Results of Short-term Exposure of Stream Organisms to Methoxychlor

James W. Merna
and
Paulette Queener

Fisheries Research Report No. 1852
November 17, 1977

MICHIGAN DEPARTMENT OF NATURAL RESOURCES
FISHERIES DIVISION

Fisheries Research Report No. 1852

November 17, 1977

RESULTS OF SHORT-TERM EXPOSURE OF STREAM ORGANISMS TO METHOXYCHLOR[1]

By James W. Merna and Paulette Queener[2]

Abstract

Experimental streams at the Saline Fisheries Research Station, Michigan Department of Natural Resources, were dosed with methoxychlor to simulate a blackfly control treatment. A series of six matched streams was used in the experiment. A 15-minute injection of methoxychlor was made into the treated streams. Two streams were treated at 200 μg/l, two at 600 μg/l, and two untreated streams served as controls.

An immediate increase in drift of benthic organisms was visually evident during treatment. Isopoda, Ephemeroptera, and Chironomidae were the most abundant organisms in the drift. Despite the significant increase in drift, the population of benthic organisms did not appear to be depleted. Hester-Dendy plate samplers showed no difference in macroinvertebrates between control and dosed streams following treatment.

Yellow perch (Perca flavescens) accumulated methoxychlor very rapidly in body tissues. Mesenteric fat samples from one of the high-level streams exceeded 200 μg/l 24 hours after dosing. The perch did not utilize the drifting benthos that were available during dosing, so it appears that the methoxychlor was not accumulated through the food chain.

Perch which were not sampled for tissue analysis remained in the streams for 3 months following dosing. The streams were then drained and all fish were collected. The perch in the high-level streams experienced a high, delayed mortality (42%) compared with perch in the low-level (18%) and control streams (8%).

---

[1] Contribution from Dingell-Johnson Project F-35-R, Michigan.

[2] Present address: Michigan State University, East Lansing, Michigan.

Introduction

Methoxychlor, a 2-bis (p-methoxyphenyl)-1, 1, 1-trichloroethane, is an analog of DDT that is biodegradable and has therefore been used in recent years as a DDT substitute. It has been used extensively for biting fly control in place of DDT. In eastern Canada, DDT was last used for biting fly control in 1968, and methoxychlor was introduced as a substitute in 1969 (Gardner and Bailey 1975). It has been used as an aerial larvicide for blackfly larvae, for ground treatment of blackfly-producing streams, and for fogging adult blackflies.

Doses of methoxychlor that have been found effective against blackfly larvae vary. In Saskatchewan River experiments, single 15-minute injections of 180 to 240 μg/l methoxychlor removed 75-99% of the larvae of Simulium arcticum from 23 to 34 km sections of the river. Injections of 300 μg/l for 15 minutes eliminated 98% of the larvae 64 km downstream, and 46% 139 km downstream. Plecoptera larvae were almost equally affected, followed by Ephemeroptera, Trichoptera, and Chironomidae in order of sensitivity (Gardner and Bailey 1975).

Populations of Chironomidae and Ephemeroptera reportedly recovered within 1 to 2 weeks, Trichoptera in 1 to 3 weeks, Plecoptera in 4 to 5 weeks, and Simulidae in 2 to 4 weeks.

Flannagan et al. (1975), as reported by Gardner and Bailey (1975), report an increase in drifting insect larvae after a 300 μg/l methoxychlor treatment. Trichoptera experienced a continued high drift rate for 4 days. The lethal range of methoxychlor to most fish is between 7.5 and 80.0 μg/l (Gardner and Bailey 1975; Merna et al. 1973). The dosages of methoxychlor used for blackfly larviciding (10-600 μg/l) covers the lethal range of all fish. Fish kills have not been associated with such treatments, probably because of adsorption by suspended solids (Freeden et al. 1975; Merna et al. 1972) and short exposure time. These levels may not cause direct mortality but may result in delayed mortalities or serious loss of benthic fish-food organisms.

The purpose of this experiment was to: (1) simulate as naturally as possible a stream treatment of methoxychlor for black fly control,

using concentrations commonly used in such operations; (2) monitor the degree of accumulation, the rate of accumulation and rate of decline in fish tissue; and (3) evaluate the effects of these concentrations on the fish and benthic organisms.

