Jerry Lee Hamelink

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THE DYNAMICS OF DDT IN THE LENTIC ENVIRONMENT

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THE DYNAMICS OF DDT IN THE LENTIC ENVIRONMENT

By

Jerry Lee Hamelink

AN ABSTRACT OF A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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Department of Fisheries and Wildlife

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ABSTRACT

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Jerry Lee Hamelink

The fate of DDT in the water of lentic ecosystems was studied in a productive farm pond and in three artificial pools. The effect of fish and food organisms on the degradation and distribution of DDT was assessed in the pools by placing both trophic levels in one pool, just food organisms in another pool and fish without food organisms in a third pool.

Methods for collection, preparation, cleanup and analysis of samples from lentic ecosystems for DDT residues were developed. Gas chromatographic methods for residue analysis were evaluated and refined.

DDT applied to the experimental units was lost from the units, apparently by codistillation with evaporating water. DDT in the water was rapidly taken up by the flora, fauna and sediments. DDT deposited on the bottom was degraded to DDD. Both DDD and DDT were recycled back into the water from the bottom.

Invertebrates and fish degraded DDT to DDE. Biological transfers of DDT facilitated its degradation to DDE.

Increasing the biological productivity of the lentic ecosystem appeared to promote DDT degradation to DDE.

The concentration of DDT-R (DDT, DDD and DDE) persisting in the flora and fauna was mediated by the concentration of DDT-R in the water. DDT-R was concentrated from the water about 1×10^3 times by algae, 1×10^4 times by invertebrates and 1×10^5 times by lean fish.

A mechanism to account for the process of biological magnification of chlorinated hydrocarbons in lentic ecosystems was proposed. The mechanism is based on the principle that the compounds are exchanged between water and fats. Exchange in fish passes through two stages, from water to blood and from blood to fats, hence a high degree of magnification is possible in fish. The mechanism accounts for the reported observations that pesticides are excreted by fish, that the body load of pesticides increases as fat content of fish increases, that pesticide magnification by fish is inverse to water solubility of the compounds, and that pesticides persist longer in oligotrophic ecosystems than eutrophic lentic ecosystems.

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The facilities of the Lake City Experimental Station were used for the experiments.

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INTRODUCTION

History of DDT:

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (Figure 1) was recognized as a powerful insecticide by Muller and his co-workers in 1939 (Frear, 1955). Widespread use of DDT against insects soon followed and grew until at least 3.5×10^9 pounds of DDT have been used world wide (Westlake and Gunther, 1966). Undesirable biological effects of DDT soon became recognized (Carson, 1962). The low water and high fats solubility, stability and action of DDT had combined to pose a serious, insidious, environmental pollution problem. Recently action has been initiated to curtail the use of DDT and other chlorinated hydrocarbon insecticides (Mitchell, 1966). However, the manner in which DDT is biologically magnified in a lentic environment, despite years of study, has only been conjectured. Since much remains to be known, and because problems of a parallel nature may develop with many other useful products or waste products, a study into the behavior of DDT in lentic environments was proposed.

Figure 1. Chemical structure of pp DDT and degradation products pp DDE, pp DDD, pp MDE and pp DDA.

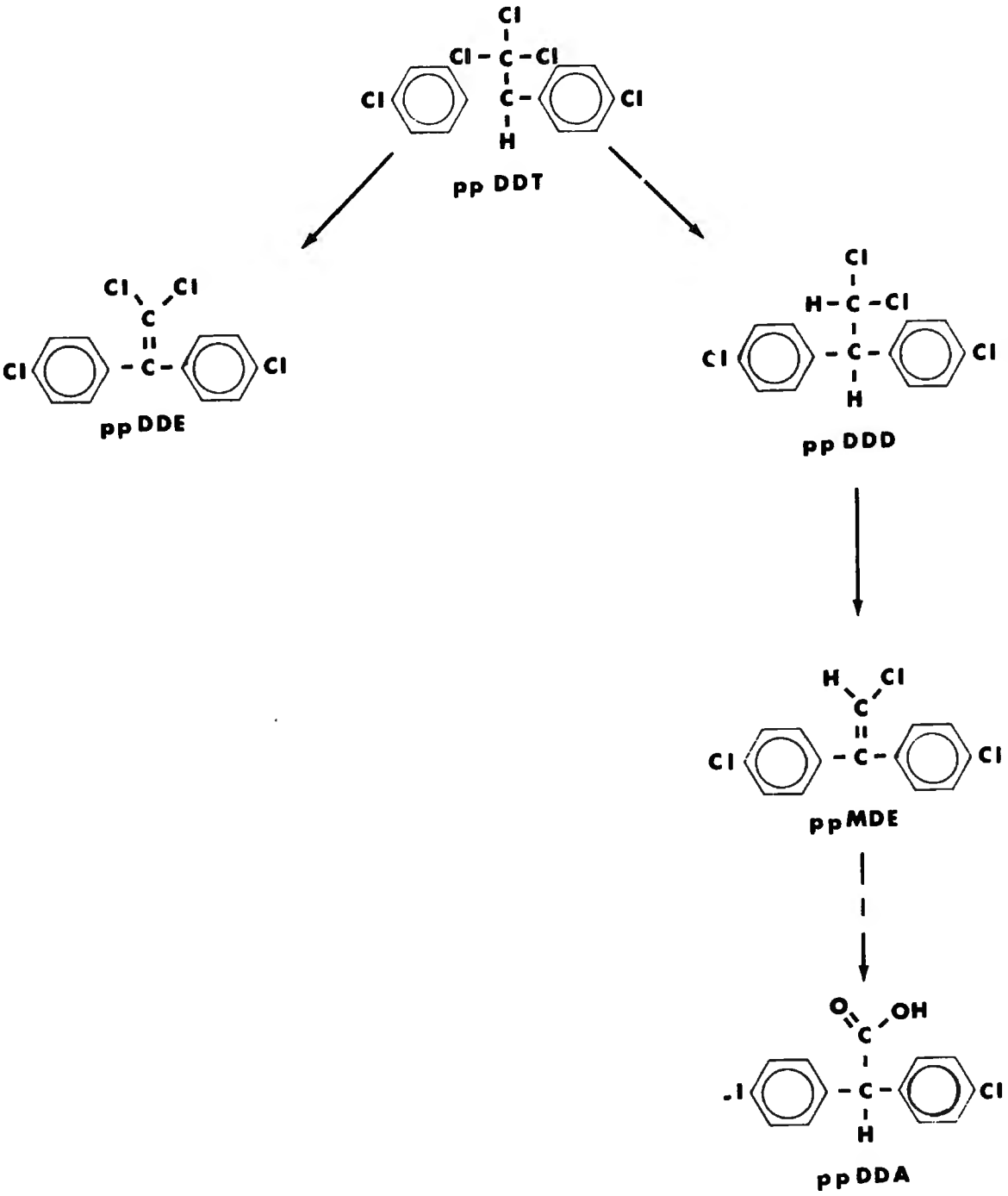


Figure 1

The Problem Posed by DDT in Lentic Environments:

DDT may be super concentrated from water by members of an aquatic biota and the consequences of this process may be disasterous. Hunt and Bischoff (1960) reported delayed mortality of water birds due to biological magnification of DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane) which had been applied to a lake for midge control. Burdick (1964) attributed hatchery losses of lake trout fry (Salvelinus namaycush) to DDT residues arising from watershed practices. Anderson and Everhart (1966) implicated DDT contamination as the major cause of poor salmon fishing in a Maine lake. Hickey et al. (1966) described the degree and possible consequences of DDT residue contamination in Lake Michigan. Their findings were reinforced when Johnson and Pecor (1969) reported DDT residues were a possible cause for the mortality observed in young coho salmon (Oncorhynchus kisutch) from Lake Michigan.

DDT and related degradation products DDD and DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethene), collectively designated as DDT-R (Ware et al., 1968), pose ecological problems far greater than just direct toxicity. The mode of toxic action of DDT and similar organo-chlorine compounds was stated by Wigglesworth (1955) to be unknown and so restated by O'Brien (1964). This still appears to be the case although research is contributing much to our knowledge of

its action. However, DDD and DDT may inhibit adrenal cortical tissue (King, 1962) and DDE may stimulate microsomal oxidase enzymes in the liver (Hart and Fouts, 1965). Thus by acting together, DDT compounds may disrupt normal hormone metabolism with catastrophic effects (Hickey and Anderson, 1968). Non-specific subtle effects, including alterations in temperature selection (Ogilvie and Anderson, 1965), growth and stress tolerance (Macek, 1968) have also been reported in fish exposed to DDT.

Biological magnification of DDT-R has been attributed to concentration through the food chain by a number of authors (Hunt, 1966; Rudd, 1964; Woodwell, 1967a) and (Woodwell et al., 1967b). Magnification in terrestrial ecosystems and in birds is certainly due to transfers through various food chains, but the mechanism involved in lentic environments is less clear (Westlake and Gunther, 1966). Controversy arises because fish may acquire DDT-R residues by eating contaminated food (Allison et al., 1964) or directly from the water (Holden, 1962). Thus, biological magnification may result from the DDT-R being concentrated from the food, the water or both.

This study proposes to establish the mechanism which controls the degree DDT-R residues may be biologically magnified by fish in a lentic environment. The manner and rate DDT-R residues enter and leave the ecosystem will govern the

supply of residues available for concentration by the biota. How and where DDT is degraded will determine what residues are magnified. How the residues are taken up, stored and lost by an organism will determine the degree of magnification.

The distribution and degradation of DDT in the flora, fauna and sediments of a small farm pond was studied first. DDT was mixed into the pond water dissolved in a small amount of acetone. Following application the water, flora, fauna and sediments were sampled and analyzed for DDT-R residues, for a period of 15 months.

Four artificial pools were then used to study the interaction of fish and food organisms on the distribution and degradation of DDT placed in the water. Three pools were treated with DDT and the fourth served as the control. The first treated pool and the control pool contained a sand hydrosoil, algal periphyton, invertebrates and fish. Fish were excluded in the second treated pool, so it contained just a sand hydrosoil, algae and invertebrates. Finally, invertebrates were almost completely excluded in the third treated pool, while fish, algae and a sand bottom were introduced. The various components in the pools were sampled for a period of 60 days following the addition of DDT.

The chemical stability and water solubility of DDT are unique for an organic compound. DDT is very resistant to oxidation and most strong acids (Frear, 1955).

On the other hand, it may be reduced to DDD by a variety of agents (Miskus et al., 1965) and dechlorinated to DDE by alkali (Frear, 1955) or by enzymatic action (Sternburg et al., 1954).

DDT is almost insoluble in water. Saturation is reached at about 1.2 ppb (Bowman et al., 1960). Concentrations exceeding 0.1 ppb dissolved in the water of lakes have rarely been observed, while concentrations in the low parts per trillion (pptr) are fairly common (Westlake and Gunther, 1966).

DDT is very soluble in non-polar organic solvents. DDT solubilities of 8 grams per 100 grams oleic acid and 7 grams per 100 grams castor oil were reported by Frear (1955). In other words, DDT is about 5×10^7 times more soluble in "fats" and oil than water. Presumably, it is the great solubility in fats which permits DDT to be highly concentrated by the members of an aquatic biota, from a low concentration of DDT in the water.

Whether a few parts per trillion of DDT in water is a high enough concentration to exert any effect on a aquatic biota has been questioned by McLean (1967). Yet, 1 pptr of DDT in water is about 1.6×10^{12} molecules of DDT per liter of water. The concentration of DDT observed in water from Lake Michigan was about 2 pptr (Mount, 1968). These same samples from the lake also contained less than 1 pptr of DDD, a trace of DDE and about 1 pptr dieldrin. Whole body

pesticide levels in 16 to 21 inch lake trout from Lake Michigan averaged 6.96 ppm DDT-R and 0.20 ppm dieldrin, while the average for all fish analyzed from the lake was about 3.5 ppm of DDT-R and 0.10 ppm of dieldrin (Reinert, personal communication, 1969). Thus, it appears fish may acquire concentrations in the parts per million of DDT-R range from water containing only a few parts per trillion.

The quantity of DDT-R contained in the various components of Lake Michigan was estimated in order to determine how much DDT-R was required to produce the concentrations observed. Lake Michigan is a big lake. It has a surface area of 22,400 square miles, a maximum depth of 923 feet (Frey, 1963) and a volume of about 1.41×10^{14} cubic feet or 87.9×10^{14} pounds of water. Suspended solids were estimated to be between 1 mg/l and 300 mg per hectare (Ruttner, 1963), or about 10×10^{9} pounds for the lake. If invertebrates like Pontoporeia sp. weighed one tenth of the suspended solids, 10×10^{8} pounds of invertebrates would be present. Fish at 10 pounds per acre (Bails, personal communication, 1969) would amount to 14×10^{7} pounds in the lake. Assuming the surface area of the basin equals twice the surface area of the lake, a sediment deposition rate of 1 mm per year (Ruttner, 1963) would amount to about one inch in 25 years. Assuming sediments weigh about the same as field soils, or 300,300 pounds per acre for a 1 inch depth (Millar et al., 1958), then about 8×10^{12} pounds of sediments

have been laid down in the last 25 years. From these values the total amount of DDT-R contained in the lake was estimated as shown in Table 1.

Some fundamental features regarding DDT-R in lentic ecosystems are presented in Table 1. First, a large percentage of the DDT-R believed to have entered the lake in the last 25 years has probably been retained in the water. Secondly, only a small percentage of the total is contained in the biotic components. Finally, though I have been unable to determine the quantity of DDT applied to the watershed, 139,290 pounds is believed to be only a small percentage of the total applied in the last 25 years.

Table 1. The amount of DDT-R estimated to be present in the various components of Lake Michigan

Component	Weight in lake (pounds)	Concentration of DDT-R (lbs per lb.)	Weight of DDT-R (pounds)	Percent of Total
Water	87×10^{14}	3×10^{-12} (A)	26,100	18.7
Sediments	8×10^{12}	14×10^{-9} (B)	112,000	80.4
Suspended solids	10×10^{9}	30×10^{-9} (C)	300	0.2
Inverte-brates	10×10^{8}	0.4×10^{-6} (B)	400	0.2
Fish	14×10^{7}	3.5×10^{-6} (D)	490	0.3
Total			139,290	

(A) Mount, 1968

(B) Hickey et al., 1966

(C) Estimated to be 1000 times the concentration in water.

(D) Reinert, personal communication, 1969

THE STUDY AREA

This study was conducted at the Lake City Experiment Station located two miles south of Lake City, Michigan (Sec 18-T22N-R7W; Reeder Twp.). It is owned and operated by Michigan State University mainly for agricultural research. The Department of Fisheries and Wildlife maintains ponds and facilities used for these investigations.

Experimental ponds were constructed during the period 1943 to 1945 adjacent to Mosquito Creek, a small stream which rises on the station property. The creek is impounded to supply water for the ponds and field irrigation. The four large ponds (Figure 2) may be filled or drained independently. Ponds E and F are supplied solely by ground water which seeps from the reservoir. Pond E was used to study the behavior of DDT in a farm pond during 1965 with pond F serving as a control.

Circular artificial pools (10' X 4') were used for another study. The four pools were placed in pond A for temperature control and were sampled from docks, as shown in Figure 3.

Figure 2. Map of the Lake City experimental ponds and laboratory facilities showing location of ponds A, B, C, D, E and F, and artificial pools 1, 2, 3 and 4 in pond A.

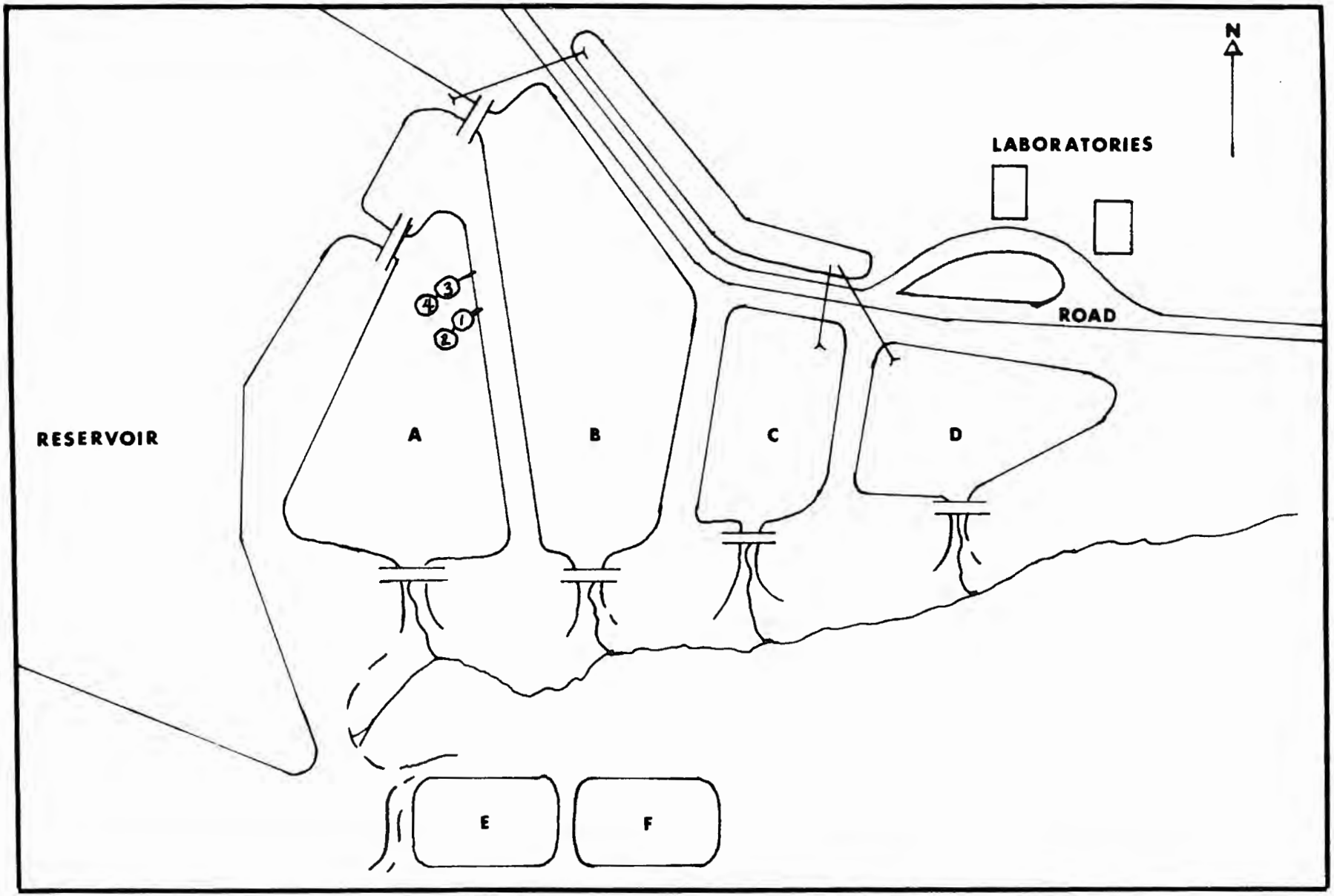


Figure 2

Figure 3. A photograph, facing east, showing four artificial pools set up in Pond A.

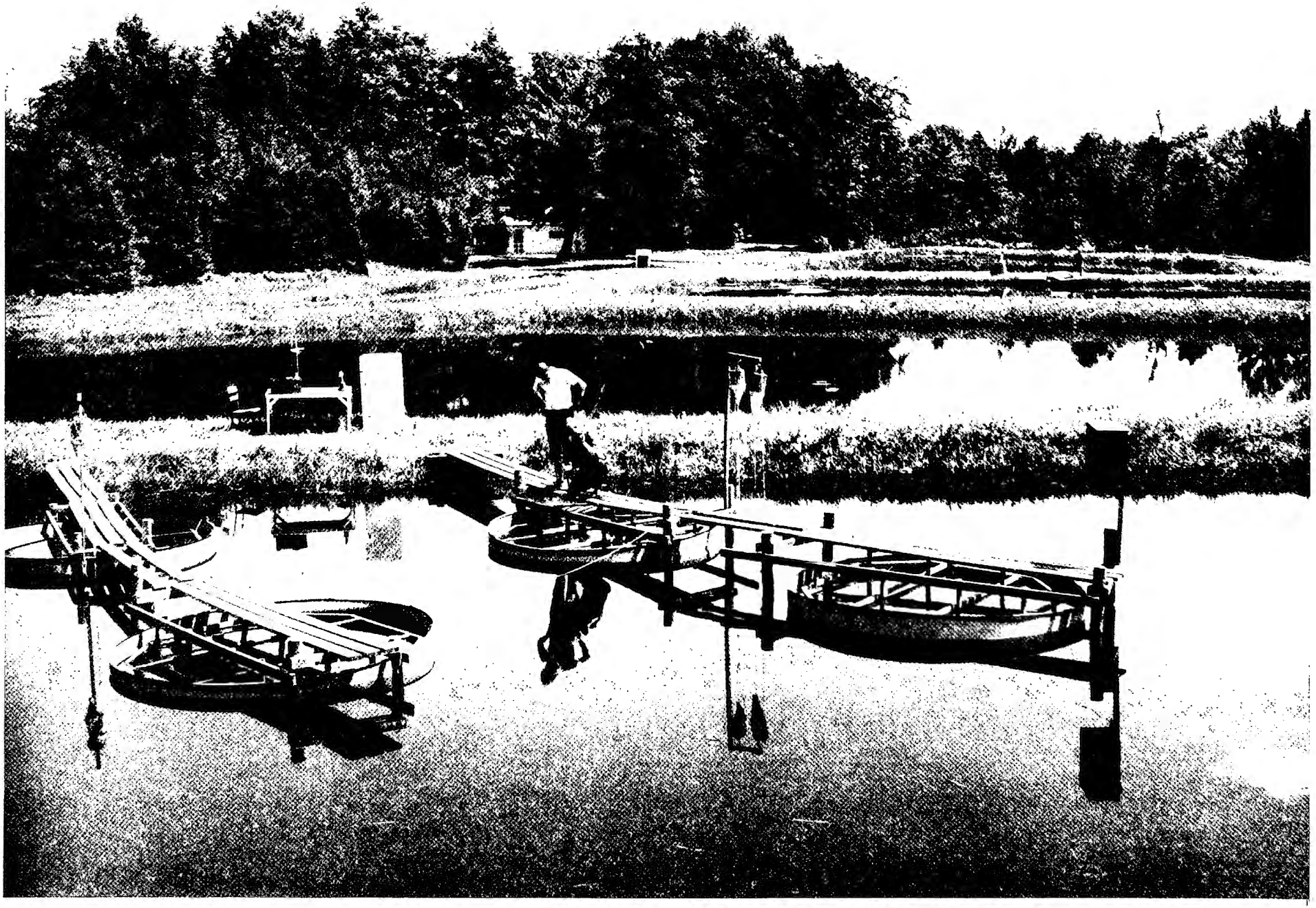


Figure 3

METHODOLOGY

General:

Specific methods used to extract and refine samples for residue analysis conform to the demands of the environment being studied, and the time, cost, identification, and sensitivity required. The methods for apolar insecticide residues were basically quite similar. Solvent systems used for extraction (Mills, 1959; Thornburg, 1965) were various combinations of non-polar and polar organic solvents that have slightly different densities, volatility, and solubilities. Cleanup procedures relied on differences in solubility between various solvents (Eidelman, 1963; Jones and Riddick, 1952) and differences in affinity for various adsorbents (Morley, 1966) which exist between the pesticide and the co-extractable substances present in a given sample (Mills, et al., 1963; Moats, 1962). Thus, while many procedures were tested, recognition of these underlying similarities facilitated developing extraction and cleanup methods which used only a few solvents and two adsorbents.

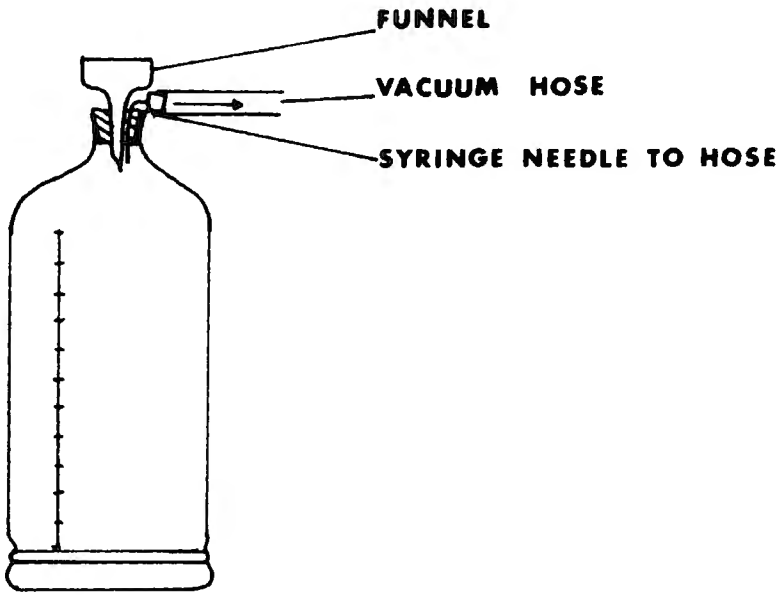
Water:

Water sampling procedures were designed to obtain a representative sample with a lower limit of sensitivity of

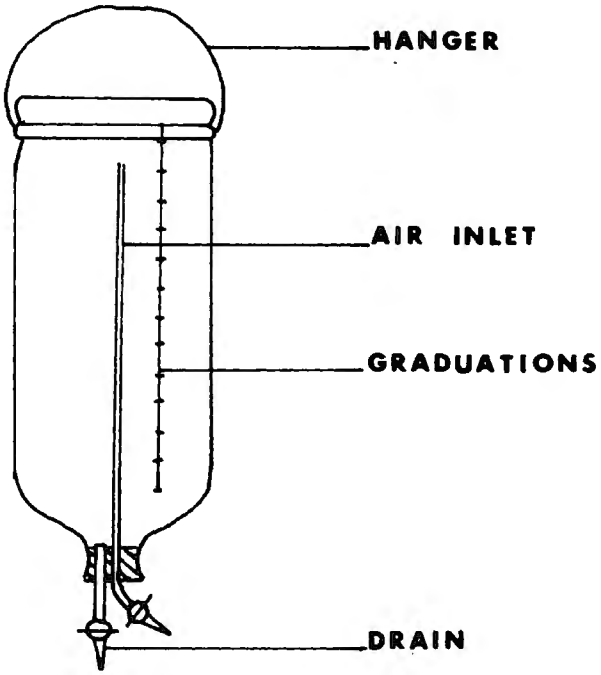
20 pptr DDT from shallow farm ponds. Sample bias due to uneven vertical distribution of DDT was minimized by taking a continuous column of water from surface to substrata with a glass tube (1" X 48"). The glass tube minimized sample modifications and permitted visual inspection, preventing contamination with substrata. Operation consisted of lowering the tube from the surface to the bottom, corking the upper end, then raising the tube to insert a stopper in the lower end, under water. During the 1965 experiments, water was drained into a jar and the operation repeated until a liter was collected. Water was drawn through a 1.2 μ Millipore filter, one liter was measured in a graduated cylinder and then extracted in a large separatory funnel.

The procedure was modified in 1966 to permit filtering water through a #1 Whatman paper filter (Keith, 1966) directly into a 1500 ml graduated solution bottle (Figure 4). The bottle was used for the extraction (Faust and Suffet, 1966) thus saving time and effort. Three columns of water were collected randomly and filtered. The filtered solution was then mixed, adjusted to one liter, and extracted by partitioning with 100 ml purified (Klein et al., 1963) petroleum ether (Warnick and Gaufin, 1965). The separatory funnel adapter (Figure 4) allowed the extracted water to be drawn off and discarded. Separated ether was dried with anhydrous Na_2SO_4 (Hindin et al., 1964) in the bottle, transferred into a 24/40 ground, round bottom flask, concentrated

Figure 4. Water sampling and extraction bottle (1500 ml).



