EFFECTS OF DIETARY METHOXYCHLOR AND REDUCED DISSOLVED OXYGEN CONCENTRATIONS ON GROWTH OF RAINBOW TROUT

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ABSTRACT

EFFECTS OF DIETARY METHOXYCHLOR AND REDUCED DISSOLVED OXYGEN CONCENTRATIONS ON GROWTH OF RAINBOW TROUT

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Two experiments were conducted; in the first experiment juvenile rainbow trout, Salmo gairdneri, were exposed to dietary methoxychlor (0.037 to 0.272 mg/kg fish/day) for 20 days at oxygen saturation and then exposed to reduced oxygen levels (40 to 95 % O_2 saturation at 11.5°C) for a following 20-day period. In the second experiment, the rainbow trout were simultaneously exposed to these levels of dietary methoxychlor and reduced oxygen over a 20-day period.

Methoxychlor had no effect on growth at 95 % O_2 saturation during 20 days exposure but growth of methoxychlor treated fish was reduced during the following 20 days. Exposure to reduced oxygen levels produced much lower growth among methoxychlor treated fish as compared with controls which also grew less than at oxygen saturation.

Residue determinations at the end of the exposure period revealed methoxychlor levels ranging from 0.02 to 0.450 µg/g, in whole fish, while the liver tissue of fish receiving 0.272 mg/kg fish/day contained between 28 to 40% of the total body burden. No residual methoxychlor was detected in treated fish 20 days after the end of methoxychlor exposure.

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INTRODUCTION

The purpose of this laboratory study is to examine the effects of dietary methoxychlor and reduced dissolved oxygen concentrations on the growth of rainbow trout, Salmo gairdneri, (Richardson).

The organochlorine insecticide methoxychlor; 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane, has been used as an alternative to DDT since 1969 primarily for the control of blackflies in streams and rivers of northern Canada (Gardner and Bailey 1975) where they are a nuisance to man; while in Africa Simulium is the vector of Onchocerca volvulus which can cause blindness. Methoxychlor has also been used in control programs for the European elm bark beetle, vector of fungal Dutch elm disease (Wallner et al. 1969) and horn fly infestations of cattle (Eschle and Miller 1968).

Wallace et al. (1973) reported that blackfly larvae were greatly reduced in numbers or eradicated in streams treated with 75 µg/L of methoxychlor (concentration used in commercial ground-level application) which also produced a heavy drift of non-target invertebrates such as Ephemeroptera, Plecoptera and Trichoptera. No fish mortality occurred from this treatment. Injections of 180 to 240 µg/L of methoxychlor into the Saskatchewan River (Fredeen 1974) removed 75 to 99% of Simulium arcticum larvae up to

34 km downstream and stronger concentrations up to 310 µg/L eliminated 98% of the larvae at 64 km downstream with reductions in Plecoptera, Ephemeroptera, Trichoptera and Chironomidae (least affected). Populations of the non-target larvae were restored within 7 to 14 days after treatment and caged rainbow trout showed no harmful effects. More recently, an injection of 600 µg/L of methoxychlor into the Saskatchewan River (Fredeen 1975) eliminated 100% of Simulium arcticum larvae (instars) up to 80 km downstream again with reductions in Plecoptera, Chironomidae, Ephemeroptera and Trichoptera while no difficulties were observed among the native fish population. Following methoxychlor treatment, the population densities of non-target larvae required 1 to 7 weeks and 2 to 10 weeks for Simulium to equal or surpass pre-treatment densities.

Methoxychlor residues in simuliid larvae caught after treatment with 0.79 µg/L of methoxychlor in the Chalk River, Ontario, ranged from 0.24 to 2.57 mg/kg (Wallace et al. 1976). Fredeen et al. (1975) reported that drifting blackfly larvae contained an average of 17.5 mg/kg methoxychlor after treatment consisting of a 15-minute injection of 309 µg/L and Ephemeroptera, Trichoptera and Plecoptera larval residues were found to be 10 mg/kg, 1 to 2 days after application of 300 µg/L of methoxychlor (Flannagan et al. 1975). Flannagan also found in this same study that methoxychlor treatment produced no

mortality or change in blood calcium of caged crayfish, Orconectes virilis, 183 m downstream, and body residues of methoxychlor had declined in 1 week after an initial increase. While mussels can concentrate methoxychlor to high levels (Lampsilis siliquoidea contained 0.07 to 0.22 mg/kg after six to ten weeks exposure to 60 - 100 µg/L of methoxychlor, Bedford et al. 1968), they are generally not affected to any extent by methoxychlor (Gardner and Bailey 1975).

Lockhart et al. (1977) found that caged rainbow trout in the Athabasca River, Alberta, treated with 300 µg/L of methoxychlor had much lower liver residues (0.96 µg/g six hours after start of treatment) than rainbow trout exposed to the same concentration in a laboratory study (13.7 µg/g after six hours). No mortality occurred in caged native fish (white sucker, longnose sucker, flathead chub, northern pike and walleye) similarly treated, but wild fish of the same species caught near the cage sites contained higher liver residues thereby reducing the reliability of cage bioassays for monitoring insecticide toxicity to fish. Methoxychlor residues in ovaries of flathead chub, Hybopsis gracilis, ranged from 0.03 to 9.33 µg/g but the reproduction response was not evaluated. Fredeen et al. (1975) reported that goldeye fish accumulated a maximum of 1.5 mg/kg in muscle tissue after treatment with 309 µg/L of methoxychlor for 15 minutes in the Saskatchewan

River and residues were no longer detectable in 17 weeks.

The acute toxicity of methoxychlor to freshwater fish has been studied by both static and continuous flow-through bioassays. Macek et al. (1969) in a static test with rainbow trout obtained 96-hour LC50 values of 30 µg/L at 1.6°C, 42 µg/L at 7.2°C and 62 µg/L at 12.7°C indicating that methoxychlor toxicity increased with a reduction in temperature. Merna and Eisele (1973) reported 96 h LC50 values of 8.63 µg/L for fathead minnows, Pimephales promelas, and 22.2 µg/L for yellow perch, Perca flavescens, in continuous flow bioassays at 20°C while the 96 h LC50 for white sucker, Catostomus commersoni, was 34.5 µg/L at 15°C (Waiwood and Johansen 1974). To date, no LD50 values for fish exposed to dietary methoxychlor have been reported.

In sublethal laboratory studies, methoxychlor has been shown to accumulate in fish tissue but to a lesser extent than DDT. Reinbold et al. (1971) showed that Tilapia exposed to 3.0 µg/L of methoxychlor and DDT in the water for a 12-day period and subsequently placed in clean water for 15 days retained 10,000 times as much DDT as methoxychlor. Tilapia also metabolized methoxychlor to a greater extent than DDT with 2-(p-methoxyphenyl)-2-(p-hydroxyphenyl)-1,1,1-trichloroethane and 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane being the major methoxychlor metabolites. Kapoor et al. (1970) constructed a model ecosystem in which methoxychlor and DDT were introduced.

Methoxychlor was concentrated 1500 times that of the water in mosquito fish, Gambusia affinis, the top of the food chain as compared with 90,000 times for DDT, and the hydroxy metabolites of methoxychlor were excreted while DDE and DDD were stored in fish tissue.

Kruzynski (1972) exposed brook trout, Salvelinus fontinalis, to dietary methoxychlor (max. intake level 2.0 mg/kg fish/day) for a 30-day period and reported no change in growth. The swimming stamina was tested with a reduction occurring at exposure levels in the range of 0.01 to 0.16 mg/kg/day while fish that received 1.00 and 2.00 mg/kg/day had an increased stamina. Residues in whole fish ranged from 2 to 44 mg/kg methoxychlor and the brook trout stored between 50 and 78 percent of the administered dose. Various pathological changes were observed in liver and kidney tissue of fish exposed to 1.00 and 2.00 mg/kg/day as well as a lowered red blood cell count in methoxychlor treated fish.

Oladimeji and Leduc (1975) showed that brook trout exposed to dietary methoxychlor at an intake level of 0.67 mg/kg fish/day had their growth rate significantly reduced at low ration levels such as 0.5 and 1.0% of wet body weight. The maintenance requirements of the fish increased with methoxychlor exposure. An increase in the food ration from 0.5 to 2.0% of wet body weight produced an increase in the fat content of the fish (from 3 to 9.5%) with methoxychlor storage being closely related to the fat

content. This relationship between organochlorine pesticide storage and fat content has also been reported by Roberts et al. (1977) who found that tissue retention of the pesticide chlordane in northern redhorse suckers, Moxostoma macrolepidotum, was directly proportional to the adiposity of the fish.

When methoxychlor is applied to streams containing blackfly larvae, fish can be subjected to the following modes of exposure: 1) direct exposure - methoxychlor uptake by the fish in the contaminated water is primarily through their gills 2) contaminated food source - once the larvae are killed through methoxychlor treatment, they detach from their substrate (vegetation or rock) and begin to drift downstream presenting fish which inhabit "methoxychlor-free" water a contaminated food supply.

Current literature agrees that the rate of breakdown of methoxychlor is rapid in natural waters. After treating a pond with 5 µg/L of methoxychlor, no traces were recorded 36 days later (Burdick et al. 1968). Merna et al. (1972) reported that methoxychlor was rapidly removed in water through adsorption to sediments and suspended solids. Therefore if fish are subjected to direct exposure, it will only be for a short period of time. In nature, blackfly larvae comprise an important part of a fish diet and since these larvae cannot metabolize methoxychlor to any great extent (Gardner and Bailey 1975), fish may be consuming

larvae containing high residue levels and accumulating methoxychlor in their tissues. "This possibility must be considered in all circumstances where the aqueous concentration of a pesticide appears to be too low to have a significant effect on fish directly" (Holden 1973).

The recent publication of insect residue data by Flannagan et al. (1975), Fredeen et al. (1975) and Wallace et al. (1976) reveals that the research conducted by Kruzynski (1972) and Oladimeji and Leduc (1975), in which the growth, swimming stamina and maintenance requirements of brook trout exposed to dietary methoxychlor were evaluated, utilized concentrations of dietary methoxychlor which were much higher than those encountered in nature.

Lockhart et al. (1977) suggest that methoxychlor may indirectly affect fish by reducing the available food supply. One must also consider the indirect effects of methoxychlor on fish subjected to an environmental stress. According to Iverson and Guthrie (1969), stress is defined as "an environmental entity which is not lethal in the toxicological sense of that term, but affects the range or scope of activity of an organism or population". The term "scope of activity" refers to Fry's concept (1947) in which environmental factors are classified according to the manner in which they influence the metabolism of the organism and hence its activity, growth being an example of activity. Modifications in the environment could present a stress to fish which may interact with sublethal

levels of methoxychlor to produce deleterious effects.