Methods

This experiment was conducted in a set of six identical stream sections at the Saline Fisheries Research Station, Michigan Department of Natural Resources. The streams are in pairs set in an old mill race (Fig. 1). They receive water from the Koch-Warner drain and empty into the Saline River. The water is piped from a small reservoir to a cement control box at the head end of the streams. Water entering the control box is filtered through a 30-mesh screen. Flow is measured as the water empties into the first pair of streams through triangular weirs; one for each stream. The weirs are at the same level, making the volume of flow the same for the two sets of streams. The first two streams are control streams and were not dosed. They flow into a mixing box where the desired concentration of methoxychlor is added and mixed with the water. Water then flows into the next two streams which are the low-level streams. These empty into another mixing box where the water is again dosed and mixed before flowing into the final two streams which are the high-level streams.

Water level in the streams is controlled by wooden planks stacked in slots in the mixing boxes. There are two sets of planks at the end of each stream. The downstream set maintains water level while the upstream set supports a plastic screen which prevents migration of organisms from one stream to another. Screens are also set at the head of each stream to prevent fish from moving upstream into the mixing boxes.

Each stream is 120 feet long and approximately 15 feet wide. The mixing boxes, which are approximately 20 feet long, also serve as fish collection boxes. Each mixing box is divided into two compartments (Fig. 1). As water moves from one compartment to the other, it passes

through a wooden box containing a series of baffles. Water flows into the bottom of these boxes where it is dosed with methoxychlor. Mixing is achieved as water flows around the baffles before exiting out the top of the box.

Before dosing, the streams were drained, the planks and screens reset, and debris removed from the bottom sediment. Water level was controlled so that there was a shallow riffle at the beginning of each stream, gradually getting deeper during the length of the stream, and ending in an approximately 3-foot pool just before emptying through the screen.

Each stream was stocked with 25 yellow perch (Perca flavescens), each approximately 50 mm in length. Fish that died due to handling were replaced within the next few days.

Dosing was set up to simulate a dosing procedure for black fly control. The low-level streams were dosed at 200 μg/l and the high-level at 600 μg/l, each for 15 minutes. Since the low-level streams empty into the high-level streams, the high-level streams were dosed at 400 μg/l. The cumulative dosings of the two sets of streams would thus equal 600 μg/l. The high-stream dosing was synchronized so that the water was being dosed at 400 μg/l just as the already dosed 200 μg/l water was flowing through the high-level mixing box. The time of passage through the streams is 20 minutes.

Water samples were collected in 500-ml BOD bottles with glass stoppers and analyzed for actual concentrations of methoxychlor during and after dosing. Upstream water samples were taken 10 and 45 minutes after dosing began. Downstream samples were collected at 30 and 60 minutes. Two composite samples, low streams combined and high streams combined, were collected 2 and 3 hours after dosing began.

Fish samples were collected by seining; one or two fish being taken from each stream. Fish samples were taken at 2 1/2 hours, 24 hours, 1 week, and 2 weeks after dosing. Crayfish were also taken from the streams 2 weeks after dosing.

Benthic drift samples were taken in approximately 1 foot of water with Surber square foot samplers. Samples were taken 30 minutes,

2 hours, and 24 hours after the start of dosing. Each sample collected drift for 30 minutes. Drift samples were also taken 8 days prior to dosing. Because there was so little drift at that time, samples were collected for 2 hours in the high north stream and for 1 hour in the high south.

Hester-Dendy multiple plate samplers also were used to collect macroinvertebrates before and after dosing. Samplers were placed in upstream and downstream locations of each stream. Upstream stations were in riffle areas with moderate current, while downstream stations were in pool areas of almost no perceptible current. Plate samples were collected from each station of the stream four times: prior to dosing, 3 hours after, 24 hours after, and 15 days after dosing. After the invertebrates were removed from the samplers they were preserved in 10% formalin solution. The samples were placed in 70% ethanol after preliminary sorting.

With the exception of the chironomid larvae, no special preparation was required for identification. The chironomid larvae were treated with 10% KOH to clear the head capsules and mounted on slides with CMC mounting media.

Water samples either were analyzed the day of collection or immediately refrigerated and processed within 24 hours. Five hundred milliliters of water were placed in a 1000-ml graduated cylinder with 50 ml of n-hexane (pesticide grade) and mixed for 1 hour with a magnetic stirrer. After mixing, the hexane was allowed to rise to the top. Ten milliliters of the hexane were extracted in a volumetric pipette and placed in a graduated centrifuge tube with a ground-glass stopper. Twenty microliters of the methoxychlor-hexane extract were injected into a gas chromatograph for analysis.