COLLECTION



EXTRACTION

Figure 4

on a rotary vacuum evaporator to about 3 ml and cleaned with approximately 0.5 g of activated Florisil. The concentrated solution was transferred into a graduated centrifuge tube, with a disposable Pasteur pipette, and thermally stabilized with 1 ml purified (Thornburg, 1966) benzene. This solution could be analyzed directly or stored in a freezer for extended periods of time without decomposition or evaporation. The method was 77 $\pm$ 8.5% efficient for pp DDT.

Algal Periphyton:

Algal periphyton was sampled as the primary producer for the study ponds. Periphyton is one of the major food supplies of the primary consumers found in northern Michigan ponds (Knight, et al., 1962). Algae grows quite steadily, depending upon temperature, sunlight and nutrient supply, and is easily cultured on most substrates (King and Ball, 1966). Algal periphyton inhibits phytoplankton growth, resulting in clear water and a further consumer dependence upon attached algae for food. Heterogeneous population composition presumably presented a natural situation, which could respond to DDT contamination and other environmental changes without undergoing catastrophic shifts in abundance.

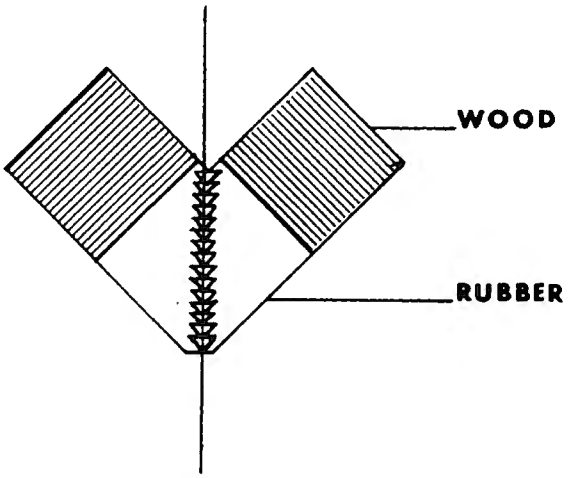
Large (10" X 30"), vertically-suspended, clear-polyethylene plastic sheets provided a substrate for an adequate sample mass (> 1 g.) of periphyton which was easily collected and quantified. The problem of DDT being adsorbed

directly to plastic sheeting was minimized by culturing periphyton on sheeting for two months before applying any DDT. Scraping periphyton off sheeting with a pair of rubber squeegees (Figure 5) minimized any possible desorption.

The periphyton mass formed a hard cake upon drying which was difficult to extract; to save time and improve accuracy a procedure to extract wet periphyton was developed. Samples were frozen immediately following collection for preservation and to facilitate detachment of algae from the sheets. Periphyton from the pools was then thawed, scraped into an enamel pan (8" X 12"), transferred to a 150 ml beaker with approximately 50 ml water and placed in a refrigerator where algae settled out. The supernatant was carefully decanted, and the periphyton vacuum filtered onto #1 Whatman paper to remove excess water. Periphyton from the natural pond was removed from sheets while still frozen and placed directly on the filter. Excess water was drawn off as the sample thawed. The algal mass was peeled off the filter paper with a spatula, weighed, and extracted in an appropriate sized mortar.

Periphyton was extracted four times with purified (Johnson, 1965) acetonitrile. The volume of nitrile used depended on the sample weight. The first extraction required 10 ml of nitrile for the first gram, plus 5 ml for each additional gram. Subsequent extractions used half as much nitrile. For samples less than two grams, a 15 ml, 10 ml, 10 ml and 10 ml extraction series was used.

Figure 5. Periphyton collection apparatus, design and operation diagram.



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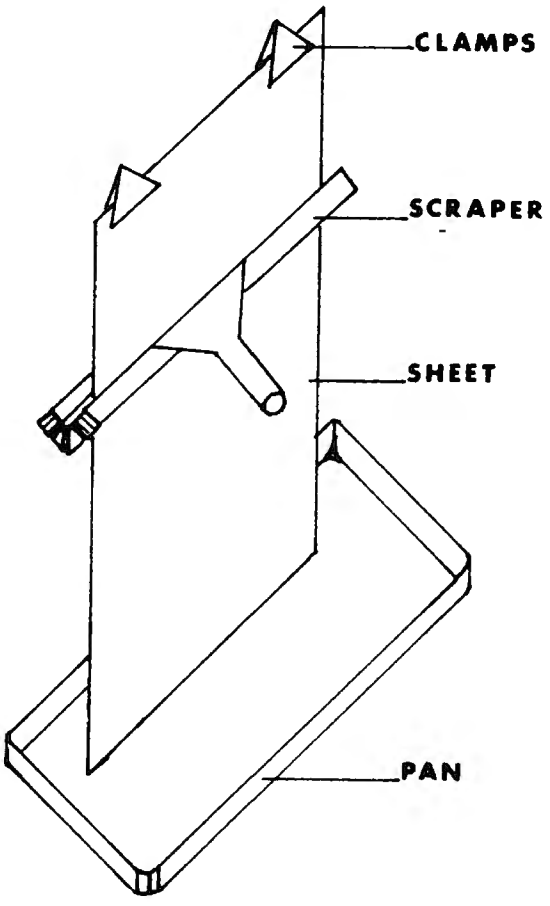

Figure 5

Acetonitrile was used as the extraction solvent for periphyton, rather than the usual petroleum ether, because nitrile did not extract a troublesome artifact as did ether. However, if periphyton had a strong musty odor, nitrile extracted a different artifact. Since acetonitrile and petroleum ether are immiscible, they may be used in liquid-liquid partition cleanup procedures (Jones and Riddick, 1952). The musty smelling artifact was very soluble in petroleum ether, so it could be partitioned from the nitrile while pesticides remained. Thus two cleanup methods were developed for periphyton; a rapid method if musty odor was absent and a complete method if musty odor was present.

Acetonitrile extracts were combined in a two liter separatory funnel to start the complete cleanup method. Extracts were partitioned for one minute with 50 percent the extract volume of petroleum ether. Nitrile was drawn off into a small separatory funnel and partitioned again with another portion of ether. When fractions separated in the second funnel, ether from the first funnel was added to the second. The first funnel was rinsed with 15 ml ether and added to the ether layer in the second funnel. A portion of DDT dissolved in ether during exchange was recovered from ether in the second funnel by partitioning with nitrile equal to 50 percent of the ether volume. Nitrile used for recovery was added to the first funnel and all portions

solivated with one liter of tap water, used because deionized water contained artifacts (Thornburg, 1966).

The rapid method did not include partitioning with petroleum ether. Instead, combined acetonitrile extracts were immediately solivated with one liter of water in the large funnel. DDT was recovered from the water-nitrile mixture by partition with 100 ml petroleum ether. The aqueous solution was drawn off and the ether dried with anhydrous Na_2SO_4. Just enough Nuchar-Attaclay (Cassil, 1962) was added to remove interfering plant pigments from solution; usually less than 0.1 gram per gram of periphyton extracted was required. Nuchar-Attaclay is a powerful decolorizing agent which also has an affinity for insecticides, however with discriminate use (using just enough to remove the color from solution) insecticide losses can be avoided. Clear ether solution was decanted from the separatory funnel, through a bed of powered anhydrous Na_2SO_4 held in a conical funnel, into a round bottom flask, and evaporated for analysis. Efficiencies for the methods are presented in Table 2. Thus, a rapid and efficient periphyton cleanup method was developed by reversing the order of extraction and partition developed by Jones and Riddick (1952) and combining it with the adsorbent cleanup procedure used by Cassil (1952).

Table 2. Percent recovery of insecticide standards from dosed periphyton samples using two methods of preparation.

	pp DDE	pp DDD	pp DDT
Complete method	67.8 ± 3.4%	91.3 ± 2.8%	87.8 ± 3.6%
Rapid method	89.7 ± 3.2%	94.0 ± 2.4%	98.1 ± 2.6%

Bottom Mud:

Natural pond bottom muds were collected with an Ekman dredge (Welch, 1948). Bottom samples from the artificial pools were collected with a device (Figure 6) which was buried at random collection points during addition of the bottom material. The device was a one pint plastic freezer bag fitted over the hanger from 1500 ml graduated hospital solution bottles (Figure 4). The wire hanger was passed through the bag held on the ring and crimped with pliers, so that it was held perpendicular to the ring. Samplers were buried flat on the bottom, about two inches deep, in such a manner that a small portion of the wire hanger protruded above the sand.

Collection consisted of locating the sampler desired, hooking the wire loop and slowly pulling the sampler vertically through the sand to the pond surface. At night and whenever visibility was poor, samplers were located by using an underwater viewing tube, a 4" X 4' PVC sewer pipe with

Figure 6. Artificial-pool, bottom sampler constructed with a spring steel band and wire loop from 1500 ml hospital solution bottles and plastic bags.

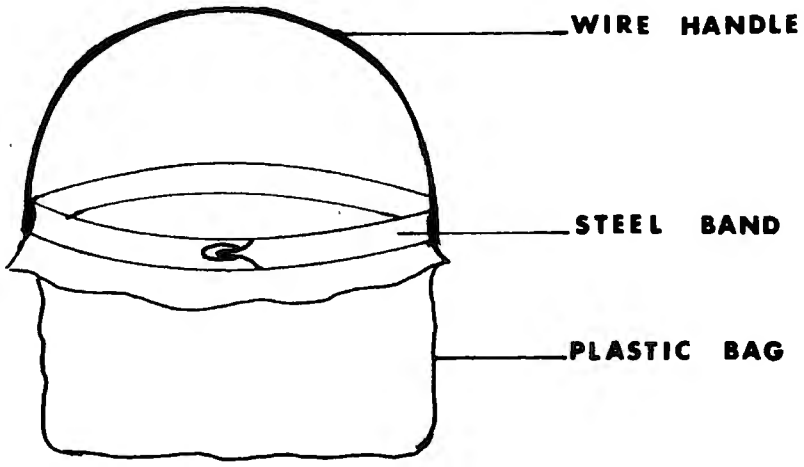

Figure 6

glass sealed in the expanded end. Small holes were pierced in the sampler bag, after being brought to the surface, to drain off excess water and retain interface particulate matter. The sampler bag was cut from the ring with scissors, placed into another plastic bag and frozen. The sample was later transferred into a 600 ml beaker and dried at 25°C in a circulating air oven.

Dried soil was granulated, weighed and placed in a 1000 ml Erlenmeyer flask. Sand was covered with 150 ml of 20 percent ether in petroleum ether, mixed and extracted for 24 hours. The extract was decanted into a 500 ml separatory funnel, and a subsequent 100 ml of ether mixture added to the flask, shaken and decanted into the funnel. Distilled water (about 150 ml) was then added to the flask, thoroughly mixed and residual ether decanted into the funnel. The water was separated from the either and discarded. The ether was dried with anhydrous Na_2SO_4, transferred into a round bottom flask, evaporated to about 10 ml, and subjected to a Florisil cleanup (Mills et al., 1963). The procedure gave good recoveries with negligible interference down to 0.1 ppb DDT residue concentrations in sand. Efficiency of the method for standard insecticides was 86.7 $\pm$ 1.0 percent for DDE, 88.0 $\pm$ 5.7 percent for DDD and 77.8 $\pm$ 2.5 percent for DDT.

Invertebrates:

Invertebrates susceptable to capture in an underwater light trap (Baylor and Smith, 1953), primarily microcrustacea, were chosen to represent the primary consumer trophic level. Light traps captured a wide variety of motile animals including some insect larvae. Ostracods and mites were frequently captured in large numbers, while Hyalella azteca which was known to occur in the pond was seldom caught. Copepods and cladocerans appeared to be captured in proportion to their abundance. Thus, traps do not provide a truly representative sample of invertebrates in a pond. Fortunately insecticide residue concentration and composition found in comparative samples collected with a light trap and a zooplankton net were essentially the same, so the trap was acceptable for this study.

Invertebrates captured were concentrated with a Foerst centrifuge (Welch, 1948), washed into a plastic vial with water and frozen until analyzed. Two preparation procedures, the complete and rapid method, were used. The complete procedure was applied to large samples and provided a wet, dry, and fats weight for each sample. A modified Bailey-Walker crude fat determination (Benne, et al., 1956) was used to quantitatively extract fat and insecticides from samples. The procedure was modified to facilitate insecticide analysis in several ways. Asbestos-mat, Gooch crucibles were prepared using a Walter crucible holder. Prepared

crucibles were weighed while still wet and again after drying. The tared crucibles were loaded in the holder so the sample could be slowly poured into each crucible, while excess water was drawn off. Wet weight of samples was obtained by weighing a loaded crucible and subtracting the wet tare. Samples were dried at 50°C for 24 hours, then held in a desiccator until a constant dry weight was obtained. This weight minus the dry tare weight gave the sample dry weight. Following extraction with ethyl ether for 24 hours, samples were again dried and crude-fat weight obtained by subtracting the weight of extracted material from its dry weight. The extract was concentrated and subjected to the Florisil cleanup procedure (Mills, et al., 1963) before being analyzed on the gas chromatograph.

The rapid procedure was developed for small invertebrate samples (one gram or less) and only provided wet weight. The sample was vacuum filtered onto a small (1" dia.) tared filter paper disc for weighing. The filter paper and animals were ground to a fine, dry powder with anhydrous Na_2SO_4 and sand (approx. 20 grams) in a mortar. The powder was placed in a 125 ml Erlenmeyer flask and extracted with 20 ml of 10% ethyl ether for 24 hours.

Cleanup consisted of filtering the extract through a bed of Florisil held in a conical funnel. The filter bed was prepared by plugging the funnel stem with a small wad of glass wool, adding about two grams of Florisil and covering

the bed with anhydrous Na_2SO_4. Florisil was taken directly from the activation oven (120°C). The filter bed was washed with 25 ml of pure, dry petroleum ether and "settled" by tapping the funnel before a sample was added. The extract was poured directly through the filter bed into a 250 ml round bottom flask. Residue in the Erlenmeyer flask was rinsed three times in succession with 10 ml of 10 percent ethyl ether and the washings passed through the filter bed. The bed was finally rinsed with 25 ml of petroleum ether to complete the transfer. This solution was concentrated and analyzed on the gas chromatograph.

The rapid procedure was successfully applied to samples as small as 0.0160 gram and to sample concentrations as low as 43 ppb. Recovery of standard insecticides was essentially 100 percent, so no corrections have been applied to results obtained with the rapid procedure.

Fish:

The ecological, physiological and chemical complexity of fish imposes immense analytical and interpretative difficulties upon the data from analysis of their pesticide residues (Durhan, 1967). Since fish were analyzed to provide information about DDT within a pond ecosystem, the behavior of residues within individual fish was not considered beyond the problem as a whole. However, insecticide residues are stored within a fish's body, while nutrients, on the other

hand, are used to build the body. Since many ecological concepts regarding DDT have been postulated from concepts about nutrients, discrepancies have arisen. Furthermore, a great deal of emphasis has been placed on ecological and ethological differences between species, while the physical and chemical similarities among fish have been neglected. These similarities are of paramount importance when describing the basic relationships between DDT residues and fish. The differences invoked by various species are considered modifying factors, not controlling factors. Thus, all fish data are titled "fish" regardless of the species involved.

Fish used during the course of the study included the following:

1. (Yearling) Common Shiner Notropis cornutus (Mitchill); natural pond study.
2. (Yearling) Redbelly Dace Chrosomus eos (Cope); natural pond study.
3. (Yearling) Green Sunfish Lepomis cyanellus (Rafinesque); Waybrant (1969).
4. (Yearling) Pumkinseed Sunfish Lepomis gibbosus (Linnaeus); Waybrant (1969).
5. (Young-of-the-Year) Largemouth Bass Micropterus salmoides (Lacépède); artificial pool study.

Fish were collected with a seine, box net, glass minnow trap, or hook and line, depending on circumstances.

After capture fish were frozen until analyzed, when the entire fish was analyzed without regard to sex.

Complete and rapid preparation procedures were also developed for fish. The procedures were essentially the same as those used on invertebrates. The only major difference was the method of preparing fish for extraction. For the complete procedure, frozen fish were finely diced and placed in a dry, tared Gooch crucible. Wet weight was taken from the loaded crucible. For the rapid procedure, fish which weighed about a gram were thawed and weighed, then cut into small pieces with clean surgical scissors. The pieces were cut into a Virtis extraction cup containing about 5 grams of anhydrous Na_2SO_4, and extracted with three 10 ml portions of 10 percent ethyl ether using a Virtis homogenizer. Efficiency of the complete method for invertebrates and fish was pp DDE 89 $\pm$ 12.8 percent, pp DDD 92 $\pm$ 12.2 percent and pp DDT 79 $\pm$ 11.7 percent. Recovery of standards from the rapid fish procedure was essentially 100 percent, so again no corrections were applied to results obtained with the rapid procedure.

Gas Chromatography:

1) Instrumentation:

Samples were analyzed by gas-liquid chromatography (Bonelli, 1965; Bevenue, 1963; Hardy and Pollard, 1960). The instruments employed were the Aerograph models 665,

600-C and 550-B oven and a Micro-Tek model MT-220 with accessory microcoulometric detector. The model 665 has a dual electrometer for simultaneously analyzing a split stream with two detectors. One side of this electrometer was used to create an independent G.L.C. out of the model 550-B oven. The model 665 oven temperature was controlled with a manual programmer and power supplied through a 1000 watt C.V.R. until 1966, when a proportional linear temperature programmer was installed. Temperature in the model 550-B was controlled with a model 325 linear temperature programmer during the entire study. A pair of model 15 Brown-Honeywell 1 mv recorders, with Disc Integrators, provided the graphic record. Characteristics of the Aerograph and Micro-Tek gas chromotographs are compared in Table 3.

2) Operation:

Chlorinated hydrocarbon insecticides are usually analyzed on a variety of silicone oils, gums or rubbers. Their basic structure is a methyl silicone chain (DC-11) which may contain phenyl (OV-17) (Menzie and Prouty, 1968) or trifluoropropyl (QF-1) substitutions or may be cross-linked to form rubbers (SE-30 and OV-1). The unsubstituted (non-polar) forms have similar separating characteristics, while substituted forms exhibit a slightly different order of selectivity among themselves and a distinct difference from non-polar forms (Anonymous, 1967) (Table 4). Generally, non-polar solutes will be separated in the order of their

Table 3. Comparison of the physical and operating characteristics of the Aerograph and Micro-Tek Gas chromatographs.

Characteristic	Aerograph	Micro-Tek
Components	All Glass	All Glass
Detector	Concentric Tube	Parallel Plate
Source	Tritium	Tritium
Voltage	90 v D.C.	50 v Pulsed (30 to 150 μsec for 0.5 μsec.)
Temperature		
Inlet	Variac	Proportional
Column	Proportional	Proportional
Detector	"Ambient"	Variac
Carrier Gas	Nitrogen	Nitrogen
Purge Gas	None	95% Argon/5% Me
Column		
Size	1/8" X 5' to 6'	1/4" X 6'
Packing	5% DC-11 cr QF-1	3% SE-30
Shape	Coiled	U-Tube

Table 4. Retention times relative to pp DDE for DDT-R on a DC-11, a QF-1 and a dual DC-11:QF-1 column.

Compound	DC-11	QF-1	DC-11:QF-1
pp DDE	1.00	1.00	1.00
pp DDD	1.29	1.85	1.49
op DDT	1.28	1.30	1.35
pp DDT	1.75	1.90	1.82
Minutes for pp DDT peak maxima	12.1	6.6	30.3
Theoretical plates for pp DDT	1936	800	2060

boiling points on non-polar liquid phases, while polar solutes with similar boiling points will be retarded less. As polarity of the liquid phase is increased polar solutes will be retarded longer. These differences in selectivity made simultaneous separation of pp DDE, pp DDD, op DDT and pp DDT possible in a dual column, consisting of 5 percent QF-1 preceded by 5 percent DC-11 on 60/80 mesh Chromosorb W, in a 50:50 or 40:60 volume ratio respectively. The dual column design exhibited less band spreading than a mixed bed design (Burke and Holswade, 1966; Henley, et al., 1966) constructed with similar components.

Preparation of high quality packing is difficult for routine analysis, so the material required was purchased in a form that could be loaded directly into the column. Good results were obtained with Chromosorb W and excellent results were obtained with Gas-Chrom Q. Mesh size ranged from 60/80 down to 100/120, depending on column diameter, with a liquid load of 3 to 5 percent DC-11, SE-30 or QF-1.

By using different columns, conditions and detectors, it was possible to qualitatively identify most common pesticides. Further verification was obtained by chemically altering the various compounds present in samples (Datta et al., 1964; Klein et al., 1963). For example, saponification with alcoholic KOH provided a rapid method for quantitatively converting DDT to DDE (Schafer et al., 1963), while dieldrin was generally unaffected by the treatment.

3) Detection:

The discussion of the G.L.C. detectors used will be limited to electron capture designs. Principles, applications and limitations of other detectors used are available in the literature (Burchfield et al., 1965; Svojanovsky et al., 1966; Westlake and Gunther, 1967) and were not comparatively evaluated.

Electron capture (E.C.) detectors contain a supply of "slow" electrons, formed by ionization of an inert gas, which can be absorbed by certain electrophilic compounds such as pesticides (Gaston, 1964). Absorption of the electrons results in a decrease in ion current across the cell, producing a signal which can be amplified, recorded and quantified. The current change is an event that can be produced or opposed by a number of additional processes. Compounds co-extracted with pesticides or contained in preparatory chemicals can also absorb electrons and appear to be insecticides. These are called artifacts. The chambers may simultaneously function in additional modes of detection, and finally, they can easily and insidiously malfunction (Westlake and Gunther, 1967). Consequently, E.C. detectors have several peculiarities and shortcomings that must be tolerated or avoided to obtain satisfactory results.

Two basic designs exist for radiometric E.C. detectors; concentric tube and parallel plate (Clark, 1964). Lovelock (1963) discussed the principles, problems and

limitations of E.C. detectors, and parallel plate design in particular. Hartmann and Oaks (1965) and Guiffrida et al. (1966) have discussed the advantages of concentric tube design, available with a fixed or movable anode, but have generally disregarded its shortcomings. Personal experience has shown that both detectors have an adequate linear range and sensitivity for the analysis of DDT.

The inexpensive Aerograph detector has a small purge volume that is advantageous with narrow bore columns and is easy to dismantle and clean. The Micro-Tek detector is more expensive to own and operate, has a large purge volume and is difficult to clean. However, the Micro-Tek detector in pulse mode operation as described by Schmit and Peters (1964) was more stable, reliable, specific and accurate than the Aerograph detector, as supported by the following discussion.

The Aerograph detector protruded out of the instrument, so the detector was subjected to room temperature fluctuations and operated considerably below column temperature. The temperature fluctuations probably contributed to the instability observed, but this was not conclusively demonstrated. The low temperature (ca. 100^{o}C) allowed many eluted products to condense onto the foil, which rapidly created a contact potential (Bonelli, 1965), reduced the standing current and sensitivity, and increased baseline noise. This problem was largely alleviated by

enclosing a 100 watt light bulb directly over the detector to serve as a heater, but the detector still required frequent cleaning. Operating time between cleaning was extended by disconnecting the cell and storing it inside the detector housing when not in use. Actually, despite the steadily decreasing current and contact potential limitations, differences in precision between the Aerograph instruments and the MT-220 could not be attributed solely to the detector.

The Aerograph detector also responded to many artifacts that were not even strong electron absorbers which greatly jeopardized validity and accuracy. This characteristic necessitated thorough cleaning of preparatory chemicals, equipment and samples, and continuous monitoring with control samples, inevitably increasing the cost and time for analysis. Accuracy cannot be obtained with any reasonable degree of confidence whenever an artifact elutes with a study compound. Analyses of water samples containing an artifact on the Aerograph (Figure 7) had a 95 percent confidence interval for pp DDT of $\pm$ 26 percent, with a mean value which was 12 percent greater than the quantity observed with the microcoulometric and Micro-Tek E.C. detector. Apparently, the Aerograph detector possessed various degrees of sensitivity to artifacts, independent of the sensitivity to insecticides, because paired observations were nearly identical, while most of the observed variability occurred between analyses conducted on different days. This implied the

Figure 7. Comparison of chromatograms obtained for same water samples on two different instruments.

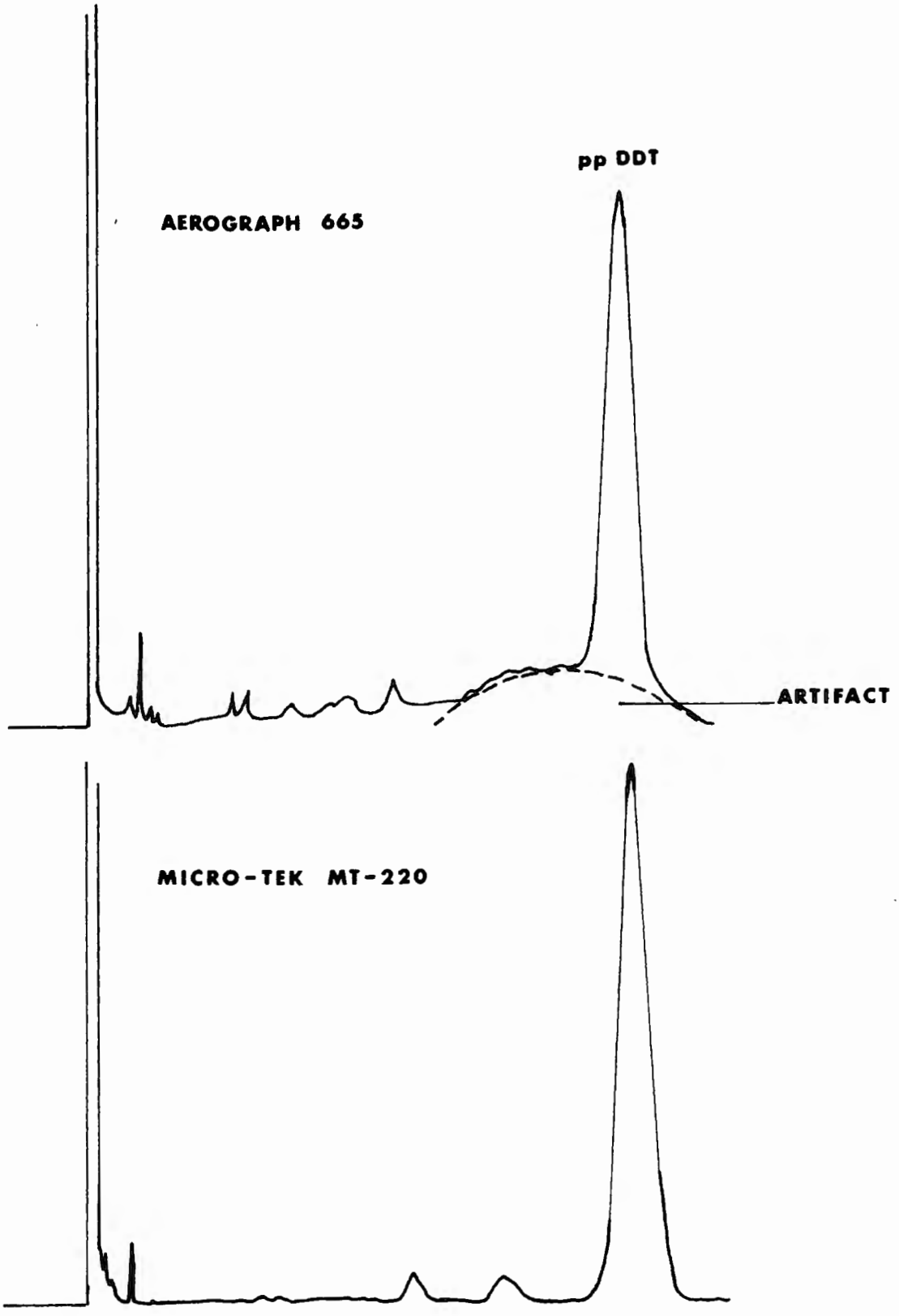

Figure 7

Aerograph may operate in additional modes of detection besides electron capture.

The Micro-Tek detector did not respond to the artifact (Figure 7) and gave consistent results for all analyses. Argon with five percent methane is the recommended carrier and purge gas for pulse-mode operation of parallel plate detectors. The argon mixture did not work well as a carrier gas for DDT and its metabolites (Figure 8). DDE and DDD exhibited band spreading and DDT was lost, as though insoluble in the gas. The loss of DDT may have been due to extreme band spreading, resulting from great differences in gas density which arose during passage through the column. Regardless of the cause, successful and less costly operation was obtained by using nitrogen as the carrier gas (60 to 80 ml/min.) and argon with 5 percent methane as the purge gas (40 to 60 ml/min.).

