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THE EFFECTS OF METHOXYCHLOR ON FISHES.[1] I. ACUTE TOXICITY AND BREAKDOWN STUDIES.

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Numerous studies in the last several years (Burdick et al., 1964; Peakall, 1970; Reinert, 1969; and Wurster, 1968) have shown a variety of environmental problems attributable to the widespread use of DDT. Interpretation of these studies, along with aroused public concern, has in some states resulted in either the complete banning of DDT (Abelson, 1969) or in restricting its use to occasions when the public health of an area is endangered (Kramer, 1969). The major compound now recommended, in place of DDT, for the control of such problems as Dutch elm disease is methoxychlor. The reasons for its selection are its: (1) similar potency for many insects; (2) cost is less than many phosphate substitutes; and (3) metabolism by

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warm-blooded animals is quite rapid (Menzie, 1969). However, virtually nothing is known of its possible effects in the aquatic environment; i. e., no studies have been made on its persistence in natural waters or its chronic toxicity to aquatic animals.

We are conducting a series of experiments designed to determine the potential dangers which may result from increased use of methoxychlor in the environment. These investigations include studies on: (1) the half life of the compound in various aquatic environments; (2) acute bioassay experiments with two species of fish--the fathead minnow (Pimephales promelas) and the yellow perch (Perca flavescens); (3) chronic laboratory and field bioassays utilizing the same two species; and (4) primary productivity experiments with natural phytoplankton populations. In this paper we present data on the first two phases of the study, i. e., breakdown experiments and acute toxicity studies.

Methods

Several authors (including Henderson et al., 1959) have indicated a lack of influence of water quality parameters, e. g., pH, alkalinity, and hardness, on the toxicity of chlorinated hydrocarbons to fishes. However, the breakdown rate of many insecticides has been found to be dependent on factors such as pH (Muhlmann and Schrader, 1957) and the presence of suitable microorganisms (Mendel et al., 1967). Therefore, our experiments on breakdown

were conducted using a variety of test waters: (1) distilled water buffered with phosphate to pH 7 and 9; (2) water from Koch Warner Creek at Saline, Michigan, which serves as the water source for our experiments on chronic toxicity; (3) aged Ann Arbor tap water which had previously held fish; and (4) water from Third Sister Lake which contained plankton. The chemical characteristics of these waters are given in Table 1.

In all experiments the methoxychlor was added to the test water from stock solutions containing ethanol and sufficient Triton X-100 to assure solution of the insecticide in the water. Highest concentrations were 500 mg/l ethanol and 0.10 mg/l Triton. Methoxychlor was extracted from the water samples with hexane, using an extraction impeller driven by a magnetic stirrer. Quantitative determination of methoxychlor was made on a gas chromatograph equipped with an electron-capture detector and a 1/8-inch by 6-foot stainless steel column packed with 5% QF1 on Varaport 30. The hexane extracts were dried with sodium sulfate and an aliquot was injected into the chromatograph. Peak areas were compared to those of standards which were run at the same time.

Four replicated 96-hour static bioassay tests were conducted, two with fathead minnows and two with perch. All tests were run in 10-gallon aquaria containing 30 liters of water. Ten fatheads or five perch were used in each aquarium. All static tests were conducted with aged Ann Arbor tap water. Continuous-flow studies were run on fatheads in Ann Arbor tap water, and on perch in water from Koch

Warner Creek. The dosing apparatus utilized in these studies was a modification of the unit described by Mount and Brungs (1967).

Results

Breakdown studies

Figure 1 shows the rate of breakdown of methoxychlor in distilled water in relation to pH. As can be seen from the figure, the half life of the compound had not been attained in 220 days. The half life estimated from these data is 270 days. Hydrogen ion concentration within the range studied (ph 7-9) had no effect on the breakdown rate. Figure 2 depicts the rate of breakdown in aged Ann Arbor tap water which had previously held fish. The half life in this case was 8 days. The results from two experiments conducted with water from Koch Warner Creek are shown in Figure 3; here, in each test, two different loss rates are apparent in the data. The initial high rate was presumably due to the adsorption of methoxychlor on particles which settled and were missed in the sampling procedure. This hypothesis is supported by the study (Fig. 4) conducted with Third Sister Lake water, where in two experiments the majority of the methoxychlor was located in the particulate fraction. Measured half life was different in the two experiments; it was 7 days in the June experiment, and (by extrapolation) 18 days in the July trial.

