

THE EFFECTS OF SUMITHION, AN ORGANOPHOSPHATE  
INSECTICIDE, ON THE BEHAVIOUR OF JUVENILE COHO SALMON  
ONCORHYNCHUS KISUTCH WALBAUM

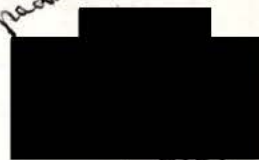
by

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to the required standard



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ABSTRACT

Bioassays based on the measurement of unconditioned behaviours reflect the energy status and general well being of the test organism. Therefore, they offer a more objective basis for establishing acceptable limits of environmental contamination than do standard, lethal concentration, bioassays. This study explores the effects of sublethal concentrations of Sumithion on a variety of unconditioned behaviours of underyearling coho salmon, mostly included in the general categories of feeding, agonistic, and comfort behaviours. The major habitat features of the test fish were duplicated in the stream aquarium in which behaviours were observed. Test concentrations of Sumithion corresponded to zero, 10, 25, 50, and 80% of the 96 hour median tolerance limit ( $TL_{m_{96}}$ ) which is 1.30 mg/liter. In concentrations ranging up to 25 to 50%  $TL_{m_{96}}$ , feeding and agonistic behaviours declined exponentially whereas comfort movements increased in frequency. In this phase, termed the motivational impairment phase, the changing patterns of behaviour appeared to be a direct response to irritation rather than to serious physiological impairment. In the next phase (concentrations up to 80%  $TL_{m_{96}}$ ), all behaviours except coughing declined in frequency. In addition, changes in spatial distribution occurred so that many fish were swept downstream and pinned against the aquarium screens. The overall pattern of behavioural changes indicates that

Sumithion concentrations greater than 25 to 50%  $TLm_{96}$  cause serious physiological impairment. Below this level feeding is substantially reduced and social behaviours, which are an integral part of territory maintenance, are altered.

Clearly prolonged exposure at such concentrations would seriously impair growth and ultimately survival. Short term exposure to such concentrations might be acceptable depending on how rapidly body levels of Sumithion, which accumulate to concentrations well above those of the surrounding water, can be eliminated. An acceptable level for prolonged exposure would be less than 10%  $TLm_{96}$ .

Additional work would be required to define this level more precisely.

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## INTRODUCTION

The advances in technology which have occurred over the last few decades have been accompanied by increased environmental contamination. One group of contaminants, the synthetic biocides, have been used in increasing quantities since their inception. For the last two decades the annual application of insecticides in the United States alone, has exceeded 100,000,000 lbs. (Stickel, 1968).

Lakes and rivers receive insecticides through direct application, aerial drift, and runoff water from surrounding land. Consequently, acceptable levels of insecticides should be established. The present study explores the possibility of using the natural behaviour of fish to establish an acceptable level for a recently developed insecticide.

Any biological method of studying contaminants should detect sublethal effects which may indirectly cause the death of an individual by inducing non-adaptive behaviour. Presently the most common method of establishing acceptable limits of contaminants, the standard bioassay (American Public Health Association, 1965), fails to detect any response except death. Suggested methods of applying safety factors to the standard bioassay tests (Burdick, 1967) have failed to offer empirically safe levels.

Warner, Peterson and Borgman (1966) suggest that behavioural methods will eventually dominate other methods of studying sublethal effects of chemicals because:

"(1) The behaviour (or activities) of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioural parameter is generally more comprehensive than a physiological or biochemical parameter;

(2) Behaviour patterns are known to be highly sensitive to changes in the steady state of an organism. This sensitivity is one of the key values for its use in exploring sublethal toxication;

(3) Behavioural measurements can usually be made without direct harm to the organism. With aquatic animals especially, implantation of detectors introduces problems of considerable complexity. Behaviour measurements can avoid this difficulty."

To be useful to resource managers, test results must be applicable to field situations. Most behavioural toxicology to date has included studies of avoidance responses (Höglund, 1961, Ishio, 1965, Hansen, 1969, and Sprague and Drury, 1969) and studies of conditioned behaviour (Warner et al. 1966, Hatfield, 1970, and Anderson and Prins, 1970) conducted under unnatural conditions. The results of these studies cannot be readily applied in field situations. The present research, on the other hand, involves observations of natural responses under conditions similar to those of the fishes home stream. To facilitate comparisons between the present work and that of others, concentrations of toxicant are given in percent of the 96 hour median tolerance limit ( $TLM_{96}$ ).

Sumithion (Fenitrothion) was chosen as the test agent chiefly because of its increasing popularity as a forest spray. Chlorinated hydrocarbon insecticides have been the most extensively used insecticides in the past. However, several studies (as reviewed by Stickel, 1968) have revealed that serious damage to non-target organisms often results

from their use. Consequently a trend has developed in recent years towards the more specific, short-lived organophosphate insecticides. Sumithion has been used extensively in New Brunswick and Ontario for controlling spruce budworm (Choristoneura fumiferana) (Freitag, Ozburn, and Leech, 1969) and its use in British Columbia is being investigated for protection against spruce weevils (Pissodes sitchensis) and possibly ambrosia beetles (Trypodendron sp. and Gnathotrica sp.). Exploratory spray experiments to test the effectiveness of Sumithion in British Columbia are planned for 1971 (Macdonald, personal communication). Consequently, it seemed to be a most appropriate test insecticide.

The value, extensive distribution, and life history of coho salmon (Oncorhynchus kisutch) all contribute to their applicability as a test species. In 1970, the value of commercially captured coho in British Columbia exceeded 12.5 million dollars, and surpassed the value of any other species of salmon (Department of Fisheries and Forestry of Canada, 1970). In addition, coho make up the majority of the sports catch of salmon in British Columbia, and in recent years more than 100 thousand coho have been captured annually by sport fishermen (Dept. of Fisheries and Forestry of Canada, 1968). In autumn, spawning runs of coho are found in innumerable coastal streams. When fry begin to emerge in April of the following year, subordinate individuals are displaced downstream (Chapman, 1962) whereas larger, more aggressive fish remain in fresh water for about

a year. Thus, adequate food and space are assured for dominant individuals.

Behavioural changes caused by sublethal concentrations of insecticides could obviously have drastic effects on coho resident in fresh water. The present study attempts to assess such effects.

## MATERIALS AND METHODS

## A. Toxic Agent

The technical grade Sumithion used in the present study contains 53% active agent, 0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate (Fig. 1) in an emulsifier consisting of 37 to 42% xylene, 2.5 to 5.0% sorpol L-550 and 2.5 to 5.0% sorpol H-770 (sorpol consists of a mixture of polyoxyethylene alkylaryl ether, polyoxyethylene sorbitan alkylate, and an anionic sulfonate). The product is produced by Sumitomi Chemical Co. Ltd., Osaka, Japan.

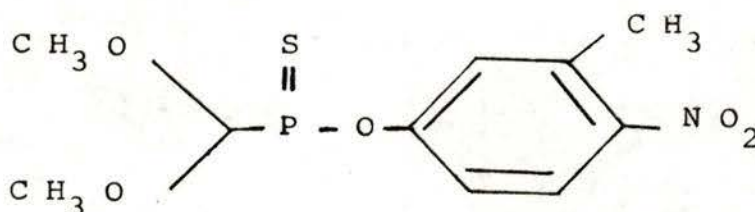


Fig. 1 The active agent in Sumithion; 0,0-dimethyl-0-(3-methyl-4-nitrophenyl) phosphorothioate

## B. Bioassay Methods

Three standard bioassays (American Public Health Association, 1965) were conducted to ascertain the 96 hour median tolerance limit (the concentration expected to kill 50% of the test animals in 96 hours) of Sumithion to coho salmon. The median tolerance limit ( $TLM_{96}$ ) was employed to establish appropriate levels of Sumithion to be used in behavioural tests.

The two preliminary bioassays were performed in September

and October, 1969. Concentrations of Sumithion used in these bioassays were chosen from a logarithmic series recommended by the American Public Health Association (1965). Results of the preliminary bioassays indicated that a third bioassay with narrower intervals between concentrations would be advantageous. It was conducted at a later date (January, 1971) to avoid interrupting the behavioural tests.

Coho salmon underyearlings of approximately equal size (Appendix, Table I) were used in all the bioassays. The number of fish and concentrations of toxicant used in each bioassay is outlined in Table V, page 28. The coho used in the two preliminary bioassays were seined from Goldstream River, the home stream of the fish used in the behavioural tests. They were not selected for size, but were removed ad hoc from the collecting seine. Conditions (water source, test temperature and handling procedures) were kept as similar to the behavioural testing conditions as possible, consequently fish were neither acclimated nor fed but were placed directly into the bioassay vessels.

Coho used in the final bioassay were seined from Cook Creek, near Qualicum, Vancouver Island on July 15th and August 20th, 1970. Between the time they were collected, and the time they were used in the bioassay, the fish were held in culture tanks at the Nanaimo Biological Station of the Fisheries Research Board of Canada. They were fed Abernathy frozen pellets twice daily, six days per week prior to testing. Temperatures and photoperiods during holding times approximated

those which occurred in streams on the east coast of Vancouver Island. Prior to testing, the holding temperature was raised 1C each day, from 4C to 10C. Fish used in the final bioassay were selected on the basis of size to resemble fish used in the behavioural tests and preliminary bioassays.

Testing methods were similar in all three bioassays, and followed the recommendations of the American Public Health Association (1965). Bioassay aquaria were glass jars containing 18 liters of water from the source which supplied the stream aquarium. The jars were immersed in a  $10.5 \pm 0.5$ C water bath. Dissolved oxygen levels remained above 8 mg/liter without aeration and the pH ranged between 6.45 and 7.00 pH units. Densities of fish in the bioassays were much greater than those in the stream aquarium (five fish were placed in each bioassay jar), but were well within the range recommended by the American Public Health Association (1965).

### C. Behavioural Test Methods

#### (i) Experimental Fish

The 96 coho salmon underyearlings used in the behavioural experiments were seined from Goldstream River, a small stream which drains into Saanich Inlet about 12 miles north of Victoria, British Columbia. Sixteen fish were collected between 0930 and 1030 hours on the first day of testing at each concentration.

Fish kept in confined areas are unlikely to show territorial or aggressive behaviour for some time after being released into larger areas (Kalleberg, 1958). For this reason, fish used in the behavioural studies were released into the

stream aquarium within one hour of capture.

Sixteen fish were placed in the aquarium, giving a density of between two and three individuals per square meter. The number was chosen for ease of observation within a range of naturally occurring densities. Allen (1969), using data from Chapman (1965), calculated that the average stream area occupied by four month old coho salmon is 0.79 square meters per individual. Hartman (1965a) found densities of 727 coho per square meter in the most suitable areas of the Chilliwack River (log jams which provided excellent cover). Thus the density used in the present study was slightly higher than Allen's theoretical stream average, much lower than maximum densities found by Hartman, and almost equal to natural densities found by Chapman (1962).

Four different sizes of fish were selected for behavioural observation (Appendix, Tables II and III), so that individuals could be recognized and the effects of size could be determined. One fish of each size was placed into each of the four sections of the aquarium. The smallest individual in each section was assigned the number one, the second smallest was given the number two, and so on. Fish of similar size were distinguished by parr marks, coloration, or other morphological features.

#### (ii) Apparatus and Test Conditions

Behavioural observations were made in a temperature controlled stream aquarium (Fig. 2) described in detail by Hartman (1965b). The aquarium was designed to represent a

short section of a small stream. Water was propelled along a tapered duct at the bottom of the unit, then upward and back along an observation flume to the pump (Fig. 3).

The aquarium was 6.3 m long, 2 m high, and 1.2 m wide, and of plywood and angle iron construction with windows of 1.6 cm thick plate glass. The axial flow pump consisted of a propeller made of welded mild steel coated with fiberglass reinforced plastic driven by a 3 hp electric motor.

The observation flume was 5 m long and 0.7 m deep, and was divided into four equal sections by galvanized screen covered by several coats of Holzon paint. So that fish were not disturbed, observations were made through horizontal slits in black polyethylene sheets arranged so that the flume was surrounded on all sides.

The substrate was similar in all sections of the observation flume and consisted of washed gravel screened to pass through 2.5 cm mesh but not 1 cm mesh. About ten rocks approximately 12 cm in diameter were aligned down the center of each section to facilitate mapping of areas occupied by fish. A large rock (about 30 cm in diameter) placed against the central screen about half way up each section, provided low velocity resting areas.

A constant rate feeding apparatus (Fig. 4) was employed to evenly distribute 10 g of freshly thawed brine shrimp (Artemia salina) into the aquarium over a 24 hour period (Leitritz, 1962 and Chapman, 1962). The apparatus consisted of a 240 liter tank from which water and feed were slowly siphoned through 4 mm i.d. plastic tubing. A Bird Kymograph

Fig. 2 The stream aquarium in which the behavioural observations were made

Fig. 3 A sketch showing the dimensions of the stream aquarium and the direction of water movement

