (1927) found that smaller fish survived longer in metallic salts than larger fish. Large rainbow trout were found to die sooner in potassium cyanide solutions than the smaller fish (Herbert and Merkens 1952). However, Post and Schroeder (1971) exposed

salmonids to insecticides and demonstrated that the increase in survival times was a function of size. Each compound appears to exert its effects in a fashion characteristic to that particular compound.

3. SWIMMING PERFORMANCE

3.1. GENERAL INFORMATION

In the swimming performance tests in this study, the critical swimming speed (Brett 1964) is the term used to describe the maximum attainable velocity by the brook trout. Webb (1975) defined three distinct biokinetic levels of swimming by the time interval (t) that a given speed can be maintained by the fish. With velocity increments increasing every 10 minutes, the level of swimming being monitored was the sustained speed, as opposed to burst speed ($t < 15$ sec) or cruising speed ($t > 200$ min). For those fish that did not fatigue at the exact beginning or end of an increment, the critical swimming speed was determined by interpolation as described by Brett (1964). The brook trout had an average critical swimming speed of 3.98 body lengths per second, or 62.61 cm/second.

Some concern has been expressed with respect to the size of the velocity increments in swimming performance tests (Beamish 1978). The 10-minute time period was chosen as the most appropriate for the objectives of this study.

The times used at each step may vary from a few minutes (Fry and Hart 1948; MacLeod 1967; Larimore and Duever 1968; Oseid and Smith 1972) to an hour or more (Brett 1964; Rao 1968; Houde 1969; Griffiths and Alderdice 1972; Kutty and Saunders 1973). Peterson (1974) tested step-test intervals from 0.5 minutes to 60 minutes. At intervals over 30 minutes, the duration of the interval had only a slight effect on the mean critical velocity. At intervals shorter than 15 minutes, the critical velocity steadily increased (Peterson 1974). Brett (1964) implemented a 75-minute period for respiratory metabolism studies and later recommended the use of 200-minute step-test intervals for true swimming performance tests (Brett 1967). Swimming performance was measured on a variety of fish species from the Mackenzie River by Jones et al. (1974) using 10-minute incremental periods. They found no statistically significant differences for either the 10- or 20-minute intervals. Dahlberg et al. (1968) also chose the 10-minute interval, given that the velocity increments are relatively small. Doubling the length of the interval did not appreciably effect the critical swimming speeds of coho salmon (Davis et al. 1963). The trend for higher critical velocities at the shorter incremental steps may be due to increasing use of the white muscles (Peterson 1974).

Jones (1971a) found no change in the critical swimming velocity of rainbow trout when velocity increments between one-sixth to one-ninth of the maximum speed were used. Brett (in Beamish 1978) was unable to find any effect using increments as large as one-fourth of the critical swimming speed. Velocity increments used in this study (11.9 cm/sec per increment) were less than one-fifth of the critical velocity of the controls. These increments satisfied both the recommendations by Brett and the time limitations imposed by the possibility of enzyme recovery. Increments of 10 cm/sec produced the highest critical speeds and therefore have been cited as a suitable choice (Beamish 1978).

In this study, most of the errors in the determination of the critical swimming speeds have been corrected for in the calculations, as described earlier. One additional source of error that cannot be removed is the variation introduced by the individual fish. In most cases, once the flow was imposed upon the fish, they immediately oriented themselves against the current and adjusted their swimming speed to equal the velocity of the water. This response to a current has also been observed by other authors (Blaxter and Dickson 1959; Harden Jones 1963; Dodson and Young 1977; Dodson and Mayfield 1979). Some individuals are able to select regions of the swimming chamber where the velocity is

minimal, or where there are standing waves or areas of turbulence (Blaxter 1969). In the present study, the control trout spent the majority of the time in mid-water or on the bottom of the chamber compared to the treated fish, which tended to swim closer to the top of the tunnel. The control fish would presumably be better able to determine which areas require the least effort to maintain position. This suggests that the mid-water to bottom region would have the most beneficial characteristics for fish to swim with the least amount of energy expended.

The lack of procedural uniformity in swimming performance studies have complicated interpretation and comparison of the results of various authors. In some instances, these variations in procedures are necessary under the restriction of the experimental design and compound involved. Such was the case in this study. The possibility of recovery from enzyme inhibition imposed limitations on the time allowed between the end of the exposure period and the commencement of the swimming performance tests. Peterson (1974) noted some recovery of the exposed fish during the lengthy swimming test and preceding 24-hour rest period; shortening the rest period to 2 hours had little effect on the critical swimming speed of control fish. To minimize any recovery from the

insecticide, a rest period of 0.5 hours was adopted in this study. Behavioural observations gave support to the suitability of this time period.

3.2. BIOLOGICAL FACTORS

Swimming performance is affected by several biological and physical factors associated with the aquatic environment. These include fish length, body weight, prehistory, maturity, water temperature and dissolved oxygen (Thomas et al. 1969; Webb 1975; Beamish 1978). Water temperature has a direct effect on the metabolic rate of the fish, with an optimum for activity at about 15^{o}C. The way in which some of the other factors affect swimming performance will be discussed below.

The length of the fish is a major component of the critical swimming velocity. Rainbow trout had a cruising speed that was dependent on length to the power of 0.4 (Fry and Cox 1970), but Bainbridge (1960, 1962) determined this speed-length exponent to be 0.58 for the same species. Boyar (1961), studying Atlantic herring, found that their endurance increased as body length increased. However, Brett and Glass (1973) found that the critical speeds of sockeye salmon relative to body length decreased with increasing body size. Some authors believe the larger fish have a higher relative scope for activity (Fry 1947;

Basu 1959) but the drag on larger fish may offset any advantages such as increased scope or body musculature (Brett 1965).

A condition factor has been proposed to provide a measure of the well-being of the fish (Carlander 1950 in Jones 1971a), and to allow for comparison between stocks of fish. The formula for the factor is $100X(W/L^3)$, and is an index relating their weight (g) and length (cm). Fessler and Wagner (1969) reported that naturally occurring stocks of trout have a condition factor equal to 1.0. The brook trout used in the present study had an average condition factor of 0.998, essentially the same as natural stocks of trout. It is difficult to relate physical fitness and swimming performance to the condition factor (Jones 1971a) but as Brett (1964) pointed out, the implication exists.

Vincent (1960) demonstrated that wild brook trout were able to sustain themselves against a current in a stamina tunnel longer than domestic trout. The hatchery brook trout were found to be heavier per unit length, compared to wild caught trout, probably because of their higher fat content. Otherwise, the two groups of brook trout were physically similar. Presumably, these hatchery trout have condition factors greater than 1, and are

predicted to be poor swimmers. This suggests that weight may be a factor in determining the critical swimming speed. Rearing wild and domestic trout from the egg stage, in the hatchery under similar conditions, produced comparable results (Vincent 1960), with wild trout performing better. Dickson and Kramer (1971) stated that the scope for activity was similar for both wild caught and hatchery reared rainbow trout.

The most influential factor creating the different critical swimming velocities between authors appears to be the genetic background of the fish stocks. In spite of efforts to standardize most of the variables, reports of significant differences in critical speeds persist in the literature. Peterson's (1974) control brook trout performed significantly better than the control brook trout, from a domestic stock, used in this study. The source of Peterson's trout was not indicated. Minor differences may have resulted from procedural inconsistencies, but genetic differences are the more probable cause. Thomas and Donahoo (1977) demonstrated that variations in swimming performance do exist between different strains of rainbow trout. More specifically, Tsuyuki and Williscroft (1977) examined the genotypically different forms of rainbow and steelhead trout and correlated the phenotype to swimming

performance. They found that the trout homozygous for liver lactate dehydrogenase (Idh $H\alpha^{A}$) swam 2.3 times as long as those trout with the homozygous allele Idh $H\alpha^{B}$. Green (1964) tested brook trout reared from wild and domestic parents and found the wild stock performed better than the domestic stock.

To what extent swimming performance is affected by the sex of the individual is uncertain. For the control brook trout used in this study, the critical swimming speed attained by the males and females were statistically similar at $\alpha = 0.05$. Brett (1965) found that the shorter males performed better than the females. The difference here is probably due to the size effect more than sex differences. Jones et al. (1974) could not attribute the intraspecific variation to maturity, condition factors or sex.

A decrease in environmental oxygen has been shown to result in decreased swimming performance in teleosts (Basu 1959; Davis et al. 1963; Jones 1971b; Kutty and Saunders 1973). Critical oxygen concentrations were determined to be 2.5 mg O_2/liter for rainbow trout at 15°C and 5.2 mg O_2/liter for Atlantic salmon at 15°C (Kutty 1968; Kutty and Saunders 1973; Dizon 1977). Schiewe (1974) tested juvenile Chinook salmon in supersaturated

water and found swimming performance decreased above 106 percent saturated. The drop in performance was attributed to the preliminary stages of gas bubble disease, where an air embolism becomes lodged in a critical organ or the red blood cells hemolyse (Rucker 1972). Dahlberg et al. (1968) determined that in general, increases in ambient oxygen did not increase critical swimming speeds.

At the commencement of all swimming performance tests in this study, oxygen levels were adjusted to between 96 and 99 percent of saturation (Elmore and Hayes 1960), thus ensuring an adequate supply of oxygen without the complications of either inadequate or excessive amounts of oxygen. Lack of sufficient oxygen at the cellular level of the contractile muscles will ultimately initiate anaerobic metabolism and eventually fatigue. Fry (1971) and Smit et al. (1971) determined that the failure to swim in hypoxic water was due to hypoxic depression of the central nervous system.