Toxicity studies

The static 96-hour TL_{50} values obtained during the experiments were 7.5 μg/l for fatheads, and 30.0 μg/l for perch. There are sizable differences in susceptibility of fish species to hydrocarbon insecticides. However, the results from static tests particularly with larger fish are often suspect. Our static tests on perch substantiate the weakness of these tests, as shown in Table 2, where the nominal dose and the measured concentrations of methoxychlor are given. It is obvious that the perch were exposed to the nominal concentration for a very short period of time.

Figure 5 shows the change in TL_{50} with time during the continuous-flow test for the fathead minnow. These results are averages of two replicate tests, involving two test chambers with ten fish per chamber at each concentration. Spot checks for methoxychlor run on the high-dose and low-dose levels at the start and end of the experiment indicated that the nominal doses were within ±10% of the measured concentrations. These results substantiate the 96-hour static TL_{50} of 7.5 μg/l. This TL_{50} was much lower than lethal levels given in the literature. Henderson et al. (1960) reported the TL_{50} for fathead minnows to be 35 ppb in soft water, and 64 ppb in hard water. The difference may be due to our use of a wetting agent to achieve better solution of methoxychlor.

Two 96-hour TL_{50} tests were run on perch. Eight fish per tank were used in Test No. I, and 10 per tank in Test No. II. Table 3

gives a summary of the nominal doses, the measured concentrations, and the accumulative mortality during the 96-hour tests. These results indicate that the 96-hour TL_{50} for perch is about 20 ppb. All fish died at 40 ppb, and 6 of 8 fish died at 20 ppb; however, there was no mortality at 15 ppb or lower levels. These results indicate that there may be a very narrow tolerance level below which perch are able to metabolize methoxychlor with no mortality.

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INSTITUTE FOR FISHERIES RESEARCH

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Table 1.--Chemical characteristics of test waters

Water source	pH	Alkalinity (mg/l)	Hardness (mg/l)	Temperature (°C)
Koch Warner Creek	8.2	180	400	20 ± 2
Third Sister Lake	8.5	60	80	24 ± 1
Distilled	7 and 9	1	1	20 ± 2
Ann Arbor tap water, aged	7	40	60	20 ± 2

Table 2.--Concentration of methoxychlor in 30 liters of water during the exposure of five yellow perch (approximately 50 g total weight) for 96 hours

Time (hours)	Nominal concentrations	Methoxychlor (μg/l)		
		40	30	20
4	Measured concentrations	35.4	21.4	15.5
24	Measured concentrations	15.4	12.3	8.3
48	Measured concentrations	5.1	2.1	2.2
96	Measured concentrations	2.0	1.3	1.0

Table 3.--Nominal and measured concentrations of methoxychlor and number of yellow perch that died during 96-hour continuous flow toxicity study (eight perch per tank were used in Test I and ten in Test II)

Time (hours)	Nominal concentrations	Methoxychlor (μg/l), and mortality						
		Test I				Test II		
		40	20	10	5	30	15	7.5
24	Measured concentration	41.4	20.7	9.6	4.6	26.9	12.9	7.1
	Accumulative mortality	7	0	0	0	5	0	0
48	Measured concentration	32.0	19.0	9.4	4.7	26.6	12.5	6.3
	Accumulative mortality	8	0	0	0	9	0	0
72	Measured concentration	39.6	21.4	9.0	2.7	27.1	11.4	6.5
	Accumulative mortality	8	2	0	0	9	0	0
96	Measured concentration	-	-	-	-	28.4	11.5	5.8
	Accumulative mortality	8	6	0	0	9	0	0