The Micro-Tek detector was extremely sensitive to temperature fluctuations, so drafts and radical room temperature changes had to be avoided. When operated with a continuous DC current and nitrogen as the only gas, many artifacts were observed and sensitivity was greatly reduced. Pulse mode operation of the E.C. detector as described above offers some additional analytical advantages. Detector sensitivity for pesticides can be decreased by shortening the pulse interval. This was an excellent technique for residue analysis where some compounds were present in much

Figure 8. Resolution of DDE, DDD and DDT using three sets of conditions in model MT-220 G.L.C.

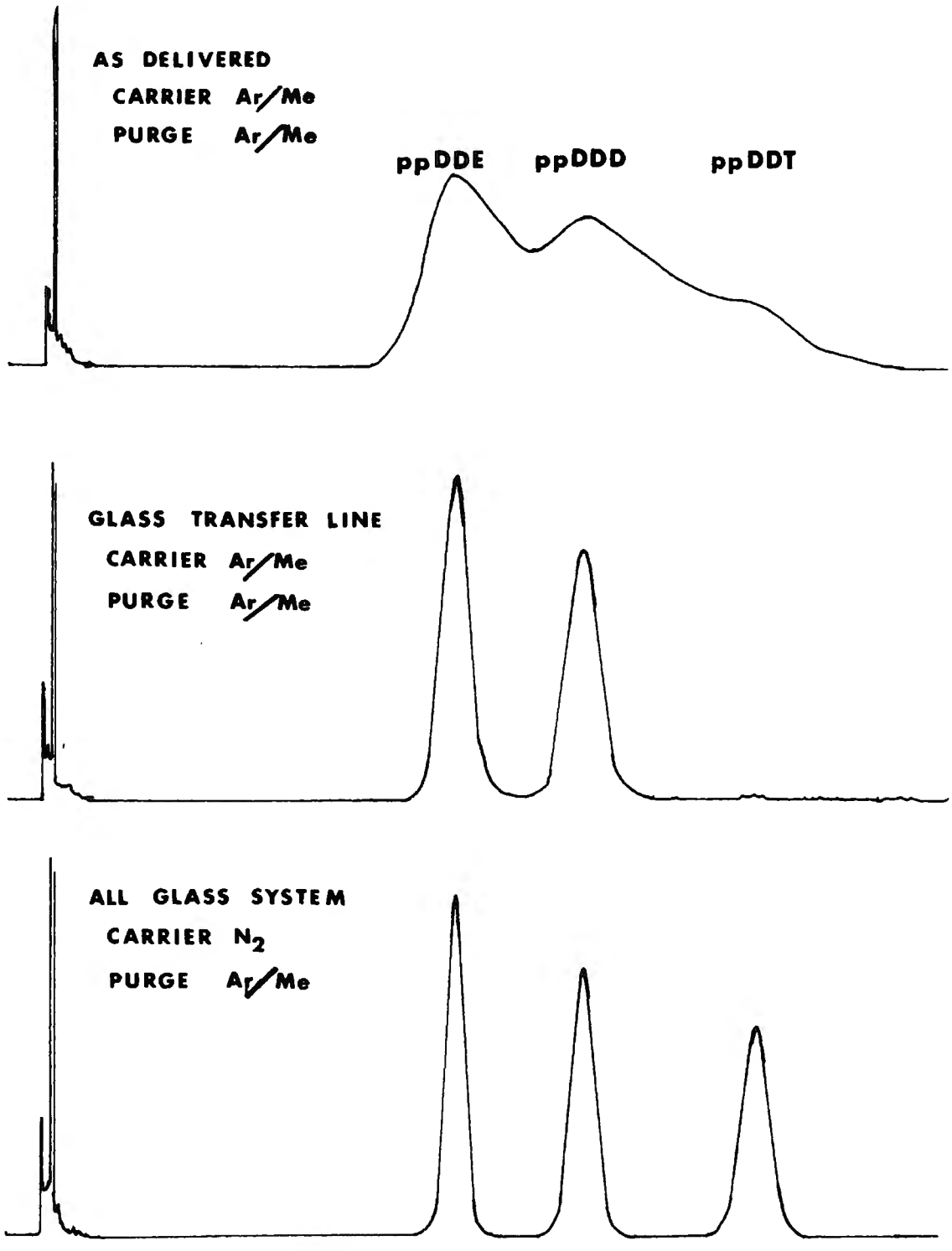
AS DELIVERED
CARRIER Ar/Me
PURGE Ar/Me
ppDDE
ppDDD
ppDDT
GLASS TRANSFER LINE
CARRIER Ar/Me
PURGE Ar/Me
ALL GLASS SYSTEM
CARRIER N_2
PURGE Ar/Me

Figure 8

greater quantities than others. Also, pulsing at 50 volts was strong enough to overcome even a high (up to 20 volts) contact potential, so this problem was rarely encountered. Finally, the detector cell could be kept clean by shutting off the carrier gas and leaving the purge gas on, whenever the instrument was not being used. This detector maintenance technique was possible because the cell quickly stabilized (ca. 30 min.) when the carrier gas was turned back on, and the packing did not fracture in the 1/4" OD columns, while it did fracture in the 1/8" OD columns used in the Aerographs.

4) Calculation and interpretation of results:

A gas chromatograph (G.L.C.) is a very sensitive qualitative instrument but not necessarily a precise quantitative instrument. Yet, its usefulness is not limited because there are ways to overcome this shortcoming. Only a few analysts have evaluated the quantitation problem (Gaul, 1966; Gill and McNair, 1965; Horning et al., 1963). I was required to measure accurately small amounts of insecticide with a high degree of precision; thus the sources of quantitative variation had to be identified and controlled. Quantitative variation has three major sources:

A) Sample preparation techniques

B) The gas chromatograph

   1) Operation

   2) Response or read-out

C) Quantification calculations

Good methods and experience minimize variation introduced during sample preparation and G.L.C. operation. Response variation depends entirely upon the G.L.C. system employed, and is minimized by using the best system available with the best conditions obtainable (Giddings, 1965). Thus, these sources of variation are seldom reduced by using an alternative method. However, different quantification techniques are available; each has certain advantages and disadvantages, and the proper method can greatly reduce analytical variation.

Quantification consists of calculating the amount of insecticide contained in an unknown by comparing the signal obtained from a known volume of unknown, to a signal obtained from a known amount of standard insecticide. Four sources of variation can be subjected to control:

A) Volumes

B) Signal recording

C) Signal measurement

D) Signal conversion

These will be discussed in order under separate headings.

A) Volume control:

1) Sample solution volume may range from microliters to liters without affecting variation, provided the vessel holding the sample is accurately graduated and appropriate volume ratios maintained. For example, a 1.0 ml sample should not be measured in a 15 ml vessel.

2) The contribution of thermal expansion to variability is usually small. With a 5°C increase, a 10 ml volume of water expands 0.1 percent, benzene expands 0.6 percent and ethyl ether expands 0.8 percent. But expansion can become a significant source of error if samples or syringe are subjected to widely different temperatures (eg. handling, drafts, sunlight, etc.).

3) Syringes should be loaded using the solvent flush technique (Anonymous, 1969). Briefly, the syringe is rinsed several times with clean solvent. About two microliters of clean solvent are drawn into the syringe, which is then removed from the solvent, and the plunger retracted until an air space is visible at the needle end of the syringe. The needle is then placed in the sample solution and the desired amount drawn into the syringe. In this manner, a portion of air is trapped between the solvent and the sample solution. The needle is removed from the solution and the plunger retracted until the volume of sample solution can be read in the syringe. The sample solution volume is measured between air drawn in the needle and the previously trapped air. Everything is injected into the G.L.C.

B) Recording control:

A monthly cleaning, oiling and performance check of the recorders effectively minimized recording errors.

C) Signal measurement:

The signal can be measured as peak height or peak area. Peak area is theoretically more precise, since it should not be altered by small changes in operating conditions. Several different devices and methods for measuring peak area exist (Gill and Tao, 1967), but for residue analysis using an E.C. detector, peak height is often more precise. Peak height is usually better because it can be measured more accurately than area when spurious responses are present. Both area and height measurements improve as separating power increases. Separating power is reported as the number of theoretical plates (Desty, 1956; Giddings, 1963), the higher the value the better the separation.

D) Signal conversion:

There are three basic methods for converting the signal into a concentration, using either peak height or peak area. Regardless of the technique used, standard response values must be close to unknown response values.

1) A standard, adjusted to approximate the unknown, is injected after each analysis. The unknown is quantified by solving a simple proportion between the standard response and the unknown response. The technique is practical for single residue analysis and precise (coefficient of variation around 2 to 3 percent), but it is a time consuming method.

2) Standards may be injected over a narrow concentration range throughout the day and a response per quantity injected obtained by division. The method relies on the assumption that the standard response for a series of concentrations forms a linear regression passing through the origin. Since in practice the regression seldom passes through the origin, large errors may be incorporated when the slope is steep or the concentration range extensive. Generally low response values relative to the range covered should not be used, because percentage change incorporated into the proportional value increases greatly as the Y intercept is approached, when the regression does not pass through the origin. However, it is a rapid technique for multiple residues, since only a few standard runs are required for a satisfactory mean and computations may be performed on a desk calculator.

3) Direct conversion from the linear regression obtained for a range of standards injected throughout the day can also be used. The regression may be drawn by eye or calculated and unknowns quantified graphically or mathematically. Desk-top computers make use of calculated regressions practical. The method works best with a broad response range from at least five standard runs. Finally, confidence limits about the regression provide a better estimate of machine precision (Linnig and Mandel, 1964), than the deviation about an average value.

Results reported were calculated from a linear regression of peak height (mm.) versus picograms of insecticide. If the 95 percent confidence interval about the $\bar{Y}$ of the standard regression for a set of analyses exceeded $\pm$ 10 percent, the results were deemed unacceptable and the samples reanalyzed on the G.L.C. This action was rarely required because the confidence interval was seldom less than $\pm$ 2 percent or more than $\pm$ 5 percent of the $\bar{Y}$. Unknowns were assumed to have the same degree of precision as standards.

Since artifacts are frequently a serious problem, sample analysis must be confirmed by using alternate columns, detectors or procedures. Unfortunately any program to maintain accuracy demands that an artifact be recognized as such when it arises, that alternative systems are operational when needed, and that samples are not destroyed during processing. Thus, it was impossible to check the accuracy of each sample and periodic checks were little better. So my method involved starting with artifact free procedures, checking any apparent deviates as they arose, and keeping remnants of every sample preserved until all results were calculated and confirmed.

Thus, having employed all of the qualitative checks and quantitative procedures described, I am confident that analytical precision, excluding sample preparation, rarely exceeded the $\pm$ 10 percent limits desired.

EXPERIMENTAL

Natural Pond Study:

Pond E was reconstructed in 1964 by hand dredging the top foot of accumulated matter and then blasting (Mathiak, 1965) to obtain a pond 35' X 70' with an average depth of 1.76 feet. The pond bottom was sand, except for a small pocket of organic material which collected in the area over 40" deep (Figure 9).

Following reconstruction the water was very turbid due to suspended clay. Turbidity was still high the following spring (April, 1965), so measures were enacted to reduce turbidity while increasing pond productivity. First the pond was treated with 5 lbs. of Mason's lime (powdered Gypsum) on 6/4/65 and on 6/6/65. Turbidity appeared to be reduced by one-half the pre-treatment level by 6/13/65. Next about two bushels of fresh cut grass were scattered over the pond on 6/15/65. The grass did not appear to reduce turbidity but it did stimulate zooplankton production. Then, water was pumped out of the pond and water from the reservoir pumped in on 6/22/65. Turbidity appeared unchanged after an estimated two pond volumes had been exchanged, so the pumping was terminated. Finally, one pound of 10-52-17 fertilizer,

Figure 9. Depth contour map of Pond E.
Area 2450 square feet
Average depth 1.76 feet

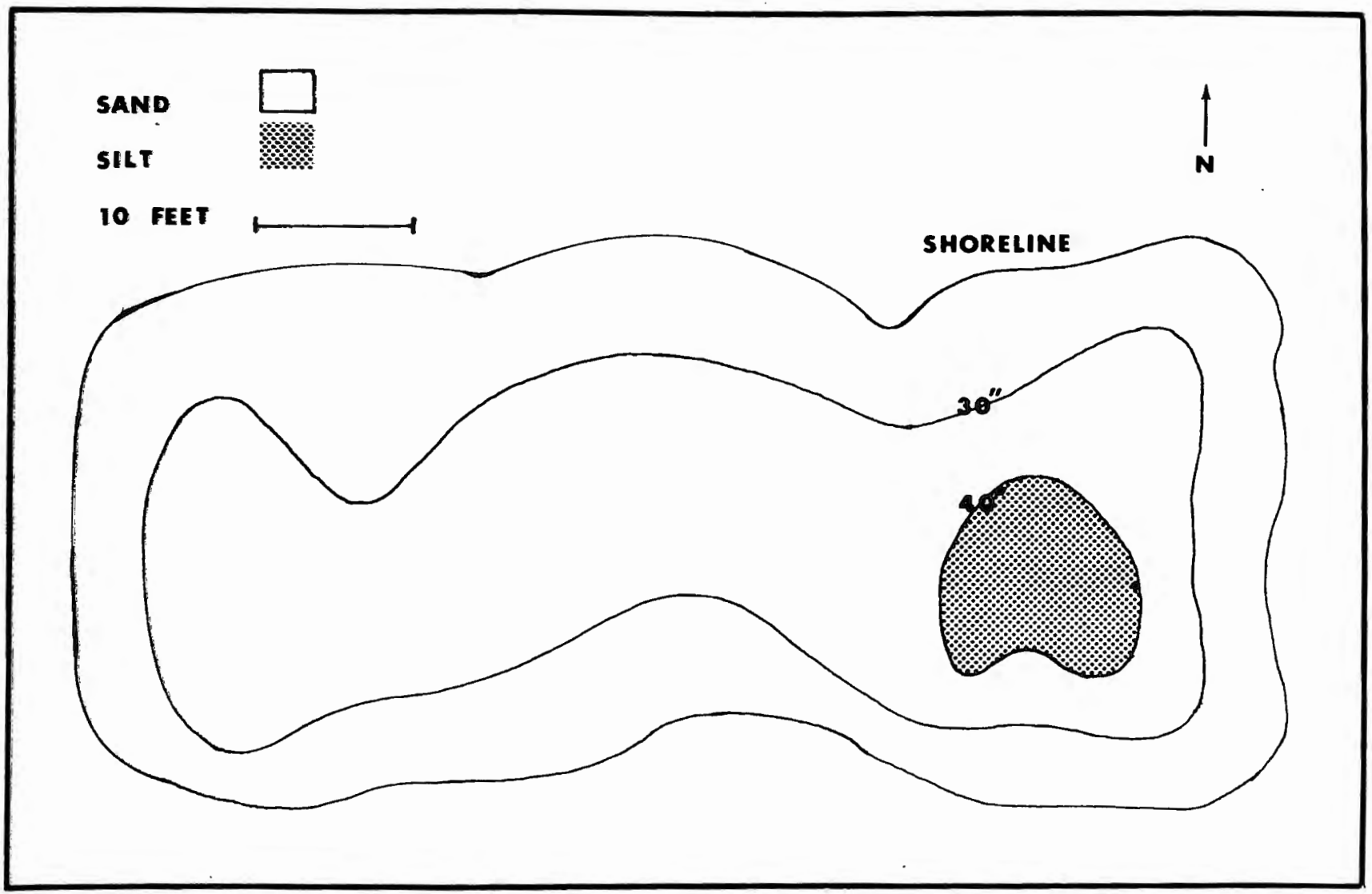

Figure 9

(about 20 lb/acre) was added to the pond on 6/23/65. The fertilizer stimulated a phytoplankton bloom, which in turn supported zooplankton started with grass cuttings, such that by 7/11/65 a still turbid but productive pond was deemed ready for treatment with DDT.

The pond containing about 120,000 liters of water, was treated with 1.800 grams of 100 percent pp DDT (ESA, 1964) at noon, on 7/11/65. The DDT was dissolved in a liter of acetone in a hand-operated garden sprayer, and diluted with a liter of pond water just prior to application. The solution was spread evenly over the water, then mixed into the water by rapidly rowing a small boat around the pond for 20 minutes. Sample collection began one hour after application was started.

Water chemistry determined just prior to treatment at 10:00 AM on 7/11/65 is presented below:

1) Water temperature 76^{o}F
2) Turbidity - secchi disk 18"
3) Color - green, due to phytoplankton abundance
4) Oxygen - 9.03 ppm.
5) Alkalinity
    a) phenolphthalein--11.0 mg/l as $CaCO_3$
    b) methyl orange--98.0 mg/l as $CaCO_3$
6) pH - 8.5

Artificial Pool Study:

Artificial pools were used to study the fate of a 5 ppb dose of pp DDT in detail. The experimental design compared the uptake, transport, degradation and dispersion of DDT in three pools, each containing a different combination of food organisms and fish. The control pool and the complete pool contained both food organisms and fish. Fish were excluded and food animals added to the no-fish pool. Finally, food organisms were nearly excluded while fish were added to the no-food pool (Table 4). A description of the study units is presented below:

1) Pool dimensions:
   - A) Circumference 922 cm
   - B) Average water depth 101.6 cm
   - C) Water volume 6875 liters
   - D) Area of bottom 72,928 cm^2
   - E) Area encompassed by bottom sampler 68.78 cm^2 or 1/1060 of the bottom.
   - F) Dry weight of sand placed in bottom 4536 grams
   - G) Average depth of sand 5 cm
   - H) Area of sides 97,231 cm^2
   - I) Area of 104 periphyton sheets 402,584 cm^2

2) Pool designations:

The experimental design and treatment used for the artificial pools is presented in Table 5. The titles

Table 5. Experimental design and treatments used for artificial pools.

POOL	TITLE	COMPONENTS			
		DDT	PERIPHYTON	INVERTEBRATES	FISH
1	CONTROL	NO	YES	YES	YES
2	COMPLETE	YES	YES	YES	YES
3	NO-FISH	YES	YES	YES	NO
4	NO-FOOD	YES	YES	NO	YES

designated for each pool will be used throughout the text to facilitate discussion of results. For example, fish from pool #4 will be referred to as fish from the no-food pool.

3) Pool components, manipulations and composition:

A) Pools:

The vinyl plastic liners used in the pools posed certain problems when working with pesticides. Plastic may absorb pesticides or give off artifacts. To minimize these two possible errors, the pools were set out to weather on 4/16/66, thoroughly cleaned from 6/20 to 6/21/66 and finally filled with water. The filling operation was completed on 6/27/66. In addition, water in all the pools except the no-food was exchanged for water in pond B from 7/18 to 7/20/66 using two large pumps. These procedures permitted a dense growth of periphyton to develop on the pool sides, which undoubtedly resulted in decreased adsorption of pesticides directly to plastic and minimized artifacts in the water from plastic.

B) Water:

Water was pumped into the pools from pond B, while pond A was filled from the reservoir. Water in the no-food pool was filtered through a large glass wool and sand filter to remove any pond animals present.

C) Periphyton:

Periphyton sheets were cultured in pond B from 5/28/66 to 6/24/66. The long culture period produced a dense

covering of algal periphyton and presumably desorbed all possible contaminants from the plastic sheeting. Sheets were attached to a support rack, then air dried prior to being placed into the pools. Drying removed attached snails and insect larvae. The attached algae quickly recovered when returned to water in the pools.

D) Bottom:

The bottom was partially-graded mortar sand taken from a gravel pit located 15 miles east of Lake City. The pit was located in a remote area and actively worked, so contamination by pesticides should have been minimal.

E) Invertebrates:

Invertebrates were taken from all the ponds, except pond E which had been treated with DDT the previous summer, using light traps and zooplankton nets. A large number and variety of animals were introduced into the pools to create a stable population well suited to the experimental environment. After thirty days, Ostracoda and Hydracarina were present in greatest abundance in all pools. Some Hyalella azteca and midge larvae were present, but cladocerans and copepods were almost completely absent.

F) Fish:

Fish used were young-of-the-year largemouth bass captured by seining ponds A and B. Fish in the no-food pool were fed laboratory raised, insecticide free

Artemia every two days to prevent starvation.

4) Treatment:

Pools were treated with 35 mg pp DDT (5 ppb) starting at 6:00 AM on 8/3/66. DDT was dissolved in 500 ml acetone in the hand-operated garden sprayer. The solution was spread evenly over the water and mixed in the water with an oar. Application of DDT required 15 minutes per pool. Sample collection began one hour after the DDT was applied, and required about 45 minutes per pool.

RESULTS AND DISCUSSION

Loss of DDT from the Water following Application to a Farm Pond and Artificial Pools:

The highest concentration of DDT-R observed in the water of the natural pond following treatment at a concentration of 15 ppb was about 6 ppb (Figure 10). Total DDT (ug of DDT in filtrate and filter residue per liter of water) declined rapidly to about 1.5 ppb. Once this level was reached, the concentration continued to decline at a slower rate, with the result that the DDT had an approximate half-life in the water of three days (Figure 11).

Dissolved DDT (ug DDT/liter of filtrate) fluctuated around 1 ppb for 48 hours, after which it began to decline. The difference between total and dissolved DDT concentrations was represented by the DDT retained by the filterable residue. The concentration of material suspended in water and the concentration of DDT associated with this material are shown in Figure 12. The concentration of suspended material and the water turbidity were high for the first 24 hours, after which both declined sharply. The concentration of DDT associated with the suspended material generally declined for the duration of the study.

Figure 10. DDT-R in water from the natural pond. Total represents the ug of DDT-R per liter in water and on filters. Dissolved is that in filtered water.

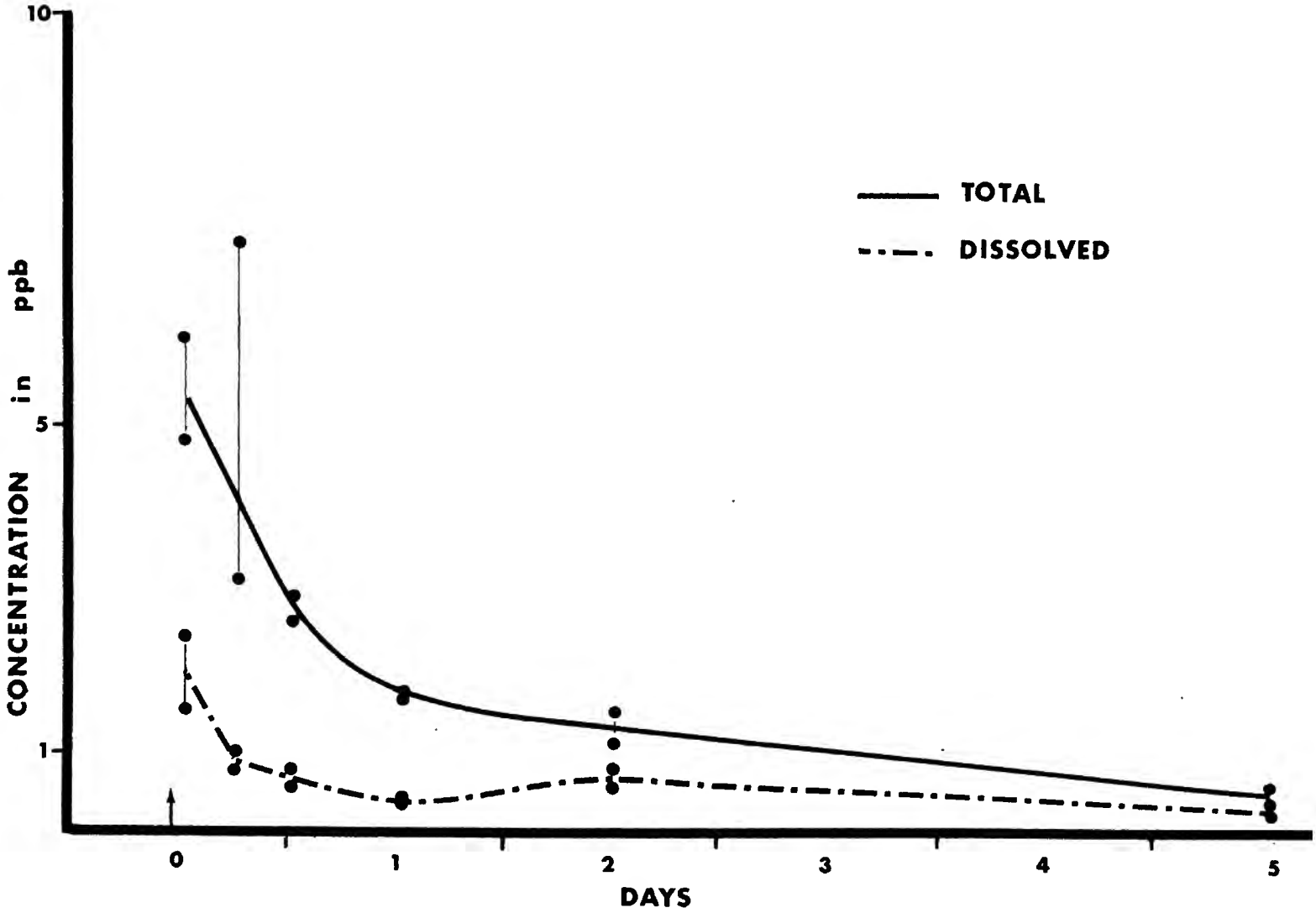

Figure 10

Figure 11. A semi-log plot of the average DDT-R concentration in water from the natural pond following a 15 ppb application of DDT.

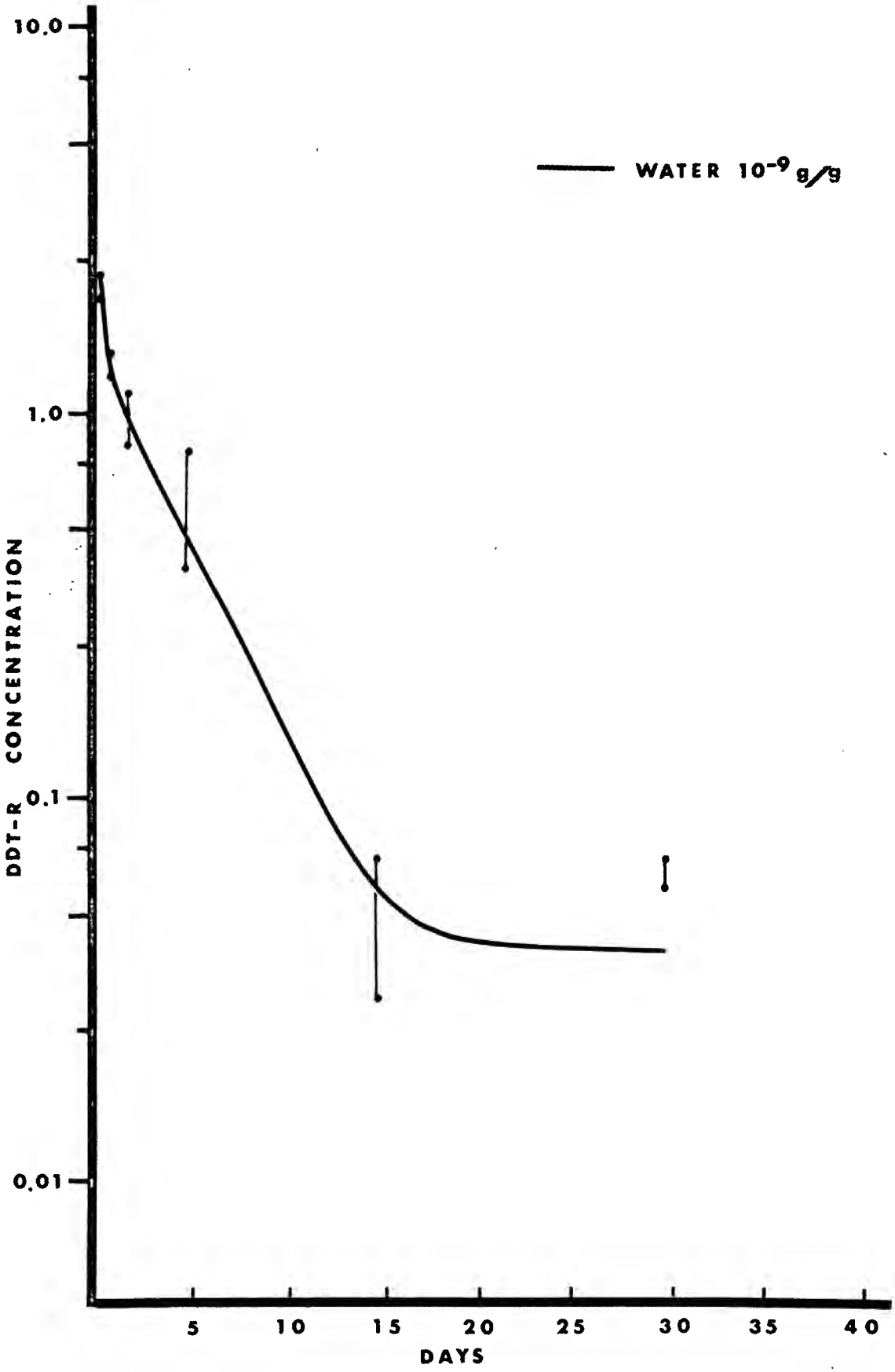
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 11

Figure 12. A comparison of the suspended matter in natural pond water and DDT-R concentration per gram of suspended matter.

