EFFECTS OF DIETARY METHOXYCHLOR ON BROOK TROUT *(SALVELINUS FONTINALIS)*

by

GEORGE M. KRUZYNSKI

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ABSTRACT

Two experiments were carried out in which continually-swimming brook trout Salvelinus fontinalis were exposed for periods of up to forty days, to an artificial diet containing from 0.5 to 100 ppm of methoxychlor. These dietary levels were chosen on the basis of methoxychlor residues found in aquatic insects which had been exposed to 0.075 mg/l of the insecticide, the standard dosage presently in use on the Quebec North Shore for blackfly larviciding.

Methoxychlor had little effect on the growth in length and weight but produced intermittent mortality and a reduction in swimming stamina at exposure levels in the range of 0.01 to 0.16 mg/kg/day, while fish that had received 1.00 and 2.00 mg/kg/day had a markedly increased stamina.

Residue determinations at the end of the exposure period revealed methoxychlor levels ranging from 2 to 44 ppm in whole fish, while the fat bodies of fish that died during the exposure period contained up to 584 ppm of the insecticide.

Histopathological observations revealed extensive degenerative changes in liver and kidney tissues and a lowered red blood cell count in fish which had been exposed to the insecticide.

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INTRODUCTION

Following the recent ban on the use of DDT in Canada and elsewhere, several chemicals have been adopted as alternate insecticides; of these, methoxychlor, a chlorinated hydrocarbon insecticide, has been used in increasing amounts since 1969 as a replacement for DDT primarily for the control of blackflies (Diptera: Simuliidae) over a widespread area of the North Shore of Quebec and Labrador (West, 1970), as shown on the map in Figure 1, inevitably reaching the waters inhabited by the brook trout, Salvelinus fontinalis and the Atlantic salmon and/or ouananiche, Salmo salar. In New York State, methoxycnlor is the only recommended blackfly larvicide, and is also used for mosquito and sand fly control (Travis, 1970). In view of the importance of the insecticide application on the one hand, and the possible harm to the fishery on the other, this study was initiated to determine in the laboratory, the possible effects that a methoxychlor-contaminated diet may have on brook trout. In the field, the methods of application of methoxychlor are quite similar to those formerly used for DDT, with aerial and ground spraying for the control of larvae in streams, and fogging against adult blackflies (West, 1970). In addition to biting insect control, methoxychlor is used against a

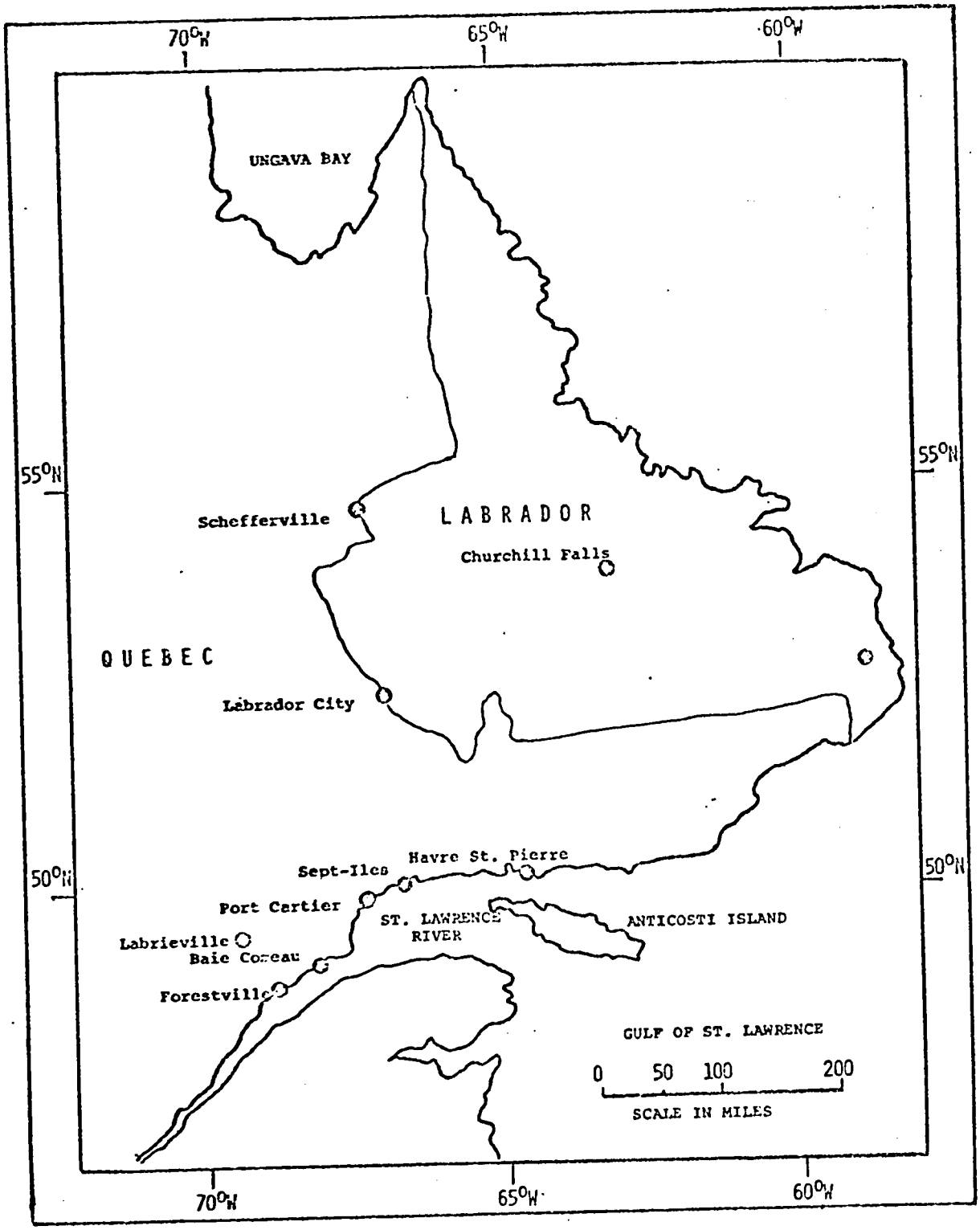

Figure 1. Map showing known locations (black dots) in the Province of Quebec and Labrador (Province of Newfoundland) where methoxychlor has recently been utilized for mosquito and blackfly control.

variety of crop pests in the U.S.; Hickey et al. (1966) indicated that about 15 tons of methoxychlor were being applied annually for the purpose of crop protection to a 400 square mile area draining into Lake Michigan. Despite its massive utilization for over twenty-five years, an estimated 50 X 10^3 tons in the U.S., only scanty information is available on metabolic pathways of detoxication, and the fate of methoxychlor in various ecosystems (Kapoor et al., 1970).

In the aquatic environment, the toxicity of methoxychlor approaches that of its close relative DDT. Ware and Roan (1970) observed an 81 percent decrease in carbon fixation by estuarine phytoplankton exposed to 1 ppm methoxychlor for four hours, as compared to 77 percent for DDT. Sanders (1969) showed that methoxychlor was more toxic to Gammarus lacustris than DDT, and earlier studies by Sanders and Cope (1966, 1968) showed that methoxychlor toxicity to daphnids and stonefly larvae was about equal to that of DDT. Eisler and Weinstein (1967) showed that following a 96-hour exposure to 4 ppb in seawater, the quahuag clam Mercenaria mercenaria accumulated 1300 ppb of methoxychlor and demonstrated a marked alteration of the metallic ion concentration in the mantle tissue. In fish, Henderson et al. (1959), Tarzwell (1963), and Sanders (1969), reported acute toxicity levels ranging

from 0.014 to 0.06 ppm, levels which are similar to those of DDT.

In the extensive literature on pesticide toxicology, the effects of chronic exposure of fish to methoxychlor have received only little attention; Eisler (1967), Grant and Mehrle (1969), Swedberg and Eller (1969) and Kennedy et al. (1970), found increased mortality, tissue damage, a higher gonadosomatic index, and accumulation of the insecticide in the tissues of fish chronically exposed to various concentrations of methoxychlor in the food and/or water.

Field observations suggest that fish kills after pesticide application are caused to a large extent by ingestion of a contaminated food supply in addition to the direct exposure of fish in the water. There is however, a definite lack of information on pesticide residue levels in insects that have been affected by the treatments and controlled studies on the effects of pesticides in the food chain under natural conditions are few in number. Schoenthal (1963) probably came the closest to duplicating field conditions; he collected aquatic insects that had been killed or paralyzed during DDT spraying and fed them to rainbow trout that had been previously exposed to DDT in the water; he found that this treatment caused the fish to swim on to the banks of

the test ponds, a phenomenon that had also been observed under field conditions after forest spraying.

Hatfield (1969) reported a fish kill after DDT larviciding (0.1 ppm for fifteen minutes) and attributed the mortality of brook trout to the consumption of poisoned aquatic insects; the stomach contents of dead fish indicated up to 139 ppm DDT, while the fish contained up to 0.5 ppm. As a result of these observations, larviciding with DDT was discontinued in that area of Labrador, and methoxychlor was selected as an alternate larvicide after the ban of DDT in Canada. There is only little information on the effects of methoxychlor larviciding on non-target invertebrates and fish. Wallace (1971) indicated that current methoxychlor treatment has severe consequences on aquatic invertebrates. Taylor (personal communication, 1971) working in Labrador during the summer 1970, observed the effects of aerial and ground larviciding operations and reported dead and dying fish, which when analyzed by gas chromatography, were found to contain 0.32 to 2.65 ppm of methoxychlor.

Only few experimental studies have been published on the effects on fish of insecticides administered through the diet, despite the well-documented extraordinary capacity of aquatic invertebrates to accumulate insecticides which find their way into natural

waters. Holden (1965) found residues of dieldrin of 20 ppm in caddisfly larvae in streams with dieldrin levels of 0.1 to 0.5 ppm in the water. Kallman et al. (1962) found residues of 16 ppm of toxaphene in insects ingested by trout. Keith (1964) found residues of DDT, dieldrin, toxaphene, and methoxychlor averaging about 5.2 ppm in invertebrates, and more recently Johnson et al. (1971) found biological magnification factors of up to 114,100 for DDT and 141,000 for aldrin in a variety of freshwater invertebrates after a three-day exposure. Andrews et al. (1966) found that bluegills fed a heptachlor contaminated diet exhibited a decreased resistance to parasitism, degeneration of liver tissue, and reduced growth. Macek (1968) fed a DDT-contaminated diet to brook trout and found fewer mature ova, higher mortality in gametes but an increased growth in males after exposure. In a recent paper by Mehrle et al. (1971), rainbow trout which were fed diets containing DDT and dieldrin exhibited a marked accumulation of serum amino acids during forced swimming. Buhler and Shanks (1970) fed a DDT-contaminated diet to coho salmon and found a selective mortality of the smaller fish in the population.

Only a few experimental studies on methoxychlor in fish food could be found in the literature. Grant and Mehrle (1969) found a hastening of gonadal development and a

trend to dilution of serum sodium concentration in cutthroat trout fed a methoxychlor-contaminated diet: Swedberg (personal communication, 1970) found a higher gonadosomatic index, higher mortality, and an accumulation of 15 ppm methoxychlor in whole cutthroat trout. Mayer et al. (1970) found residues of 774 ppm in adipose tissue of rainbow trout, and Grenier (personal communication, 1970) observed no external effects in rainbow trout which had been fed a diet containing up to 100 ppm methoxychlor while they were kept in renewed water but under otherwise static conditions. The only published account of residual methoxychlor in fish food organisms was that of Burdick et al. (1968) who reported no detectable methoxychlor in invertebrates sampled from a test pond thirty-six days after a treatment with 0.005 ppm of methoxychlor, however no samples had been taken from the time of treatment to thirty-six days. In the same study, no residue levels were given for organisms from streams that had actually received methoxychlor treatment in blackfly larviciding, although a heavy drift of stonefly and mayfly larvae was reported.

Like other organochlorine insecticides, methoxychlor may tend to accumulate quite significantly in aquatic invertebrates and thus be passed on to the fish consuming these food organisms in a manner similar to that of DDT during larviciding operations, as observed by Hatfield

(1969). Thus it is quite possible that fish could be exposed to low levels of the insecticide in the water, as well as to a contaminated food supply for some time after larviciding, especially where multiple sprayings are undertaken. It appears very doubtful that methoxychlor can be classified as a "safe" insecticide as far as aquatic organisms are concerned, and it was the purpose of this study to investigate the possible effects of sub-lethal levels of methoxychlor in the diet of brook trout on two physiological functions of ecological importance, namely growth and swimming ability; some incidental observations were also made on the behavior of the fish, on tissue histopathology, some blood characteristics, and methoxychlor accumulation in the body.

Note

Because of a conspicuous lack of published information on the effects of methoxychlor on non-target organisms, both invertebrates and fish, attempts were made to gather what little information was available, by means of personal letters to government agencies, suppliers, and individuals who have been involved in studies and/or application of methoxychlor. Because of the importance of the information gathered, and since it is not otherwise

accessible through normal means of publication, the sources of the various documents are presented in Appendix B.

MATERIAL, APPARATUS AND METHODS

Underyearling brook trout, Salvelinus fontinalis (Mitchill) ranging from 108 to 157 mm in total length, were obtained from La Pisciculture du Lac a L'Eau Claire, St. Alexis-des-Monts, Maskinonge County, Quebec. Upon arrival at the laboratory, the fish were kept in 1400-litre insulated fiberglass holding tanks (Frigid Unit, Ohio, Model MT-700) equipped with a cooling unit (Min-O-Cool, Model BHL 9098) for at least three days before the experiments began. During this period, the fish were not fed and were held at the same temperature that they would be exposed to during the experiments.

APPARATUS

The experimental equipment consisted of a series of six paddle wheel equipped annular growth chambers and one apparatus for measuring swimming stamina.

Growth Chambers

The growth of brook trout subjected to various dietary levels of methoxychlor was observed in annular growth chambers in which a constant water velocity was maintained. The annular growth chambers, one of which is illustrated in Figure 2, were molded in fiberglass placed

Figure 2: Photograph of one annular growth chamber in which brook trout were held, swimming against a constant water velocity while being exposed to various dietary levels of methoxychlor.

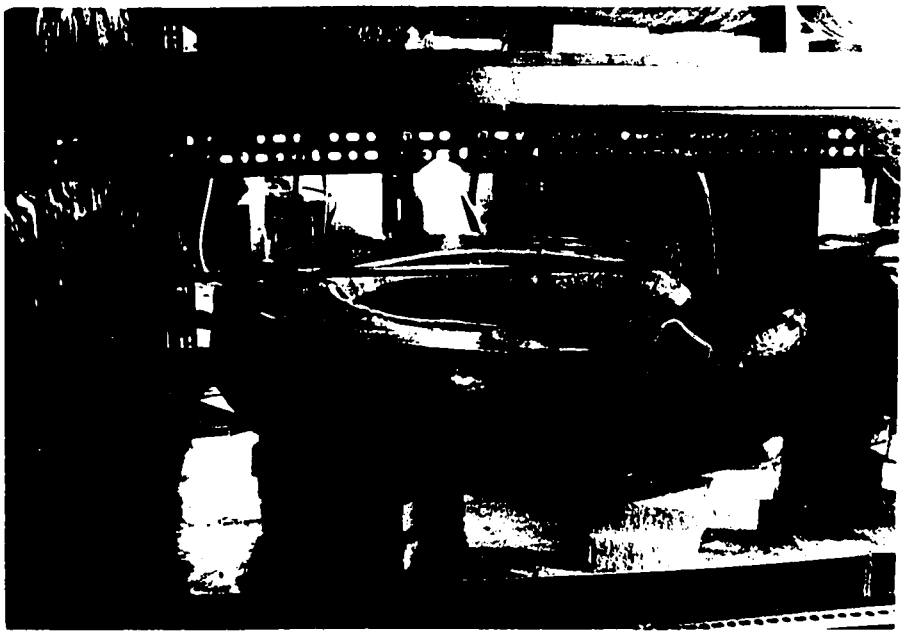

Figure 2: Photograph of one annular growth chamber in which brook trout were held, swimming against a constant water velocity while being exposed to various dietary levels of methoxychlor.

on a truck tire tube of 20-inch radius and 9-inch cross section. The inflated tube was first coated with several layers of liquid floor wax, and upon drying, with one coat of "Vibrin" mold release compound. A 3-inch wide fiberglass tape was then wrapped around the inflated tube with about one inch overlap; two coats of liquid epoxy resin were applied and allowed to harden for half an hour. When set, the inner tube was deflated and a cut made around the top surface of the annular tank with an electric jig saw. The inner tube could then be easily pulled out through the slit, as the walls of the tank at this stage were quite flexible, but stiff enough to maintain shape after removal of the tube. The outer surface of the empty shell was then reinforced with one layer of fiberglass matting (1-1/2 ounce weight) and two coats of resin. Once set, the tank was washed out thoroughly with floor wax remover and hot water to remove all trace of wax and Vibrin; one coat of resin was then applied to the inside surface to ensure a smooth finish. The top opening of the tank was widened to 6 inches with a jig saw and the rough edges were fitted with 1/4-inch plastic tubing to provide a smoother surface for gluing the cover screening. To prevent fish from jumping out of the tank, lengths of fiberglass mosquito screening were glued to the inside circumference, while the outer edges were clamped with short pieces of slit 1/2-inch plastic tubing.

The tank was then equipped with various fittings as illustrated in Figure 3; a composite diagram. A standpipe and waste trap were fitted through holes drilled in the tank wall as shown in Figure 3b, and fine adjustment of water level was achieved by a moveable piece of plastic tubing fitted to the top of the standpipe. During the experiment, the waste trap outlet tubing (Figure 3b) was held slightly higher than the water level so that in the event of a standpipe clogging, the water would drain through the trap tube, thus preventing overflowing. The tanks were periodically cleaned by lowering the trap tubing for several minutes; emptying the waste accumulated in the trap as well as any drifting around the tank.

To provide a constant water velocity, each tank was fitted with a paddle wheel driven by a common shaft rolling on pillow blocks bolted to the supporting frame. The paddle wheel assembly consisted of a pair of circular vanes about 5-inches in diameter, held to the shaft by a plexiglass base (see Figures 3a and 4). The two shafts were chain driven by a 1/3-hp electric motor fitted with a reductor, the speed being regulated by the ratio of drive to shaft sprockets. To prevent the fish from jumping out, the paddle wheel assemblies were covered with fiberglass screening on a wooden frame (Figure 2). The filled tanks contained approximately 90

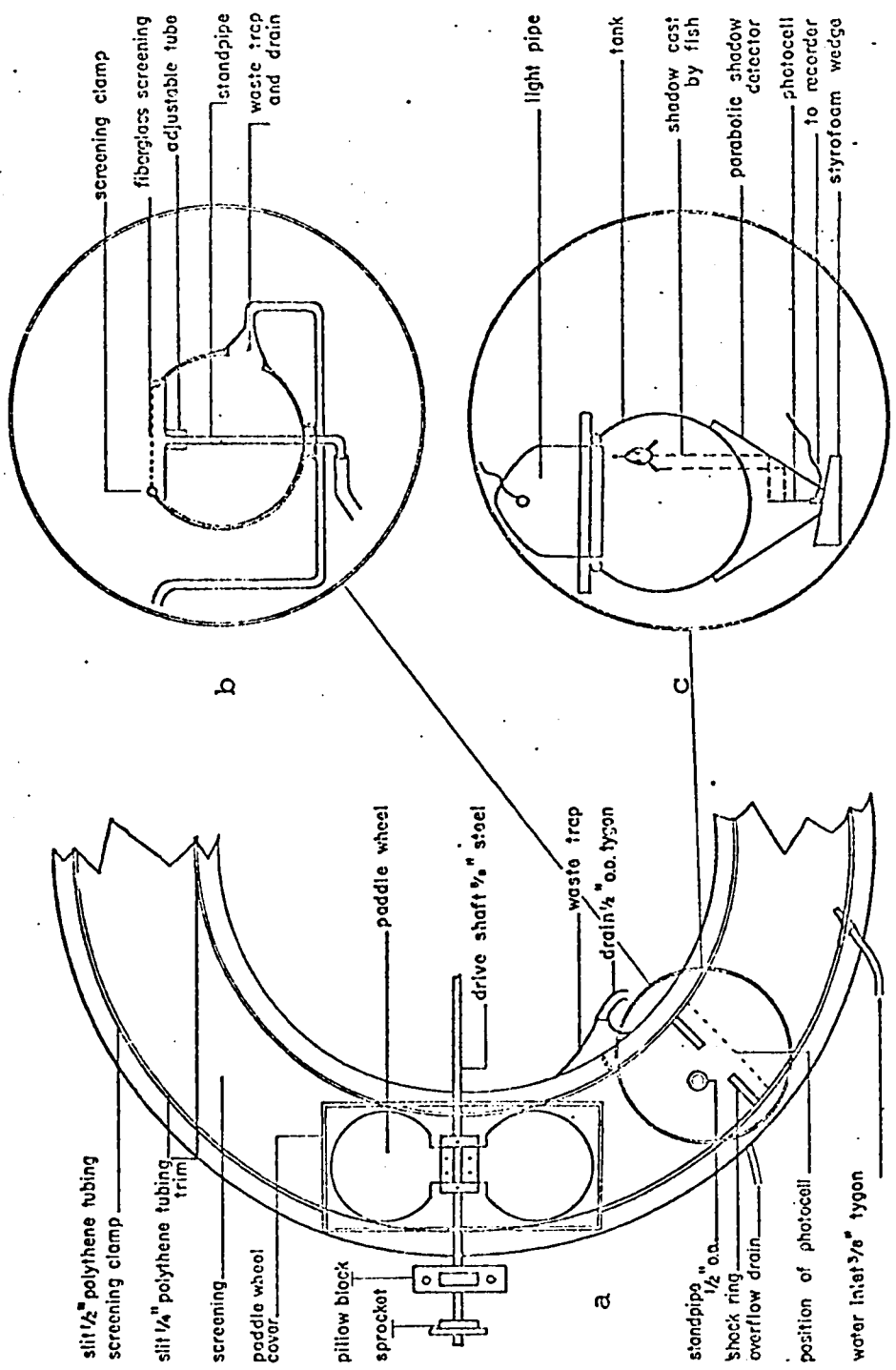

Figure 3: A composite diagram showing a section of one annular growth chamber including details of the waste trap and fish shadow detector.

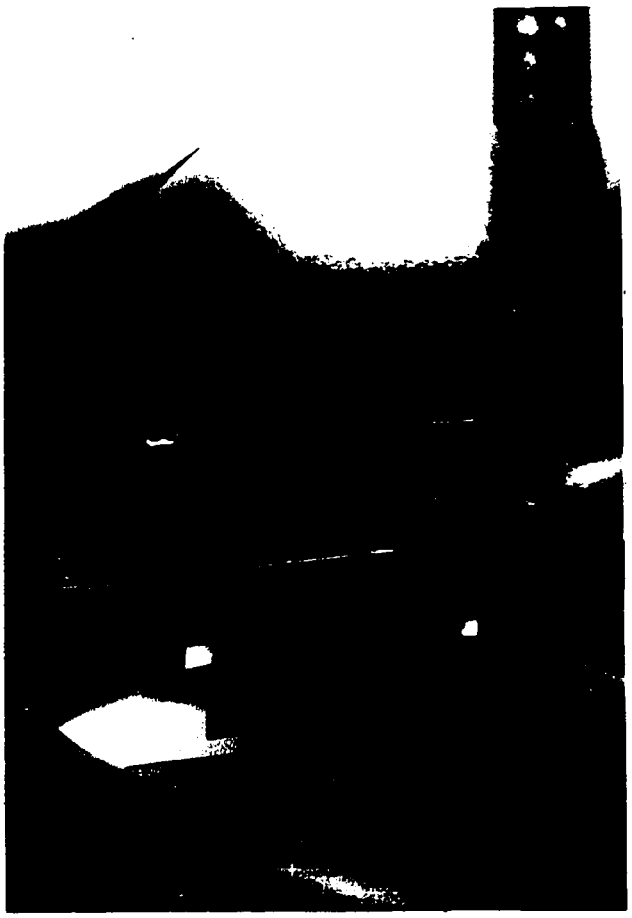

Figure 4: Photograph of one paddle wheel showing the attachment of the vanes to the driveshaft.

Figure 4: Photograph of one paddle wheel showing the attachment of the vanes to the driveshaft.

litres of water and a constant velocity of 0.4 fps was maintained by the paddle wheels. The water velocity measured at three points in the tank with a small current meter (OTT Small Current Meter C 1, A. OTT Kempten, Bayern) varied from 0.45 fps along the outer circumference to 0.35 fps along the inner circumference; fine adjustments were made by varying the height of the standpipe.

To prevent fish from resting behind the standpipe and to minimize free drifting with the current, an electric shock ring was installed in each tank, slightly behind the standpipe (see Figure 3a). A strip of aluminum tape (Scotch Pressure Sensitive No. 425) 1/2-inch wide, was stuck to the inside wall with a break of about 2-inches at the bottom (Figure 3a) and a potential difference of 3 volts A-C was maintained using a small potentiometer for each tank. Any fish passing through the electric field would experience a slight shock and thus be kept away from the area. The shock ring served to discourage drifting of fish and its efficiency could be checked visually during the daytime but not at night. To overcome this difficulty, a photocell was designed to detect the movement of fish. The photocell was supplied with a light source of low intensity by means of a parabolic light pipe (Figures 3c and 5). The light pipe was made from 3/8-inch

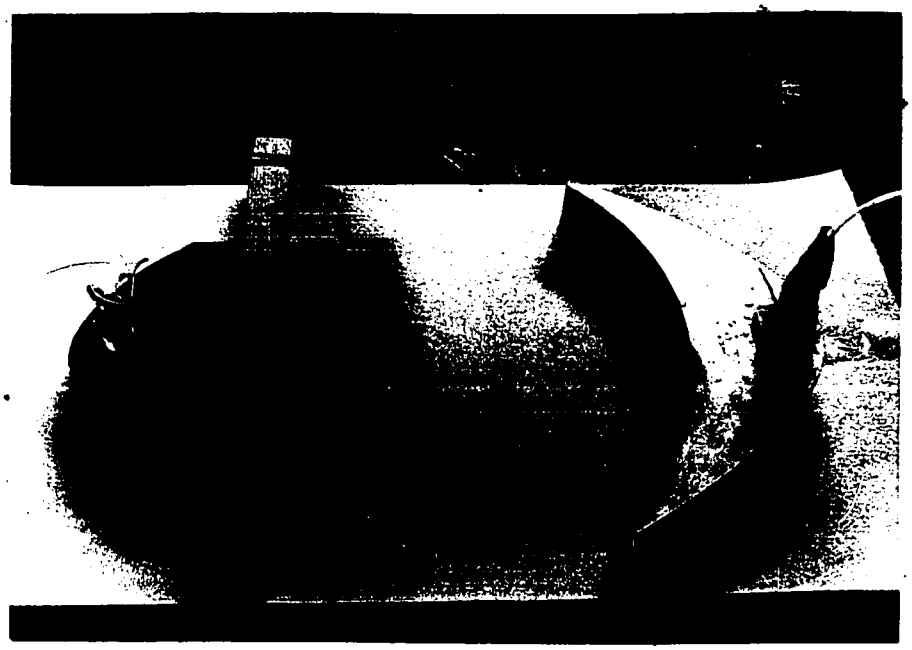

Figure 5: Photograph of the fish shadow detector which was installed under the annular growth chambers showing the light pipe on the left and the parabolic light detector on the right.