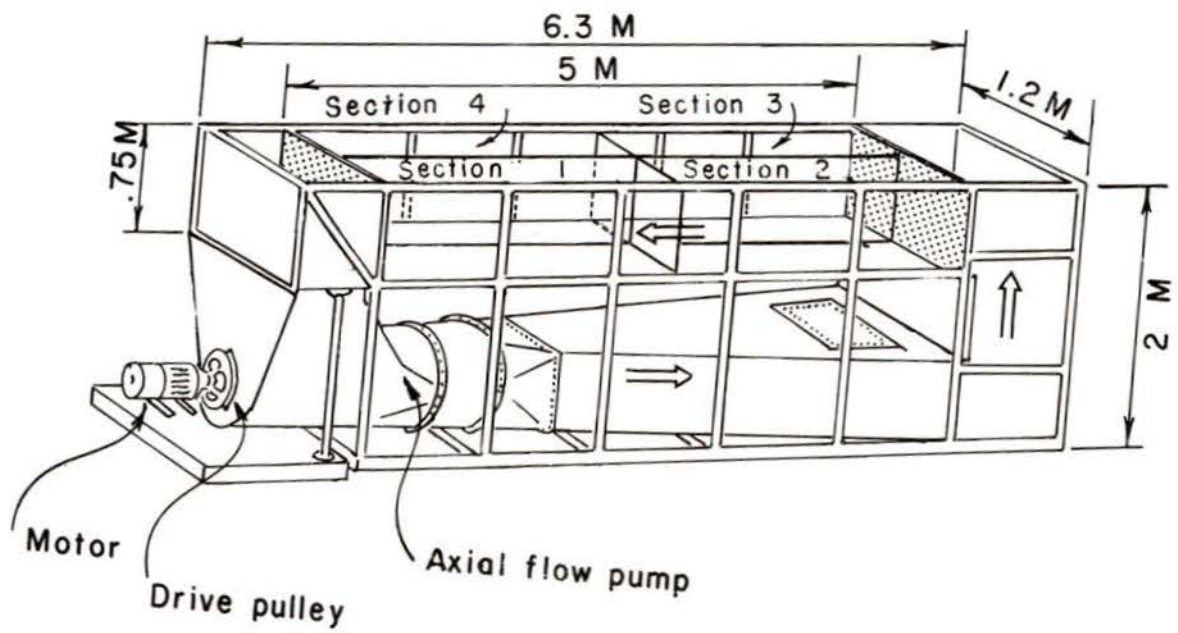
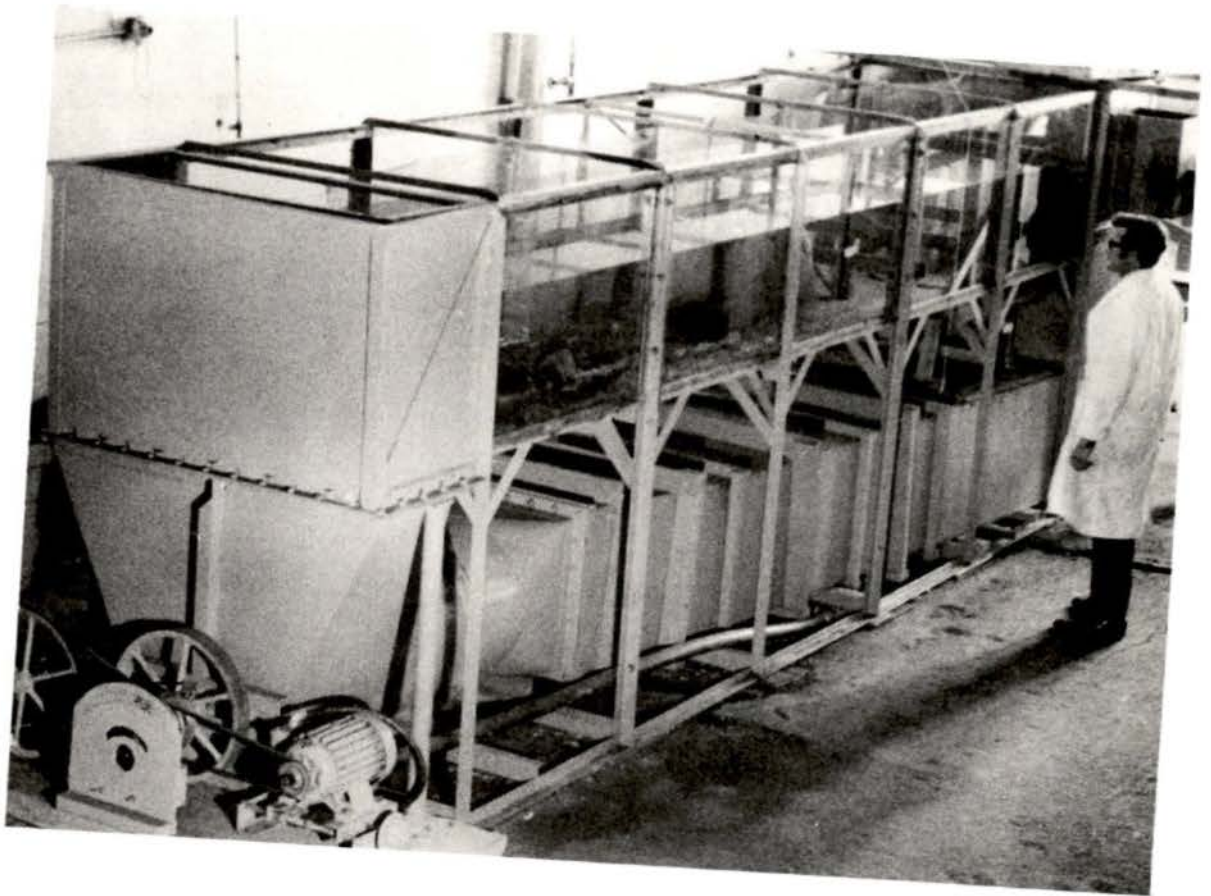
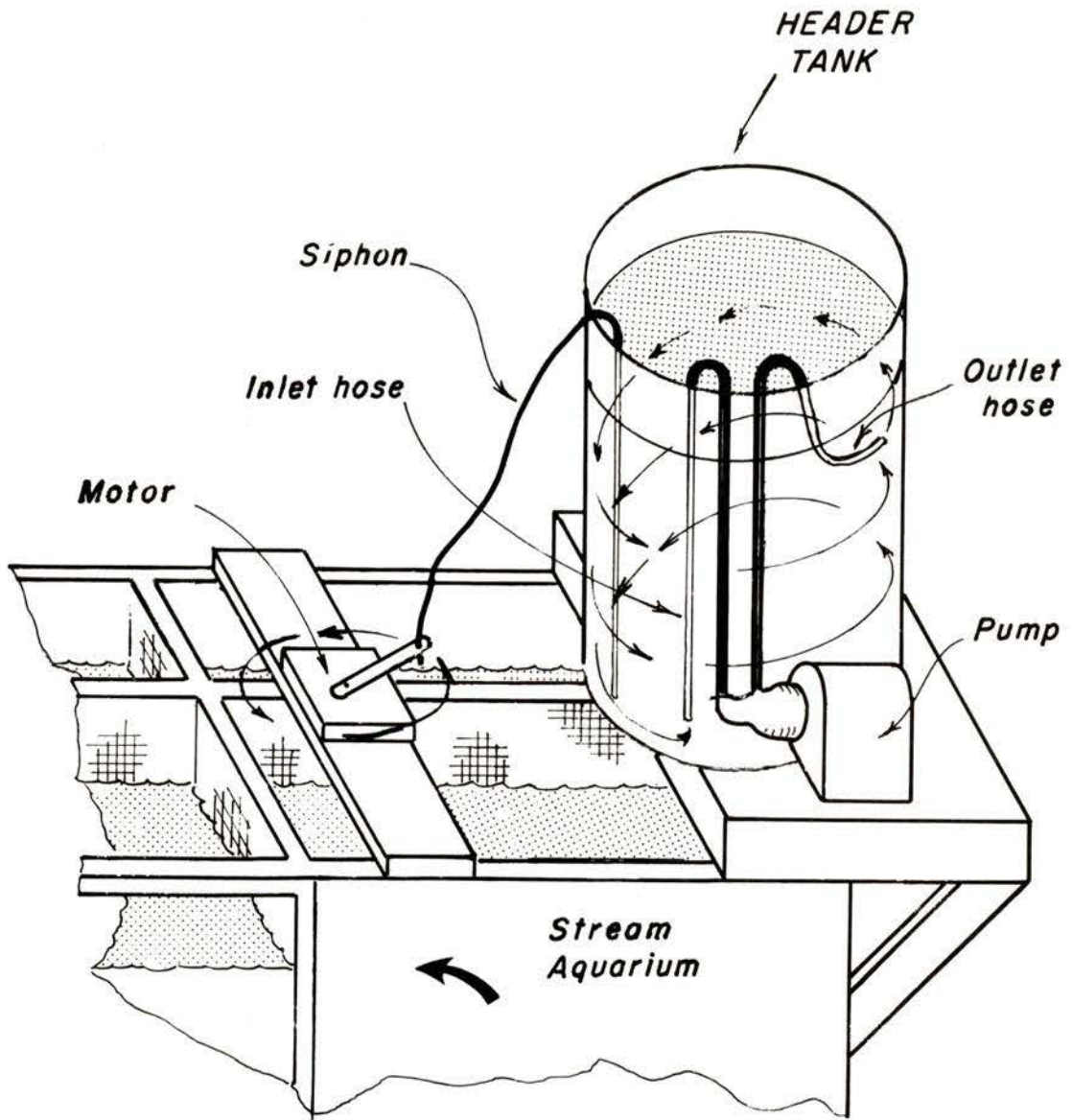


Fig. 4 Constant rate feeding apparatus



motor with a rotating arm was used to move the tubing from one side of the aquarium to the other in order to distribute the food. Each rotation required 13 seconds. Every 12 hours the tank was filled from the stream aquarium and 5 g of brine shrimp were added. A Cole-Parmer 1/35 hp polyethylene centrifugal pump moved water from the bottom of the food tank to the top and created a constant spiralling current which maintained even distribution of food within the header tank.

Water used in the stream aquarium came via the City of Victoria mains from Humpback Reservoir which also supplies the home stream (Goldstream) of the experimental fish. Analysis of water from the stream aquarium (Appendix, Table IV) indicated normal levels of all constituents except zinc. Because of plumbing modifications utilizing galvanized steel pipe, the zinc content was 0.119 mg/liter at the beginning of the experimental period and thereafter decreased exponentially to 0.050 mg/liter. It is unlikely that zinc ions affected the Sumithion experiments, since no mortalities or behavioural abnormalities were noted during 88 hours of preliminary observation when the zinc concentration was six times greater than that of the present study.

Water temperatures in the aquarium were recorded several times daily and were maintained at  $11 \pm 1$  C during each test. Temperatures within Goldstream River were recorded during each collecting trip and varied between 9 and 13 C. Water depth was maintained at 43 cm. This corresponded to a volume of 8,200 liters for the entire apparatus.

Current velocities were recorded in several locations of the aquarium (Table I) with a Gurley flow meter (number 625). Velocities varied from nearly zero behind large rocks to a maximum of 25.4 cm/sec. Water moved slightly faster in the upstream sections of the observation flume than in the downstream sections. Likewise, velocities within a section were slightly higher at the upstream end. Surface water moved almost twice as fast as water near the substrate. The screen which divided the tank in half longitudinally, slowed water flowing adjacent to it.

Dissolved oxygen levels in the stream aquarium were checked periodically with a Hach kit and were close to 10 mg/liter during every test. Hach kits are limited in accuracy, but precise oxygen determinations were not required in the present study.

Two 200 watt floodlights, symmetrically placed 1.5 m above the water surface, were switched on at 0830 hours and off at 1730 hours each day. Natural light entering through windows before and after these times provided a natural photoperiod. Light intensity was monitored with a Kahlsico (Kahl Scientific Instrument Corp.) submarine photometer, and varied between 45 and 482 lux in different sections. All sections were similar in light intensity, but section 4 was the brightest followed by sections 1, 3 and 2 (Table II).

The aquarium was filled and circulation started at least 24 hours before each test to permit the elimination of any chlorine present in the water supply (Holland, Lasater,

Table I Current velocities (cm/sec) at various  
locations in the stream aquarium

		Section 1		Section 2		Section 3		Section 4	
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Upstream	Inside	14.7	9.7	24.4	12.2	23.9	11.7	16.3	12.2
	Middle	21.3	10.2	24.4	8.1	24.4	7.6	22.4	13.2
	Outside	21.3	17.3	25.4	8.6	23.4	8.1	20.3	11.7
Center	Inside	12.7	4.6	13.2	2.0	17.3	6.6	14.2	3.0
	Middle	20.3	8.1	22.4	13.7	24.4	5.1	19.3	12.2
	Outside	18.2	9.7	21.3	14.2	22.4	13.2	16.3	12.2
Down- Stream	Inside	10.2	10.7	9.7	8.6	14.2	9.1	11.2	10.2
	Middle	18.3	13.2	21.3	9.1	22.4	11.7	19.3	11.7
	Outside	15.2	12.2	20.3	13.2	22.4	10.7	15.2	12.2

Table II Light intensities (lux) at various locations in the stream aquarium

		Section 1		Section 2		Section 3		Section 4	
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Upstream	Inside	211	150	60	45	57	54	241	180
	Middle	211	180	66	57	57	57	241	180
	Outside	180	180	66	63	69	66	241	180
Center	Inside	452	241	332	180	302	180	482	302
	Middle	392	271	271	180	302	211	452	302
	Outside	362	331	271	211	302	241	392	302
Down-stream	Inside	60	57	180	121	211	150	60	48
	Middle	60	60	211	180	241	150	57	52
	Outside	60	54	211	150	241	180	66	60

Neuman, and Eldridge, 1960). Sumithion was mixed in approximately 30 liters of water before it was added to the aquarium. The water containing the toxicant was then intermittently poured into both sides of the aquarium over a 15 to 20 minute period while the water within the aquarium flowed by. Toxicants were added approximately 15 hours before fish were placed in the aquarium. This time interval was thought to be long enough to allow toxicant dispersion but short enough to avoid substantial toxicant breakdown.

At the completion of a test, the aquarium was emptied and then rinsed by refilling, running the equipment, and emptying. Unless the next test involved a higher concentration of the same toxicant, the entire apparatus was scrubbed with clear water and rinsed a second time.

The amount of toxicant mixed into the aquarium was based on the assumption that a prescribed water level would correspond to a predetermined aquarium volume. Four replicate volume determinations were within 2.5% of the predicted volume.

Analysis of Sumithion content within the stream aquarium was not possible during the actual experimental work because methods of analysis and standard preparations of Sumithion were not available. When these were obtained in June 1970, certain experimental conditions were reestablished. Sumithion levels were then checked to ascertain whether predicted levels of Sumithion actually occurred, whether residues remained after the tank was cleaned, how quickly Sumithion became degraded, and whether Sumithion accumulated within the test fish.

Water samples (4500 ml) were extracted directly into hexane, and fish samples (five coho per sample) were frozen. Gas chromatography analyses were performed by the British Columbia Department of Agriculture Pesticides Laboratory in Vancouver.

(iii) Procedure

The stream aquarium contained the following concentrations of Sumithion during the following dates:

October 16 - 19	0 mg/liter
October 22 - 25	0.322 mg/liter (25% TLM <sub>96</sub> )
October 28 - 31	0.645 mg/liter (50% TLM <sub>96</sub> )
November 4 - 7	1.030 mg/liter (80% TLM <sub>96</sub> )
November 10 - 13	0.129 mg/liter (10% TLM <sub>96</sub> )
November 25 - 28	0 mg/liter

Sixteen different coho were subjected to each test concentration and their behavioural responses and distribution were quantified. A standard testing procedure involving 11 observation periods (Table III) was repeated at each concentration. The observation periods lasted about 2 hours and started at 1300 and 1500 hours Pacific Daylight Standard time on day 1, and 0900, 1300, and 1500 hours on days 2, 3, and 4. Each observation period began with section 1 of the stream aquarium. The behaviour of a single fish was recorded for exactly 5 minutes, and then another individual was watched. After all four fish in a section had been observed, the procedure was repeated in another section. The observation period was complete when each fish in all four sections had been studied.

For each individual, the following behavioural responses

Table III An outline of the test design, which was repeated for each test concentration

Test Day	Time (Pacific Daylight Standard)	Observation Period	Aquarium Section 1				Aquarium Section 2				Aquarium Section 3				Aquarium Section 4				
			Fish Number				Fish Number				Fish Number				Fish Number				
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
1	1300	1																	
	1500	2																	
2	0900	3																	
	1300	4																	
	1500	5																	
3	0900	6																	
	1300	7																	
	1500	8																	
4	0900	9																	
	1300	10																	
	1500	11																	

were recorded; feeding, chewing, coughing, yawning, nipping, chasing, displaying, displacing, vacating, flight, appeasement, flicking, thrusting, swimming rate, and interstation distance.

Tugendhat (1960) describes feeding behaviour as being a sequence of two responses; an initiated response in which the fish fixates on the food object, and a completed feeding response in which the fish goes to and grasps the food object. In this study, only completed feeding responses were recorded due to the difficulty of consistently recognizing fixations.

Chewing responses were repeated shallow movements of the lower jaw. These were recorded whether or not they were associated with feeding.

Coughing (Schaumberg, Howard, and Walden, 1967) was a quick gulp which occurred independently of feeding. The complete coughing movement was usually rapid, but sometimes the mouth and operculum was opened slowly at first then quickly snapped completely open and shut. Isolated coughing movements were more common than a rapid series of coughs.

Yawning differed from coughing in the amount of time required to execute the response. When yawning, fish extended the jaw and operculum relatively slowly.

Nipping (Hoar, 1951) occurred whenever the fish being observed made a quick darting movement toward another fish and appeared to try to bite it.

Chasing (Hartman, 1965a) occurred when a fish darted after another and chased it past the point from which it fled.

Aggressive displays included lateral, frontal and wig wag

displays as described for juvenile salmon by Hartman (1965a) and Kalleberg (1958).

Displacing (the tail first approach described by Jenkins, 1969) occurred when the fish being observed passively caused another fish to vacate its station. Aggressive displacing was scored as chasing.

Vacating, the reciprocal response to displacing (above), was recorded whenever the observed fish passively drifted from its station because of the presence of another fish. Active swimming from a station was scored as a flight rather than a vacating movement.

Appeasement (Jenkins, 1969) or submission without flight (Chapman, 1962) occurred when the fish being observed was approached by a more dominant fish, and responded by folding its fins.

Flicking occurred when a fish quickly moved its head to one side and then whipped the body over to the same position. Frequently a cough and flick occurred together.

Thrusting occurred when a fish propelled itself forward with one big push of the tail then stiffened its body in a frontal display position but with a raised dorsal fin. Thrusting did not seem to be a social behaviour.

Swimming rate (the total distance, in feet, moved during a five minute recording session) was noted by pressing a counter each time the fish appeared to move a distance of one foot. Movements which involved distances less than one foot were not recorded.

All behavioural parameters noted were recorded on a multiple key tally counter or a tape recorder.

When a 5 minute recording session ended, the station of the recorded fish was marked on maps of the side and bottom of the aquarium section. A station (Kalleberg, 1958) was the location which a fish, by subjective evaluation, occupied during most of the recording session. A fish may have occupied a different station in the next recording session. If so, the distance between the two stations (the interstation distance) was calculated. Since stations are fixed locations within territories (Kalleberg, 1958), interstation distance was assumed to be inversely related to territory maintenance. If a fish maintained a territory it would presumably occupy the same location most of the time. Its interstation distance would then be very small.

In order to calculate interstation distance, a two step procedure was followed. First, locations of stations were transferred into X Y Z coordinates by laying a plastic grid system over maps of the side and bottom of the aquarium section. Next, Pythagorus' Theorem was used to calculate the distance between two sets of X Y Z coordinates. The formula used to compute interstation distance D which occurred between times t and t-1 would be

$$D = \sqrt{(X_t - X_{t-1})^2 + (Y_t - Y_{t-1})^2 + (Z_t - Z_{t-1})^2}$$

A program was written for the IBM Systems 360 to compute this distance for all fish, time periods and test conditions.

In addition to interstation distance, the location of stations was noted to ascertain whether these depended upon the concentration of Sumithion.