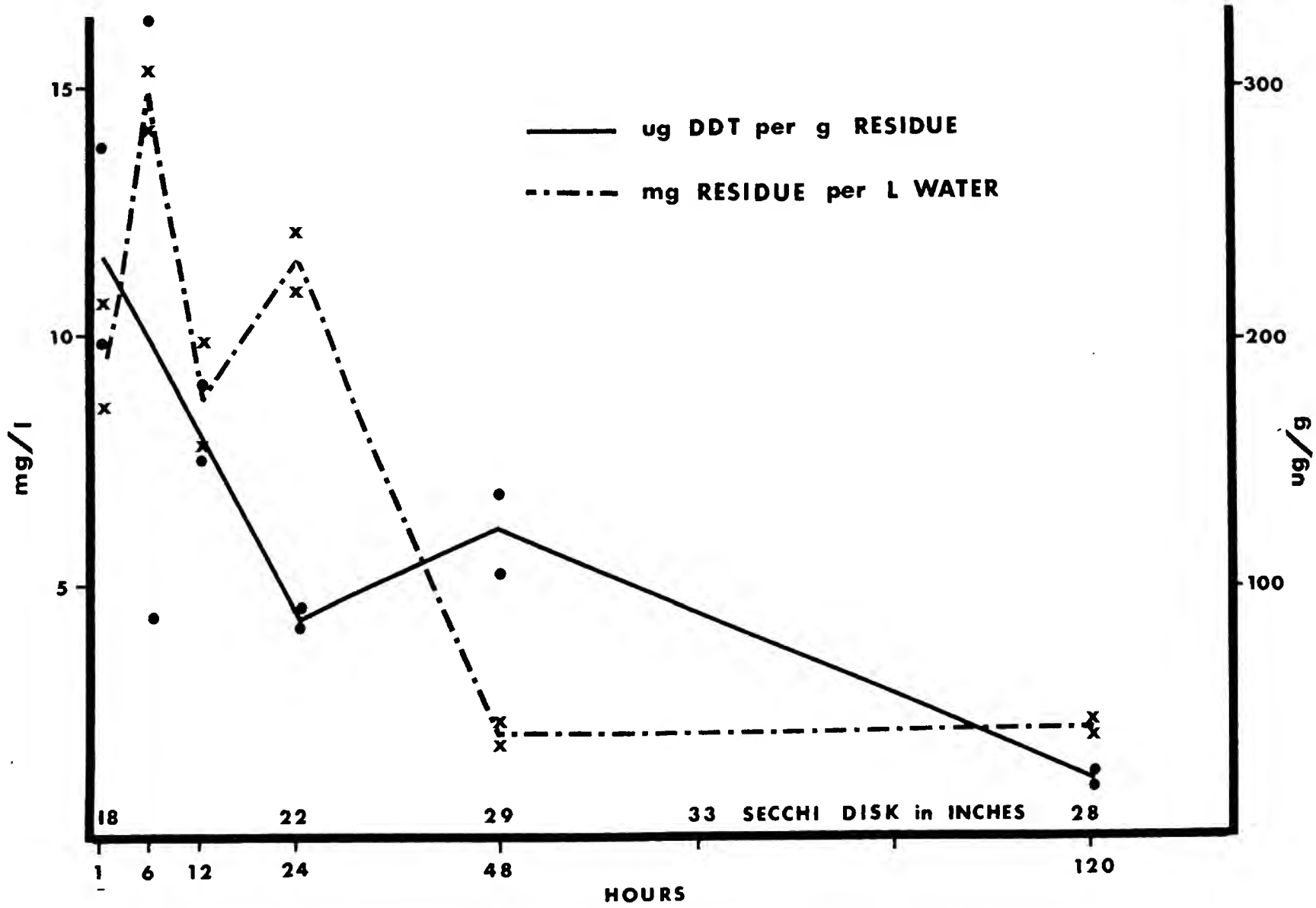
ug DDT per g RESIDUE
mg RESIDUE per L WATER
mg/l
ug/g
15
10
5
300
200
100
18
22
29
33
SECCHI DISK in INCHES
28
1
6
12
24
48
120
HOURS

Figure 12

The cause for the sudden reduction in turbidity was not clear, but appeared to be associated with the DDT application. Perhaps mixing of the pond water, accomplished with a boat when the DDT was applied, produced a change in water chemistry which promoted precipitation of the suspended material.

An average of 11.1 mg suspended material filtered from a liter of water collected within 24 hours after the DDT was applied. By the next day turbidity had declined and only 2.1 mg of material was filtered from a liter of water. In a pond volume of 120,000 liters, a difference of 9 mg/l in filterable solids would amount to a sediment deposition of 1080 grams. The average concentration of DDT in filtered residue during this same 48 hour period was 177 ug/g. Though 177 ug/g is actually about twice the concentration of DDT observed in the filtered residue when the turbidity was decreasing, it does provide a maximum estimate of the amount of DDT deposited on the bottom associated with these sediments. 177 ug/g of DDT on 1080 grams of sediment would amount to 191 mg of DDT, or about 10 percent of all the DDT added to the pond. Water samples collected 48 hours after DDT was added and after the turbidity had declined, contained a total DDT concentration of 1.31 ug/l. The water thus held another 157 mg of DDT.

Differences in the concentrations of DDT in the water samples from the artificial pools were not significant.

The combined results are presented in Table 6 and Figure 13. The amount of suspended material present in the water from the pools was small (between 0.1 and 1.0 mg per liter). The amount of DDT associated with this suspended material was too low to be routinely quantified, but trial analyses and laboratory tests demonstrated that less than 10 percent of the DDT in the water could have been associated with suspended material.

The disappearance of the DDT from the water could be attributed to a number of causes:

1) The amount of exchangable DDT in the sediments, flora and fauna of the system was large.
2) DDT was being continuously and irreversibly adsorbed to some substance, such as the plastics or the bottom material, and not being exchanged with the DDT in the water.
3) DDT was being degraded to products which went undetected by the methods of analysis used.
4) DDT was being lost from the study units to the atmosphere.

Table 6. Concentration of DDT-R in the water, all treated pools combined.

Sample		Number	ppb DDT-R	Standard
Day	Hour		(mean)	error
0	1	3	3.01	0.252
0	3	2	2.65	0.249
0	9	3	2.49	0.182
0	12	4	2.56	0.219
0	16	4	2.87	0.025
0	20	4	2.39	0.106
1	30	4	2.28	0.236
1	36	3	2.14	0.215
1	42	1	1.30	
2	49	2	1.29	0.179
2	60	4	0.77	0.040
4	106	4	0.86	0.087
5	120	2	0.72	0.009
7	177	5	0.48	0.061
9	216	2	0.265	0.015
12	288	2	0.130	0.014
15	360	2	0.085	0.007
25	600	2	0.038	0.000
30	720	2	0.026	0.000
40	960	1	0.010	

Figure 13. Semi-log plot of the DDT-R concentration in water from the artificial pools following a 5 ppb application of DDT.

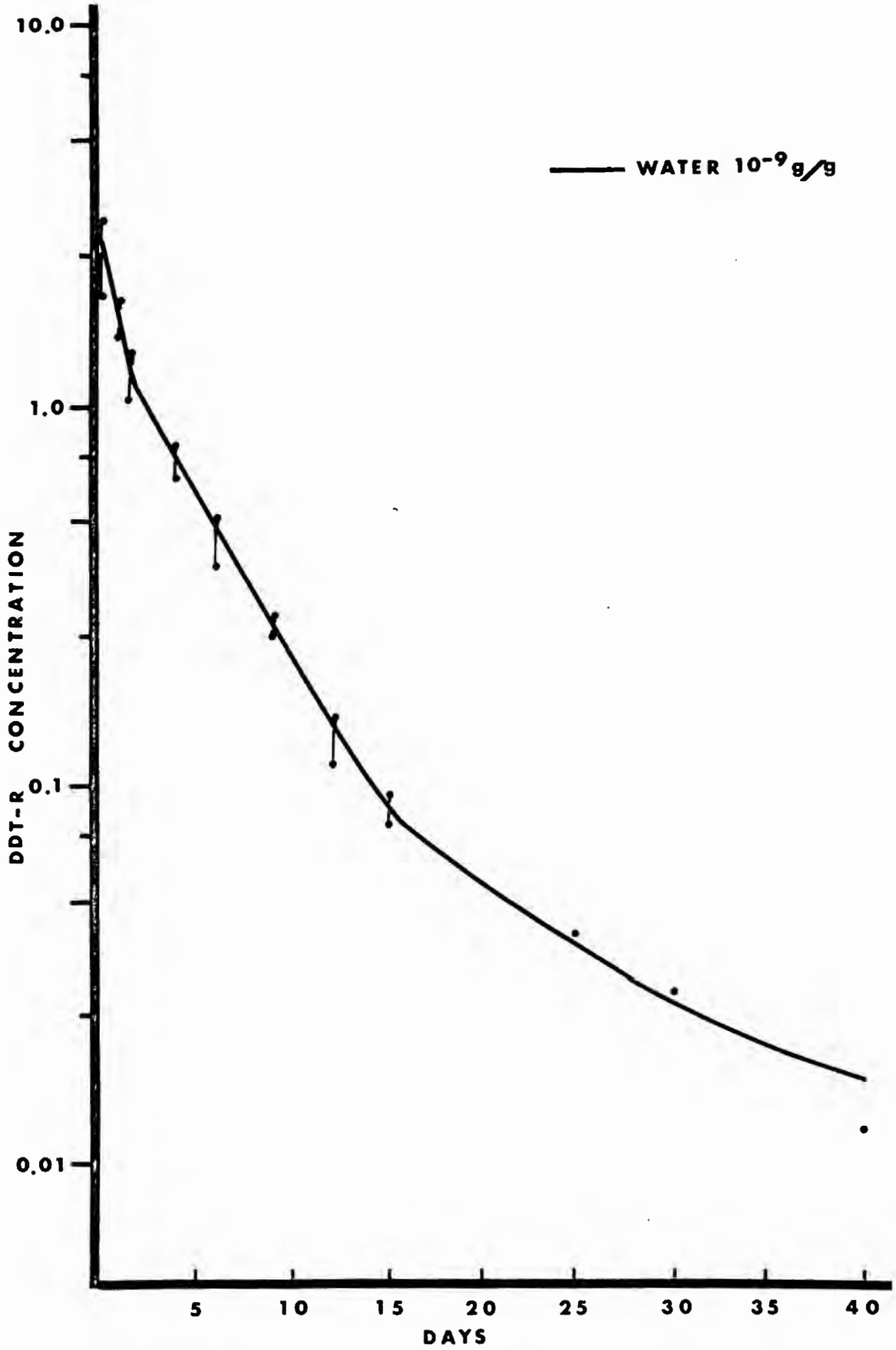
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 13

Concentration and Quantity of DDT-R in the Flora, Fauna and Sediments:

1) Invertebrates:

The invertebrates captured in the natural pond were primarily Cyclops sp. (Copepoda) and Chaoborus sp. (Culicidae) and some mites (Hydracarina), dragonfly naiads (Odonata) and Scapholeberis mucronata (Cladocera). DDT-R concentrations in the invertebrates from the natural pond are shown in Figure 14. The invertebrates are plotted on a scale 1 X 10^4 times higher than the water, showing the animals accumulated DDT-R more than 10,000 times the concentration in the water. The concentration of DDT-R in the invertebrates also appeared to be closely associated with the concentration of total DDT-R in the water.

The invertebrates captured in the pools were primarily ostracods, mites, dragonfly naiads, and some Hyalella azteca and Chaoborus sp. DDT-R concentrations in the invertebrates from all the treated pools combined are presented in Table 7 and compared to the concentration in the water in Figure 15. Mites contained about 20 percent more DDT-R residues than ostracods (Figure 16), but odonates contained about half the residues in ostracods (Table 2A. Appendix 2). The difference between the concentration of DDT-R in ostracods and mites is presumably real. However, data on dragonflies may have been biased, since they were usually crushed during handling and storage. Presumably the

Figure 14. Semi-log plot of the DDT-R concentration in invertebrates from the natural pond compared to the concentration in the water.

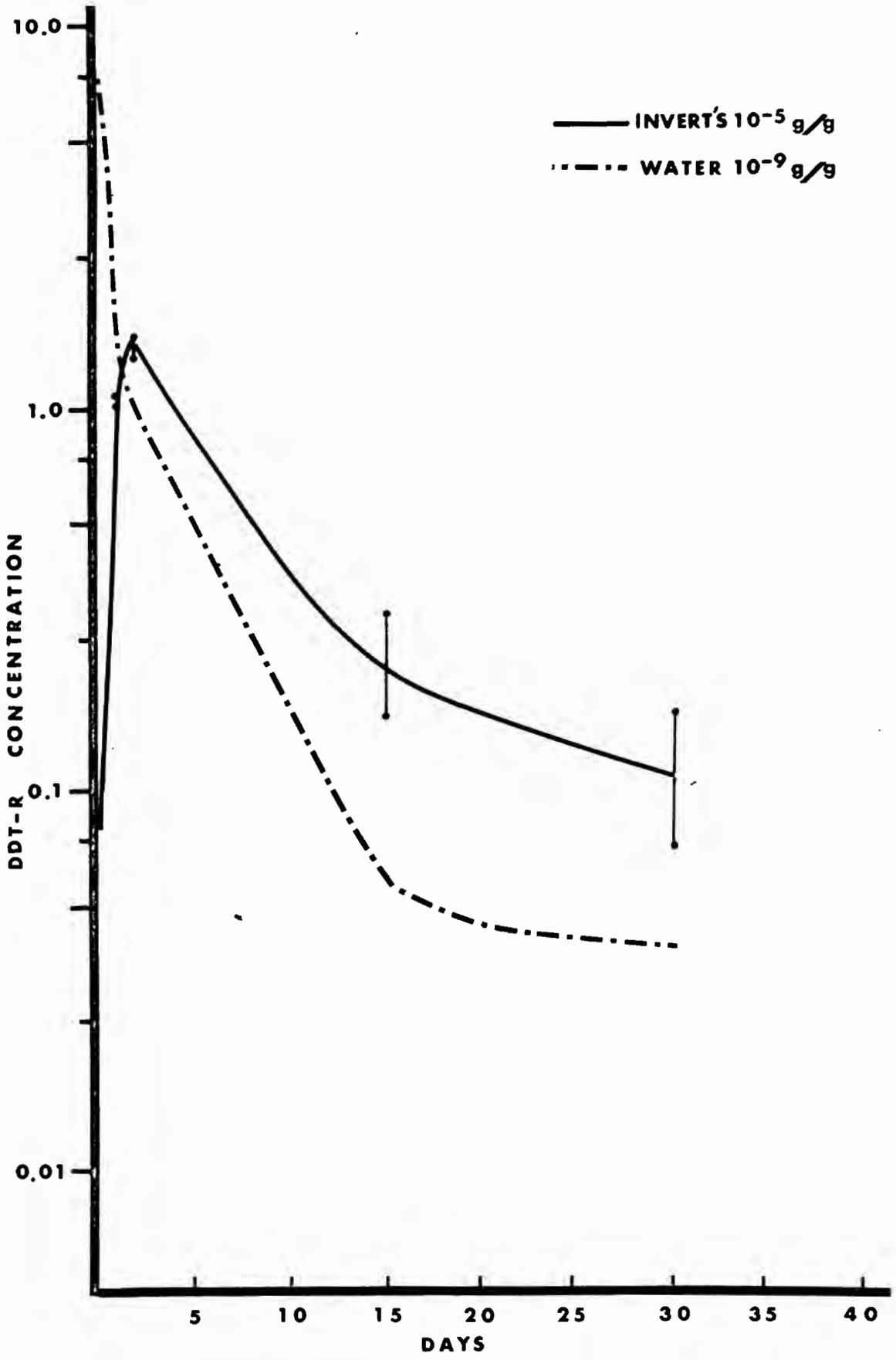
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
INVERT'S 10^{-5} g/g
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 14

Table 7. Concentration of DDT-R in invertebrates from the pools combined.

Sample Day	Sample Hour	Number	ppb DDT-R (mean)	Standard error
Pretreat.		3	355	61
0	1	2	2641	759
0	6	1	4424	
0	12	4	4097	744
1	24	2	6461	1023
1	36	4	4880	351
2	48	2	6181	2692
3	72	2	7091	1584
5	120	3	10568	943
7	177	3	6176	883
12	216	3	2269	320
15	360	5	1991	405
25	600	5	1059	159
30	720	4	587	175
35	840	2	475	89
40	960	3	388	110

Figure 15. Semi-log plot of the DDT-R concentration in all invertebrates from the pools compared to the concentration in the water.

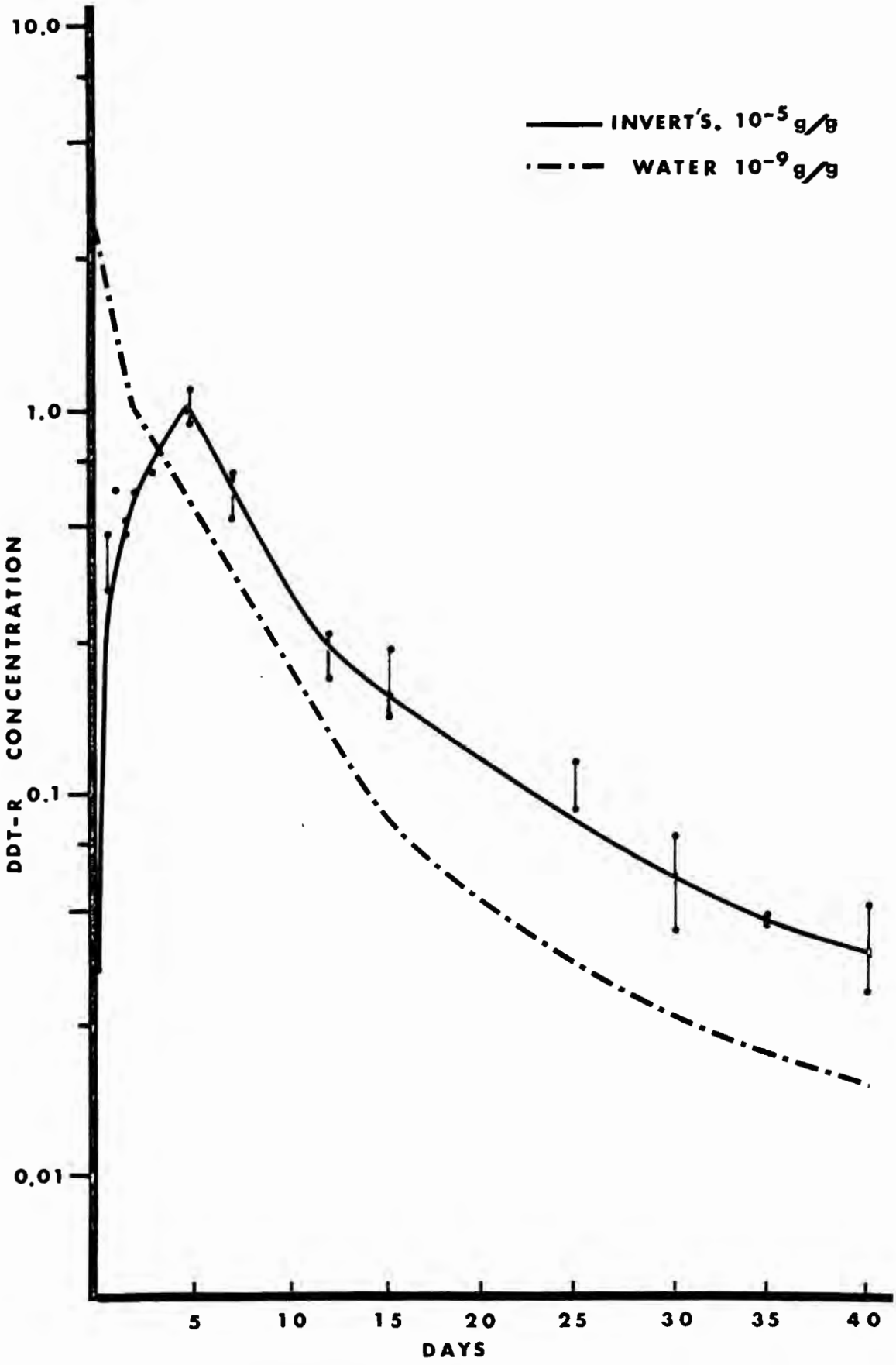
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
INVERT'S. 10^{-5} g/g
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 15

Figure 16. Concentration of DDT-R found in mites and ostracods compared to the concentration in the water.

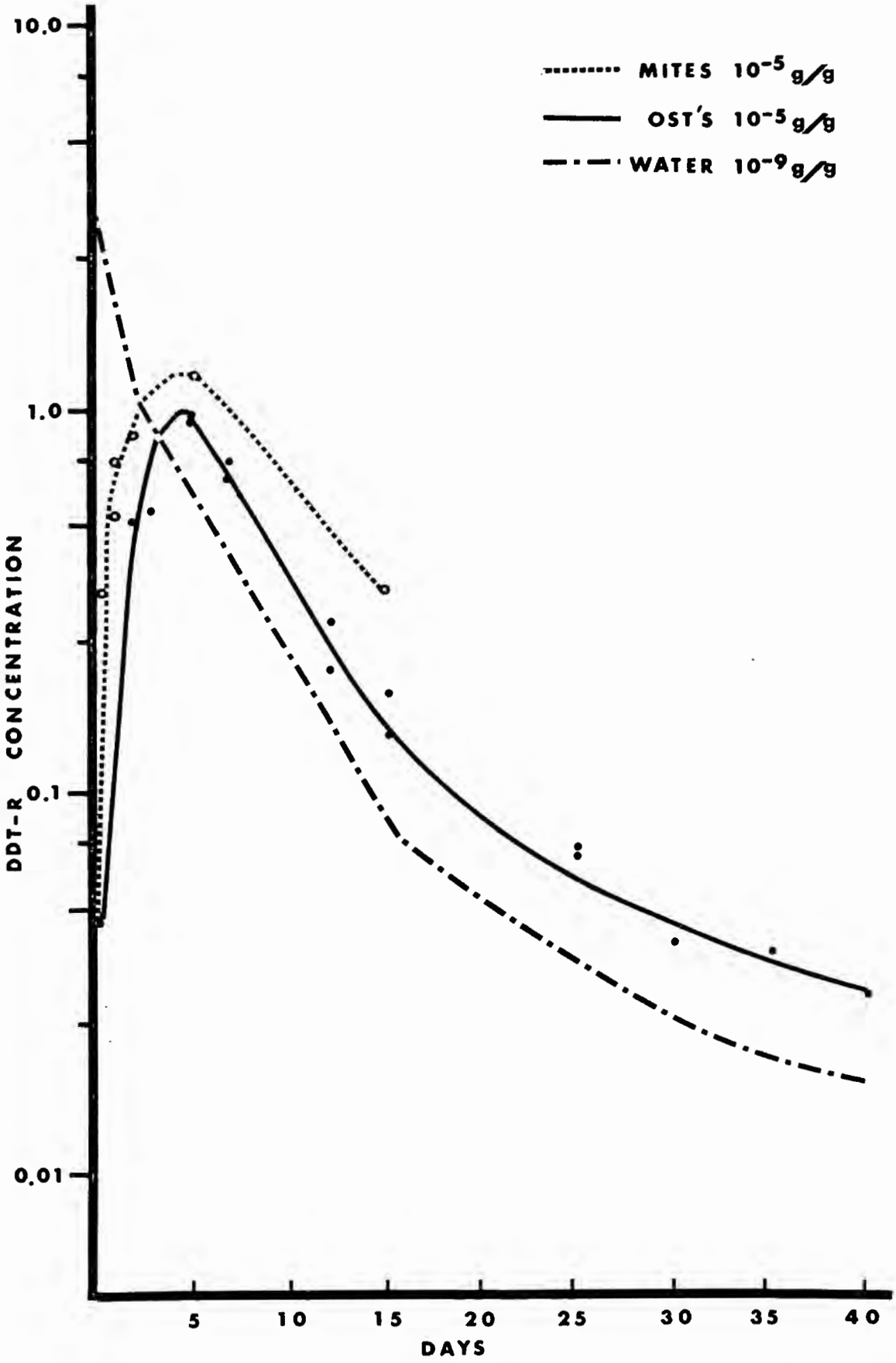
MITES 10^{-5} g/g
OST'S 10^{-5} g/g
WATER 10^{-9} g/g
DDT-R CONCENTRATION
10.0
1.0
0.1
0.01
5
10
15
20
25
30
35
40
DAYS

Figure 16

crushing resulted in body fluids and DDT-R residues being lost from the naiads. Therefore, DDT-R levels in the three types of invertebrates may not have been as different as shown by these data.

The DDT-R concentration found in the invertebrates was constantly changing. Whether individual animals exchanged residues with the water or the changes observed in concentration resulted from changes in the populations was not determined. The concentration of DDT-R in the animals was correlated with the DDT-R concentration in the water once equilibrium was reached five days after the DDT was applied (Figure 17). Thus, invertebrates appear to take up and retain DDT-R residues in direct proportion to the concentration dissolved in the water.

The light traps proved to be efficient collecting devices for those invertebrates susceptible to capture; notably ostracods, mites and odonates. Under normal field conditions the traps are not acceptable for making population density measurements, but they were used in the pools to provide a unit of measure for comparing invertebrate densities among the pools. Traps were rotated among the pools and placed in randomly selected positions within the lattice work of periphyton sheets. Differences in trapping efficiency among the traps was observed only when the colored filters had faded or the traps were damaged in some way. Two traps were usually placed in a pool at the same time in

Figure 17. Concentration of DDT-R in invertebrates versus concentration in water.