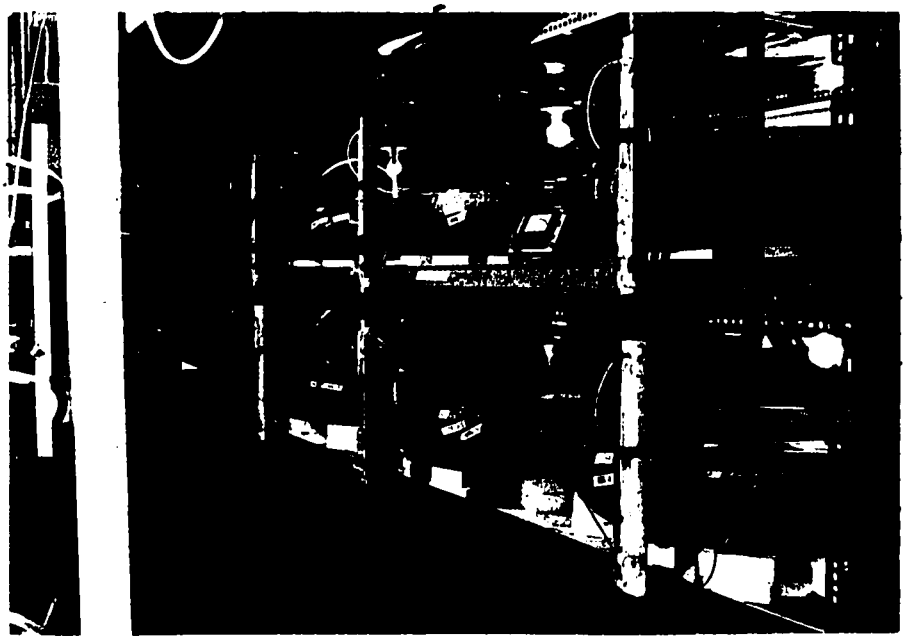

Figure 6: Photograph showing the assembly of the six annular growth chambers in operating condition, as they were used to study the effects of various dietary levels of methoxychlor in brook trout.

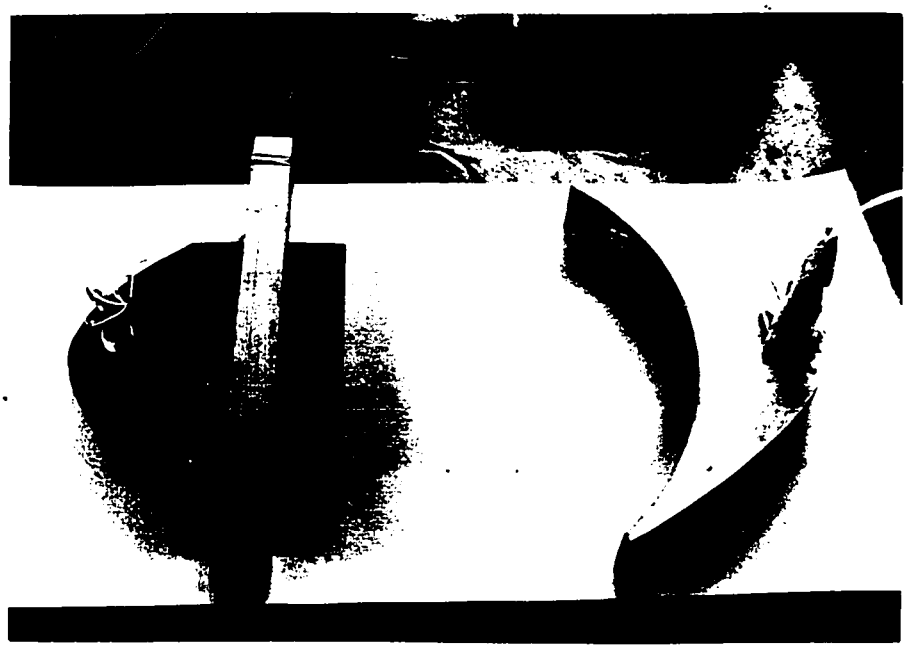

Figure 5: Photograph of the fish shadow detector which was installed under the annular growth chambers showing the light pipe on the left and the parabolic light detector on the right.

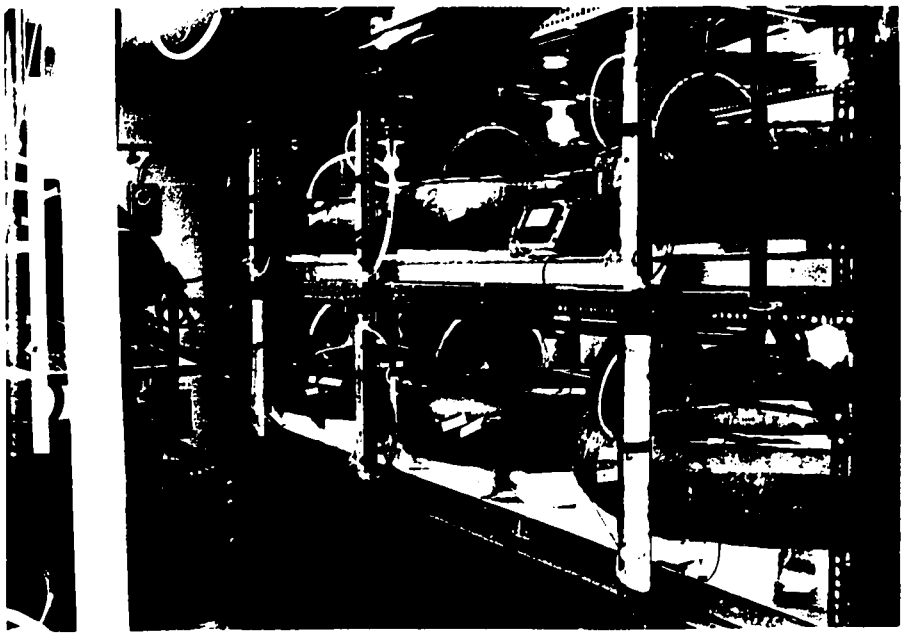

Figure 6: Photograph showing the assembly of the six annular growth chambers in operating condition, as they were used to study the effects of various dietary levels of methoxychlor in brook trout.

thick plexiglass with the upper portion shaped in the form of a parabola with a 1-1/2 watt light bulb fitted with a red filter, mounted at the focus. The free upper and side edges were covered with reflecting tape and the rest of the surface painted flat black, except for the bottom edge which was highly polished. With the bulb on, all light emitted was concentrated in a thin curtain shining from the polished edge, and by immersion just below the water surface, extraneous shadows caused by water ripples were eliminated. The light pipe was clamped between two pieces of wood with a rubber band so that its height could be adjusted for each tank.

The shadow detector consisted of a CdS photocell mounted in the region of the focus of a parabolic light gathering device formed from plexiglass 7/8-inch thick, coated with reflecting silver paint, and having a polished concave surface which fit tightly under the tank. (Figures 3c and 5). The light pipe was aligned directly above the detector and changes in photocell resistance due to changes in incident light caused by passing fish were obtained via a balanced bridge circuit connected to a chart recorder. The same set up was used for daytime recording after adjustment of the bridge balance.

Each tank was illuminated with a 40-watt light bulb placed 8 inches above it and a photoperiod of 12

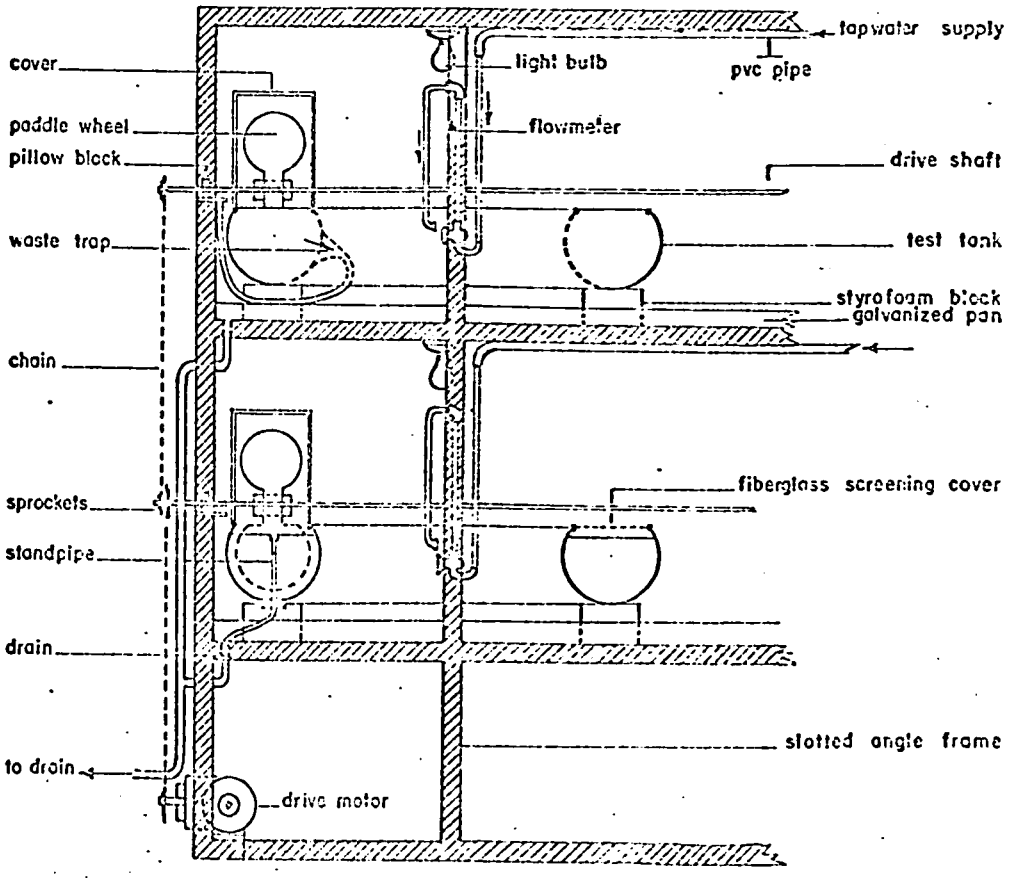

Figure 7: Cross-sectional view of two annular growth chambers showing paddle wheel drive mechanism and the position of the standpipe and waste trap.

hours was controlled by a time-switch. The complete apparatus consisted of a double-tiered slotted angle frame which served to support the annular growth chambers and the paddle wheel driveshaft mechanism (Figures 6 and 7); the entire assembly was enclosed in black plastic sheeting to minimize disturbance from the outside.

Swimming Stamina Chamber

The swimming ability of brook trout subjected to various dietary levels of methoxychlor was tested in an apparatus that is a modification of the one described by Smith and Newcomb (1970). It consists of two concentric tubes through which water is circulated by a motor driven impeller (Figure 8). The outer tube 8-5/8 inches OD by 26 inches long was constructed from fiberglass using a steel drain pipe as a mold and fitted with a plexiglass viewing window on one side. The inner tube 6 inches OD by 24 inches long was of plexiglass. Wall thickness of both tubes was 1/8-inch. Full length plexiglass vanes were glued to the outside surface of the inner tube to minimize water turbulence, and an electrified grid backed by plastic screening was fitted inside the tube just ahead of the impeller. To prevent fish from resting against the retaining screen, a potential difference of 0-10 volts A-C could be applied to the grid by a switch completing a

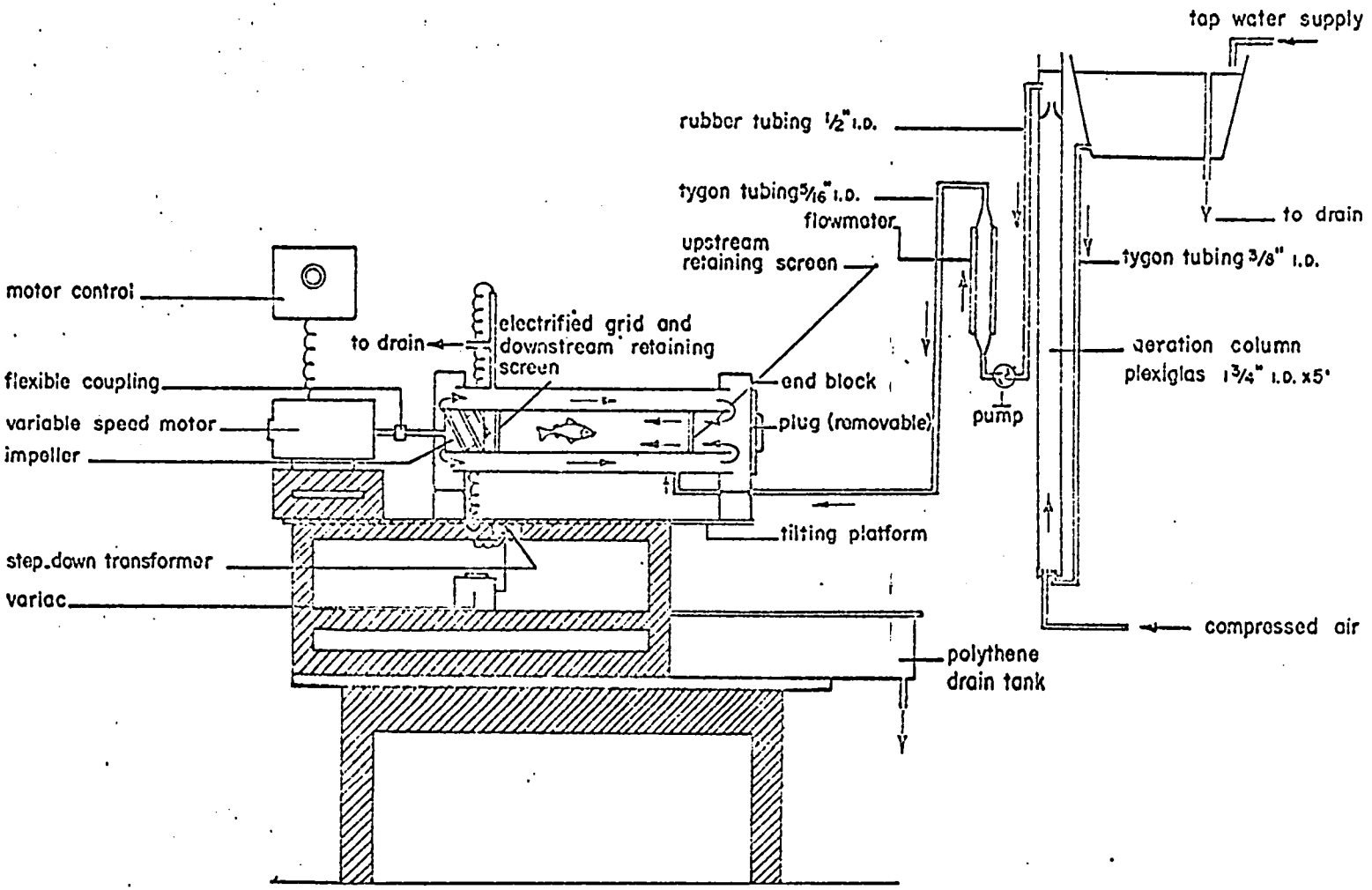

Figure 8: Diagram of the experimental apparatus used for testing the swimming ability of brook trout exposed to dietary methoxychlor, showing the water supply arrangement, motor and impeller assembly, and the direction of water flow within the swimming chamber.

circuit through a Variac and a step-down transformer. Another plastic screen was fitted at the upstream end of the inner tube to keep fish from swimming out during a test. The end blocks were made of wood coated with epoxy resin for waterproofing; the upstream block was fitted with an opening to permit insertion and removal of fish. Water was circulated by a jet outboard impeller (Model OJ-206 Specialty Mfct. Co., California) machined to 3 9/16-inch OD, fitted with a 5/8-inch stainless steel drive-shaft turned by a variable speed electric motor of 3/4-hp. An OTT current meter was used to calibrate water velocity in the chamber in relation to the speed of the motor and the maximum velocity obtainable was 2 fps.

Water Supply

The growth and swimming chambers were supplied with City of Montreal tap water of pH 7.8 through plastic (PVC) pipe after dechlorination to less than 0.03 mg/l total chlorine and an exchange rate of 1 litre/min was controlled with predictability flowmeters. Temperature regulation was accomplished using stainless steel jacket heaters and a water chiller fitted into the line. Water temperature was controlled at 10 $\pm$ 1°C for the first experiment and at 7.5 $\pm$ 0.5°C for the second. Oxygen was

measured regularly using an EIL Model 15A Dissolved Oxygen Meter and stayed at or above saturation throughout the experiments.

METHODS

In this study, two experiments were performed during which brook trout were fed various dietary levels of methoxychlor while swimming continuously against a current of fixed velocity. As a result of problems encountered during the course of the first experiment, several modifications were incorporated into the methods followed in the second experiment.

In the first experiment, conducted in December 1970, brook trout were exposed to dietary methoxychlor concentrations of 8.0, 4.0, 2.0, 1.0, 0.5, and 0.0 ppm for a period of forty days, during which growth and swimming ability were measured. In the second experiment, conducted in April 1971, brook trout were exposed to dietary concentrations of 100.0, 50.0, 25.0, 12.5, 6.25 and 0.0 ppm over a period of forty-three days. Swimming tests were performed on fish that had been exposed for twenty-three days, growth and whole body methoxychlor residue levels were measured after thirty-three days of exposure to the experimental diet; while blood and histological observations were made on fish after an exposure period of forty-three days.

Preparation of the diet

In the absence of information on possible residue levels in non-target aquatic invertebrates after exposure to methoxychlor in stream larviciding operations, it was considered desirable to estimate these concentrations to lend some significance to the dietary levels of exposure of fish to the insecticide in the laboratory. Several test sprayings were conducted on small streams in the provincial park of Mont Tremblant, province of Quebec, in attempts to collect poisoned organisms in drift nets and establish the methoxychlor residue levels by gas chromatography. Treatments were done at the concentration that is currently being used in ground blackfly larviciding operations on the North Shore of Quebec and Labrador, i.e. 0.075 mg/l maintained in the stream for fifteen minutes (West, personal communication, 1970).

These tests could not be completed however, because of the disturbance of drift nets by flooding and campers in the period following methoxychlor treatment. Following these unsuccessful attempts, an experiment was performed in the laboratory where aquatic invertebrates were exposed to methoxychlor and then analyzed for residues. Stream bottom organisms; mostly caddisfly and some stonefly larvae, were collected with a Surber sampler, brought to

the laboratory in ice-water and placed in one of the annular tanks previously described. Two hours later, the water supply was turned off and enough methoxychlor emulsion (25% Emulsifiable Concentrate, Green Cross) was added to establish a concentration of 0.075 mg/l in the water. Fifteen minutes after addition of the insecticide, the water supply was resumed at 200 ml/min and the organisms were collected twenty-four hours after the initial exposure. Insecticide residue determination by gas chromatography revealed concentrations ranging from 1.5 to 3.0 ppm in these invertebrates. These levels of methoxychlor served as a basis for the choice of dietary methoxychlor concentrations that were incorporated into the test diet. In order to determine what dosage a hypothetical fish could be exposed to if faced with a heavy drift of aquatic invertebrates contaminated with the insecticide after stream treatment, one brook trout swimming in an annular growth chamber was fed to satiation with invertebrates which were collected two hours after initial exposure to the insecticide in the laboratory test outlined above. The fish was then weighed, measured and its stomach contents analyzed for residual methoxychlor by gas chromatography. In this way a relation of amount of methoxychlor to body weight of fish was obtained.

The diet used throughout the experiments was made up of a mixture which contained two parts of beef liver, one part of beef heart and one part of Ewos trout chow (Ewos F. 48, Sweden). The liver and heart were passed through a meat grinder and thoroughly mixed with the trout chow. The mixture was then put through the meat grinder once again, spread on a tray, oven dried at 70°C and ground to a powder in a Waring blender. The methoxychlor was incorporated into the diet following a modification of a gelatin-bonded diet suggested by Grenier (personal communication, 1970) and which consisted of five parts (by weight) of water, five parts of methoxychlor emulsion, four parts of dry powdered chow and one part of gelatin.

The methoxychlor used, 1,1,1,-trichloro-2,2-bis (p-methoxyphenyl) ethane, was 89.5 percent technical grade Pesticide Reference Standard (Entomological Society of America), and was obtained from the City Chemical Corporation, N.Y. The insecticide emulsion was prepared by dissolving the required amount of methoxychlor in one millilitre of xylene, adding five drops of an emulsifying agent (Atlox 3335, Ciba-Geigy, Montreal) and diluted to one litre with distilled water.

The required dietary concentrations of methoxychlor were prepared with the control receiving the same amount of emulsion as the highest concentration, but

without the methoxychlor. The food was prepared by dissolving the gelatin in warm water to which the methoxychlor emulsion and dry chow were added; the mixture was mixed well to break up any lumps, poured into a shallow pan and placed in the refrigerator to set. Once set, the preparation was cut into small pieces (about 2 by 2 by 5 mm) and frozen until used. The final preparation contained 31 percent dry matter and the determination of methoxychlor by gas chromatography revealed a concentration close to 90 percent of the anticipated levels.

First experiment

In preparation for the experiment, brook trout were graded according to length and thirty fish were placed in each growth chamber in an order determined from a table of random numbers. On the following day, individual fish were anesthetized with MS-222 (tricaine methanesulphonate, Sandoz), weighed in water to the nearest 1/10 gram, measured to the nearest millimeter and marked with a V-shaped branding tool chilled with liquid nitrogen (Mighell, 1969). The dietary methoxychlor levels (0 to 8 ppm) were assigned to the six tanks in an order determined from a table of random numbers. Food was weighed out each day and fed to the test fish at the rate of 2% of their body weight; adjustments for growth were made

at ten day intervals. This feeding rate corresponds to a dosage of 0.00, 0.01, 0.02, 0.04, 0.08 and 0.16 mg of methoxychlor per kg of fish per day. No problems were encountered with acceptance of the contaminated diet by test fish and the food pellets were quickly consumed as they drifted around in the current, most being consumed before one circuit of the tank. All fish were fed the test diet for ten days and the swimming ability of five fish chosen at random from each tank was tested after a one-day starvation. These fish were weighed and measured as above, while the remaining fish in each tank were weighed in groups. The same procedure was followed for two more ten-day intervals except that for the swimming tests, ten previously untested fish were chosen from each tank. After the last swimming test, the fish were fed for another ten days, individually weighed, and then left in the tanks for a starvation period of twenty days, during which the fish were observed.

Swimming test procedure

On the day of the swimming tests, the fish were herded between two plastic screens placed in the upright position in an area comprising about one-third of the annular growth chamber. One test fish was taken from the tanks with a net and inserted into the swimming chamber

(see Figure 8) which had already been partially filled with water. The lid was bolted on, the chamber was filled completely and the air bubbles were eliminated through the outlet tube by tilting the apparatus. As soon as the chamber was filled, the water velocity was turned up to 1.54 fps and the duration of swimming was measured with a stopwatch, with no preliminary swimming practice. Most fish settled down to face the current immediately and made no effort to escape. When a fish drifted back against the screen, it was given single electric shocks of short duration, and was judged exhausted when it could no longer move off the screen and was pressed tightly against it by the water current. With the swimming chamber in the tilted position, the end plug was removed, and the chamber emptied into the drain tank, with the fish being swept out with the flow of water. The fish was then anesthetized, weighed, measured and placed in a holding tank for recovery.

Second experiment

To ensure the continuous swimming of fish during the second experiment, the shock rings and fish detector (see Figures 3a, 3c, and 5) were added to the growth chambers. Thirty-five fish were placed in each of the six growth chambers and kept there without feeding for three

days. This period was provided so that the fish would become accustomed to their flowing water environment, and the shock rings were not put into operation until the second week of the experiment. All fish were then pooled, graded according to length, and thirty fish assigned to each tank in an order determined from a table of random numbers. After one day in the tanks, at least twelve fish from each group were tested for swimming ability at a velocity of 1.28 fps; of these, ten were chosen to be repeatedly tested at ten-day intervals during the experiment. All fish were anesthetized, marked, weighed and measured, with individual records being kept for each swimming-test fish. This procedure was very time consuming but was necessary since the same fish were used in consecutive swimming tests and their identity had to be known. The condition of each fish could thus be followed during the course of the experiment. Feeding was started on the following day when no problems were encountered, and all fish swam readily against the current (0.4 fps) accepting food as soon as it was offered. The dietary methoxychlor levels (6.25 to 100.00 ppm) were randomly assigned to the six test tanks, but were not fed immediately as in the first experiment. Instead, the control diet was fed to all groups at a rate of 2 percent of the body weight for five days to ensure acceptance before

administration of the contaminated diets. On the sixth day, the water supply was shut off, and a sufficient amount of methoxychlor emulsion was added to produce a concentration of 0.075 mg/l in all tanks except in the control group; fifteen minutes later, the water supply was resumed. Three hours after the application of methoxychlor to the water, the fish in tank number one (not a control group) were fed to satiation with the experimental diet; i.e. until several pellets of food drifted around the tank with no attempt by the fish to consume them. The amount of food consumed was converted into percent body weight and the fish in the five other tanks were fed proportionately. In the following two days, the fish of tank number one were fed first again, and the same procedure repeated. While not a true unrestricted feeding, this procedure had to be followed to subject all the test fish to similar amounts of food per body weight. The feeding levels administered to all tanks on days 1, 2 and 3 were 3.55, 3.24, and 4.50 percent of body weight respectively.