Reduced dissolved oxygen concentrations which freshwater fish are subjected to through organic pollution such as sewage waste in rivers and also in the hypolimnion of eutrophic lakes were selected as a possible environmental stress factor. Fish growth may not be impaired by oxygen levels below 100 % saturation when fish are kept on a low restricted diet; however impairment does occur when the dissolved oxygen is reduced from saturation levels with an unrestricted diet (Warren et al. 1973). The more food a fish consumes, the more oxygen it needs; because the assimilative processes require energy. Brungs (1971) reported that growth of fathead minnow fry was significantly reduced below 7.9 mg/L of dissolved oxygen. Growth studies by Herrmann et al. (1962) on juvenile coho salmon, Stewart et al. (1967) on juvenile largemouth bass and Adelman and Smith (1970) on northern pike have shown that the growth rate of fish decreased with a reduction in oxygen concentration and an extreme decrease occurred below oxygen levels of 3 mg/L . "As growth is an expression of the net product of metabolic functions, it is a good indicator of an overall effect of low oxygen" (Davis 1975).

Lloyd (1961) showed that oxygen levels below 60 % saturation combined with monohydric phenols, zinc, lead, copper and ammonium chloride produced increased toxicity in rainbow trout at 17.5°C . The additive effects are due to increased water volume passed over the gills as well as an

increase in the concentration of the toxicant at the surface of the gill epithelium.

Since previous research has shown that methoxychlor utilized in larviciding operations can indirectly affect fish in an adverse manner by either reducing the food supply or contaminating remaining food sources (Fredeen et al. 1975, Lockhart et al. 1977 and Wallace et al. 1973, 1976), this study at sublethal levels continues research into the indirect effects of methoxychlor on fish by evaluating the potential ecological stress produced through an interaction between dietary methoxychlor and reduced dissolved oxygen concentrations which will be reflected by the growth rate of rainbow trout. Determinations of residual methoxychlor in fish tissue will also give some information on bioaccumulation and clearance at various oxygen levels.

MATERIAL, APPARATUS AND METHODS

MATERIAL

The test organisms used in this study were juvenile rainbow trout, Salmo gairdneri (Richardson), which were obtained from Pisciculture Mont Sutton, Sutton, Québec. They were transported in large plastic bags to the Water Pollution Research Laboratory of Concordia University (Sir George Williams Campus) in Montreal. These bags were pressurized with oxygen and also contained ice. The fish were pre-sorted at the hatchery so that most of them were of uniform size; the initial average wet weight of the fish used in Expt. No. 1 was 25.14 g while the initial average wet weight of the fish in Expt. No. 2 was 29.07 g .

In the laboratory, the fish were held in large fiberglass holding tanks (200 L) equipped with a flow-through system. The temperature of the water was maintained at 12.0 C $\pm$ 1°C , and the oxygen saturation of the inflowing water was 100%. The fish were fed daily with Ewos trout chow (No. 3) ad libitum. While being kept in these holding tanks, the fish seemed to be healthy and less than 1% mortality occurred.

APPARATUS

The City of Montreal supplies the water for the laboratory which is dechlorinated through charcoal filters. This water was then monitored for residual chlorine levels which were found to be always less than 0.01 mg/L (Orthotolidine Method - Standard Methods 1971). The water

chemistry during both experiments (data provided by Service des Travaux Publics, Div. des Eaux et de l'Assainissement, Ville de Montreal) is as follows: 1) pH 7.9 2) alkalinity 86 mg/L $CaCO_3$ 3) total hardness 127 mg/L $CaCO_3$ 4) carbon dioxide 0.5 mg/L CO_2 . The dechlorinated water was thermally controlled at 11.0°C and delivered to the test apparatus via plastic (PVC) piping.

The test tanks used in this study were cylindrical polyethylene tanks (Rosedale Plastics, Model 325-1) with a 82 L capacity. A flow of 1.5 L/min. was controlled by individual plastic stopcocks. The water temperature in Experiment 1 was maintained at 11.5°C ± 0.5°C , while in Experiment 2 the temperature was kept at 12.0°C ± 0.5°C . The test area was subjected to a 12-hour photoperiod (08:00 to 20:00 h) which was controlled by a time switch.

The test apparatus consisting of 2 support benches each with 10 test tanks is illustrated in Figure 1, and the water supply system designed to control the concentration of dissolved oxygen to the apparatus in Figures 2 and 3. Water from the laboratory entered the head tank (90 L capacity) suspended from the ceiling, and then flowed directly into the top of 4 PVC columns (1.524 m x 10.16 cm ID), packed with Rashig rings. Compressed nitrogen was diffused through the column via air stones located near the base, thus producing a "nitrogen stripping" column to remove dissolved oxygen from the water (Fry 1951). A 0.635 cm plexiglass grille was positioned just above the water outlet pipes in order to keep the Rashig rings from obstructing them. The various

Figure 1. Photograph of test tank assembly in which rainbow trout were held while being exposed to various dietary levels of methoxychlor in conjunction with different concentrations of oxygen.

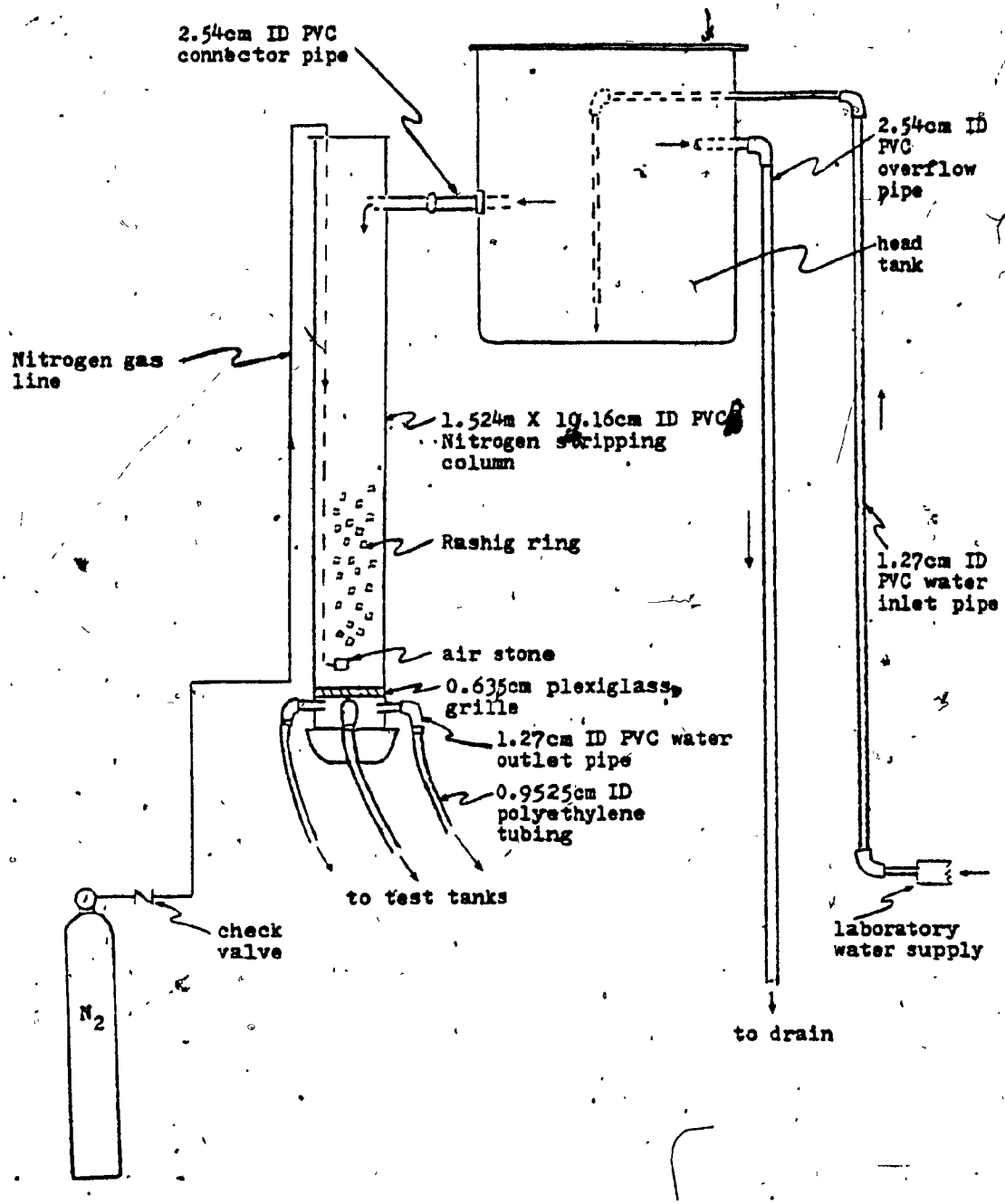

Figure 2. Diagram of water supply system designed to supply water of varied oxygen concentrations. Only one "nitrogen stripping" column is illustrated. (Arrows indicate the direction of water flow)