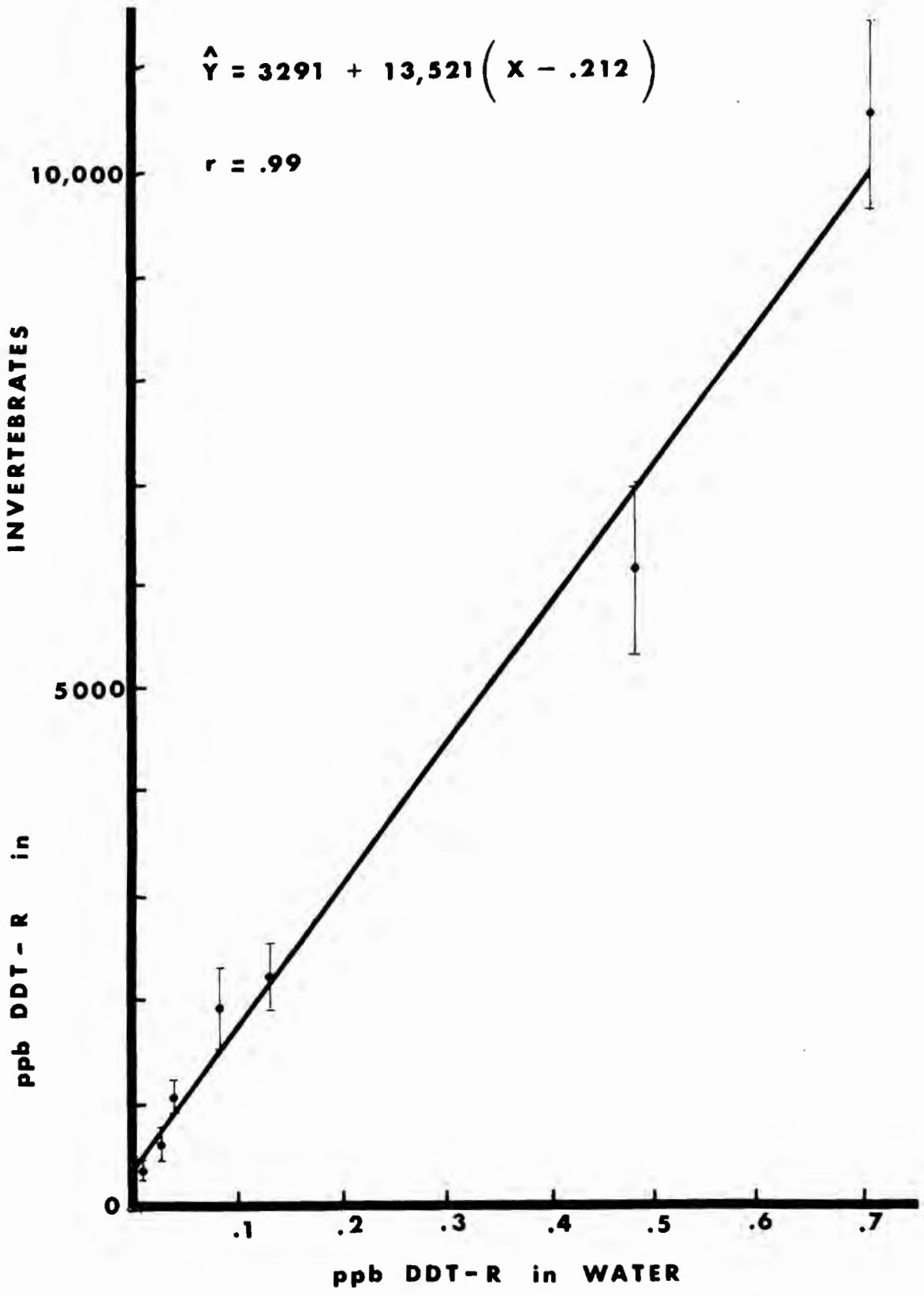
$\hat{Y} = 3291 + 13{,}521\left(X - .212\right)$
r = .99
10,000
5000
0
INVERTEBRATES
ppb DDT - R in
.1
.2
.3
.4
.5
.6
.7
ppb DDT-R in WATER

Figure 17

order to obtain an adequate sample mass. The average weight per trap provided a means for comparing the abundance of invertebrates in each pool.

There was an indication that the concentration of DDT-R residues in the invertebrates may have been influenced by their abundance in the pools. The average wet weight and DDT-R concentration in ostracod samples from the three treated pools are compared in Table 8. The weight of ostracods captured in the complete pool was at least 10 times the weight captured in the no-fish pool. Conversely, the DDT-R concentration in the ostracods from the complete pool was about one-half the concentration in the no-fish pool. The weight and DDT-R concentration observed in ostracods from the no-food pool were intermediate between the other two pools. Thus, the concentration of DDT-R in ostracods appeared to be inversely related to their abundance in a pool.

The total biomass of invertebrates and the quantity of DDT-R contained in the invertebrates was estimated for each pool. The estimates were made by assuming one-tenth the biomass of invertebrates in a pool was captured per trap during a night long collection period. Under this assumption, the highest biomass recorded for any pool was 20 grams or about 3 mg per liter. This was observed in the complete pool 30 days after the DDT was applied (Table 9). The maximum amount of DDT-R contained in all the invertebrates living in one pool was estimated to be 10.43 ug or

Table 8. Average sample weight and DDT-R concentration for ostracods in the three treated pools.

Sample	No-Fish Pool		No-Food Pool		Complete Pool	
day	Grams	ppb DDT-R	Grams	ppb DDT-R	Grams	ppb DDT-R
15	0.0143	2319			0.8556	1655
25	0.0291	1527	0.0437	1130	0.9433	703
30	0.1218	1112			2.0342	400
35			0.2529	565	1.1207 (A)	368
40	0.2184	485	1.1370	390	1.7672	291

(A) Only one trap used in the complete pool on day 30.

Table 9. Biomass in grams of invertebrates and total micrograms of DDT-R contained in invertebrates estimated to be in the complete pool and no-fish pool.

Sample	Complete Pool		No-Fish Pool	
(day)	Biomass in grams	Micrograms of DDT-R	Biomass in grams	Micrograms of DDT-R
5	0.879	9.12	0.123	1.206
7	1.400	10.43	0.358	2.194
12	2.945	6.68	0.230	0.422
15	8.725	10.32	0.376	0.559
25	9.433	6.61	0.531	0.742
30	20.342	8.11	2.109	1.743
35	11.207	4.32	---	---
40	17.672	5.14	2.360	1.170

about 0.03 percent of the DDT added to the pool. This was recorded in the complete pool 7 days after the DDT was added (Table 9). Based on observations on the pools and attempts to quantify invertebrates by conventional means, the estimates made are felt to be reasonable. However, even if the biomass of invertebrates was 10 times greater than I have estimated, the amount of DDT-R they could have contained would still be only a small fraction of the total DDT added to each pool.

2) Quantity and concentration of DDT-R in algal periphyton:

If biological magnification of DDT-R is due to the greater solubility of DDT-R in body fluids than in water, then, after a period of time, the concentration of DDT-R contained in biotic components should be related to the concentration of DDT-R in water. The concentration of DDT-R in invertebrates was directly correlated with the concentration of DDT-R in water (Figure 17). Presumably the concentration of DDT-R in algal periphyton would also be correlated with the concentration of DDT-R in water, particularly since algae take up DDT-R solely from the water. There was some evidence for such a relationship in the algae from the natural pond (Figure 18). But this is only an approximation based on the four samples analyzed.

The concentration of DDT-R in the algae from the pools (Table 10) is compared to the concentration of DDT-R

Figure 18. DDT-R concentration in algae from the natural pond compared to the concentration in the water.

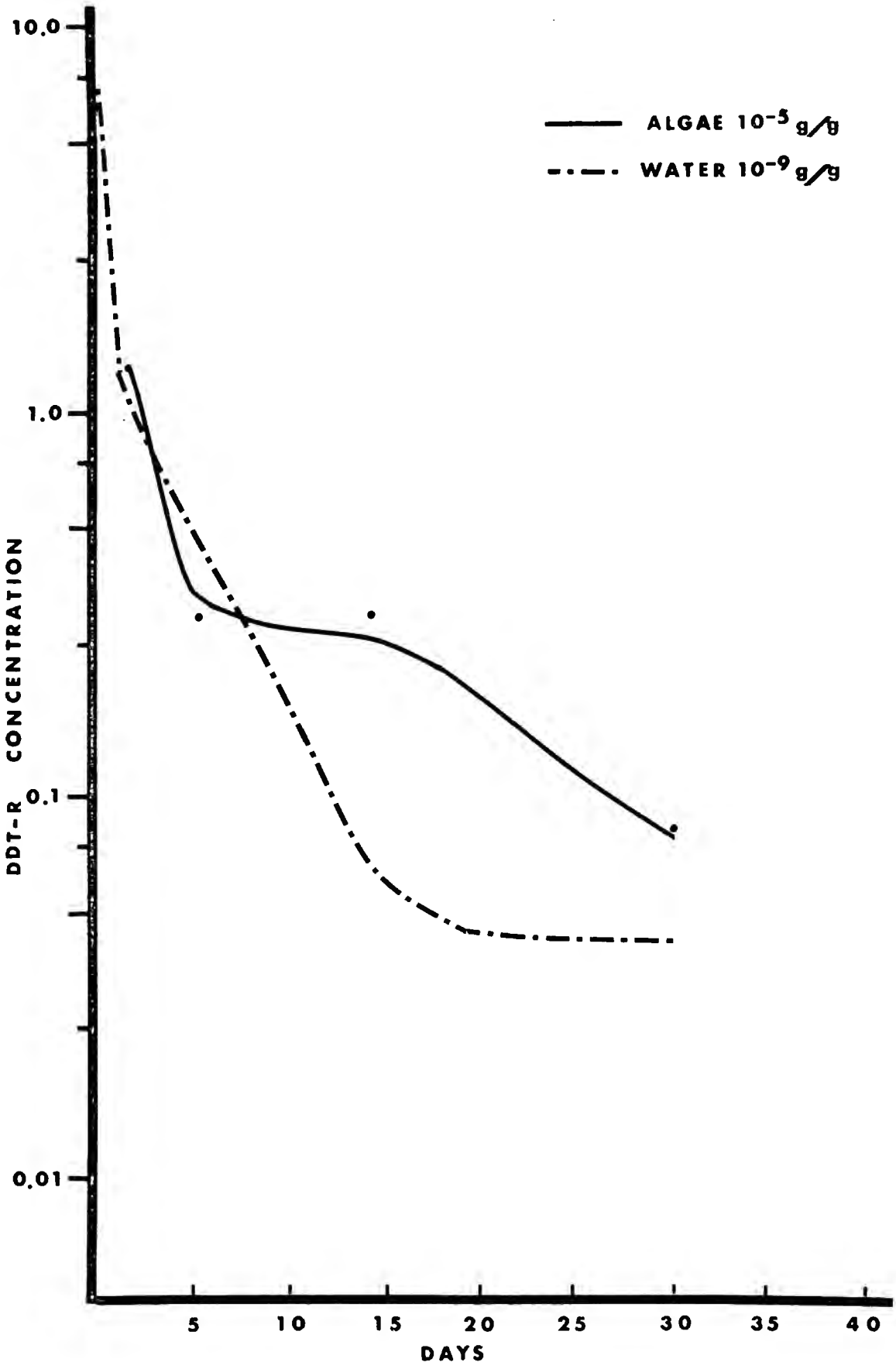
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
ALGAE 10^{-5} g/g
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 18

Table 10. Algal periphyton wet weights and DDT concentrations from all treated pools combined.

Sample		Wet weight in grams			DDT Concentration 1×10^{-9} g/g		
Day	Hour	N	$\bar{X}$	SE	N	$\bar{X}$	SE
Pretreat.		4	2.2252	0.2767	2	24	
0	1	6	1.6161	0.2356	3	136	86
	6	5	1.5054	0.4186	3	178	87
	12	6	1.6219	0.1633	4	207	21
1	24	6	3.0750	0.4617	5	227	33
	36	5	1.8729	0.2902	5	420	43
2	48	6	2.0808	0.2051	6	624	106
3	72	6	2.1178	0.2409	4	380	54
5	120	5	2.4458	0.5339	5	444	32
7	177	6	2.0053	0.2145	5	463	77
9	216	6	2.3563	0.2936	6	451	44
12	288	6	2.1717	0.1869	6	275	35
15	360	6	2.7223	0.5181	6	284	32
25	600	6	1.8794	0.3412	6	220	29
30	720	6	1.2439	0.2597	6	285	44
40	960	6	1.2080	0.2089	6	174	27

in the water in Figure 19. Data for algae in the pools does not show a linear correlation between DDT-R concentration in the water and in the algae (Figure 20). Since the relationship between the concentrations was not a direct correlation, some other factor besides solubility must have affected the concentration of DDT-R contained in algae samples.

The relationship observed between the concentration of DDT in the water and the algae may have arisen because DDT was bound to the surface of the algae instead of being dissolved in cellular fluids. Some evidence exists for this type of relationship as a result of an error during the preparation of some samples. Six algae samples were stored in a refrigerator for two days just after being scraped off the sheets. Algae was usually separated from water used during the scraping process in a few hours; but, in this case, the algae were held in the water. The cell lysis which occurred during storage permitted cellular fluids to escape into the water overlying the algae. The supernatant containing the cellular fluid was drained off and analyzed for DDT-R. The amount of DDT-R contained in the supernatant was insignificant compared to the amount retained by algae. Also, data from these algae samples were indistinguishable from comparative data attained from other samples. Thus, the concentration of DDT in algal samples may depend on DDT adsorbed to the surface of cells, instead of DDT dissolved in cellular fluids.

Figure 19. DDT-R concentration in algae from the pools compared to the concentration in the water.

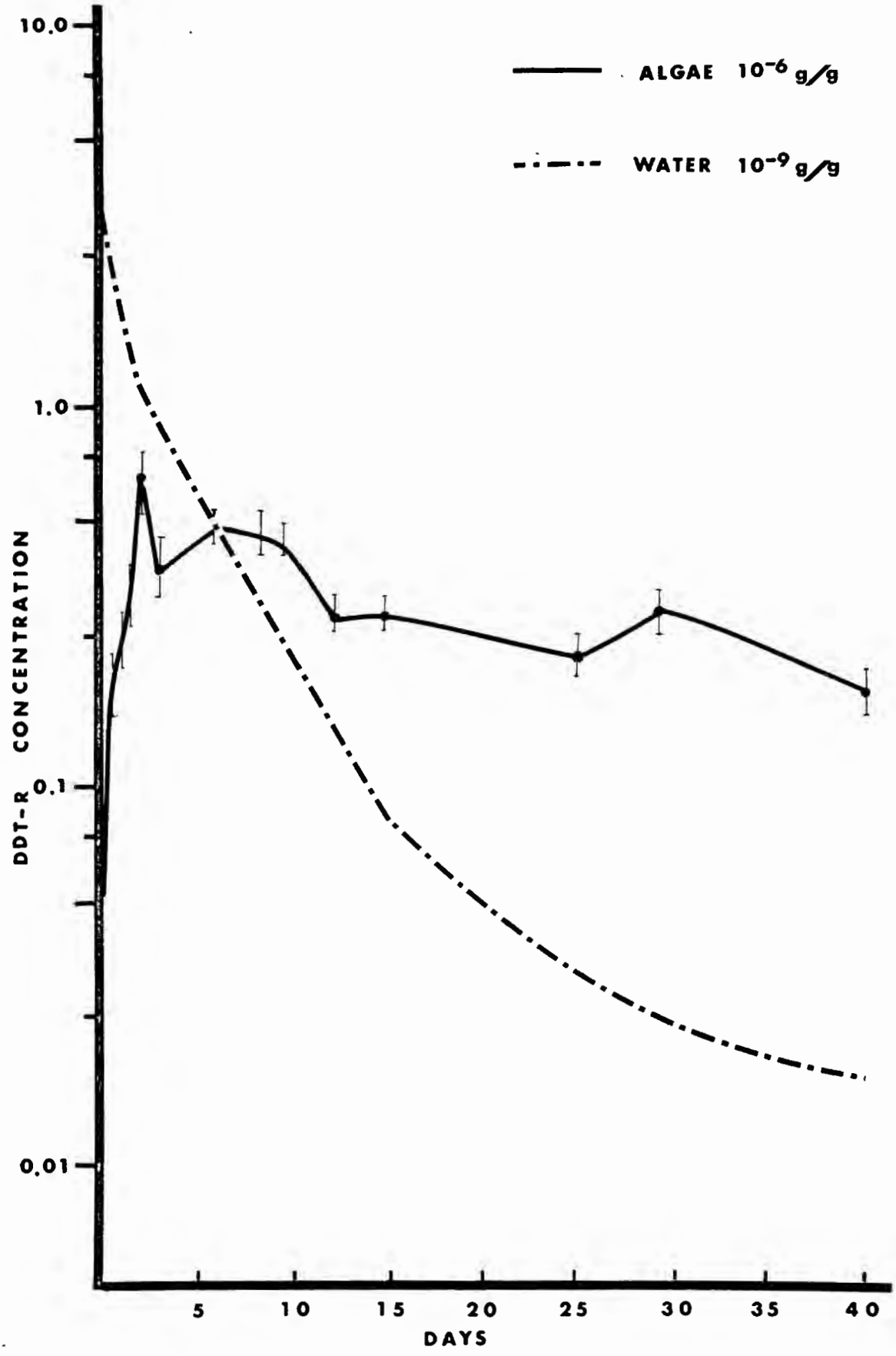
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
ALGAE 10^{-6} g/g
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 19

Figure 20. Concentration of DDT-R in algae versus concentration in water.
Number of days after treatment is indicated for each distribution. Line was fitted by inspection.

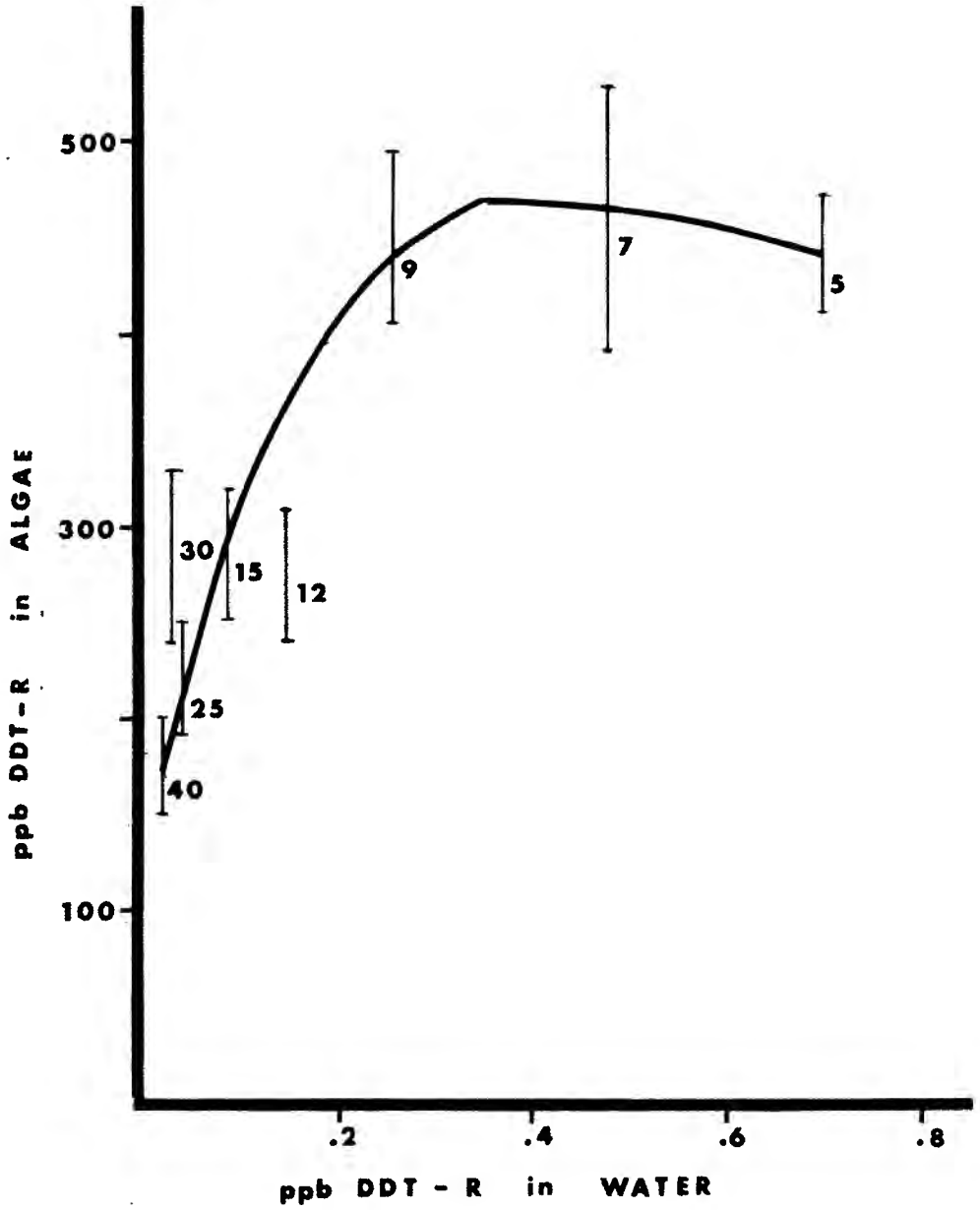
500
300
100
ppb DDT-R in ALGAE
30
15
12
25
40
9
7
5
.2
.4
.6
.8
ppb DDT - R in WATER

Figure 20

Uniform algal substrate size permitted the biomass of algae in the pools to be estimated. The biomass was estimated from the average wet weight of algae collected per unit area, times the surface area of the substrates and the pool sides. Amounts of DDT contained in the algae of a pool were estimated from the average concentration of DDT and biomass for each sampling period. As shown in Table 11, the greatest amount of DDT held by the algae was 149 ug or 0.4 percent of the DDT added to each pool.

3) Quantity and concentration of DDT-R in fish:

Fish in the natural pond, common shiners and dace, rapidly took up and retained about 15 ppm DDT-R (Table 12). Food organisms in the pond appeared to be affected by the DDT applied. Large numbers of chironomid midge larvae were observed to come out of the bottom and swim erratically to the surface. Chaoborus sp. larvae, mites and dragonfly naiads were also observed swimming erratically. The fish fed heavily on the affected food organisms for a few days after the application. However, the food supply was reduced by the fish and the DDT. Consequently, within a week the fish began to catabolize their stored fat. Since the water content of fish tissues is inversely related to fat content, lipid catabolism not only accompanied growth but the wet weight of samples was further increased by tissue hydration. These weight changes presumably account for the fluctuation

Table 11. Quantity of algae and the concentration and quantity of DDT in the algae per pool.

Sample Day	Sample Hour	Biomass (grams)	Concentration DDT in algae (1×10^{-9} g/g)	Weight of DDT in algae (1×10^{-6} g)	Percent of DDT added in algae
Pretreat.		278	24	6	0.019
0	1	201	136	27	0.078
0	6	184	178	32	0.093
0	12	186	207	38	0.110
1	24	365	227	82	0.236
1	36	219	420	91	0.262
2	48	239	624	149	0.426
3	72	238	380	90	0.258
5	120	271	444	120	0.343
7	177	218	463	100	0.288
9	216	251	451	113	0.323
12	288	228	275	62	0.179
15	360	280	284	79	0.227
25	600	189	220	41	0.188
30	720	122	285	34	0.099
40	960	116	174	20	0.057

Table 12. Average concentration of DDT-R residues in fish from natural pond.

Sample (days)	ppm Concentration (wet weight) MDE	DDE	DDD	DDT	DDT-R
Pretreat.	.01	.03	.01	N.D.	.05
1	N.D.	4.95	2.09	2.34	9.38
5	.05	14.16	1.75	3.78	19.74
15	.34	4.05	1.71	1.36	7.46
30	.39	11.62	2.08	.29	14.38

N.D. = None detected.

in DDT-R concentration observed in the fish from the pond compared to the concentration in the water (Figure 21).

An abundant food supply did not develop in the complete pool. Consequently, except for being associated with some subtle differences in the amounts of each residue, the supply of natural food available in the pools did not appear to influence the DDT-R concentration in the fish. Thus, the DDT-R concentrations in the fish from the complete pool and the no-food pool did not differ significantly from each other.

Data for the fish from both the complete pool and no-food pool are presented combined in Table 13 and compared to the concentration of DDT-R in the water in Figure 22. Again, as in the natural pond, the fish rapidly took up and retained about 1.5×10^{-5} g/g DDT-R. However, in agreement with the other biotic components, the fish were estimated to contain less than one percent of the DDT added to the pools.

4) Quantity and concentration of DDT-R in bottom material:

The hydrophobic nature of DDT in water causes DDT to be attracted to surfaces (Bowman et al., 1959). DDT can be removed from water by adsorption to soil particles (Berck, 1953) which may result in an accumulation of DDT on stream (Barthel et al., 1966) and lake bottoms (Hickey et al., 1966). It was postulated that a large portion of the DDT

Figure 21. Average DDT-R concentration in the fish from the natural pond compared to the concentration in the water.

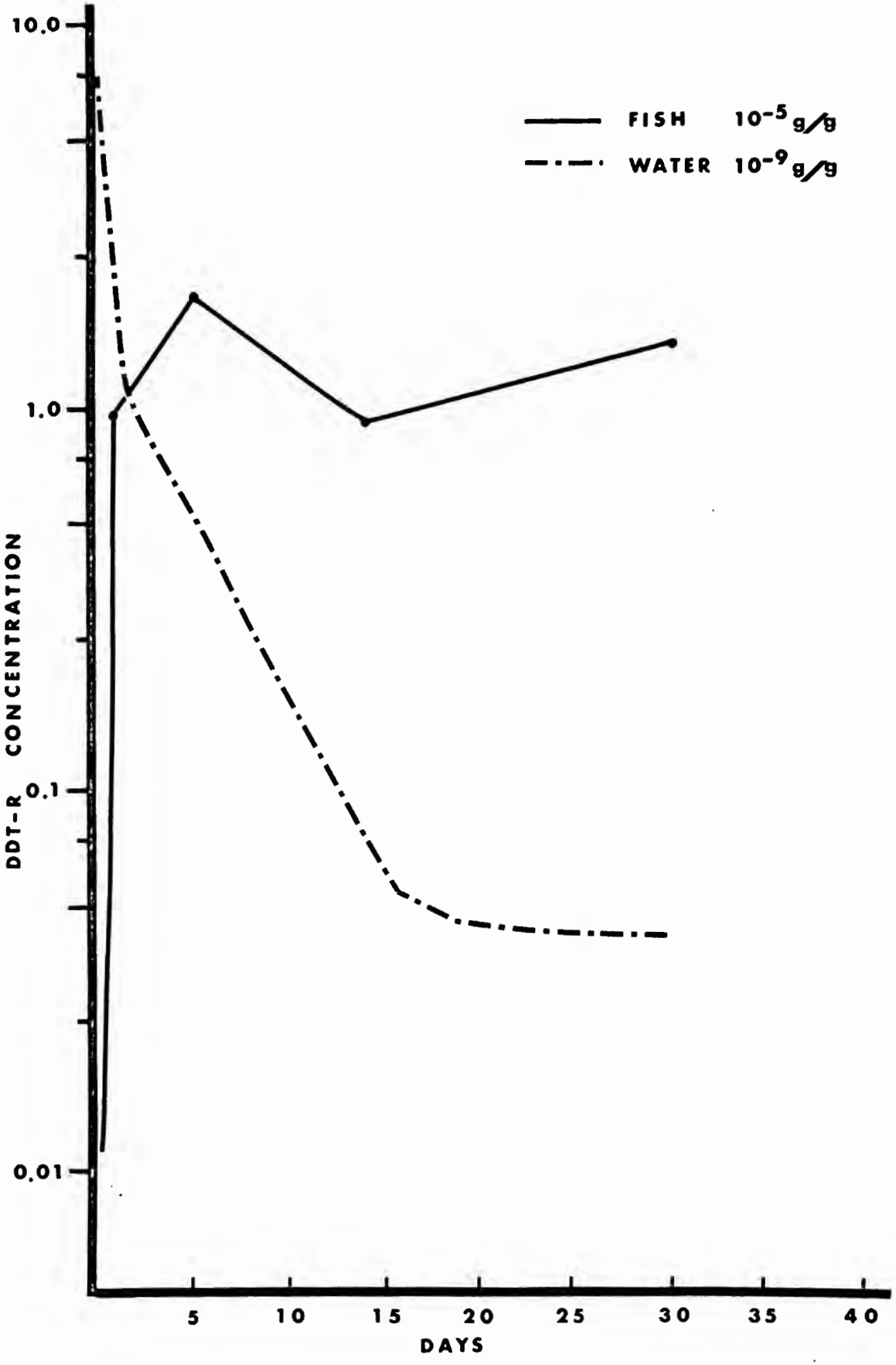
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
FISH 10⁻⁵ g/g
WATER 10⁻⁹ g/g
5
10
15
20
25
30
35
40
DAYS

Figure 21

Table 13. Concentration of DDT-R in fish from the no-food pool and complete pool.