Dahlberg et al. (1968) also found that levels of carbon dioxide, up to 48 mg/liter, did not alter the swimming performance of largemouth bass (Micropterus salmoides). Similar increases in carbon dioxide did affect coho salmon (Oncorhynchus kisutch) (Dahlberg et al. 1968).

3.3 SWIMMING PERFORMANCE AND INSECTICIDE EXPOSURE

The control brook trout had an average critical swimming speed of 3.98 body lengths per second. This value is comparable to the range of values for other teleosts (Brett et al. 1958; Boyar 1961; Dahlberg et al. 1968; Beamish 1970; Jones et al. 1974; Otto and Rice 1974), but less than the critical swimming speeds for other salmonids (Brett and Glass 1973; Webb and Brett 1973; Jones et al. 1974; Beamish 1980). Compared to other salmonids, brook trout are not outstanding swimmers (Beamish 1980).

The brook trout exposed to sublethal concentrations of fenitrothion in the present study showed a decrease in swimming performance similar to that reported by Peterson (1974). The shapes of the two curves are identical up to approximately 1.5 mg/liter, the highest concentration that Peterson (1974) tested . Statistically significant decreases in critical swimming speeds occurred at 0.3 mg/liter in this present study, which represented a level of fenitrothion less than 1 order of magnitude higher than levels commonly found in streams sprayed with the insecticide (Banks and Hall 1972; Hall and Eaton 1973; Peterson and Zitko 1974). Fenitrothion concentrations measured in forest streams subsequent to aerial spraying ranged from less than 0.001 mg/liter up to 0.08 mg/liter (Penney 1971; Flannagan 1973; Sundaram 1974a; Eidt and Sundaram

1975). The spray application rates varied from 138 to 275 g/hectare (2-4 oz./acre) (NRCC 1975). Fluctuations in the concentration of the insecticide are due to methods of application, droplet size, atmospheric and meteorological conditions, tree cover, stream size, water quality, plus many more factors influencing the amount of insecticide reaching bodies of water. Given the almost limitless number of variables, the possibility of higher concentrations beyond the no effect level is not so remote. After a spraying operation, elevated levels of fenitrothion may persist in the water from a few hours to several days (Flannagan 1973; Peterson and Zitko 1974). A comprehensive discussion of the proposed underlying mechanisms by which fenitrothion affects the critical swimming speed will follow in the discussion regarding acetylcholinesterase inhibition and oxygen consumption.

There is no consensus as to the factors which precipitate swimming failure or fatigue in fish. Brett (1967, 1972) believed that sustained swimming was limited by metabolite supply and that fatigue was brought about by energy demands exceeding the oxygen supply at higher velocities (Brett 1964). Fish invariably indulge in burst-type swimming during incremental velocity tests, most often after an increase in water velocity and as the critical swimming speed is approached. Weihs (1974)

demonstrated how burst swimming has theoretical energetic savings, which can be efficiently used to prolong sustained swimming. Burst swimming was determined by Stevens and Black (1966) to be almost entirely anaerobic suggesting that the failure of muscle activity was brought about by the high blood lactate levels and low muscle glycogen. Burst swimming was confirmed as a component of incremental tests, and a factor necessary to push blood pH to detrimental levels (Driedzic and Kiceniuk 1976; Jones and Randall 1978).

3.4. TAILBEAT FREQUENCY

Bainbridge (1958) developed an equation relating swimming speed to body length and frequency of tail beats per second. Tailbeat frequency was monitored as an indicator of swimming efficiency in this study. The type of body undulation used in locomotion changed from primarily caudal fin and peduncle oriented locomotion, to total body involvement as the critical velocity was approached. This shift in the mode of locomotion appeared at lower velocities with increasing concentrations of fenitrothion. The total body involvement seemed to be a last attempt by the fish to remain in the current and was less efficient in terms of effort expended to maintain a constant velocity. Webb (1977) found that for salmonids,

large increases in propeller efficiency were possible with moderate decreases in specific wavelengths. This would also bring about a concomitant decrease in drag coefficients and thereby an increase in swimming efficiency. The erratic type of swimming was similar to burst swimming, relying heavily on white muscle involvement. White muscle cells are generally anaerobic, thus producing large amounts of lactate and contributing to fatigue (Walker and Emerson 1978; Beamish 1966). White muscle in skipjack tuna, Katsuwonus pelamis, becomes functional at a speed of 3 body lengths per second (Rayner and Keenan 1967). This may not apply to all teleosts since the multiple innervation of the white fibers may elicit a graded response (Bone 1964; Hudson 1973). Biochemical and physiological differences between red and white muscle have been documented (Johnston 1975; Johnston et al. 1975; Wardle 1977), suggesting physical separation of the two muscle types. With the shift in locomotion to whole body undulations, white muscle involvement has increased dramatically, and presumably produced large amounts of lactate.

4. RESPIRATION

4. 1. OXYGEN CONSUMPTION

Oxygen consumption is used as a indicator of stress, efficiency of respiration and as an expression of

the effort dissipated through locomotion. When measuring the critical swimming speed, the extent to which aerobic and anaerobic processes contribute at the maximum velocity has not been thoroughly investigated (Beamish 1980). Aerobic metabolism appears to be functional up to 93 percent of the critical swimming speed (Driedzic and Kiceniuk 1976). Below this speed, there is no accumulation of lactate because it is oxidized at the gills. Above this speed, aerobic processes are inadequate to oxidize the lactate and the fish enters oxygen debt. Metabolic requirements are temporarily being met by anaerobic means. However, with increasing levels of lactate present in the body and muscles, swimming efficiency decreases rapidly. Delivery of oxygen to the locomotory muscles as well as the supportive organs is heavily dependent on the well-being of every other organ system of the fish body. It is this dependence that makes the measurement of swimming performance a sensitive test.

Oxygen consumption by the control brook trout in this study was determined by methods similar to that used by Evans (1972) and that of other authors (Brett 1964; Beamish 1980). The standard rate of oxygen consumption was well above that determined for brook trout (Beamish 1980) and for rainbow trout (Brett 1965; Skidmore 1970;

Webb 1971a). The difference was probably due to the shortened recovery period allowed, handling, and the general increased activity. The maximum oxygen consumption for brook trout in this study was 610 mg/kg/hour. This value is higher than any other value given in the literature for the active oxygen consumption for brook trout (Gramham 1949; Job 1955; Basu 1959; Beamish 1980). The difference in oxygen consumption may also be attributable in part to the genetic background (Green 1964) and to the time of year (Beamish 1964). Sockeye salmon have been documented as having consumption rates as high as 895 mg/kg/hour (Brett 1964). Klyashtorin (1975) found sockeye to be the least resistant to oxygen deficiency and the charrs, including brook trout, to be the most resistant of the groups examined.

Maximum oxygen consumption has been predicted to occur when the oxygen supply to the tissues is optimal (Jones 1971a), which occurs at 15^{o}C for salmonids (Basu 1959; Brett 1964). Below this temperature, activity is not limited by oxygen availability given normoxic conditions, but instead by inadequacies in the oxygen delivery systems within the fish. Therefore, in the swimming performance tests run at 11^{o}C, the oxygen content in the water is sufficient to facilitate the maximum possible scope for

activity. Jones (1971b) and Kiceniuk and Jones (1977) reported that at 15°C, the maximum oxygen consumption is 7.5 to 10 times the resting rates, and can be as high as 15 times basal levels (Brett 1964, 1965, 1972). The rate of consumption for the brook trout in this study increased only by a factor of 4, presumably due to the relatively high standard rate of consumption. Fish are assumed to be making their maximum effort immediately before the critical swimming speed, and as a result, they are also consuming oxygen at a maximum rate (Alexander 1974). Gordon and Chow (1974) noted a sharp increase in oxygen uptake in two marine teleosts just prior to their critical swimming speeds. No similar sharp increases were detected in the present study.

For the control brook trout, the linear relationship between the logarithm of oxygen uptake and swimming speed was similar to that found in rainbow trout (Brett 1964; Kiceniuk and Jones 1977). This relationship broke down with increasing concentrations of fenitrothion. The general effect of swimming is to increase oxygen uptake (Muir and Niimi 1972) possibly by facilitating venous return (Randall 1970a). Oxygen consumption was better correlated to swimming performance than to insecticide concentration, as exemplified by those trout exposed to 50 percent of

the 24-hour LC50. Any direct effect by fenitrothion on oxygen uptake was masked by its close association with activity. The reduced scope for activity in trout exposed to the insecticide is also an indication of a lower potential for survival (Alexander 1974).

Oxygen availability to the fish and to the tissues has a major effect on endurance and swimming performance in general. Less than optimal conditions regarding oxygen transport within the fish will eventually contribute to fatigue. At steady swimming speeds, Kutty (1968) determined the respiratory quotients were constant and below unity, suggesting aerobic swimming conditions. Under normal conditions, swimming is limited by the inability to supply enough oxygen to the gills and/or tissues, inability to remove metabolic wastes or, inadequate activation of enzymatic processes (Jones 1971b). Resistance to oxygen debt and the ability to mobilize glycogen reserves depends on preconditioning (Hochachka 1961). During steady activity the limiting factors are the supply and removal of oxygen and carbon dioxide at the gills and tissues (Jones 1971b). Failure to satisfy these demands results in the immediate collapse of the swimming ability of the fish. Rainbow trout are asphyxiated when the respiratory quotient reaches 1.4 (Kutty 1968).

Presumably, a similar respiratory quotient would be observed as the fish approach the critical swimming speed, and anaerobic processes begin to replace aerobic sources of energy.

The maximum weight-specific oxygen consumption of rainbow trout was found to be approximately 528 mg/Kg/hour (Kiceniuk and Jones 1977), slightly below that of Rao (1968) and Webb (1971b) and the maximum rate of 610 mg/Kg/hour for the control trout in the present study.