In field conditions, it has been observed (Hatfield, 1969) that brook trout are faced with a marked increase of invertebrate drift shortly after blackfly larviciding operations. The feeding procedure outlined above was used to simulate a sudden rise in consumption

of food which has been contaminated with methoxychlor. After two days of starvation, swimming tests were performed on ten fish from each group. For the next ten days, all fish were fed the test diet at a rate of 2 percent of their body weight; on the eleventh day, they were starved, and on the twelfth day they were tested for swimming ability, weighed and measured; this procedure was then followed for another ten-day period. This feeding rate corresponds to a dosage of 0, 0.125, 0.250, 0.500, 1.000 and 2.00 mg. of methoxychlor per kg of fish per day. After another ten-day feeding period all the fish were individually weighed and measured, ten fish were taken from each tank to determine the percent dry weight, ten were frozen at -20°C for methoxychlor residue analysis, and the remainder were fed for another ten-day period. These fish were then starved for one day and sacrificed to collect blood samples and preserve kidney and liver tissue for histological examination.

In summary, swimming tests were conducted on fish exposed to the methoxychlor diet for 23 days, growth measurements and residue analysis were done on fish exposed for 33 days, and blood and histological work was done on fish that had been exposed for 43 days.

Swimming test procedure

During the first experiment, bubble formation due to supersaturation of the water supply was encountered in the growth chambers but was more pronounced in the swimming chamber because of the churning action of the impeller. In order to avoid this problem during the second experiment, a stripping column was added to the swimming chamber and the water temperature was lowered from 10±1°C to 7.5±0.5°C. In addition to a lower test temperature, there were two differences in swimming tests conducted during the second experiment. Upon filling of the chamber, the test fish was subjected to a current of the same velocity as in the annular growth chambers (0.4 fps) for five minutes; then the current was increased within three seconds to 1.28 fps and the duration of swimming was measured as in the first experiment. This procedure was followed in all swimming tests and varied only in the case where fish swam for more than one hour. In these cases, the water velocity was increased from 1.28 to 1.78 fps after the first sixty minute period, and was maintained until exhaustion of the fish. This change in procedure was deemed necessary in order to accelerate the time to exhaustion, as there were sixty fish to be

tested one at a time at ten day intervals. To permit a comparison of swimming ability of fish tested at these two velocities and that used in the first experiment, swimming times were converted into distance swum in feet.

Determination of residual methoxychlor

Following their exposure to methoxychlor in the annular chamber, the insects were spread on a paper towel and left to dry at room temperature for about ten minutes before weighing; this treatment removed any water that was present on the external surface of the organisms. The samples were then weighed to the nearest milligram and the methoxychlor extracted according to the method outlined by Johnson (1970, personal communication, see Appendix A). The same procedure was followed for determination of methoxychlor in the test diet.

Residual levels of methoxychlor were determined only on fish of the second experiment. At the end of the thirty three-day exposure period, ten fish from each of the six groups were frozen at -20°C. Before the analysis, the fish were thawed out, blotted dry and homogenized in a Waring blender as pooled samples of ten fish; a sample of approximately 20 grams weighed to the nearest 10 milligrams was used for the extraction procedure (see Johnson 1970, Appendix A).

In addition to the determination of residual methoxychlor in surviving fish at the end of the thirty-three day exposure period, some determinations were made on fish that died during the course of the second experiment. In cases where the fish were preserved for histological examination, only the pyloric caeca were analyzed for residues; in other cases, whole body residues were determined as previously described. The pyloric caeca were removed from the fish immediately after death and frozen at -20°C; whole fish that died were also frozen. Before analysis, the samples were thawed out, blotted dry, and weighed to the nearest 10 milligrams. The pyloric caeca were ground with anhydrous sodium sulphate in a mortar and pestle and the methoxychlor was extracted following the same method as previously described for the insect samples.

The amounts of methoxychlor in the insect and fish extracts were determined by gas chromatography following a modification of the method described by McCully and McKinley (1964) using a Hewlett-Packard 5750 Gas Chromatograph equipped with an electron capture detector. A helical glass column (8mm OD by 6mm ID by 6 ft long) was packed with 10% stationary phase [4%OV-17 + 6% D.C. QF-1 (FS-1265) fluorosilicone] on 60/80 mesh Chromosorb WAW.

The operating parameters were the following:

column temperature	250°C
injection temperature	225°C
detector temperature	300°C
carrier gas	5% methane/95% argon at 90 ml/min
injection volume	2µl
retention time	14 minutes

The reference standards were prepared from pesticide reference standard methoxychlor and a standard curve was drawn from peak areas measured with a planimeter. The peak areas from samples of unknown concentration were compared to the standard curve and expressed in nanograms per microlitre; the concentrations of methoxychlor in the samples of insects, fish and food were then calculated in ppm on a wet weight basis.

Tissue sampling

Samples of blood, kidney, and liver tissue were taken from fish in the second experiment only. The fish were anesthetized in 0.01 percent MS-222, weighed, measured and the blood collected in heparinized microhematocrit tubes (Capilets) after severance of the caudal peduncle. The tubes were sealed at one end with Critocaps, and centrifuged for ten minutes at 3100 rpm in an International Clinical Centrifuge equipped with a microhematocrit head. Hematocrit values were determined by comparing the height of the plasma meniscus to the height of the red blood cell layer.

Blood was also collected in a standard RBC diluting pipet, diluted with Hendrick's solution (Hesser, 1960) and the erythrocyte counts were made using a Neubauer Bright Line Haemacytometer. At least three fish from each of the six groups were used, and four readings were made for each blood sample.

Kidney and liver tissues were taken from fish that died during the course of the experiment and also from at least three fish from each of the six groups after forty-three days exposure to methoxychlor. The tissues were immediately fixed in Bouin's solution, held for twenty-four hours, and then cleared with eight

washes of 70% ethanol. Slides were prepared from paraffin infiltrated tissue (Paraplast m.p. 56.5°C) and ten-micron sections were stained with Harris's hematoxylin and Bowie's eosin.

RESULTS

Behavior of fish in the growth chambers.

In both experiments, all groups of fish readily accepted the diet and no differences in feeding behavior were noted. Mortalities in the first experiment were restricted to fish that jumped out of the annular holding tanks and could not be related to methoxychlor exposure; improvements to the cover screening minimized this problem, but did not completely eliminate it during the first experiment. During the second experiment some mortalities apparently attributable to methoxychlor poisoning were observed and these will be considered later.

During the first experiment, no significant behavioral differences could be observed throughout the entire exposure period, and in the second experiment the behavior of all six groups of fish was similar for the first two weeks. The fish swam together in a compact school, maintaining position against the current and occupying an area of about one-quarter of the annular growth chamber (Figure 9A). Two or three of the largest brook trout headed each group and nipped at other fish that attempted to move ahead of them. For the first few days, the food was dropped into the annular chambers from the same point, and it was thought that this spatial distribution could be

GROUP A

GROUP B

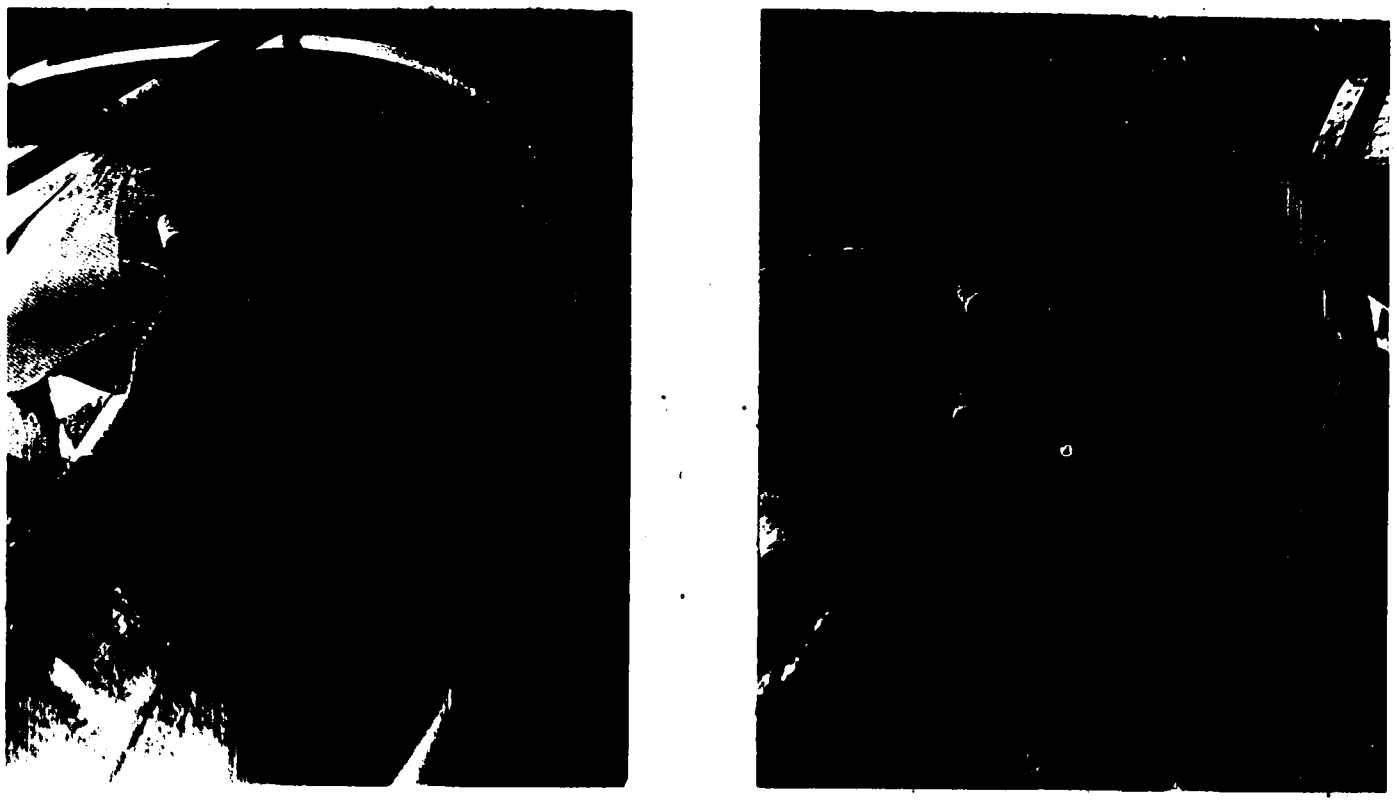

Figure 9. Photographs illustrating the difference in spatial distribution between two groups of brook trout in annular growth chambers after group A had fed on a control diet for 18 days, and group B on a diet containing 2 mg of methoxychlor/kg of fish/day, while the fish were kept swimming against a current of 0.4 fps at 7.5°C.

GROUP A

GROUP B

Figure 9. Photographs illustrating the difference in spatial distribution between two groups of brook trout in running growth chambers after group A had fed on a control diet for 12 days, and group B on a diet containing 2 mg of methoxychlor/kg of fish/day, while the fish were kept swimming against a current of 0.1 fps at 7.5°C.

related to food availability, with the more aggressive individuals taking up position near the point of food introduction. However, even when the food was evenly distributed around the tank, similar positions were maintained by the lead fish; the tracings obtained from the fish shadow detector photocell indicated similar movements of fish across one point in the tank, at all exposure levels and no free drifting was observed in any of the growth chambers. At night, the tracings indicated somewhat less movement, suggesting that the fish maintained position during darkness as well.

In the third week of the experiment, a different spatial distribution of fish in two of the six growth chambers was noted. The association of fish in the control group and the three lowest methoxychlor intake groups remained similar to that already described, but a change in behavior was noted in fish exposed to the highest level (2.00 mg/kg/day) and to a lesser extent in the group fed at the rate of 1.000 mg/kg/day. The fish-to-fish distances were greater in all planes (Figure 9B) and the school was spread over more than two-thirds of the annular tank. While the size hierarchy remained unchanged, much less defence of territory was noted, and the brook trout were seen to maintain essentially the same relative positions for periods of thirty minutes or more. In spite of this

difference, which persisted until the end of the experiment, no changes in feeding activity were noticeable.

Growth in weight and length.

Growth, as measured by changes in wet weight and total length, of all brook trout subjected to various dietary levels of methoxychlor in both experiments, is presented in Tables 1 and 2. A comparison of the specific growth rates (Table 1) indicates that the fish showed poorer weight gains in Experiment 1 in spite of the higher temperature. The larger initial size of fish and the occurrence of air supersaturation in the water may explain the lower growth rate in the first experiment. In both experiments 1 and 2, various methoxychlor intake levels ranging from 0.01 to 2.000 mg/kg of fish/day had little effect on the wet weight gain of brook trout, with five of the insecticide-exposed groups showing better growth than the control, and five showing poorer growth. It should be noted however that the highest methoxychlor concentration appears to have noticeably reduced the growth in weight of brook trout during Experiment 2; this tendency became even more pronounced in fish that were repeatedly tested for swimming ability. Swim-tested fish fed 2 mg/kg/day of methoxychlor showed a 17.73% wet weight gain as compared to a 23.22% gain in control swim-tested fish.

Table 1. Changes in wet weight of brook trout during two growth experiments in which the fish were kept in annular growth chambers swimming against a current of 0.4 fps and fed various dietary levels of methoxychlor.

	Dietary methoxychlor intake level (mg/kg of fish/day)	Number of fish at beginning of experiment	Mean wet weight at beginning of experiment (g) 1/	Number of fish at end of experiment	Mean wet weight at end of experiment 2/ (g) 1/	Weight gain 2/ (g)	(%)	Specific growth rate in weight 2/
Expt. No. 1 40 days 10°C	0.00	30	19.89 (2.71)	28	24.20 (3.72)	4.19	20.94	0.475
	0.01	30	18.40 (2.90)	23	22.81 (3.66)	4.13	22.11	0.499
	0.02	30	19.54 (2.79)	25	23.52 (3.73)	3.96	20.25	0.461
	0.04	30	19.77 (2.90)	24	23.28 (4.22)	3.32	16.63	0.385
	0.08	30	19.23 (3.26)	27	23.08 (3.24)	4.27	22.70	0.512
	0.16	30	19.27 (2.58)	25	23.63 (3.35)	4.18	21.49	0.492
Expt. No. 2 33 days 7.5°C	0.000	30	15.55 (3.13)	28	19.16 (3.54)	3.73	24.17	0.656
	0.125	30	17.07 (3.36)	25	21.28 (4.28)	4.04	23.43	0.638
	0.250	30	16.33 (3.19)	28	20.81 (3.70)	4.35	26.43	0.711
	0.500	30	15.01 (2.51)	29	18.00 (2.95)	3.03	20.24	0.599
	1.000	30	15.75 (3.58)	29	19.64 (4.12)	3.86	24.46	0.663
	2.000	30	16.36 (3.36)	24	19.55 (3.77)	3.35	20.68	0.570

1/ The figures in parentheses indicate the standard deviations of the means.

2/ Mean values based on fish alive at the end of the experiment.

The growth in length presented in Table 2 shows slight impairment at all dietary exposure levels of methoxychlor during Experiment 1, while the results of Experiment 2 show no noticeable effects that can be related to insecticide poisoning. If however, a similar calculation is made for fish from the 2 mg/kg/day exposure group which were repeatedly tested for swimming ability, it yields a length gain of 4.10% as compared to 5.12% for control swim-tested fish. These data suggest that at this concentration, a greater reduction of growth occurred in fish subjected to the stress imposed by the repeated swimming tests than in the overall sample of fish exposed to the same concentrations of insecticide in the diet. While these differences in growth are small, they may have become more pronounced over a longer exposure period.

In addition, for Experiment 2, the total biomass of fish at 33 days was corrected for losses due to fish that jumped out of the annular chambers and the percent gain of biomass was calculated. The biomass of the control group increased by 18.97% while the group exposed to 2 mg/kg/day, due to the combined loss of five fish attributable to poisoning and reduced growth, increased by only 4.63%. These results indicate that as a population, brook trout in the highest exposure group were severely affected.

Table 2. Changes in total length of brook trout during two growth experiments in which the fish were kept in annular growth chambers swimming against a current of 0.4 fps and fed various dietary levels of methoxychlor.

	Dietary methoxychlor intake level (mg/kg of fish/day)	Number of fish at beginning of experiment	Mean total length at beginning of experiment (cm) 1/	Number of fish at end of experiment	Mean total length at end of experiment 2/ (cm) 1/	Length gain 2/ (cm)	Length gain 2/ (%)	Specific growth rate in length 2/
Expt. No. 1. 40 days 10°C	0.00	30	13.12 (0.69)	28	13.81 (0.69)	0.69	5.26	0.128
	0.01	30	13.02 (0.65)	23	13.66 (0.77)	0.64	4.92	0.120
	0.02	30	13.20 (0.67)	25	13.78 (0.70)	0.58	4.39	0.108
	0.04	30	13.29 (0.60)	24	13.76 (0.69)	0.47	3.54	0.087
	0.08	30	13.11 (0.72)	27	13.69 (0.67)	0.58	4.42	0.108
	0.16	30	13.24 (0.66)	25	13.74 (0.59)	0.54	4.08	0.093
Expt. No. 2. 33 days 7.5°C	0.000	30	11.92 (0.78)	28	12.41 (0.72)	0.55	4.64	0.137
	0.125	30	12.25 (0.80)	25	12.79 (0.81)	0.52	4.24	0.126
	0.250	30	12.08 (0.93)	28	12.64 (0.70)	0.60	4.98	0.147
	0.500	30	11.76 (0.70)	29	12.36 (0.67)	0.61	5.19	0.153
	1.000	30	11.97 (0.84)	29	12.60 (0.81)	0.62	5.18	0.153
	2.000	30	12.02 (0.70)	24	12.63 (0.73)	0.56	4.64	0.137

1/ The figures in parentheses indicate the standard deviations of the means.

2/ Mean values based on fish alive at the end of the experiment.

Percent dry weights.

The percent dry weights of brook trout that had been exposed to methoxychlor for 33-days in the second experiment are presented in Table 3, and it appears from these results that methoxychlor had no effect on this condition of the body of fish over the exposure period. All six groups showed a slightly lower percentage of dry weight than the control fish at the start of the experiment; this change possibly reflects a change in body composition attributable to the continuous swimming activity in the growth chambers during the experimental period.

Starvation period.

When brook trout were left in the annular growth chambers for 20 days after a 40-day exposure to methoxychlor in Experiment 1, no mortalities occurred that could be attributable to prior exposure and the behavior of the six groups of fish remained unchanged during this period.

Table 3. Percent dry weight of brook trout that had been fed various dietary levels of methoxychlor for 33 days while they were kept in annular growth chambers swimming against a current of 0.4 fps at 7.5°C.

Dietary methoxychlor intake level in mg/kg of fish/day	Dry weight as percent of wet weight
0.000	25.63 1/
0.000	24.36
0.125	24.27
0.250	24.32
0.500	24.05
1.000	23.37
2.000	24.30

1/ Determined at the beginning of the experiment.

Swimming performance.

In both experiments, the brook trout swam readily when subjected to the test velocity in the swimming stamina chamber; in the second experiment, the preliminary five minute low velocity period (0.4 fps) given to the fish before raising the water velocity to the test level (1.28 fps) produced a more uniform swimming performance than in the first experiment in which there was no pre-test period. The fish that were given the five-minute pre-test period quickly oriented themselves in the position that they would maintain during the entire swimming test, while the fish tested in the first experiment swam irregularly for about ten seconds before taking up their final position. The fish that showed the best performance quickly assumed a position a few inches from the upstream retaining screen and swam with a steady movement until near exhaustion. At that time, they would drift back until they touched the downstream retaining screen and then moved forward again; only when a fish began to rest against the screen, was an electric shock given. Just before exhaustion, the fish would swim in powerful bursts against the current, drift back and burst forward several times; a few times fish swam through the upstream retaining screen in these powerful bursts of swimming. Fish showing the poorest

swimming performance would start burst-like swimming shortly after the test velocity was attained, or would turn around and drift with the current against the screen, exhibiting the same symptoms of exhaustion as normal fish, but much sooner.

The symptoms of exhaustion were similar in both experiments; when the water current was stopped, a fish would fall to the bottom breathing heavily and often lie on its side. If the water velocity was turned up again the fish was immediately swept against the screen and could not move off. Thus the end point of swimming endurance could be determined to the nearest ten seconds during which the fish made an attempt to move off the screen. Once returned to the holding tank, the exhausted fish regained equilibrium within five minutes and seemed to behave normally fifteen minutes after the end of the swimming test.

The swimming performance of the tested fish was corrected for size using the equation ($Y = 2.49 + 1.12\ X$) of the regression line obtained from the relationship between the natural logarithms of the swimming times in seconds and the wet weights in grams of 82 brook trout tested before the start of the growth experiment. The corrected swimming times were transformed into distance

swum and the median values were used.

The results of the first experiment in which brook trout were tested for swimming ability after 10, 20 and 30 days of feeding, are presented in Table 4 and indicate that the performance of fish of all groups decreased from the first test to the third with greater decreases occurring in the methoxychlor exposed groups than in the control. In addition all the insecticide-exposed fish showed a poorer performance than the controls in the last swimming test. When the swimming performance of fish at thirty days is expressed as a percent of that at ten days (Figure 10) a steady decrease in swimming ability with increasing methoxychlor concentration can be seen. At the two highest exposure levels, the brook trout swam only 50 and 25-percent of the distance covered at ten days, while control fish covered 94-percent of their initial swimming distance. As the same fish could not be retested at each trial, the observed variation in the control appears to be well within individual differences. From these results it appears that a thirty-day exposure to dietary methoxychlor levels as low as 0.01 mg/kg of fish/day can affect swimming performance and that a level of 0.16 mg/kg of fish/day can, under laboratory conditions, cause a drastic reduction in the swimming

Table 4. Swimming performance of brook trout as tested in a stamina chamber at water velocities ranging from 1.28 to 1.78 fps after different periods of feeding on a diet containing various levels of methoxychlor while the fish were kept swimming against a current of 0.4 fps.

	Methoxychlor concentration (mg/kg of fish /day	Number of days fed	Number of fish tested	Median wet weight (grams)	Median observed distance swum (feet)	Median corrected distance swum (feet)
Expt. 1. 10°C	0.00	10	5	21.9	486	486
		20	10	23.6	402	402
		30	10	21.7	457	457
	0.01	10	5	17.4	773	907
		20	10	23.9	542	533
		30	10	22.8	474	440
	0.02	10	5	19.8	454	494
		20	10	23.6	433	433
		30	10	20.1	306	354
	0.04	10	5	18.8	686	778
		20	10	21.9	381	433
		30	10	20.8	425	453
	0.08	10	5	19.0	707	793
		20	10	19.9	434	546
		30	10	19.0	321	401
	0.16	10	4 1/	20.6	679	718
		20	10	19.3	371	501
		30	10	28.8	378	141
Expt. 2 7.5°C	0.000	0	10	16.0	357	357
		3	10	17.5	312	312
		13	10	17.8	382	382
		23	9 2/	22.2	526	526
	0.125	0	10	19.3	445	365
		3	10	17.6	490	488
		13	10	20.1	503	446
		23	10	20.2	544	594
	0.250	0	10	19.6	429	342
		3	10	17.6	392	390
		13	10	22.7	942	820
		23	10	21.3	476	498
	0.500	0	10	17.4	365	331
		3	10	18.9	446	412
		13	10	16.6	493	522
		23	10	17.8	488	597
	1.000	0	10	21.0	462	340
		3	10	22.2	672	556
		13	10	21.9	1946	1844
		23	10	21.8	1196	1206
	2.000	0	10	20.0	477	380
		3	10	16.3	694	823
		13	10	25.1	782	599
		23	8 3/	18.8	4021	4105

1/ one fish swam through the retaining screen.

2/ one fish died during the exposure period.

3/ two fish died during the exposure period.

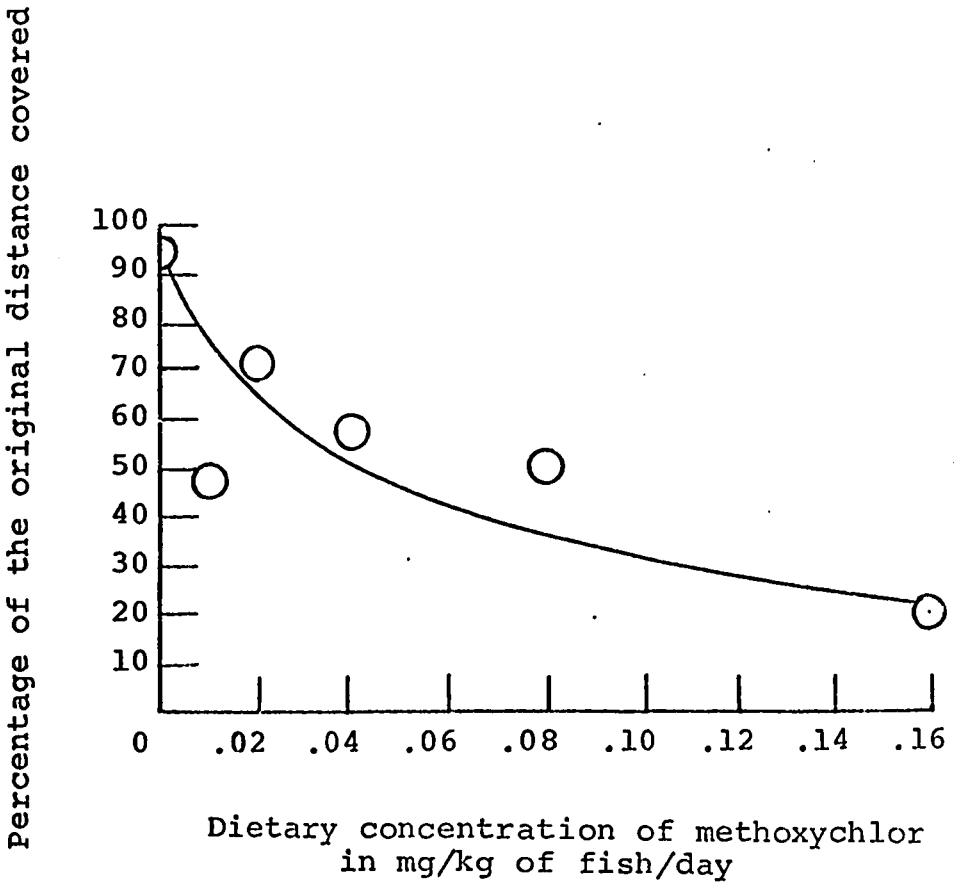

Figure 10. Changes in swimming performance of brook trout from 10 to 30 days of exposure to various dietary levels of methoxychlor fed to the fish while they were kept in annular growth chambers swimming against a current of 0.4 fps at 10°C.

performance of brook trout.