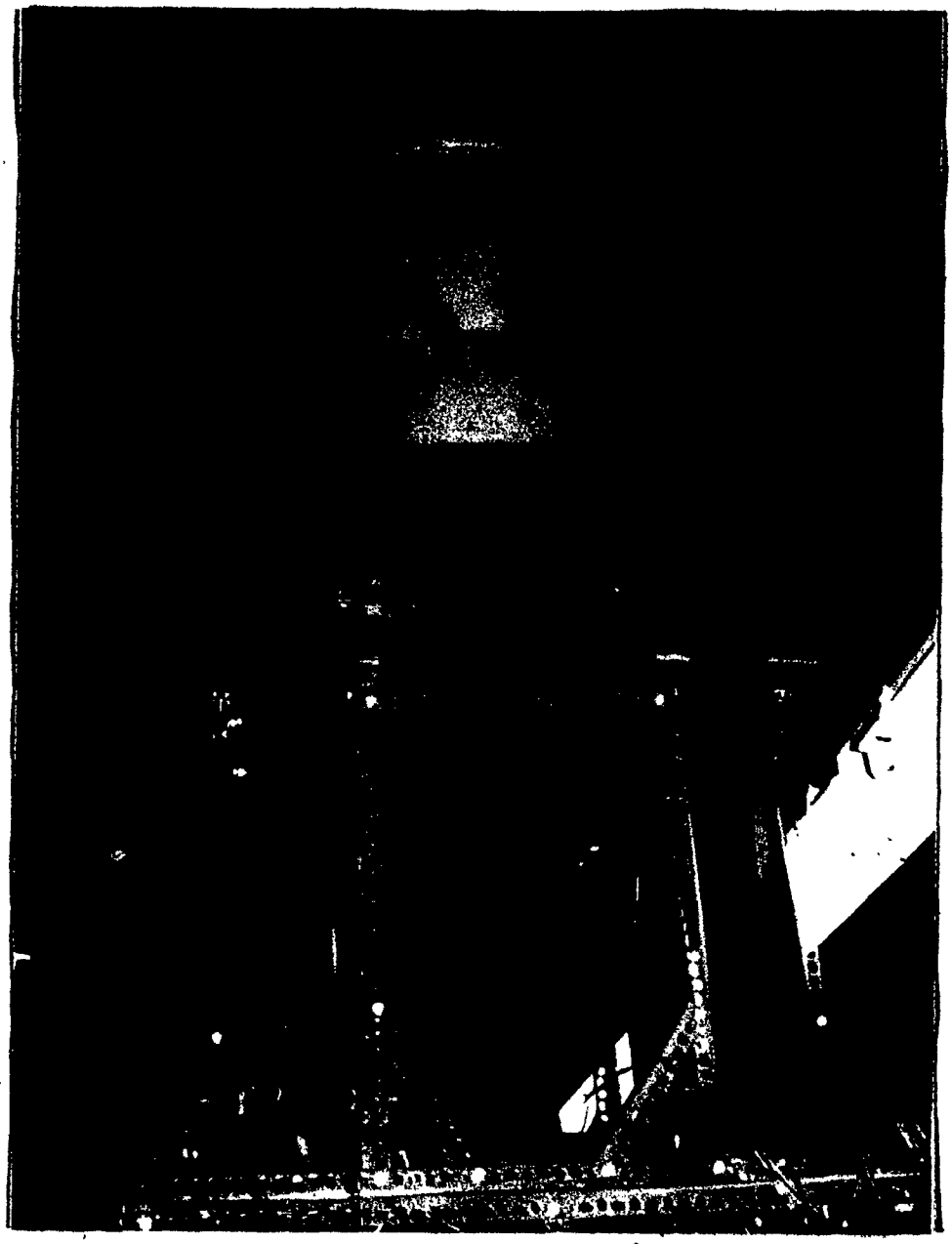

Figure 3 . Photograph of the water supply system showing the head tank and PVC "nitrogen stripping" columns.

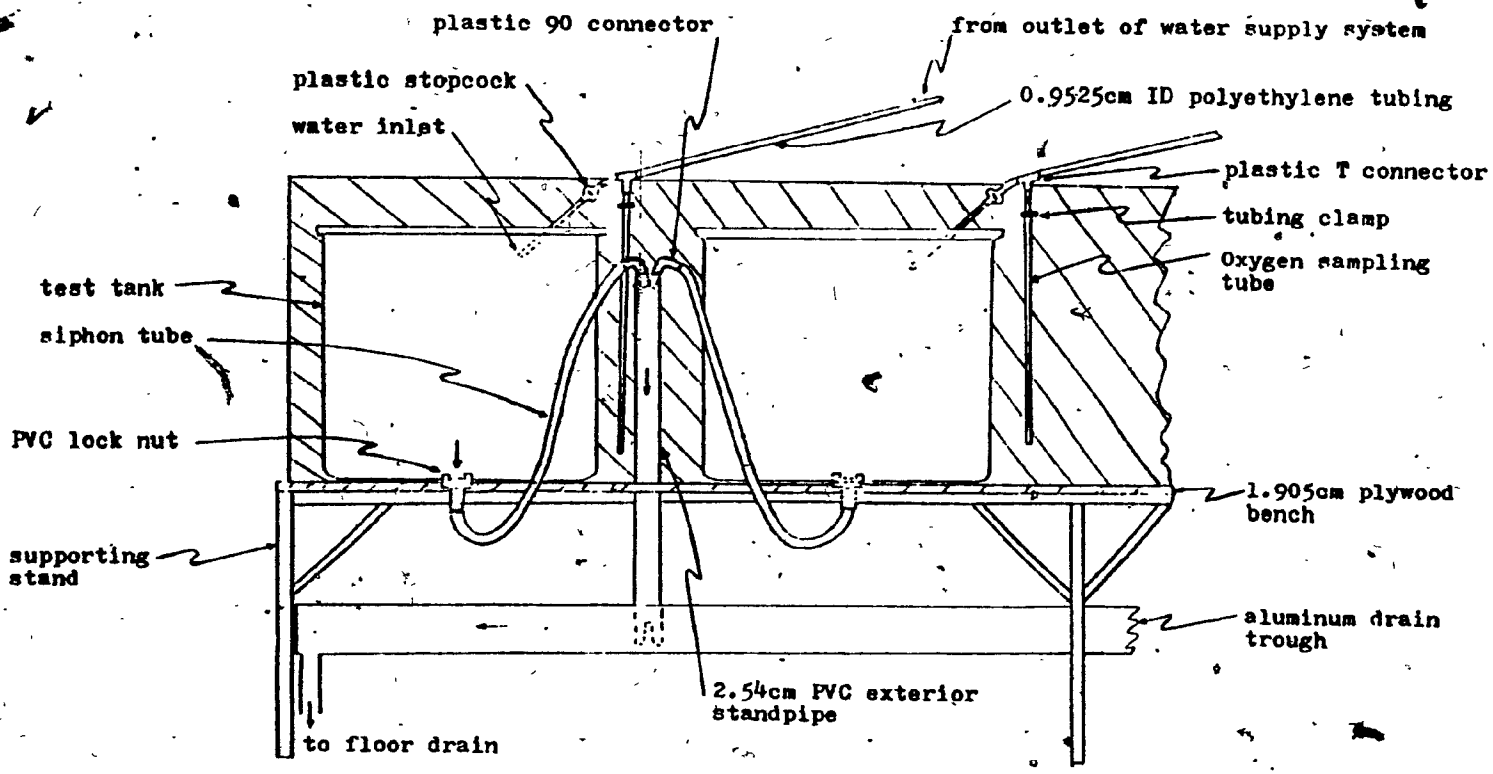

Figure 4. Diagram of test tank assembly illustrating the siphon drainage system. (Arrows indicate the direction of water flow)

Figure 5 . Photograph showing a close-up view of the siphon drainage system used to maintain water level and continuous cleaning of test tanks.

oxygen concentrations used in the experiments were controlled by maintaining pre-determined flow rates of nitrogen. The oxygen-depleted water was delivered through five outlets and polyethylene tubing (0.9525 cm ID) entering the test tanks below the water surface. A T-connector was inserted into the water delivery tubing so that water could be drawn off to measure its dissolved oxygen concentration without removing the water inlet tube from the tank.

The test tanks had no standpipe (Figure 4, 5) rather a siphon drainage system was used. A length of latex tubing connected the bottom of each tank with an exterior standpipe thus producing a slight current insuring continuous cleaning. The drain hose may be lowered to further clean out tanks or to change the water with minimum disturbance to the fish. Another advantage of this system is the flexibility with which different water levels may be chosen in the tanks. This task is easily accomplished by adjusting the height of the exterior standpipe to the desired level. During the experiments, the sides of each tank were covered with a black plastic covering, again to minimize outside disturbance.

METHODS

Preparation of the diet

The diet used in these experiments was prepared according to Kruzynski (1972). This diet consists of a mixture of two parts of beef liver, one part of beef heart and

one part of Ewos trout chow (No. 3). The beef liver and heart were ground in a meat grinder, mixed with the trout chow, then oven dried and finally ground to powder in a Waring blender. The methoxychlor emulsion was prepared by dissolving the required amount in 1 ml of xylene and adding 3 drops of emulsifying agent (Atlas g-3404 F, ICI, United States Inc., Wilmington, Del.) and making it up to 250 ml with distilled water. Methoxychlor was added to the diet with the other constituents in the proportions shown in Table 1 .

Table 1 . Ingredients of the artificial diet containing methoxychlor.

	Weight (g)
distilled water (warm)	250
gelatin (binding agent)	50
dry powder chow	200
methoxychlor emulsion	250

This mixture was poured into a shallow tray, placed in a refrigerator to set and then passed through a meat grinder, the small pieces being stored frozen until used. The control diet was prepared in an identical manner to the methoxychlor test diet and the same amounts of xylene and emulsifying agent were added.

Technical grade methoxychlor was used containing 88 percent 1,1,1-trichloro-2,2-bis(p-methoxyphenyl)ethane and 12

percent other isomers and reaction products. It was obtained from the E.I. du Pont de Nemours & Co., Wilmington, Del. The methoxychlor concentrations in the diet, shown in Table 2, were determined by gas chromatography from the final diet preparations to be approximately 85 to 95 percent of the theoretical values which were 2, 4, 8 and 16 ppm methoxychlor. The methoxychlor concentrations were chosen according to residue levels measured in drifting insects following larviciding operations from field data reported by Fredeen et al. (1975), Flannagan et al. (1975) and Wallace et al. (1976).

Table 2. Concentrations of methoxychlor in test diet and corresponding intake levels in test fish.

Methoxychlor content of diet (ppm)	Methoxychlor intake level (mg/kg fish/day)
1.84	0.037
3.80	0.076
7.20	0.144
13.60	0.272

The diet was analyzed for protein, fat and ash content (Table 3). The protein was determined by the Micro Kjeldahl method as described by Bailey (1967); the fat content was determined by ether extraction with a Labconco Goldfisch Fat Extractor (Model 35003), using about 2 g of dry material

subjected to a four hour reflux distillation. The ash content was determined by incineration of 1.5 g of food at 1000°C.

Table 3. Analysis (based on dry weight) of the experimental diet used to study the effects of dietary methoxychlor and reduced oxygen levels on the growth of rainbow trout.

Dry matter	percent	34.92
Protein	"	58.75
Fat	"	12.56
Ash	"	4.93

Experimental design

Two experiments were conducted. In both these experiments, the fish were randomly chosen and distributed in each of the 20 test tanks where they underwent a two-week acclimation period. At the beginning of each experiment, the fish were anesthetized in MS 222 (tricaine methane sulphonate), Sandoz Ltd., Switzerland, and individually branded using the liquid nitrogen technique of Mighell (1969). Each fish was then blotted dry and weighed to the nearest hundredth of a gram. During the experiments, the fish were weighed at ten-day intervals and the food ration (2% of wet body weight/day for all test groups) readjusted accordingly. In all cases fish were not fed 24 hours prior to weighing.

Experiment No. 1 : The first experiment was composed of two 20-day periods (40 days total duration) in which 400 fish were utilized, see Table 4. In the first 20-day period, the

Table 4. Distribution of methoxychlor and reduced oxygen levels in Expt. No. 1 where rainbow trout received various amonts of dietary methoxychlor in the first 20 days followed by a methoxychlor-free diet but exposed to reduced oxygen levels in the second 20-day period.

Test Period (days)	Number of tanks	Number of fish/tank	O_2 Saturation (%)	Methoxychlor concentration in diet (ppm)
0 - 20	4	20	95	0 (control)
	4	20	95	1.84
	4	20	95	3.80
	4	20	95	7.20
	4	20	95	13.60
20 - 40	4 (0.00) 1/	10	(95,80,60,40)	0 (control)
	4 (1.84)	10	(95,80,60,40)	0
	4 (3.80)	10	(95,80,60,40)	0
	4 (7.20)	10	(95,80,60,40)	0
	4 (13.60)	10	(95,80,60,40)	0

1/ Concentration of dietary methoxychlor (ppm) to which rainbow trout were previously exposed during day 0 - 20.

fish were fed a methoxychlor contaminated diet while the dissolved oxygen concentration was maintained at 95% saturation. There were four groups of fish for each concentration of dietary methoxychlor. All fish (20 fish per test tank) were weighed on day 0, 10 and 20. In addition, ten fish from each tank were removed on day 20, killed by a lethal concentration of MS 222 and frozen at -15.0°C until the various analyses were undertaken. Five of these fish were pooled for determination of residual methoxychlor and five were pooled to first obtain the dry weight after one week in a drying oven at 70°C. The samples were weighed to the nearest hundredth of a gram. The pooled samples were then ground in a Waring blender and the fat content was determined as previously described for the diet.