The proportion of the increase in oxygen consumption delivered to the locomotory muscles during activity is a matter of speculation. Stevens (1968) failed to demonstrate any shunting of blood away from visceral organs of rainbow trout to locomotory muscles during exercise. At speeds above 90 percent of the critical velocity, blood pH decreases markedly as a result of either respiratory or metabolic acidosis (Kiceniuk and Jones 1977). The erratic swimming behaviour observed in this study and by Kiceniuk and Jones (1977) at the higher velocities, was symptomatic of white muscle activity and desperate attempts by the fish to maintain its position in the swimming chamber. White muscle metabolism, being anaerobic, greatly increases the amount of blood lactate and further reduces the pH of the blood, contributing to fatigue.

Trout in hypoxic conditions have been shown to have greatly reduced critical velocities, thereby demonstrating the dependence of swimming performance on oxygen availability. Cruising speeds of brook trout were appreciably reduced when levels of oxygen were lowered below 50 percent of air saturation (Graham 1949). Davis et al. (1963) also reported a drop in swimming performance with a decrease in oxygen concentration in the water. Eventually, a critical concentration of oxygen is reached when all swimming ceases. As this level of oxygen is approached, Kutty and Saunders (1973) observed a progressive decrease in locomotor behaviour, as indicated by reduced tailbeat frequency. Under exposure conditions to toxicants which may impair oxygen uptake at the gills, fish may experience the phenomenon of internal hypoxia, and respond to this situation as if it had been initiated externally. Lunn et al. (1976) and Skidmore (1970) also noted how the responses of fish to some pollutants resembled those of fish subjected to hypoxia, which strongly suggested impaired gill function.

Swimming performance is not always affected by pollutants, but when an effect is clear it is often related to impaired respiration (Sprague 1971). Since swimming performance would seem to be easily reduced by the toxic

effects of pollutants, its measurement would be one of the best means of evaluating sublethal effects (Cairns 1966). Surprisingly, swimming ability is not as badly affected by all toxicants as initially believed (Sprague 1971). Swimming performance was not influenced at all by high concentrations of ABS detergents (Cairns and Scheier 1962; Lemke and Mount 1963) in spite of concomitant damage to the gills by the detergents. MacLeod and Smith (1966) showed that swimming performance was reduced by sublethal levels of pulpwood fibers. The cause was found to be the impairment of oxygen uptake at the gills, probably the key mechanism for other toxicants (Sprague 1971). Alexander (1974) determined that the reduced oxygen consumption and critical swimming speed was due to reduced oxygen uptake across the gills. This may be due to damage and increased mucus accumulation on the gill epithelia caused by sublethal levels of DDT. These factors effectively reduced the area of gas exchange and the mucus increased the diffusion distance for the gases. Evidence for similar occurrences have been noted in this study, but the anticipated effects on oxygen uptake are difficult to relate with fenitrothion concentration.

4.2. RESPIRATORY PARAMETERS

4.2.1. VENTILATION RATES

The gills of fishes are in intimate contact with the aquatic environment and thus are particularly susceptible to the effects of toxic materials. As a result, changes in respiratory parameters have been suggested as indicators of the presence of aquatic toxicants and their sublethal effects (Schaumburg et al. 1967; Lunn et al. 1976). Parameters used as indicators of toxic exposure include ventilation rate, buccal amplitude and cough frequency (Schaumburg et al. 1967; Davis 1973; Lunn et al. 1976; Majewski and Klaverkamp 1976). Significant changes in ventilation rates occurred at levels of fenitrothion equal to 6.25 percent of the 24-hour LC50 and higher. This represents a significant change at 0.30 parts per million of fenitrothion. The initial increase in ventilation rates was followed by decreases at the highest concentrations compared to control rates. The probable mechanism underlying this response will be discussed best in relation to the acetylcholinesterase inhibition. Increases in ventilation rates after exposure to pollutants have been recorded by Skidmore (1970) for zinc and Davis (1973) for bleached kraft pulp mill effluent.

A further complicating component in relating respiration and toxicity is the mechanical factor in

toxicity proposed by Lloyd (1961). It was found that increases in the respiratory flow could be correlated to increased toxicity. Weiss and Botts (1957) found that an increase in oxygen uptake could be related to a decreased survival time in toxic solutions. The mechanism behind this was, that as the need for oxygen increased, respiratory flow increased, bringing more toxicant in contact with the gills. As a result, any environmental or physical change which affected the rate of respiratory flow of a fish would also affect the concentration of the poison at the gill surface (Sprague 1970). Data obtained by Herbert and Shurben (1963) was consistent with Lloyd's hypothesis on the effect of increased respiratory flow (Sprague 1970). In addition, further work with other toxicants has reinforced the hypothesis and has improved the predictability of the toxicity of compounds to fish (Brown 1968).

4.2.2. COUGH FREQUENCY

Cough frequency has been used as a sensitive indicator of exposure to a wide range of toxicants, but it is not a response initiated by all chemical compounds. Davis (1973) and Walden et al. (1970) observed increases in the cough frequency in fish exposed to pulp mill effluent. Schaumburg et al. (1967) found the cough rate increased significantly when fish were exposed to DDT at 0.2 - 0.35

parts per million for 5 hours. A concentration dependent increase in cough frequency was observed in the brook trout in this present study in response to exposure to fenitrothion. Similar responses to this insecticide have been documented by several authors (Bull and McInerney 1974; Majewski and Klaverkamp 1976; Duangsawasdi 1977; Klaverkamp et al. 1977). Bass and Heath (1977) demonstrated a pronounced increase in cough frequency in rainbow trout exposed to 0.4 - 0.5 mg/liter.

For the cough to be used as a respiratory parameter, the toxicant must be an irritant to gill epithelia. Hughes (1975) states that the cough response is the result of some unspecific mechanical stimulation causing a general level of irritation and increased mucus production by the gills. The cough reflex serves to clean debris or other adhering particles from the gill lamellae (Ballintijn 1969). Observations made in this present study during the preparation of the gill tissue samples for enzyme analysis, tended to support the theories of increased mucous production. Gill homogenates of those fish treated with fenitrothion had a definite layer of mucous at the top, compared to isolated occurrences of mucus in homogenates of control tissues. In this study, gills removed from fish which had undergone extensive coughing also displayed filaments which were noticeably disorganized and inconsistent

with a functional gill sieve. Hughes and Nyholm (1979) showed how rainbow trout with damaged gills participated in compensatory behaviours to correct for the inadequacies of the gas exchange tissues. Skidmore (1970) found that zinc interfered with gill function and contributed to an internal hypoxic condition. Trout were demonstrated to cough less frequently when subjected to low oxygen concentrations simulating hypoxia. Therefore, coughing is not the primary response to hypoxia (Hughes 1975).

The muscular coordination of a cough is highly characteristic, and is not a rigid reflex initiated by stimulation of some specific receptor (Hughes 1975). It is also distinguishable from the yawn reflex (McCutcheon 1970). The nature of the receptors involved in the cough response is not known (Hughes 1975). Under normal circumstances, rainbow trout have been observed to cough to remove strings of mucous and entangled particulate matter from the gills. Under conditions of exposure where severe coughing is persistent, the anatomical dead space in the gill sieve (Hughes 1966) would be increased, effectively reducing the gas exchange surface area of the gills.

4.2.3. GILL INVOLVEMENT IN TOXICITY

The gills are believed to be involved in the uptake of toxicants from the aquatic environment. Gill surface area may be a major factor in the amount of the toxicant

entering the fish tissues. No estimates of the gill lamellae surface area for brook trout have been documented (Power 1980), but estimates for the gill area for an active salmonid, Salmo trutta, range from 275 mm^2/g (Gray 1954) to 339 mm^2/g (Hughes 1966). Muir (1969) found that gill area was a function of the body weight to the power of 0.8 to 0.9. The relatively smaller surface area in larger fish may be a handicap in stressful situations and contributing to the reduced scope for activity in the fenitrothion-treated fish.

Circulation of blood through the gills is labile (Hughes et al. 1978), being influenced by many nervous and hormonal factors (Rankin and Maetz 1971; Wood 1974, 1975; Payan and Girard 1977). Oxygen uptake is ultimately restricted by the morphometric limitations of the gills (Jones and Randall 1978). During hypoxia, either externally induced or internally detected as a result of severe exercise or problems associated with the respiratory mechanisms, several changes take place to offset these adverse conditions. Among these changes there is a large increase in water pumped over the gills which corrects for the drop in uptake efficiency and offers considerable gain in oxygen to the fish (Saunders 1962; Randall 1970b).

Recruitment of secondary lamellae and gill shunts are other mechanisms which lessen hypoxic effects (Cameron et al. 1971; Davis 1972; Hughes 1972; Booth 1978).

Evidence in the literature is sporadic with regard to documented cases of physical damage to gill or other tissues by organophosphorus insecticides. Matton and LaHam (1969) found histological changes in the gills of rainbow trout exposed to Dylox, an organophosphate insecticide. Miyamoto et al. (1976) were unable to detect any histochemical changes in muscle tissue from rabbits exposed to fenitrothion. However, mammals are better equipped to detoxify the insecticide than poikilotherms. Matton and LaHam (1969) found that the pseudogills of treated fish had disrupted cell rows and the epithelial cells were abnormally elongated and contained swollen nuclei. In addition, blood vessel walls were distorted, blood cells appeared irregular and shrunken, with extravasated blood present in some tissues. Matton and LaHam (1969) attributed these changes to an electrolytic imbalance that produced an increase in cell membrane permeability. Ionic changes were recorded in fenitrothion-treated rainbow trout by Duangsawasdi (1977). These changes resembled the ion fluxes which followed acetylcholine-stimulated muscle contractions (Hoar 1975).