Sample (Day)	Number	ppm DDT-R (mean)	Standard error
1/2	4	1.688	.310
2	4	4.115	.247
3	2	8.183	.357
5	5	11.904	.971
7	4	12.015	.384
9	2	13.692	3.262
12	2	13.654	.215
15	2	14.937	.754
25	2	12.046	.447

Figure 22. DDT-R concentration in fish from two treated pools compared to the concentration in the water.

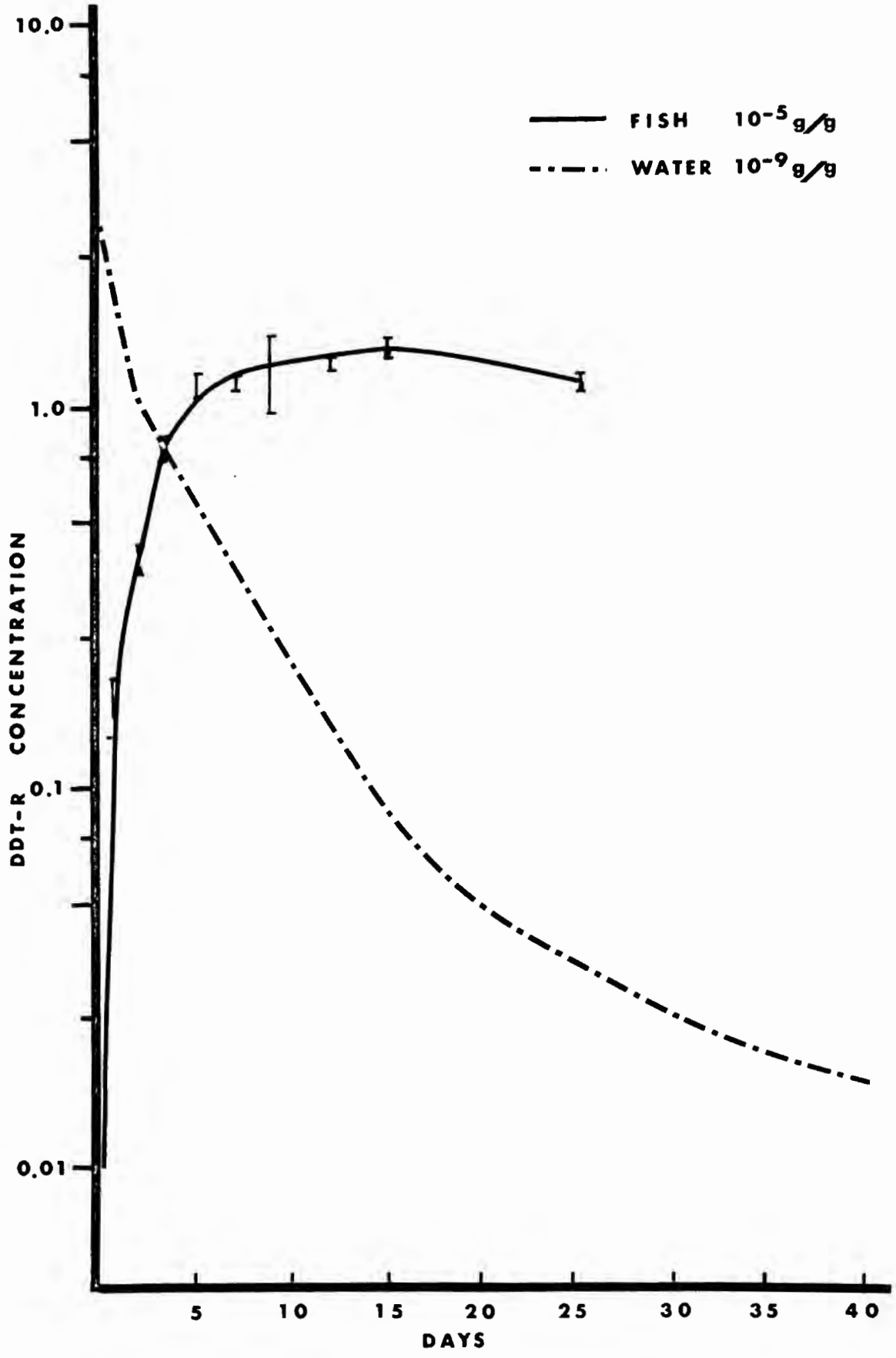
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
FISH 10^{-5} g/g
WATER 10^{-9} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 22

introduced into the experimental units would be deposited on the bottom. Furthermore, the DDT-R associated with a thin, uppermost layer of sediments would establish some sort of equilibrium with DDT-R in the water. In this manner, a continuous supply of DDT-R into the system would be provided and a relatively high concentration of DDT-R would be sustained in the biota for a long time.

DDT-R residues deposited on the bottom of the natural pond were released from the sand during the course of the study. About 14 ppb DDT-R accumulated in the bottom the first 5 days and then disappeared from the bottom during the first year following treatment (Figure 23). DDD and DDT were the primary residues found in the bottom material from the pond.

The sand used for the bottom material in the pools contained about 1 ppb real or artifact DDT. Also two artifacts eluted from the G.L.C. on either side of DDD which prevented quantification of less than 0.5 ppb of DDD. These chromatographic responses are anologous to the "background" radiation encountered when conducting radioactive tracer studies. Likewise, quantification depends on the "background" either being eliminated or exceeded to a significant degree by the "tracer" employed. In this case, stable DDT was the tracer employed. I was unable to eliminate the artifacts, but if over 25 percent of the DDT added to the pond was deposited on the bottom, the DDD and DDT residues

Figure 23. Average concentration of DDT-R and each residue (DDT, DDD and DDE) found in the sandy bottom material of the natural pond.

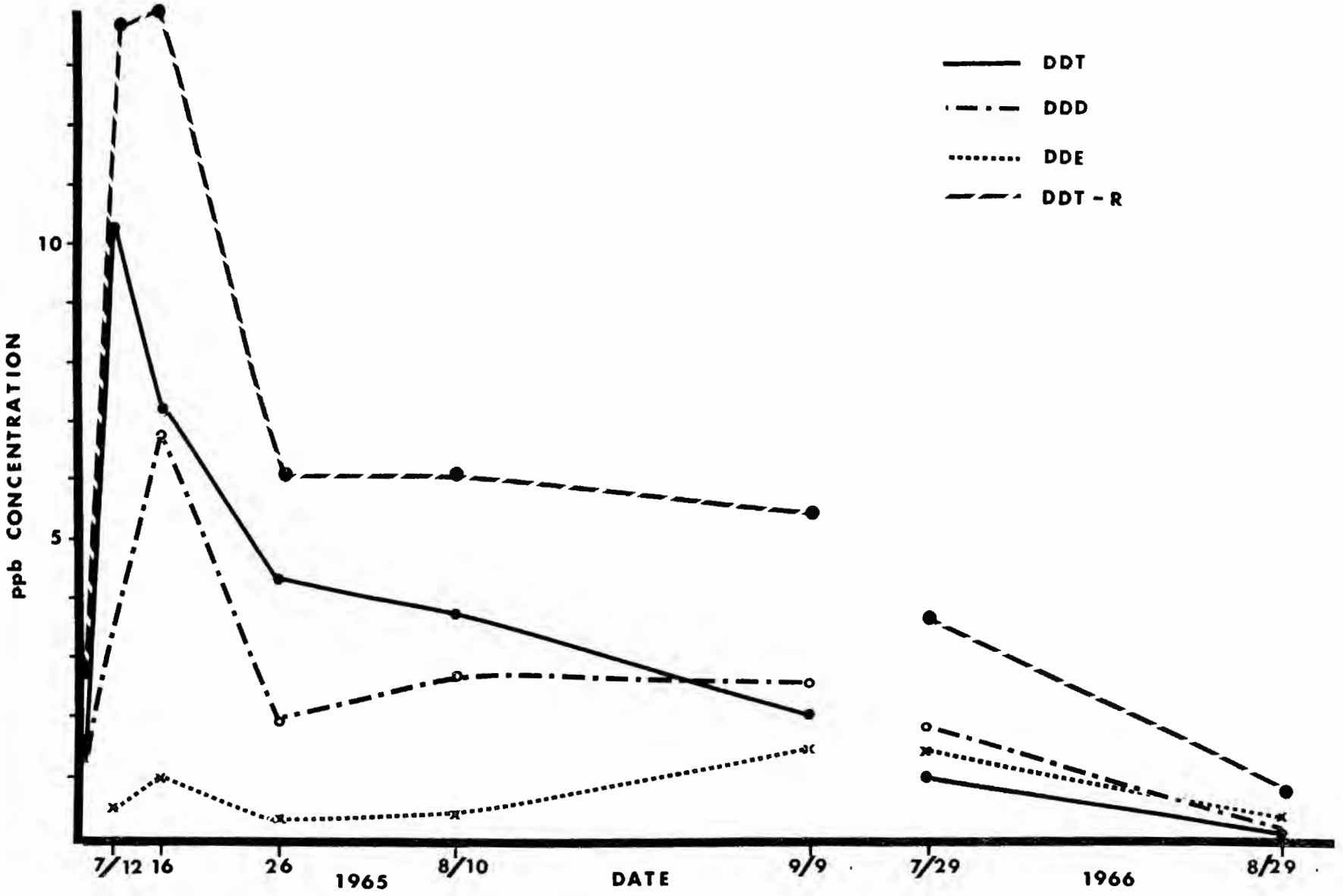
DDT
DDD
DDE
DDT - R
10
5
ppb CONCENTRATION
7/12
16
26
8/10
9/9
7/29
8/29
1965
DATE
1966

Figure 23

combined for the samples taken should have exceeded pretreatment concentrations by 1.75 ppb.

A concentration increase of 1.75 ppb in the sand would have been sufficient for accurate quantification on a G.L.C. with an E.C. detector. This residue concentration would have also permitted analysis to be confirmed on the specific, but much less sensitive, microcoulometric detector. The combined DDD and DDT residue concentrations in a few samples reached or exceeded the 1.75 ppb level, but the average residue concentration for all bottom samples from the treated pools combined did not at any time differ significantly from the concentration in pretreatment samples (Table 14). Thus, less than 25 percent of the DDT added to the pools appeared to be deposited on or in the bottom.

Inadequate amounts of DDT-R residues for quantitative analysis prompted a review of analytical procedures used for bottom samples. Five different extraction procedures were compared to test for DDT-R which may have been tightly bound to the soil and consequently missed by the procedure employed. The procedures tested were:

1) Air dried and extracted with 20 percent methanol in benzene.
2) Moistened with 25 percent water and extracted with a mixture of equal parts methanol and benzene.
3) Air dried and extracted with 20 percent ethyl ether in petroleum ether; the standard procedure.

Table 14. Concentration of DDD and DDT combined, in bottom material from pools.

Sample Day	Hours	Number	ppb DDD and DDT (mean)	Standard error
Pretreat.		5	1.168	.246
0	1	1	.557	
0	6	1	.595	
0	12	1	1.116	
1	24	4	1.012	.134
1	36	2	1.687	.169
2	48	2	2.330	1.70
3	72	6	1.54	.26
5	120	5	1.52	.16
7	177	5	1.35	.16
9	216	5	1.86	.29
12	288	1	3.36	
15	360	3	2.25	.627
25	600	5	1.50	.440
30	720	1	1.50	
40	960	3	1.31	.66

4) Moistened with 25 percent water and extracted with 20 percent ethyl ether in petroleum ether.
5) Moistened with 25 percent dilute formic acid and extracted with a mixture of equal parts methanol and benzene.

No significant differences were observed in DDT-R recovery, except greater analytical interference was encountered in moist samples than in the dry ones. These tests did not exclude the possibility that the DDT-R residues were lost prior to extraction during the drying process. Significant amounts of DDT can be lost from bioassay test containers due to codistillation (Bowman et al., 1959), but Lichtenstein (1966) reported that DDT losses were not observed when water was evaporated from test soils.

DDD and DDT residues contained in bottom samples from the pools were combined for presentation, because the actual quantities of each residue could not be given with certainty while the combined total could. All the samples analyzed on the E.C. detector and SE-30 column appeared to contain both DDD and DDT. When the samples were analyzed with the microcoulometric detector inconsistent results were obtained. The irregular results were due to reduction decomposition of DDT to DDD in the injection port. The reduction of the DDT was catalyzed by impurities contained in the highly concentrated sample solutions required for analysis. Finally, analytical confirmation was made using an E.C.

detector and a QF-1 column from which DDD and DDT eluted simultaneously. Consequently, the actual amounts of each DDD and DDT was not determined, but the combined DDD and DDT residue concentration was determined.

Disappearance of the DDT Added to the Study Units:

The amount of DDT-R found in the flora, fauna and sediments was compared to the amount of DDT added to the system and a large percentage of the DDT added to the pools could not be accounted for as the study progressed (Figure 24). DDT added would not be accounted for if DDT-R residues were being:

1) Irreversibly bound to some component in the system.
2) Degraded to unidentified products.
3) Lost from the system to the atmosphere.

Each of these possibilities will be discussed in the order stated above.

1) Irreversibly bound DDT-R in the system:

DDT-R could have been irreversibly bound to some component in the pools which was not being sampled. Plastic used for the pools and periphyton substrates constituted a component which may have bound DDT-R, though it was not believed to be a major problem. DDT-R residues were not extracted from plastic placed in the pools. Periphyton which covered the pool liners and substrates presumably minimized

Figure 24. The percentage of DDT added to the pools estimated to be in all components sampled as the study progressed.

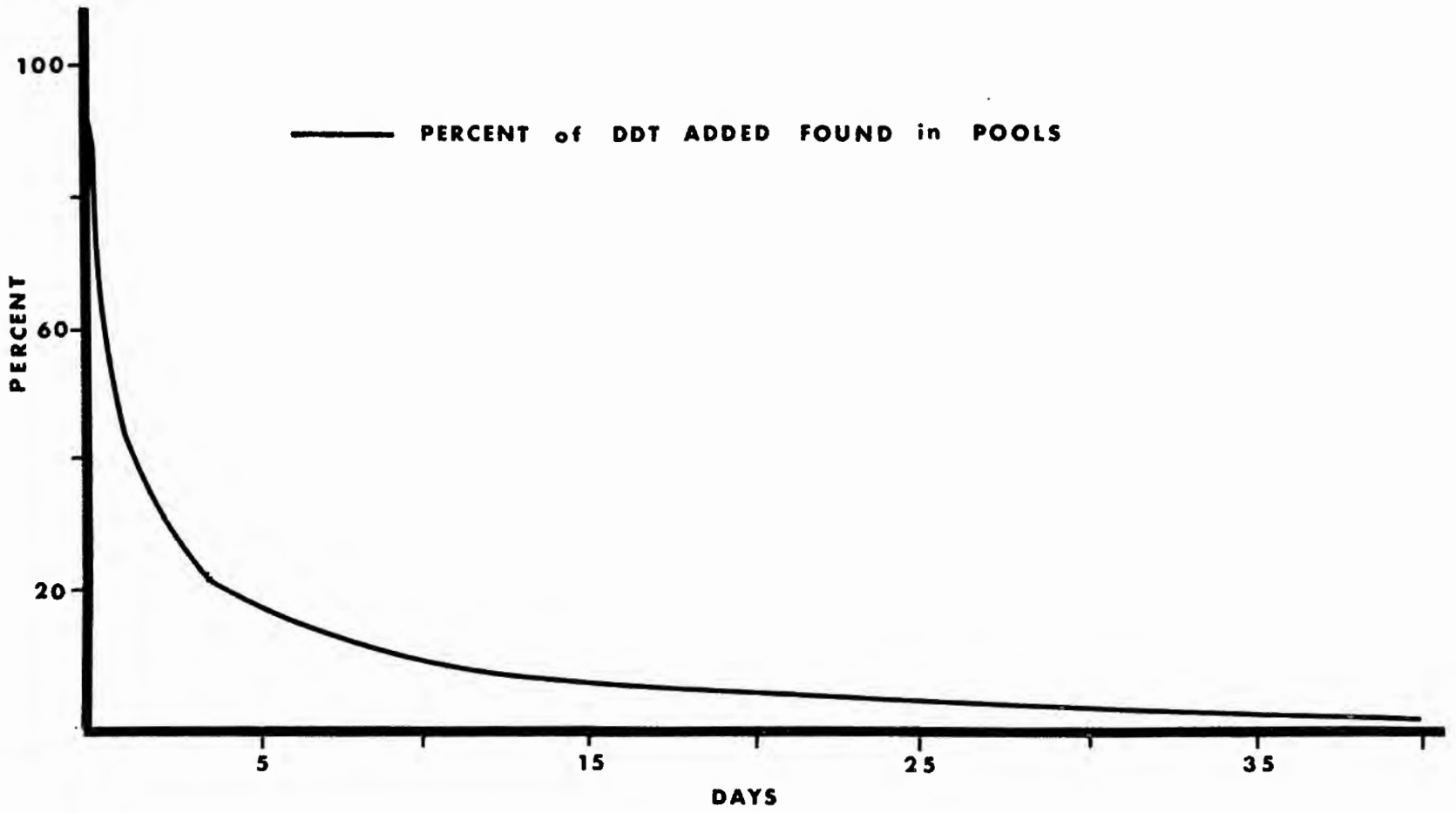
100
60
20
PERCENT
PERCENT of DDT ADDED FOUND in POOLS
5
15
25
35
DAYS

Figure 24

direct adsorption. Finally, a similar percentage of DDT-R appeared to be lost in the natural pond experiment and in natural pond experiments conducted by others (Andrews et al., 1966; Edwards, 1964). Since all other components of the system were evaluated and found to be free of bound residues, it is believed only a small portion of DDT-R residues were being irreversibly bound to some component within the study units.

2) DDT degradation to unidentified products:

DDT can be degraded to a number of compounds by reduced porphyrins (Castro, 1964), lake water (Miskus et al., 1965), bacteria (Wedemeyer, 1967), insects (Sternburg, et al., 1954) and fish (Greer and Paim, 1968). In the aquatic environment DDT may be degraded to DDE or DDD. DDD in turn may be degraded to MDE. Degradation of DDT to DDA, the product commonly formed by mammals (Jensen, et al., 1957), has not been reported in the aquatic environment.

Factors controlling degradation are complex (Alexander, 1966) and the products which may be formed are numerous. DDE or DDD is the first stable intermediate formed in the pathways presently known. Thus, the presence of either one in a sample would indicate degradation has occurred somewhere in the ecosystem. Both DDE and DDD were found in different concentrations in a variety of samples taken during the study. Only DDE and DDD were found in the

pool samples, despite a thorough search for additional compounds. Bridges et al. (1963) also reported the presence of these degradation products in a comparable pond study.

The large decrease over time in the amount of DDT-R accounted for in a pool (Figure 24) may have been due to rapid degradation of the DDT-R to unidentified products. A hypothetical case estimates the rate of degradation required to produce the observed decrease in DDT-R. The decline observed in the amount accounted for could have resulted if the half-life for the degradation of the DDT to DDD and DDE was four days, and their half-life for degradation to non-detectable products was one day. Since DDD and DDE are very persistent in aquatic environments (Johnson, 1968) the chance these conditions could have developed appears remote. Thus, degradation of the DDT to products which went undetected by the methods employed does not appear to account for the disappearance of the DDT applied.

3) DDT-R losses to the atmosphere:

Suffering unaccountable losses of DDT in experimental aquatic systems is not a new problem (Cope, 1965). Bowman et al. (1959) evaluated the losses of DDT from larvicide suspensions and observed that a large percentage was lost by volatilization with the evaporating water. A detailed study of DDT codistillation with water from small test jars was conducted by Acree et al. (1963). In the

latter study about 77 percent of the DDT was lost when about 5 percent of the 250 ml of distilled water was evaporated (average for 3.6 to 0.36 ppb range at 25°C). I conducted a comparable study using gallon jars containing 3.0 liters of a 5 ppb DDT solution and recorded a loss of about 50 percent of the DDT with 5 percent of the water. Though the difference between gallon jars and pools is great, DDT must still be lost to the atmosphere from the pools. Thus codistillation could have accounted for a share of the DDT-R lost during the course of these studies.

The quantity of DDT-R lost by codistillation was difficult to estimate. Weight of DDT codistilled depends upon the weight of water evaporated, vapor pressure of the water and DDT, molecular weight of the two phases (Bowman, et al., 1959), and finally, the solubility of DDT in the liquid phase (Acree et al., 1963). The unusual affinity of DDT for the water surface layer produces a localized concentration at the interface which causes the codistillation rate to be roughly proportional to the concentration of DDT above the solubility level for DDT. Codistillation was also demonstrated to be proportional to the concentration below the level of solubility (op. cit.). Assuming codistillation to be proportional to concentration, less than 20 percent of the DDT would be lost in 10 days, which was the time required for 5 percent of the water in the pools to evaporate.

Assuming the surface affinity of DDT produced a localized concentration at the surface (op. cit.), and the

large pool size permitted enough DDT to be contained in the water to sustain at least saturated concentrations at the surface for a few days, then the amount codistilled would have depended primarily on factors other than concentration. Applying these assumptions, a temperature of 73°F and a DDT vapor pressure of 2×10^{-7} mm Hg (Bowman et al., 1959), about 40 percent of the DDT applied could have been lost in two days. If the actual amount codistilled was between the two estimated values, then most of the DDT-R missing from the pools would be accounted for by the amount lost to the atmosphere.

The problem of codistillation stimulated the thought that DDT could be removed from contaminated lakes by aeration. The affinity of the DDT for the air-water interface would make air bubbles an excellent vehicle to transport DDT up from the depths. Robeck et al. (1965) demonstrated that the ease with which various chlorinated hydrocarbons were removed from water was inversely proportional to their solubility. One of the ways DDT was removed from water was by treating the water with ozone (op. cit.), presumably by bubbling the gas through the water. Since DDT is extremely resistant to oxidation, removal from the water was probably due to the bubbles rather than chemical action by the ozone.

Once DDT is displaced into the atmosphere (Mitchell, 1966) it may undergo photodecomposition (Fleck, 1966). DDT adsorption bands all occur below 300 mu (Frear, 1955), so

lack of radiation with sufficiently short wave lengths in the environment would restrict auto-photodecomposition to the laboratory. Suitable solvents (alcohols or aldehydes) to serve as the catalyst required for photodecomposition at higher wave lengths via conjugated intermediates (Fleck, 1949) would also be absent in the environment. Thus, DDT would only be displaced temporarily and eventually be returned to earth in rain (Cohen and Pinkerton, 1966) or snow (Westlake and Gunther, 1966). However, aeration may provide a means for removing other pesticides from lakes (eg. Toxaphene) which readily undergo photodecomposition in the environment.

Relationships between Degradation and Distribution of DDT-R in the Lentic Environment:

The cause, rate and products of degradation may effect the distribution of DDT-R in lentic ecosystems. Degradation of DDT could not be attributed to any single component of the experimental units; rather, most components degraded DDT to some degree depending on certain conditions.

1) Degradation of DDT in components studied:

A) Degradation of DDT in algal periphyton:

DDT was not degraded in the algal periphyton sampled. Since the amount of DDT found in the samples appeared to be associated with surface area, perhaps DDT failed to

enter the algae cells and become exposed to a reaction site. Wurster (1968) presumed DDT was absorbed by marine phytoplankton prior to affecting photosynthesis, but equated cell surface areas between species in order to obtain comparable results. DDT is rarely toxic to plants, so effects and degradation of DDT in plants and algae may partially depend on adsorbed DDT being absorbed into the cells.

Phytotoxicity from DDT has been described in some varieties of barley (Hayes, 1959). San Antonio and Wiebe (1963) reported similar amounts of DDT were absorbed by both resistant and susceptible varieties of barley. No metabolites of DDT were found in treated plants, so resistance was not associated with degradation as observed in insects (op. cit.). However, prior exposure to DDE did prevent DDT from acting on susceptible barley plants (Lawler and Rogers, 1967). The site of action appeared to be the chloroplasts, where the Hill reaction may have been blocked (op. cit.). Perhaps DDT substituted for the normal electron acceptor, thus disrupting photosynthesis which led to chlorosis and eventual death. The mechanism by which DDT inhibits photosynthesis in phytoplankton has not been reported.

B) Degradation of DDT in the pond bottom:

DDT deposited in the pond bottom appeared to be degraded to DDD. Low concentration levels and recycling

made precise definition of mechanisms difficult. However, it appeared that degradation somehow accompanied recycling, since in the natural pond DDD did not accumulate while DDT was lost (Figure 23). Rather, DDD increased as DDT was being deposited but both were lost from the bottom simultaneously. Waybrant (1969) clearly demonstrated DDT degradation to DDD in the bottom, but was unable to clarify the importance of microorganisms and invertebrates in the recycling of residues.

C) Degradation of DDT in invertebrates:

DDE concentrations found in invertebrates from the natural pond (Figure 25) and in ostracods from the complete pool (Figure 26) were correlated with DDT concentrations when the DDT-R concentration was declining ($r = .99$). The concentration and the percentage of DDD the invertebrates contained was independent of DDT and DDE (Figure 27). Presumably DDT was degraded to both DDE and DDD within the animals, but DDD was also taken up independent of DDT.

The percentage DDD would increase when DDD was being taken up from the environment by the animals. The percentage of DDD in ostracods from the complete pool increased for 15 days following the DDT application and then decreased (Figure 27). Presumably, the changes in DDD concentration observed in the animals were due to the amount of DDD being recycled from the bottom.

Figure 25. Concentration of MDE, DDE, DDD and DDT in invertebrates from the natural pond in the period following application.

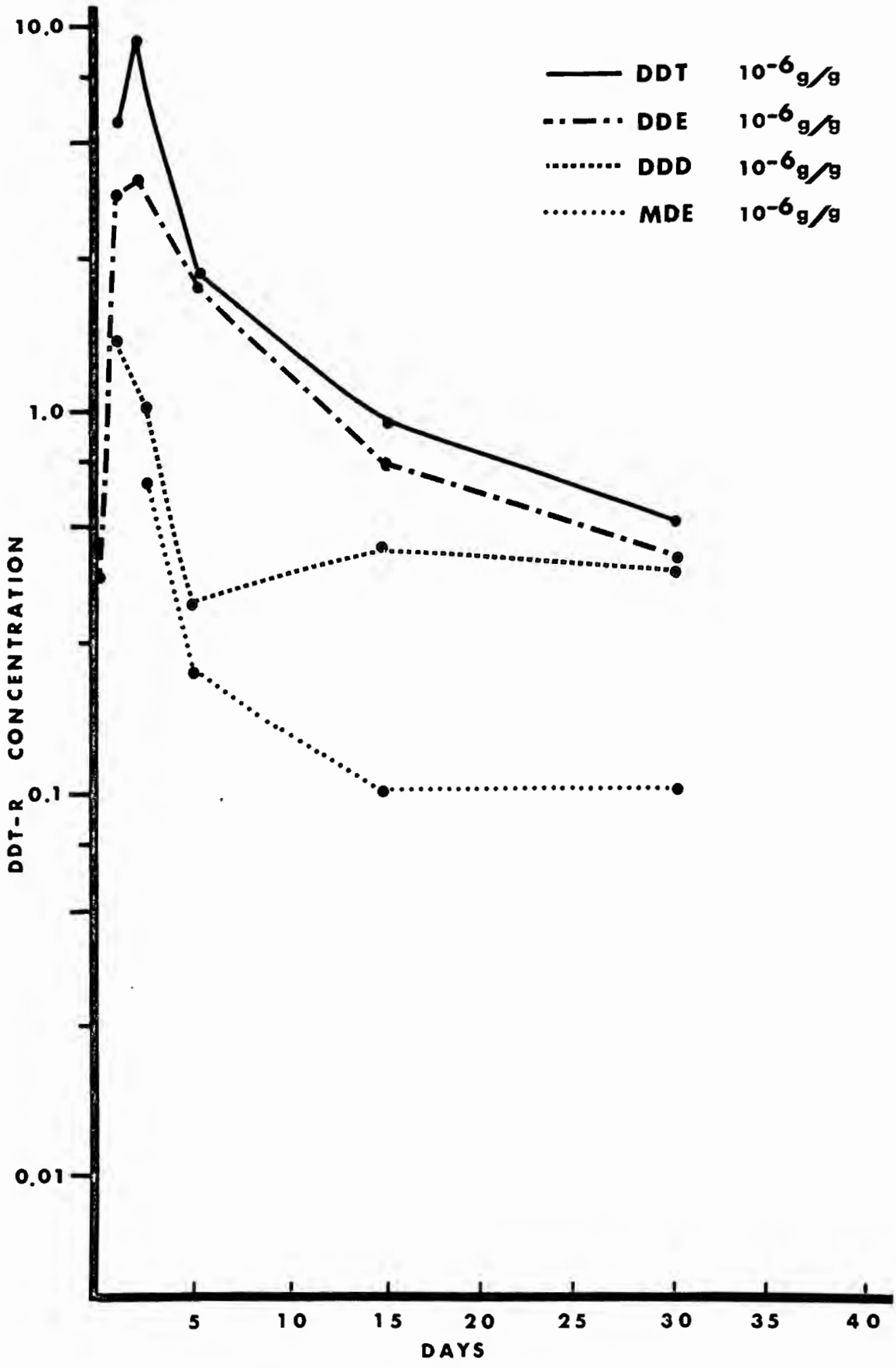
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
DDT 10^{-6}g/g
DDE 10^{-6}g/g
DDD 10^{-6}g/g
MDE 10^{-6}g/g
5
10
15
20
25
30
35
40
DAYS

Figure 25

Figure 26. Concentration of DDE, DDD and DDT in ostracods from the complete pool, in the period following application.