For the following test period (day 20-40), the remaining ten fish per tank were fed a methoxychlor-free diet; but the dissolved oxygen concentrations were adjusted to 40, 60, 80 and 95% saturation at 11.5°C . These oxygen levels were monitored daily in each tank using the Winkler method (Azide Modification - Standard Methods 1971). Thus each of the four groups of fish exposed to a particular concentration of dietary methoxychlor during day 0-20 was assigned a different oxygen level. All fish were weighed again on day 30 and at the end of the experiment (day 40) when they were killed by an overdose of MS 222 and then kept frozen (-15.0°C) for analysis. The ten fish in each tank at the end of the experiment were pooled for analysis as in the previous test period.

Table 5. Distribution of methoxychlor and reduced oxygen levels in Expt. No. 2 where rainbow trout were simultaneously exposed to dietary methoxychlor and reduced oxygen over a 20-day period at 12.0°C .

Number of tanks	Number of fish/tank	O_2 Saturation (%)	Methoxychlor concentration in diet (ppm)
4	10	(95,80,60,40)	0 (control)
4	10	(95,80,60,40)	1.84
4	10	(95,80,60,40)	3.80
4	10	(95,80,60,40)	7.20
4	10	(95,80,60,40)	13.60

Experiment No. 2 : In the second experiment which was 20 days in duration (Table 5), the fish (10 fish per tank) were fed a methoxychlor contaminated diet while being simultaneously exposed to the same reduced oxygen levels as during day 20 - 40 of Expt. No. 1 . The fish were weighed on day 0, 10 and 20 and the dissolved oxygen concentrations were monitored daily. On day 20, the fish were killed by an overdose of MS 222 and then kept frozen (-15.0°C). The analyses conducted on pooled samples consisted of determinations of residual methoxychlor, dry weight and fat content which were performed in the same manner as previously described in Expt. No. 1 .

Determination of residual methoxychlor

Determinations of residual methoxychlor were conducted on whole fish samples from all test groups including the control group. In addition, all fish samples from test tanks fed the highest concentration of methoxychlor in the diet (13.60 ppm) were assayed for methoxychlor residue in their liver tissue. The extraction procedure used was the method outlined by Boileau (1979) which was used to verify the methoxychlor content of the test diet, whole fish and liver tissue samples.

The residual methoxychlor from these extracts was assayed by gas chromatography using a Microtek/Tracor (model 220) gas chromatograph equipped with an electron capture detector. A glass column (0.635 cm OD, 4.0 mm ID

by 1.8288 m long) was packed with 0.66% OV1 + 0.66% OV210 + 0.06% OV17 on 100 - 120 mesh Gas-Chrom Q.

The operating parameters were as follows:

Injection port temperature	215°C
Column temperature	190°C
Detector temperature	305°C
Carrier gas	4.8 methane, 95.2 argon
Carrier gas flow rate	75 ml/min.
Retention time	7 minutes
Injection volume	1 µl

The peak areas obtained from the injection of samples of reference standard methoxychlor were used to establish standard curves at the various levels of attenuation on the gas chromatograph. The peak areas of the unknown samples were then compared to these standard curves. The concentrations of methoxychlor were expressed as micrograms/gram (ppm) on a wet weight basis in whole fish samples.

RESULTS

The fish adapted quite well to the experimental conditions during the acclimation period. Throughout Expt. No. 1 and Expt. No. 2, the fish readily accepted and consumed either the control or experimental diets. Also no behavioural changes were observed in any of the methoxychlor treated fish.

During both experiments a small number of fish exhibited symptoms of tail rot, and once the symptoms were noticed the diseased fish was removed from its particular test tank to minimize further contamination. In addition, some fish died during both experiments with the number of mortalities being shown in Table 6. These fish, which were usually the smallest ones, exhibited no disease symptoms which would permit the cause of death to be ascertained. Fish mortality and occurrence of tail rot in control and methoxychlor treated fish appeared random and therefore could not be attributed to either dietary methoxychlor and/or reduced oxygen levels.

Wet weight gain

The response of the fish was measured as changes in growth rate through the calculation of the average relative growth rate (Warren, 1971).

$$\text{growth rate (\%/day)} = \frac{W_2 - W_1}{0.5\,(W_1 + W_2)\,(t_2 - t_1)} \times 100.$$

Table 6. Mortality and disease observed in yearling rainbow trout exposed to control or experimental diets containing methoxychlor and held at various levels of dissolved oxygen.

Expt. No. and temp.	Test Period (days)	No. of fish at start of test period	O_2 Saturation (%)	Methoxychlor intake level (mg/kg fish/day)	Number of diseased fish	Number of fish mortality
1 (11.5°C)	0 - 20	400	95	0.076	3	4
			95	0.144	2	0
			95	0.272	1	1
1 (11.5°C)	20 - 40	200	95	0.037	0	1
			60	0.076	2	0
			95	0.144	1	0
			60	0.144	1	0
			80	0.272	1	0
			60	0.272	1	0
2 (12.0°C)	0 - 20	200	95	0.000	1	1
			95	0.037	0	2
			60	0.037	1	0
			40	0.037	1	1
			60	0.076	1	0
			60	0.144	1	0
			40	0.144	1	1
			40	0.272	0	2

Table 7. Growth rate based on wet weights during day 0 - 20 of Expt. No. 1 , in which rainbow trout were exposed to dietary methoxychlor at 95 % O_2 saturation and at 11.5°C .

Methoxychlor intake level (mg/kg fish/day)		Mean Growth Rate (%/day)	Student's t 1/ (P = 0.05)
0 (control)		0.47	
		0.33	
		0.41	
		0.41	
	Mean	0.41	-
0.037		0.36	
		0.47	
		0.28	
		0.45	
	Mean	0.39	ns
0.076		0.43	
		0.50	
		0.28	
		0.18	
	Mean	0.35	ns
0.144		0.36	
		0.35	
		0.39	
		0.23	
	Mean	0.33	ns
0.272		0.45	
		0.28	
		0.40	
		0.44	
	Mean	0.39	ns

1/ t-test comparing growth rates of methoxychlor treated fish to controls.

ns = Not significant

Table 8. Growth rate based on wet weights during the last 20 days of Expt. No. 1 , in which rainbow trout previously exposed to dietary methoxychlor were subjected to reduced oxygen levels at 11.5°C while being fed a control diet.

O_2 Saturation (%)	Methoxychlor intake level (day 0 - 20) (mg/kg fish/day)	Mean Growth Rate (%/day) Day 20-30	Day 30-40	Day 20-40
95	0.000	0.45	0.87	0.66
	0.037	0.11	0.10	0.09
	0.076	0.60	0.60	0.60
	0.144	0.12	0.37	0.30
	0.272	0.26	0.41	0.33
80	0.000	0.44	0.76	0.60
	0.037	0.20	0.36	0.28
	0.076	0.48	0.73	0.61
	0.144	-0.05	0.42	0.18
	0.272	0.35	0.55	0.49
60	0.000	0.53	0.70	0.62
	0.037	0.29	0.51	0.39
	0.076	-0.15	0.04	-0.02
	0.144	0.15	0.22	0.18
	0.272	0.26	0.19	0.22
40	0.000	0.23	0.59	0.41
	0.037	0.41	0.48	0.44
	0.076	-0.05	0.15	0.05
	0.144	0.16	0.14	0.15
	0.272	0.14	0.05	0.09

where:

W_1 = initial wet weight of fish,

W_2 = final wet weight of fish,

$(t_2 - t_1)$ = duration of the experiment in days.

The effects of various dietary levels of methoxychlor on the wet weight gain of rainbow trout during day 0 - 20 of Expt. No. 1 are shown in Table 7. Each growth rate value in this table represents a mean of all the individual growth rates in each group of fish (numbering 20 fish per group). The results indicate that a 20-day exposure of rainbow trout to dietary methoxychlor at 95 % O_2 saturation (11.5°C) did not produce any significant change ($P = 0.05$) in growth rate as compared with control fish. The mean growth rate of control fish was 0.41 %/day and ranged from 0.33 to 0.39 %/day for methoxychlor treated fish.

During day 20 - 40 of Expt. No. 1, the fish were fed a methoxychlor-free diet while the dissolved oxygen concentrations were reduced. The results were calculated in the same manner as for the first 20 days and Table 8 shows the effects of reduced oxygen and previous exposure to dietary methoxychlor on the wet weight gain of rainbow trout during the last 20 days. The data suggest that the growth of fish previously exposed to methoxychlor was more reduced as compared with control fish at all levels of dissolved oxygen tested. Because of high variation it was not possible to show a dose response, and the data was therefore treated in terms of an all-or-none effect.

Table 9. Analysis of variance to compare the effect of dietary methoxychlor with reduced oxygen levels on the growth of rainbow trout based on wet weight gain during the second 20-day period of Expt. No. 1 .

Source of variation	Mean square	Degree of freedom	Significance
Total	0.135	193	
Main effects	0.610	7	
A (Methoxychlor)	0.766	4	* *
B (Oxygen)	0.402	3	*
AB (Interaction: Methoxychlor/Oxygen)	0.329	12	* *
Residual	0.102	174	

* = Significant ($P<0.05$)

* * = Significant ($P<0.01$)

Table 10. Comparison, using a t-test, of the effect of dietary methoxychlor (pooled methoxychlor data) at different oxygen levels on the wet weight gain of rainbow trout during the second 20-day period of Expt. No. 1 .

O_2 Saturation (%)	Mean Growth Rate (%/day)		
	Control	Methoxychlor Treated	
95	0.66	0.34	**
80	0.60	0.38	*
60	0.62	0.20	**
40	0.41	0.18	*

* = Significant ($P < 0.1$)

** = Significant ($P < 0.05$)

There were a few methoxychlor treated groups which grew faster than the control fish at 95, 80 and 40 % O_2 saturation during day 20 - 30 and at 80 and 40 % O_2 saturation during day 20 - 40. Also contrary to all other results obtained at the other methoxychlor intake levels, at 0.037 mg/kg fish/day growth rates increased with a reduction in percent O_2 saturation.