(iv) Statistical Methods

Behavioural experiments were arranged in a factorial design with two between-group variables (size of fish and concentrations of Sumithion), and one within-group variable (time periods). For each type of behaviour, the null hypothesis that the frequency of occurrence was affected by neither the concentration of Sumithion, nor fish size, was to be tested by analysis of variance. However, due to mortalities and lack of responses, the data was skewed, non-homoscedastic, and many cell values were missing. Consequently, parametric statistics were inappropriate. As an alternative, Friedman's two-way analysis of variance (Siegel, 1956) was employed to test the hypothesis that the frequency of occurrence of a behaviour depended upon the amount of Sumithion present. For this test the data were cast into a two-way table with the rows representing time periods and the columns representing Sumithion concentrations. A cell value for the table was computed as the average of the responses of all surviving subjects at a particular time period and concentration.

Matching on the basis of time of observation is unorthodox, but in this case justified on the following basis. In higher test concentrations some fish died before the end of the test so they did not contribute scores during late time periods. Therefore the average score would consist mainly of scores

from early time periods. The procedure of matching by time period avoided such a bias.

While matching by time period precluded a bias due to averaging over time, it necessitated averaging the scores for all subjects. If the subjects which died at the higher concentrations behaved abnormally prior to their death, a bias would result. Graphic analysis indicated that differences between subjects were less striking than differences between time periods. Consequently, the best method of matching was on the basis of time.

For the types of behaviour whose frequency of occurrence was shown by Friedman's two-way analysis of variance to vary significantly ( $p < 0.05$ ) with the concentration of Sumithion, a series of sign tests (Siegel, 1956) was used to show at which concentrations the changes occurred. Pairs of scores used in the sign tests were matched on the basis of time at which the observation was made. This is the same matching procedure as used in the Friedman tests.

Fish which were swept downstream against the screen were eliminated from the experiment. Since only fish in high concentrations of Sumithion were incapacitated in this manner, some error was introduced by eliminating them. The measurements obtained at higher concentrations were biased because only resistant fish contributed to the score. This bias would probably result in a Type II error (Li, 1964), if any. To compensate for a Type II error the level of significance ( $\alpha$ ) should have been lowered, but  $\alpha$  was assigned subjectively

and thus it was difficult to know how much to compensate. The 95% level of confidence was used as a guide.

## RESULTS

## A. Sumithion Analyses

Samples of fish and water analysed by gas chromatography showed (Table IV) that actual concentrations of Sumithion within both the bioassay vessels and the stream aquarium did not vary greatly from predicted amounts. In other words, the method of calculating concentrations was accurate and unknown factors such as adsorption by the substrate, hydrolysis, and breakdown within containers, were negligible. Water samples from the post-test control indicated that the cleaning process was adequate.

Fish within the bioassay vessels accumulated Sumithion quickly (Fig. 5), so that after one day of exposure they contained more than 20 times the amount present in the surrounding water. Fish exposed for 96 hours contained slightly less Sumithion than those exposed for only 24 hours.

## B. Bioassays

Survival records of coho in both preliminary and final bioassays are given in Table V. Median tolerance limits, found by graphical interpolation on semilogarithmic graph paper (Fig. 6), were 1.34 and 1.1 mg/liter in the preliminary bioassays and  $1.30 \pm 0.12$  mg/liter in the final bioassay. Further experimental results are based upon a  $TLm_{96}$  of 1.30 mg/liter.

In all bioassays Sumithion caused fish to swim near the surface of the bioassay jars and in some cases fish swam on an angle with head up and tail down (Fig. 7 and 8). A more vertical position was assumed at higher concentrations with

Table IV Actual concentrations of Sumithion (in mg/liter)  
as compared with predicted values

Experimental condition	Predicted Concentration	Actual Concentration					
		Hours after Dilution					
		1	16	24	39	96	111
Bioassay control	0.00	0.00					
Stream aquarium pre-test control	0.00	0.00					
Stream aquarium post-test control	0.00	0.00					
Fish in bioassay control		0.00					
Fish in 0.56 mg/liter bioassay conditions		1.29		8.71		7.52	
Bioassay vessel without fish	0.56	0.55		0.45		0.43	
Bioassay vessel with fish	0.56			0.40		0.39	
Stream aquarium	0.56		0.42		0.42		0.41

Fig. 5 Quantities of Sumithion present in bioassay fish and their water supply after 1, 24, and 96 hours of exposure. Five fish constituted a sample.

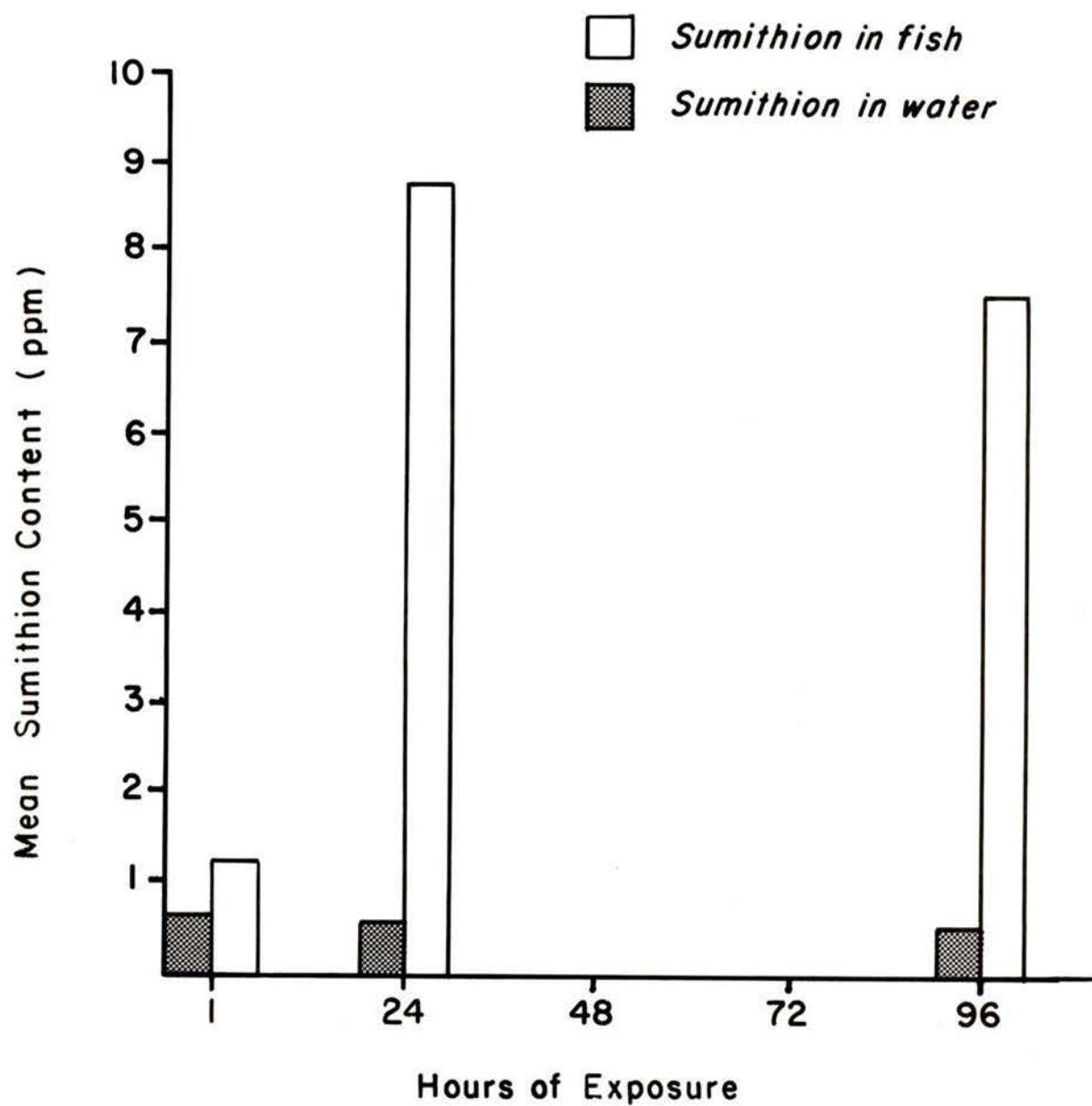


Table V Numbers of coho surviving in various bioassay conditions

Concentration of Sumithion (mg/liter)	Bioassay 1 (Sept.30-Oct.4,1969)			Bioassay 2 (Oct.16-Oct.20,1969)			Bioassay 3 (Jan.14-Jan.18,1971)		
	Hours of Exposure			Hours of Exposure			Hours of Exposure		
	0	48	96	0	48	96	0	48	96
0.00	5	5	5	5	5	5	10	10	10
0.10	5	5	5						
0.32	5	5	5						
0.56	5	5	5	5	5	5			
1.00	5	5	5	5	5	4			
1.15							10	10	10
1.19							10	10	10
1.34							10	10	2
1.39							10	5	2
1.43							10	2	0
1.61							10	0	0
1.80	5	0	0	5	0	0	10	0	0
3.20	5	0	0	5	0	0			

Fig. 6 Graphic interpolation of bioassay results

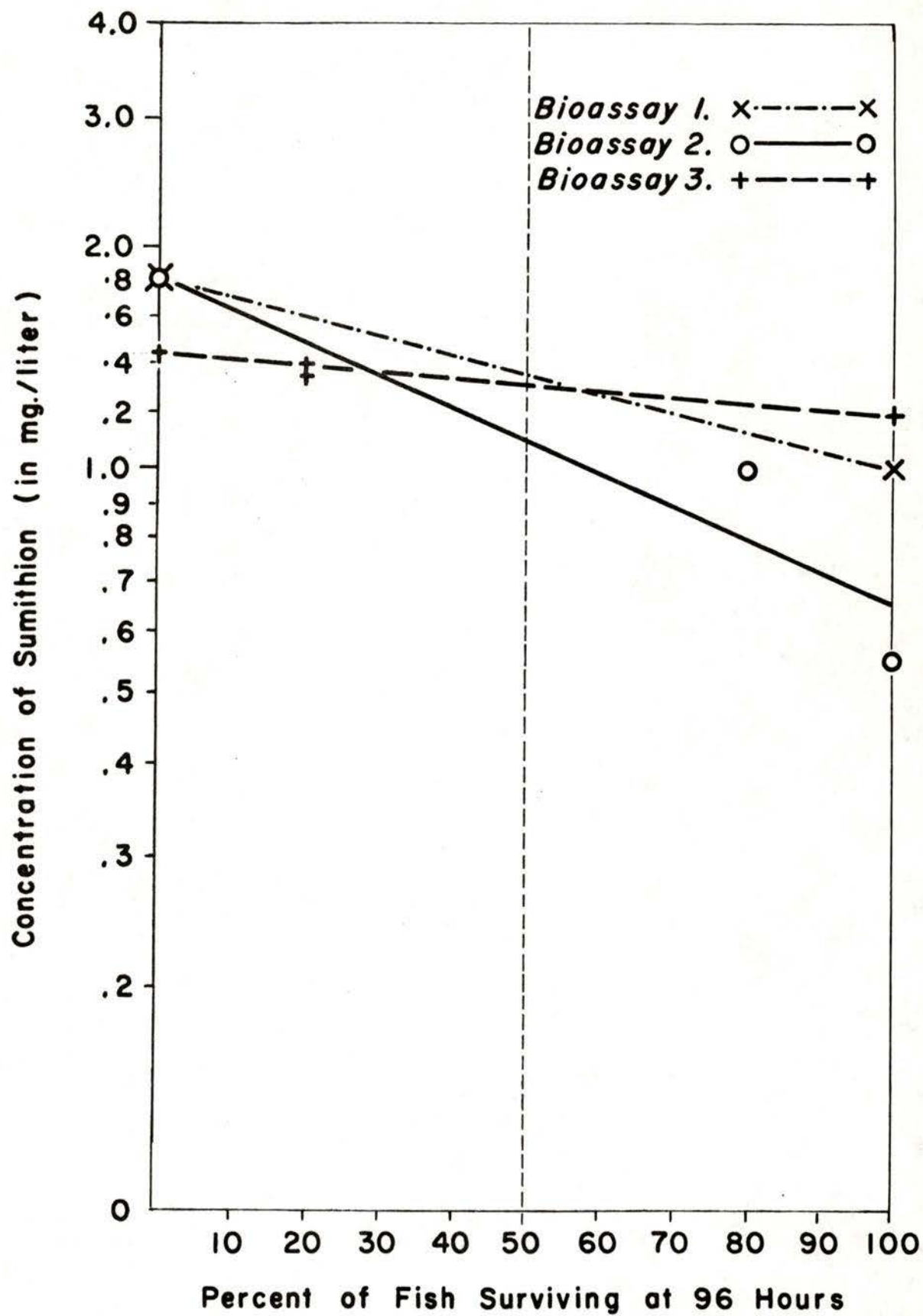
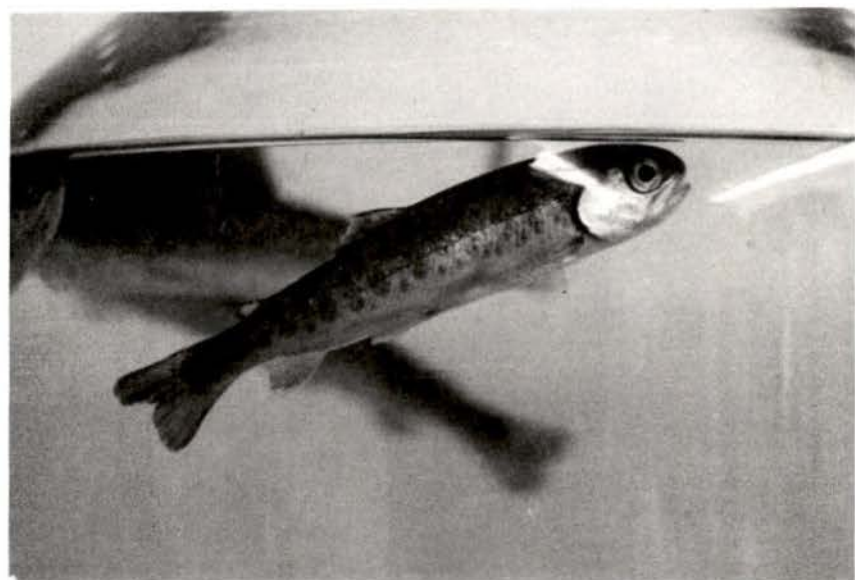
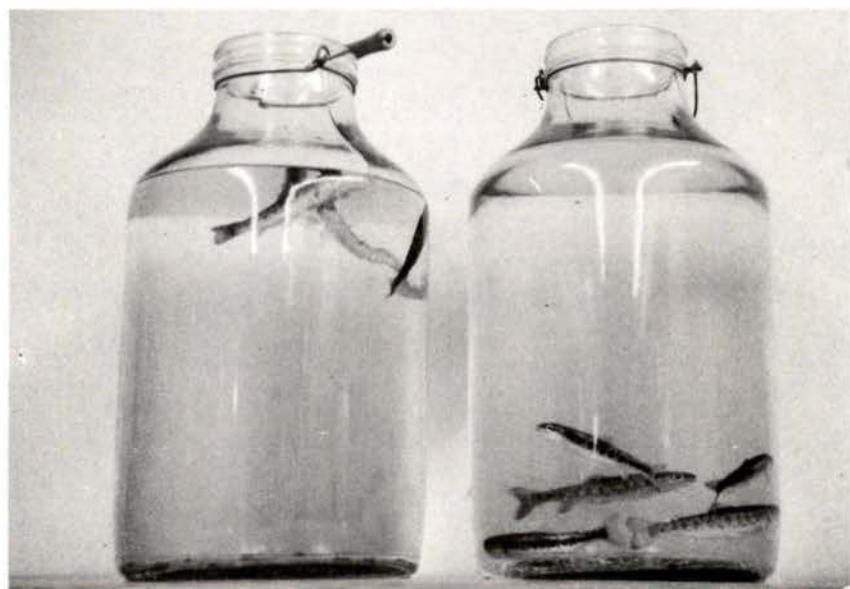


Fig. 7 Positioning of fish after 18 hours of exposure to Sumithion concentrations equal to 80%  $TLm_{96}$  (left jar) and to clear water (right jar)

Fig. 8 Head up, tail down posture



some fish adopting a completely vertical position. In addition, gill ventilation rate increased with increasing Sumithion concentration. An attempt was made to quantify these behavioural changes in the final bioassay (Table VI). Movement of bioassay fish during the procedure of counting opercular movements made it impossible to keep track of all individuals. Consequently, ventilation studies may be subject to slight errors due to the possibility of recounting individuals.