The results of the second experiment also presented in Table 4, indicate that the swimming ability of fish in all groups increased from the first test to the last with the greatest increase observed in the fish in the highest methoxychlor exposure levels. After a twenty-three day exposure period to methoxychlor, brook trout which received 1.000 and 2.000 mg/kg of fish/day respectively swam approximately two and eight times further than the control fish. If, as in the first experiment, the median performance at the end of the exposure period is expressed as a percent of that after the first ten days of the experiment, (Figure 11), a marked increase in distance covered is obtained. Fish exposed to the highest methoxychlor intake level (2.00 mg/kg of fish/day) swam four times further than after the first ten days (corresponding to three days of feeding to satiation), while the fish at the three lowest concentrations show progressively less increase than the control. This represents a situation comparable to what was observed in the first experiment (see Figure 10) where the highest concentration tested overlaps the lowest tested in the second experiment. As these changes in swimming ability cannot be attributed to a smaller size of fish at the lower exposure levels (see Table 4), these results suggest

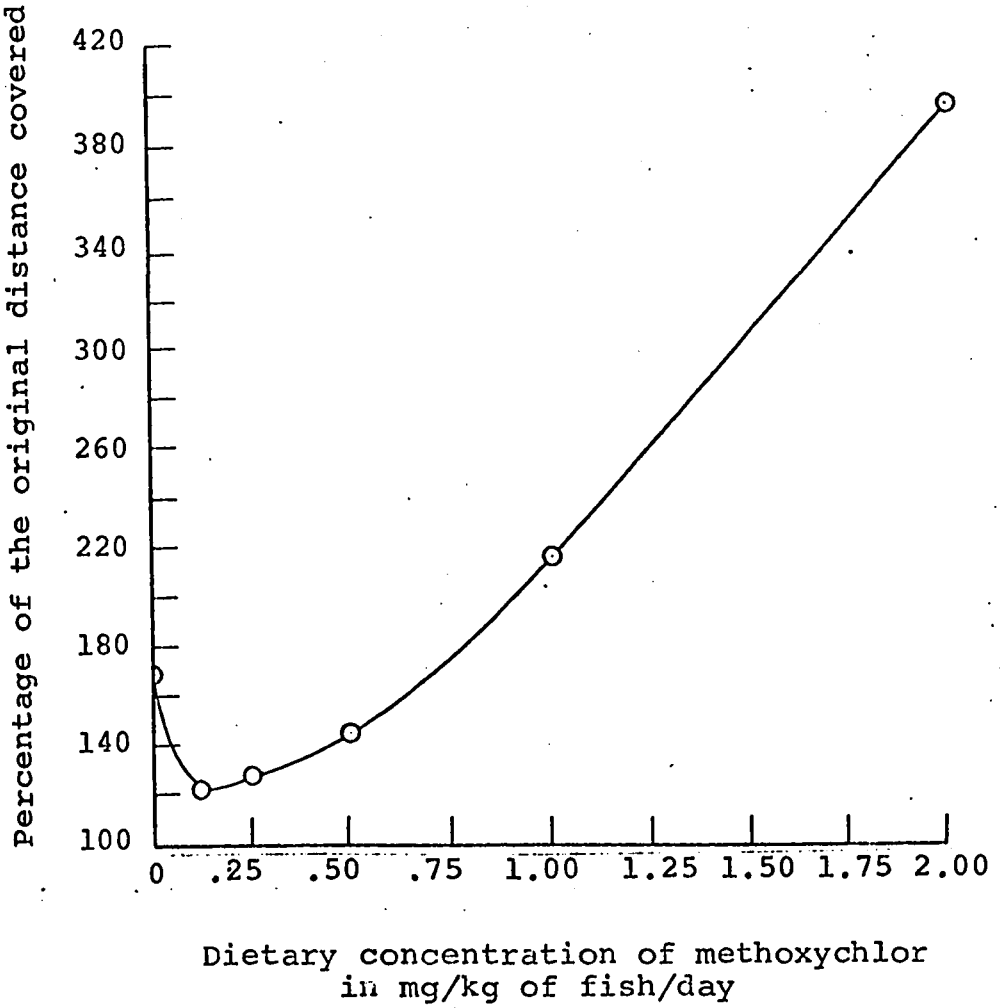

Figure 11. Changes in swimming performance of brook trout from 3 to 23 days of exposure to various dietary levels of methoxychlor fed to the fish while they were kept in annular growth chambers swimming against a current of 0.4 fps at 7.5°C.

that low levels of methoxychlor in the diet of brook trout can decrease the swimming performance, while higher levels can stimulate it under the conditions of this experiment.

Residual methoxychlor

In aquatic insect larvae

The amounts of methoxychlor accumulated by aquatic insect larvae which had been exposed to 0.075 mg/l of the insecticide for 15 minutes in the laboratory are presented in Table 5. These results indicate that methoxychlor was rapidly taken up from the water, and after 24-hours, a mixture of fish food organisms consisting of larvae of caddisfly, stonefly and cranefly accumulated the insecticide to a level about nineteen times that of the initial concentration in the water.

One brook trout weighing 13.6 grams was fed to satiation with caddisfly and stonefly larvae which had been exposed to the same methoxychlor treatment as the other insect larvae, but for a period of only two hours. The residue determination on the stomach content (0.7270 g wet weight) revealed a concentration of 3.10 ppm methoxychlor which was equivalent to an intake level of 0.17 mg of methoxychlor/kg of fish. This value falls well within the experimental intake levels of 0.01 to

2.000 mg/kg of fish/day applied in the feeding experiments. The higher residual methoxychlor observed in this fish stomach sample is probably attributable to the shorter sampling interval (2 hours after the initial treatment) than in the other samples (Table 5) which were collected after 24-hours, an interval which may have allowed for some degradation and/or excretion to take place.

Table 5. Concentrations of methoxychlor in aquatic insect larvae which had been exposed to 0.075 mg/l of the insecticide for 15 minutes at 7.5°C in 90 l of water followed by a progressive dilution at a rate of 200 ml/min for 24 hours.

Organisms	Sample No.	Wet weight of sample (grams)	Concentration of methoxychlor (ppm)
caddisfly larvae	1	4.431	1.02
	2	2.142	1.05
mixture of caddisfly stonefly and cranefly larvae	1	0.880	1.42

1/ The estimated methoxychlor concentration in the water after 24 hours is 0.0038 mg/l.

In live fish

The concentrations of residual methoxychlor in whole body homogenates (10 fish) of brook trout that survived the 33-day feeding period at various dietary levels of the insecticide in Experiment 2 are presented in Figure 12. These results indicate that when brook trout were fed a daily ration containing methoxychlor, an increase in the dietary concentration was accompanied by an increase in the amount of stored insecticide, up to a dietary level of 2.00 mg/kg/day, where some levelling off seems to have occurred. The food consumption was not measured, but since the fish in all exposure groups quickly consumed the food given, a comparison of the total calculated amounts of methoxychlor ingested during the 33 days of feeding with the amounts retained in the body seemed appropriate. The results, also presented in Figure 12, indicate that the accumulation of the insecticide increased with the intake level, up to 78 percent at 1.00 mg/kg/day, and then dropped at the highest (2.00 mg/kg/day) level. These results show that brook trout can store more than half of the methoxychlor they have absorbed through the diet. While some of this accumulated insecticide may have been taken up directly from the water in the initial exposure to 0.075 mg/l at the beginning of

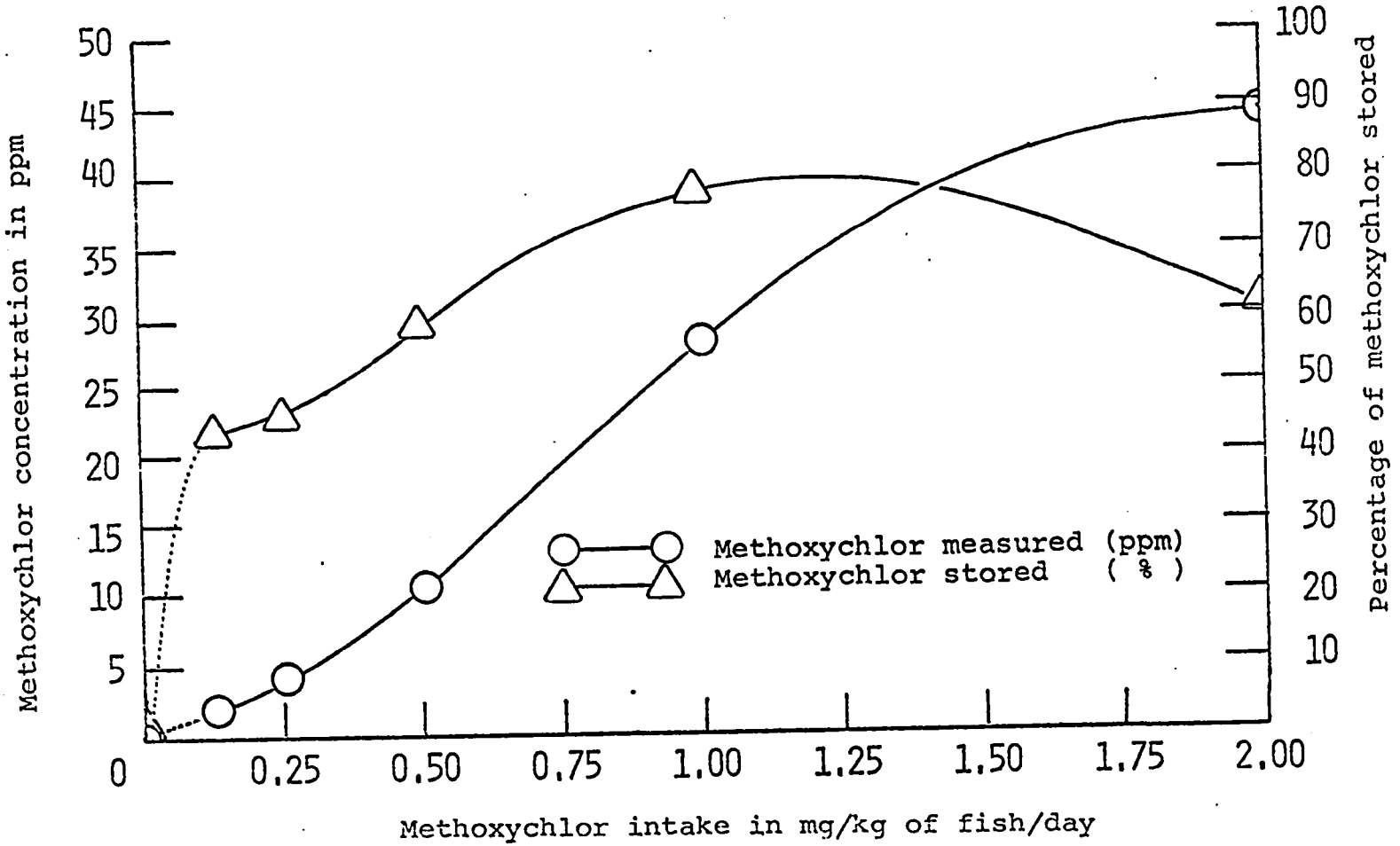

Figure 12. Relationships between the concentrations of methoxychlor in whole brook trout and the relative amounts of the insecticide retained in the whole body of brook trout that had been fed various dietary levels of methoxychlor for 33 days while swimming in the annular growth chambers against a current of 0.4 fps at 7.5°C.

the growth experiment, the dietary intake probably accounted for most of the residue.

In dead fish.

Determinations of residual methoxychlor were also made on fish that died during the course of the second experiment, using either the whole fish or the pyloric caeca. The results, presented in Table 6 indicate that the pyloric caeca of the fish that died during the exposure period contained higher concentrations of methoxychlor than whole fish analyzed at the end of the experiment. After 24 days of feeding at the lowest intake level 0.125 mg/kg/day, the pyloric caeca of a dead fish contained 9 times as much methoxychlor as a whole fish after 33 days (see Figure 12). After 23 days of feeding at 2.000 mg/kg/day, the methoxychlor content of the pyloric caeca of one fish was 13 times higher than in the pooled samples of whole fish analyzed at the end of the experiment (see Figure 12). On the other hand, whole body residues of one fish that died after 31 days (Table 6) contained about 6 times less methoxychlor than what was found in the pooled sample of ten fish at 2.000 mg/kg/day at 33 days. This suggests considerable individual differences in accumulation of methoxychlor among fish and that very high concentrations may be reached in certain organs which can store the lipophilic insecticide.

Table 6. Concentrations of residual methoxychlor in samples of brook trout which died during a growth experiment in which they were fed various dietary levels of methoxychlor while swimming against a current of 0.4 fps in annular growth chambers at 7.5°C.

Methoxychlor intake level (mg/kg of fish /day)	Days of feeding	Fish Number	Nature of sample	Wet weight of sample (grams)	Methoxychlor concentration (ppm)
0.125	24	1	pyloric caeca	1.437	17.65
0.250	18	2	whole fish	13.280	1.17
0.500	19	3	whole fish	16.825	1.78
2.000	23	4	pyloric caeca	1.197	584.70
2.000	31	5	whole fish	16.687	7.61

Histopathological observations

Blood

The hematocrit values and the red blood cell counts of brook trout exposed to various levels of methoxychlor for 43-days are presented in Table 7. These results indicate decreasing values in both measurements with increasing methoxychlor intake. It therefore appears that methoxychlor has a definite deleterious effect on the blood of brook trout, reducing both the volume of corpuscles and the number of the red blood cells.

Liver and kidney of living fish

The only apparent difference between the liver tissue of the control fish and the fish which had been exposed to the three lowest methoxychlor levels (0.125, 0.250, and 0.500 mg/kg/day) was a change in staining characteristics from the normal basophilic to acidophilic, but no evidence of tissue damage was observed in the liver or kidney tissue of these fish.

In fish that had been exposed to 1.000 mg/kg/day of methoxychlor, in addition to the histochemical change mentioned above, the normal cord arrangement of the liver parenchyma shown in Figure 13A was disturbed (Figure 13B) and the cells showed extensive cytoplasmic vacuolisation

Table 7. Mean hematocrit and red blood cell counts of brook trout which had been fed various dietary levels of methoxychlor for 43 days while swimming against a current of 0.4 fps in annular growth chambers at 7.5°C.

Methoxychlor intake level (mg/kg of fish/day)	Number of fish sampled	Number of hematocrit determinations[1]	Mean hematocrit (%)	Number of red blood cell counts[1]	Mean red blood cell count (million/mm^3)
0.000	4	22	41.2	16	1.15
0.125	4	20	38.9	16	1.09
0.250	3	19 [2]	39.8	12 [2]	1.07
0.500	4	18	34.3	16	1.08
1.000	5	22	36.9	20	0.98
2.000	4	19	35.4	16	0.86

1/ At least 4 determinations were made per fish.

2/ Data for one dying fish was omitted from mean.

(Figure 13C). In one fish, degenerative changes were observed in erythrocytes and blood vessels; in areas where erythrocytes were breaking down, some blood vessels contained large droplets (Figure 13D).

In two of the four brook trout that had been previously exposed to 2.000 mg/kg/day of methoxychlor, a generalized breakdown of the cords of liver parenchyma was observed, and in one fish, this was accompanied by extensive cytoplasmic vacuolisation (Figures 13E and 13F). The liver tissue of the other two fish examined was strongly acidophilic but showed no evidence of structural damage. The kidney sections showed a slight alteration of the normal structure (Figure 13G) of the tubules (Figure 13H) and in one fish, a loss of cytoplasm and an irregularity in the shape of the nuclei was observed in cells of the head kidney (Figure 13I).

Liver and kidney of dead or dying fish

Histopathological changes in fish that died during the experiment were similar but somewhat more pronounced than those in fish that survived the test period. The residual methoxychlor levels that were found in these fish have been presented in Table 6 and in order to relate the histopathological symptoms of the dead or dying fish to their methoxychlor contents, the fish

Figure 13. Photomicrographs of liver and kidney sections of brook trout which had been fed various dietary levels of methoxychlor for 43-days while they were kept swimming against a current of 0.4 fps at 7.5°C.

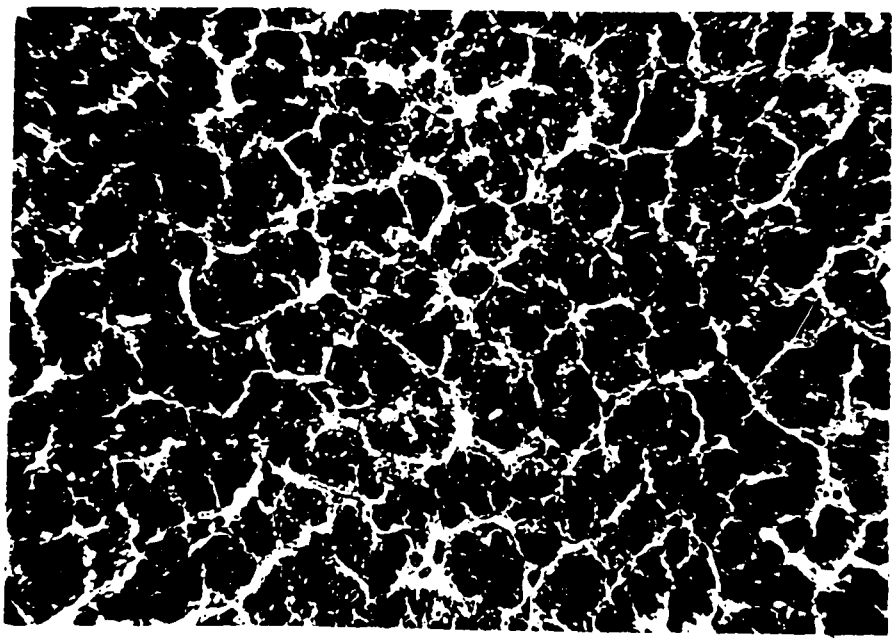

Figure 13A. Control fish. Liver section showing cord-like arrangement of parenchymal cells. x 450.

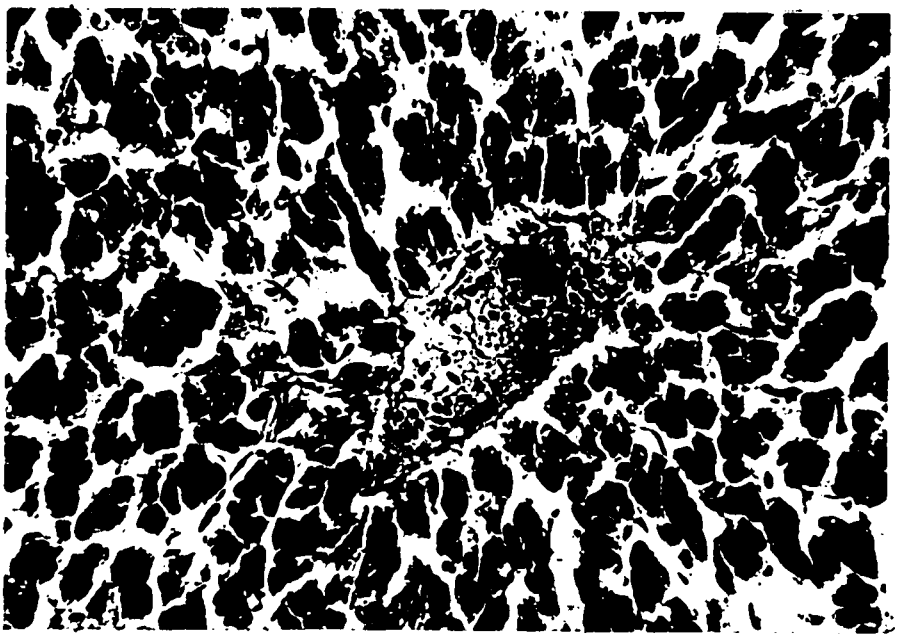

Figure 13B. Fish exposed to 1.00 mg/kg/day of methoxychlor. Liver section showing disturbance of cord structure some breakdown of erythrocytes, and accumulation of debris in a blood vessel. x 450.

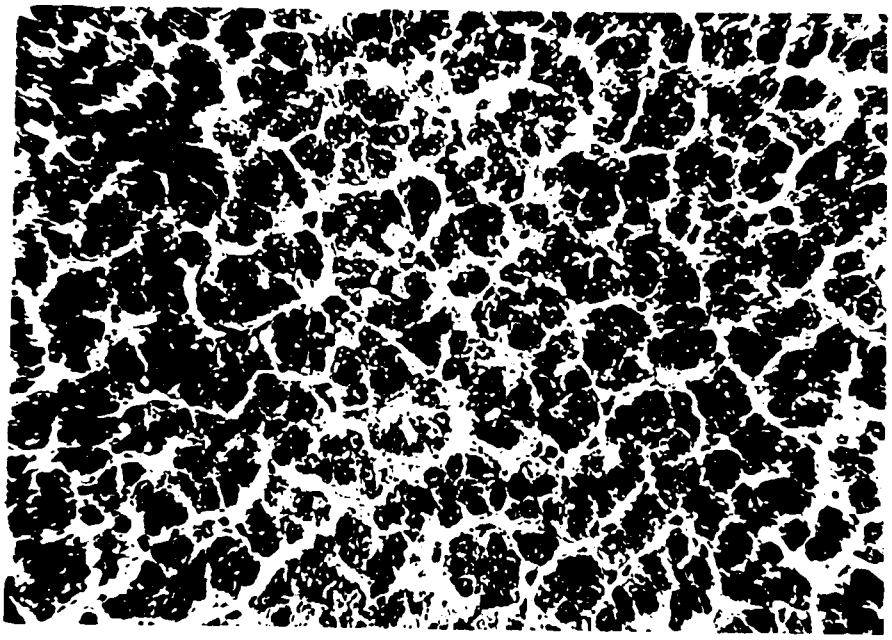

Figure 13A. Control fish. Liver section showing cord-like arrangement of parenchymal cells. x 450.

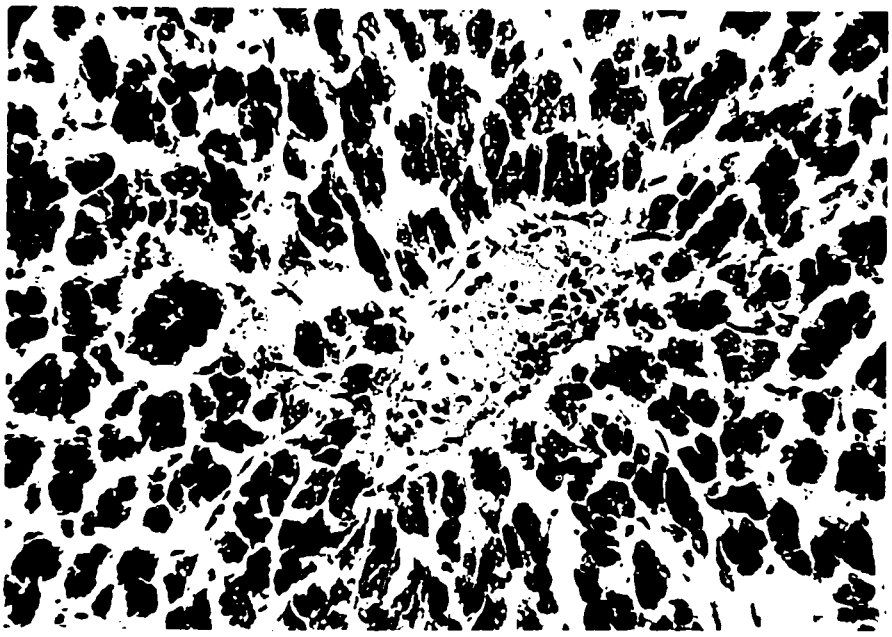

Figure 13B. Fish exposed to 1.00 mg/kg/day of methoxychlor. Liver section showing disturbance of cord structure some breakdown of erythrocytes, and accumulation of debris in a blood vessel. x 450.

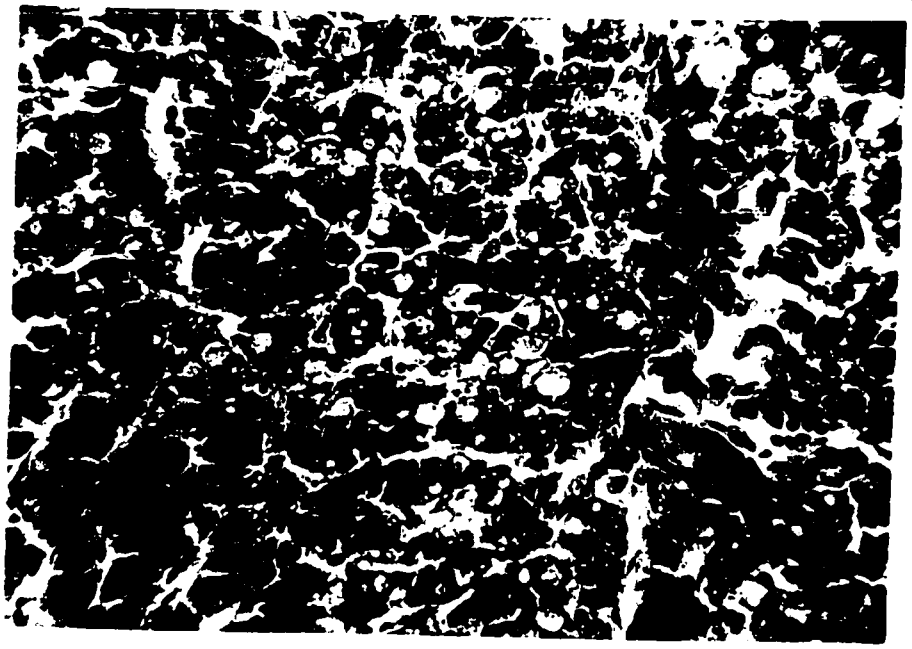

Figure 13C. Fish exposed to 1.00 mg/kg/day of methoxychlor. Liver section showing extensive cytoplasmic vacuolisation in parenchymal cells. x 450.

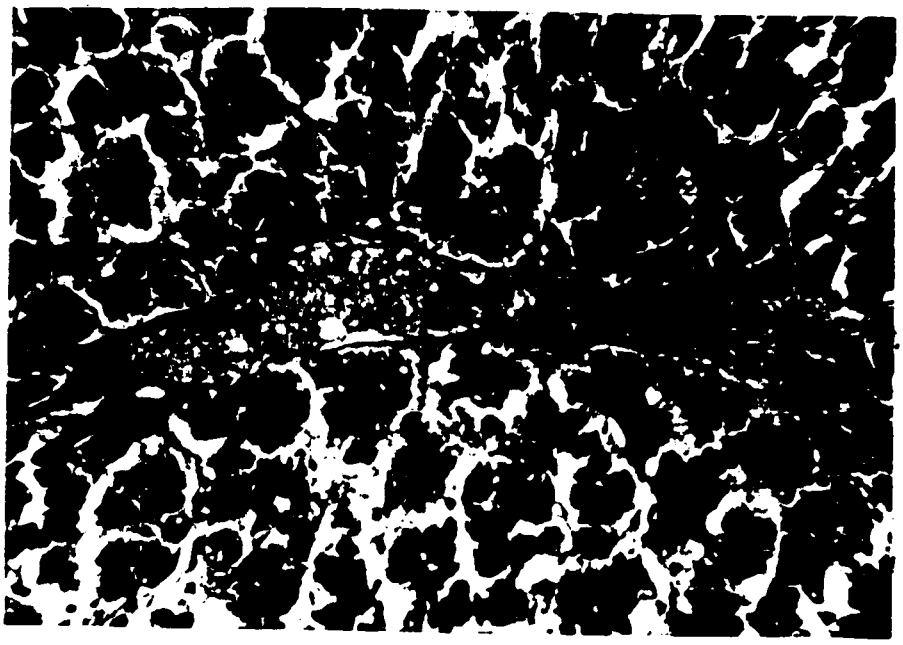

Figure 13D. Fish exposed to 1.00 mg/kg/day of methoxychlor. Liver section showing a blood vessel containing droplets and debris possibly resulting from breakdown of erythrocytes. x 450.