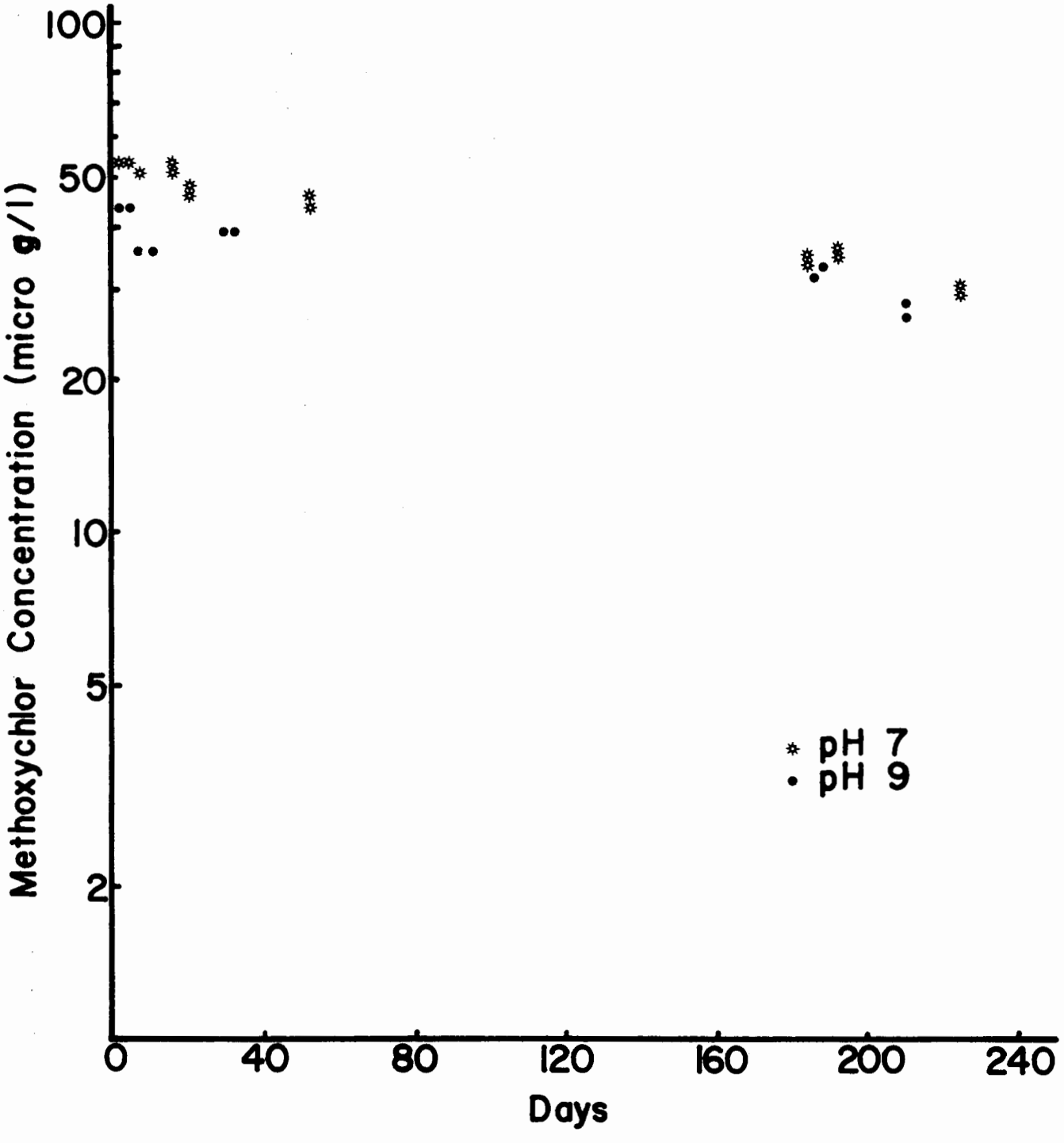

Figure 1. --Concentration of methoxychlor in replicated samples of buffered distilled water at pH 7 and pH 9 during 220 days of hydrolysis.