A two-way analysis of variance based on changes in wet weight carried out to verify any possible interaction between methoxychlor and reduced oxygen showed that each does contribute significantly to a reduction in growth of rainbow trout, see Table 9. A highly significant interaction ($P < 0.01$) exists between methoxychlor and reduced oxygen resulting in an even further reduction in growth. A paired data t-test showed that the growth rates of all control fish during day 20 - 40 had nearly doubled as compared with day 0 - 20 while the growth rates of 9 out of 16 methoxychlor treated groups of fish were significantly reduced ($P < 0.05$) during day 20 - 40 as compared with the results from day 0 - 20.

Since the results were treated in terms of an all-or-none effect, the data representing methoxychlor treated fish (Table 8) were pooled at each oxygen level in order to obtain a larger sample number and a t-test conducted in which the growth of methoxychlor treated fish (40 fish) was compared to the controls (10 fish). The analysis showed that the previous methoxychlor treatment significantly reduced growth when compared to controls (Table 10) at 95 and 60 % O_2 sat-

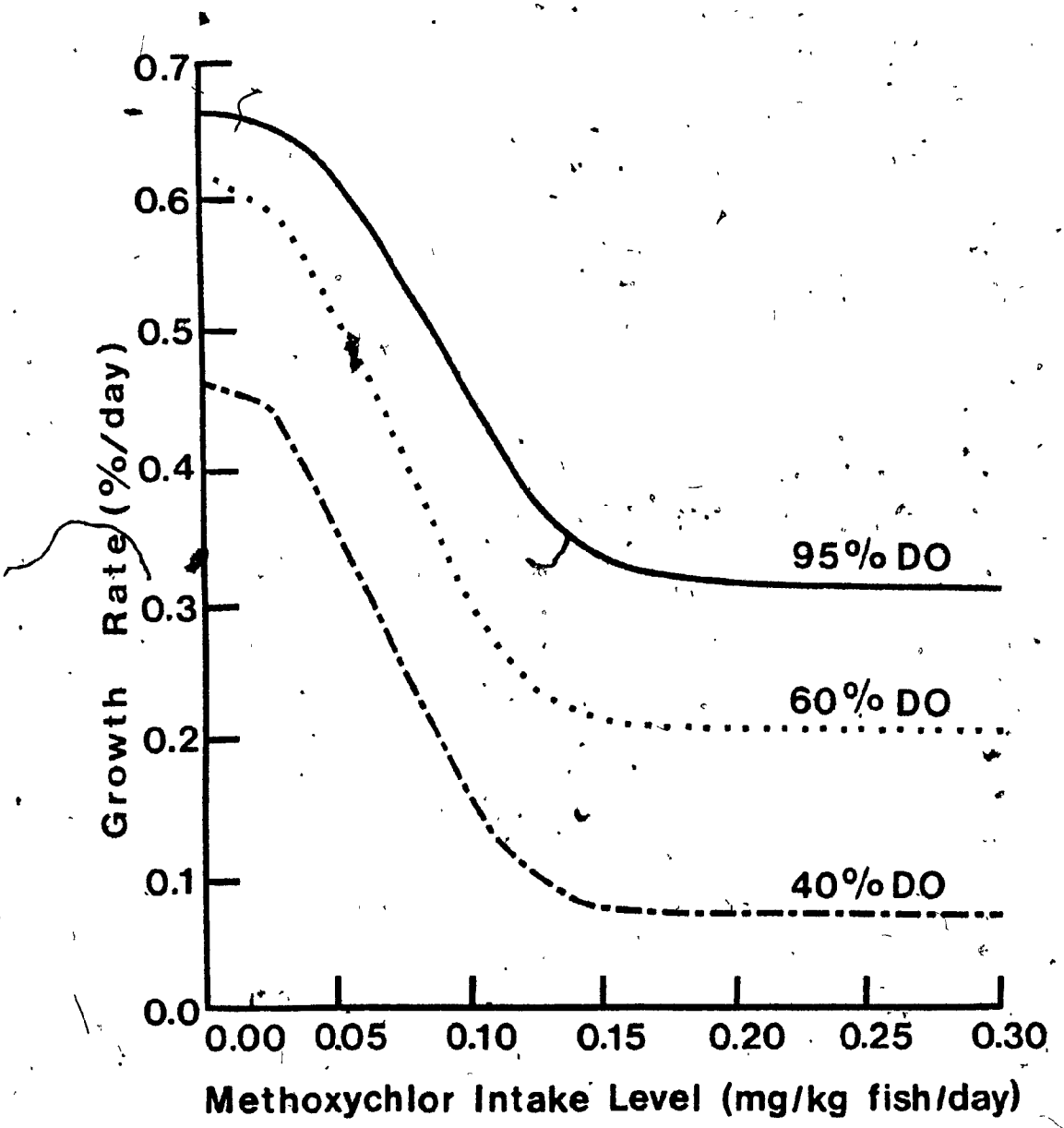

Figure 6. The relationship in Expt. No. 1 between the wet weight gain at various levels of dissolved oxygen saturation during day 20 - 40 and dietary methoxychlor to which rainbow trout had been previously exposed to during day 0 - 20.

Table 11. Growth rate based on wet weights during Expt.No.2 in which rainbow trout were simultaneously exposed to dietary methoxychlor and reduced oxygen over a 20-day period at 12.0°C .

O_2 Saturation (%)	Methoxychlor intake level (mg/kg fish/day)	Mean Growth Rate (%/day) Day 0-10	Day 10-20	Day 0-20
95	0.000	-0.09	0.13	0.04
	0.037	-0.05	-0.02	0.00
	0.076	0.14	0.14	0.14
	0.144	-0.11	-0.09	-0.10
	0.272	0.09	0.21	0.15
80	0.000	0.27	0.44	0.36
	0.037	-0.07	0.12	0.03
	0.076	-0.08	-0.03	-0.06
	0.144	-0.01	0.15	0.07
	0.272	0.06	0.04	0.05
60	0.000	0.14	0.20	0.17
	0.037	0.12	0.15	0.14
	0.076	-0.18	0.08	-0.05
	0.144	-0.04	0.06	0.01
	0.272	-0.01	0.06	0.02
40	0.000	0.17	0.24	0.21
	0.037	-0.07	-0.09	-0.08
	0.076	-0.07	0.11	0.03
	0.144	-0.24	-0.23	-0.24
	0.272	0.09	-0.09	0.00

Table 12. Comparison, using a t-test, of the effect of simultaneous exposure of dietary methoxychlor and reduced oxygen on the wet weight gain of rainbow trout during day 0 - 20 of Expt. No. 2 (pooled methoxychlor data used at each oxygen level).

O_2 Saturation (%)	Mean Growth Rate (%/day) Control	Methoxychlor Treated	
95	0.04	0.05	ns
80	0.36	0.02	**
60	0.17	0.03	*
40	0.21	-0.07	**

ns = Not significant ($P > 0.05$)

* = Significant ($P < 0.2$)

** = Significant ($P < 0.05$)

uration ($P < 0.05$) and at 80 and 40 % O_2 saturation ($P < 0.1$).

This response is graphically illustrated in Figure 6 which suggests a growth reduction threshold around 0.05 mg/kg fish/day at 95, 60 and 40 % O_2 saturation. The data at 80 % O_2 saturation in Table 8 was not included in Figure 6 because of variation which made it impossible to eye-fit a curve. Another threshold occurred around 0.15 mg/kg fish/day where an increase of dietary methoxychlor did not further reduce fish growth. The effect of reduced oxygen alone is illustrated by the lower position of the 40 and 60 % O_2 saturation growth curves compared to the 95 % O_2 saturation curve.

In Expt. 2 where rainbow trout were simultaneously exposed to dietary methoxychlor and reduced oxygen over a 20-day period, no effect was discernable at 95 % O_2 saturation; however at 80, 60 and 40 % O_2 saturation the growth rate of methoxychlor treated fish, calculated in the same manner as in Expt. No. 1, was reduced as compared with control fish during day 0 - 10 and day 10 - 20 (Table 11). No dose response was obtained and the variable data was treated in terms of an all-or-none effect. A t-test was conducted to compare the growth of methoxychlor treated fish (pooled sample of 40 fish) to the controls (10 fish) at each oxygen level, see Table 12. While there was no significant reduction in growth of methoxychlor treated fish when compared to controls at 95 % O_2 saturation, methoxychlor treatment did significantly reduce growth ($P < 0.05$) over controls at 80 and 40 % O_2 saturation and at 60 % ($P < 0.2$).

Table 13. Analysis of variance to compare the effect of dietary methoxychlor with reduced oxygen levels on the growth of rainbow trout based on wet weight gain during day 0 - 20 of Expt. No. 2 .

Source of variation	Mean square	Degree of freedom	Significance
Total	0.079	189	
Main effects	0.235	7	
A (Methoxychlor)	0.343	4	*
B (Oxygen)	0.092	3	ns
AB (Interaction Methoxychlor/Oxygen)	0.108	12	ns
Residual	0.070	170	

ns = Not significant ($P > 0.05$)

* = Highly significant ($P < 0.01$)