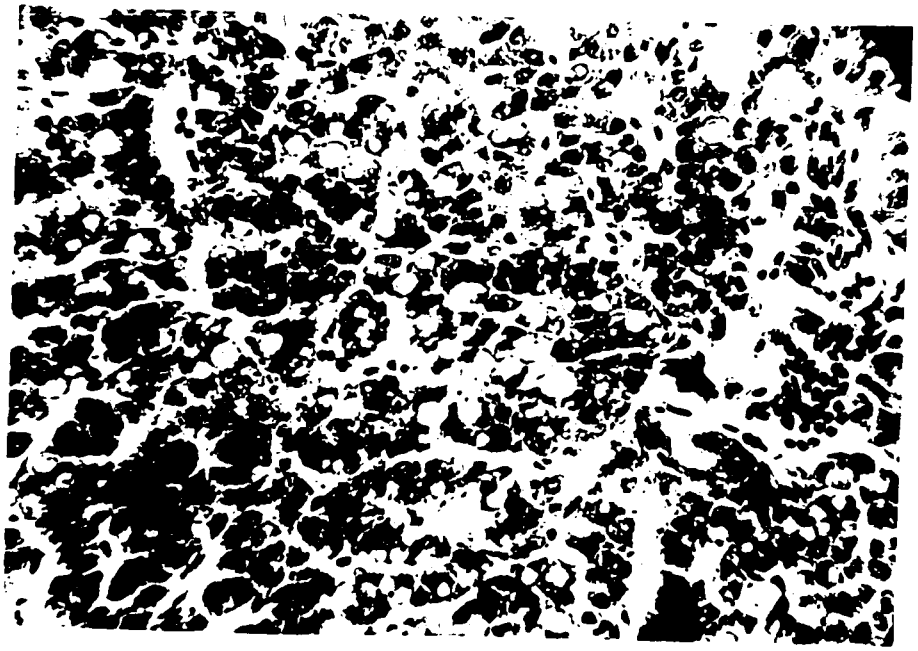

Figure 13C. Fish exposed to 1.00 mg/kg/day of methoxychlor. Liver section showing extensive cytoplasmic vacuolisation in parenchymal cells. x 450.

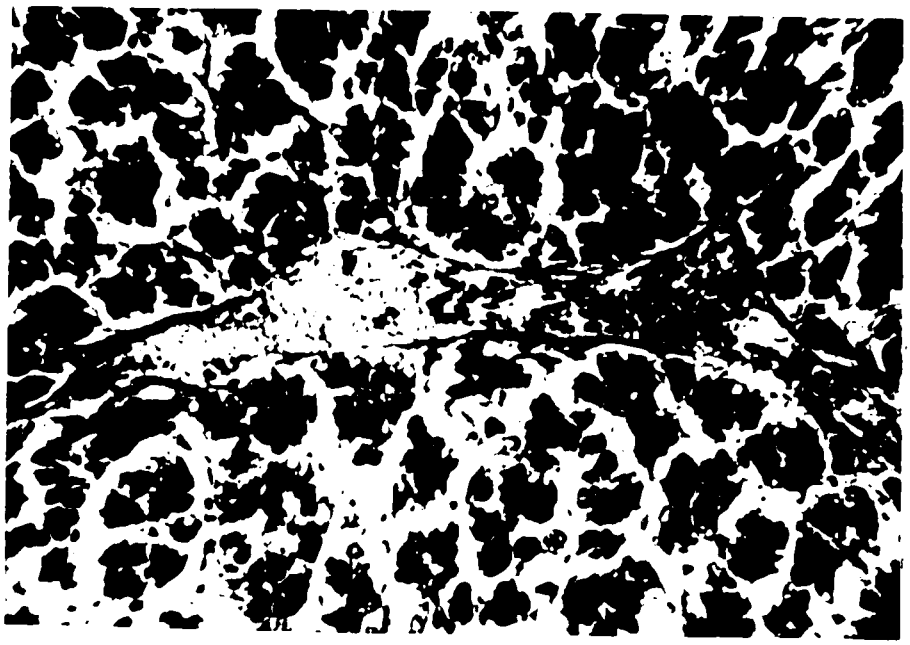

Figure 13D. Fish exposed to 1.00 mg/kg/day of methoxychlor. Liver section showing a blood vessel containing droplets and debris possibly resulting from breakdown of erythrocytes. x 450.

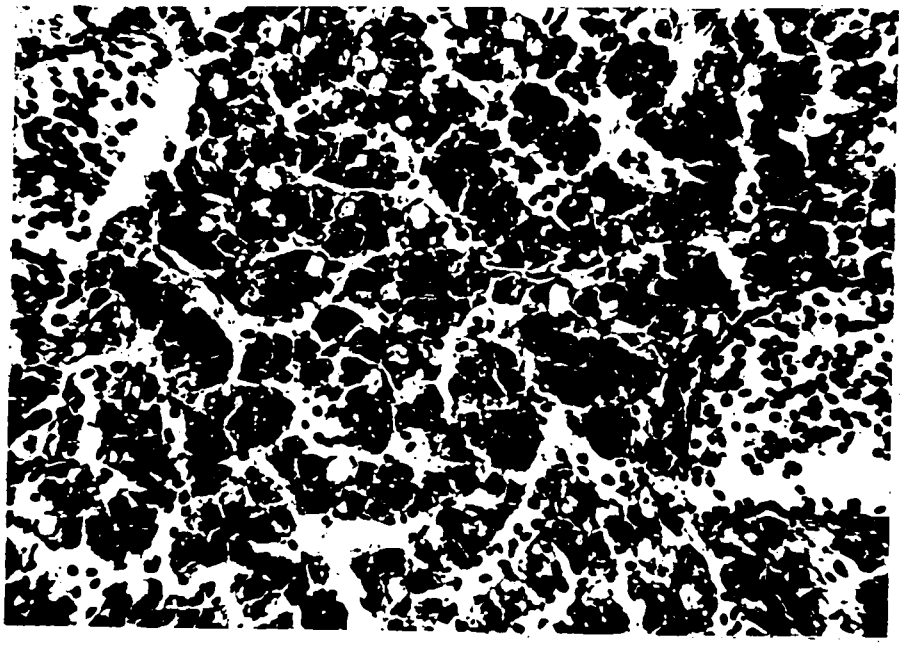

Figure 13E. Fish exposed to 2.00 mg/kg/day of methoxychlor. Liver section showing extensive vacuolisation of the cytoplasm in parenchymal cells. x 450.

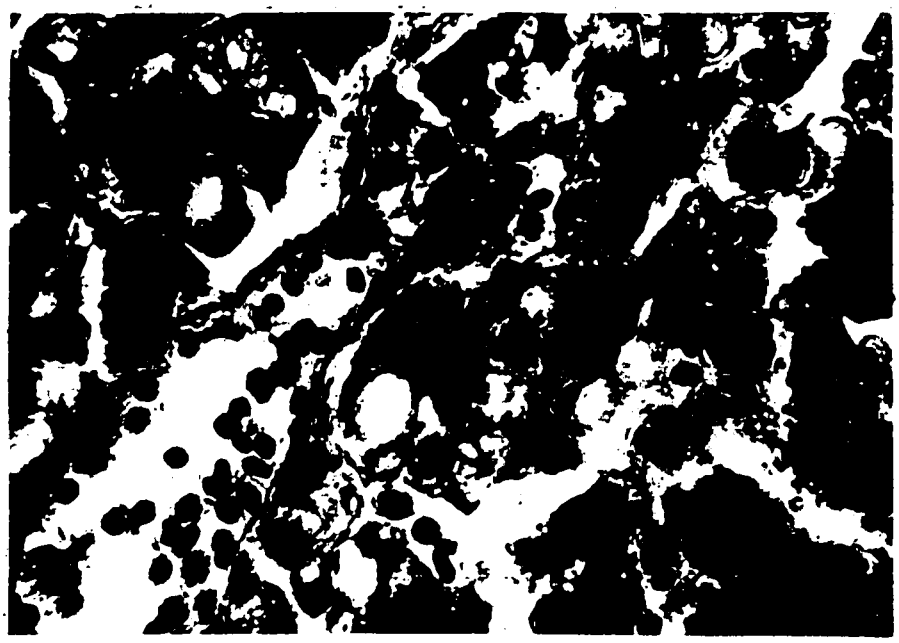

Figure 13F. Same as above. x 1000.

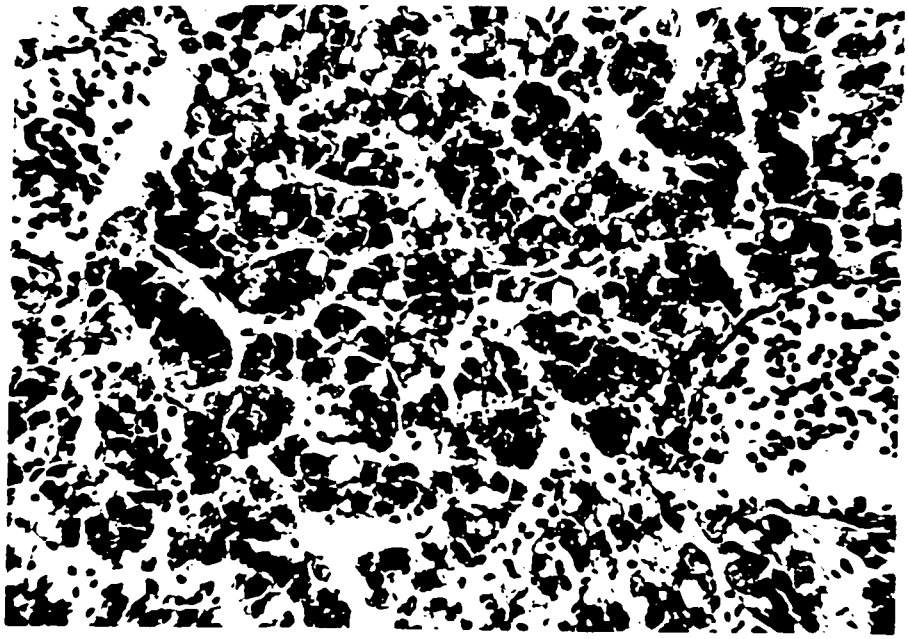

Figure 13E. Fish exposed to 2.00 mg/kg/day of methoxychlor. Liver section showing extensive vacuolisation of the cytoplasm in parenchymal cells. x 450.

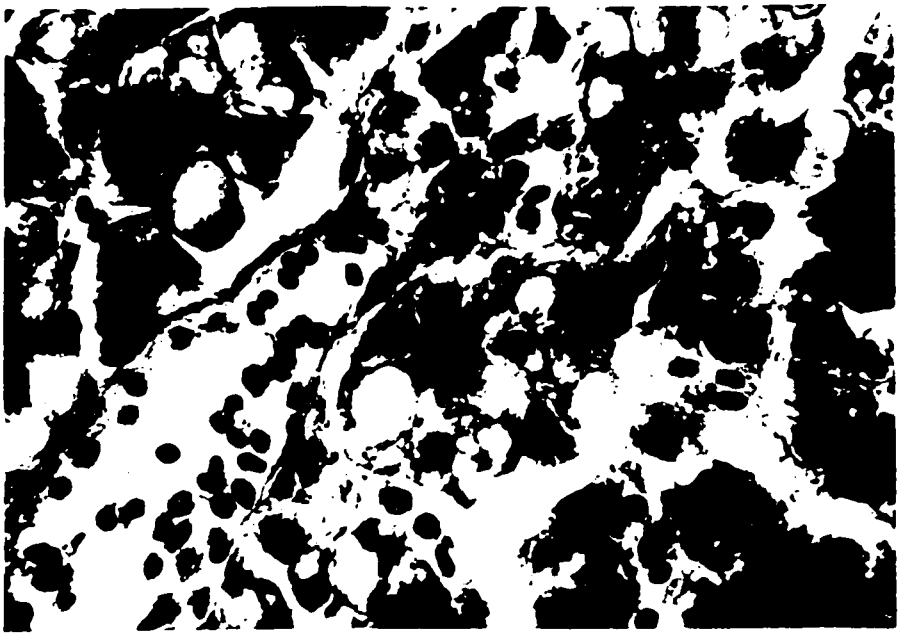

Figure 13F. Same as above. x 1000.

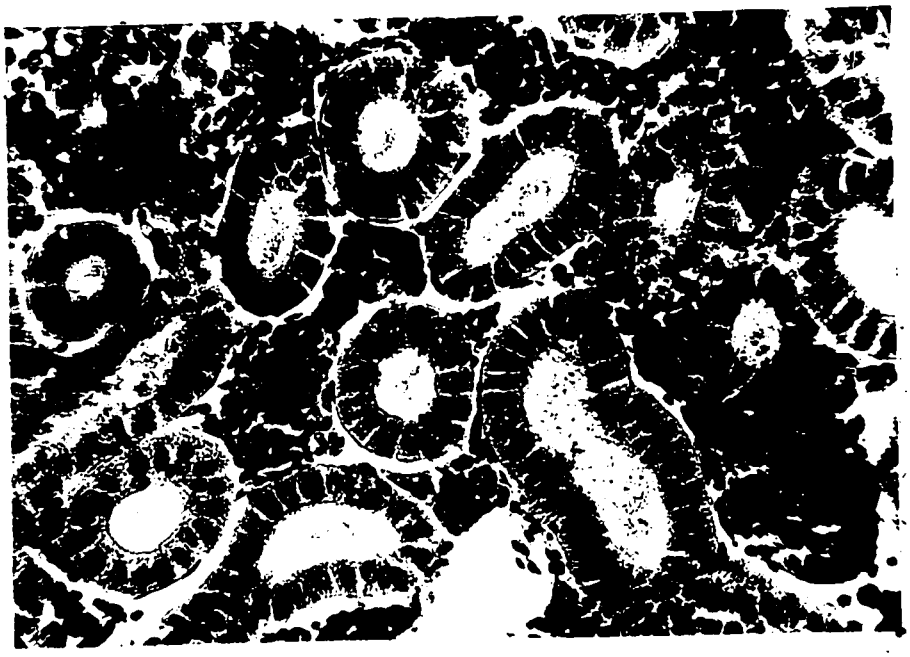

Figure 13G. Control fish. Kidney section showing regular shape and cellular detail of the tubules. x 450.

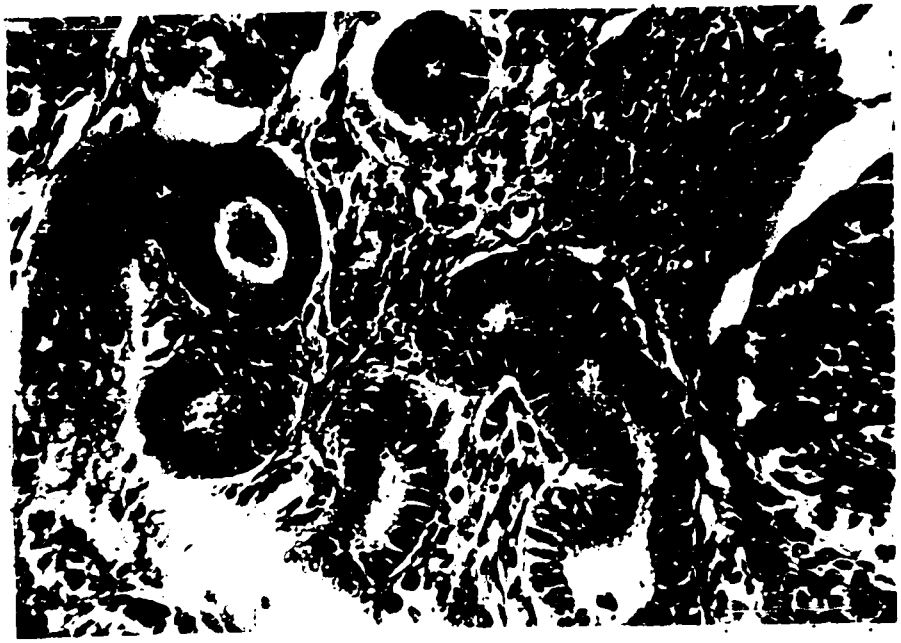

Figure 13H. Fish exposed to 2.00 mg/kg/day of methoxychlor. Kidney section showing shrinkage of tubules and changes in the cells lining the tubules. x 450.

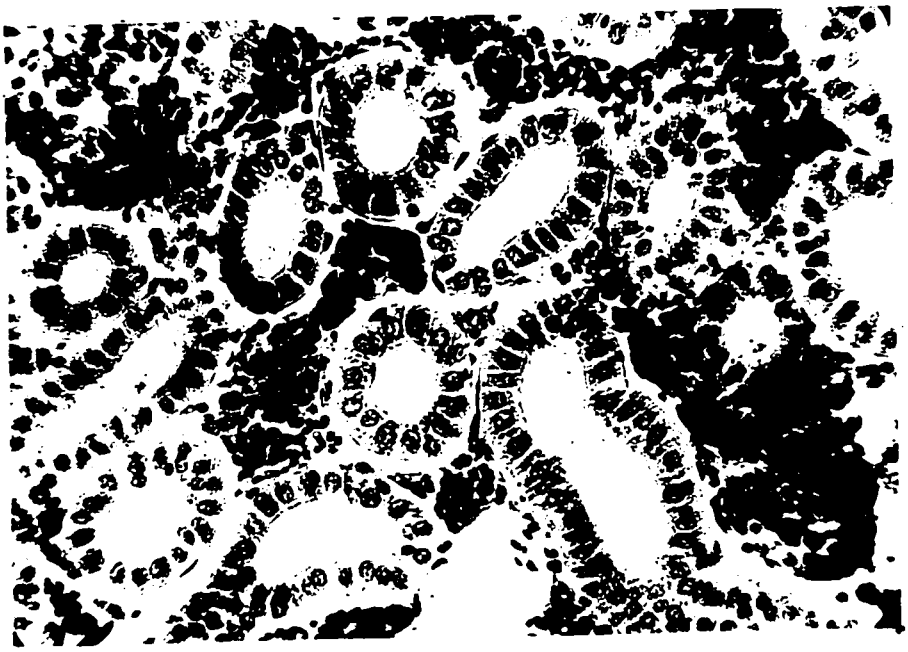

Figure 13G. Control fish. Kidney section showing regular shape and cellular detail of the tubules. x 450.

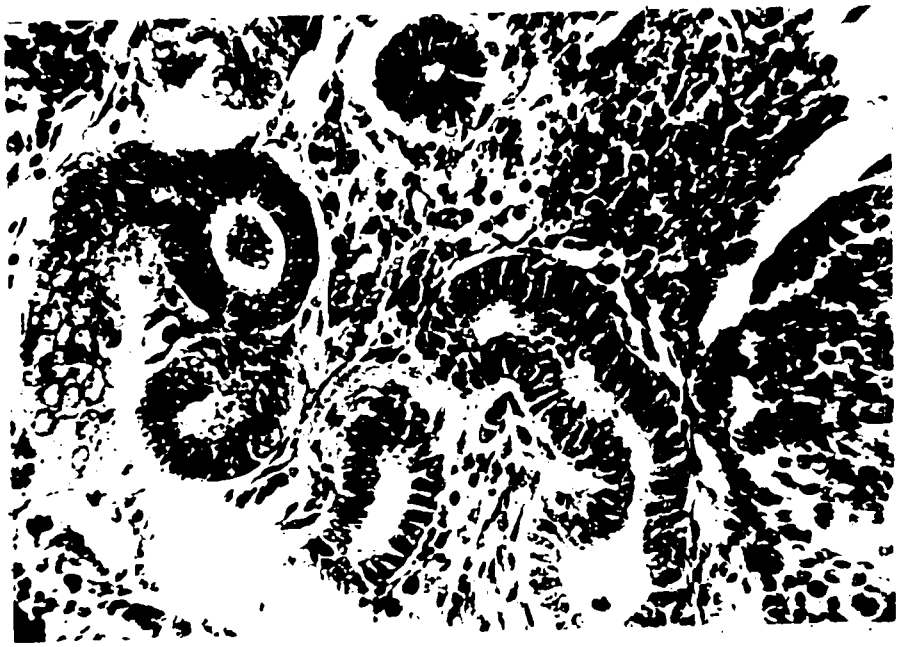

Figure 13H. Fish exposed to 2.00 mg/kg/day of methoxychlor. Kidney section showing shrinkage of tubules and changes in the cells lining the tubules. x 450.

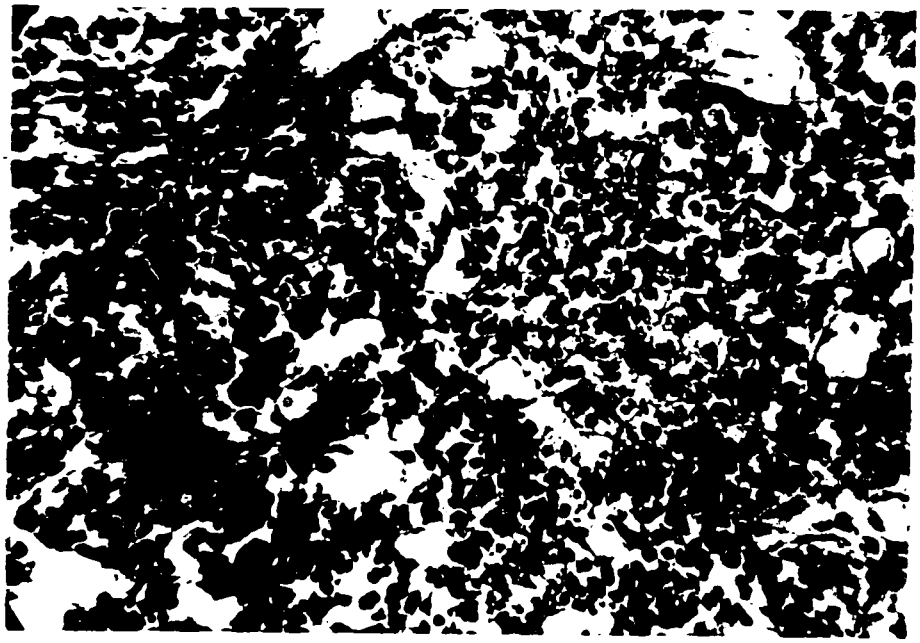

Figure 13I. Fish exposed to 2.00 mg/kg/day of methoxychlor. Kidney section showing loss of cytoplasm and irregularity in the shape of nuclei in cells of the head kidney. x 450.

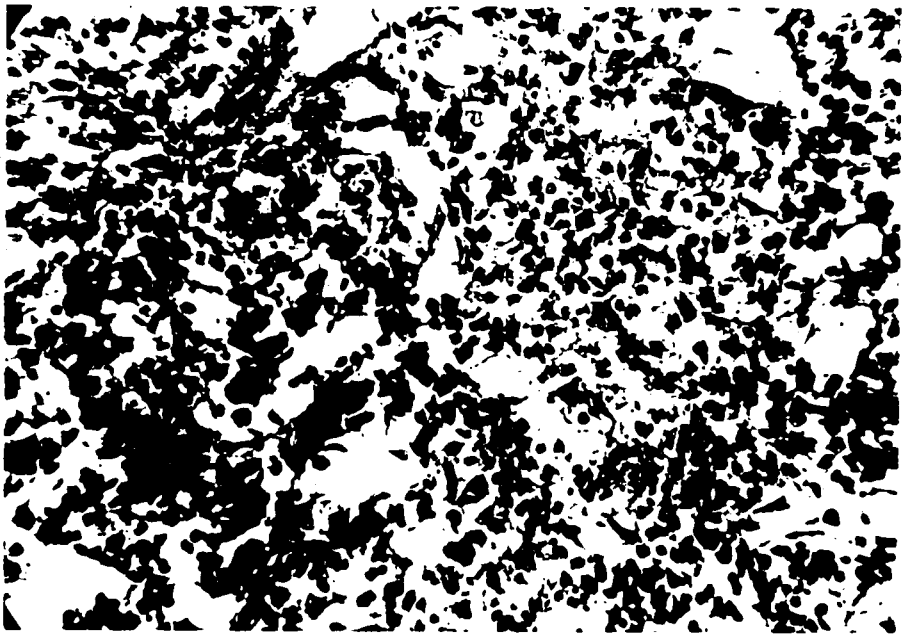

Figure 13I. Fish exposed to 2.00 mg/kg/day of methoxychlor. Kidney section showing loss of cytoplasm and irregularity in the shape of nuclei in cells of the head kidney. x 450.

described here are identified by numbers which also appear in Table 6. During the second experiment, no mortality occurred in any of the six groups of fish until the second week of exposure. Out of a total of 180 fish at the start of the experiment, ten fish died, five of which were in the highest intake level group. One fish died in the control tank a month after the start of the experiment after showing a slight loss in weight between the initial and second weighing.

At 0.125 mg/kg/day, one fish died after 24 days of feeding. The fish was found drifting around with the current in an inverted position, breathing regularly, but except for slight tremors, was motionless and made no effort to regain equilibrium. This fish had a swollen appearance with an unusual length-weight relationship (12.7 cm, 27.8 g) some eight grams over the weight of fish of similar length (obtained from a length-weight regression before the start of the experiment). Dissection revealed a greatly enlarged liver, with definite signs of hemorrhage; when an attempt was made to section the liver tissue after embedding, the sections crumbled, suggesting advanced destruction of the tissue. Histological examination of the kidney of the same fish showed a massive degeneration, characterized by an almost complete

loss of cytoplasm in connective tissue cells, leaving large spaces between the kidney tubules which in many areas, were infiltrated by erythrocytes. In addition a loss in cytoplasm by cells lining the kidney tubules was observed which gave the tubules a shrunken appearance. (Figure 14A). The pyloric caeca of this fish (No. 1) were frozen for residue analysis (see Table 6).