In the presence of an acetylcholinesterase inhibitor, there would be an accumulation of acetylcholine in the synaptic clefts and generally within certain regions of the body controlled by the parasympathetic nervous system. Cellular atropy would follow these prolonged periods of stimulation and possibly result in the histological changes reported by Matton and LaHam (1969).

4.2.4. COMPENSATORY RESPIRATION

Interruptions in the breathing cycles of fenitrothion-treated fish were observed while they were being tested in the swimming tunnel. One possible explanation for these cessations of breathing was proposed by Peyraud and Serfaty (1964 in Lomholt and Johansen 1979). They noted that the interruptions in ventilation were associated with reduction in the heart rate. This implied that changes in gill ventilation were reflexly coupled with altered gill perfusion which probably ensured efficient distribution and optimal diffusion gradients across the respiratory surfaces (Lomholt and Johansen 1979). Another explanation for the ventilation interruptions may be the adoption of ram ventilation, a form of passive gill irrigation by means of open-mouth swimming (Roberts 1974). The change in ventilation methods is not initiated by chemosensitive mechanisms as in the former explanation,

but rather by mechano-receptor detection of water flow velocity and swimming movements (Roberts 1974).

Ram ventilation seems to be the more probable mechanism since the interruptions in ventilation occur only at intermediate to high velocities, and ram ventilation is a velocity-dependent phenomenon (Freadman 1979). Roberts (1975) also stressed how size was a prerequisite for ram ventilation. The advantages of adopting ram ventilation are to reduce the cost of breathing and to increase swimming efficiency (Roberts 1974). In addition, the transfer of ventilatory work to myotomal musculature produces energy savings up to 15 percent (Cameron and Cech 1970; Shelton 1970; Hughes 1973). The onset of ram ventilation occurs between 35-82 cm/sec, or a velocity at which respiratory volume can be supported by the head pressure across the gills (Roberts 1978). Ram ventilation has been recorded in anadromous salmonids such as sockeye salmon (Onchorhynchus nerka) (Smith et al. 1967), striped bass (Morone saxatilis) and bluefish (Pomatomus saltatrix) (Freadman 1979). Webb (1975) observed a form of ram ventilation in the shiner seaperch (Cymatogaster aggregata). In this fish, the propulsive system made a significant contribution to ventilation of fish that continued to show buccal and opercular movements while swimming (Shelton 1970;

Webb 1975). Observations of ram ventilation in freshwater salmonids are sporadic. Freadman (1979) found that rainbow trout actively ventilated their gills over the range of velocities tested. Roberts (1975) concluded that some fish lack the reflex controls necessary to convert to ram ventilation.

The gills play a role in the toxic effects of fenitrothion. Significant changes in respiratory parameters such as ventilation rates and cough frequency occur at concentrations as low as 0.3 mg/liter, approximately 6 percent of the 24-hour LC50. Some of these changes are presumably attempts to compensate for the inadequacies of the respiratory mechanisms under the influence of fenitrothion.

5. HEMATOCRIT

Only those brook trout exposed to fenitrothion for four days displayed a significant increase in hematocrit compared to control fish. Increased hematocrit appear to be a normal and successful acclimation to stress but the variability of it makes the test less sensitive than survival or histopathology (Snieszko 1960). Davis et al. (1963) found that there was a high incidence of anemia in hatchery reared juvenile fish. No evidence to support this claim was found among the brook trout used in

this study. Schiffman and Fromm (1959) reported that the average hematocrit value for rainbow trout was 31.8, well below the control value of 37.3 for the brook trout in this study. Under stress or hypoxic conditions, it has been demonstrated that the hematocrit will increase, not always as a result of an increase in the number of red blood cells, but instead due to the swelling of the cells with extra hemoglobin (Holeton and Randall 1967). The hemoglobin level in trout blood may increase by 35 percent over basal levels under the influence of stress. Randall (1970a) reported that violent bursts of swimming result in small increases in body weight of trout. Evidence suggested that there was a net influx of water across the gills, followed by a shift of water to the muscles. The initial hemodilution was followed by a hemoconcentration as exercise continued (Stevens 1968). Exercise alone may therefore be responsible in part for the increased hematocrit readings in the swimming performance tests. Soivio and Hughes (1978) attributed the increase to swelling of the erythrocytes. The relatively rapid adaptation of trout blood to these changing variables is made possible by the nucleus being retained by the blood cells. This enables the red blood cells to adapt their hemoglobin to changing environmental conditions, a facility which has been lost by mammalian

erythrocytes (Eddy 1977). Leopard frogs (Rana pipiens) exposed to fenitrothion displayed progressive anemia and leucopenia until mortality occurred (Lyons et al. 1976). The inability to establish a relationship between swimming performance and the hematocrit negates the possibility that this parameter is a major contributing factor to fatigue and the reduction in swimming performance.

6. ACETYLCHOLINESTERASE INHIBITION

Fenitrothion and other organophosphorus compounds are recognized as potent inhibitors of cholinesterase. As a group, the organophosphate insecticides are the most potent and selective enzyme inhibitors known. The primary mode of action of this type of insecticide is to create a biochemical lesion or a related biophysical effect, such as the disruption of nerve membrane permeability (Casida 1973). The toxic action of the insecticide is initiated by inactivation of acetylcholinesterase (EC 3.1.1.7, acetylcholine acetylhydrolase) at localized sites in the nervous system (Casida 1973). Inhibition is accomplished by the insecticide complexing with the enzyme and by the subsequent phosphorylation of the serine hydroxyl at the esteractic site (Aharoni and O'Brien 1968; Fukuto 1969; Casida 1973). Numerous other studies have confirmed the fact that acetylcholinesterase is inhibited by organophosphates (Knowles and Casida 1966; Hogan and

Knowles 1968; Gibson et al. 1969; Coppage 1971, 1972; Lockhart et al. 1973; Hart and O'Brien 1974, 1976; Duangsawasdi 1977; Klaverkamp et al. 1977; Hyde et al. 1978; Duangsawasdi and Klaverkamp 1979). The enzyme acetylcholinesterase, reacts with acetylcholine and with organophosphorus insecticides in essentially the same way, as described by Casida (1973). The inhibition of acetylcholinesterase reduces the rate of hydrolysis of the neurohormone, acetylcholine (O'Brien 1967).

Cholinesterases are present in the brain, plasma, liver, pancreas, intestine, gill and heart tissues and are more or less sensitive to acetylcholinesterase inhibition (Casida 1973; Pilz 1974). This enzyme is essential for nerve conduction and its inhibition interferes with vital neurophysiological functions (Klaverkamp et al. 1977).

The inhibition of the acetylcholinesterase enzyme may involve a decrease in cytochrome P-450 content. Yoshida et al. (1975) determined that the inhibition of the drug metabolizing enzymes in rats by fenitrothion was due to a decrease in cytochrome P-450 in the hepatic microsomal components. NADPH-cytochrome c reductose was only slightly reduced.

The degree of inhibition necessary for disruption of normal nerve function, or for a lethal effect is

difficult to determine because of its localized nature (Gibson et al. 1969; Coppage 1972; Casida 1973). In addition, interpretation of the relationship between inhibition and fish mortality is controversial. Weiss (1958, 1959, 1961) found that inhibition of brain acetylcholinesterase of 40 to 70 percent below controls was lethal. Enzyme activities have been reported to range from 5 to 92 percent of the normal values in fish having died from exposure to organophosphorus insecticides (Weiss 1958, 1961) but Gibson et al. (1969) and Weiss (1961) also documented how some fish survived with enzyme activities as low as 10 to 20 percent of normal. Coppage (1972) arrived at a critical acetylcholinesterase activity level of 17.7 percent of normal as the lethal level. This value is well below the threshold value in this study. The mortalities in the acute lethal bioassays generally had brain acetylcholinesterase activities in the range of 40 percent of the controls. One group of surviving brook trout had an average enzyme activity of 23 percent of the controls, confirming the variability recorded in the literature.

Holland et al. (1967) considered the amount of natural variation in enzyme activity and recommended that 80 percent of the control values should fall within the

90 to 110 percent boundaries of the norm, otherwise evidence suggests that the organism has been exposed to an anti-cholinesterase agent. Gibson et al. (1969) demonstrated how some tissue handling methods can create significant variations in enzyme activity. As a result, Nicholson's (1967) 10 percent inhibition criterion was discounted and standardization of tissue handling was recommended.

The results of the enzyme activity tests from this study suggested that the trout exposed to the lethal concentrations of fenitrothion may have been affected by the rate at which the acetylcholinesterase was inhibited. Tissue samples from dead trout at the highest concentrations tested in both of the acute bioassays, displayed enzyme activities higher than the activities of tissue exposed to lower concentrations. Coppage (1971) suggested that the rate at which the pesticide penetrated certain membranes and the rates at which it is metabolically converted to a more toxic compound may be responsible for the inconsistent threshold values. The differential enzyme inhibition in the three tissues sampled in the present study, supports Coppage's (1971) theory.