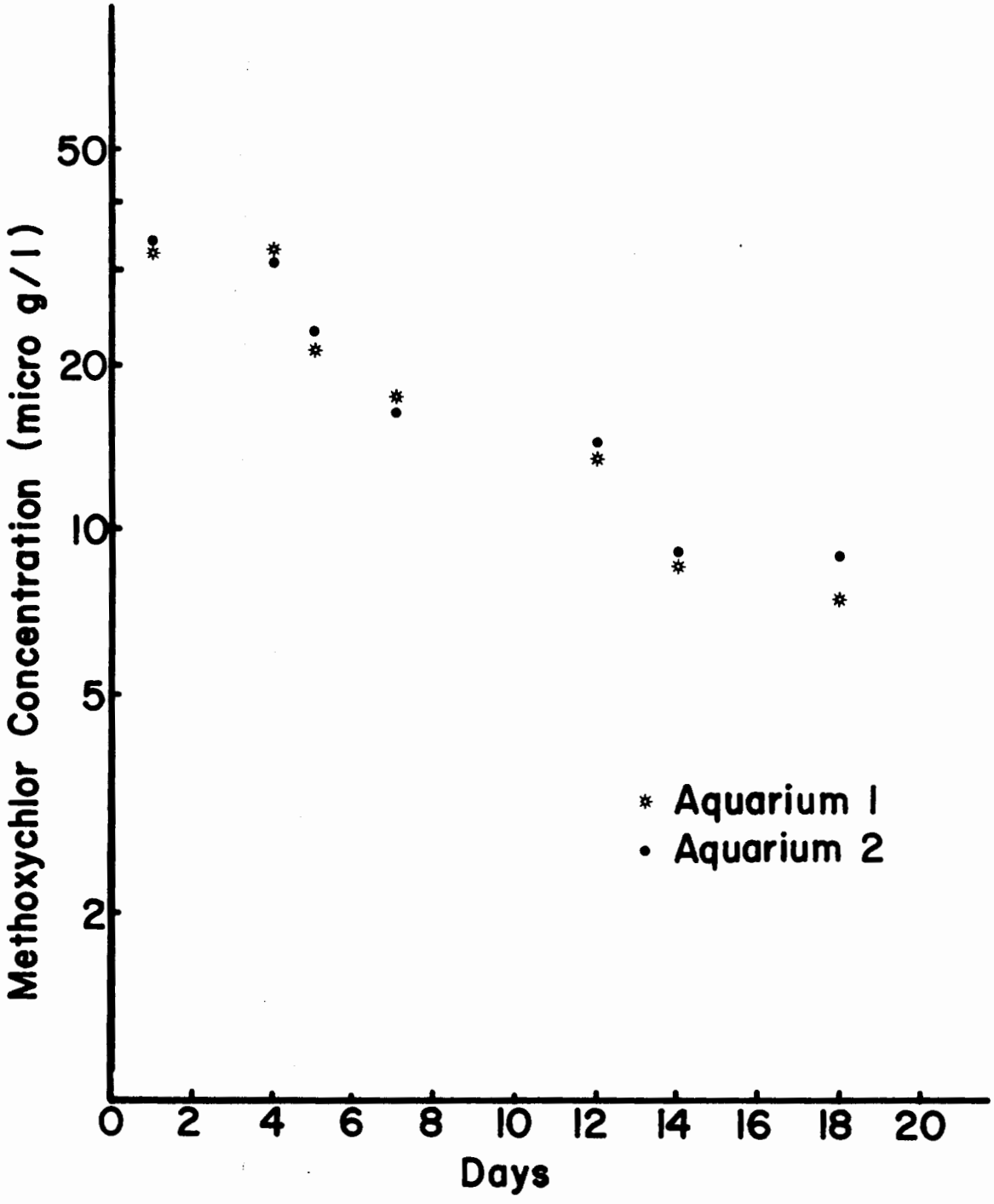

Figure 2. --Concentration of methoxychlor in replicated samples of aged Ann Arbor tap water, which had previously held fish, during 18 days of breakdown.