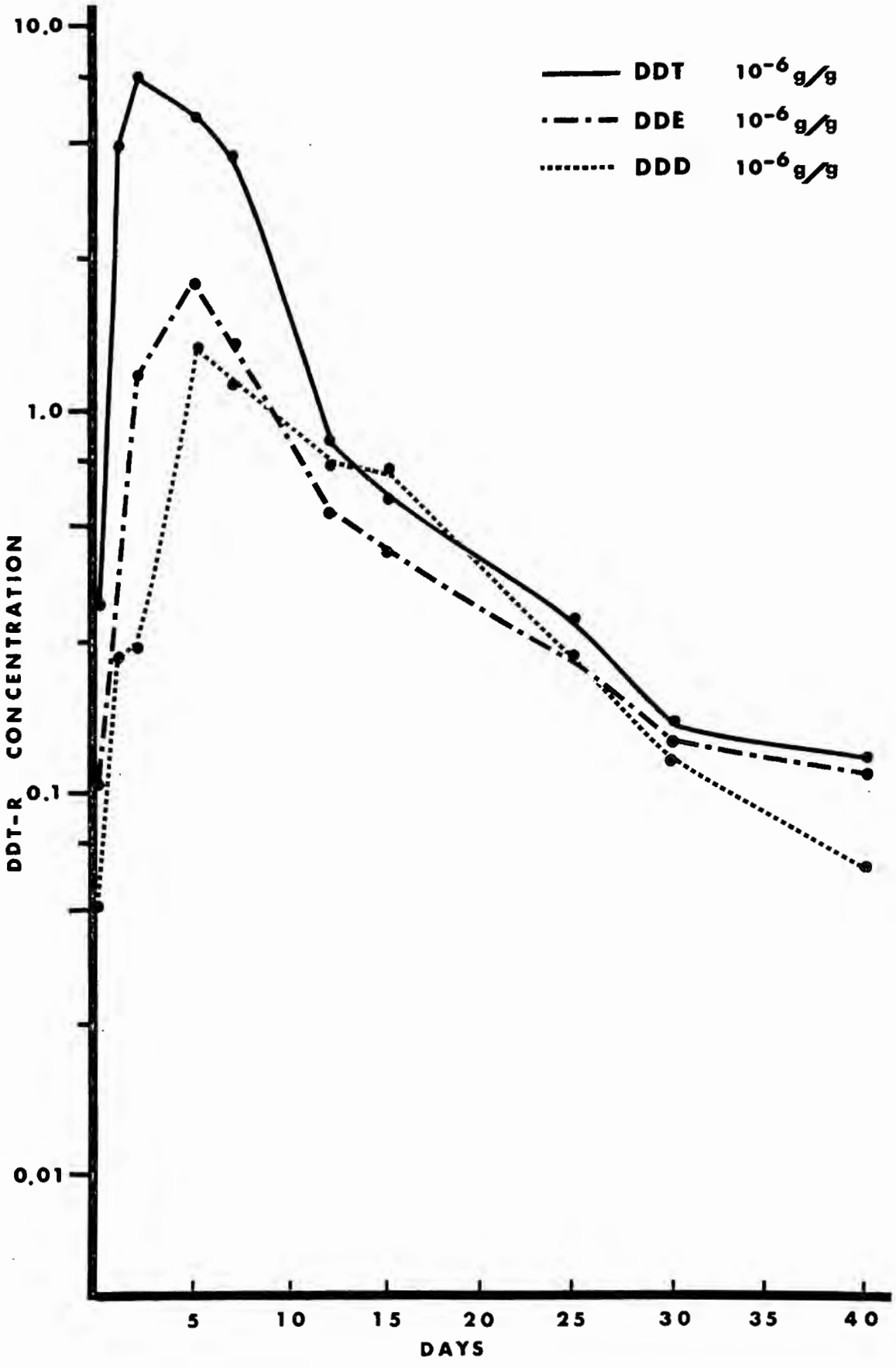
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
DDT 10^{-6} g/g
DDE 10^{-6} g/g
DDD 10^{-6} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 26

Figure 27. Percentage of the total DDT-R made up of DDE, DDD and DDT in invertebrates from the natural pond and ostracods from the complete pool and the no-fish pool.

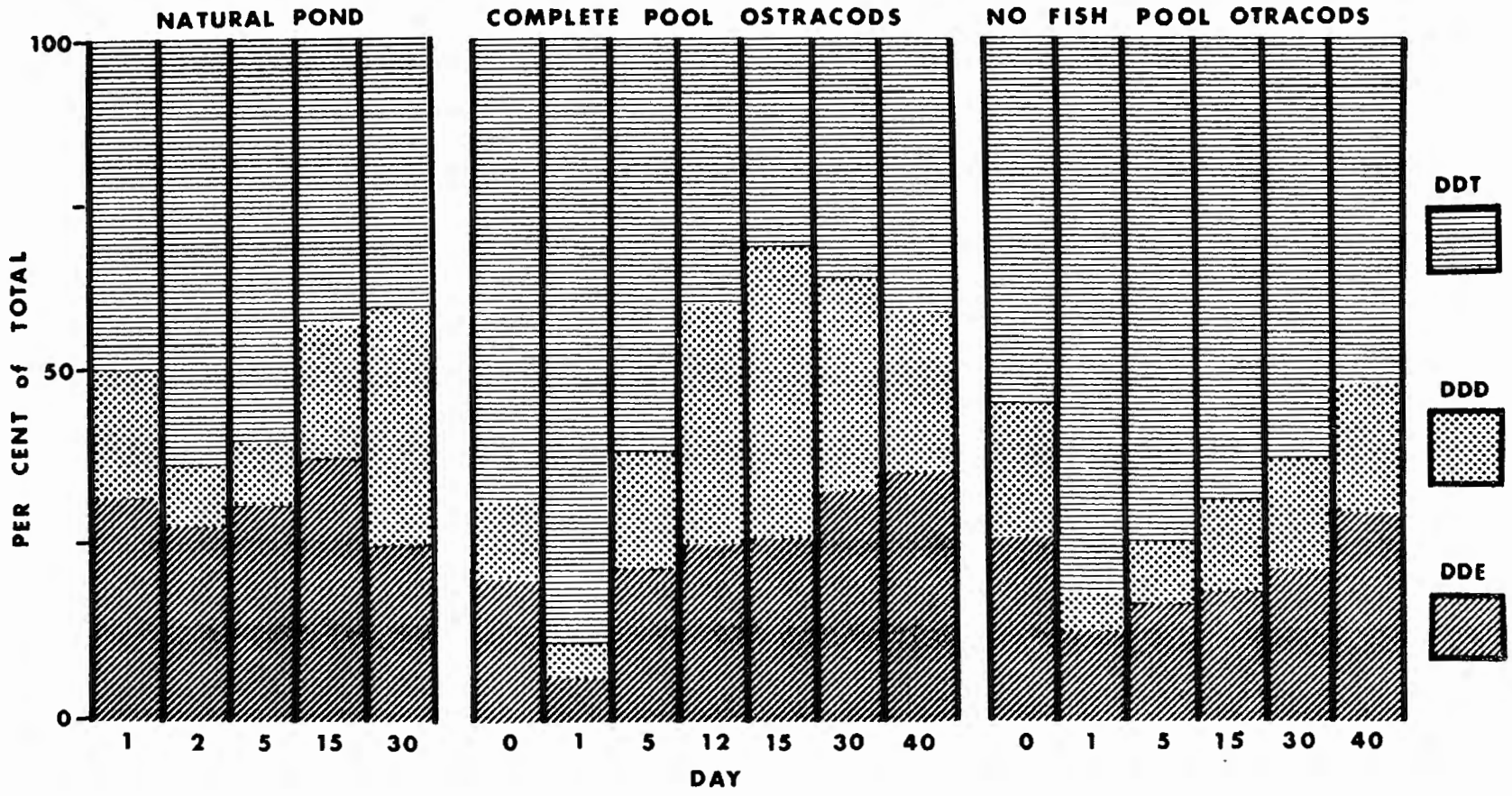
NATURAL POND
COMPLETE POOL OSTRACODS
NO FISH POOL OTRACODS
PER CENT of TOTAL
100
50
0
1
2
5
15
30
0
1
5
12
15
30
40
0
1
5
15
30
40
DAY
DDT
DDD
DDE

Figure 27

A change in the DDD concentration in invertebrates does not mean the animals caused the DDD to be recycled from the bottom. The invertebrates contained only a small amount of DDT-R, so unless turnover was extremely fast, they would not have been able to translocate a significant quantity of residue out of the bottom. This, and the observations of Waybrant (1969), suggests the invertebrates concentrated DDD from the water, after DDD was released from the bottom as a result of other factors. Thus, changes in the percentage of DDD observed in invertebrates, appears to reflect changes in the amount of DDD being recycled into the water from the bottom over a period of time.

DDT was probably degraded to DDE within invertebrates. The combined DDT and DDE concentration in ostracods from the complete pool was correlated with the concentration of DDT in the water ($r = .99$), starting 5 days after the DDT application and continuing until the study was terminated. Presumably DDT was taken in from the water and degraded to DDE within the ostracods and both compounds were lost back into the water, resulting in the correlation between DDT in water and both DDT and DDE in ostracods.

The relative percentage of DDE and DDT was associated with the abundance of invertebrates. Invertebrates were found in increasing abundance in the no-fish pool, the complete pool and the natural pond, respectively.

Generally, the percentage of DDE increased and DDT decreased as invertebrate abundance increased (Figure 27), implying that DDT degradation to DDE increases as biological productivity in a lentic system increases.

D) Degradation of DDT in Fish:

DDT comprised only a small percentage of the DDT-R in fish from the natural pond while DDE was the most abundant residue found (Figure 28). The percentage of DDE in the fish was about twice and DDT about one-half the percentage found in invertebrates (Figure 27). This indicates DDT was degraded to DDE in the fish. As shown in Figure 29, the half-life for DDT in the fish from the pond was only seven days. The short half-life was attributed to growth of the fish and degradation of DDT to DDE within the fish.

The percentage of DDE was smaller and DDT greater in the fish from the pools than in fish from the natural pond. DDE steadily increased in fish from the pools until it amounted to about 15 percent of the total DDT-R 25 days after DDT was applied. Since the amount of DDE-contaminated food available was small, the steadily increasing concentration of DDE observed (Figure 30) must have been due to degradation of DDT in the fish. If all the DDE formed was retained by the fish, the degradation rate of DDT to DDE in fish from the pools was about 0.1 ug of DDT per gram of tissue per day, or about one percent of the DDT contained in the fish per day.

Figure 28. Percentage of the total DDT-R made up of DDE, DDD and DDT in fish from the natural pond, no-food pool and a pool with a DDT-treated-sand-bottom (Waybrant, 1969).
RC in time scale denotes "recycled" for those fish placed in the experimental units to access recycling of residues.

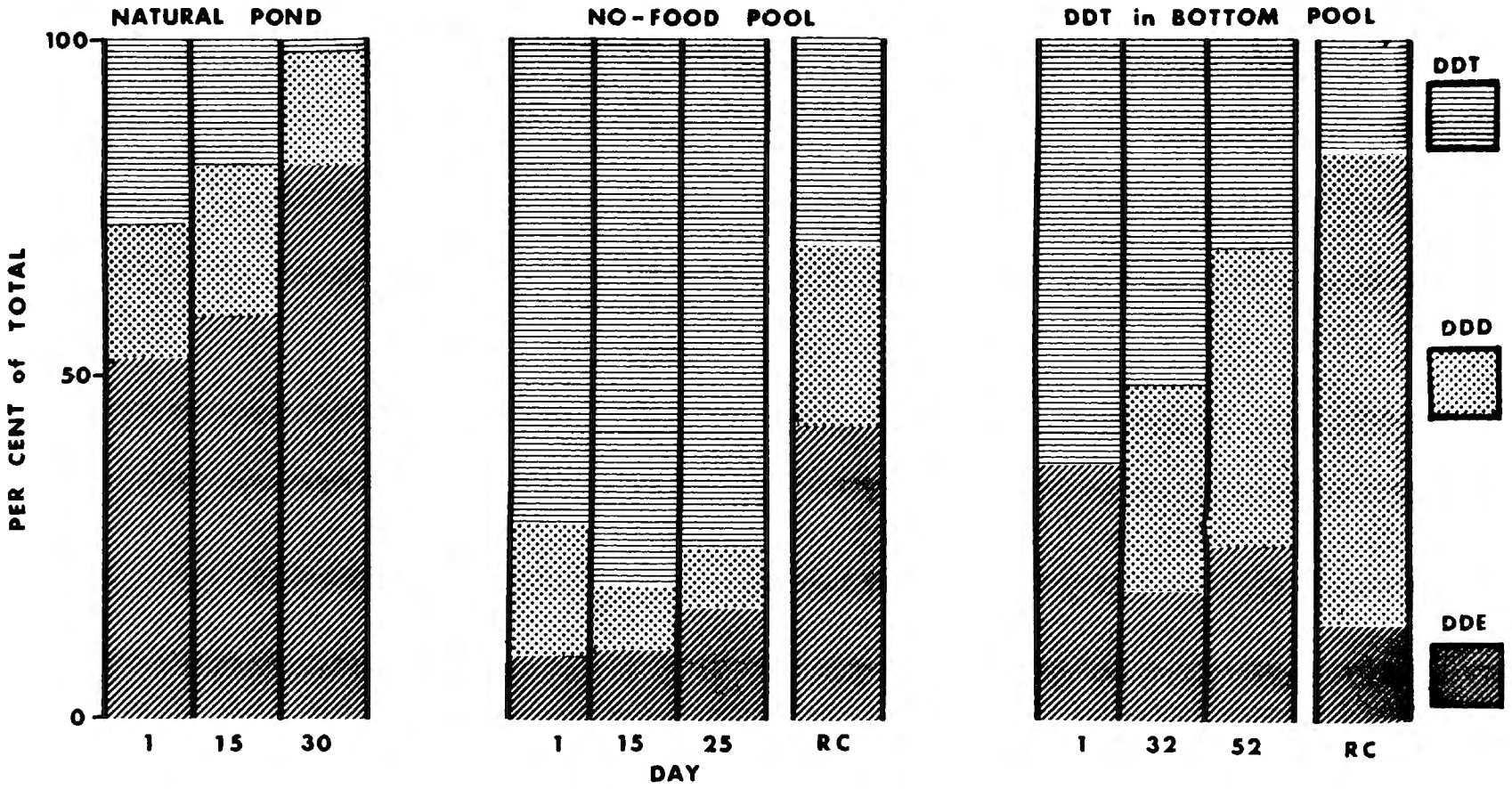
NATURAL POND
NO-FOOD POOL
DDT in BOTTOM POOL
PER CENT of TOTAL
100
50
0
1
15
30
1
15
25
RC
DAY
1
32
52
RC
DDT
DDD
DDE

Figure 28

Figure 29. Concentration of MDE, DDE, DDD and DDT in fish from the natural pond.

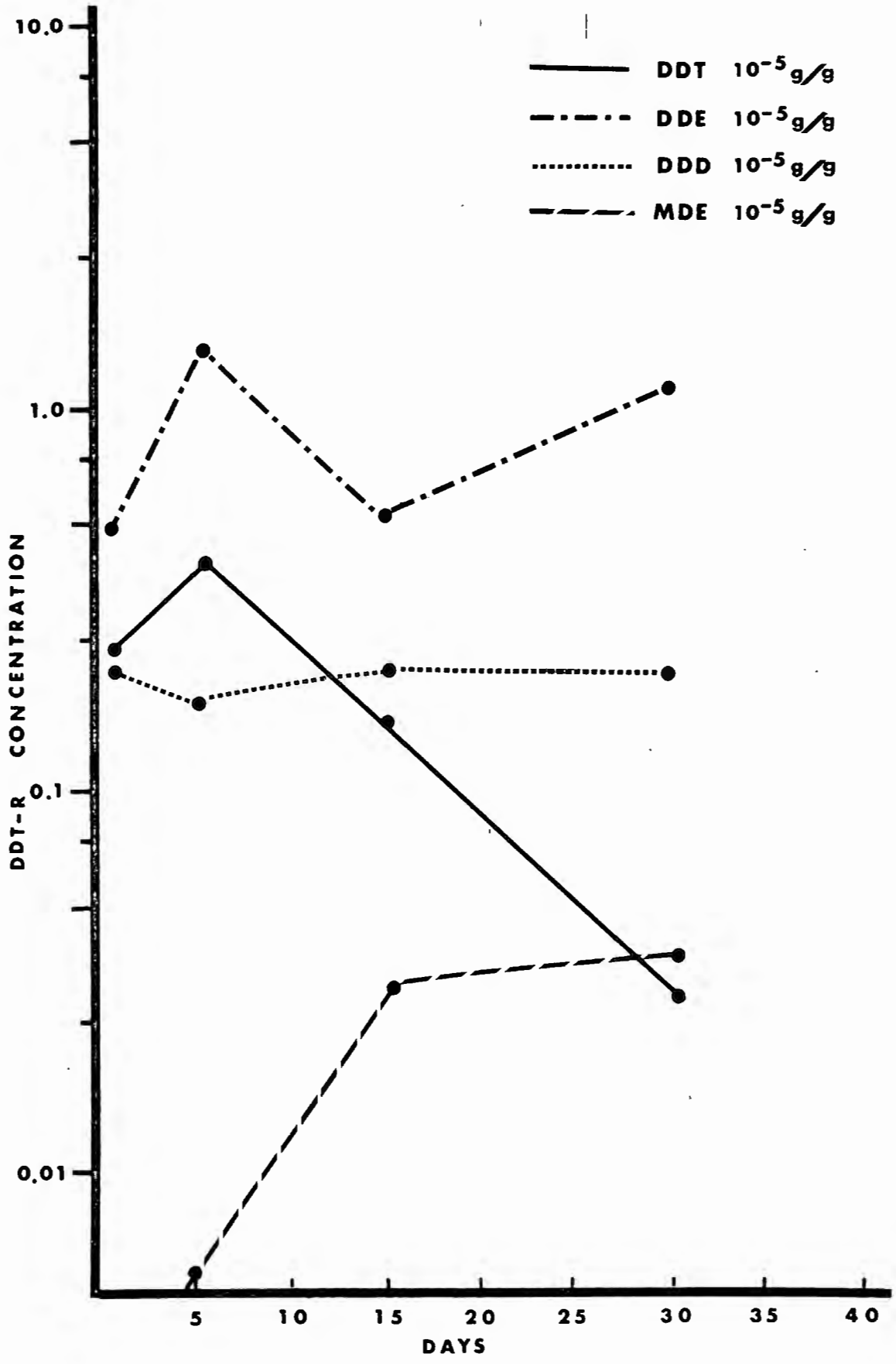
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
DDT 10^{-5} g/g
DDE 10^{-5} g/g
DDD 10^{-5} g/g
MDE 10^{-5} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 29

Figure 30. Concentration of DDE, DDD and DDT in fish from the complete pool and no-food pool combined.

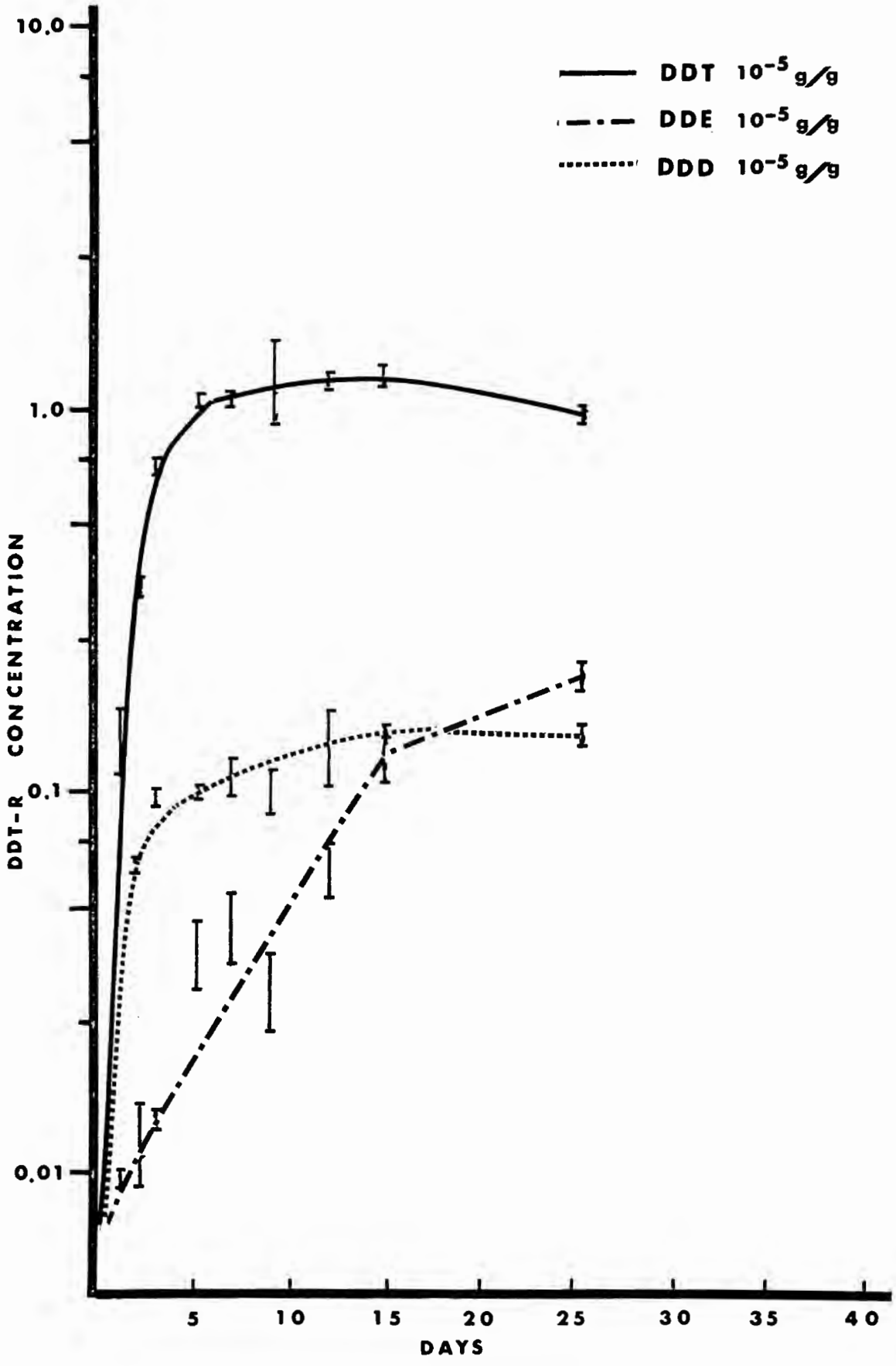
10.0
1.0
0.1
0.01
DDT-R CONCENTRATION
DDT 10^{-5} g/g
DDE 10^{-5} g/g
DDD 10^{-5} g/g
5
10
15
20
25
30
35
40
DAYS

Figure 30

The persistence of DDT in fish in the pools was in sharp contrast to the rapid degradation and disappearance of DDT observed in fish from the natural pond. Some of the difference may be attributed to the particular species of fish analyzed in each case. However, some of the difference in the persistence of DDT must have resulted from the different environmental conditions involved in each experiment. The fish in the pond were in good condition and took up DDT-R both directly from the water and in their food. The fish in the pools were in poor condition and took up DDT-R almost solely from the water. Consequently, both the way DDT is taken up and the physical condition of fish may influence the degradation and disappearance of DDT in fish.

Compounds pp DDD and op DDT were not separated by the column used in the G.L.C. to analyze the residues in fish from the pool study. The apparent correlation between DDT and DDD (Figure 30) was believed to be due to the 9 percent op DDT contained in stock pp DDT used for all studies. The dual column used in the G.L.C. to analyze fish from the natural pond did separate pp DDD and op DDT, and the concentration of pp DDD was consistently about 2 ppm or 20 percent of the total DDT-R. The small amount of DDD in fish from the pools, its abundance in fish from the natural pond, and data from Waybrant (1969) indicates little DDD is formed *in vivo*.

Rather, DDD appears to be taken up by fish from the water and in food and stored in body tissues.

2) The relationship between environmental conditions and the degradation of DDT in lentic environments:

A) DDT degradation to DDD:

DDT degradation to DDD is a simple reduction reaction, with DDT serving as the terminal hydrogen acceptor for a reduced electron carrier (O'Brien, 1967). The reaction simulates the anaerobic reduction of nitrates and complexed ferric hydroxides observed in the bottom of eutrophic lakes (Ruttner, 1963). Presumably, bacteria (Wedemeyer, 1967) and reduced porphyrins (Castro, 1964) are the electron carriers of greatest importance in degrading DDT to DDD in the lentic environment.

Bacteria and porphyrin compounds are abundant in lentic environments, so reduction of DDT to DDD would be limited by availability of DDT and alternative terminal electron acceptors. Assuming DDT is always in a form which can be used by a carrier, alternative acceptors become limiting factors. Oxygen is a better acceptor than DDT, so the reaction is anaerobic. Perhaps, as the redox potential declines, nitrates or some other compound become a better acceptor than DDT, again limiting the amount of DDT reduced to DDD. Thus, DDT may be degraded to DDD under anaerobic conditions or remain

stable, depending on factors other than oxygen which have not been completely defined.

B) DDT degradation to DDE:

Enzymatic degradation of DDT to DDE may constitute the major pathway leading to DDE found in aquatic environments. Such an enzyme, DDT dehydrochlorinase, has been studied in insects (Sternburg et al., 1954). Its presence in insects is correlated with resistance to the toxic action of DDT, but both resistant and susceptible insects can degrade DDT to DDE. The reaction has not been studied for a variety of animals, but I believe most aquatic animals are capable of degrading DDT to DDE.

The enzyme prepared from resistant flies which degraded DDT exhibited some general characteristics. Presumable these characteristics apply to similar enzymes in other animals. Purified enzyme required glutathione for activation but crude extracts did not necessarily require activation. No other activators or cofactors were required, so the enzyme may act independently. The enzyme attacked DDT coated on glass beads, a solution of DDT was not required. In fact, poor results were obtained when an emulsifier was used to "dissolve" DDT, either the emulsifier inhibited the enzyme or DDT was bound to the emulsifier and unavailable to the enzyme. Presence or absence of oxygen had an insignificant effect on the reaction, while the primary reaction

limitations were surface area of DDT exposed and the enzyme concentration.

Translating these enzyme characteristics into ecological factors permits another mechanism for the degradation of DDT to be postulated for lentic ecosystems. The pathway for degradation would not be confined by any normal environmental conditions, such as oxygen concentration. Rather, since most aquatic animals may be able to degrade DDT to DDE, the mechanism would be almost ubiquitous. Whether DDT was degraded would depend on DDT being in a free or unbound form while in close proximity to the enzyme. Thus, the amount of degradation of DDT to DDE would depend on the enzyme concentration and frequency DDT was released at a reaction site.