The gas chromatograph used was a Varian Aerograph, series 1200. The carrier gas was nitrogen and the column was 5 feet long, composed of 11% OV-17 + QF - 1 on 100/120 mesh gas-Chrom. Q. The method of detection was electron capture, and the peaks were measured by triangulation. All samples were run against a standard solution of methoxychlor.

Fish samples were frozen when collected and not thawed until they were analyzed. The fish were weighed and their lengths measured in centimeters. They were then autopsied and various internal organs were separated for analysis. In all cases, the intestinal fat was analyzed and in most cases the liver also. If the digestive tract appeared full, it was selected for analysis to see if the method of accumulating methoxychlor was through the diet. When the digestive tract was empty, a small piece of fillet was run as a substitute sample.

Three samples were run at one time from the same fish. If two fish were taken from a stream, their separate tissues were combined. Samples were placed in tarred glasses used with the tissue grinder and weighed. Samples were homogenized with the grinder in 30 ml hexane three times to extract the methoxychlor. Anhydrous sodium sulfate was added during grinding to absorb excess water. The extracts were filtered through filter paper and placed in separatory funnels. This solution was then extracted with 10-ml aliquots of redistilled acetonitrile saturated with redistilled hexane. The bottom layer was then drained directly into a 500-ml separatory funnel. The acetonitrile partitioning was repeated a total of three times and each time the bottom layer was drained into the second separatory funnel. After the three extracts were collected in the 500-ml funnel, 20 ml of redistilled hexane were added without mixing. One hundred milliliters of 10% NaCl solution were then added and mixed. The bottom layer was drained off (disposed of) and the hexane and NaCl wash was repeated. The remaining hexane layer was evaporated to 10 ml and placed on a prepared column for separation.

The column was prepared as follows: A small plug of glass wool was placed at the bottom of a 25-ml burette, 3/4- to 1-inch Na_2SO_4 was added, and the column was tapped gently to settle the contents. Five grams of Florisil were placed over the glass wool on the column. The Florisil had been previously heated in an oven to activate it, then mixed with water to make a 20% H_2O solution to slightly deactivate the Florisil. This was also packed lightly, then a 1/2-inch to 1-inch layer of Na_2SO_4 was added and packed. The entire column was then wetted with hexane.

The 10 ml of sample were added, allowed to drain to the top of the column, and followed with 85 ml of hexane. The first 10 ml of hexane to flow through were discarded and the remainder was saved until the column drained dry. The remaining 75 ml were evaporated to 10 ml and 20 μl were injected into a gas chromatograph for analysis.

Results

Water samples taken at upstream stations in the low-level streams contained methoxychlor concentrations higher than expected 10 minutes after dosing began (Table 1). This was probably due to uneven mixing that far upstream while dosing was still in progress. Forty minutes after dosing the concentrations upstream had lowered to 30 ppb or less, while 15 minutes later (60 minutes after dosing) the concentrations downstream were still comparatively high.

Concentrations in the high-level streams exhibited less variation between streams with levels closer to those desired. The initial concentrations (10 minutes) upstream were close to 600 ppb but dropped to around 200 ppb downstream 30 minutes after dosing. The 45-minute samples varied widely at the upstream stations. However, the 60-minute samples showed the two streams were fairly consistent in concentrations.

In both the low- and high-level streams, methoxychlor concentrations were reduced significantly by 2 hours, and the streams were completely flushed within 3 hours.

Of the various tissues analyzed from the fish samples no traces of methoxychlor were found in the fish taken from the control and low-level streams (Table 2).

Fish from the high north stream contained undectable methoxychlor levels in the intestinal fat in the 2 1/2-hour sample, 202.0 μg/l at 24 hours, and again undetectable in the 1-week and 2-week samples. Also in the 2 1/2-hour sample, a level of 43.0 μg/l was found in a partially full digestive tract with no reoccurrence in the remaining digestive tracts analyzed (1-week and 2-week samples). The liver showed a similar pattern with a level of 46.0 μg/l in the 2 1/2-hour sample and no reoccurrence.

In the south high-level stream, the concentration of methoxychlor in the intestinal fat from the 2 1/2-hour sample was 68.0 μg/l. The level increased to 154.0 μg/l at 24 hours and 410.0 μg/l at 1 week. Concentrations were undetectable at 2 weeks. Concentrations in the liver were 41.0 μg/l at 2 1/2 hours, but undetectable in the 24-hour and 1-week samples. There was a reoccurrence to a level of 116.0 μg/l in the 2-week sample. The digestive tract at 2 1/2 hours had a concentration of 32.0 μg/l but no other digestive tracts were analyzed because they were not full. The variations seen in methoxychlor concentrations in digestive tracts and fish tissues are probably due to varying feeding reactions to the increased drift resulting from dosing. Most stomachs, when examined, were empty or nearly so. The major food items were midge larvae, pupae, and unidentified adult insects.