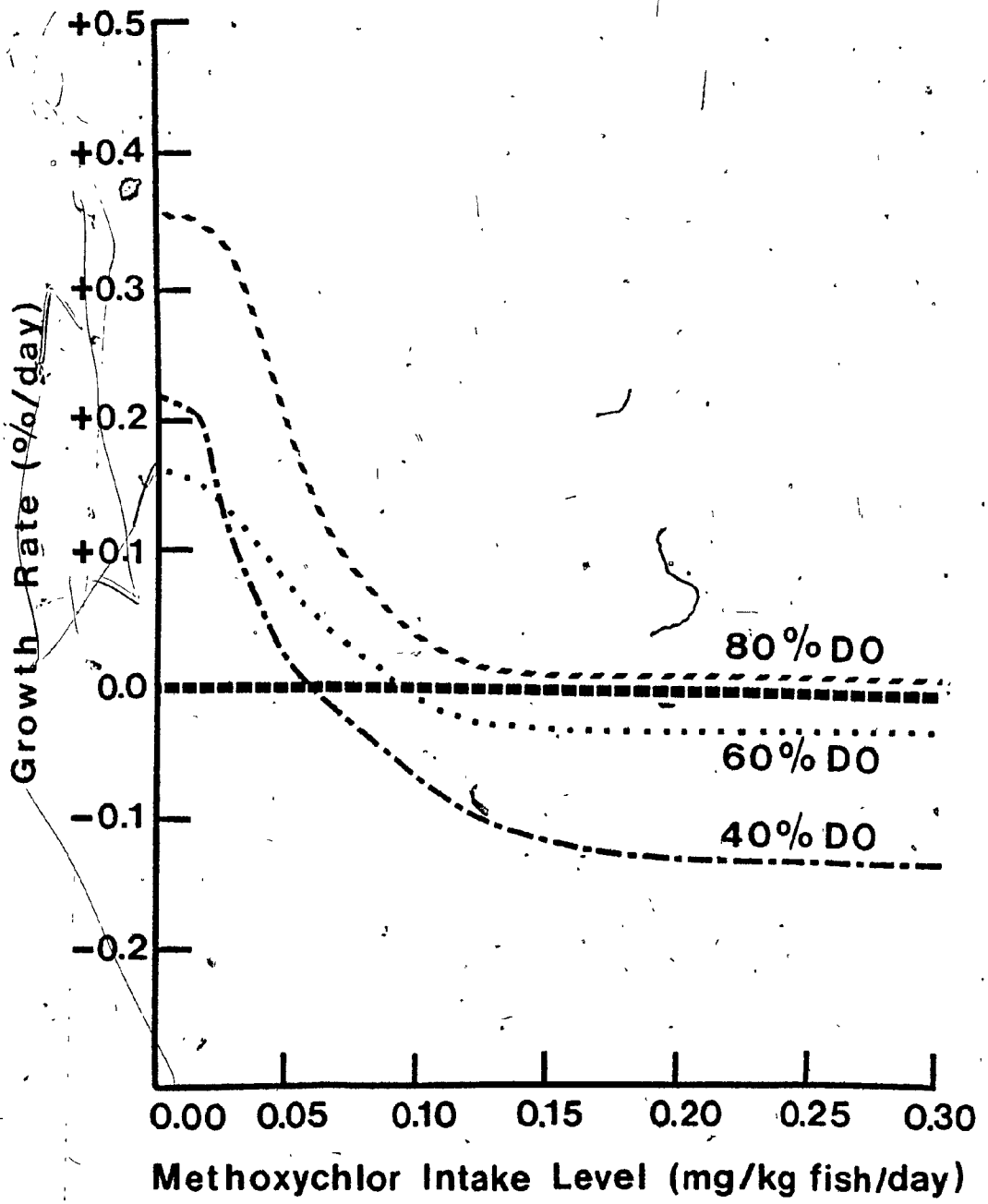

Figure 7. The relationship between the wet weight gain at various levels of dietary methoxychlor and dissolved oxygen saturation to which rainbow trout had been exposed over a 20-day period in Expt. No. 2 .

A two-way analysis of variance indicates that there has been no interaction between methoxychlor and oxygen in producing an additional reduction in growth, see Table 13. This analysis also shows that reduced oxygen did not significantly affect growth rates in this experiment while methoxychlor's role in growth reduction was highly significant ($P < 0.01$).

Figure 7 illustrates the general trend of the growth rate where methoxychlor exposure has been shown to be significant by means of a t-test and analysis of variance in Expt. No. 2 . An initial drop occurred around 0.05 mg methoxychlor/kg fish/day and another threshold was encountered around 0.15 mg/kg fish/day where an increase in the concentration of methoxychlor in the diet did not produce any further reduction in growth at 80, 60 and 40 % O_2 saturation. A decrease in growth due to reduced dissolved oxygen concentrations, while not significant, is suggested by the lower position of the growth curves at 40 and 60 % O_2 saturation as compared with the curve at 80 % O_2 saturation.

Dry weight gain

The effects of dietary methoxychlor and reduced oxygen levels on the percent dry weights of rainbow trout are shown in Table 14. The results indicate no differences due to methoxychlor nor to reduced oxygen in both experiments. The dry weight gain is parallel to the wet weight gain, meaning differences in growth as seen in wet weight

Table 14. Percent dry weight of rainbow trout exposed to dietary methoxychlor during day 0 - 20 of both Expt. No. 1 and No. 2 and reduced oxygen levels during day 20 - 40 of Expt. No. 1 and day 0 - 20 of Expt. No. 2 .

Methoxychlor intake level	O_2 Saturation	Dry Weight (% of wet weight)		
		Expt. No. 1		Expt. No. 2
(mg/kg fish/day)	(%)	Day 0-20	Day 20-40	Day 0-20
0 (control)	95	19.47	21.09	22.33
	80	19.53	20.59	20.65
	60	20.46	21.41	21.57
	40	20.86	20.17	21.12
0.037	95	20.32	21.11	21.43
	80	20.05	20.95	20.88
	60	20.50	20.73	20.05
	40	19.83	21.51	20.74
0.076	95	20.48	20.58	20.93
	80	20.37	22.09	19.85
	60	19.42	19.61	21.59
	40	18.67	20.92	21.93
0.144	95	20.24	20.57	20.84
	80	20.57	21.34	20.92
	60	19.69	20.96	21.60
	40	20.04	21.09	20.69
0.272	95	20.61	21.04	21.18
	80	20.29	20.74	21.57
	60	20.53	21.62	21.20
	40	19.84	21.62	22.12

Table 15. Fat gains of rainbow trout in Expt. No. 1 after exposure to dietary methoxychlor during day 0 - 20 at 95 % O_2 saturation and reduced oxygen during day 20 - 40.

Methoxychlor intake level (day 0 - 20) (mg/kg fish/day)	O_2 Saturation (day 20-40) (%)	Mean Fat Gain (%/day) Day 0 - 20	Day 20 - 40
0 (control)	95	-1.04	3.83
	80	-1.42	2.71
	60	0.77	2.43
	40	1.78	-1.66
	Mean	0.02	
0.037	95	0.61	2.11
	80	-0.23	2.12
	60	1.01	0.79
	40	0.12	2.83
	Mean	0.42	
0.076	95	1.03	0.53
	80	1.39	3.25
	60	-1.62	0.68
	40	-0.87	1.90
	Mean	0.05	
0.144	95	-0.68	1.39
	80	2.59	-0.35
	60	-1.06	3.39
	40	0.16	1.50
	Mean	0.25	
0.272	95	1.36	0.16
	80	1.16	0.83
	60	1.22	1.83
	40	-0.45	3.07
	Mean	0.85	

gain are not due to changes in water retention by the fish.

Fat gain

The fat gain of rainbow trout exposed to dietary methoxychlor and reduced oxygen was measured using the average relative growth rate with the following modifications:

W_1 = initial fat content of fish estimated from pre-experimental sample,

W_2 = final fat content of fish.

During day 0 - 20 of Expt. No. 1 , the fat gains of methoxychlor treated fish at 0.037, 0.144 and 0.272 mg/kg fish/day increased as compared with control fish while the fat gain at 0.076 mg/kg fish/day (0.05 %/day) was similar to control data (0.02 %/day), see Table 15. Higher methoxychlor concentrations in the diet during the following 20-day period produced a decrease in fat gains of methoxychlor treated fish as compared with controls at 95, 80 and 60 % O_2 saturation and an increase in fat gains of methoxychlor treated fish over controls at 40 % O_2 saturation. Reduced oxygen levels did not produce any change in fat gain at 0.037, 0.076 and 0.144 mg/kg fish/day ; however a decrease in fat gains of control fish occurred with a corresponding reduction in oxygen saturation while the fat gains of fish at 0.272 mg/kg fish/day increased with a reduction in oxygen saturation.

An all-or-none response prevailed throughout these results and a t-test was conducted in order to ascertain their level of significance. Thus for day 0 - 20 of Expt.

Table 16. Comparison, using a t-test, of the effect of dietary methoxychlor (pooled methoxychlor data) during day 0 - 20 and reduced oxygen levels (day 20 - 40) on the fat gain of rainbow trout in Expt. No. 1.

Test Reriod (days)	O_2 Saturation (%)	Mean Fat Gain (%/day) Control	Methoxychlor Treated
0 - 20	95	0.02	0.39 **
20 - 40	95	3.83	1.05 **
	80	2.71	1.49 **
	60	2.43	1.73 *
	40	-1.66	2.32 **

* = Significant ($P < 0.1$)
** = Significant ($P < 0.05$)

Table 17. Fat gains of rainbow trout which were simultaneously exposed to dietary methoxychlor and reduced oxygen during 20 days in Expt. No. 2 .

Methoxychlor intake level (mg/kg fish/day)	O_2 Saturation (%)	Mean Fat Gain (%/day)
0.000	95	2.15
	80	-0.68
	60	0.70
	40	0.33
0.037	95	0.54
	80	-0.03
	60	-0.81
	40	0.18
0.076	95	-0.06
	80	-2.84
	60	0.74
	40	1.47
0.144	95	-0.80
	80	-1.03
	60	0.43
	40	-0.44
0.272	95	0.73
	80	0.31
	60	0.42
	40	1.43

Table 18. Comparison, using a t-test, of the effect of simultaneous exposure of dietary methoxychlor (pooled methoxychlor data) and reduced oxygen levels on the fat gain of rainbow trout during 20 days in Expt. No. 2 .

O_2 Saturation (%)	Mean Fat Gain (%/day)		
	Control	Methoxychlor Treated	
95	2.15	0.26	*
80	-0.68	-0.95	ns
60	0.70	0.20	*
40	0.33	0.66	ns

ns = Not significant ($P > 0.05$)

* = Significant ($P < 0.05$)

No. 1, the pooled control groups (20 fish) and the pooled methoxychlor treated groups (80 fish) were compared, see Table 16, with the result that the fat gain of methoxychlor treated fish was significantly greater ($P < 0.05$) than that of control fish. For the following test period (day 20 - 40), a similar test was performed between the control group and the pooled methoxychlor treated fish at each oxygen level revealing higher fat gains by control fish than by methoxychlor treated fish at 95, 80 and 60 % O_2 saturation. At 40 % O_2 saturation the fat gain of control fish was however significantly less ($P < 0.05$) than that of methoxychlor treated fish.

In Expt. No. 2, the fat gains of methoxychlor treated fish were reduced as compared with controls at 95 % O_2 saturation, see Table 17 ; however no trend was apparent due to methoxychlor treatment at the other oxygen levels. Reduced oxygen also did not produce any changes in fat gain, therefore a t-test was conducted on pooled data as in Expt. No. 1 (day 20 - 40) at each oxygen level. The fat gains of control fish were significantly greater ($P < 0.05$) than that of methoxychlor treated fish at 95 and 60 % O_2 saturation (Table 18) while there was no difference between control and methoxychlor groups at 80 and 40 % O_2 saturation.

Residual methoxychlor

No residual methoxychlor was detected in any group of fish at day 40 of Expt. No. 1, nor in any of the

Table 19. Fat content and residual methoxychlor in rainbow trout exposed to dietary methoxychlor in both Expt. No. 1 and No. 2 and also to reduced oxygen levels in Expt. No. 2 .