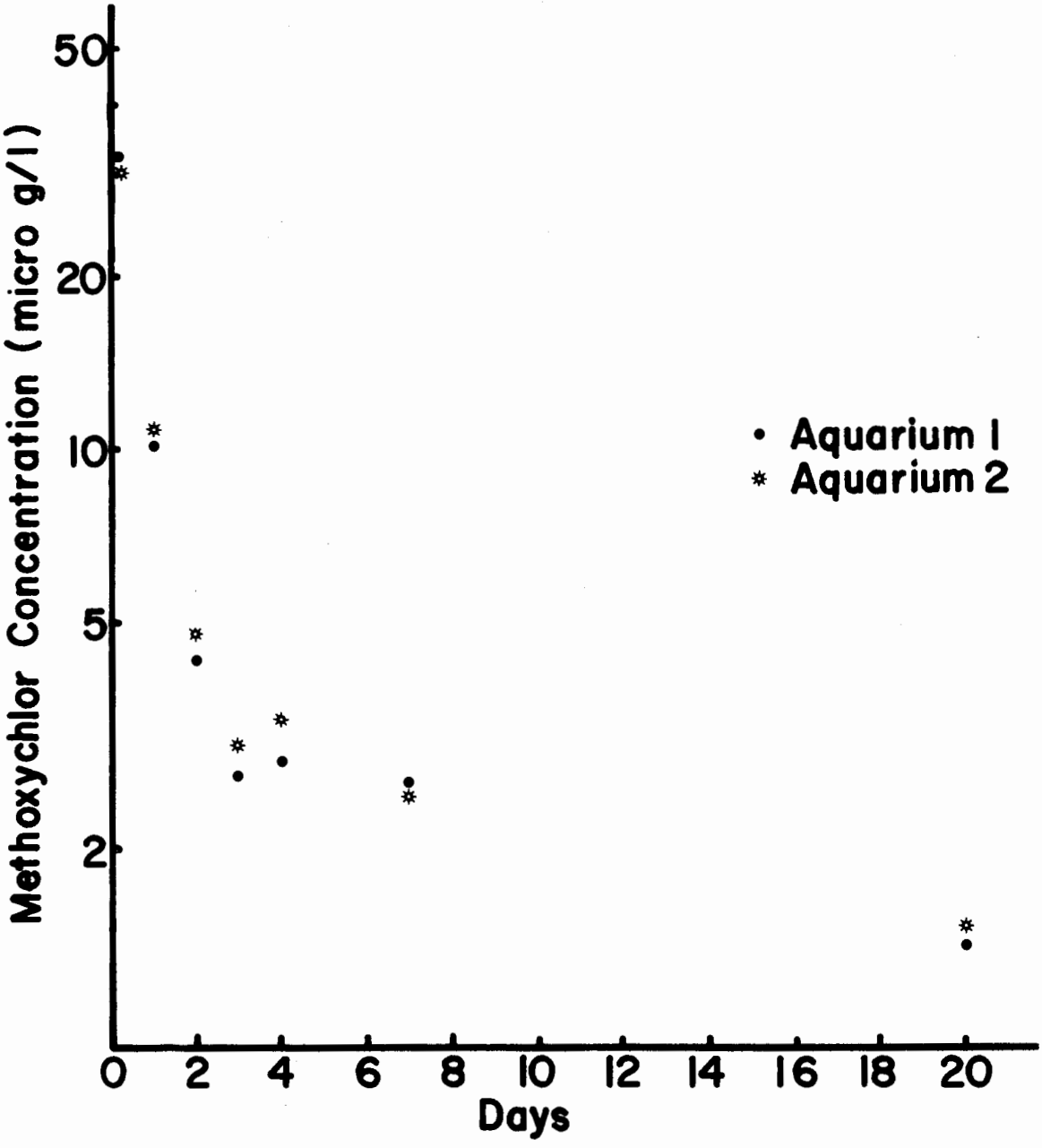

Figure 3. --Concentration of methoxychlor in replicated samples of Koch Warner Creek water during 20 days of breakdown.