Drift samples taken 8 days prior to dosing showed very little drift in any of the streams. The few organisms in the drift consisted of gastropods and chironomidae. The gastropods were almost certainly associated with drifting filamentous algae. There was a drastic increase in drift in the high-level streams approximately 30 minutes after dosing started (Table 3) but no increase in drift in the control or low-level streams. The organisms most affected were the isopods, tendipedids, and Ephemeroptera. Two hours after dosing the drift was back to the level exhibited prior to dosing.

The results of the enumeration and identification of the four sets of plate samples are summarized in Table 4. Chironomids and Asellus were the only organisms abundant on the plates. Ephemeropterans were only present on three of the 48 samplers yet they were present in all drift samples from the dosed streams. They were present on the samplers only immediately after dosing. Their presence at that time was probably the result of the high numbers in the drift at the time of dosing. The sample taken 15 days after dosing was greatly reduced in numbers and taxa. This is almost entirely due to the reduced chironomid population. Emergence of the adult midges may have been the cause for this reduction since the population was also reduced in the control streams.

Three months after dosing, the streams were drained and the surviving fish collected. Six fish had previously been taken from each control and high-level stream and five fish from each low-level stream for methoxychlor analysis. Based on the number of yellow perch stocked in each stream (25) less the number removed, the average percentages of mortality for the control, low-, and high-level streams were calculated at 8%, 18%, and 42%, respectively (Table 5).

Discussion

Variations in methoxychlor concentration in the water could have been due to uneven mixing or uneven flow at the time of sampling. However, most of the samples were consistent enough to assume a fairly even dosing close to the levels desired. It was also seen that significant concentrations of methoxychlor in the water were absent 3 hours after dosing. The sediments were not analyzed for methoxychlor concentration but it is possible that some was adsorbed by organic material in the sediments. It has been noted that disappearance of methoxychlor is much more rapid in water containing particulate matter and microorganisms (Gardner and Bailey 1975; Merna, Bender, and Novy 1972).

The concentrations in the low-level streams were not high enough nor present long enough to accumulate in the tissues of the fish. Also, there was no dramatic increase in benthic drift in the low-level streams as compared with the high-level. There was no difference in exposure time so concentration is the only parameter that could produce the differences in the methoxychlor accumulation in the fish tissues and the extreme benthic drift. The drift was readily visible from the surface and occurred within 30 minutes after dosing started.

We assumed that the fish would take advantage of the increased drift and feed heavily, thus attaining some of the methoxychlor through the digestive tract. This did not seem to happen, since most of the fish sampled 2 1/2 hours after dosing had very little food in their digestive tracts. Analysis of the stomachs showed the fish feeding primarily on midge larvae, pupae, and unidentified adult insects. These organisms

were not in the drift so the fish did not increase their feeding activity. Isopods constituted most of the drift, and they were not taken by the perch. Even though the perch did not utilize the drifting organisms, they did accumulate methoxychlor in body tissues.

Concentrations tend to be high in the liver due to its function as a filter of toxic substances. Following storage in the liver, methoxychlor is metabolized by means of multifunction oxidase enzymes (Gardner and Bailey 1975). The highest accumulation in fish tissue was 410 ppb in the intestinal fat of a perch from the high-level south stream 1 week after dosing. The levels in the fat followed a regular pattern of increasing accumulation up to 1 week after dosing then a rapid decline within a week. This was expected since fish, like most vertebrates, are able to metabolize methoxychlor (by the processes previously mentioned) and excrete the derivatives. Burdick et al. (1968) found 41.3% loss of the methoxychlor in fish tissue in 1 week and complete elimination within 36 days in a pond system.

Even with the ability to metabolize methoxychlor, the perch experienced a high average mortality (42%) in the high-level streams. This is compared to only 8% average mortality in the control streams and 18% average mortality in the low-level streams. No dead fish were seen in the streams, and it is presumed that there was a considerable delay in the mortality following dosing.

The Hester-Dendy samplers indicated there was little loss of organisms despite the excessive rate of drift resulting from dosing. In a few cases, molluscs have initially concentrated methoxychlor to the same or higher levels than observed for DDT but are themselves generally insensitive to the pesticide (Gardner and Bailey 1975). On all plate samples examined, Physa was thriving and reproducing.