Expt. No. and test period	O_2 Saturation	Methoxychlor intake level	Methoxychlor residue in whole fish	Fat Content	Methoxychlor stored
	(%)	(mg/kg fish/day)	(µg/g)	(% of dry weight)	(%)
1 (day 0 - 20)	95	0.000	0.000	4.85	-
			0.000	4.61	-
			0.000	6.75	-
			0.000	8.09	-
		0.037	0.010	6.57	1.42
			Trace	5.58	-
			Trace	7.25	-
			Trace	5.99	-
		0.076	Trace	7.07	-
			0.010	7.52	0.69
			0.002	4.47	0.14
			0.010	5.55	0.69
		0.144	Trace	5.14	-
			0.050	9.90	1.83
			0.060	4.88	2.19
			0.040	6.28	1.46
		0.272	0.070	7.35	1.39
			0.060	5.43	1.18
2 (day 0 - 20)	95	0.000	0.000	14.56	-
		0.037	Trace	11.10	-
		0.076	0.065	9.79	4.28
		0.144	0.075	9.78	2.60
		0.272	0.124	11.25	2.28
	80	0.000	0.000	8.40	-
		0.037	Trace	10.10	-
		0.076	0.018	6.04	1.18
		0.144	0.081	8.17	2.81
		0.272	0.301	10.36	5.53
	60	0.000	0.000	10.96	-
		0.037	Trace	8.81	-
		0.076	0.034	11.58	2.24
		0.144	0.114	10.71	3.96
		0.272	0.345	10.88	6.34
	40	0.000	0.000	10.24	-
		0.037	Trace	10.85	-
		0.076	0.133	12.98	8.75
		0.144	0.210	9.92	7.29
		0.272	0.450	12.88	8.27

control groups in both experiments. The concentrations of residual methoxychlor based on wet weights in whole body homogenates during Expt. No. 1 and No. 2 , see Table 19, indicate that at 95 % O_2 saturation an increase in the concentration of dietary methoxychlor produced an increase in the accumulation of methoxychlor by fish. In Expt. No. 1 (day 0 - 20) the residue levels of methoxychlor ranged from 0.002 µg/g at 0.076 mg/kg fish/day to 0.07 µg/g at 0.272 mg/kg fish/day while in Expt. 2 they ranged from 0.065 to 0.124 µg/g at 95 % O_2 saturation. The residue data from Expt. No. 2 also shows that a reduction in oxygen saturation produced a further increase in accumulation of methoxychlor. At 0.272 mg/kg fish/day , residues ranged from 0.124 µg/g at 95 % O_2 saturation to 0.450 µg/g at 40 % , this trend being repeated at the other methoxychlor intake levels.

The fat content of fish (% of dry weight) has been included in Table 19 because of the established link between the storage of organochlorine pesticides and fat content. The fish in Expt. No. 2 contained nearly twice as much fat in comparison to Expt. No. 1 (day 0 - 20). The percentage of methoxychlor stored, also in Table 19, was calculated in the following manner:

$$\text{methoxychlor stored} \quad (\%) = \frac{\text{residue in whole fish } (\mu g/g)}{\text{total dietary intake } (mg/kg)} \times 100$$

The percentage of methoxychlor stored as compared to the

given dose is very low ranging from 0.14 to 2.19 % in Expt. No. 1 and from 1.18 to 8.75 % in Expt. 2 , the highest values being obtained at 40 % O_2 saturation.

Levels of residual methoxychlor determined on fish liver in Expt. No. 2 at 0.272 mg/kg fish/day followed the same trend as the results of whole fish samples in that a reduction in oxygen saturation also increased the methoxychlor accumulated in the liver, see Table 20. The liver residue values ranged from 0.027 µg/g at 95 % O_2 saturation to 0.129 µg/g at 40 % O_2 saturation.

Table 20. Residual methoxychlor in liver and whole fish samples of rainbow trout simultaneously exposed to 0.272 mg methoxychlor/kg fish/day and reduced oxygen over a 20-day period in Expt. No. 2 .

O_2 Saturation (%)	Methoxychlor residue in liver sample (µg/g)	Methoxychlor residue in whole fish sample (µg/g)
95	0.027	0.124
80	0.075	0.301
60	0.075	0.345
40	0.129	0.450

DISCUSSION

In this present study of the effects of dietary methoxychlor and reduced dissolved oxygen concentrations on the growth of rainbow trout, there was no change in the growth of fish exposed to dietary methoxychlor for 20 days at 95 % O_2 saturation as compared with controls but a reduction in the growth of methoxychlor treated fish occurred during a following 20-day period while the fish were fed a control diet. Exposure to reduced oxygen levels produced a much lower growth rate among methoxychlor treated fish as compared with control rates which were also lower than at oxygen saturation. The results of the effects of dietary methoxychlor and reduced oxygen levels on fat gain were inconclusive due to a marked variation of the data; however the analysis of residual methoxychlor has shown that a greater percentage of the given dose of methoxychlor was stored at reduced oxygen levels and a significant portion of the methoxychlor body burden was associated with liver tissue.

Physiological significance

An error in calculating the amount of methoxychlor required for the various intake levels proved to be beneficial. Twenty-four hours after the first feeding, an approximate LD50 of 78.43 mg of methoxychlor/kg of fish was obtained. This value represents a 288 fold increase

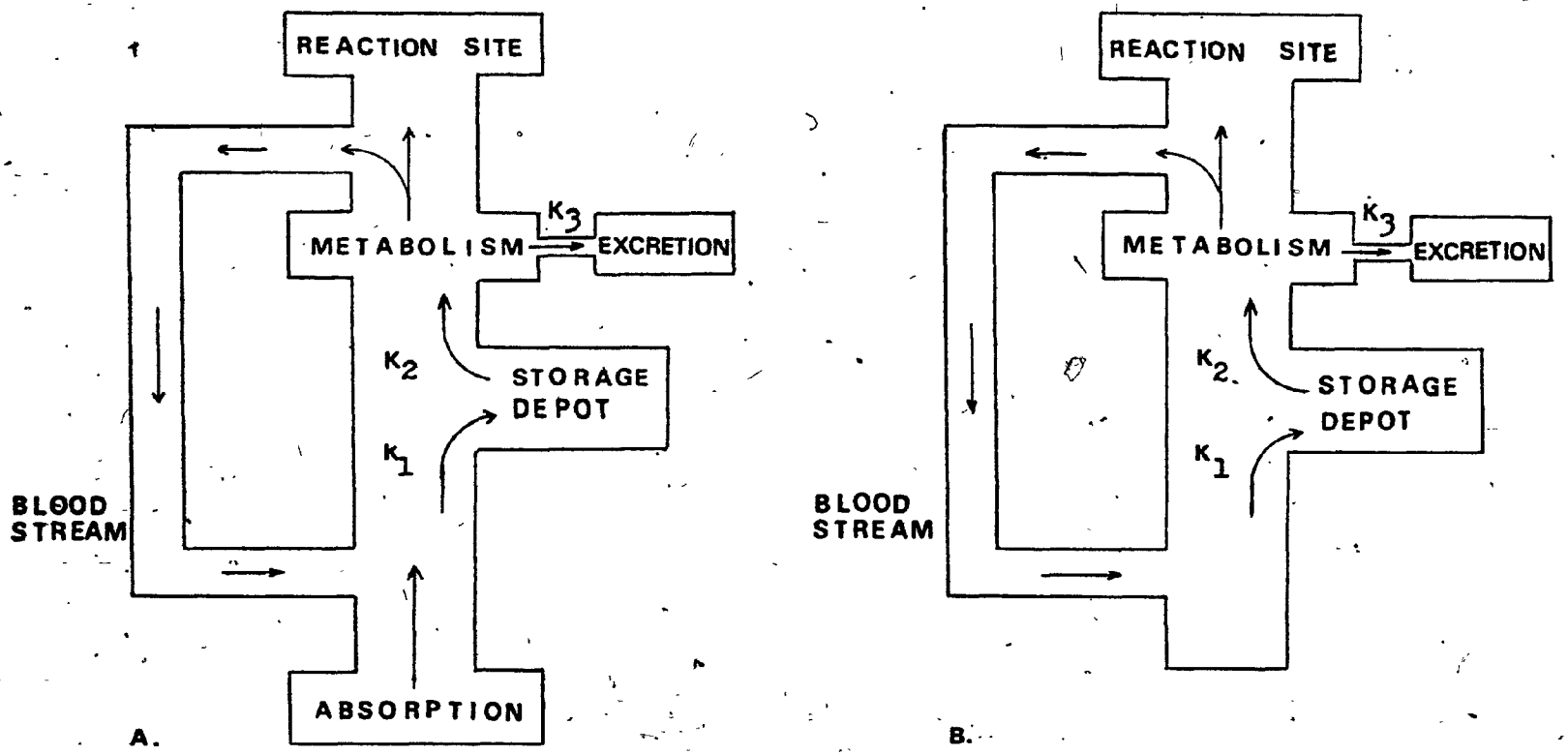

Figure 8. Schematic representation of the pathways involved in methoxychlor uptake, metabolism, mode of action and depuration during A) day 0 - 20 of both experiments, B) day 20 - 40 of Expt. No. 1 .

over the highest concentration used in this study (0.272 mg/kg fish/day) and places the experimental concentrations (0.037 to 0.272 mg/kg fish/day) in perspective to the lethal dose. These concentrations may also represent the incipient effect threshold and therefore at such low levels not all the fish respond to methoxychlor exposure which may in part explain the large variation in the data of wet weight gain and fat gain. Despite the large variance, the all-or-none effect obtained in the wet weight gain of methoxychlor treated fish has permitted the use of a t-test with pooled data to statistically validate the results. The fact that a majority of the fish were affected in a detrimental manner in the two experiments suggests that these low levels of dietary methoxychlor are capable of contributing stress to an aquatic organism.

While this study does not actively investigate the biochemical nor all the physiological aspects of methoxychlor metabolism, a general discussion of pesticide metabolism is useful in order to understand the effects of methoxychlor on growth as well as its capacity to accumulate in fish tissue. Figure 8-A illustrates possible pathways of methoxychlor uptake, metabolism and depuration at 95 % O_2 saturation during day 0 - 20 of both experiments. According to Walker (1975) lipophilic pesticides bind with plasma lipoprotein and are distributed throughout the fish. This binding is reversible and functions as a

transport mechanism. Since methoxychlor is a lipophilic compound, it may also bind with plasma lipoprotein once it is absorbed via the gastric membrane into the blood stream.

In the organism, lipophilic pesticides tend to associate with the various lipid pools and are thus removed from circulation (Pocock and Vost 1974). This greater affinity of the lipid pools for methoxychlor as compared with lipoprotein may upset the natural tendency towards the establishment of an equilibrium and the rate at which methoxychlor in the plasma is bound (k_1) and stored in fat tissue may be greater than the rate at which it is returned (k_2) to the plasma. In this manner, a large portion of the methoxychlor may be removed from circulation. The multifunction oxidase enzymes (MFO) of the liver which are responsible for the metabolism of methoxychlor in fish (Gardner and Bailey 1975) may metabolize the low level present in the plasma before it comes in contact with a reaction site.

The combination of the removal of a large percentage of methoxychlor from circulation and its efficient metabolism by the MFO system may account for the lack of effect of methoxychlor exposure on the wet weight gain of rainbow trout at 95 % O_2 saturation during the first 20 days of both experiments. Kruzynski (1972) reported no reduction in growth of brook trout, Salvelinus fontinalis,

exposed to dietary methoxychlor levels ranging from 0.01 to 1.00 mg/kg fish/day for 33 days at 7.5°C and Oladimeji and Leduc (1975) found that a level of 0.67 mg/kg fish/day for 30 days at 10-12.5°C also had no effect on growth of brook trout fed a 2% ration (percent of wet body weight). But the fish in these two studies were forced to swim against a current and the accompanying increase in the metabolic rate could have aided the detoxification process.