The secondary effects of organophosphorus insecticides can be more easily observed than the enzyme

inhibition, but they can be just as detrimental. With the removal of acetylcholinesterase, there is a concurrent accumulation of endogenous acetylcholine in nervous tissue and effector organs (Murphy 1975). Acetylcholine is the neurotransmitter of nerve impulses at the endings of post-ganglionic parasympathetic nerve fibers, somatic motor nerves to skeletal muscles, preganglionic fibers of both parasympathetic and sympathetic nerves and certain synapses in the central nervous system (Florey 1967; Murphy 1975). The absence of acetylcholinesterase in the synaptic cleft brings about abnormal excitation of the nervous system, resulting in its deterioration (Sakai 1978). Koundinya and Ramamurthi (1978) reported an accumulation of acetylcholine in the liver, gill, intestine and kidney of fish exposed to fenitrothion. Kutty et al. (1976) indicated the role that acetylcholinesterase plays in the maintenance of the integrity of the structure and function of cellular membranes. The inhibition of the enzyme in these tissues may cause structural and functional disturbances which may augment the primary effects of the insecticide on brain and muscle tissues.

The general symptoms of organophosphate poisoning include muscle fasciculation and severe dyspnea preceding death (Murphy 1975; Hyde et al. 1978). Acetylcholine is an

extremely effective vasoconstrictor and thereby increases vascular resistance to blood flow through the gills (Keys and Bateman 1932; Wood 1975) and directs it away from the secondary lamellae (Ostlund and Fange 1962; Steen and Kruysse 1964; Richards and Fromm 1969; Wood 1975; Booth 1979). Acetylcholine also has a negative chronotropic effect on the teleost heart (Falck et al. 1966). The heart is controlled in part by the vagus nerve, which is cholinergic (Helgasson and Nilsson 1973), and would therefore be affected by the anti-cholinesterase properties of organophosphorus insecticides. Majewski and Klaverkamp (1976) reported a cardiac arrythmia in trout exposed to fenitrothion. There was also a significant increase in the Q-T interval of the heart rhythm, representing the time required for the ventricle to depolarize then repolarize.

Lehotzky and Ungvary (1976) found histological changes in the nervous system of small mammals exposed to organophosphate insecticides. The decreased conduction velocity was attributed to the destruction of mylinated fibers of the nerve and the creation of concentric intramyelinic vacoules. The delay in impulse velocity down a nerve may be contributory to other secondary effects of organophosphate exposure such as the heart arrythmia noted by Majewski and Klaverkamp (1976). Hyde et al.

(1978) found no electrocortical disturbances in the rat presumably due to the failure of the compounds reaching the higher brain centers. This may be due to the rapid, peripherally-induced inactivation typical in homeotherms, and/or inadequate penetration of the compounds into cortical neurons (Sharma et al. 1973; Joy 1976). Teleosts do not have the same capacity as mammals with respect to detoxification capabilities, therefore the residence time of the active compounds would be relatively longer. Fenitrothion is non-ionic at physiological pH (Windholz 1976), therefore the lipid-soluble insecticide would readily pass through biological membranes, including the blood-brain barrier (Oldendorf 1974).

A detailed analysis of how acetylcholinesterase inhibition influenced swimming performance and respiration will be presented in the following sections.

7. SWIMMING PERFORMANCE AND ENZYME INHIBITION

The mechanism through which fenitrothion reduces swimming performance is uncertain to date (Beamish 1978). Schneider and Weber (1975) investigated the importance of cholinesterase to neuromuscular transmission in largemouth bass after exposure to diisopropylfluorophosphate, an organophosphate insecticide. They concluded that destruction of neuromuscular function did occur, but it was not

responsible for the observed mortalities. Peterson (1974) suggested that the effects of fenitrothion on the critical velocity of trout could be due to direct action of the insecticide on the muscle, effects on areas of the nervous system concerned with muscle activity, or by indirect effects through motivational disturbances.

Peterson (1974) found it difficult to relate the decrease in swimming performance to acetylcholinesterase inhibition, because the rate of recovery of brain acetylcholinesterase was much slower than recovery of swimming ability. Swimming performance may follow blood plasma cholinesterase recovery more closely than recovery of brain enzyme activity, since Pickering and Pickering (1971) reported that plasma cholinesterase levels recover within 24 hours. Swimming ability also returned to near normal levels within 24 hours. To date, little more than speculation has been offered to explain the initial decrease in swimming performance. Considering the available information and the present data collected over a more comprehensive range of concentration, a theory has been proposed.

According to Stein (1978), the central nervous system produces a specific pattern of motor neuron impulses during a coordinated movement. Activation of

a single interneuron, or command neuron, in some fish is sufficient to elicit an entire coordinated behaviour. One class of these command neurons called trigger cells elicit swimming in invertebrates (Willows and Hoyle 1969). Single reticulospinal neurons, called Mauthner cells, are the equivalent command neurons in fish (Diamond 1971; Eaton, Bombardieri and Meyer 1977; Eaton et al. 1977; Zottoli 1977). Stimulation of the Mauthner cells produces rapid tail flexes. Mauthner cells also serve as the final common pathway for speedy coordination of complex swimming movements, and are functionally similar to the giant axons in invertebrates.

Normally, in the intact nervous system of fish, an adequate amount of acetylcholinesterase is present to regulate the inhibitory impulses for swimming from the brain. As the fish are exposed to anti-acetylcholinesterase compounds, the enzyme is removed from the synaptic clefts resulting in increased levels of acetylcholine, and gradually increasing inhibitory impulses from the brain. This situation was evident in the present study at fenitrothion concentrations below 40 percent of the 24-hour LC50. A strong correlation existed between enzyme inhibition and the initial decrease in swimming ability. At the intermediate concentrations, the acetylcholinesterase levels

within the brain tissues are well below 50 percent of the normal values. This data suggests that the fish are entering a transition phase regarding regulation of swimming behaviour, as demonstrated by those trout displaying the biphasic response at 37 percent of the LC50. Nervous impulses from the higher centers have presumably deteriorated sufficiently to effectively remove any inhibitor effect the brain may have upon swimming. The swimming performance and behaviour of the brook trout exposed to fenitrothion at 50 percent of the LC50 support this hypothesis. Muscle tissue cholinesterase remains relatively high at comparable concentrations of the insecticide (Duangsawasdi 1977), indicating that the peripheral nervous system may still be functional. Given that the swimming behaviour of fish is a natural reflex to a current being imposed upon them, the higher centers of the nervous system need not be involved in the initiation of swimming. Grillner (1976) demonstrated that chemical stimulation of the spinal cord produced rhythmic alteration between extensor activity and flexor activity in paralyzed cats, without rhythmic sensory input (Grillner and Zangger 1974). This information confirms the existence of an oscillatory mechanism probably in the lower or dorsal region of the spinal column, and not associated with the brain.

At the highest concentration, swimming behaviour was severely depressed. Brain acetylcholinesterase has been inhibited by approximately 70 percent and the level of acetylcholine in the nervous system has presumably contributed to its deterioration.

The relatively high acetylcholinesterase values obtained from some of the mortalities may seem at first inconsistent with the outcome. Current theories regarding cellular compartmentalization of the enzyme refer to an extracellular and an intracellular pool of cholinesterase (Koelle 1970). The extracellular pool is responsible for pharmacologic functions and the intracellular pool is a nonfunctional reserve for the enzyme. The distribution of the enzyme is approximately 20 percent external and functional, and 80 percent nonfunctional. Inhibition of the external pool creates the anti-acetylcholinesterase effects (McIsaac and Koelle 1959). Tissue homogenization will release the intracellular pool and possibly give misleading results with respect to the cholinergic functioning of the cell. The type of enzyme assay performed in the present study is actually a measure of total cellular activity. The higher total activity in those fish that died early at high concentrations was probably the result of rapid inhibition of the extracellular pool contributing to the fish's death,

but little intracellular inhibition. At the other extreme, some fish had very low enzyme activities but were still alive. This was probably because the insecticide had more time to penetrate the cell and inhibit the intracellular pool. One cannot ignore the possibility that the fish may be adapting to the anti-cholinesterase effects by either synthesizing new enzyme or building up a tolerance to the excessive amounts of acetylcholine.

The impaired motor ability seen in the swimming performance tests may be the result of the over-stimulated post-synaptic membranes and accompanying dystrophic changes of the muscles. Matton and LaHam (1969) observed these changes in fish muscle following exposure to organophosphorus insecticides. The muscular dystrophy was characterized by fiber fragmentation and proliferation, and a central displacement of sarcolemmal nuclei. These observations may be due to the lack of proper differentiation rather than a positive degradation (Matton and LaHam 1969). Muscle fatigue, as a result of continuous contraction, where the muscle ultimately becomes exhausted, dies and degenerates, may also be a contributing factor. A final possible source for the muscle degradation is from the direct action of the insecticide, or its by-products, on the locomotory muscle tissue (Matton and LaHam 1969).

8. RESPIRATION AND ENZYME INHIBITION

The loss of enzyme activity in muscle tissue would presumably also occur in the branchial muscles and be a source of complications for the swimming fish. Visual observations confirmed the labored breathing as predicted when the muscles are operating below peak efficiency. Oxygen consumption data discounted any evidence that suggested oxygen uptake may be a factor in the decreased swimming performance. The disruption of normal breathing rates and amplitudes implies a muscular basis for the observed changes, which in turn are precipitated by physiological alteration at the cellular level. Although some evidence suggest that the lethal effects of organophosphorus insecticides are different in fish compared to mammals (Schneider and Weber 1975), the physiological mechanisms are as yet uncertain, leaving the area open for speculation. Duangsawasdi and Klaverkamp (1979) suggested that death was a result of hypoxia, involving direct effects on the gills. Gill enzyme activity was severely inhibited in the present study, supporting the theory that the gills may play a role in causing death. However, the mechanics of respiration are also involved to some extent. Howard (1975) found that fingerling coho salmon were unable to attain normal critical swimming speeds after exposure to bleached kraft pulpmill effluent

at 10-20 percent of the 96-hour LC50. The reason for the drop in swimming performance was attributed to gill impairment by the effluent interferring with the transfer of oxygen across the gills. Davis (1973) stated that the effluent produced ventilatory abnormalities and reduced the level of oxygen in the dorsal aorta.