The isopod present in the samples, Asellus miliataralis, like the snails, continued to thrive and reproduce after their initial drifting reaction to the injection of the methoxychlor. This is important as it has been suggested that, although drifting organisms are not physically dead, they may be ecologically dead as they are removed from their habitats.

The only effect on the chironomid population also was the drift immediately following injection. No genera were eliminated from any of the samples and numbers remained relatively stable until the last sample on August 26, 1976, when a decrease was noted. As stated previously, the stage in the life cycle most likely caused this decline as the decline was similar in the control streams.

The extensive drift of benthos and delayed mortality of fish seen in this study indicate that there is a severe potential danger of using methoxychlor as a stream larvicide for control of biting insects. The increased drift did not deplete the benthic community, however blackfly control in trout streams might be more damaging. Trout stream benthos consist of mayflies, stoneflies and caddisflies which are more sensitive to toxicants than the isopods that constituted the majority of the drift in our streams.

It is also significant that the delayed mortality of fish would probably be unnoticed in a natural stream treatment.

Table 1.--Measured concentrations of methoxychlor ($\mu g/l$) in streams during and following dosing.

Dose, stream, and station	Time in minutes from start of dosing					
	10	30	45	60	120*	180*
Low North						
Upstream	380		30			
Downstream		280		110	60	0
Low South						
Upstream	370		0			
Downstream		20		150		
High North						
Upstream	590		3			
Downstream		230		290	190	0
High South						
Upstream	540		260			
Downstream		130		380		

* Composite samples of water from both north and south streams, collected downstream.

Table 2.--Methoxychlor concentrations in fish tissue ($\mu g/l$).

Dose, and stream	Time after dosing			
	2.5 hrs	24 hrs	1 week	2 weeks
Mesenteric fat				
Control				
North	0	*	0	*
South	*	0	*	*
Low				
North	0	*	0	*
South	0	0	0	*
High				
North	0	202	0	0
South	68	154	410	0
Liver				
High				
North	46	0	*	0
South	41	0	0	116
Digestive tract				
High				
North	43	*	0	0
South	32	*	*	*
Crayfish				
Low				
North	*	*	*	0
South	*	*	*	0

* Data not available.

Table 3.--Number of organisms in drift samples from high-level streams before and after dosing.

Dose, stream, and organism	8 days prior to dosing	Time after dosing		
		0.5 hr	2 hrs	24 hrs
	2-hr sample	30-minute samples		
High				
North				
Chironomidae	0	24	1	21
Corixidae	1	29	6	0
Ephemeroptera	0	29	6	0
Gastropoda	5	12	2	28
Isopoda	2	137	5	0
	1-hr sample	30-minute samples		
High				
South				
Chironomidae	17	16	0	5
Corixidae	2	22	2	0
Ephemeroptera	0	130	7	0
Gastropoda	16	1	0	0
Isopoda	3	154	1	7

Table 4.--Number of taxa and total number of organisms present on Hester-Dendy plate samplers prior to and at various times following dosing with methoxychlor.

	Control		Low-level		High-level	
	Up-stream	Down-stream	Up-stream	Down-stream	Up-stream	Down-stream
Prior to dosing						
Number of taxa	12	4	6	7	10	7
Total number of organisms	106	121	180	135	104	120
3 hrs after dosing						
Number of taxa	7	4	10	8	7	12
Total number of organisms	112	26	233	112	103	121
2 days after dosing						
Number of taxa	11	9	7	10	10	10
Total number of organisms	184	88	324	126	201	205
15 days after dosing						
Number of taxa	3	2	5	6	5	5
Total number of organisms	28	15	140	166	298	350

Table 5.--Fate of 25 fish stocked in each stream as a result of dosing with methoxychlor.