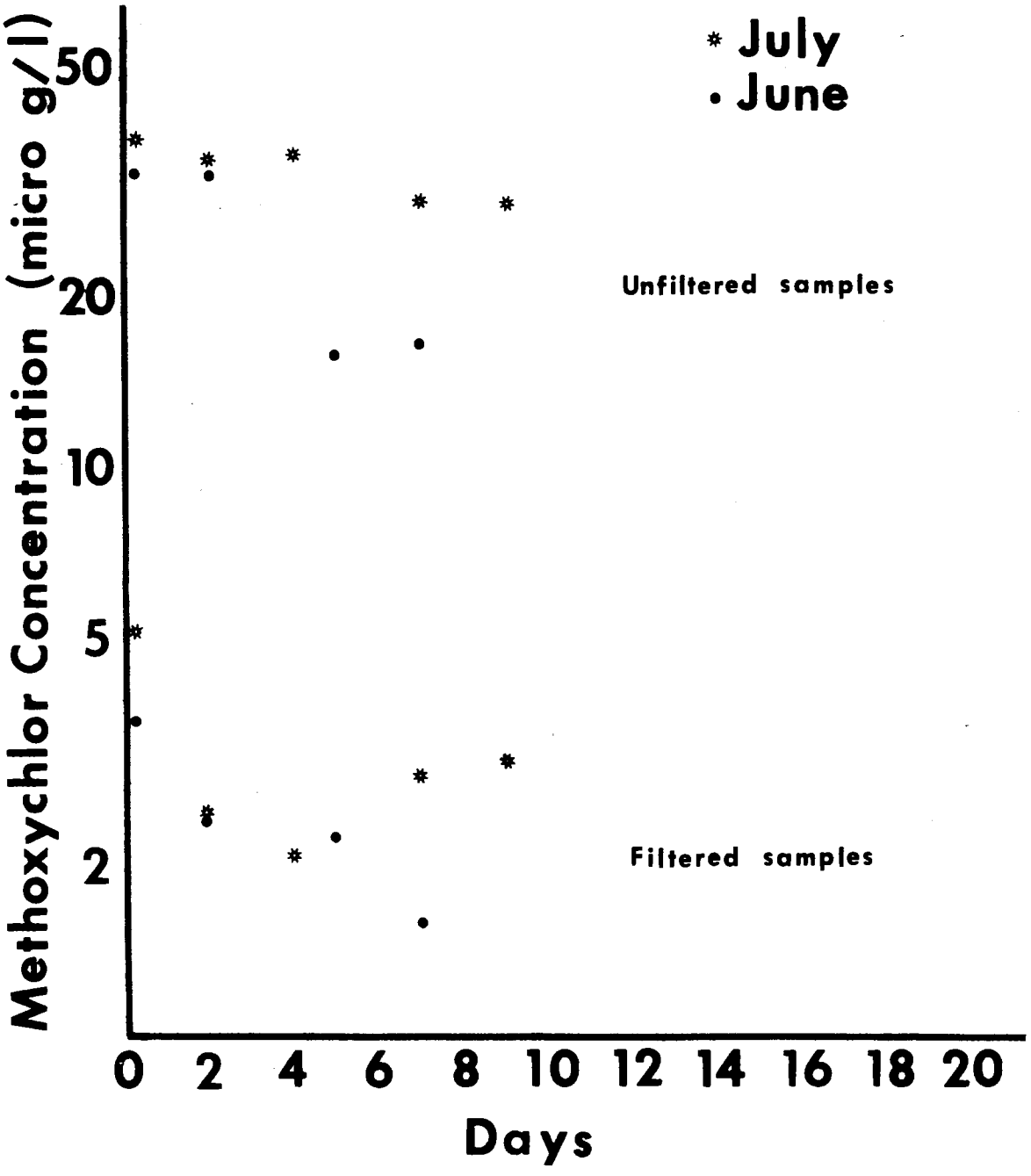

Figure 4. --Concentration of methoxychlor in filtered and unfiltered samples of Third Sister Lake water during 10 days of breakdown.