Kapoor et al.(1970) have reported the chemical pathway involved in methoxychlor metabolism in mouse and housefly but it is assumed that metabolism of methoxychlor in fish follows the same pathway. According to Kapoor, a major difference between DDT and methoxychlor metabolism in fish is that the metabolites of DDT are lipophilic and hence can be stored while the water soluble hydroxy metabolites of methoxychlor are readily excreted primarily through the urine but possibly through biliary excretion. Addison et al.(1977) have found that exposure to dietary DDT and DDE in brook trout, Salvelinus fontinalis, failed to induce the activity of the MFO system. It is not known whether methoxychlor exposure can induce MFO activity in fish.

"Storage of a toxic compound may be protective in the short term yet hazardous in the longer term" (Walker 1975 p.83). The reduction in wet weight gain of methoxychlor treated fish at 95 % O_2 saturation during the second test period in Expt. No. 1 is an example of this phenomenon.

Findlay and deFrietas (1971) have shown with DDT that the duration of storage is not permanent because of slow leakage into circulating fluids accelerated by starvation and energy expenditure resulting in the mobilization and turnover of lipids.

Since in the second test period of Expt. No. 1 there was no longer any influx of methoxychlor into the lipid pool through absorption from the gut, see Figure 8-B, there was a shift in methoxychlor flux ($k_2 > k_1$) so that the stored methoxychlor could re-enter the plasma and be transported in the blood stream bound to the lipoprotein in order to re-establish an equilibrium. The MFO system, no longer able to cope with the elevated level of methoxychlor in the plasma allowed it to reach the various reaction sites. At high doses, methoxychlor acts in the same manner as DDT in altering the permeability of the nerve axon and interfering with the production of action potentials. It has also been suggested that methoxychlor can inhibit mitochondrial ATPase which is utilized in ATP synthesis (Gardner and Bailey 1975). Any reduction in ATP would seriously affect the energy requirements involved in the growth process.

While a decrease in fish density in the test tanks might have been responsible for the increase in growth of control fish during day 20 - 40 of Expt. No. 1, it must be remembered that the treated fish were also exposed to this

same density reduction yet their growth rate either remained constant or was reduced during this test period. These growth data represent to date the lowest concentrations of dietary methoxychlor found to have an effect on fish growth at oxygen saturation.

In general, reduced dissolved oxygen concentrations may restrict fish growth by influencing the metabolic rate. According to Beamish et al.(1975), the active metabolic rate of fish such as brook trout, Salvelinus fontinalis, and sockeye salmon, Oncorhynchus nerka, is dependent on dissolved oxygen to over 100% saturation but is independent of O_2 saturation for fish such as carp, Cyprinus carpio, largemouth bass, Micropterus salmoides, and goldfish, Carassius auratus; and basal metabolism is independent of dissolved oxygen over a wider range than active metabolism. Warren et al.(1973) reported that growth of juvenile coho salmon, Oncorhynchus kisutch, fed small restricted diets was not affected by low oxygen concentrations except at levels lower than 3 mg/L and Stewart et al.(1967) found that food conversion efficiency was reduced at oxygen concentrations of 4 mg/L and lower at 26°C .

Food conversion efficiency may have been reduced at the lowest oxygen levels in Expt. No. 2 but it seems that methoxychlor uptake was not affected by reduced oxygen levels as evidenced by the higher levels of residual methoxychlor at low oxygen saturation. Stewart et al.(1967)

also reported a lower content of lipids in the tissue of fish exposed to low oxygen concentrations (less than 4 mg/L at 26°C). Thus with a smaller lipid pool, the methoxychlor could not be stored to any great extent and levels in the plasma would rise. Oxygen along with a flavoprotein NADPH and cytochrome P 450, a heme protein are the principal components in the MFO system (Nakatsugawa and Morelli 1976)

$$XH + NADPH + H^+ + O_2 \xrightarrow{P\ 450} XOH + NADP + H_2O \quad (1)$$

This equation (Walker 1975) briefly summarizes the main events in the oxidation process. Any reduction in O_2 will hamper the effectiveness of cytochrome P 450 to catalyze the O-dealkylation of methoxychlor. Therefore a combination of increased levels of methoxychlor in the plasma ($k_2 > k_1$) and a reduced efficiency of the MFO system allowed methoxychlor to reach the reaction sites.

Kruzynski (1972) reported that brook trout fed dietary methoxychlor (0.01 - 2.00 mg/kg fish/day) retained between 40 and 50% of the administered dose while Oladimeji and Leduc (1975) obtained about 12% of the given dose of 0.67 mg/kg fish/day. In this present study, 1 - 2% was retained at oxygen saturation (Table 19). More methoxychlor was accumulated in fish treated with higher doses of dietary methoxychlor although the relative percentage of the administered dose remained constant.

Norstrom et al.(1976) have formulated a bioaccumulation

model equation in order to predict uptake and depuration of various pollutants in the environment.

$$dP/dt \approx AW^{0.7 \pm 0.03} - k_{cl}PW \qquad (2)$$

where W = body weight,
A = sum of all coefficients involved in uptake,
P = body burden of pollutant in fish,
k_{cl} = clearance coefficient.

In this equation, the rate of change of pollutant body burden in the fish is approximately equal to the rate of uptake from food and water minus the clearance rate. Their model equation makes use of various coefficients which represent factors (environmental modifications) which could alter metabolism and growth. While equation (2) cannot be applied to this study, it is quite clear that events which slow down the depuration rate allow a pollutant to accumulate to a greater extent. An increase in the percentage of methoxychlor stored from 1 - 2% at 95 % O_2 saturation to a maximum of 8.75% at 40 % O_2 saturation suggests that lower oxygen levels have reduced the depuration rate (k_3) by limiting the ability of the MFO system to metabolize methoxychlor.

The residue levels found in the liver of fish exposed to dietary methoxychlor (0.272 mg/kg fish/day) in this study (Table 20) represent 28 to 40% of the total body burden. This suggests that a large percentage of the body burden is undergoing metabolism by the various detoxifi-

cation mechanisms located in the liver.

The relation between fat content and residual methoxychlor storage is seen at 95 % O_2 saturation in this study. The fish in Expt. No. 2 contained nearly twice as much fat as compared with Expt. No. 1 and a greater amount of methoxychlor (absolute) accumulated in the fish in Expt. No. 2 . Roberts et al.(1977) also observed that tissue retention of chlordane in northern redhorse suckers, Moxostoma macrolepidtum, was directly proportional to the adiposity of the fish at the start of the experiment.

No reasonable explanation can be given for the marked variation among the fat gain results in both experiments. During day 0 - 20 of Expt. No. 1, the methoxychlor treated fish at 95 % O_2 saturation had much higher fat gains than controls but under similar experimental conditions in Expt. No. 2, the controls had significantly higher fat gains than the methoxychlor treated fish. Macek et al.(1970) reported increased lipogenesis in rainbow trout, Salmo gairdneri, exposed to DDT and dieldrin over 140 days and Buhler et al. (1969) also observed an increase in the lipid content of coho salmon, Oncorhynchus kisutch, with an accompanying increase in the concentration of dietary DDT. The variation of fat gain data in this study makes it very difficult to ascertain their significance.

The significant interaction between dietary methoxychlor and reduced oxygen levels (Table 9) in Expt. No. 1

may be explained by multiple toxicity. This term is usually applied to the interaction of two or more toxicants on an organism. Obviously oxygen is not a toxicant but a lack of oxygen can be just as detrimental to a fish as the effect of a toxicant. Multiple toxicity involves various physiological interactions which alter the sequence of events regarding the binding of a toxicant to the target tissue. These interactions may also alter the rate of uptake, metabolism and depuration of the toxicant (Anderson and d'Apollonia 1978).

The kinetic processes illustrated in Figure 8 have shown that reduced oxygen levels influenced the amount of methoxychlor in the plasma, the percentage of the given dose stored and reduced efficiency of the MFO system to metabolize methoxychlor. While the differences in growth due to exposure to methoxychlor and reduced oxygen levels have not been quantified, the growth curves in Figure 6 suggest an additive response to the interaction of methoxychlor and oxygen depletion. At 0.25 mg/kg fish/day methoxychlor reduced growth by about 3.5 % at 95 % O_2 saturation and reduced oxygen lowered growth of control fish by about 2.0% at 40 % O_2 saturation. The growth rate value at 0.25 mg/kg fish/day (40 % O_2 saturation) has been reduced by about 5.5% as compared with control fish at 95 % O_2 saturation.

Ecological significance

In nature, fish in streams and rivers undergoing larviciding operations to control blackflies are subjected to stress when the organochlorine pesticide methoxychlor is utilized. There is no consensus on whether exposure to a toxicant in the water or via the food chain will result in a greater body burden. Macek et al.(1977) have defined the term bioconcentration as the process in which toxicants can enter aquatic organisms through gills directly from the water while the term bioaccumulation includes bioconcentration and any uptake from dietary sources. Their study revealed that fish exposed to toxicants such as kepone, leptophos, di-2-ethyl hexyl phthalate (DEHP) and 1,2,4-trichlorobenzene (TCP) would obtain a majority of their body burden through bioconcentration. They have only considered the situation when the toxicant is in the water and in the food at the same time. Larviciding operations with methoxychlor may present fish which inhabit clean water with a contaminated food supply caused by the drift of dead and dying insects who have accumulated methoxychlor in their bodies. Thus investigating the effects of methoxychlor exposure via the diet only was a valid approach.

Reduced oxygen conditions occur in the environment due to organic pollution such as sewage waste and also in the hypolimnion of eutrophic lakes. The findings of this

study increase the necessity to control organic pollution and other causes of man-made reduced oxygen levels.

While this study has used concentrations of methoxychlor as found in insect residues as the basis for selecting the various intake levels, it is highly unlikely that fish would be continuously exposed in nature for 20 days duration. They however may be exposed for shorter periods at intermittent intervals since any blackfly control program requires repeated applications. Over the blackfly season they may be exposed to and accumulate more dietary methoxychlor than that utilized in this study.

The possibility that methoxychlor inhibits ATP synthesis would mean that energy required for feeding activity and in metabolism (conversion of food to body tissue) would be greatly reduced with the result of decreased growth and ultimately the reduction of the population.

This laboratory study suggests that low levels of dietary methoxychlor in combination with an environmental stress such as reduced oxygen could impair the growth of fish in streams and rivers undergoing larviciding operations. Growth is not the most sensitive parameter which can be measured, but if a toxicant is capable of reducing fish growth, it may affect more sensitive parameters such as reproduction. Dixon (1975) has shown with cyanide (HCN) that at concentrat-

ions which did not affect growth, inhibition of oogenesis (Lesniak 1977) and spermatogenesis (Ruby et al.1979) did occur.

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