Two mortalities occurred in the 0.250 mg/kg/day test group, one of which was due to an overdose of anesthetic at the second weighing, while the other fish (No. 2) was found after eighteen days of feeding, drifting with the current in a way already described for fish (No. 1). The liver was a pale yellowish color, but was not sectioned as the entire fish was frozen for whole body residue analysis.

No mortality occurred in the 1.000 mg/kg/day test group, while five fish died at the highest intake level (2.000 mg/kg/day) with two on the 21st day of feeding. One fish (No. 3) died overnight, so no symptoms could be observed; examination of liver sections indicated a breakdown of liver cells and the presence of large vacuoles or droplets. The kidney tissue showed cytoplasmic deterioration and a breakdown of cells lining the tubules. The second mortality, exhibited the typical

symptoms of tremors and periodic strong convulsions; examination of liver tissue showed a marked degeneration of the liver parenchyma and the presence of large droplets or vacuoles in the cytoplasm of these cells (Figure 14 B). The kidney tissue also showed some degeneration, but not as severe as in the following brook trout (No. 4), which was found in distress during the third swimming test after 23-days exposure. When placed in the swimming test chamber, the fish swam in vertical circles with no current applied; when the water velocity was turned up, it was immediately swept against the retaining screen. When removed from the chamber, this fish could no longer maintain equilibrium and its opercula were trembling. Externally, it had a slightly bloated appearance and dissection revealed an enlarged liver, very pale in color, and a heavy deposition of fat in the pyloric caeca. Histological sections of the liver indicated severe tissue damage with a complete loss of structure in the cells as well as an absence of erythrocytes (Figure 14C). The kidney structure was severely disrupted by a breakdown of blood vessels and erythrocytes, vacuolisation of the cytoplasm in tubule cells, and an almost complete loss of cytoplasm in the connective tissue cells between the kidney tubules (Figure 14D). A fourth fish (No. 5) died after 31 days of feeding, no symptoms could be observed and it was frozen

for whole-body residue determination. The fifth fish died during the night after 33-days of feeding but as it looked normal externally and since enough samples had already been taken from this concentration, it was not examined histologically; no further mortalities were observed in the remaining fish until the completion of the experiment.

Figure 14. Photomicrographs of liver and kidney sections of brook trout which died during exposure to various dietary levels of methoxychlor while they were kept swimming against a current of 0.4 fps at 7.5°C.

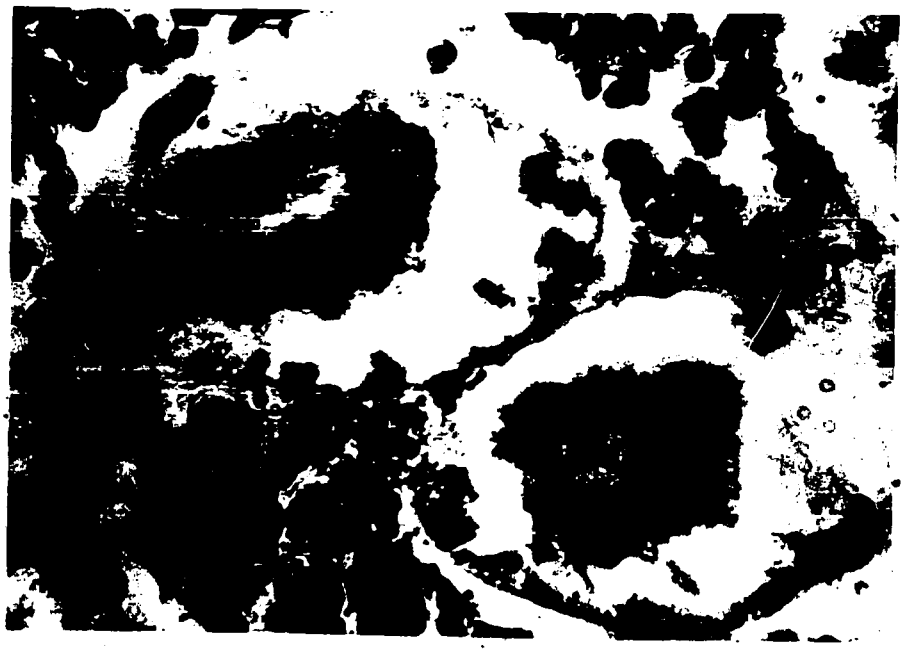

Figure 14A. Fish exposed to 0.125 mg/kg/day of methoxychlor; died after 24 days. Kidney section showing loss of cytoplasm and shrinking of the cells lining the tubules. x 1000.

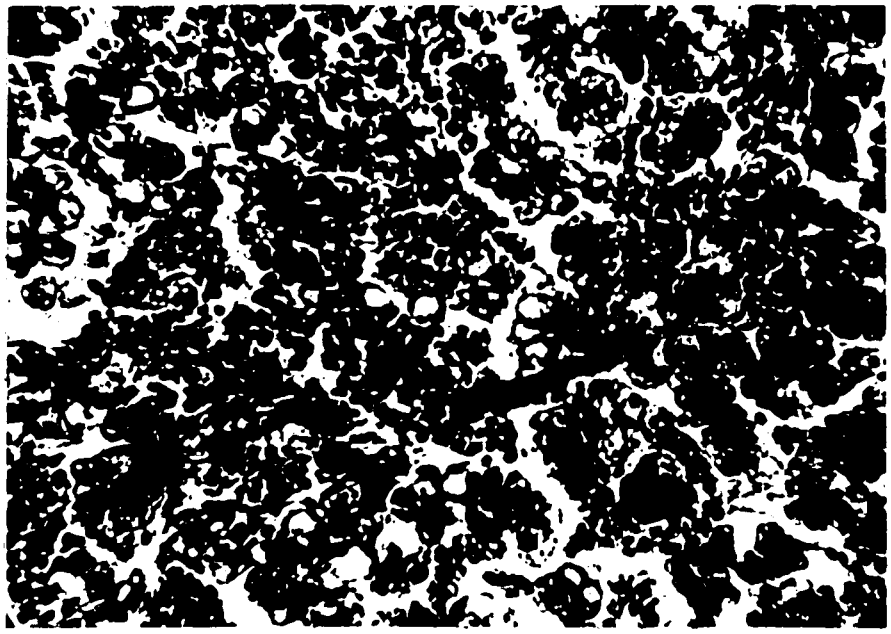

Figure 14B. Fish exposed to 2.00 mg/kg/day of methoxychlor; died after 21 days. Liver section showing cord disarray and cytoplasmic vacuolisation in parenchymal cells. x 450.

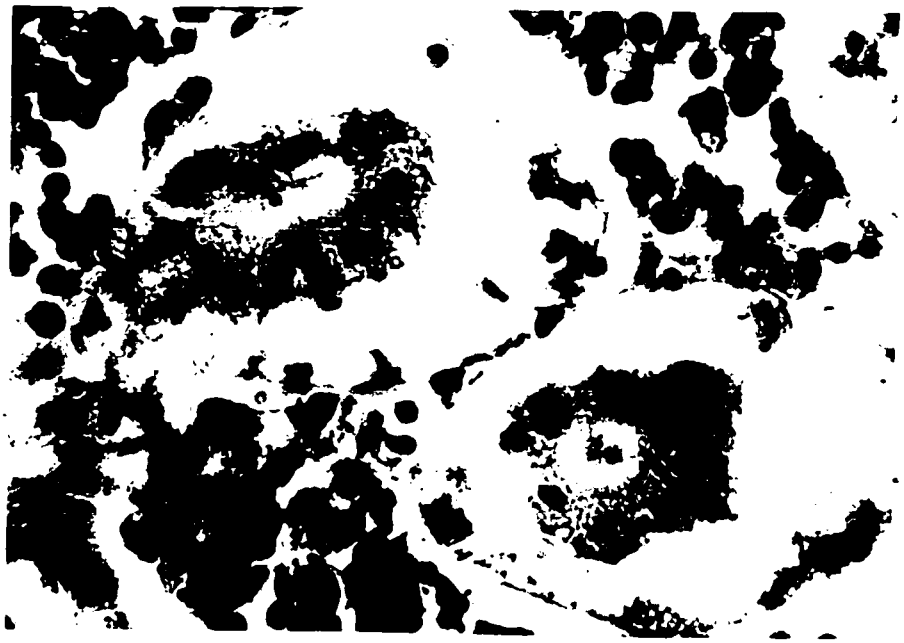

Figure 14A. Fish exposed to 0.125 mg/kg/day of methoxychlor; died after 24 days. Kidney section showing loss of cytoplasm and shrinking of the cells lining the tubules. x 1000.

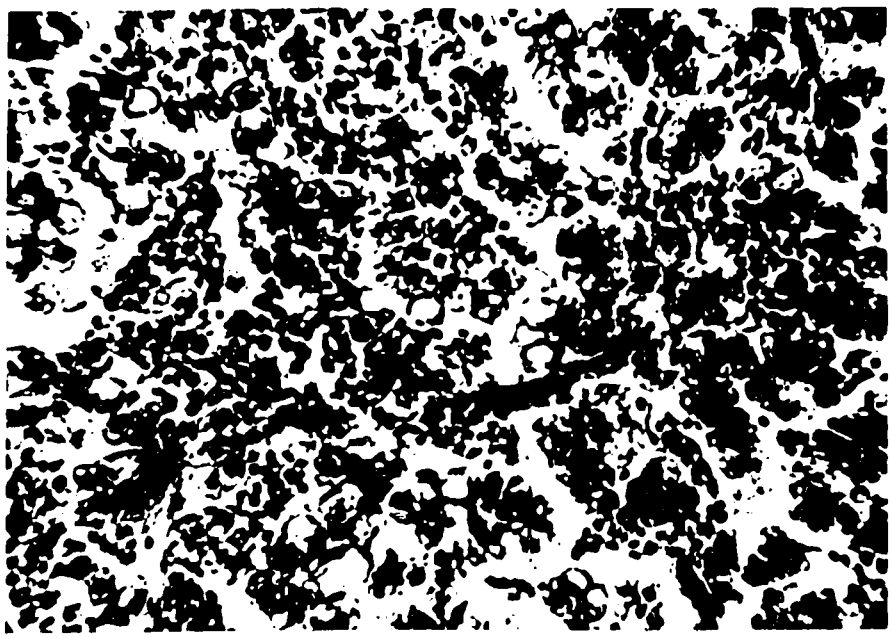

Figure 14B. Fish exposed to 2.00 mg/kg/day of methoxychlor; died after 21 days. Liver section showing cord disarray and cytoplasmic vacuolisation in parenchymal cells. x 450.

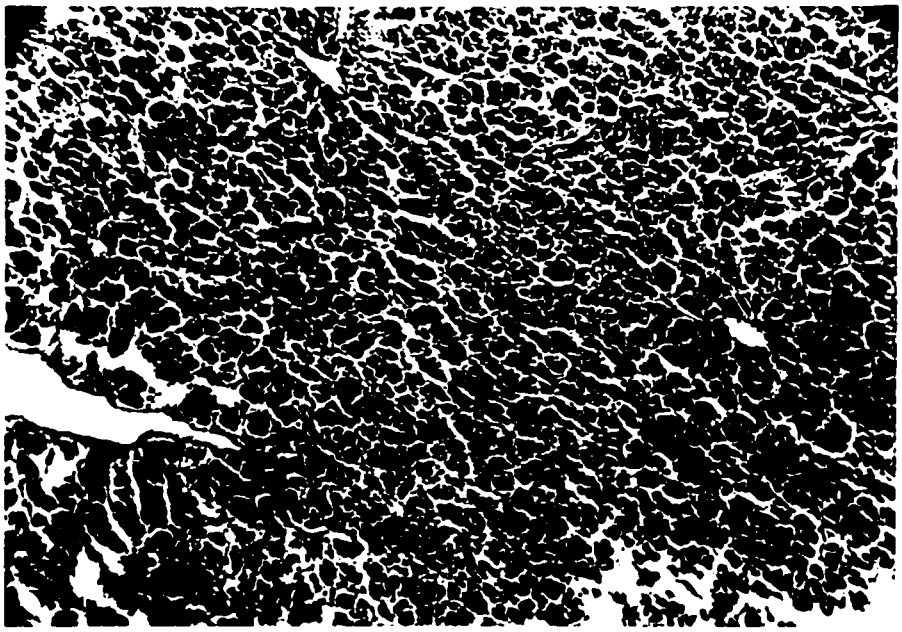

Figure 14C. Fish exposed to 2.00 mg/kg/day of methoxychlor; died after 23 days. Liver section showing loss of cord structure and absence of erythrocytes in blood vessels. x 100.

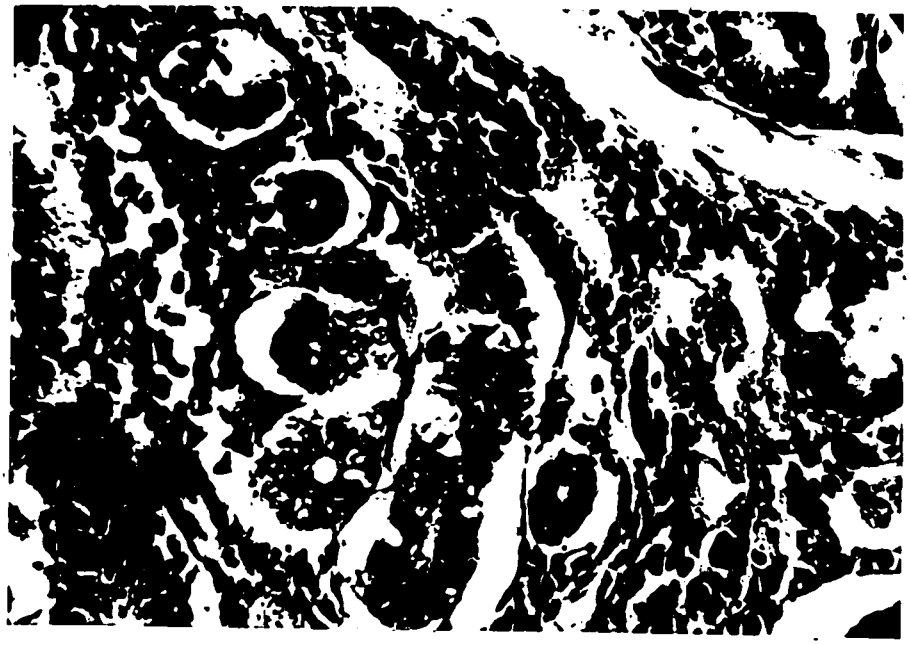

Figure 14D. Fish exposed to 2.00 mg/kg/day of methoxychlor (same as 14C); died after 23 days. Kidney section showing vacuolisation, loss of cytoplasm, and shrinking of cells lining the tubules. x 450.

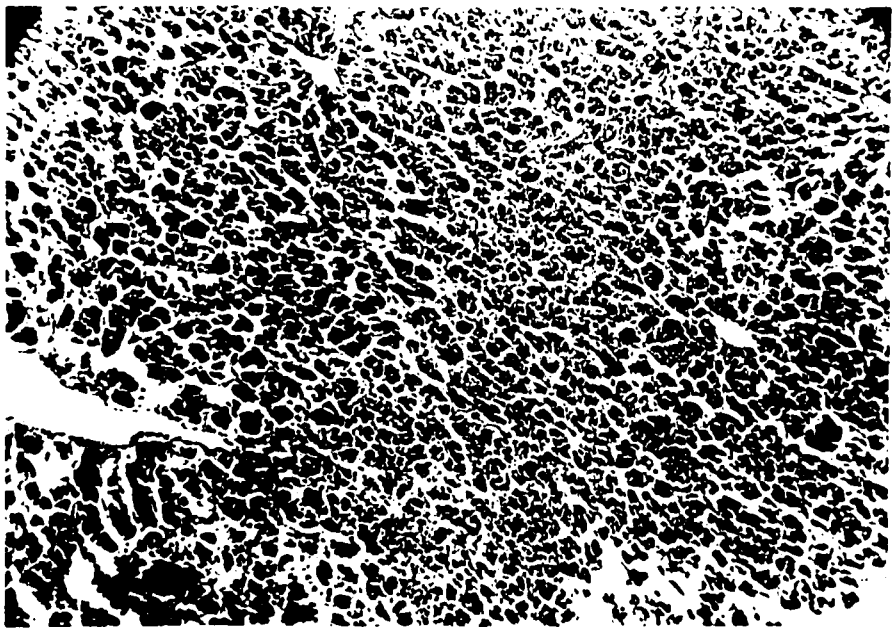

Figure 14C. Fish exposed to 2.00 mg/kg/day of methoxychlor; died after 23 days. Liver section showing loss of cord structure and absence of erythrocytes in blood vessels. x 100.

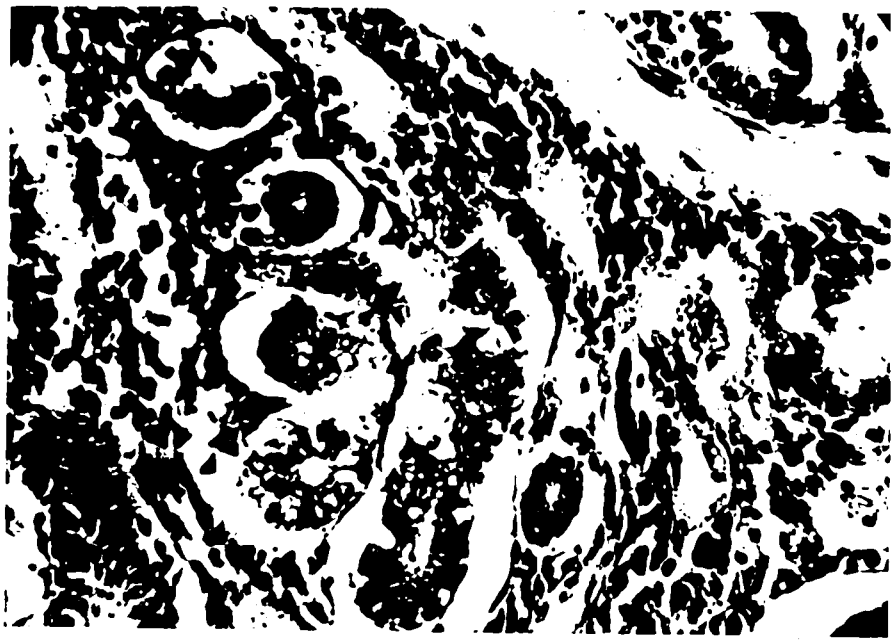

Figure 14D. Fish exposed to 2.00 mg/kg/day of methoxychlor (same as 14C); died after 23 days. Kidney section showing vacuolisation, loss of cytoplasm, and shrinking of cells lining the tubules. x 450.

DISCUSSION AND CONCLUSIONS

While the acute toxicity of many of the organochlorine insecticides to fish and aquatic invertebrates is well known, most of this information has been obtained by laboratory assays in which the organisms were exposed to fixed concentrations of the toxicant until death, due to direct absorbance from the water.

In the natural environment, however, fixed concentrations are rarely encountered, and in addition to the direct intake from water, aquatic organisms are exposed to these insecticides in the diet. Larvae of aquatic insects, which comprise the main food source of stream-dwelling salmonids, have been shown to possess remarkable abilities to concentrate the small amounts of insecticide that can be found in surface waters after forest spraying operations, and pass these doses on to fish feeding on them (Meeks, 1968; Burdick et al. 1968; Hatfield, 1969; Brown and Brown, 1970; Johnson et al., 1971).

While this has been shown both in the field and in laboratory experiments for many chlorinated hydrocarbons, most often for DDT, which has been linked directly to adverse effects on the aquatic community, no study has been published on the possible environmental consequences of sublethal methoxychlor contamination of water, fish

food organisms or fish, resulting from current pest control practices. Thus far, there appears to have been no attempt to determine the extent of this contamination in nature, nor to study the effects of the insecticide in the diet of fish at levels that might be encountered during spraying operations.

The ecological relevance of a laboratory experiment is based largely on how close its conditions come to the actual field situation. In flowing water, salmonids must maintain position, seek and capture food, avoid predators, migrate, reproduce, and in general, be more active than under usual laboratory conditions, where they are kept in still water and have food given to them while they are immersed in a toxicant emulsion or solution.

In an effort to make this study at least partly relevant to the natural situation, brook trout were kept in flowing water and fed a diet containing levels of methoxychlor which were related to possible levels of contamination resulting from present blackfly larviciding practices in Quebec and Labrador, with the purpose of detecting any deleterious physiological changes that might be related to such exposure. This study has shown that methoxychlor, administered at levels ranging from 0.01 to 2.00 mg/kg of fish/day, could, over a relatively short exposure period,

bring about deleterious effects bearing considerable ecological significance.

Growth

The present study revealed that dietary methoxychlor had no significant effect on the growth of brook trout except perhaps at the exposure level of 2 mg/kg of fish/day, where some reduction occurred (see Tables 1 and 2).

The literature presents widely differing results on the effects of sublethal exposure to chlorinated insecticides on the growth of fish; these can be traced to variations in modes of action and to different experimental conditions. Eisler (1967) found no differences in the growth of adult northern puffers during a 45-day exposure to a weekly-restored concentration of 0.03 mg/l methoxychlor in non-renewed water. Kennedy et al. (1970) found that a single exposure of bluegills to 0.04 mg/l of methoxychlor in ponds had no effect on their growth during a thirteen week test period. Swedberg (personal communication, 1970) observed a reduced growth of cutthroat trout which were periodically exposed, at 28-day intervals, to 0.1 and 0.3 mg/l of methoxychlor; however, the fish that were fed a diet containing 0.24 and 0.79 mg of methoxychlor/kg of fish/day in clean water showed no reduced growth.

Insecticides may also have a stimulatory effect on the growth of fish. Grant and Mehrle (1970) found that 0.004 to 0.043 mg/kg/day of dietary endrin improved the growth of goldfish; Macek (1968) observed that male brook trout receiving 2 mg/kg/week of DDT were significantly longer than the controls at the end of a 156-day experiment.

Deleterious effects of organochlorine insecticides on the growth of fish are usually linked to an interference with the normal body metabolism, increasing maintenance requirements to such a degree that growth is affected. Andrews et al. (1966) found such a relation for bluegills exposed to dietary levels of heptachlor ranging from 5 to 25 mg/kg/day. Dieldrin at a concentration of 0.05 µg/l in the water, greatly reduced the growth of sculpins by increasing the amount of food necessary for maintenance, and at the same time, reducing the food consumption of these fish (Warren, 1971, p. 163). It also appears that the fish seeks to increase its food consumption in an effort to compensate for an impaired food conversion efficiency. In experiments in which cichlids were exposed to 0.2 mg/l of pentachlorophenol, no difference in final size of the control and poisoned fish was observed, but the poisoned fish had markedly increased their food consumption during the experiment (Warren, 1971, p. 163).

A toxicant may act in such a way that no apparent differences in growth occur until food becomes limited. When exposed to 0.05 µg/l of dieldrin and fed a restricted diet, the growth of sculpins fell far behind that of the control fish due to the decreased efficiency in energy utilization (Warren, 1971, p. 163).

In the present study, the absence of any marked effect of dietary methoxychlor may be explained by the constant rate of feeding of all the groups of fish tested, i.e. 2 percent of the body weight per day. More pronounced effects could have been observed had the fish been forced to reduce their food consumption, as may occur in nature in cases where the fish-food fauna is decimated by insecticide application (Ide, 1956; Graham and Scott, 1958; Elson and Kerswill, 1967). The resulting increase in the energy cost of obtaining food in such a situation may well have a marked effect on growth under natural conditions. In the case of the highest exposure level (2 mg/kg/day), some reduction in the growth of brook trout was observed (Table 1); perhaps this decrease may not have occurred had the fish been given more food. It is of interest to note that the fish fed 2 mg/kg/day which had been repeatedly tested for swimming ability showed a smaller weight gain than fish

from the whole group. This may be an indication of a subtle effect that was enhanced by the additional stress of the swimming tests. In addition, this level of exposure may represent a threshold, above which more definite changes could occur, and this threshold could possibly be reached at lower levels of methoxychlor but over a longer exposure period.

Another consequence of the constant food supply maintained during this study may be the lower growth rate of brook trout in Experiment No. 1 at 10°C than in Experiment No. 2 at 7.5°C. The growth rate of fish tends to increase with temperature due to an increased food consumption which not only fulfills the energy load imposed by a higher metabolic rate, but is adequate to increase the energy available for growth. In Experiment No. 1 however, while the same feeding rate (2 percent of body weight per day) was maintained as in Experiment No. 2, the brook trout were subjected to a higher temperature. While the energy required for swimming probably remained quite similar, the cost of standard metabolism was raised without a concurrent rise in food supply. Consequently these fish had less energy available for growth at the higher temperature of Experiment No. 1 than at the lower temperature of Experiment No. 2. In addition, it is possible that the occurrence of air supersaturation in the water supply

contributed to a lower growth rate of brook trout in Experiment No. 1.

Another mode of action of a toxicant can be the elimination of the weaker members of a population, resulting in an apparently better growth in the survivors. Allison et al., (1964) found no effect of DDT on the growth rate of cutthroat trout but noted that the overall size of exposed fish was larger due to the elimination of the smaller fish in the population. In the case of field insecticide application, the growth of the surviving fish must depend largely on the balance between the reduced numbers of fish and the reduced numbers of fish-food organisms, and such changes in growth as observed in the laboratory may have little bearing on fish production when compared to the serious reduction in fish biomass due to mortality. Schoenthal (1963) found that when wild rainbow trout which had been forced to swim continuously in a hatchery head trough for three weeks were exposed to 1 mg/l of DDT, they showed a mean mortality of 5.6, whereas fish that had been kept in a holding pond, with no appreciable current, showed a mortality of only 0.7. In the present study, a slight reduction in the growth of continually swimming brook trout was accompanied by a sharp drop in biomass over a relatively short period of exposure

to 2 mg/kg/day of methoxychlor. At lower methoxychlor concentrations, the severe liver and kidney damage that was observed in many of the poisoned fish suggests a less subtle effect but one which did not affect the growth of fish in the exposure period used in this experiment. It is doubtful however, that fish so affected could maintain normal body functions much longer without serious consequences, and more pronounced changes in the growth of fish from the lower exposure levels would have certainly occurred with progressively increasing damage to the liver and the kidney.

Swimming performance

Although this study was undertaken with no prior knowledge of what effects, if any, could be expected from the exposure of brook trout to dietary methoxychlor, it was designed to enable such effects to be related to possible field conditions. For this purpose, brook trout were forced to maintain position against a moderate current in the annular growth chambers during the exposure period and were subjected to occasional swimming tests at a higher velocity, conditions which are often encountered in small, rapidly flowing streams. This offers a more realistic approach than the flowing bioassay design where the fish are exposed to a toxicant while kept in test

tanks with a water exchange but no appreciable current.