Fish exposed to fenitrothion often displayed a head-up, tail-down posture (Warner 1967; Bull 1971) which may be a response to respiratory distress. Respiratory depressant chemicals produced a similar response in Siamese fighting fish (Betta splendens) (Abramson et al. 1958). The swimbladder gases that were observed being released by fish in the swimming tests contributed to negative bouyancy and to the head-up, tail-down posture at the surface. The loss of equilibrium may also have an enzyme mediated component, brought about by depression of the acetylcholinesterase activity in the cerebellum, and the nervous system in general (Bernstein 1970). Rosic et al. (1974) obtained evidence suggesting a direct causal relationship between loss of equilibrium and enzyme depression. Wildish et al. (1971) reported surface swimming and the presence of gas bubbles in fenitrothion-treated salmon, but concluded that it was not neurotoxic in origin.

9. OTHER BEHAVIOUR CHANGES

Pesticides induce behavioural changes at sublethal concentrations. The central nervous system is the initial area where these changes in complex behaviour occur (Anderson and Peterson 1969). Although aerial spraying programs with fenitrothion produce little direct mortality (Hatfield and Riche 1970), behaviour abnormalities have been observed. At 1.0 mg/liter, fenitrothion suppressed aggressive and territorial behaviour (Bull 1971), necessary mechanisms to ensure adequate food distribution and utilization. Territories were not reclaimed for 2-3 weeks (Symons 1973). At 10 mg of fenitrothion per gram of food, Wildish and Lister (1973) reported that dominant fish became subordinate and did not recover for approximately three weeks. They dismissed the possibility of lethal and sublethal effects arising from the consumption of poisoned aquatic and terrestrial insects, although the possibility does exist. Symons (1973) demonstrated that mealworms injected with 2-5 microliters of fenitrothion were almost all regurgitated after being force-fed to Atlantic salmon. The non-dietary uptake of fenitrothion contributes to and prolongs the behavioural changes in fish even though the ambient concentrations are low (Bull and McInerney 1974). Tissue bioaccumulation of

fenitrothion has been documented at nearly 16 times that of the concentrations in the surrounding water (Bull 1971). Hatfield and Johansen (1972) demonstrated how learning was completely inhibited after exposure to fentirothion at about 1.0 mg/liter for 24 hours. Hatfield and Anderson (1972) showed that treated fish were more susceptible to predation than unexposed salmon. Goldfish were shown to actively avoid fenitrothion at a threshold of 10 µg/liter (Scherer 1975). Temperature selection may also be affected by insecticides. Peterson (1976) reported that Atlantic salmon displayed a non-significant drop of 2°C in temperature preference from 0.2 - 1.0 mg/liter fenitrothion.

Saunders (1969) noted how brook trout moved downstream following an agricultural pesticide spill. Cote and Tetrault (1973) also reported a downstream movement of salmon following fenitrothion spraying, but these movements may be attributed to increases in water velocity following heavy rains. Major shifts in stream position may be in response to large decreases in the biomass of aquatic insects (Elson et al. 1972), necessitating the movement in search of food. Symons and Harding (1974) stated that the observed decrease in fish biomass by 24 percent was due to the drop of nearly 100 percent in insect biomass following a spraying program using fenitrothion.

The loss of color in exposed trout is suggestive of a neural imbalance, since color is under neural control in fish. Lyons et al. (1976) reported a similar color loss in leopard frogs after exposure to fenitrothion.

SUMMARY

(1) The broad spectrum organophosphorus insecticide, fenitrothion, has been used extensively in Canada since the late 1960's, primarily for the control of the spruce budworm. Brook trout (Salvelinus fontinalis) were used in this study because of their presence in forest ecosystems sprayed with the insecticide and because of their economic importance. Laboratory exposures to fenitrothion were used to determine the acute lethality of the insecticide and the effects on several physical and physiological parameters. Sublethal concentrations were used to determine the mechanism(s) of action of fenitrothion with respect to swimming performance as well as other behavioural patterns.

(2) The median lethal concentrations of fenitrothion to the brook trout in this study were 2.26 $\pm$ 0.18 mg/liter for 96-hour exposure and 4.85 $\pm$ 0.42 mg/liter for the 24-hour exposure. Mortality pressure in both bioassays appears to be from the same mechanism. The emulsifier, Atlox 3409, did not contribute to the acute lethal effects of fenitrothion, although it may have been a minor factor in enzyme inhibition in the brain and gill tissues. Inherent impurities in the technical formulations of the fenitrothion were assumed to be less toxic to the trout than fenitrothion and its oxygen analog fenitrooxon.

(3) Fenitrothion is a lipid soluble compound and can therefore penetrate cell membranes. Within the liver and other

tissues in the body, fenitrothion is transformed by the mixed-function oxidases to fenitrooxon, the more toxic analog of the insecticide. This biotransformation process is a complicating factor, because the new compound has a greater effect on acetylcholinesterase than fenitrothion. Degradation products were not considered to be responsible for any mortality in the acute lethality test nor for the decreased ability in the swimming performance tests, because of the rapid turnover of water and toxicant in the continuous flow design.

(4) There is no relationship between acetylcholinesterase inhibition and mortality in the brook trout from the acute lethality studies. The best explanation for the incongruent data may be the two pool theory, where only 20 percent of the total cellular acetylcholinesterase is extracellular and functional, with the remainder being intracellular and nonfunctional. The rate at which the extracellular enzyme was inhibited may be responsible for the early mortalities at high concentrations. This will also explain why those same fish exhibited relatively high enzyme activity levels due to the enzyme being released from intracellular reserves after homogenization. Adaptation, and/or an ability to tolerate the effects of the fenitrothion,

cannot be ignored as another possible source of variation, and contributed to the lack of correlation between enzyme activity levels and death.

(5) The swimming tunnel used to determine the critical swimming speeds of the brook trout in this study was similar in design to Brett's respirometer. The tunnel had high horsepower, high head and low volume, favorable traits for its use as a swimming tunnel and for measuring oxygen consumption. The rectilinear flow created by the turbulence grids and honeycombs produced the desired flow profile throughout the range of velocities required. The experimental procedures for the pre-exposure and for the actual swimming performance tests were best suited to the objectives of this study.

(6) The control group of brook trout had an average critical swimming speed of 62.61 $\pm$ 5.97 centimeters per second or 3.98 body lengths per second. Fish exposed to 0.3 mg/liter and higher were unable to swim as well and showed significant decreases in their critical swimming speeds. The initial decrease in swimming performance, for trout exposed to concentrations up to 25 percent of the LC50, was strongly correlated with the inhibition of brain acetylcholinesterase. Brook trout exposed to the

intermediate concentrations, from 40 to 60 percent of the 24-hour LC50, displayed a transition stage in acetylcholinesterase inhibition. Apparently, as the brain enzyme activity was further reduced, the regulatory impulses from the brain to the locomotory centers in the lower vertebrae were also inhibited, resulting in an elevated swimming performance. As the concentration of fenitrothion increased, the enzyme activity of the peripheral nervous system and/or locomotory muscles was sufficiently inhibited to result in a generalized reduction in swimming performance and critical swimming speeds. The regulatory impulses from the brains of the fish are apparently inhibited prior to the collapse of the entire peripheral cholinergic system. This theory would account for the exceptional swimming ability recorded by some of the brook trout exposed to this intermediate range of fenitrothion. The mechanical-like behaviour of these fish was conspicuous, with swimming being their only recognizable behaviour. A curvilinear relationship existed between tailbeat frequencies and swimming speed, but became more variable with increasing insecticide concentrations.

(7) Exposure to the insecticide fenitrothion, produced behavioural changes in these brook trout. The severity

of the change was proportional to insecticide concentration. The occasional fin twitching at low concentrations progressed to body spasms and generalized fasciculations at high concentrations. Tail flexes and tetanic muscle contractions were common. Fish in the swimming performance tests often displayed tetanic mouth movements or lock-jaw. Loss of equilibrium at the higher levels involved body rolling and the head-up, tail-down posture. The latter behaviour may have been further complicated by the negative bouyancy created by an internal hypoxia and respiratory distress, and by the loss of air being burped up during velocity increments in the swimming performance tests. Spontaneous behaviour was progressively depressed with increasing fenitrothion concentration.

(8) Coughing was correlated with the concentration of fenitrothion. The possibility exists that this insecticide is a gill irritant eliciting the observed cough response. However, concrete data to prove this theory is lacking or inconsistent. The cough response may also be a secondary effect of the acetylcholinesterase inhibition in the respiratory centers of the brain.

(9) Ventilation rates of fenitrothion-treated fish used in the swimming performance tests differed significantly

from the controls, except for the lowest concentration tested. A significant change in ventilation rates occurred at 0.3 mg/liter, the same concentration at which swimming performance decreased significantly. Trout exposed to 6.25 to 37.5 percent of the LC50 displayed ventilation rates higher than the controls during one or more of the velocity increments. This supports the possibility that the fish were experiencing a form of internal hypoxia, and respiratory distress in general. At the higher concentrations of fenitrothion, ventilation rates are initially similar to the controls but they failed to increase with control rates as the critical velocities were approached. This may have been a factor in reducing the critical speeds these fish achieved. The differential suppression of gill and brain enzyme activities may be responsible for the ventilation irregularities observed. There was some evidence to suggest that the stressed fish in the swimming tests may have adopted ram-jet ventilation as an alternative to rhythmic respiration.