### C. Behaviour

The hypothesis that the frequency of a behavioural response was independent of the amount of Sumithion present in the stream aquarium was tested, for each type of behaviour studied, by Friedman's two-way analysis of variance (Siegel, 1956). Results (Table VII) showed that the frequency of behavioural response varied significantly ( $p < 0.05$ ,  $df = 5$ , for all comparisons) with the concentration of Sumithion for the following behaviours; swimming rate, feeding, chewing, coughing, yawning, displaying, chasing, flights, and vacating. Frequency of response was not shown to vary with Sumithion concentration for appeasement, flicking, thrusting, nipping, displacing or interstation distance. These behaviours occurred very infrequently (less than once per hour) and may have shown insignificant results because of the small number of recorded scores.

Table VI Location, swimming position, and gill ventilation rate of coho in the final bioassay

Hours of Exposure	Concentration of Sumithion (mg/liter)						
	0	1.15	1.19	1.39	1.61	1.80	
4	Number of fish	10	10	10	10	10	10
	Per cent in upper half of bioassay jar	0	10	10	50	80	50*
	Per cent in head up position	0	0	0	10	80	70
	Mean gill ventilation rate	67	103	75	101	144	160
24	Number of fish	10	10	10	5	0	0
	Per cent in upper half of bioassay jar	40	100	100	100	-	-
	Per cent in head up position	0	20	10	20	-	-
	Mean gill ventilation rate	84	123	125	144	-	-
48	Number of fish	10	10	10	5	0	0
	Per cent in upper half of bioassay jar	0	100	100	100	-	-
	Per cent in head up position	0	0	0	0	-	-
	Mean gill ventilation rate	58	-	118	116	-	-

\*The fish in the bottom half were dying (motionless except for gill ventilation)

Table VII Results of Friedman's two-way analyses of variance

Behaviour	$\chi^2_r$	
Swimming Rate	40.6	
Feeding	49.6	
Chewing	39.7	
Coughing	41.0	Within the region of rejection ( $\chi^2_r > 11.07$ )
Yawning	33.9	
Displaying	12.7	
Chasing	11.1	
Flights	20.9	
Vacating	11.2	
<hr/>		
Appeasement	8.3	
Flicking	8.3	
Thrusting	6.4	Beyond the region of rejection ( $\chi^2_r < 11.07$ )
Nipping	3.2	
Displacing	3.1	
Interstation Distance	5.6	

## (i) Swimming Rate

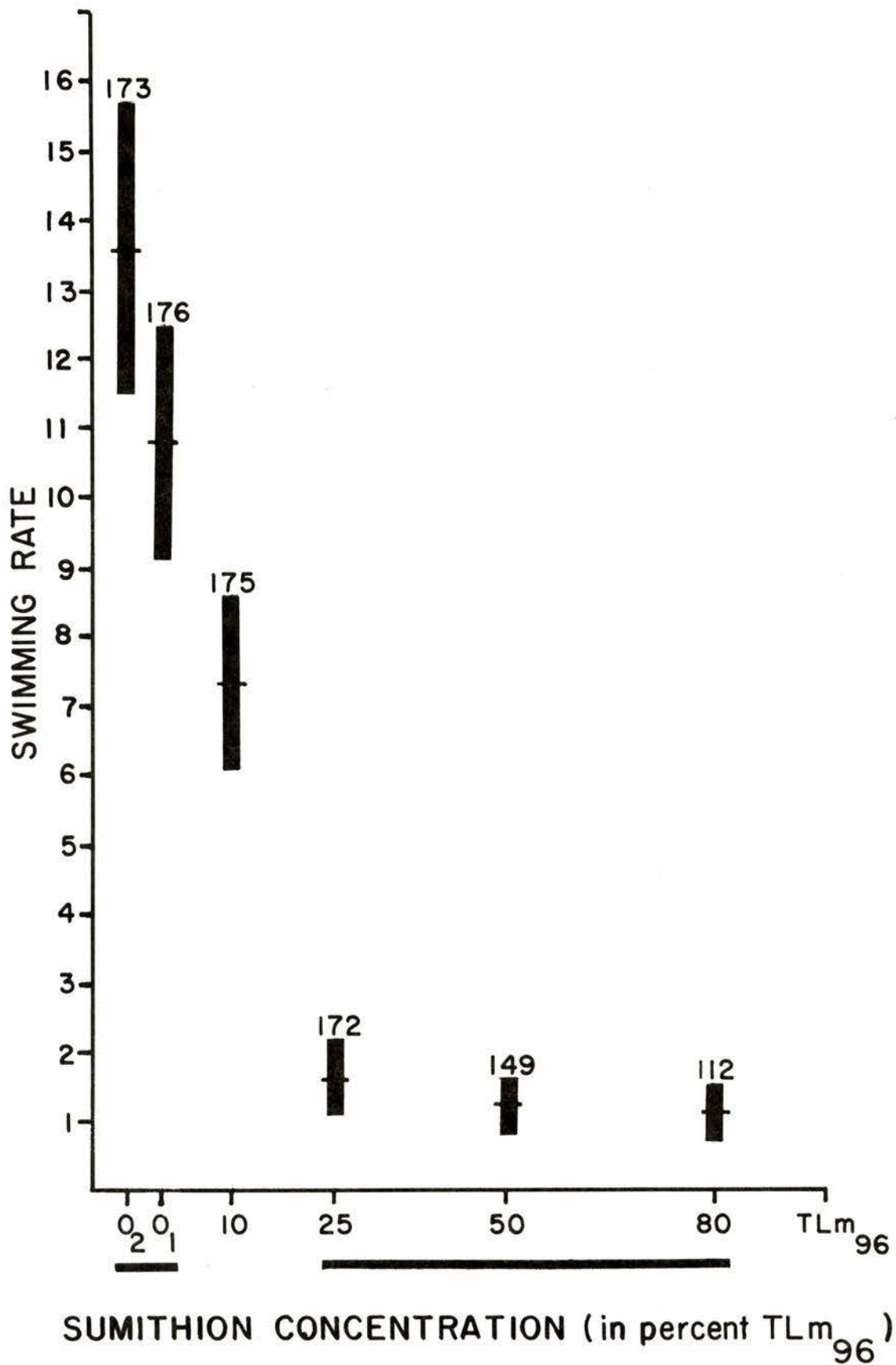
Movement of fish within the stream aquarium was initially restrained. Typically when fish were introduced to the tank they swam straight down to within 6 cm of the substrate and remained grouped together. Within an hour they spread out and explored the aquarium. By the first observation period (two hours after fish were introduced) many fish were feeding and defending territories.

Pre-test and post-test controls showed that in the absence of Sumithion, fish moved at a fairly constant rate. With increasing Sumithion concentrations, however, swimming rate was reduced in an exponential fashion as illustrated in Fig. 9. (In figures 9 to 14 pre-test control,  $O_1$ , may have followed post-test control,  $O_2$ , or vice versa. The order was arranged to avoid reversing the curve).

## (ii) Feeding and Chewing

Large fish seemed to dominate the most suitable feeding

Fig. 9 Swimming rate (distance moved in feet per 5 minute observation period) at various concentrations of Sumithion. Horizontal lines represent means for all fish at each time period. Vertical lines represent 95% confidence intervals. The number of scores comprising each mean appears above the confidence interval. Concentrations not underscored by the same line are significantly different (sign test,  $p < 0.05$ ).  $O_1$  denotes pre-test control.  $O_2$  denotes post-test control.



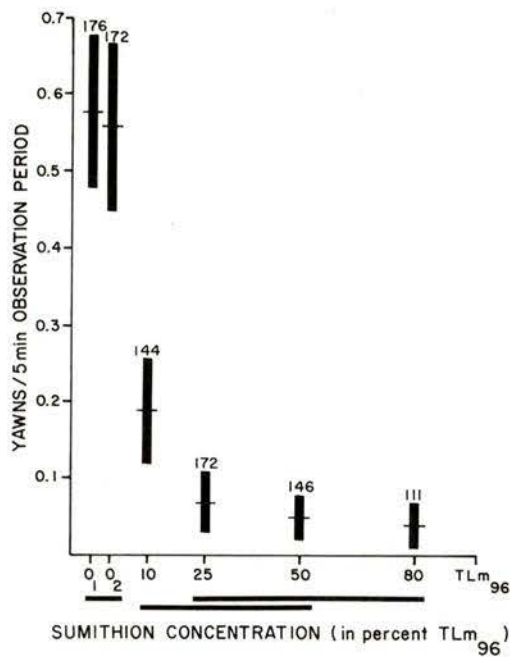
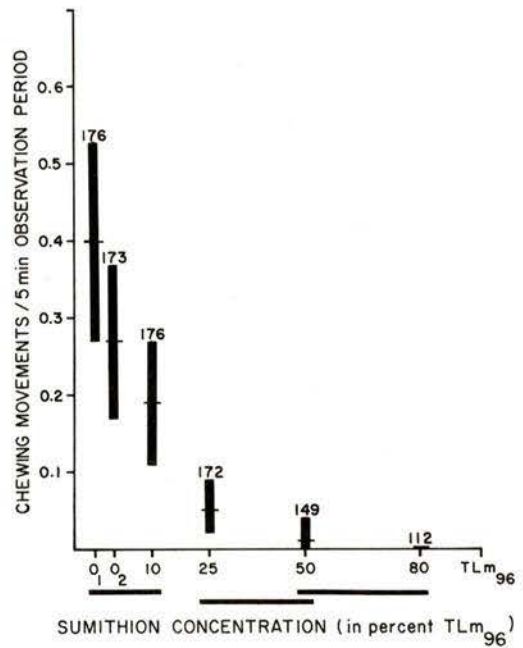
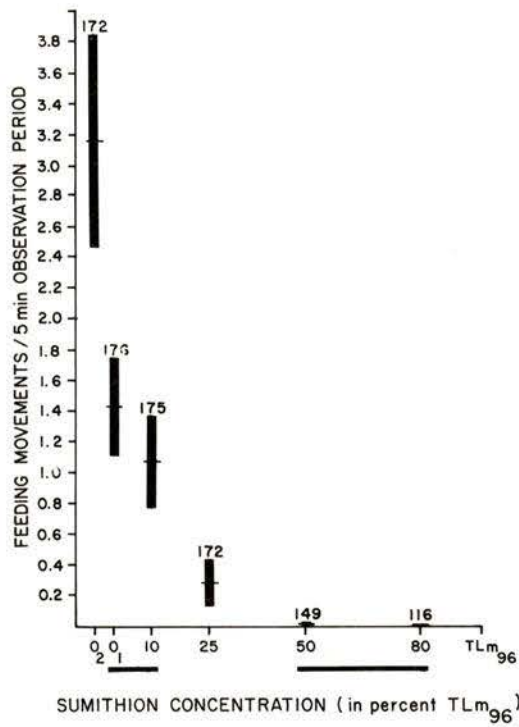
areas. Fringe areas close to screens or glass seemed to be less desirable than more open areas for two reasons; feeding movements were restricted in certain directions, and less food was available due to lower current velocities (Chapman and Bjornn, 1968). These areas were usually occupied by small fish. Large fish spent most of the time near the bottom and swam up for food, but they seldom went all the way to the surface. Fish which dwelt near the surface (generally small fish) swam laterally and upward but seldom moved down for food. Feeding movements ranged in distance from a fraction of an inch to 3 feet. Average movements seemed to be about 6 inches in length.

Feeding was usually, though not always, followed by chewing, but chewing movements sometimes occurred without feeding movements. Feeding and chewing movements both became less frequent as the concentration of Sumithion increased (Fig. 10). At high test concentrations both ceased completely. To confirm these findings, excessive quantities of food were offered to fish after observations at a particular test concentration were complete. Feeding activity was intense in the absence of Sumithion and non-existent at high toxicant concentrations. At intermediate concentrations (10% and 25% of the  $TLm_{96}$  level) some fish fed actively while others refrained.

(iii) Yawning

Yawning, like feeding and chewing, diminished in frequency as the concentration of Sumithion increased (Fig. 10). The diminution occurred in an exponential

Fig. 10 Frequency of feeding movements, chewing movements, and yawns at various concentrations of Sumithion. Horizontal lines represent means for all fish at each time period. Vertical lines represent 95% confidence intervals. The number of scores comprising each mean appears above the confidence interval. Concentrations not underscored by the same line are significantly different (sign test,  $p < 0.05$ ).  $O_1$  denotes pre-test control.  $O_2$  denotes post-test control.



fashion so that it was most noticeable at low concentrations.

#### (iv) Comfort Behaviours

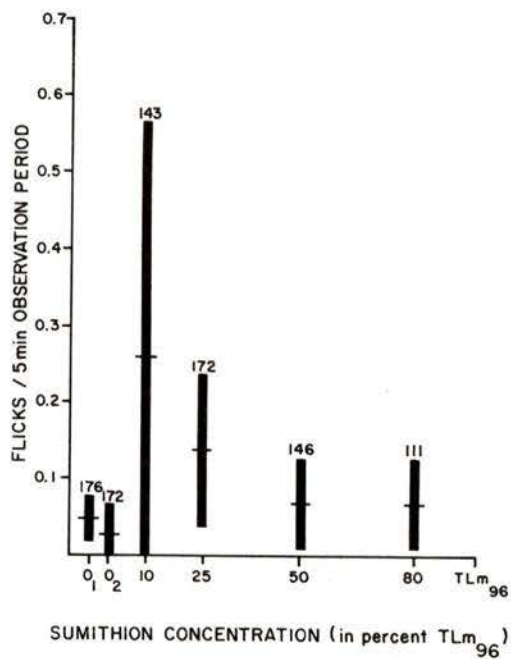
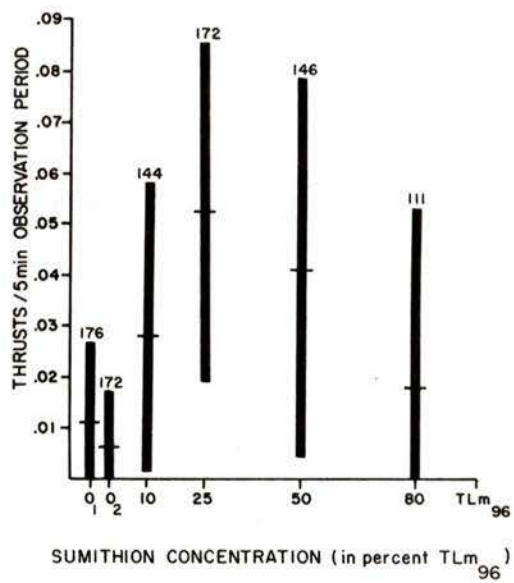
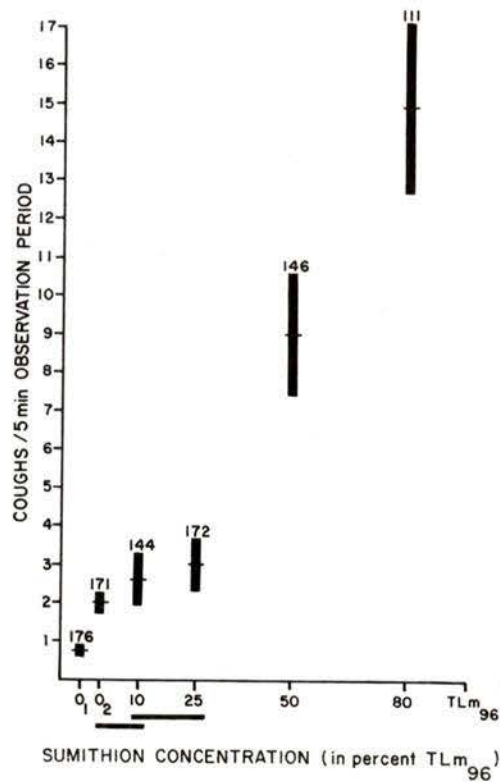
Coughing, thrusting and flicking were all termed comfort behaviours on the basis of the few available reports by others. Coughing is probably an attempt by fish to clear their gills of an irritating substance. Servizi, Gordon and Martens (1969), Schaumberg, Howard and Walden (1967) and Walden, Howard and Froud (1970) have indicated the usefulness of this type of behaviour in evaluating toxicants. Coughing was the only type of behaviour studied which increased progressively with increasing concentrations of Sumithion (Fig. 11). Unlike most other types of behaviour, the greatest rate of change occurred at the two highest concentrations.

Thrusting and flicking have seldom, if ever, been reported by researchers of salmonid behaviour, but they closely resemble S-bending and stretching reported as comfort behaviours by researchers working with sticklebacks (Gasterosteus aculeatus) (Tugendhat, 1960). Both flicking and thrusting increased somewhat at low concentrations of Sumithion and then decreased (Fig. 11). Both behaviours occurred more frequently at all concentrations of Sumithion than in clear water.