Dose, and stream	Number		Percent mortality	Average percent mortality
	Sampled for analysis	Collected 3 months after dosing		
Control				
North	6	19	0	8
South	6	15	16	
Low				
North	5	13	28	18
South	5	18	8	
High				
North	6	5	56	42
South	6	12	28	

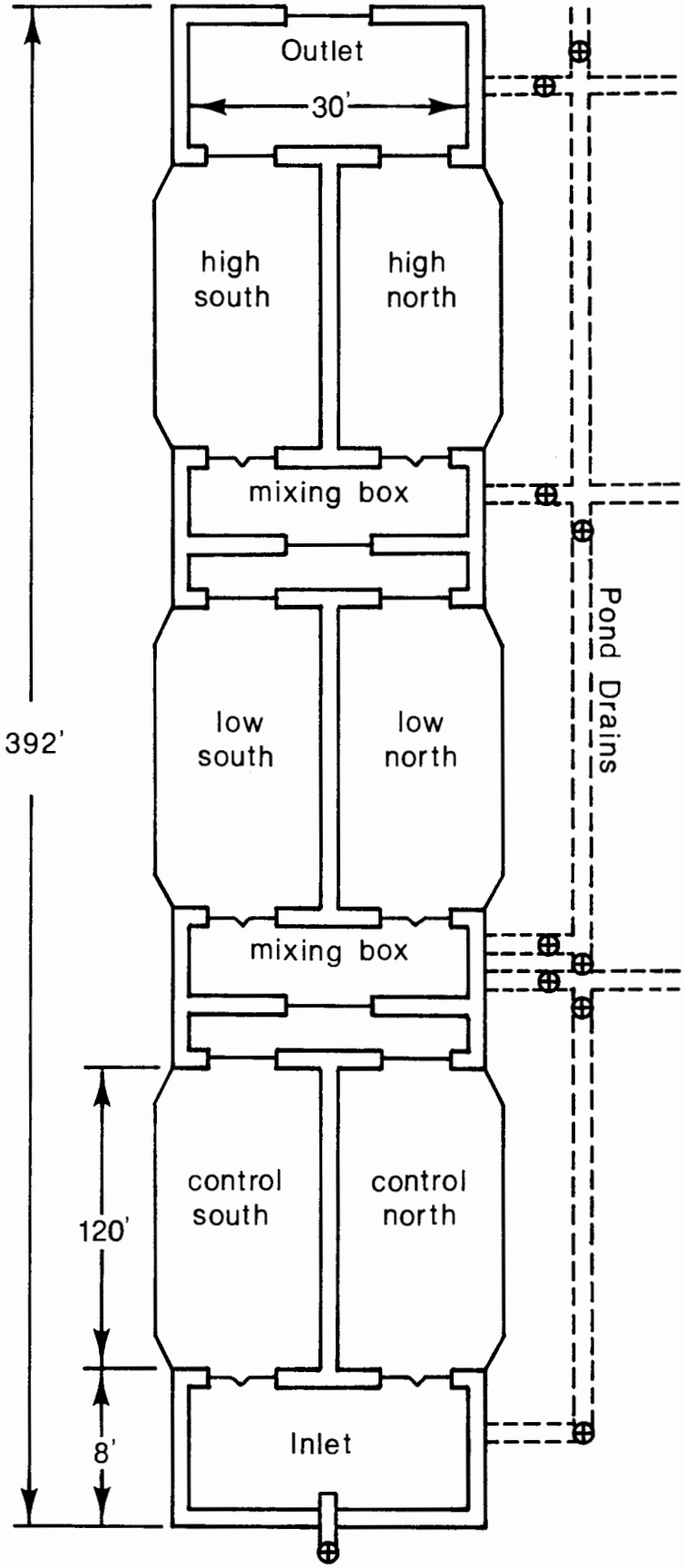

Figure 1.--Experimental streams at Saline Fisheries Research Station, Saline, Michigan

Literature cited

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 Game J. 15(2): 121-142.

Flannagen, J. F., M. Friesen, S. Leonhard, B. de March, and B. Townsend. 1975. Preliminary report on studies to determine the effects of methoxychlor on aquatic invertebrates of the Athaleasha River, Alaska. (manuscript, 39 pp.)

Fredeen, F. J. H., J. G. Saha, and M. H. Balba. 1975. Residues of methoxychlor and other chlorinated hydrocarbons in water, sand and selected fauna following injections of blackfly larvicide into the Saskatchewan River, 1972. Pestic. Monit. J. 8(4): 241-246.

Gardner, D. R., and J. R. Bailey. 1975. Methoxychlor: its effects on environmental quality. National Research Council of Canada, NRCC No. 14102.

Merna, J. W., M. E. Bender, and J. R. Novy. 1972. The effects of methoxychlor on fishes. I. Acute toxicity and breakdown studies. Trans. Am. Fish. Soc. 101: 298-301.

Merna, James W., and Paul J. Eisele. 1973. The effects of methoxychlor on aquatic biota. U.S. Env. Protection Agency, EPA-R3-73-046, pp. 1-59.

Report approved by W. C. Latta

Typed by M. S. McClure