(10) Oxygen consumption was reduced at all concentrations of fenitrothion tested compared to control fish. This strongly supported the fact that the insecticide may interfere with oxygen uptake at the level of the gills,

but oxygen consumption rates seemed to follow the swimming ability of the brook trout more closely. The highest rate of consumption by the treated fish was recorded by those trout exposed to 50 percent of the LC50. This was the same group that performed extraordinarily well in the swimming performance tests. The reduced rate of oxygen consumption and reduced swimming ability of treated trout are concomitant outcomes of exposure to fenitrothion.

(11) Only the hematocrit values of those fish exposed to fenitrothion for 96 hours showed a significant increase over control values. The increase was dependent on the length of exposure, and appeared to be a response to stress. The increased values are presumed to be as a result of red blood cells swelling in response to insecticide exposure.

(12) Comparison of brain weights, corrected for body weight of the brook trout used in the acute lethality studies revealed that some swelling of the brain did occur, symptomatic of Reye's syndrome. This swelling increased with fenitrothion concentration. The highest brain weight to body weight ratio occurred in those fish surviving after a full 96 hours of exposure. The smallest

increases were found in those fish which died prior to the completion of the 24-hour exposures. The length of exposure to the insecticide had a definite effect on the corrected brain weight ratio. Similar analyses for heart and liver weights were inconclusive.

(13) The magnitude of acetylcholinesterase inhibition in the brain, gill and heart tissues was different for each tissue. The differential inhibition was attributable to the physicochemical properties of the insecticide, the different rates of membrane permeability, and proximity to, or distribution of, the insecticide in the body. The overall pattern of enzyme inhibition was similar over the range of concentrations tested.

(14) The ecological implications of the inability of fish to attain their maximum critical swimming speeds are obvious. Concentrations of fenitrothion necessary to significantly reduce the critical speeds are less than one order of magnitude higher than levels of the insecticide recorded in bodies of water after aerial spraying programs. It is not inconceivable that a slight error in application rates or a drop in dilution rate in natural waters may bump the levels up to the threshold levels. The margin for error is uncomfortably small.

APPENDICES

APPENDIX A

Fenitrothion

Chemical name: 0,0-dimethyl-0-(3-methyl-4-nitrophenyl)-phosphorothioate

Chemical Abstract Number: 122-14-5

Synonyms - Sumithion (Sumitomo Chemical Company, Japan), Accothion, Folithion, Agrothion, Novathion, Nuvanol, Bayer 41,831, Bayer S 5660, Metathion, Methylnitrophos, Danathion and MEP.

Structural Formula:

0,0-dimethyl-0-(3-methyl-4-nitrophenyl)-phosphorothioate

(see figure 28)

Molecular formula: $C_9H_{12}NO_5PS$

Molecular weight: 277.2

C 38.99%

H 4.36%

N 5.05%

O 28.85%

P 11.17%

S 11.57%

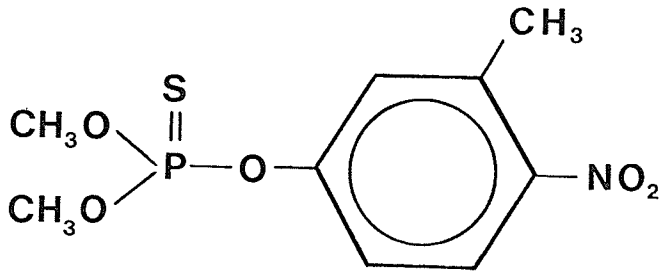
Physical state, color and odor: Yellowish, bronze oil

Unpleasant organic odor

Boiling Point: 118°C at 0.05 mm Hg (Merck Index)

Figure 28. Chemical structure of the organophosphorus insecticide fenitrothion (Sumithion ®).

FENITROTHION



O,O-DIMETHYL-O-(3-METHYL-4-NITROPHENYL)-

PHOSPHOROTHIOATE

Decomposition: 140-145°C at 0.10 mm Hg

Vapour Pressure: 5.4×10^{-5} mm Hg at 20°C

Density $d_4^{25} = 1.3227$

Refractive Index: $n_4^{25} = 1.5528$

Solubility: Practically insoluble (Sumitomo Chemical 1963)

- 20 mg/l distilled water (Zitko and Cunningham 1974)
- soluble in alcohols, ethers, ketones and aromatic hydrocarbons.

U.V. maximum: 269.5nm (ϵ 6756)

Toxicity: LD50 orally in rats = 250 mg/kg

Preparation:

Belgian patent 594,669 (1960 to Sumitomo)

Belgian patent 596,091 (1960 to Bayer)

Half life: 672 minutes at 20°C in ethanol - pH 6.0 buffer solution (Ruzicka et al. 1967)

Half life in equeous solutions:

- 720 to 5000 minutes in natural forest aquatic environment (Sundaram 1974a)
- 272 minutes determined at 30°C in 0.01 N NaOH as 40% ethanolic solution (Nishizawa et al. 1961)
- 1957 minutes at 25°C, pH 10.99 borate buffer (Kovacicova et al. 1973)

Isomerization: Fenitrothion is converted to S-methyl isomer under conditions of light,

heat and/or polar solvents (Eto et al. 1968; Truchlik et al. 1972; Ohkawa et al. 1974).

Use: Insecticide

Caution: Cholinesterase Inhibitor.

APPENDIX B

FENITROTHION IMPURITIES

Impurities inherently associated with technical grade formulations of fenitrothion have been shown to influence the toxicity of the insecticide in some situations (Kovacicova et al. 1973; Miyamoto et al. 1978). Metcalf and March (1953) found that the technical grade samples of many phosphorothioate insecticides are stronger inhibitors of cholinesterase than the pure substances. The thiolate isomers have been shown to be more potent cholinesterase inhibitors (Marshall et al. 1974; Truchlik and Kovacicova 1977). S-methyl fenitrothion is the most common impurity in technical grade fenitrothion, and is also found in analytical standards of the insecticide (Marshall et al. 1974; Zitko and Cunningham 1974). Three sources of the S-methyl isomer have been identified and they are all associated with the starting materials or are necessary components in the formulation of the insecticide (Truchlik and Kovacicova 1977). Methods are available (Kovacicova et al. 1971) which can produce technical grade fenitrothion containing toxicologically insignificant amounts of S-methyl fenitrothion.

The S-methyl isomer has been shown to be highly toxic to some animal groups (Miyamoto 1977; Miyamoto

et al. 1978; Kovacicova et al. 1973). Miyamoto (1977) demonstrated that all of the impurities found in technical grade Sumithion, including S-methyl fenitrothion, were less toxic to killifish (Oryzios latipes) than the active ingredient, fenitrothion. Zitko and Cunningham (1975) found the acute toxicity of S-methyl fenitrothion to juvenile Atlantic salmon (Salmo salar) to be approximately equal to that of fenitrothion. Other common impurities in the technical grade insecticide include 3-methyl-4-nitrophenol, fenitrooxon, S-methyl-bis-fenitrothion and bis-fenitrothion. In fresh stocks of the insecticide, Marshall et al. (1974) were unable to detect any of the impurities, except for bis-fenitrothion, measured at 0.94 percent m/m. Thus, stock solutions prepared from freshly obtained technical grade Sumithion were assumed to contain toxicologically insignificant amounts of the impurities.