#### (v) Agonistic Behaviours

Fish size was a major factor influencing agonistic behaviours. Ninety per cent of chases, 75% of displacing movements, 69% of nips, and 61% of aggressive displays were directed toward smaller fish. Alternatively, 90% of appeasements, 90% of flights and all vacating movements were in

Fig. 11 Frequency of comfort movements (coughs, thrusts, and flicks) at various concentrations of Sumithion. Horizontal lines represent means for all fish at each time period. Vertical lines represent 95% confidence intervals. The number of scores comprising each mean appears above the confidence interval. Concentrations not underscored by the same line are significantly different (sign test,  $p < 0.05$ ). If no horizontal lines appear under the X axis, individual comparisons were precluded by a lack of significance in the overall test.  $O_1$  denotes pre-test control.  $O_2$  denotes post-test control.



response to larger individuals. Social interactions were most frequent among fish of nearly equal size.

The intensity of conflicts varied from time to time for no apparent reason. Even after territories were established, individuals occasionally came within 8 cm of each other without expressing aggressiveness. The same fish may have, at another time, travelled the length of the aquarium section to chase another individual.

Aggressive displays were more than twice as frequent as chases. On the other hand, the number of submissive displays (appeasements) recorded was only half that of flights. This might indicate that in many situations mere lack of aggression represents an appeasement.

All the agonistic behaviours studied, except nipping, decreased in frequency as the concentration of Sumithion increased (Fig. 12 and 13). In the case of displacing movements and appeasements the decrease was not shown to be statistically significant. Neither aggressive types (chasing and display) nor submissive types (flights and vacating) of agonistic behaviour were altered significantly at Sumithion concentrations between 10 and 80%  $TLm_{96}$ . Vacating movements ceased completely in the presence of Sumithion.

(vi) Interstation Distance and Distribution of Stations

Interstation distance, the distance between stations occupied from one time period to the next, decreased as the concentration of Sumithion increased (Fig. 14), though the decrease was not statistically significant.

Fig. 12 Frequency of aggressive behaviours (displays, chases, displacing movements, and nips,) at various concentrations of Sumithion.

Horizontal lines represent means for all fish at each time period. Vertical lines represent 95% confidence intervals. The number of scores comprising each mean appears above the confidence interval. Concentrations not underscored by the same line are significantly different (sign test,  $p < 0.05$ ). If no horizontal lines appear under the X axis, individual comparisons were precluded by a lack of significance in the overall test.  $O_1$  denotes pre-test control.  $O_2$  denotes post-test control.

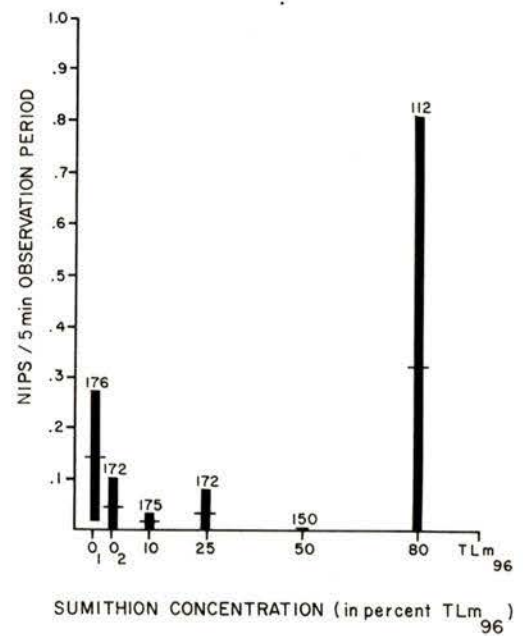
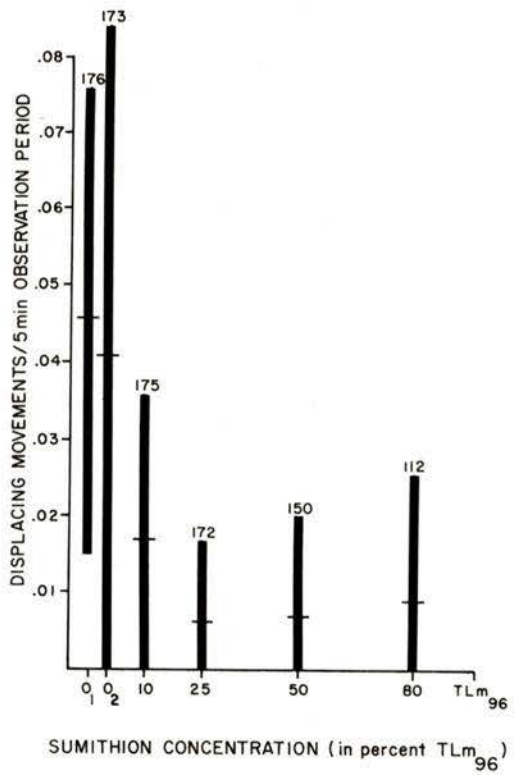
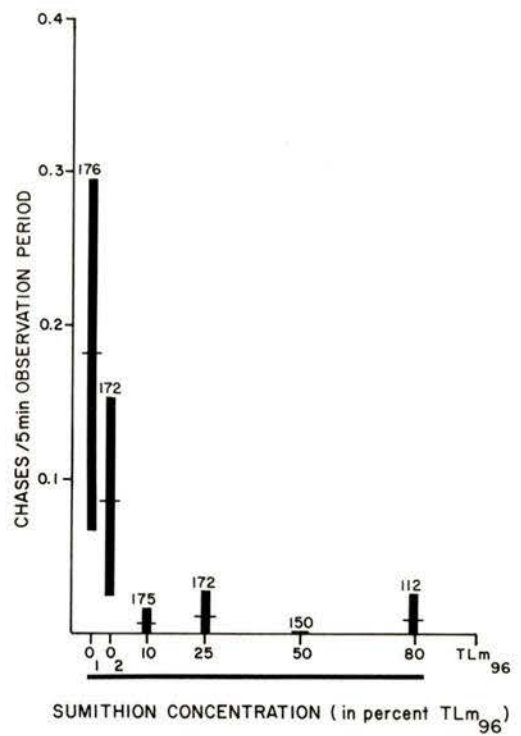
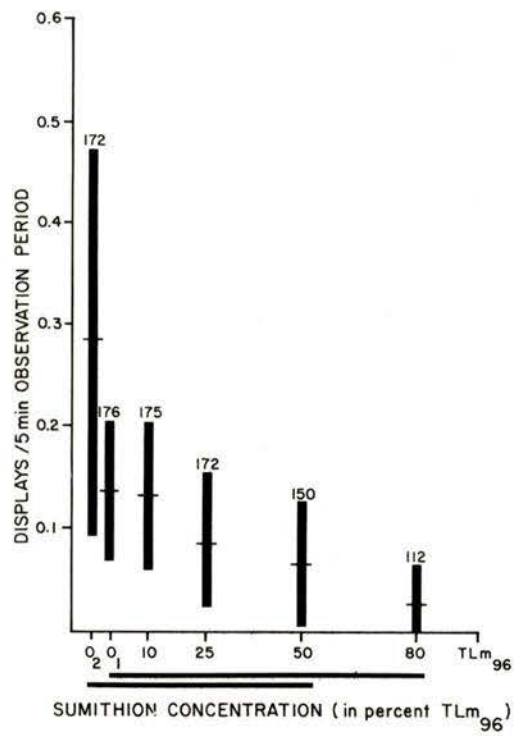


Fig. 13 Frequency of submissive behaviours (flights, vacating movements, and appeasements) at various concentrations of Sumithion. Horizontal lines represent means for all fish at each time period. Vertical lines represent 95% confidence intervals. The number of scores comprising each mean appears above the confidence interval. Concentrations not underscored by the same line are significantly different (sign test,  $p < 0.05$ ). If no horizontal lines appear under the X axis, individual comparisons were precluded by a lack of significance in the overall test.  $O_1$  denotes pre-test control.  $O_2$  denotes post-test control.

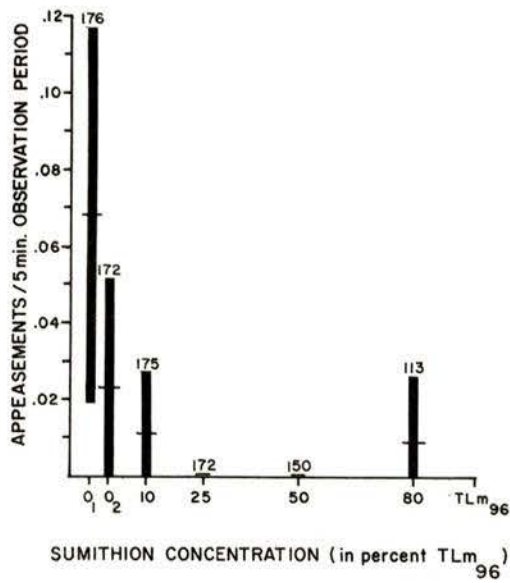
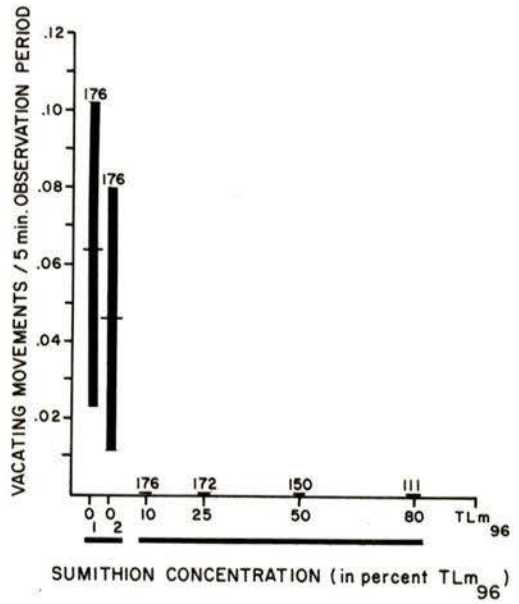
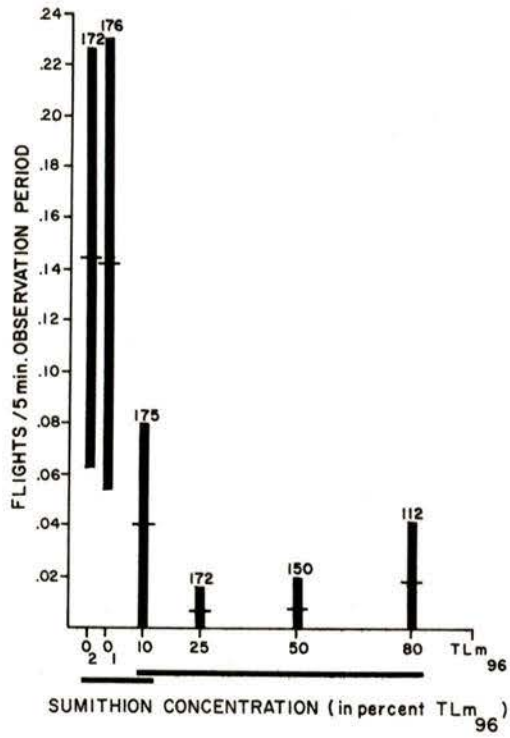
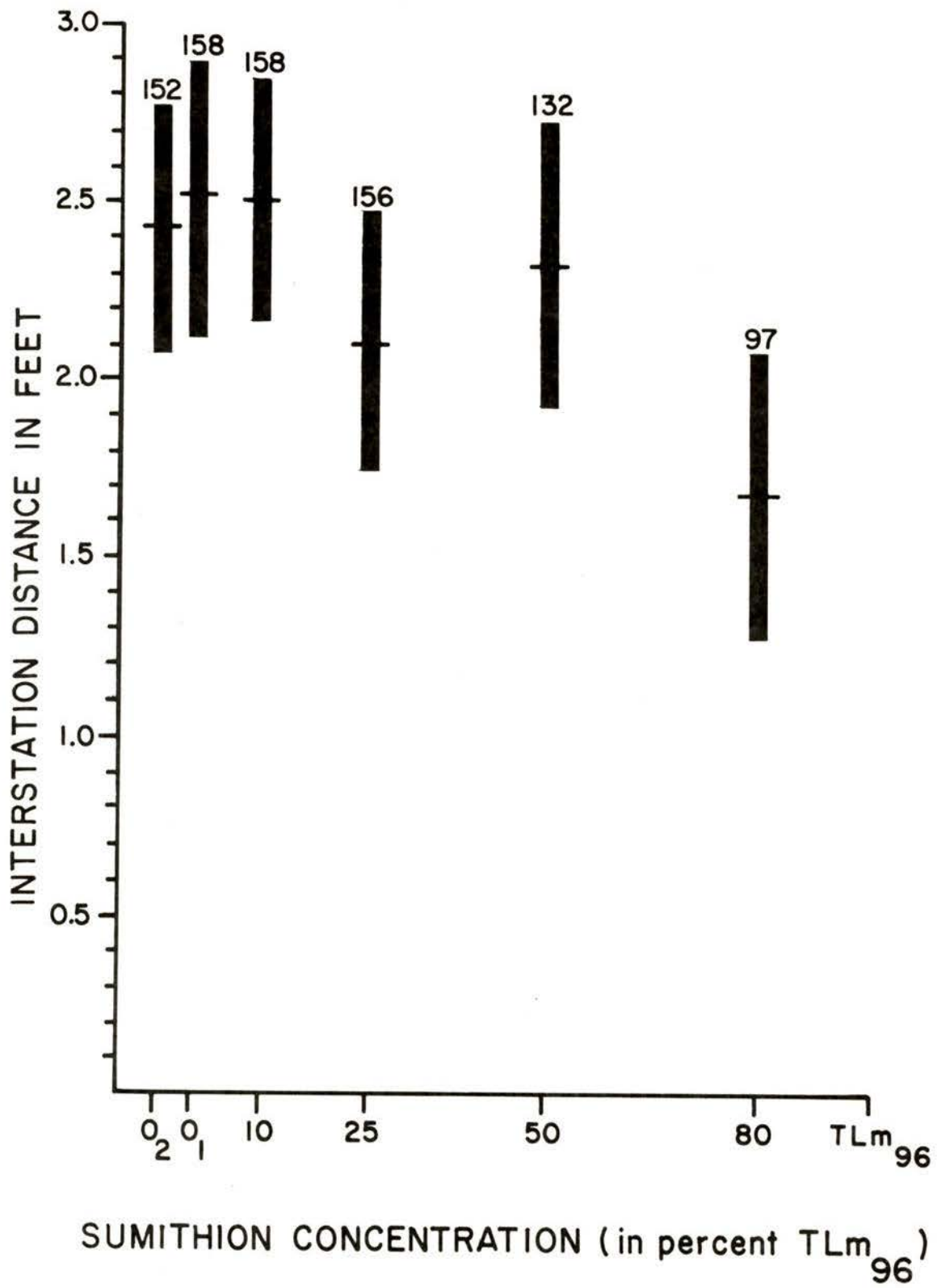


Fig. 14 Interstation distance at various concentrations of Sumithion. Horizontal lines represent means for all fish at each time period. Vertical lines represent the 95% confidence intervals. The number of scores comprising each mean appears above the confidence interval.  $O_1$  denotes pre-test control.  $O_2$  denotes post-test control.