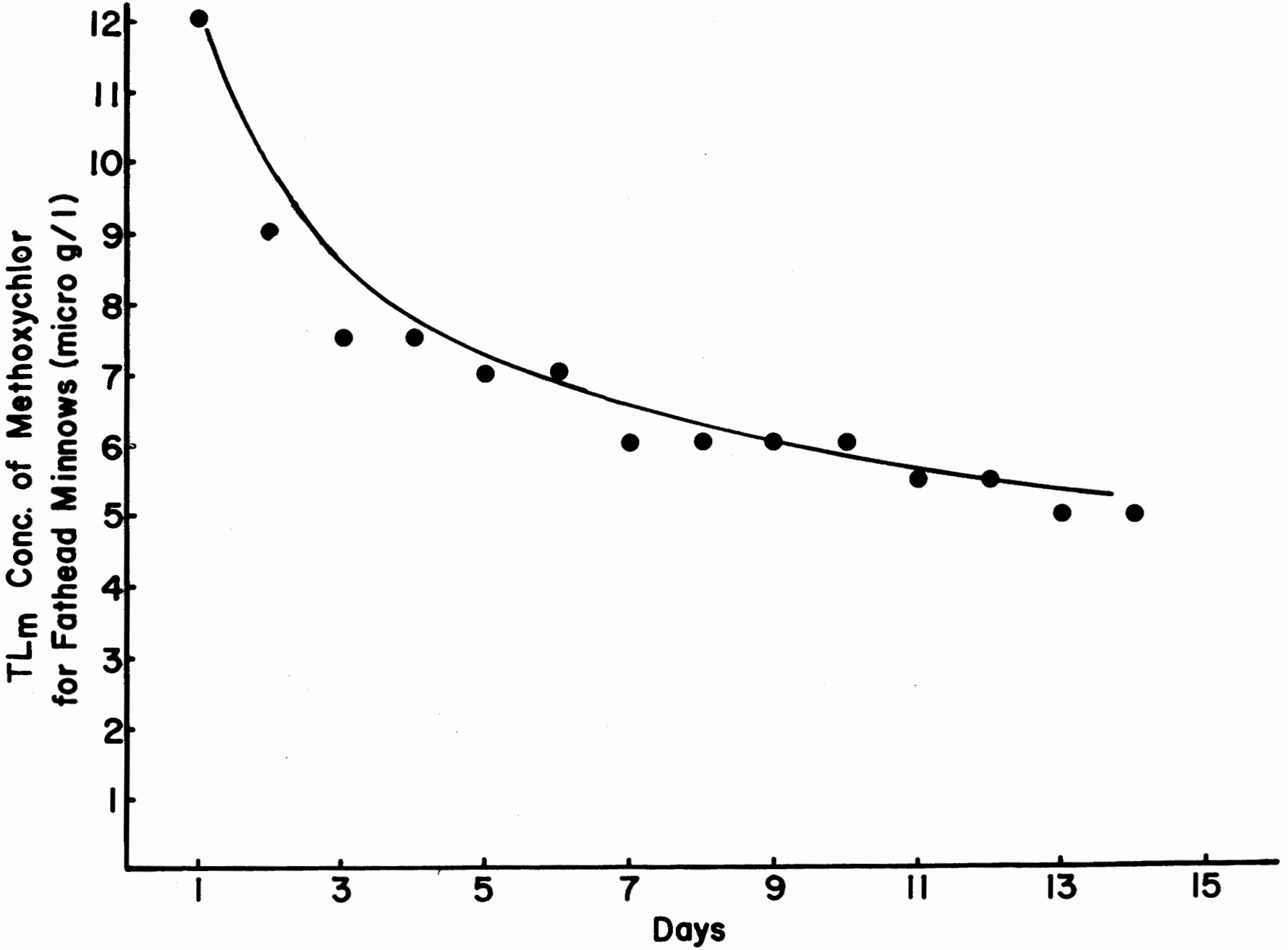

Figure 5. --Changes in median tolerance limit of methoxychlor for fathead minnows with time of exposure.