Although it would appear that a swimming test is relatively easy to apply, this ecologically important measure of performance has not often been used to determine the sublethal effects of toxicants (Sprague, 1971) and in the few cases where it has (Neil, 1957; MacLeod and Smith, 1966; Leduc, 1966) reduction of performance has usually been linked to an interference with normal gill ventilation by some physical agent such as pulp fiber or with internal tissue respiration by chemical agents such as cyanide.

The results of the present study have shown that dietary methoxychlor had a pronounced effect on the swimming ability of brook trout and suggested a different physiological response to low and high concentrations of the insecticide in the diet. On the one hand, in the first experiment, low dietary methoxychlor concentrations (0.01 to 0.16 mg/kg of fish/day) caused a marked reduction of swimming ability (Figure 10). Similarly, in the second experiment, brook trout which received a daily dose of 0.125 to 0.500 mg/kg of fish/day showed less of an increase in swimming stamina than the controls; on the other hand, at the higher concentrations (1.00 and 2.00 mg/kg of fish/day), a markedly increased swimming performance was observed,

up to eight times higher than the control fish (Figure 11 and Table 4). However, it must be noted that the methoxychlor exposed brook trout of Experiment No. 2., all had a lower hematocrit and red blood cell counts than the control, the lowest values occurring at the higher concentrations.

One would expect that such a decrease in the number of erythrocytes would lower the oxygen carrying capacity of the blood and thus limit active metabolism. Instead, the group of fish with the lowest blood counts achieved a striking increase of swimming ability; such a performance may be possible through some mechanisms activated by the higher methoxychlor dosages which promote an increase of the energy available for swimming. The increased swimming ability observed in brook trout which were exposed to dietary methoxychlor levels of 1.00 and 2.00 mg/kg/day may have been brought about by an increase in anaerobic metabolism. While Mehrle et al. (1971) state that little is known of the biochemical effects of organochlorine insecticides on fish, a brief consideration of swimming activities may lead to a possible explanation of the results obtained in this study.

Bilinski (1969) suggests that for prolonged swimming, such as undertaken by migrating salmon, fish

rely on the aerobic metabolism of fat in the red muscle as a source of energy; however, for short periods of vigorous swimming, fish can rely on anaerobic metabolism of glycogen in the white muscle. Studies by Black et al. (1962) on the rainbow trout, and by Beamish (1968) on the cod, showed in both cases, that these fishes utilized more than 80 percent of their muscle glycogen during fifteen minutes of strenuous exercise. While glycolysis has been traced as the energy source for burst swimming and aerobic metabolism for cruising speed, it is not clear what the situation is in water velocities between these two extremes. The test velocities used in the present study cannot be called burst or cruising speed, but rather a maximum sustained speed; this term has been usually applied to the maximum velocity that a fish can sustain for a given period of time when subjected to a step-wise increase. In this experiment, the time to exhaustion was measured and the swimming velocities used were in the 3.1 to 4.4 body length/second range, which lies at the lower limit of the division between maximum cruising and burst speed assumed by Brett (1964). Smit et al. (1971) suggested from their experiments with goldfish, that when a fish exceeds a swimming speed of 3.4 lengths per second, it maintains its sustained swimming speed through both aerobic

and anaerobic metabolism in the red and white muscle fibers. The observation that these fish could increase their swimming speed fourfold without a concurrent increase of oxygen consumption supports these assumptions. It is quite probable that an increased anaerobic metabolism, induced by exposure to 1.00 and 2.00 mg of methoxychlor/kg of fish/day would allow the marked increase of swimming ability observed in this study.

On the other hand, the results of Experiment No. 1 and the results of the exposure of brook trout to methoxychlor levels ranging from 0.125 to 0.50 mg/kg/day in Experiment No. 2, indicated a decrease in the swimming ability of these fish (see Figures 10 and 11). Only two references could be found on the effects of prior exposure to a chlorinated hydrocarbon insecticide on the swimming performance of fish. Mount (1962) observed no change in the swimming endurance of bluntnose minnows which had been exposed to acute concentrations of endrin, although several fish died during the swimming test. Cairns and Scheier (1964) observed a decrease in oxygen consumption and in the cruising speed of sunfish which had been exposed to 1.68 µg/l of dieldrin for 12 weeks, but did not suggest a reason for this reduction. Several authors have suggested that organochlorine insecticides act by

interfering with enzymatic processes.

DDT and several of its analogs selectively inhibit the action of ATP-ases (Matsumara et al., 1969, Janicki and Kinter, 1971), and the extent of inhibition has been found to be greater in muscle than in nervous tissue (Koch et al., 1969). Colvin and Phillips (1968) showed that the toxicity of endrin to catfish could be traced to the disruption of electron transport caused by the association of the insecticide with the lipid component of the mitochondrial membrane. Recently, Mehrle et al. (1971) found that rainbow trout which had been fed a diet containing 0.146 mg of DDT/kg/day could not utilize selected amino acids in the blood during swimming, and suggested the inhibition of specific enzymes which were responsible for the utilization and energy transformation of these amino acids.

In view of the tendency of lipophilic organochlorine insecticides to combine with the lipid components of the mitochondrial membrane with consequent disruption of the enzymatic processes involved in aerobic metabolism, it appears that low levels of methoxychlor could have similar effects on brook trout. The reduced swimming performance of brook trout at low dietary levels of methoxychlor may be explained by a lower oxidative metabolism in the red muscles which contain a much greater abundance of

mitochondria than the white muscles (Bilinski, 1969). At higher methoxychlor levels, the balance between aerobic and anaerobic energy sources for swimming has apparently been disturbed, and the glycolytic activity of the white muscles may be selectively enhanced thus allowing for a better performance at the velocity tested. Whatever the exact nature of the mechanisms responsible for the reduced swimming ability at low dietary levels of methoxychlor, it is important to realize that in nature, low levels of insecticides in food organisms may be more prevalent over extended periods of time than high concentrations.

Spatial distribution

Hoar (1957) suggested that thyroxine may be associated with glycolysis in fish, and indicated that studies showed that the decreased growth rate of brown trout during the summer months was related to maxima of thyroid gland activity which stimulates the spontaneous activity of these fish, thus reducing the amount of energy available for growth. Hoar et al. (1955) indicate that a number of studies suggest that thyroxine and gonadal steroids stimulate swimming activity in fish and Stanley and Tescher (cited in Hoar et al., 1955) reported a 400 percent increase in the activity of goldfish fed

on testicular material. Hoar et al., (1952 and 1955) working with young chum and sockeye salmon, observed increased spontaneous activity and swimming speed as well as a reduced grouping of the fish that had been treated with thyroxine and testosterone; Woodhead (1970) observed a similar effect on the swimming speed of cod treated with thyroxine.

There is a scarcity of information concerning the effects of insecticides on the endocrinology of fishes. There is, however, indirect evidence of a disturbance caused by methoxychlor reported by Swedberg (personal communication, 1970) who found that mature female cut-throat trout which had been fed a diet containing 0.79 mg of methoxychlor/kg of fish/week had a higher gonado-somatic index and began ripening earlier than those exposed to the lower insecticide level and those of the control groups. Welch et al. (1969) showed that methoxychlor could enhance estrogen and androgen metabolism in the rat; Beach (1948) suggested that androgens and estrogens act as chemical sensitizers which increase the responsiveness of the central nervous system to external stimuli.

The results of the present study, in which methoxychlor exposure of 2 mg/kg/day caused a change in the spatial distribution and an increase of swimming

residues are no more likely to occur in fishes than in warm blooded animals."

Kapoor et al. (1970) presented further evidence of rapid elimination by mammals showing that 98.3 percent of the methoxychlor administered orally to mice was excreted within 24 hours. In the same study, however, when methoxychlor was applied to a model aquatic ecosystem, mosquito fish and snails were found to contain methoxychlor residue levels respectively 1500 and 120,000 times those in the water.

Taylor (personal communication, 1971) referring to a fish kill in Labrador after methoxychlor larviciding, stresses that "what is needed is more work to determine the effects on fish and non-target invertebrates to prevent a possible repeat of the DDT story.... there are many conflicting reports regarding methoxychlor toxicity to fish and it would be wise to find answers before this chemical is acknowledged as "safe"."

In the present experiment, when brook trout were fed a diet containing from 6.25 to 100 ppm of methoxychlor, they retained more than 40 percent of the dosage administered. In addition, it was shown in Figure 7 that the percentage of methoxychlor retained apparently increased with increase in dietary concentration up to 50 ppm (1.00 mg/kg/day), then showed a drop at the highest exposure level

(2.00 mg/kg/day). Buhler et al. (1969) found a similar increase in the storage of DDT in fingerling coho salmon which were fed dietary levels of 6.25 to 100 ppm DDT; however at 400 ppm, there occurred a drop in the amount of the insecticide retained. While the authors did not offer an explanation for the increase in storage with increase in dietary concentration, they suggested that the apparent excretion of DDT by fish in the highest exposure group could be explained by the selective death of fish which contained higher than average pesticide burdens. In the present study, the presence of high methoxychlor residues in fish that died during the exposure period would tend to support such an explanation for the apparent decrease in storage that was observed in the 2.00 mg/kg/day group (see Figure 7). In addition, since a depression in the growth of these fish was observed, it could be conceivable that a drop in food utilization might have occurred, with the consequent passage of some methoxychlor out with the feces before absorption had taken place.

Chadwick and Brocksen (1969) obtained the opposite results with sculpins that had been fed tubificid worms containing from 25 to 350 ppm of dieldrin; the proportion of dieldrin retained by the fish was the greatest in those groups that had received rations of worms

containing smaller amounts of the insecticide. These authors suggested that perhaps an enhanced metabolism and excretion of dieldrin at higher tissue concentrations could account for this observation.

Macek and Korn (1970) found that 35.5 percent of the DDT fed to brook trout in a diet containing 3.0 ppm could be accounted for at the end of a 120-day exposure period, similarly, Buhler et al. (1969) could account for 37.8 percent of DDT fed to fingerling chinook salmon in a diet containing 6.25 ppm, and for 76.1 percent of that ingested in a diet containing 100 ppm of the insecticide after a 39-day exposure. In the present study, corresponding figures for the retention of ingested methoxychlor were 42 percent at 6.25 ppm and 78 percent at a feeding level of 100 ppm. It appears therefore, that the results of a continuous intake of DDT and methoxychlor are almost identical; the main difference between the two insecticides lying in the time to elimination after a return to uncontaminated food, with DDT taking considerably longer to leave the body. In another laboratory study, Swedberg (personal communication, 1970) found residues of methoxychlor in excess of 15 ppm in adult cutthroat trout that had been receiving 0.79 mg of methoxychlor/kg of fish/day for three months.

Only one study (Mayer et al., 1970) could be found which mentioned methoxychlor levels in specific tissues of fish that had been exposed to various dietary levels. These authors showed that when methoxychlor was fed to rainbow trout at a rate of 2.5 mg/kg/day for 14 days, these fish accumulated 100 ppm in their fat bodies, and at a rate of 12.3 mg/kg/day, a level of 774 ppm was reached in the same tissue. Burdick et al. (1968) exposed four brook trout to 0.005 mg/l methoxychlor for 7 days and found residues of 142.4 ppm of the insecticide in the oil of one fish that died after 4 days of exposure. In the present experiment, a methoxychlor level close to 600 ppm in the pyloric caeca of one of the dead brook trout (see Table 6) is readily comparable to the observations of Mayer et al. (1970).

The ability of fish to concentrate chlorinated insecticides in adipose tissue has been well established. Grzenda et al. (1970) found that goldfish which were exposed to a dietary level of 0.1 mg/kg/day of DDT for 32 days had residues of 14.4 ppm in mesenteric adipose tissue, 8.1 ppm in nerve, 4.8 ppm in liver, and 3.4 ppm in kidney tissue; after 128 days of feeding, these levels were 42.7, 18.7, 6.4, and 6.7 ppm respectively.

Although limited in its scope, the present study has indicated that in addition to its retention in whole

fish, methoxychlor can accumulate in selected tissue to an extent comparable to that of the better known chlorinated hydrocarbon insecticides such as DDT, and the presence of methoxychlor residues of up to 2.65 ppm in a sample of fish found dead in Labrador after the 1970 blackfly larviciding operation (Taylor, personal communication, 1971) adds evidence to the possibility of the serious danger resulting from the short-term cumulative properties of this insecticide.

Histopathological changes

While a number of histopathological changes in fish have been linked to various chlorinated insecticides, (Johnson, 1968) very few studies related to sublethal methoxychlor exposure have been reported in the literature. In many cases of poisoning, pronounced liver and kidney damage occurred within a short time after initial exposure.

Kennedy et al. (1970) observed some liver parenchymal shrinkage, increased cytoplasmic granularity, and a partial loss of the radial orientation of liver cords in bluegills within three days after a single exposure to 0.04 mg/l methoxychlor in ponds. Mount (1962) observed vacuolisation of liver cells in bluntnose minnows chronically exposed to endrin, and Eller (1971) observed liver

lesions in cutthroat trout after chronic exposure to endrin in the diet. In both studies the livers of these fish were characterized by liver cord disarray and swelling and vacuolisation of cells undergoing necrosis.

In the present study, identical changes were observed in the liver tissue of brook trout after chronic exposure to methoxychlor in the diet.

At the lower methoxychlor exposure levels (0.125 to 0.500 mg/kg/day) while no liver damage was observed, there still occurred a distinct increase in eosinophilia in these tissue sections.

In mammals, the liver cell has been shown to respond to the administration of lipid soluble drugs by a sharp increase in the amount of smooth endoplasmic reticulum (S.E.R.), and correlated with this increase, a substantial increase in drug metabolizing enzymes has been noted. These changes are interpreted as adaptations to increase the efficiency of the liver in the elimination of these drugs and the hypertrophy of the S.E.R. may be so extensive that it occupies nearly all of the available cytoplasm (Fawcett, 1966, p. 165-66). Porter and Bruni (1959) found that in liver cells in which such proliferation was taking place, extensive areas of the cytoplasm were occupied by large lipid vacuoles, and suggested that in addition to the role of detoxification, the S.E.R. appears

to play some role in glycogen storage and mobilization. Wang and Matsumara (1969) indicated that the liver cells of rats treated with insecticide started producing S.E.R. shortly after administration. Porter and Bruni (1959) have found that such proliferation of S.E.R. gives liver cells a marked eosinophilia when stained with hematoxylin and eosin. It would seem therefore, that methoxychlor, which is a lipid soluble insecticide, may stimulate the proliferation of S.E.R. in liver cells of brook trout, imparting to them the eosinophilic colour and by interfering with normal glycogen metabolism, may lead to the extensive cytoplasmic vacuolisation that characterized the liver cells of fish exposed to 1.00 and 2.00 mg methoxychlor/kg of fish/day.

Kidney pathology often accompanies liver damage related to exposure of fish to chlorinated hydrocarbon insecticides. Mathur (1962) observed a degeneration in the kidney epithelium in four species of fish that had been exposed to DDT concurrent with vacuolar degeneration and necrosis of liver cells, and King (1962) observed similar effects of DDT exposure on brown trout and guppies. Buhler et al., (1969) found lesions in the renal epithelium of fingerling coho salmon which had been fed a diet containing from 6.25 to 400 ppm of DDT over a

period of 60 days; these lesions led to a complete degeneration of the epithelial lining of the distal convoluted tubule. This author suggests that the marked degenerative changes could cause severe damage of the nephron with resultant osmoregulatory failure. Kennedy et al. (1970) observed the congestion of kidney glomeruli but found no structural damage in kidneys of bluegills exposed to a single dose of 0.04 mg/l of methoxychlor.

In the present study, methoxychlor exposure of 1.00 and 2.00 mg/kg/day led to vacuolisation of cells in the head kidney, a marked tubule shrinkage and vacuolisation of the lining cells to the point of causing, in some fish, a complete destruction of the tubules (Figure 14A and 14D). Although somewhat less pronounced than the observed morphological changes in the liver, changes in the kidney would undoubtedly seriously impair the proper functioning of this organ.

In addition to structural changes in the liver and kidney, methoxychlor appeared to have affected the vascular system, as characterized by red-blood-cell breakdown and the presence of hyaline globules or inclusions in the blood vessels of the liver (Figure 13B and 13D). Within 24-hours after exposure to methoxychlor, Kennedy et al. (1970) observed changes in the vascular system in

the form of precipitated material in the blood which became multivacuolated and remained in the blood vessels for 4 weeks. These authors identified this material as altered serum protein or damaged erythrocytes and suggested that it might severely affect circulatory function and lead to a state of anoxia. In the present study, similar vacuolated inclusions were observed in the blood vessels of the liver in a number of fish exposed to methoxychlor in the diet, and seemed to be related to erythrocyte breakdown. Kennedy et al. (1970) also observed higher hematocrit values in the poisoned fish as compared to the controls but gave no erythrocyte counts, which should have been lower if destruction was occurring. Eisler (1967) found that the mean hematocrit values of methoxychlor-exposed puffers were significantly lower than the controls but noted no differences in erythrocyte number; this might suggest smaller erythrocyte volume. In the present study, both the erythrocyte number and the hematocrit value of brook trout were reduced by the dietary methoxychlor, (Table 7) with the lowest values appearing at the highest (2.00 mg/kg) exposure level. These effects may be indicative of liver damage, as it has been reported that active hematopoeisis is a typical feature of a normal trout liver (Simon et al., 1967).

From this study it appears that after a month of exposure to dietary methoxychlor, brook trout as a group, could still maintain position against the current of the annular growth chambers, and showed no external symptoms of poisoning except for a slight change in their spatial distribution. However, the death of several individuals with obvious symptoms of insecticide poisoning, and the advanced degenerative changes in the liver, kidney and vascular system in dead and surviving fish suggest that, had the experiment been maintained longer, progressively more fish would have died. The observed mortality of brook trout under the present laboratory conditions is noteworthy. Grenier (personal communication, 1970) had fed a diet containing 100 ppm methoxychlor to rainbow trout for 3-1/2 months and observed only one mortality, after 29 days. The different effects can reasonably be ascribed to differing experimental conditions, as Grenier exposed his fish in aquaria with renewed but still water, whereas in the present study, brook trout were forced to swim against a current; a condition which better approximates that of the natural environment of a stream-dwelling fish.

Ecological significance of the study

Aerial spraying with DDT for mosquito and black-fly control on the Quebec North Shore (see Figure 1) started in 1952, at Forestville and Labrieville; the extent of spraying increased yearly with the establishment of mining, lumber and hydro power dam construction camps, and soon was done on a regular basis around townsites. Since that time, several areas in Labrador, Province of Newfoundland, have been included in this spraying program (Figure 1). For several years, the standard DDT dosage was 1.0 lb/acre but was eventually reduced to 0.2 lb/acre, and in 1969, methoxychlor was substituted for DDT for these control measures.

In spite of more than 15 years of insecticide application over this large area, there is no indication that any systematic study of the possible contamination of that environment has been undertaken. In 1969, Hatfield reported DDT residues of up to 139 ppm in the stomach contents of brook trout which had died during ground black-fly larviciding operations in Labrador; later, Taylor (personal communication, 1971) reported a fish kill and a concentration of 2.65 ppm of methoxychlor in minnows after a standard air spray with methoxychlor (0.2 lb/acre) in Labrador. As a result of such disturbing evidence,

except for ground fogging, the use of methoxychlor has been discontinued in Newfoundland on the advice of fishery biologists.

Brown and Brown (1970) presented the results of a detailed study on DDT residues in various members of an ecosystem in Northern Manitoba which had received 22 air-sprays of DDT at 0.22 lb/acre between 1947 and 1964 for the purpose of mosquito control. Fish food organisms (chironomid and tabanid larvae) contained up to 1.25 ppm of residual DDT. It is quite possible therefore, that the areas of the Quebec North Shore and Labrador that have been subjected to repeated DDT airsprays have comparable levels of contamination in the food organisms of fish species such as the brook trout and the Atlantic salmon that inhabit these areas. At the present time, there is no information available on methoxychlor residue levels in fish food organisms following three years of field application in this area, and the presence of combined DDT and methoxychlor contamination cannot be discounted.

The results of the present study, while not obtained under actual field conditions, can at least furnish an order of magnitude to the previously unknown possible methoxychlor levels in fish food organisms resulting from current ground blackfly larviciding practice. A mixture of caddisfly, stonefly and cranefly larvae were

found capable of concentrating methoxychlor present in the water at the concentration used for ground larviciding (0.075 mg/l) by a factor of 19 during a 24-hour exposure period to yield levels of methoxychlor ranging from 1.02 to 1.42 ppm (see Table 5). In the case of the natural situation, in addition to the amount of methoxychlor that these organisms extract from the water, a large proportion could be ingested by detritus feeders, as organochlorine insecticides are known to bind to such material (Meeks, 1968) and once bound, may serve as a continued source of contamination.

Wallace (1971) studied the effects of methoxychlor larviciding on non-target fish food organisms and reported severe drifts and subsequent mortality of Ephemeroptera, Plecoptéra and Trichoptera larvae after blackfly larviciding of streams near Baie Comeau (see Figure 1) in 1968 and 1969. The author showed that methoxychlor seriously disturbed these aquatic communities which constitute the primary food source of the brook trout and the Atlantic salmon inhabiting these waters. Current methoxychlor larviciding operations have been shown to reduce the abundance of food organisms, and the results of the present experiment suggest that those organisms that survive can serve as a continued source of methoxychlor contamination due to its accumulation.

Under field conditions, fish mortality has definitely been linked with feeding on an insecticide-contaminated diet (Graham and Scott, 1958; Bridges, 1961; Schoenthal, 1963; Welch and Spindler, 1964) but due to the inherent difficulty in conducting field experiments, the effects of chronic exposure to chlorinated hydrocarbon insecticides in nature are little known, although gross effects such as the decline of fish populations in the years following application have been noted.

Fish kills probably occur as a result of feeding on a large, suddenly available biomass of killed and/or drifting aquatic insects, containing significant amounts of methoxychlor after the insecticide applications. In the case of aerial blackfly control, which involves two treatments within a space of two or three weeks, it is possible that the residual levels reached in fish food organisms could rise well above those obtained in the laboratory exposure done in this study. It is not improbable, therefore, that the levels of methoxychlor contamination in fish food organisms could reach those found by Hatfield (1969) after similar treatment with DDT. However, immediate fish mortality is a very crude and unacceptable measure of environmental toxicity, and if it is occurring at all, sub-lethal effects are almost certainly present.

While no study on the possible effects of current methoxychlor spraying operations on fish populations is available, DDT applied at 1/2 lb/acre was largely responsible for the catastrophic reduction of the Atlantic Salmon fishery in New Brunswick; at 1/4 lb/acre, significant effects were still being felt and were attributed to a severe depletion of food supply in the streams (Ide, 1956; Elson and Kerswill, 1964, 1967; Keenleyside, 1967). In New Brunswick, because of the importance of the fishery to the economy, extensive work was being done on fish populations at the time that these effects became apparent. Many rivers and lakes of the Quebec North Shore and Labrador harbor the Atlantic salmon and/or ouananiche, but little is known about the stocks they support; consequently, alterations in their abundance following insecticide applications may well go unnoticed. The results of the present laboratory study suggest that rather than experiencing a sudden dramatic mortality, salmonid populations exposed to low levels of methoxychlor in the diet would undergo a slow, insidious, and intermittent decimation, as individual fishes reach a threshold at which they can no longer physiologically meet their natural environmental needs, and die.

In spite of its limited scope, this study has shown that all is not well with brook trout exposed to low dietary levels of methoxychlor which was found to accumulate

readily in the body tissue of these fish. Such accumulation has, under otherwise favorable laboratory conditions brought about a slight depression in growth, anemia, as well as extensive damage to two vital organs; the liver and kidney. In this unhealthy condition, one can anticipate a lower resistance to disease and parasitism (Andrews et al., 1966) and probable osmoregulatory failure at the time of seaward migration. The maintenance of optimum swimming performance is of vital importance to a salmonid living in a lotic environment. Maintenance of position, pursuit and capture of prey, migration and spawning are some functions of extreme importance that could be seriously affected by the lowering of swimming ability caused by a toxicant. The changes in swimming performance and in behavior that were observed in this experiment, while perhaps not of extreme ecological importance in themselves, may rather suggest a metabolic upset which could have serious consequences under the more rigorous conditions in nature.

There is evidence that the current application of methoxychlor for blackfly and mosquito control can have severe deleterious effects on aquatic ecosystems, leading to an eventual destruction of the fish stocks and perhaps of other forms of life in the treated areas. It appears

that methoxychlor is unsafe for these purposes, and unless other safe and economically feasable control measures are found, perhaps the nuisance of the mosquito and the black-fly may be the price that man must pay for living and working in this area if a balance between environmental quality and economic goals is to be achieved.

SUMMARY

Two experiments were carried out in which underyearling brook trout Salvelinus fontinalis were exposed to various dietary levels of methoxychlor. The insecticide was incorporated into an artificial diet and the dietary levels ranging from 0.5 to 100 ppm were chosen on the basis of methoxychlor residues found in aquatic insects which had been exposed to 0.075 mg/l of the insecticide, the standard dosage presently in use on the Quebec North Shore for blackfly larviciding.

The experimental apparatus consisted of six annular chambers in which the fish swam continuously against a current of 0.4 fps produced by a motor driven paddle wheel system. The fish were fed daily; at ten day intervals, they were weighed, measured, and the swimming ability of selected individuals was tested in a swimming chamber at velocities ranging from 1.28 to 1.78 fps.

In the first experiment, performed at 10°C, the fish were given food designed to provide dietary levels of 0.01, 0.02, 0.04, 0.08, and 0.16 mg of methoxychlor/kg of fish/day for 40 days. Methoxychlor had no apparent effect on length and weight gain, but produced a marked reduction in swimming stamina; no mortality could be ascribed to the insecticide treatment.

In the second experiment, performed at 7.5°C, the fish were exposed to dietary levels of 0.125, 0.250, 0.500, 1.000, and 2.000 mg/kg/day of methoxychlor for 43 days. During this experiment, several fish died showing obvious symptoms of insecticide poisoning. In addition, a definite disturbance of the spatial distribution of the fish in the annular chambers was observed in the group treated with 2.000 mg/kg/day of methoxychlor.

After 33 days of feeding, lower length and weight gains occurred only in the fish that had been exposed to 2.000 mg/kg/day of methoxychlor and which had been repeatedly tested for swimming ability. The swimming performance was also markedly affected by the insecticide; at low levels, lower performance was observed, whereas at 1.000 and 2.000 mg/kg/day, the fish swam 2 and 4 times longer than the controls.

Residual methoxychlor was measured in fish that died during the experiment as well as in those that survived the test period. Whole body residue analysis of fish made after 33 days of feeding, showed that 45 to 78% of the methoxychlor fed had been stored in their tissues, while analysis of pyloric caeca showed that methoxychlor was stored at very high concentrations in the fat.