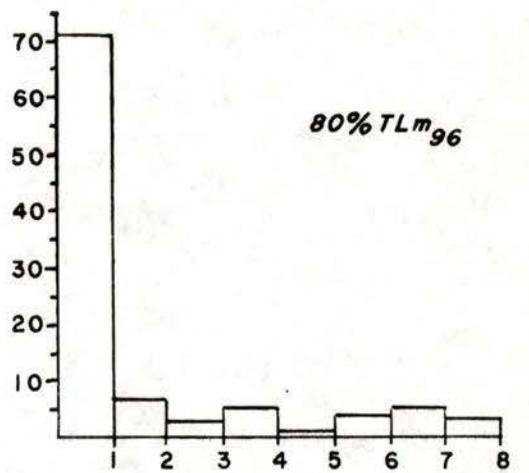
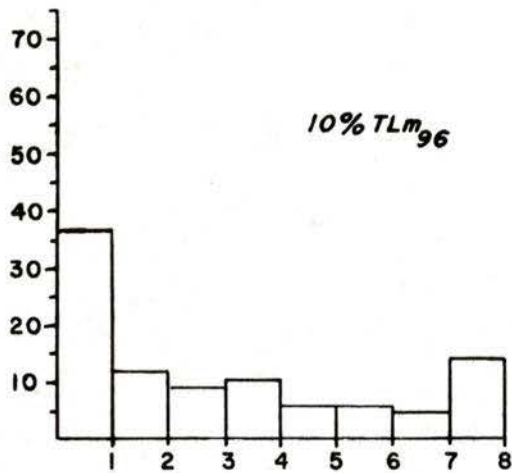
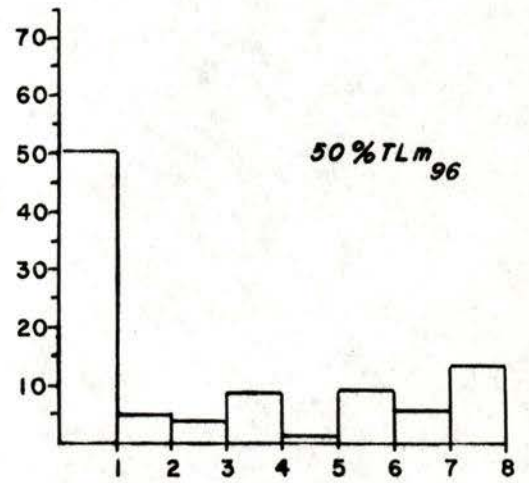
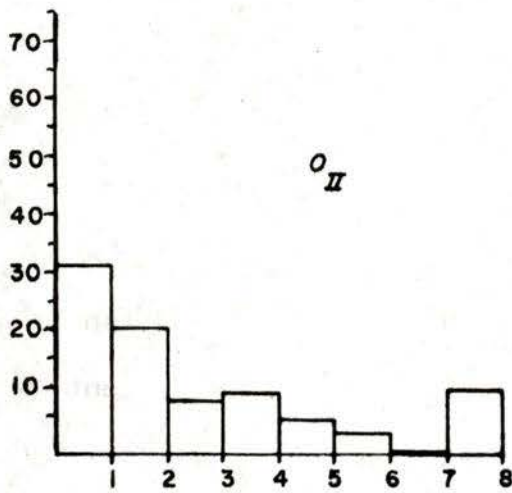
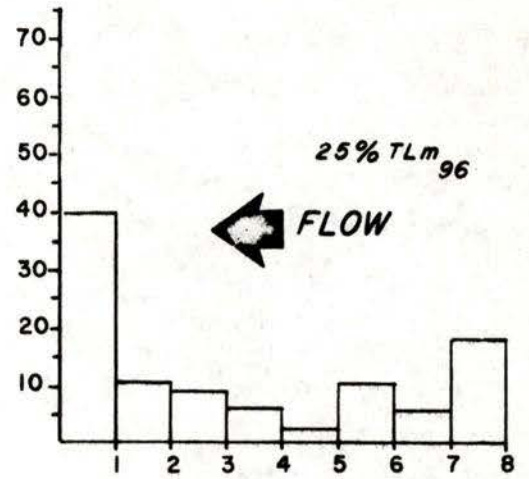
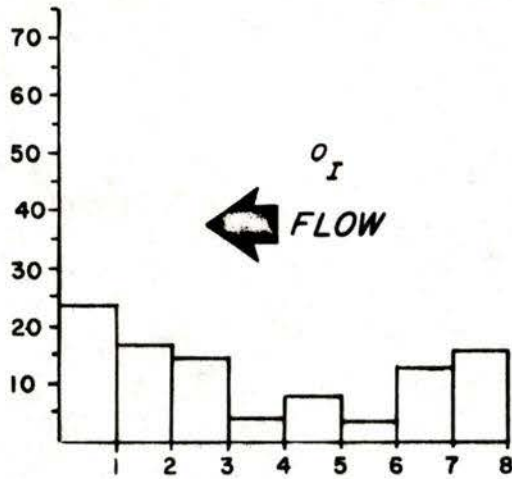
Locations where fish dwelt most of the time shifted in response to the amount of Sumithion present. As the concentration of Sumithion increased, more fish moved to downstream, surface and lateral boundaries of each section (Fig. 15, 16, and 17). The hypothesis that the number of fish in the downstream, surface and lateral boundaries of the aquarium (Fig. 18) did not change with increasing concentrations of Sumithion was tested using Page's L test (Page, 1963). For this test, as with Friedman's two-way analysis of variance, data were cast into a two-way table with columns representing treatment conditions (concentrations of Sumithion) and rows representing time periods. The hypothesis was rejected at the 1% level of significance. Consequently the shift in distribution of stations with increasing concentrations of Sumithion was considered significant.

(vii) Irregular Behaviour and Morphological Changes

In behavioural tests involving high concentrations of Sumithion, some fish were unable to maintain their position against the current and were swept downstream against the screen. They usually remained motionless while pinned against the screen and the force of the current held their operculae shut so that it was impossible to tell whether they were dead or alive. Some fish eventually recovered from this position and resumed swimming against the current. Others remained pinned against the screen and eventually died. Twenty-eight per cent of pinning incidents occurred at a Sumithion concentration equal to 50%  $TLm_{96}$  and 70% occurred at a concentration equal to 80%  $TLm_{96}$ . The remaining 2%

Fig. 15 Longitudinal distribution of fish in the stream aquarium at various concentrations of Sumithion

PERCENT OCCURRENCE OF FISH



LENGTH OF SECTION (in feet)

Fig. 16 Per cent of fish in the upper half of  
the stream aquarium at various  
concentrations of Sumithion

Fig. 17 Per cent of fish in the outer half of  
each aquarium section at various  
concentrations of Sumithion

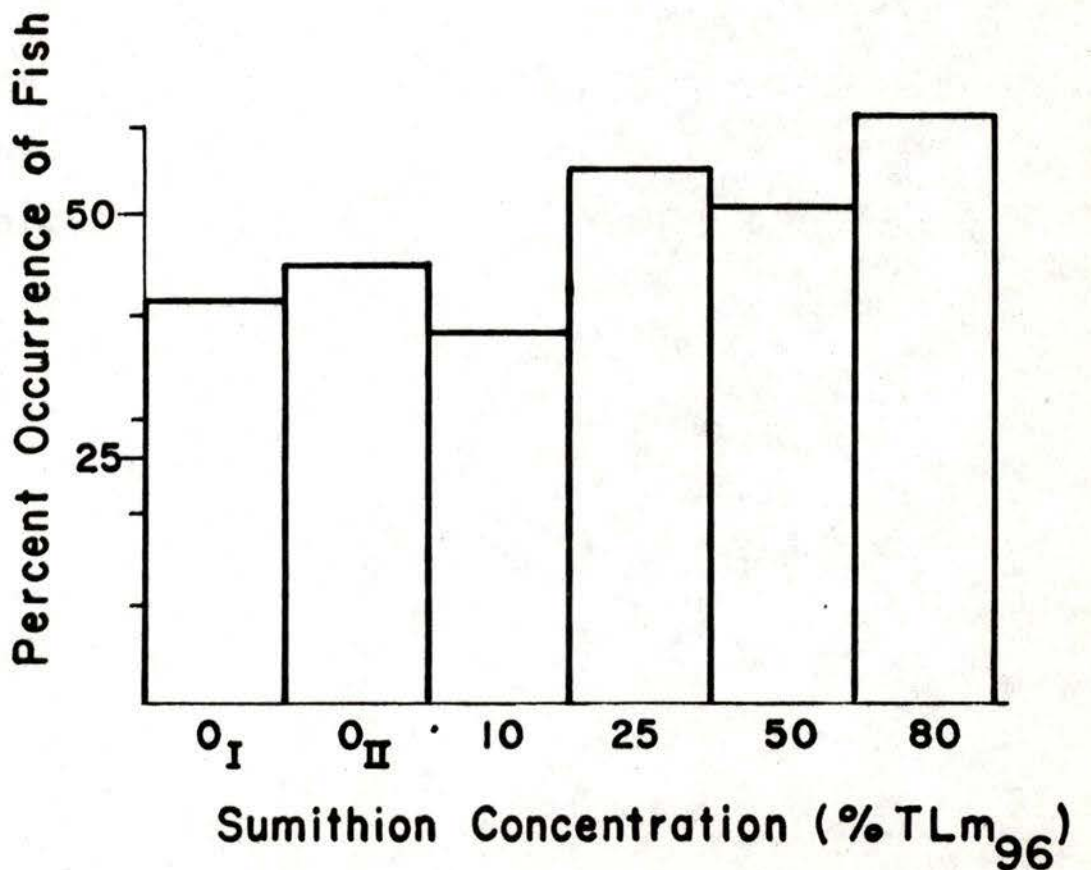
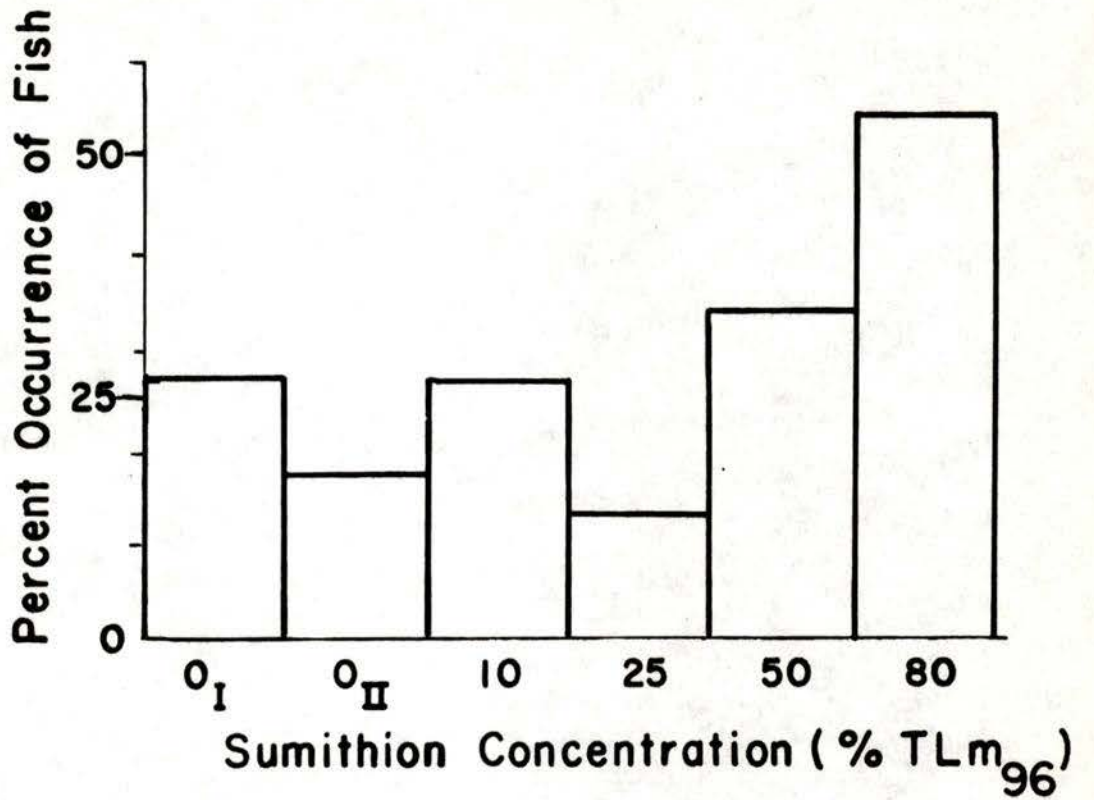
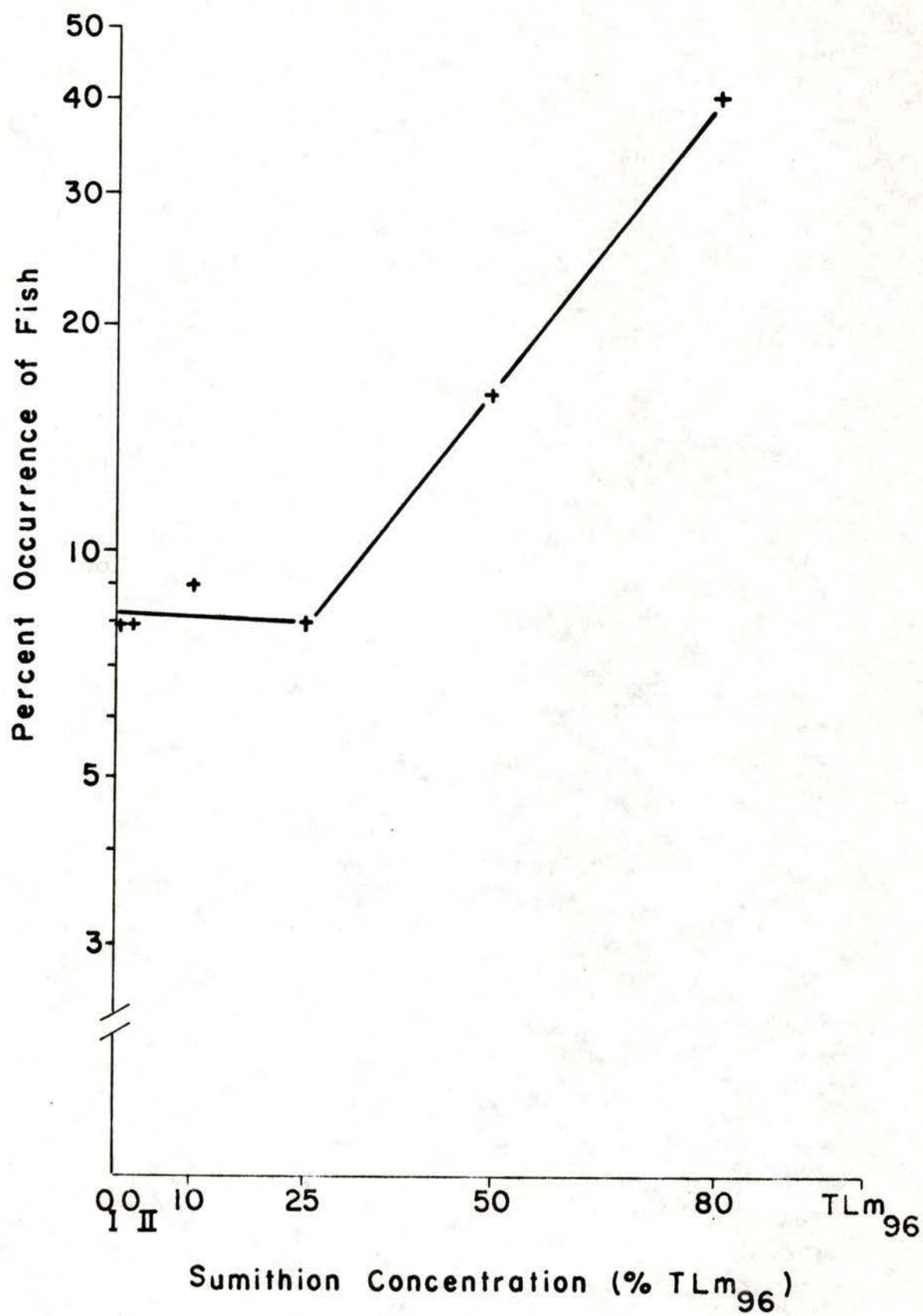


Fig. 18 Combined percentage occurrence of fish  
in downstream, surface and lateral  
boundaries of each section in various  
concentrations of Sumithion



occurred at Sumithion concentrations less than 50%  $TLm_{96}$ . Pinning was evident during the first time period (2 hours after initial exposure to the toxicant), but more fish became incapacitated as exposure time increased (Table VIII). Fish which were pinned were excluded from the experiment until they resumed swimming.