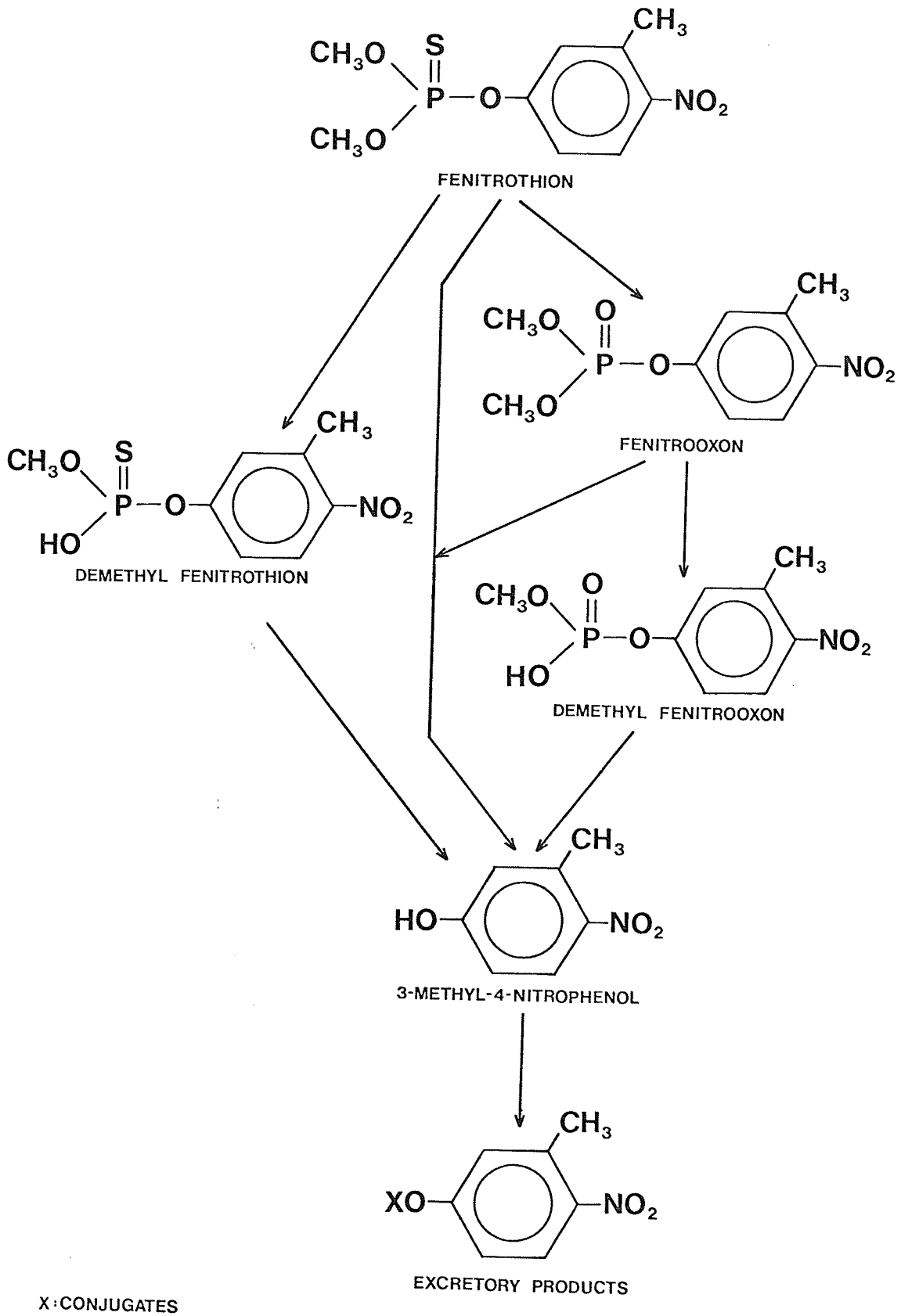
APPENDIX C

FENITROTHION DEGRADATION PRODUCTS

Some concern arises regarding the relative toxicity of the degradation products (Fig. 29) of fenitrothion to fish. Fenitrothion is readily taken up by fish in a manner similar to that described in Hunn and Allen (1974) for lipid soluble compounds. It is metabolized by rainbow trout (Salmo gairdneri) into fenitrooxon, desmethylfenitrothion, desmethylfentrooxon 3-methyl-4-nitrophenol and its glucuronide (Takimoto and Miyamoto 1976). According to Miyamoto et al. (1978), all of the potential degradation products encountered in natural waters are less toxic to fish than the original compound. However, fenitrooxon toxicity is highly variable depending on the species of fish involved and the mode of application. Its anticholinesterase capacity is 600 to 1000 times more potent than that of fenitrothion (Miyamoto et al. 1978).

Fenitrothion is converted to fenitrooxon by the mixed-function oxidase enzyme system of the mitochondria in the liver of fish and other animal groups (Buhler and Rasmusson 1968). The biotransformation process is common with the majority of the organophosphorus insecticides. These metabolic reactions are usually detoxifications,

Figure 29. Summary of the degradation products of the insecticide fenitrothion with respect to freshwater fish (after Miyamoto et al. 1978).


FENITROTHION
FENITROOXON
DEMETHYL FENITROTHION
DEMETHYL FENITROOXON
3-METHYL-4-NITROPHENOL
EXCRETORY PRODUCTS
X : CONJUGATES

but some reactions including desulfuration, can activate compounds to more toxic ones (Buhler and Rasmusson 1968; Miyamoto 1969; Zitko et al. 1970; Chambers and Yarborough 1976). Biotransformation of phosphorothioate compounds to their active analogs by liver preparations have been observed in brook trout and several other species of freshwater teleosts (Potter and O'Brien 1964; Murphy 1966). Ludke et al. (1972) treated mosquitofish (Gambusia affinis) with sesamex, a mixed-function oxidase system inhibitor, and increased the 48-hour median lethal concentration by almost 11-fold. Presumably, conversion of fenitrothion to its oxygen analog is a significant factor in the determination of the median lethal concentration.

In the aquatic environment, organophosphates were thought to degrade rapidly and were not considered a chronic hazard (Sundaram 1974b). Similar compounds have retained their anticholinesterase activity for a year or more while in aqueous solution (Weiss and Gakstatter 1965). Zitko and Cunningham (1974) found fenitrothion to be stable in tap water for 45 days. Acidic conditions lengthen the time this insecticide can remain stable in water (Sundaram 1973). Ultimately, hydrolysis will degrade the active compound into less toxic byproducts (O'Brien 1960; Ruzicka et al. 1967). Under controlled conditions,

fenitrothion follows first order kinetics as it disappears from aqueous systems (Sundaram 1974a). In natural waters, hydrolysis is more dynamic and proceeds more rapidly due to the type of water (Zitko and Cunningham 1974), microorganisms (Yasuno et al. 1965), and photo-induced degradation by sunlight or U.V. light (Lockhart et al. 1973; Brewer et al. 1974 Ohkawa et al. 1974). Fenitrothion is also rapidly adsorbed on suspended solids (Peterson and Zitko 1974) and sediments (Zitko and Cunningham 1974).

The observed enzyme inhibition therefore is in part the result of direct action of fentirothion after being taken up by the fish, and in part due to the metabolic by-product, fenitrooxon. Organophosphorus insecticides degrade more rapidly in the environment than do the chlorinated hydrocarbons they are replacing, but the presence and effect of the organophosphates in the environment may be unexpectedly high due to the necessity of larger doses and/or more frequent applications (Coppage and Matthews 1974).

The dynamics of fenitrothion in aquaria water, as summarized in Figure 30, are governed by essentially the same principles as in natural waters. Differences occur only in the absolute quantities involved such as the amount of fenitrothion lost in the laboratory due to photodecomposition, compared to natural conditions.

Figure 30. Proposed model for the dynamics of fenitrothion in laboratory aquaria with special reference to freshwater fish (after Lyons et al. 1976).

Symbols:

A - ingestion and gill ventilation

B - removal by gills

C - coprophagy

D - adsorption of walls and fish body

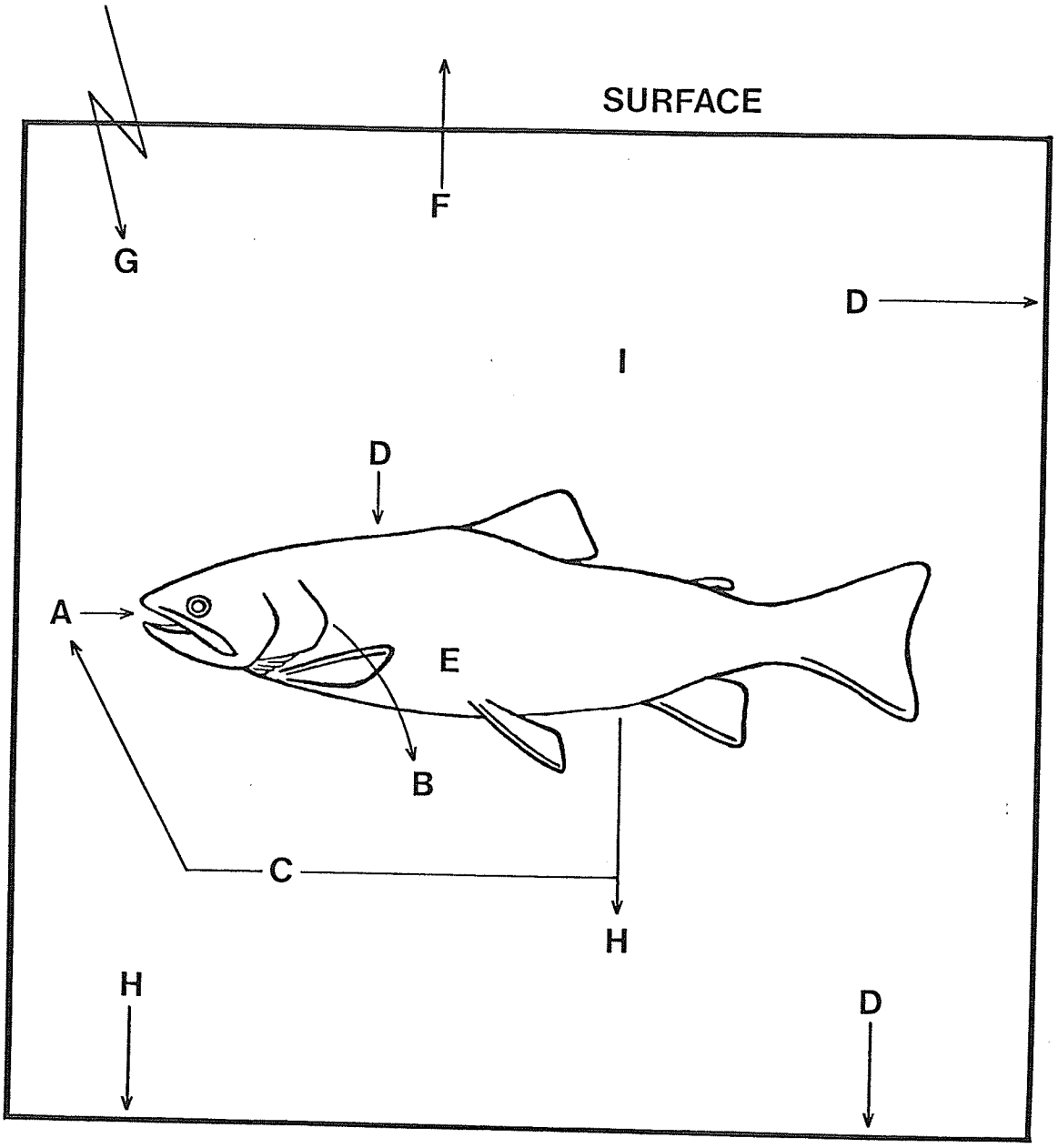
E - biotransformation

F - volatilization

G - photodecomposition

H - sedimentation

I - hydrolysis



AQUARIA BOTTOM

AND WALLS

LITERATURE CITED

LITERATURE CITED

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