Histopathological observations also revealed serious deleterious effects of methoxychlor. The red blood cell count and hematocrit were reduced, and histological examination of the liver and kidney of dead and/or dying fish revealed extensive tissue damage.

The overall results of this study suggest that continued blackfly larviciding with methoxychlor may have disastrous ecological effects on the fish populations in the treated areas.

BIBLIOGRAPHY

1. Allison, D.T., B.J. Kallman, O.B. Cope, and C. Van. Valin. 1964. Some chronic effects of DDT on cutthroat trout. U.S. Fish. Wildl. Serv., Res. Rept. No. 64. 30 p.

2. Andrews, A.K., C.C. Van Valin, and B.E. Stebbings. 1966. Some effects of heptachlor on bluegills Lepomis macrochirus. Trans. Amer. Fish. Soc. 95: 297-309.

3. Beach, F.A., 1948. Hormones and Behavior. Paul B. Hoeber Inc., New York. 368 p.

4. Beamish, F.W.H. 1968. Glycogen and lactic acid concentrations in Atlantic cod (Gadus morhua) in relation to exercise. J. Fish. Res. Bd. Canada. 25: 837-851.

5. Bilinski, E. 1969. Lipid catabolism in fish muscle. In: Fish in Research, edited by O.W. Neuhaus and J.E. Halver. Academic Press, New York. 135-152.

6. Black, E.C., A.R. Connor, K-C. Lam, and W-G. Chiu. 1962. Changes in glycogen, pyruvate, and lactate in rainbow trout (Salmo gairdneri) during and following muscular activity. J. Fish. Res. Bd. Canada. 19: 409-436.

7. Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Canada. 21: 1183-1226.

8. Bridges, W.R. 1961. Disappearance of endrin from fish and other materials of a pond environment. Trans. Amer. Fish. Soc. 40: 332-334.

9. Brown, N.J. and A.W.A. Brown. 1970. Biological fate of DDT in a sub-arctic environment. J. Wildl. Mgmt. 34: 929-940.

10. Buhler, D.R., M.E. Rasmusson, and W.E. Shanks. 1969. Chronic oral DDT toxicity in juvenile coho and chinook salmon. Toxicol. Appl. Pharmacol. 14: 535-555.

11. Buhler, D.R., and W.E. Shanks. 1970. Influence of body weight on chronic oral DDT toxicity in coho salmon. J. Fish. Res. Bd. Canada 27: 347-358.

12. Burdick, G.E., H.J. Dean, E.J. Harris, J. Skea, C. Frisa and C. Sweeney. 1968. Methoxychlor as a blackfly larvicide, persistence of its residues in fish and its effect on stream arthropods. N.Y. Fish and Game J. 15: 120-142.

13. Cairns, J., and A. Scheier. 1964. The effect upon the pumpkinseed sunfish, Lepomis gibbosus (Linn.) of chronic exposure to lethal and sublethal concentrations of dieldrin. Notul. Nat. No. 370. 1-10.

14. Chadwick, G.G., and R.W. Brocksen. 1969. Accumulation of dieldrin by fish and selected fish-food organisms. J. Wildl. Mgmt. 33: 693-700.

15. Colvin, H.J. and A.T. Phillips. 1968. Inhibition of electron transport enzymes and cholinesterases by endrin. Bull. Environ. Contam. and Toxicol. 3: 106-115.

16. Eisler, R. 1967. Tissue changes in puffers exposed to methoxychlor and methyl parathion. U.S. Bureau of Sport Fish. and Wildl., Tech. Paper No. 17. 15 p.

17. Eisler, R., and M.P. Weinstein. 1967. Changes in metal composition of the quahaug clam, Mercenaria mercenaria, after exposure to insecticides. Chesapeake Sci. 8: 253-258.

18. Eller, L.L. 1971. Histopathologic lesions in cutthroat trout Salmo clarki exposed chronically to the insecticide endrin. Amer. J. Path. 64: 321-336.

19. Elson, P.F., and C.J. Kerswill. 1964. Forest spraying and salmon angling. Atlantic Salmon J. No. 3: 20-29.

20. Elson, P.F., and C.J. Kerswill. 1967. Developing criteria for pesticide residues important to fisheries. Canadian Fisheries Report No. 9: 41-45.

21. Fawcett, D.W. 1966. The Cell. W.B. Saunders Company, Philadelphia, 448 p.

22. Graham, R.J., and D.O. Scott. 1958. Effects of forest insect spraying on trout and aquatic insects in some Montana streams. Final Report. Mont. Fish and Game Dept. 32 p.

23. Grant, B.F., and P.M. Mehrle. 1970. Chronic endrin poisoning in goldfish Carassius auratus. J. Fish. Res. Bd. Canada 27: 2225-2232.

24. Grant, B.F. and P. Mehrle. 1969. Serum analysis of cutthroat trout exposed chronically to methoxychlor. Reprint from: Progress in Sport Fishery Research,1968 United States Bureau of Sport Fisheries and Wildlife, Resource Publication 77: 99-100.

25. Grzenda, A.R., D.F. Paris, and W.J. Taylor. 1970. The uptake, metabolism, and elimination of chlorinated residues by goldfish Carassius auratus fed a ^{14}C-DDT contaminated diet. Trans. Am. Fish. Soc. 99: 385-396.

26. Hatfield, C.T. 1969. Effects of DDT larviciding on aquatic fauna of Bobby's Brook, Labrador. Can. Fish. Cult. No. 40: 61-72.

27. Henderson, C., Q.H. Pickering, and C.M. Tarzwell. 1959. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Trans. Amer. Fish Soc. 88: 23-32.

28. Hesser, E.F. 1960. Methods for routine fish hematology. Prog. Fish-Cult. 22: 164-171.

29. Hickey, J.J., J.A. Keith, and F.B. Coon. 1966. An exploration of pesticides in a Lake Michigan ecosystem. J. of Applied Ecol. 3(Suppl.): 141-154.

30. Hoar, W.S. 1957. Endocrine Organs. In: The Physiology of Fishes, Vol. 1: Metabolism, edited by M.E. Brown. Academic Press, New York. 245-285.

31. Hoar, W.S., M.H.A. Keenleyside, and R.G. Goodall. 1955. The effects of thyroxine and gonadal steroids on the activity of salmon and goldfish. Can. J. Zool. 33: 428-439.

32. Hoar, W.S., D. MacKinnon, and A. Redlich. 1952. Effects of some hormones on the behavior of salmon fry. Can. J. Zool. 30: 237-286.

33. Holden, A.V. 1965. Contamination of fresh water by persistent insecticides and their effects on fish. Ann. Appl. Biol. 55: 332-335.

34. Ide, F.P. 1956. Effect of forest spraying with DDT on aquatic insects of salmon streams. Trans. Am. Fish. Soc. 86: 208-219.

35. Janicki, R.H. and W.B. Kinter. 1971. DDT: Disrupted osmoregulatory events in the intestine of the eel Anguilla rostrata adapted to seawater. Science 173: 1146-1148.

36. Johnson, D.W. 1968. Pesticides and fishes- a review of selected literature. Trans. Am. Fish. Soc. 97: 398-424.

37. Johnson, B.T., C.R. Saunders, H.O. Sanders, and R.S. Campbell. 1971. Biological magnification and degradation of DDT and aldrin by freshwater invertebrates. J. Fish. Res. Bd. Canada. 28: 705-709.

38. Kallman, B.J., O.B. Cope, and R.J. Navarre. 1962. Distribution and detoxification of toxaphene in Clayton Lake, New Mexico. Trans. Amer. Fish. Soc. 91: 14-22.

39. Kapoor, I.P., R.L. Metcalf, R.F. Nystrom, and G.K. Sangha. 1970. Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem. J. Agr. Food Chem. 18: 1145-1152.

40. Keenleyside, M.H.A. 1967. Effects of forest spraying with DDT in New Brunswick on food of young Atlantic Salmon. J. Fish. Res. Bd. Canada 24: 807-822.

41. Keith, J.O. 1964. Relation of pesticides to waterfowl mortality at Tule Lake refuge. U.S. Fish Wildl. Serv., Denver Wildlife Res. Center, Ann. Prog. Rept. 14 p.

42. Kennedy, H.D., L.L. Eller, and D.F. Walsh. 1970. Chronic effects of methoxychlor on bluegills and aquatic invertebrates. U.S. Bureau of Sport Fisheries and Wildlife, Technical Paper 53. 18 p.

43. King, S.F. 1962. Some effects of DDT on the guppy and the brown trout. U.S. Fish Wildl. Serv. Spec. Sci. Rept. (Fisheries) 339: 1-22.

44. Koch, R.B., L.K. Cutkomp, and F.M. Do. 1969. Chlorinated hydrocarbon insecticide inhibition of cockroach and honey bee ATP-ases. Life Sci. 8: 289-297.

45. Leduc, G. 1966. Some physiological and biochemical responses of fish to chronic poisoning by cyanide. Ph.D. thesis. Oregon State University. 146 p.

46. Lehman, A.J. 1952. Chemicals in foods: Part II, Sect. IV. Biochemistry. Quart. Bull. of Assn. Food & Drug Officials. 16: 85-91.

47. Lehman, A.J. 1953. Pesticides: Some chemical considerations and toxicological interpretations. Quart. Bull. of Assn. Food & Drug Officials. 17: 8-12.

48. Lehman, A.J. 1956. The minute residue problem. Quart. Bull. of Assn. Food & Drug Officials. 20: 95-99.

49. Macek, K.J. 1968. Growth and resistance to stress in brook trout fed sublethal levels of DDT. J. Fish. Res. Bd. Canada 25: 2443-2451.

50. Macek, K.J. and S. Korn. 1970. Significance of the food chain in DDT accumulation by fish. J. Fish. Res. Bd. Canada 27: 1496-1498.

51. MacLeod, J.C., and L.L. Smith, Jr. 1966. Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, Pimephales promelas. Trans. Amer. Fish. Soc. 95: 71-84.

52. Mathur, D.S. 1962. Studies on the histopathological changes induced by DDT in the liver, kidney and intestine of certain fish. Experientia 18: 506-509.

53. Matsumara, F., T.A. Bratkowski, and K.C. Patil. 1969. DDT: Inhibition of an ATP-ase in the rat brain. Bull. Environ. Contam. and Toxicol. 4: 262-270.

54. Mayer, Jr., F.L., J.C. Street, and J.M. Neuhold. 1970. Organochlorine insecticide interactions affecting residue storage in rainbow trout. Bull. Environ. Contam. and Toxicol. 5: 300-310.

55. McCully, K.A., and W.P. McKinley. 1964. Determination of chlorinated pesticide residues in fat by electron capture gas chromatography. J. Assoc. Off. Agr. Chem. 47: 652-659.

56. Meeks, R.L. 1968. The accumulation of ^{36}Cl ring-labeled DDT in a freshwater marsh. J. Wildl. Mgmt. 32: 376-398.

57. Mehrle, P.M., D.L. Stalling, and R.A. Bloomfield. 1971. Serum amino acids in rainbow trout Salmo gairdneri as affected by DDT and dieldrin. Comp. Biochem. Physiol. 38B: 373-377.

58. Metcalf, R.L. 1966. Metabolism and fate of pesticides in plants and animals. In: Scientific Aspects of Pest Control; A Symposium arranged and conducted by The National Academy of Sciences National Research Council at Washington, D.C. 230-250. Publication 1402. N.A.S. - N.R.C.

59. Mighell, J.L. 1969. Rapid cold-branding of salmon and trout with liquid nitrogen. J. Fish. Res. Bd. Canada 10: 2765-2769.

60. Mount, D.I. 1962. Chronic effects of endrin on bluntnose minnows and guppies. U.S. Fish Wildl. Serv. Res. Rept. 58: 38 p.

61. Neil, J.H. 1957. Some effects of potassium cyanide on speckled trout Salvelinus fontinalis. In: Papers presented at the Fourth Ontario Industrial Waste Conference, Honey Harbor, Ontario. Water and Pollution Advisory Committee of the Ontario Water Resources Commission. 74-96.

62. Porter, K.R., and C. Bruni. 1959. An electron microscope study of the early effects of 3'-Me-DAB on rat liver cells. Cancer Res. 19: 997-1009.

63. Sanders, H.O. 1969. Toxicity of pesticides to the crustacean Gammarus lacustris. U.S. Bureau of Sport Fisheries and Wildlife, Technical Paper 25. 18 p.

64. Sanders, H.O., and O.B. Cope. 1968. The relative toxicities of several pesticides to naiads of three species of stoneflies. Limnol. Oceanogr. 13: 112-117.

65. Sanders, H.O., and O.B. Cope. 1966. Toxicities of several pesticides to two species of cladocerans. Trans. Amer. Fish. Soc. 95: 165-169.

66. Schoenthal, N.D. 1963. Some effects of DDT on cold water fish and fish-food organisms. Proceedings of the Montana Academy of Sciences 23: 63-95.

67. Simon, R.C., A.M. Dollar, and E.A. Smuckler. 1967. Descriptive classification on normal and altered histology of trout livers. In: Trout Hepatoma Research Conference Papers; U.S. Fish Wildl. Serv., Res. Rept. No. 70: 18-27.

68. Smit, H., J.M. Amelink-Koutstaal, J. Vijverberg and J.C. Von Vaupel-Klein. 1971. Oxygen consumption and efficiency of swimming goldfish. Comp. Biochem. Physiol. 39A: 1-28.

69. Smith, L.S., and T.W. Newcomb. 1970. A modified version of the Blazka respirometer and exercise chamber for large fish. J. Fish. Res. Bd. Canada 27: 1321-1324.

70. Sprague, J.B. 1971. Measurement of pollutant toxicity to fish-III. Sublethal effects and "safe" concentrations. Water Research 5: 245-266.

71. Stanley, L.L., and G.L. Tescher. 1931. see Hoar et al., 1955.

APPENDIX A

Extraction of methoxychlor from animal tissue.

For insect samples 0.5 g to 2.0 g the following method should work well. Grind the sample with four times its weight of anhydrous sodium sulfate with a mortar and pestle. It is not necessary to grind to a fine powder as long as the sample is well broken. Let sample stand for about 1/2 hour with occasional mixing so that the sodium sulfate will take up the moisture in the sample. Pack the sample mixture in a small chromatographic column with an I.D. of about 1 cm. Extract the methoxychlor with about 100 ml of 5% diethyl ether in petroleum ether. Control the flow rate to no more than 3 ml/min. The 100 ml extraction volume should be more than enough to quantitatively remove any methoxychlor present but you might have to adjust the volume to your needs. The flow rate is important. If it is too fast poor recovery will result. After the extracting solvent has passed through the column, evaporate it to a small volume (2-3 ml). Rinse the sample onto a 1 cm I.D. column with petroleum ether (small volume) containing 1 gm of anhydrous sodium sulfate, two grams of 5% deactivated Florisil and two grams of anhydrous sodium sulfate in that order. Elute methoxychlor with 75-100 ml of 5% diethyl ether in petroleum ether. Most of the interfering material in insects will be adsorbed on the

Florisil. The Florisil is prepared by heating it to 1200°F for two hours. After cooling it is deactivated with 5% water v/w. Mix the Florisil well and store in a sealed container and let equilibrate for a day before using.

For extraction of fish tissue take 20 g of fish and blend in small blender with 4 times weight of sample of anhydrous sodium sulfate. Mix salt and tissue together. Let stand for at least 30 minutes with occasional stirring. Pour into chromatographic column with 19-22 mm I.D. Pack with glass rod. Rinse sample container with cyclohexane into column. Let solvent run into column. Extract pesticide with 200 ml of cyclohexane. Note: flow rate will depend on how tightly column is packed and will control extraction efficiency. Most pesticides will come out in the first 100 ml including methoxychlor but the extra 100 ml is for a safety margin. Evaporate solvent to small volume. Partition sample between 30 ml of hexane saturated with acetonitrile and 30 ml of acetonitrile saturated with hexane. Withdraw the acetonitrile bottom layer and extract the hexane layer again with 30 ml of acetonitrile saturated with hexane. Combine the acetonitrile fractions and evaporate to a small volume. Use 5 ml of toluene to evaporate sample into a chromatographic column 19-22

I.D. containing 2 g of anhydrous sodium sulfate, 10 g of 5% deactivated Florisil, and 10 g of anhydrous sodium sulfate in that order. Rinse sample and container into the Florisil with minimal amount of petroleum ether. Elute pesticide with 200 ml of 5% diethyl ether in petroleum ether. Evaporate to desired volume suitable for the gas chromatograph.

Johnson, J.L. Personal Communication, 1970. U.S. Fish and Wildlife Service, Fish-Pesticide Research Laboratory, Columbia,Missouri.

APPENDIX B

List of the authors of personal communications.

Grenier, F. Chemist, Quebec Wildlife Service, Quebec City, Quebec.

Johnson, J.L. Fishery Biologist, U.S. Fish and Wildlife Service, Fish-Pesticide Research Laboratory, Columbia, Missouri.

Swedberg, D., Fishery Biologist, U.S. Fish and Wildlife Service, Fish-Pesticide Research Laboratory, Jackson, Wyoming.

Taylor, V.R., Department of the Environment, Fisheries Service, St. John's, Newfoundland.

West, A.S. , Professor of Zoology, Queen's University, Kingston, Ontario.

APPENDIX C

Wet weights (g) and distances (ft) covered by individual brook trout in swimming stamina tests performed during Expt. 1.

	CONTROL		0.01 mg/kg/day		0.02 mg/kg/day	
10 days	21.9	486 1/	15.9	1218 1/	24.9	804
	16.9	719	17.4	773	21.3	371 1/
	21.6	410	18.6	140 1/	21.5	365
	23.1	309 1/	20.8	1782 1/	19.8	454
	23.9	573	21.4	100	26.0	686 1/
20 days	25.3	736	22.0	832	22.3	357
	19.3	550	16.9	554	19.5	615
	24.2	223	19.2	1363	18.0	348
	26.5	1796	12.6	208	24.3	166
	23.1	169	22.6	585	21.6	619
	20.3	416	25.2	534	21.5	197
	20.5	373	22.6	550	24.9	508
	21.4	416	17.9	219	22.7	562
	25.8	388	21.4	142	17.4	701
	20.0	234	15.5	454	19.1	199
30 days	25.5	380	23.5	547	23.5	927
	18.7	407	26.9	665	18.3	385
	21.9	662	17.1	177	23.9	166
	18.6	431	16.4	604	22.6	316
	20.9	473	21.0	296	18.8	205
	20.7	578	18.2	153	17.0	180
	23.5	539	27.6	547	22.3	704
	18.8	916	22.1	402	18.4	370
	16.9	416	23.6	270	27.1	214
	23.2	442	20.2	687	21.7	242

1/ These fish were tested at 1.4 fps; all others at 1.54 fps.

0.04 mg/kg/day		0.08 mg/kg/day		0.16 mg/kg/day	
18.8	686 1/	25.8	281 1/	23.7	790 1/
21.1	1232	19.0	707	22.1	251
26.4	2078 1/	22.2	473	22.5	743 1/
29.4	302 1/	22.7	4625	18.6	615
17.2	547	22.1	888 1/	-	- 2/
23.0	382	19.5	354	20.7	408
24.6	320	15.4	296	23.7	303
20.8	380	17.7	390	22.7	117
18.6	354	22.0	477	22.5	832
27.2	548	21.1	357	21.1	558
22.4	388	22.1	1170	20.2	316
22.7	408	28.2	1440	24.6	739
27.9	293	18.5	397	15.8	385
21.2	701	24.3	4772	18.0	300
22.6	347	20.4	856	17.9	334
17.3	148	26.1	647	18.3	327
21.0	1120	18.1	283	23.3	496
23.0	770	19.9	359	25.1	809
22.6	653	20.3	373	29.7	408
23.6	477	20.6	360	20.9	191
17.9	373	23.7	253	24.8	955
27.3	594	26.1	154	21.3	202
21.8	345	26.6	169	27.8	348
13.7	74	18.1	137	23.4	770
17.5	325	23.6	454	21.7	325

1/ These fish were tested at 1.4 fps.

2/ This fish swam through the retaining screen.

Changes in wet weights (g) and endurance (sec) of individual brook trout in swimming stamina tests performed during Expt.2. 1/

	0 days		3 days		13 days		23 days	
	16.0	460	17.2	257	18.3	278	19.4	192
	18.8	282	20.4	285	22.6	505	23.6	307
	21.5	344	22.3	217	20.5	187	-	-
	17.7	216	17.8	240	19.2	1890	20.0	3780
	18.4	240	19.5	225	20.4	395	21.5	305
0.00	23.0	327	23.2	374	24.6	3747	24.9	3750
	13.2	276	13.6	275	14.8	265	15.9	596
	15.5	120	14.9	108	16.2	300	17.4	299
	14.0	294	14.5	135	15.0	250	15.1	245
	17.3	268	17.7	230	13.8	297	20.7	514
	18.5	436	19.7	498	19.6	432	20.0	605
	23.1	468	25.3	628	26.7	1166	29.0	548
	17.3	235	18.9	268	19.6	288	20.8	400
	18.4	343	19.3	453	20.0	530	19.8	450
	20.1	352	21.0	315	22.2	440	23.6	526
	17.6	282	18.9	272	19.8	212	20.3	420
0.125	17.1	195	17.5	372	19.1	375	19.6	325
	17.8	307	19.1	465	21.1	410	21.8	402
	16.1	300	17.6	394	19.2	330	20.1	430
	17.3	480	18.5	267	19.3	277	20.0	217
	19.0	210	20.2	090	20.9	150	21.7	253
	15.7	298	16.7	168	16.6	317	17.9	379
	23.5	373	25.4	035	26.6	142	26.7	0
	15.4	291	15.6	275	16.2	357	16.9	215
	14.5	199	16.1	245	16.7	565	17.5	552
	20.8	545	22.7	410	23.8	1433	24.7	365
0.250	25.2	477	26.9	358	28.6	907	30.6	395
	15.5	447	16.9	750	18.7	1184	19.5	310
	18.7	440	19.9	992	21.4	922	22.6	495
	18.7	174	19.6	337	20.8	3630	21.7	3757

1/ After 3600 seconds, the water velocity was increased from 1.28 fps to 1.78 fps.

	20.8	339	21.4	354	22.1	496	22.7	363
	15.4	448	16.4	235	16.8	1520	17.5	525
	21.6	488	22.3	570	24.0	1533	25.5	561
	13.8	200	14.4	360	15.2	380	16.3	388
	16.1	345	16.4	343	17.0	326	17.7	1120
	14.2	202	14.9	355	15.7	390	16.9	459
0.500	15.2	230	15.2	243	16.2	364	17.5	260
	20.0	300	20.1	520	21.4	408	23.0	360
	14.7	270	15.2	310	16.6	344	17.8	374
	16.1	189	16.5	147	17.3	140	18.2	200
	17.2	251	18.2	425	18.9	732	20.2	270
	18.3	281	19.0	835	20.0	2302	21.6	3600
	21.4	1178	21.2	499	21.4	382	23.2	215
	18.3	902	19.4	600	20.2	3787	21.9	3770
	21.2	368	23.2	550	24.1	3780	24.5	3872
	22.3	255	23.9	1130	25.5	863	27.1	450
1.000	15.2	235	17.1	277	18.3	2177	20.1	878
	17.2	591	18.1	1110	19.0	2687	20.5	3750
	15.9	180	16.4	285	17.5	256	18.9	200
	20.7	354	20.8	442	21.5	520	23.4	990
	23.3	415	24.2	375	24.4	632	24.6	3754
	18.6	757	20.3	1090	21.2	3838	21.5	3768
	23.8	570	24.5	544	23.2	380	22.8	-
	23.9	360	24.6	360	25.7	590	26.5	3747
	17.8	426	18.9	625	20.3	2734	21.3	2085
	16.1	375	17.6	544	18.8	1483	21.2	-
2.000	14.0	280	14.8	455	15.9	584	16.8	470
	18.9	708	19.7	633	21.4	3810	22.3	3820
	13.9	265	15.0	540	15.8	524	16.0	2532
	17.1	210	18.4	170	19.5	464	18.2	205

APPENDIX E

The wet weights and swimming times of brook trout that were used for calculating the regression Y= 2.49 + 1.12x. 1/

wet wt. g.	time sec.	wet wt. g.	time sec.
12.9	270	17.5	267
18.4	343	16.1	345
18.5	436	15.2	230
17.3	235	16.1	189
17.8	307	15.9	180
23.8	580	15.2	235
23.9	360	17.2	251
17.8	426	15.4	340
15.0	350	21.2	368
14.0	280	16.4	140
14.2	202	22.3	255
21.6	488	14.7	155
20.0	300	25.1	067
15.4	448	15.1	384
17.4	373	13.3	388
13.8	200	15.0	065
15.4	291	13.9	184
18.7	174	23.3	415
25.2	477	18.9	708
14.6	347	15.2	326
15.5	317	18.6	757
17.2	591	16.1	375
14.3	060	13.9	265
20.7	354	17.1	210
21.4	1178	23.5	373
18.3	902	19.0	210
13.3	281	18.7	440
13.2	276	15.7	298
23.0	327	15.5	447
21.2	2100	14.5	199
17.7	216	18.1	090
15.5	120	20.8	545
17.1	375	17.3	268
17.8	331	18.8	062
20.1	352	20.3	489
17.3	480	18.8	282
23.1	468	18.4	240
17.6	282	21.5	344
16.1	300	16.0	460
20.8	339	14.0	294
14.8	270	16.3	065

1/ Y= ln time in seconds, x= ln wet weight in grams. Test temperature 7.5°C, oxygen 100-102% saturation, water velocity 1.28 fps.

APPENDIX E cont'd...

Method used for obtaining corrected swimming distance using the regression Y = 2.49 + 1.12x.

Actual time swum seconds	Wet weight grams
120	
216	
240	
268	
276	13.2
282	18.8
294	
327	
344	
460	

276 x 1.28 fps = 353 ft. 13.2 g.
282 x 1.28 fps = 361 ft. 18.8 g.

Median distance 357 ft. by a median fish of 16.0 g.

From the regression equation, a fish of 16.0 g wt.would cover 1.28 fps x 269.5 sec. = 345 ft.

For example; a fish of 20.0 g. wt. covered 477 ft.
- from the regression, for its size, this fish should have covered 442 ft.
- in order to correct this fish for size and make it comparable to the control
- subtract the theoretical control distance from the theoretical exposed fish distance i.e. 442- 345 = 97 ft.
- how subtract 97 ft from the observed 477 ft to obtain a corrected distance of 380 ft.
- therefore the corrected distance covered by this the median fish is 380 ft.

This example corresponds to Table 4, Expt.2., 2.000 mg/kg/day at time 0 days.