Table VIII Number of fish pinned against the downstream screen under various test conditions

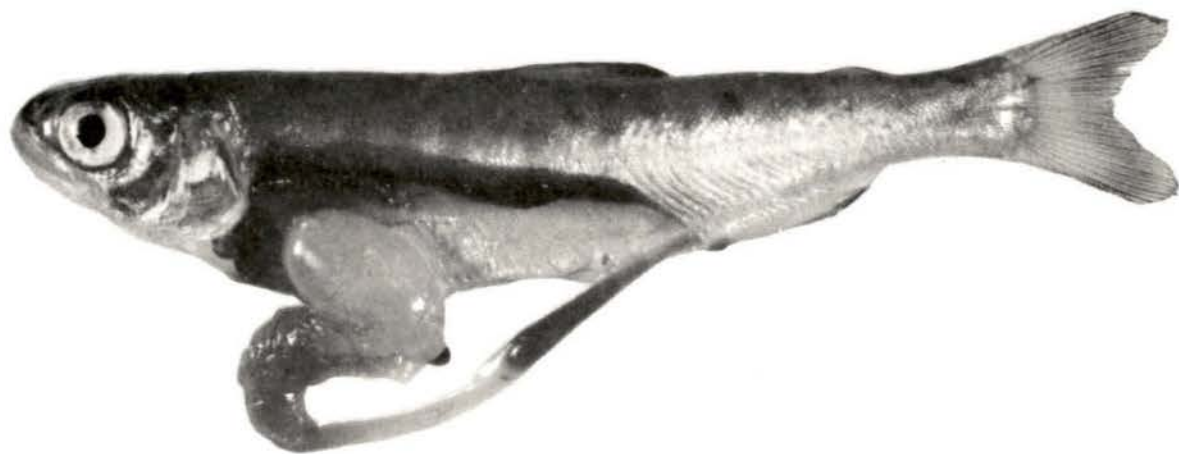
Test Day	Time Period	Concentration					
		$0_1$	$0_2$	10	25	50	80
1	$T_1$	0	0	0	0	1	4
	$T_2$	0	0	0	0	2	4
	$T_3$	0	0	0	0	2	5
2	$T_4$	0	0	0	0	1	7
	$T_5$	0	0	0	0	2	5
	$T_6$	0	0	0	0	2	7
3	$T_7$	0	0	1	0	4	6
	$T_8$	0	0	0	0	3	8
	$T_9$	0	0	0	0	3	6
4	$T_{10}$	0	0	0	0	3	6
	$T_{11}$	0	1	0	0	3	6
Total		0	1	1	0	26	64

Certain kinds of behaviour occurred only at high concentrations of Sumithion (50 and 80%  $TLm_{96}$ ). Occasionally a fish lay stiffly on the bottom with all fins outstretched and remained motionless as if paralyzed. No respiratory movements were apparent, in fact, the only noticeable movement was a tremble of pectoral and pelvic fins. After 30 seconds or so, the fish usually resumed swimming for a few seconds and then returned to its former conditions.

Another behavioural condition peculiar to high Sumithion concentrations was one in which a fish swam very quickly, but aimlessly, coughing and contorting its body, sometimes swimming on its side, often banging into the bottom or sides of the aquarium. This type of behaviour was usually terminated with the fish either being pinned against the downstream screen, or burrowing between the rocks in the substrate. Quite often burrowing resulted in the fish dying well within the substrate.

Some morphological changes occurred at 80%  $TLm_{96}$ , but these were not quantified. Five of the 16 fish appeared bloated after 5 hours of exposure to Sumithion. The bloated appearance disappeared within 48 hours. Similar bloating occurred frequently in bioassay fish exposed to lethal and near lethal concentrations of Sumithion (Fig. 19). Dissection revealed the presence of clear watery liquid in the corpus (Barrington, 1957) portion of the stomach.

Fig. 19 External and internal views of normal coho (uppermost fish in both cases), and coho subjected to 1.3 mg/liter of Sumithion (the  $TLm_{96}$ ) for 18 hours



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## DISCUSSION

## A. Bioassays

Very little information is available in the literature to substantiate the 96 hour median tolerance limit of juvenile coho salmon in Sumithion. However, Sprague (1966-unpubl.) reported the  $LD_{50}^*$  (lethal dose for 50% of the test animals) of Sumithion to juvenile Atlantic salmon (Salmo salar) to be 1.4 mg/liter after 48 hours. This level closely approximates the 96 hour median tolerance limit (1.30 mg/liter) found in the present study for juvenile coho salmon.

Technical grade Sumithion used in the present studies contains 53% active agent and 47% emulsifier. The question arises as to whether toxicity of the mixture might be due to the emulsifier rather than the active agent. Iyatomi, Tamura, and Itazawa (N.D.) found that this was not so. They reported that Sumithion was three times more toxic to carp (Cyprinus carpio) than the emulsifier.

Sumithion caused many bioassay fish to swim near the surface in a head up, tail down position. Abramson, Weiss, and Baron (1958) found that respiratory depressant chemicals (potassium cyanide, sodium azide and LSD-25) caused identical behaviour in Siamese fighting fish (Betta splendens). The suggestion that surfacing behaviour and head up swimming of coho resulted from respiratory distress is strengthened by the fact that Sumithion substantially increased gill

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\*TLM is recommended (American Public Health Association, (1965) rather than the corresponding measure  $LD_{50}$  often seen in toxicology literature.

ventilation rate. Swimbladder gases may have been lost under hypoxic conditions causing negative bouyancy and therefore a head up, tail down posture. Swimming movements executed by a fish which adopted such a posture would result in fish moving to the surface.

The possibility that surfacing occurred as an avoidance of Sumithion near the bottom of bioassay vessels was over-ruled when Sumithion analyses showed concentrations at the surface and substrate to be identical. It is possible, however, that surfacing and head up swimming was not due to respiratory causes. Further study would be required to delineate mechanisms responsible for surfacing behaviour.

#### B. Sumithion Analyses

Concentrations of Sumithion in the bioassay vessels decreased rapidly during the first day of the test, then slowly during the subsequent three days. This type of decrease is typical of chemical reactions in general (Sienko and Plane, 1966), and other workers (Miller, Zuckerman and Charig, 1966) have found that organophosphates similar to Sumithion (parathion and diazinon) degrade in a similar manner. Miller et al. (1966) attributed the breakdown to the action of microorganisms. It is unlikely that degradation products of Sumithion were more toxic than the parent compound, since most bioassay fish died during the first 24 hours.

Sumithion concentrations in bioassay vessels which contained fish were slightly lower than the concentration in the stream aquarium and the bioassay vessel without fish.

This was as expected, since fish accumulated large concentrations of Sumithion. The stream aquarium contained slightly less Sumithion than the bioassay vessel without fish perhaps because the rocky substrate in the stream aquarium probably supported a greater population of microorganisms than glass bioassay vessels. However, concentrations of Sumithion in all experimental vessels were sufficiently similar that differences may have been due to dilution, sampling or measurement errors.

Sumithion was found to accumulate in the bioassay fish to levels many times the concentration in surrounding water. The accumulation rapidly reached a maximum level and then slowly declined. Miller et al. (1966) reported similar findings with parathion and diazinon accumulations in Fundulus heteroclitus.

The mechanism by which organophosphates accumulate in fish may be similar to that described by Fromm and Hunter (1969) for the organochloride pesticide dieldrin:

"We suggest that dieldrin and other related insecticides diffuse through the gills of fishes and are dissolved in the lipid portion of plasma lipoprotein, in which form they are transported to and become dissolved primarily in the lipid portion of various tissues. Since the insecticide is much more soluble in lipid than in water the tissue concentrations attain levels far above that of the environmental water independent of any active transport mechanisms."

A similar uptake mechanism may have operated in the present study since Sumithion has solubility properties similar to organochloride pesticides (Sumitomo Chemical Co., Ltd., 1967).

If this explanation of pesticide accumulation is correct, the question arises as to why the accumulation does

not continue until the fish eventually dies. Grzenda, Paris, and Taylor (1970) found with  $C^{14}$  labelled DDT that intact DDT first reached a maximum level in fish, and subsequently that metabolites of DDT continued to accumulate. Their interpretation was that DDT was being metabolised by induced enzymes. Presumably a similar degradation of Sumithion occurred in the present study.

### C. Behaviour

Behavioural results obtained in the present study parallel the findings of several other researchers. The decline in swimming rate agrees with work of Hatfield (1970) in which activity was reported to decrease when Atlantic salmon (Salmo salar) were exposed to Sumithion. The decline in feeding rate is supported by the work of Misu, Segawa, Kuruma, Kojima and Takagi (1965) who found that rats fed Sumithion treated food consumed less than rats fed uncontaminated food. Furthermore, according to Hatfield (1970), a decline in feeding caused by organophosphates has been shown for fish by Rongsriyam, Prownebon, and Shusmosuke (1968). Since feeding behaviour is in part dependent upon overall activity (Swift, 1964) and aggressiveness is in turn dependent upon feeding (Newman, 1956), both feeding and social behaviours would be expected to decline because of the general decrease in locomotion.

The increase in coughing frequency is supported by the work of Servizi, Gordon and Martens (1969); Schaumberg, Howard and Walden (1967); and Walden, Howard and Froud (1970) who reported similar increases in salmonids exposed to

sublethal concentrations of pollutants. Likewise, the increase in flicks noted at lower concentrations of Sumithion is similar to findings of Abramson and Evans (1954) who report that Siamese fighting fish (Betta splendens) "kinked" their bodies when subjected to lysergic acid diethylamide (LSD-25). Finally, the increase in thrusts is substantiated by the work of Matton and LaHam (1969) who have described a similar reaction in rainbow trout (Salmo gairdneri) larvae exposed to the organophosphate Dylox.

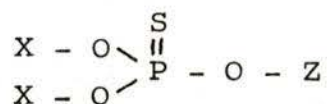
(i) Mode of Action of Organophosphates

The following is an attempt to integrate the observed behavioural effects of Sumithion with what is known of physiological and biochemical effects of organophosphates.

Organophosphates exhibit a common mode of anticholinesterase action, and there is no reason to believe that Sumithion is exceptional. In fact, in vivo and in vitro studies involving Sumithion (Hatfield, 1970 and Warner, 1967) have shown that it affects fish in a similar manner to other phosphorothioates\*. The mode of action of organophosphates as will be outlined has been demonstrated in studies of insects (Reay, 1969), fish (Weiss, 1961) and mammals (O'Brien, 1960 and Holmstedt, 1959). The following account of physiological effects of Sumithion is based mainly

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\*Phosphorothioates are organophosphates with the generalized structural formula:

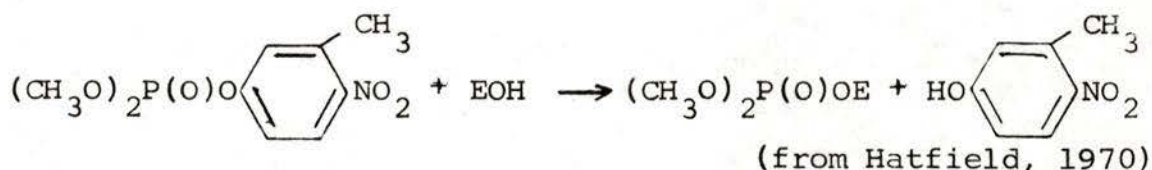


Where X and Z symbolize a variety of chemical groups.

upon comprehensive reviews of the subject by Holmstedt (1959) and O'Brien (1960).

After an organophosphate enters the body it is changed in form by microsomal enzymes (especially NADPH<sub>2</sub> in the liver) to its oxygen analog and to various other phosphorates. Conversion in mammals is generally complete within 15 to 30 minutes.

Oxygen analogs rather than phosphorothioates themselves are usually responsible for physiological and behavioural changes in vertebrates. Consequently it is probably Sumioxon [0,0 dimethyl 0-(3-methyl - 4 -nitrophenyl) phosphate] rather than Sumithion that phosphorylates acetylcholinesterases (and other enzymes) and thus destroys their ability to catalyse the breakdown of acetylcholine. The reaction is as follows:



where EOH symbolized the cholinesterase.

The rate of reaction is dependent upon an equilibrium between those enzymatic reactions which produce Sumioxon from Sumithion and those which break down Sumioxon into degradation products of low toxicity such as desmethylsumithion (0-methyl 0-hydrogen 0-3-methyl-4-nitrophenyl phosphorothioate), dimethylphosphorothioic acid and dimethylphosphoric acid.

As a result of the phosphorylation process, acetylcholine accumulates, causing repeated firings and eventual

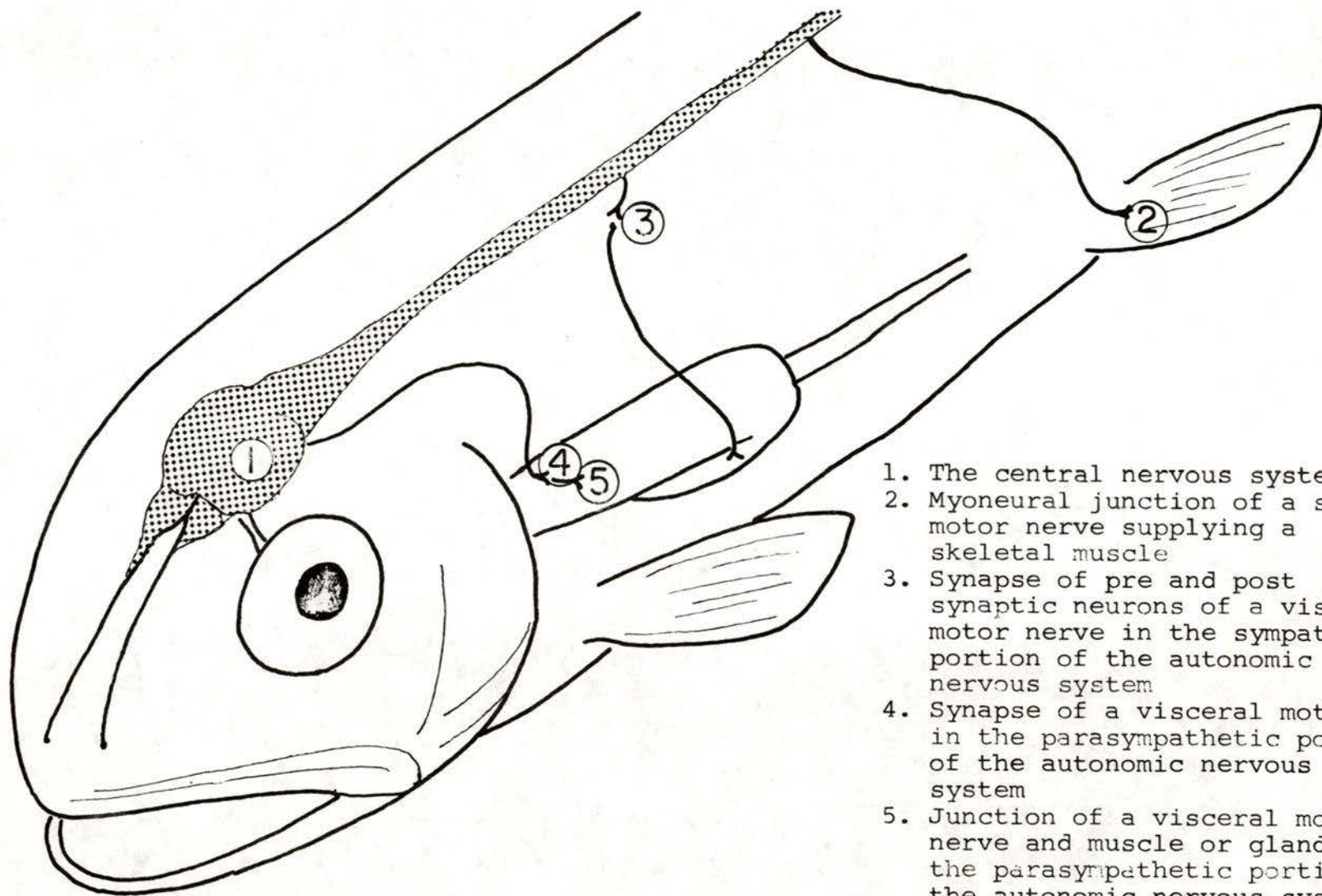
fatigue of cholinergic junctions of the nervous system. Known cholinergic junctions (Fig. 20) include; sites within the central nervous system, myoneural junctions between somatic motor nerves and skeletal muscles, synaptic gaps between pre and post ganglionic neurons and parasympathetic nerve-organ junctions, both of the visceral motor system. Afferent (sensory) portions of the somatic peripheral nervous system are unaffected by anticholinesterase chemicals.

Warner (1967) suggested that certain tissues of the central nervous system are especially sensitive to organophosphates, and O'Brien (1960) states that anticholinesterase chemicals result in lack of coordination and partial paralysis in the mammalian central nervous system. Death by asphyxia eventually results from muscle paralysis.