3) Relationships between binding, degradation and biological magnification of DDT-R in lentic environments:

I previously assumed DDT was able to react freely in order to establish the decomposition mechanisms presumably operating in lentic environments. It was a false assumption because DDT residues are usually found bound in or on some substrate, and this binding has a profound effect on the degradation and distribution of DDT-R.

Before discussion of the relationships among binding, degradation and biological magnification of DDT can progress,

certain terms must be defined. Bound DDT is used to describe DDT in any physical state which prevents the DDT from being physically or chemically active. Conversely, free DDT may be physically or chemically active. Dissolved DDT cannot be removed from a solution by high speed centrifugation and may be either bound or free. Dissolved DDT in a free form can be exchanged between two solutions. Finally, "active" DDT produces the toxic action of DDT but is not believed to have any effect on the behavior of DDT in lentic ecosystems.

DDT entering a lentic ecosystem may be bound to sediments. It may be released from sediment by being dissolved directly into the water, or indirectly released into the water or an animal body through biochemical action. If DDT is released where anaerobic conditions prevail it will probably be degraded to DDD. If anaerobic conditions generally prevail in the sediments, organic material deposited on the bottom will only be partially decomposed. Presumably, if organic material which contains DDT is deposited on the bottom and only partially decomposed, less DDT and DDD will be released than if the material had been completely broken down. Furthermore, organic deposits probably have a greater affinity for DDT contained in water than mineralized deposits (Harris, 1966). Thus, while DDT may be bound to organic or inorganic sediments and released by physical or biochemical action, less DDD and DDT will be returned to the water from sediments as anaerobic conditions become progressively more severe.

DDT may be bound to various plant and animal tissues. If these tissues are consumed and digested by an animal, DDT associated with the tissues may be taken up by the animal. Also, DDT within an animal may be transferred from one tissue to another. Thus, there are times during biological transfers of DDT both between and within organisms when bound DDT may become free DDT.

If degradation of DDT to DDE may take place in biotic components when DDT is free, then degradation may take place in conjunction with the transfer of DDT through a food chain. If this is true, then greater amounts of DDE than DDT would be expected in animals from successively higher trophic levels. DDE was the most abundant residue found in the tissues of fish eating birds (Hickey and Anderson, 1968) and coho salmon (Johnson and Pecor, 1969) from Lake Michigan, yet less than one part per trillion DDE was found in the water (Mount, 1968). Woodwell et al. (1967) observed proportionately more DDE and less DDT in samples representing the upper trophic levels than in samples representing the lower trophic levels of a marsh ecosystem. Waybrant (1969) observed substantial quantities of DDE in fish from DDT treated pools although DDE was only found in trace quantities in the water. A trace in his work was less than 10 pptr of DDE in the water (Waybrant, personal communication, 1969). Fish from the natural pond, which were believed to have taken up a large portion of their body load

from food, contained a higher percentage of DDE and a lower percentage of DDT than food organisms in the pond. Fish from the natural pond also contained much higher percentages of DDE and much lower percentages of DDT than fish from the no-food pool. Thus, biological transfers of DDT appear to be associated with the degradation of DDT to DDE.

The percentage DDE increased in the fish from the no-food pool with time following the start of exposure (Figure 30), although none was observed in the water. Greer and Paim (1968) demonstrated a substantial portion of the DDT taken up from the water by salmon was degraded to DDE within the fish. DDE also accumulated in trout exposed to DDT (Allison et al., 1964) in amounts sufficient to indicate the metabolite was formed within the fish. Presumably, DDT is degraded to DDE by some sort of an enzyme system confined to one site within the body, such as the liver. A similar enzyme system would account for the progressive epoxidation of aldrin to dieldrin observed in goldfish exposed to a single dose of aldrin (Gakstatter, 1968).

If degradation of DDT to DDE is confined to one site within the fish, then DDT residues must be transferred from various storage sites to the site of degradation via the blood. Since all body tissues are constantly being borken down and renewed, it is conceivable that residues stored in various tissues are also constantly released into and taken up from the blood (Holden, 1962). Hence, some of the DDT-R

residues contained within a fish are probably dissolved in the blood at all times. The concentration and physical state, whether free or bound, of residues in blood would depend on many factors, but some of the dissolved residues would undoubtedly be free to react chemically and to physically exchange into other mediums. In other words, free DDT in blood may be exchanged between the blood and body tissues or, out of the body via the gills, with the surrounding water. If this is true, then a mechanism which controls the degree of biological magnification of DDT-R by fish in a variety of lentic environments can be postulated.

Factors Controlling the Degree of Biological Magnification of DDT-R in Fish:

The concentration of DDT-R contained in a fish must have been obtained, renewed and sustained by virtue of some mechanism operating in the fishes environment. At least two factors must be operating in any proposed mechanism to accomplish the above actions. (1) The body load must be taken up and retained. (2) The DDT-R must initially be supplied and often resupplied or recycled in the environment, in order for any uptake to take place.

1) Uptake and retention of DDT-R residues by Fish:

The maximum DDT-R concentration observed in fish from each study was quite similar, even though the way

residues were taken up and the amount of each residue was different. The maximum DDT-R concentration based on wet weight in fish from the natural pond was 19.8 X 10^{-6} g/g. It was 16.9 X 10^{-6} g/g in fish from the complete pool and 15.7 X 10^{-6} g/g in fish from the no-food pool. The maximum concentration Waybrant (1969) observed in fish from his medium pool was 12.3 X 10^{-6} g/g. The concentration of DDT placed in and persisting in the water and other components during the course of each of these studies was also different. However, the whole body concentration of DDT-R observed in the fish appeared to be mediated by the concentration of DDT-R in the water.

A body load of residue results from some mechanism operating over a period of time. Fish in the natural pond took up DDT-R until the concentration in the water declined to about 0.5 X 10^{-9} g/g, when the body load stabilized at about 15 X 10^{-6} g/g (Figure 21). Fish in the pools also took up residues until the concentration in the water reached about 0.5 X 10^{-9} g/g and the body load stabilized at about 15 X 10^{-6} g/g, or 3 X 10^{4} times the concentration in water (Figure 22). Uncontaminated fish placed in the no-food pool 30 days after DDT was added took up between 0.5 to 0.6 X 10^{-6} g/g in water containing about 10 X 10^{-12} g/g DDT-R, or about 5 X 10^{4} times the concentration in the water (Figure 31). The water in Waybrant's (1969) medium pool (1.0 ppm in bottom) contained between 0.1 to 0.2 X 10^{-9} g/g DDT-R

Figure 31. Concentration of DDT-R in fish placed in the no-food pool 30 days after the DDT was applied.

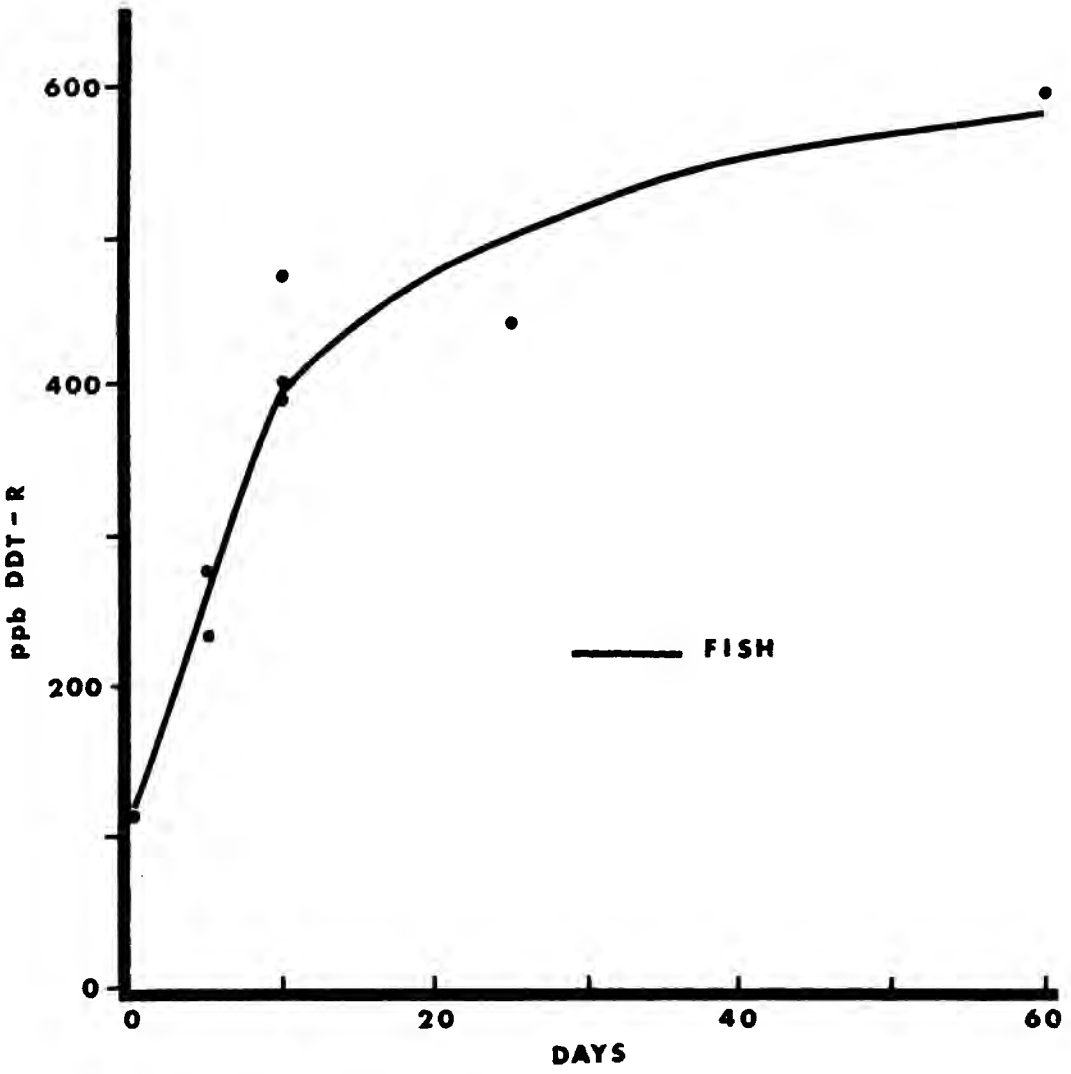

Figure 31

throughout most of his study, yet the fish in that pool eventually took up over 10 X 10^{-6} g/g DDT-R, or 1 X 10^{4} times the concentration in the water. Thus, all fish studied appeared to concentrate DDT-R directly from the water about 1 X 10^{4} times the concentration in the water.

DDT (Holden, 1962), endrin (Ferguson et al., 1966), dieldrin and lindane (Gakstatter and Weiss, 1967) can be taken up directly from the water via the gills. Gill tissue constitutes a passive membrane between water and an organic solvent, the blood (Fromm, personal communication 1969). Presumably, the compounds are passively taken up into blood from water because they are much more soluble in blood.

If compounds are passively taken up from water, they must be lost back into water from the blood when the concentrations favor exchange in the outward direction. Endrin can be "excreted" by resistant fish in sufficient quantities to kill susceptible fish (Ferguson et al., 1966). Gakstatter and Weiss (1967) reported dieldrin and DDT were eliminated from exposed fish respective of their increasing solubility in water. Lindane was hardly taken up and readily lost, while DDT was readily taken up and largely retained by the fish. Finally, Gross (personal communication 1968) observed fish lost dieldrin primarily via the gills and only small amounts were eliminated in the feces, urine and mucus.

The concentration of DDT-R retained by fish in the no-food pool had declined slightly 25 days after the DDT

application to about 12 ppm, but was still 3.2 X 10^5 times the 37.5 ppb concentration found in the water. The 14.5 ppm concentration of DDT-R found in the fish from the natural pond 30 days after the DDT application, was 2.4 X 10^5 times the 60 ppb concentration found in the water on that day. If we assume the control fish Gakstatter (1967) placed in the recovery tank concentrated DDT 1 X 10^4 times the concentration in the water in 24 days, then the water contained about 12 to 16 pptr DDT. Since the fish in both of his experiments still contained about 3 ppm of DDT-R after 32 days, they were able to retain about 2 X 10^5 times the concentration of DDT presumably contained in the water. Thus, the concentration of DDT-R retained by a fish appears to be at least an order of magnitude greater than the concentration taken up directly from the water.

Fish in a natural environment may take up DDT-R directly from the water or in their food. Fish are capable of taking up a large percentage of the DDT-R contained in their food (Allison *et al.*, 1964) and in the water passed over their gills (Gakstatter and Weiss, 1967; Waybrant, 1969). The amount of DDT-R potentially available from both sources appears adequate to produce concentrations an order of magnitude higher in fish than in food organisms. In fact, if uptake from the water and the food are both quite efficient, then the amount of DDT-R potentially available for uptake is several times greater than the amount required to sustain

a body load an order of magnitude higher than the food organisms. Consequently, whether DDT-R residues are taken up directly from the water or from the food probably does not determine the degree DDT-R residues are biologically magnified by fish. Instead, the body load appears to represent a balance between the amount of DDT-R being stored and the amount being lost at any given time.

Holden (1962) stated DDT entering the body of a fish is almost certainly transferred within the body by solution in blood lipids and gradually accumulated in fat deposits of various organs. He also reported turnover of lipids within the body lead to a fairly uniform DDT concentration in fats extractable with carbon tetrachloride. Anderson and Everhart (1966) found the ratio of DDT contents corresponded to the ratio of fat contents between combined samples of "good-condition" and "poor-condition" salmon (Salmo Salar). Reinert, 1969) has found DDT-R and dieldrin residue concentrations in fish from Lake Michigan increase as the fat content of the fish increases. Thus, fat deposits appear to be a primary site for storage of DDT-R within the body of fish.

DDT-R, entering or leaving a fish must be transferred between the water and fats via the blood. Hence DDT-R must be exchanged twice; once between the water and blood, and again between the blood and fats.

Presumably fish blood has an affinity for DDT-R about 10,000 times that of water. This was indicated by the

degree DDT was concentrated directly from the water by fish in the different studies we conducted. Similar differences in affinity for DDT-R by body fluids compared to water presumably account for invertebrates being able to concentrate DDT-R about 1.3×10^4 times the concentration in the water. Oysters can concentrate DDT 7×10^4 times the concentration in sea water (Butler, 1966). Thus, expressing the exchange of DDT-R between water and blood by an equilibrium reaction, blood is favored over water by a factor of at least 1×10^4.

Direct reference to a comparison between the concentration of DDT-R in blood and fats of fish was not found. Dieldrin and aldrin residues in fish eight days following a single exposure to aldrin were about 80 times higher in the fats than the blood (Gakstatter, 1968). Since these cyclodienes are about 100 times more soluble in water than DDT and equally soluble in organic solvents, it is reasonable to assume fats have an affinity for DDT-R about 1000 times greater than blood.

Expressing the transfer of DDT between water and body fats as a series of exchange equilibriums, total uptake and retention of DDT in the fats is favored by a sum 1×10^7 times greater than water, as shown below.

$$\text{DDT in Water} \underset{\leftarrow}{\xrightarrow{1 \times 10^4}} \text{DDT in Blood} \underset{\leftarrow}{\xrightarrow{1 \times 10^3}} \text{DDT in Fats}$$

Consequently, if a fish contained one percent by weight body fats, the equilibrium body load of DDT-R would be about

1×10^5 times the concentration in the water, and as the fat content of the fish increased the body load of DDT-R would increase.

The exchange equilibrium depends on the difference in solubility of the DDT-R in the water and fats. If we assume DDT, dieldrin, toxaphene and lindane have similar solubilities in fats, then differences in the degree of biological magnification of the compounds observed in fish would be due to differences in their solubility in water.

Water solubility of various pesticides varies greatly. DDT has the lowest solubility at 1.2 ppb or less at 25°C (Bowman et al., 1960). The solubility of dieldrin and endrin is still low, between 140 to 180 ppb, but about 100 times greater than DDT (Robeck et al., 1965). Toxaphene and rotenone, common fish toxicants, have solubilities of about 1.0 ppm (Spencer, 1968; Frear, 1955) or 1000 times that of DDT. Finally, lindane has a solubility of about 10 ppm, or 10,000 times that of DDT (op. cit.). They are all very soluble in most organic solvents (op. cit.). Consequently, DDT would be expected to have the highest partition coefficient between water and an organic solvent and lindane one of the lowest. The effective or actual concentration difference observed in biological systems may differ markedly from the theoretical because of binding, active transport or inhibition, and other processes, but the magnitude represented by the theoretical values should provide a basis for comparison.

The degree of biological magnification observed in fish for the different pesticides agrees with the magnitude expected based on their solubility in water. Fish in Lake Michigan contain about 1×10^6 times the concentration of DDT-R and about 1×10^5 times the concentration of dieldrin (Reinert, personal communication, 1969) found in the water (Mount, 1968). Fish placed in two different lakes concentrated toxaphene about 1×10^4 times the concentration found in the water (Terriere et al., 1966). Finally, the equilibrium concentration of lindane in fish appeared to be about 1×10^2 times the concentration in the water (Gakstatter and Weiss, 1967).

2) Supply and persistence of DDT-R in lentic environments as related to biological magnification by fish:

The concentration of DDT-R persisting in the water of a lentic ecosystem may be controlled by many factors. The concentration in the experimental waters was controlled primarily by codistillation. Presumably, codistillation is a minor factor in most natural lakes, because both the percentage of the total water volume evaporating per year and the concentration of DDT-R in the water are much lower than in the experimental systems. Hence, the pond and the pools were not good models for larger lentic ecosystems with regard to the persistence of DDT-R residues. However, sufficient evidence is available to postulate what factors

might control the concentration of DDT-R in the water of lakes.

In accordance with the definitions previously given for the various physical forms of DDT, it is the concentration of free DDT-R in the water which appears to determine the equilibrium concentration observed in fish from lentic ecosystems. If this is true, then any factor which binds DDT-R and reduces the concentration of free DDT-R in the water will eventually reduce the concentration of DDT-R found in the fish.

Many factors bind and remove DDT-R in lentic environments. A few of these factors were defined and quantified during the course of these experiments. Fish bind DDT-R about 1×10^5 to 1×10^6 times the concentration in water. Invertebrates bind DDT-R about 1×10^4 and algae about 1×10^3 times the concentration in water. However, the amount of DDT-R bound in these components was small compared to the total contained in the ecosystem.

Most of the DDT-R in a natural lentic ecosystem appears to be contained in the water and bottom material. This was illustrated in Table 1 given in the Introduction. Sand has a low affinity for DDT-R (Harris, 1966), so the sandy bottom material used in the experiments did not take up and retain a large amount of the DDT added. Organic soils have a high affinity for DDT-R (op. cit.) (Lichtenstein, 1966). Consequently, if bottom material rich in

organic matter had been employed in the pools, presumably it would have taken up and retained large amounts of DDT-R.

In natural lakes, the amount of DDT-R held in bottoms rich in organic material, combined with the many other biological and physical factors usually associated with lakes of this nature, may result in reduced concentrations of free DDT-R in the water. If this is true, then fish from a fertile lake contaminated with a similar amount of DDT as an infertile lake, should contain less DDT-R than fish from the infertile lake. Kleinert et al. (1968) has reported a case to support this sort of a relationship. They found nearly an order of magnitude lower DDT-R concentrations in fish from Pewaukee Lake than nearby and similarly developed Lake La Belle and Pine Lake. They attributed the difference to Pewaukee Lake being "more shallow, more fertile and having extensive mud flats covering most of the lake bottom." A similar relationship would account for the observation that in a survey of the Great Lakes only Lake Superior fish contained lower concentrations of DDT-R than fish from Lake Erie (Reinert, 1969).

The rate DDT-R and other pesticides were removed from the water by binding to sediments, codistillation and decomposition would determine persistence time. Burdick (personal communication, 1969) stated the DDT-R residue concentrations in fish from the oligotrophic lakes he studied between 1960 and 1962 (Burdick et al., 1964) have remained

essentially unchanged since that time. These levels have persisted despite a complete ban on the use of DDT on the watersheds of the lakes since 1962. Contrary to this observation, the concentration of DDD in fish from relatively shallow and fertile Clear Lake, California appeared to be declining, based on samples collected in 1963 (Hunt, personal communication, 1966) compared to samples collected in 1958 (Hunt and Bischoff, 1960). Terriere et al. (1966) observed toxaphene persisted about four times longer in an oligotrophic lake compared to a eutrophic lake in the same area. Thus, pesticide residues in the water and fish appear to persist longer in oligotrophic lakes than in eutrophic lakes, and DDT-R residues may persist for many years in oligotrophic lakes.

Finally, Terriere et al. (1956) made a conclusion based on toxicity data alone which demonstrates the importance of associating persitence and biological magnification with the water solubility of the various pesticides. They observed, based on the information acquired from their study with toxaphene, "freshwater lakes and streams would be nontoxic if they contained less than 100 parts per trillion of endrin, 3 p.p.b. of dieldrin, and 5 p.p.b. of DDT." A concentration of 5 ppb of DDT is about 2000 times the concentration of DDT observed in the water of Lake Michigan (Mount, 1969) which held fish containing over 3.5 ppm of DDT-R (Reinert, 1969). Since, DDT-R residues have been implicated

as the cause for the death of young coho salmon (Johnson and Pecor, 1969) and herring gulls (Ludwig and Ludwig, 1969) from Lake Michigan, the concentration of DDT-R in the water of the lake can not be designated as nontoxic. Consequently, a concentration of 5 ppb of DDT in the water of a lake appears to be about 1000 times the amount required to be toxic, which is surprisingly similar to the difference between the solubility of toxaphene and DDT in water.

3) Conclusion on the factors controlling the degree of biological mangification of DDT-R in fish:

The uptake and retention of DDT-R residues by fish depends primarily on the difference between their solubility in water and body fluids. The degree of biological magnification and persistence of the compounds in lentic ecosystems appears to be promoted by oligotrophic conditions and retarded by eutrophic conditions. The primary factor controlling these relationships appears to be the concentration of free or unbound DDT-R in the water.

SUMMARY

1) The distribution and degradation of DDT added to the water of a farm pond and three artificial pools was studied.
2) Much of the DDT added was lost from the pond and the pools apparently due to codistillation with evaporating water.
3) The flora and fauna rapidly took up and retained a high concentration of DDT-R, however, these environmental components held just a small portion of the DDT added to the study units.
4) Some DDT was deposited on the bottom and degraded to DDD. Both DDD and DDT were recycled out of the bottom.
5) The concentration of DDT-R in invertebrates from the pools was about 1×10^4 times and directly correlated with the DDT-R concentration in the water. Presumably, a correlation was observed because the residues in invertebrates and water were constantly being exchanged which resulted in a concentration equilibrium between water and invertebrates.
6) The concentration of DDT in algae was not correlated with the concentration in the water. The concentration

relationship observed between water and algae may have been due to binding of DDT to the surface of algae cells. The effect on DDT concentration of the algal population being heterogenous was not evaluated.

7) DDT was degraded to DDE in invertebrates and fish.

8) The proportional amounts of DDE increased and DDT decreased as biological productivity of the lentic ecosystem increased. Degradation of DDT to DDE appeared to be associated with biological transfers of the DDT.

9) Fish took up DDT-R residues directly from the water and in their food. The concentration taken up and retained appeared to depend on a balance between the amount being stored in fats and the amount being lost into water. The exchange equilibrium is believed to pass through two stages in fish, from water to blood and blood to fats, resulting in a total concentration magnification in the fats of 1×10^7 times the concentration in the water.

10) The magnitude of the exchange factor for chlorinated hydrocarbons presumably decreases as the water solubility of the compounds increases. Such a relationship would account for the different degrees of biological magnification reported for other pesticides.

APPENDICES

APPENDIX I

Reagent Purification Procedures:

1) Petroleum ether:

Fisher brand, reagent grade, 30 - 60°C boiling range petroleum ether was used throughout most of the study. Several other brands were tried, but they frequently smelled foul and contained artifacts which could not be removed. The petroleum ether was purified by refluxing 3 liters of the solvent with 10 grams of sodium-lead granules (Dri-Na) for 3 to 4 hours. The solvent was slowly distilled in an all glass system, discarding the first 50 ml of distillate and collecting the remaining fractions up to 50°C (Klein et al., 1963).

2) Ethyl ether:

Fisher brand, reagent grade ethyl ether was redistilled in an all glass system prior to use. Peroxide formation was inconsequential and purification with an aqueous-salt system ineffective (Eidelman, 1963). However, it was observed that the addition of 2 percent by volume ethyl alcohol, ostensibly to prevent peroxide formation, greatly facilitated the elution of dieldrin from Florosil columns but had little effect on the elution of DDT-R.

3) Acetonitrile:

Fisher brand, reagent grade acetonitrile was purified weekly as required. The solvent was purified by refluxing for 4 hours with 85% H_3PO_4 and P_2O_5, added in the proportions of 1 ml acid and 30 grams pentoxide to 4 liters of nitrile, slowly distilling and collecting the fractions distilling at 81 to 82°C (Klein et al., 1963).

4) Benzene:

Fisher brand reagent grade benzene was refluxed over chips of freshly cut sodium, decanted and slowly distilled at 80°C in an all glass system (Thornburg, 1966).

5) Sodium sulfate:

Artifacts were removed from the sodium sulfate by heating overnight at 250°C.

APPENDIX II

Raw Data:

Table 1A. Sample wet weight, species composition and residue concentration for invertebrates from the complete pool.

Sample	Species	Grams	ppb Concentration			
			DDE	DDD	DDT	DDT-R
Pre $\bar{X}$	C	------	104	49	302	455
1 hour	C	0.0513	81	50	3270	3401
6 hour	M	0.0214	418	453	3553	4424
12 hour	M	0.2095	164	223	2419	2806
12 hour	C	0.1842	462	268	2182	2912
24 hour	C	0.0838	312	226	4903	5441
24 hour	M	0.0465	466	271	6744	7481
36 hour	S	0.1029	673	565	3885	5123
36 hour	S	0.0610	544	436	2956	3939
2 day	M	0.0300	1242	222	7434	8898
3 day	C	0.0430	989	545	3978	5512
5 day	M	0.0529	2154	1946	8384	12448
5 day	S	0.1230	2147	1384	5953	9484
7 day	S	0.1400	1521	1280	4656	7457

Table 1A (cont'd)

Sample	Species	Grams	ppb Concentration			
			DDE	DDD	DDT	DDT-R
12 day	S	0.4479	549	708	817	2074
12 day	C	0.1410	594	649	1651	2894
15 day	M	0.0338	309	530	2511	3350
15 day	S	1.2990	383	609	465	1457
15 day	S	0.4123	445	676	732	1853
25 day	S	0.6066	207	214	288	709
25 day	S	1.2800	216	218	264	698
30 day	S	2.0342	136	120	143	399
35 day	S	1.1207	133	112	141	368
40 day	S	1.7672	110	62	119	291

Key for species composition:

C = Combination
M = Mites
S = Ostracods
O = Odonates

Table 2A. Sample wet weight, species composition and residue concentration for invertebrates from the no-fish pool.

Sample	Species	Grams	ppb Concentration			
			DDE	DDD	DDT	DDT-R
Pre $\bar{X}$	M & S	.2871	61	43	129	233
1 hour	M & S	.0455	209	105	1568	1882
12 hour	C	.0686	833	250	3743	4826
12 hour	M	.0719	752	366	4727	5845
36 hour	C	.0614	877	404	3575	4856
36 hour	C	.0438	728	236	4643	5607
2 day	C	.0417	586	253	2709	3548
3 day	C	.0332	1020	578	7072	8670
5 day	C	.0247	1703	749	7320	9772
7 day	O & M	.0160	1718	978	1793	4489
7 day	C	.0557	1118	415	5057	6590
12 day	O	.0460	980	262	596	1838
15 day	O	.0465	546	208	222	976
15 day	S	.0287	429	318	1571	2318
25 day	O	.0481	576	291	367	1234
25 day	S	.0582	256	166	1110	1532
30 day	S	.2436	251	163	698	1112
30 day	O	.1783	190	102	145	437
40 day	S	.4369	149	87	248	484
40 day	O	.0351	236	187	226	649

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