#### (ii) Basis of Behavioural Changes

Since anticholinesterase chemicals are not believed to affect sensory pathways (O'Brien, 1960), Sumithion must have interfered centrally within the nerve cord and brain, or peripherally at nerve-muscle junctions. Present results suggest that low concentrations of Sumithion cause motivational changes within the central nervous system, whereas high concentrations cause physical impairment probably both centrally and peripherally (Warner, 1967 and Matton and LaHam, 1969). The division between these two types of effects occurs somewhere between Sumithion concentrations of 25 and 50% TLM<sub>96</sub>. Concentrations below this level are said to be

Fig. 20 Types of cholinergic nerve junctions  
in fish



1. The central nervous system
2. Myoneural junction of a somatic motor nerve supplying a skeletal muscle
3. Synapse of pre and post synaptic neurons of a visceral motor nerve in the sympathetic portion of the autonomic nervous system
4. Synapse of a visceral motor nerve in the parasympathetic portion of the autonomic nervous system
5. Junction of a visceral motor nerve and muscle or gland in the parasympathetic portion of the autonomic nervous system

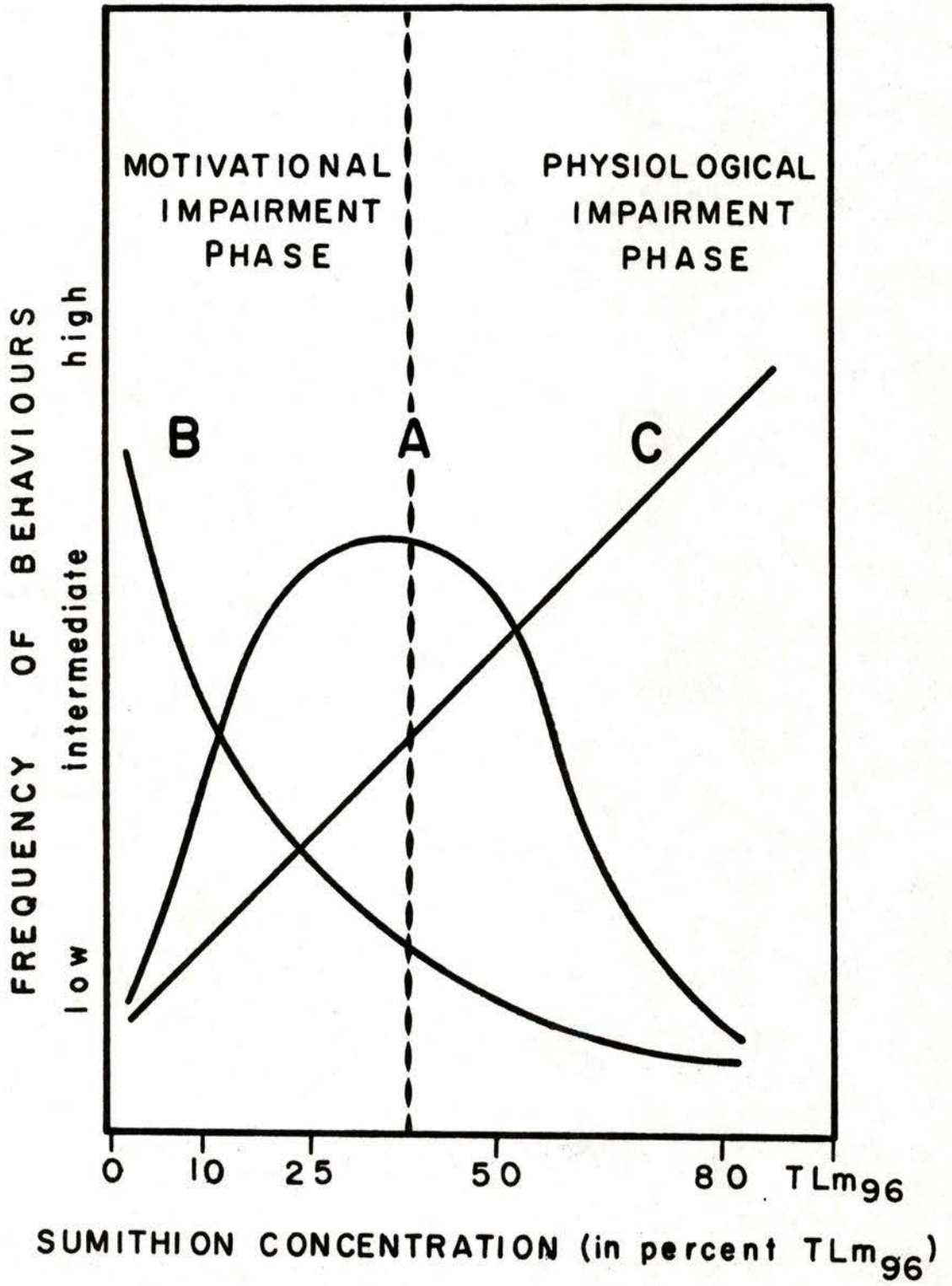
within the motivational impairment phase (Fig. 21). Higher concentrations are considered to be within the physiological impairment phase.

Evidence that behaviour was not physiologically affected within the motivational impairment phase is provided as follows. If nerve pathways and musculature required for locomotion had been impaired by Sumithion, fish would not have been able to swim constantly against the current and would have been displaced downstream. However, such a displacement was not evident until the physiological impairment phase (Sumithion concentrations in excess of 25 to 50%  $TLm_{96}$ ) was reached (Fig. 18, page 46). Although locomotion was not physically hampered in the motivational impairment phase, the frequency of many behaviours involving locomotion (e.g. feeding and agonistic behaviours) was altered. Consequently the alteration must have been due to motivational changes. The occurrence of motivational changes is further illustrated by the cessation of vacating movements. Since these movements were executed by drifting passively with the current, they would not have ceased because of physical impairment.

Physiological impairment was apparent at concentrations above 25 to 50%  $TLm_{96}$ . Many fish were unable to maintain their position against the current, so that they were frequently pinned against the downstream screen and many died (Fig. 18, page 46 and Table VIII, page 47).

Three general patterns of response were observed as

Fig. 21 General patterns of behavioral response



Sumithion concentrations increased (Fig. 21); pattern A behaviours increased and then declined, pattern B behaviours declined exponentially, and pattern C behaviours increased continuously. These varying patterns may be reconciled with the concept of motivational and physiological phases of behavioural change as follows.

Comfort behaviours not involving branchial musculature (flicking and thrusting) were included in pattern A. These behaviours increased in frequency throughout the motivational phase of change but decreased thereafter. Presumably these behaviours initially increased in response to irritation caused by Sumithion, but physiological impairment reduced the frequency of these movements at higher concentrations.

Pattern B behaviours included swimming rate, feeding, chewing, yawning and all the agonistic behaviours. All these behaviours declined rapidly during the motivational impairment phase, but showed little change during the physiological impairment phase. These changes would be expected if pattern B behaviours are inversely related to comfort movements. The increase in pattern A behaviours may have caused a decrease in pattern B behaviours. A similar relationship has been described by Van Iersel and Bol (1958) who note that brooding behaviour inhibits nest-building, escape, aggression and preening of terns.

Coughing (pattern C, Fig. 21) and gill ventilation continued to increase in frequency even at high levels of Sumithion, and they appear to contradict the physiological

impairment hypothesis. However, both involve the use of branchiometric muscles which respond to chemicals quite differently than muscles involved in locomotion. Servizi, Stone and Gordon (1966) noted that when sockeye salmon were exposed to pulp mill effluent, behaviours involving locomotory musculature were curtailed while coughing increased. The difference between the two types of behaviours as documented in this study and those of others, is probably due to differences in the neural circuitry controlling the two sets of musculature. Respiratory musculature in teleosts is partially controlled by a medullary centre, but this form of control may be dominated by reflex patterns initiated by a variety of physical stimuli (Black, 1951). Whereas the central nervous system is generally dependent on cholinergic synapses which are presumably impaired at higher Sumithion levels, branchiometric musculature bypasses impairment by employing these reflexes.

The increasing frequency of coughing in response to elevated levels of Sumithion provides a possible basis for understanding another apparently anomalous result. Up to 50% TLm<sub>96</sub> nipping showed a pattern of declining response similar to other agonistic behaviours. At 80% TLm<sub>96</sub>, however, it dramatically increased in frequency.

The groups of muscles used in nipping appear to be much the same as those employed in coughing. Possibly at concentrations of 80% TLm<sub>96</sub> coughing behaviour was in some instances mistakenly recorded as nipping because of the

proximity of previously subordinate fish. This interpretation is supported by the observation that nipping, which in control tests and lower Sumithion concentrations was normally preceded by threats and chases, occurred at 80%  $TLm_{96}$  in the virtual absence of these preliminary activities. An alternate explanation is that nipping recorded at 80%  $TLm_{96}$  Sumithion concentrations was generated by some form of neural short-circuitry in the context of high levels of coughing.

(iii) Distribution and Interstation Distance

With increasing concentrations of Sumithion, coho were observed to redistribute to the surface, downstream and lateral boundaries of the stream aquarium. The shift in distribution was not correlated with changes in light intensity or current velocity. Evidence that a shift in distribution could occur under field conditions is provided by Saunders (1969) who noted that brook trout (Salvelinus fontinalis) and juvenile Atlantic salmon (Salmo salar) surfaced and moved downstream following a spillage of agricultural pesticides.

An examination of the vertical distribution of fish (Fig. 16, page 45) reveals that at low Sumithion concentrations most fish occupied positions near the substrate, whereas at high concentrations fish were nearly evenly distributed. Apparently rather than causing active migrations toward the surface, Sumithion resulted in a failure of fish to maintain contact with the substrate. Hoar (1951) stated

that coho fry remain in streams by relating to localities and objects in the daytime and by resting quietly on the bottom at night. He showed that coho are displaced downstream when they rise to the surface at night and when nipping, chasing and territorial behaviour are lessened. Since high concentrations of Sumithion caused surfacing and reduced all aspects of social behaviour it is not surprising that fish were displaced downstream.

Accompanying the relocation to the surface and downstream was a shift in distribution laterally. Fewer fish were located near the screen in the center of the tank as Sumithion concentrations increased. A plausible explanation is that fish prefer lower velocity areas near the screen, but their ability to select these areas was impaired by high concentrations of Sumithion.

The decrease in interstation distance may not have been due to chance, despite the fact that the change was not statistically significant. Many fish shifted to a downstream location and (judging from the swimming rate results) tended to remain there. Consequently, it is not surprising that station locations were restricted.

The preceding explanations for the effect of Sumithion on the behaviour of coho are offered as possible avenues for further research, rather than as firm conclusions. In fact, there is some recent evidence that factors as yet unknown may be involved in organophosphate poisoning (Matton and LaHam, 1969).

Levine (1971) has recently found that when an animal senses stress, pituitary and adrenal glands secrete hormones which greatly influence behaviour. Future testing may reveal that behavioural changes are due to this mechanism or an entirely different one.

(iv) Irregular Swimming Behaviour and Morphological Changes

The irregular, often spastic, swimming behaviour which was observed in the highest concentrations of Sumithion was probably due to neuromuscular blockages. An interesting facet of the irregular swimming patterns was the burrowing behaviour which sometimes occurred. Since this behaviour has been noted under field conditions by others (Saunders, 1969) it appears that many fish killed by insecticides may escape observation.

The external morphological changes evident in some of the test fish were caused by edemata of the corpus portion of the stomachs. Fluid in the stomachs may have been secretions resulting from hyperactivation of the gastric glands, or water which was swallowed by the fish. The former explanation seems most reasonable since similar effects of organophosphates have been noted in mammals (O'Brien, 1960).

D. Management Implications

Results of the present study indicate that quantification of unconditioned behaviours is a most useful bioassay tool. The method is applicable in field situations because spontaneous behaviour of the organism is studied under

simulated natural conditions. Behavioural responses reflect the effects of all factors affecting the organism. Therefore toxicant levels established by behavioural bioassays are more meaningful and objective than those which involve subjectively choosing a level of toxicant below the lethal concentration (Burdick, 1967). The behavioural bioassay methods used in this study should be applicable for a variety of species and toxicants.

An acceptable concentration of toxicant for a particular species would not lower the long term carrying capacity of the environment for that species. Present results indicate that concentrations of Sumithion within the motivational phase (below 25 to 50%  $TLm_{96}$ ) would be acceptable for coho underyearlings for short time periods. Test fish were able to adjust to these concentrations by altering their behaviour. At higher concentrations, behavioural compensation was incomplete, and physiological impairment was evident. The test fish stopped feeding completely, many were swept downstream, and some died. Such concentrations of Sumithion would therefore be unacceptable for even short periods of time.

Even the lowest concentration of Sumithion used in the present test (10%  $TLm_{96}$ ) would be unacceptable for prolonged exposure. It is readily apparent that some of the behavioural changes at this level (e.g. the decrease in feeding) would eventually cause a reduction in numbers of fish. However, Warner, Peterson and Borgman (1966) warn that

"all normal processes are of significance to the organism, and toxicant-induced aberrations of any sort are likely to be deleterious". The decrease in frequency of agonistic behaviours is a good case in point. Stream dwelling fish which feed on invertebrate drift select positions which will maximize food intake while minimizing energy expenditure (Jenkins, 1969). Territorial behaviour prevents all fish from congregating in the most desirable area, thus providing an adequate food supply for more aggressive members of the population (Chapman, 1962 and Kalleberg, 1958), while eliminating subordinate fish (Allen, 1969). Since territory maintenance is dependent upon agonistic behaviours, a reduction in frequency of these behaviours would be seriously detrimental.

A level of toxicant acceptable for prolonged exposure should not alter the frequency of behavioural responses of the test organism. Nor should it affect the numbers of food organisms, predators, and other animals and plants which affect the species of interest (Jernelov, 1970). An acceptable level of Sumithion for coho underyearlings will be less than the lowest concentration tested in the present study. Further research to delineate an acceptable level would be worthwhile, but is beyond the scope of the present study.

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APPENDIX TABLE I Fork lengths (mm) and weights (g)

of fish used in Sumithion bioassays

Sumithion Concentration mg/liter	Mean Fork Length Bioassay			Mean Weight Bioassay		
	1	2	3	1	2	3
0	51	47	63	1.2	1.0	2.8
0.10	50			1.3		
0.32	49			1.1		
0.56	50	47		1.2	1.0	
1.00	49	46		1.2	0.9	
1.15			65			2.7
1.19			67			2.9
1.34			65			2.7
1.39			66			3.2
1.43			65			3.1
1.61			67			3.8
1.80	50	48	64	1.5	1.4	3.2
3.20	48	45		1.4	1.1	
5.60	56			2.5		
Mean	50	47	65	1.4	1.0	3.0

APPENDIX TABLE II Fork length (mm) of experimental fish

Section	Fish No	Sumithion Concentration (%TLM96)					
		O <sub>1</sub>	O <sub>2</sub>	10	25	50	80
1	1	49	44	42	41	41	43
1	2	55	49	49	57	50	49
1	3	63	51	54	62	61	75
1	4	78	70	66	79	73	78
2	1	-	40	41	48	40	-
2	2	53	54	52	58	45	56
2	3	61	62	61	67	65	59
2	4	74	71	66	82	72	73
3	1	45	42	46	44	37	46
3	2	64	48	47	46	45	52
3	3	69	59	55	58	60	52
3	4	73	65	65	71	72	71
4	1	55	41	44	45	42	47
4	2	60	50	50	45	50	48
4	3	69	59	59	57	61	49
4	4	79	72	63	68	70	73
Mean		63	55	54	58	55	58

APPENDIX TABLE III Weight (g) of experimental fish

Section	Fish No	Sumithion Concentration (%TLMg6)					
		0 <sub>1</sub>	0 <sub>2</sub>	10	25	50	80
1	1	0.9	0.8	0.7	-	0.6	1.2
1	2	1.6	1.2	1.0	1.7	1.3	-
1	3	2.8	1.6	1.7	2.4	2.6	2.7
1	4	5.1	3.4	4.6	4.6	5.4	3.8
2	1	-	0.8	0.6	-	0.9	1.1
2	2	1.3	1.5	1.3	1.5	1.0	1.6
2	3	2.0	2.7	2.2	2.8	2.5	-
2	4	3.5	3.8	4.1	3.4	4.4	3.7
3	1	0.9	0.8	1.0	0.8	1.0	-
3	2	2.2	1.1	1.1	1.8	1.6	-
3	3	3.1	2.4	3.0	1.9	2.7	2.6
3	4	4.7	3.3	4.8	4.3	5.0	3.8
4	1	1.4	0.7	0.7	0.6	0.7	0.4
4	2	2.0	1.5	1.1	1.3	-	1.1
4	3	3.0	2.5	2.6	2.8	2.8	2.8
4	4	4.4	4.3	3.2	3.9	3.7	1.9
	Mean	2.5	2.0	2.2	2.4	2.4	2.2

APPENDIX TABLE IV Chemical analysis of the water supply

(in mg/liter unless otherwise noted)

Colour .....	10 (units)
Turbidity .....	0.3 (units)
pH .....	7.3 (units)
Chloride (Cl) .....	2.5
Alkalinity (Total) .....	14.5
Total Hardness (CaCO <sub>3</sub> ) .....	17.8
Total Solids .....	33
Dissolved Solids .....	29
Fixed Solids .....	18
Iron (Total) (Fe) .....	Nil
Calcium (Ca) .....	4.48
Magnesium (Mg) .....	1.6
Nitrate Nitrogen (N) .....	0.11
Silica (SiO <sub>2</sub> ) .....	3.0
Sodium (Na) .....	1.4
Potassium (K) .....	0.6
Total Phosphate (PO <sub>4</sub> ) .....	<0.05
Fluoride .....	Nil
Sulfate (SO <sub>4</sub> ) .....	2.4
Copper .....	0.006

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