

TRANSLOCATIONS AND STORAGE EQUILIBRIA
INVOLVING SUBLETHAL LEVELS OF DIELDRIN IN
AQUATIC ECOSYSTEMS

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This is to certify that the

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Sublethal Levels of Dieldrin in Aquatic
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presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Fisheries and Wildlife

A handwritten signature in cursive script that reads "Peter I. Tack".

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ABSTRACT

TRANSLOCATIONS AND STORAGE EQUILIBRIA INVOLVING SUBLETHAL LEVELS OF DIELDRIN IN AQUATIC ECOSYSTEMS

by Herbert Lee Lenon

Dieldrin has been shown to be taken up from water primarily via direct uptake as opposed to food chain uptake. By feeding bluntnose minnows, Pimephales notatus, with daphnia, Daphnia spp., treated with sublethal levels of dieldrin, only low storage equilibrium levels were obtained through 30 days. These levels did not vary significantly with different amounts of dieldrin fed through daphnia or with different amounts of daphnia containing a constant insecticide concentration. Hence, biological magnification through an aquatic food chain alone does not appear likely when sublethal levels are involved. However simple exposure of the minnows to the same sublethal levels in water resulted in high storage levels at equilibrium and closely reflected the concentrations in the water. This suggests that the equilibrium levels are most closely related to the insecticides' solubility differential between the water and the oil and fat content of the fish. Loss of dieldrin from minnows that had reached equilibrium levels occurred when they were transferred to water lacking this chemical and the rate of loss corresponded to the rate of uptake, thus further confirming the direct uptake-loss as the major mechanism. Interaction of direct uptake with normal levels of feeding yielded a storage level not much different from the former mechanism acting alone. With excessive feeding of treated food,

however, the food chain appears to become important. With the fish storing high levels due to direct uptake, the excessive feeding was then enough to produce mortality. From field studies of an area intensively treated, dieldrin was found to persist in the streams through three years of sampling following treatment. Consequently, the possibility for direct uptake as well as its movement along the food chain exists.

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By

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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENT	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
INTRODUCTION	
General	1
Historical Background	1
Occurrence, Persistence, and Solubility	1
Toxicity Characteristics	5
Biological Magnification	8
Uptake and the Aquatic Ecosystem	10
Objectives	11
MATERIALS AND METHODS	
Instrumentation	13
Reagents Used	14
Sampling, Extraction, and Clean-up Procedures	14
Water	14
Plants	15
Invertebrates	17
Fish	18
General Techniques	19
Quantitation	22
FIELD STUDIES	
Description of the Study Area	23
Results	25
Water	25
Green Filamentous Algae	28
Higher Aquatic Plants	30
Animals	32
Discussion	32

	Page
LABORATORY STUDIES	
General	35
Experiment 1: Dieldrin Treatment of Daphnia	37
Experiment 2: Food Chain Study	40
Experiment 3: Extended Food Chain Study	46
Experiment 4: Food Chain Uptake Versus Direct Uptake . .	51
Experiment 5: Food Chain Uptake Related to Feeding Rates	62
Experiment 6: Dieldrin Elimination Study	69
GENERAL DISCUSSION	72
LITERATURE CITED	81

LIST OF TABLES

Table	Page
1. Field Collected Water Samples	26
2. Field Collected Samples of Green Filamentous Algae . . .	29
3. Field Collected Samples of Higher Aquatic Plants	31
4. Field Collected Samples of Animals	33
5. Dieldrin Concentration in Daphnia at the End of Three Day Exposure, Using 1.5 µg/L Initial Dieldrin	37
6. Dieldrin Concentration in the Daphnia Culture Water at the End of Three Day Exposure, Using 1.5 µg/L Initial Dieldrin	38
7. Dieldrin Concentration in Daphnia at the End of Three Day Exposure, Using 3.0 µg/L Initial Dieldrin	39
8. Results of Feeding Treated Daphnia to Bluntnose Minnows	43
9. Daphnia Analyses for Food Chain Experiment, Sampled Every Three Days	44
10. Feedings for the Extended Food Chain Study	47
11. Daphnia and Minnow Analyses for the Extended Food Chain Study	48
12. Smallmouth Bass Tissue Analysis After 14 Days of Feeding	49
13. Daily Amounts of Daphnia Fed Utilizing Three Feeding Rates	64

LIST OF FIGURES

Figure	Page
1. Field Study Area, Monroe County, Michigan	24
2. Dieldrin Treatment of Daphnia Cultures	41
3. Results of Feeding Treated Daphnia to Bluntnose Minnows	45
4. Dieldrin Concentration in the Water of the Fish Tanks .	54
5. Rate of Dieldrin Feeding to Minnows	58
6. Dieldrin Uptake by Bluntnose Minnows	60
7. Rates of Dieldrin Feeding to Minnows	65
8. Food Chain Uptake with Three Feeding Rates	67
9. Dieldrin Elimination Curve	70

INTRODUCTION

General

With increasing use of insecticides there is an urgent need to know more about the behavior of these chemicals in our environment. Such knowledge can no longer be obtained by merely observing the number and types of organisms that die after a widespread application of these materials, or by taking large numbers of samples from such an area and analyzing them for residue levels. Carefully designed laboratory studies are required to explain the specific functional relationships involved within our complex ecosystem. Although an increasing number of such ecological studies have appeared recently, there is still a dearth of information in some areas. For example, how are insecticides translocated within an ecosystem? How are they picked up by the various members of the biotic community? To what extent are they stored in the various trophic levels? Under what conditions do they increase mortality rates in wild species? These general questions prompted my field study of an area intensively sprayed with dieldrin. This was correlated with a series of laboratory experiments. I confined my research to the aquatic ecosystem and to the insecticide dieldrin.

Historical Background

Occurrence, Persistence, and Solubility

It is generally accepted that many of the chlorinated hydrocarbon insecticides, especially DDT, are found all over the earth, carried by wind, water, and migrating animals (Woodwell et al., 1967).

Risebrough et al. (1968) has demonstrated transatlantic movements of DDT and dieldrin in airborne dust carried by the trade winds. Dieldrin is a chlorinated hydrocarbon and, like DDT, it has been widely used. It is more specifically a cyclodiene (1,2,3,4,10,10-hexachloro-6,7,-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo,exo-5,8-dimethanonaphthalene), and therefore, very closely related to several other common insecticides (Negherbon, 1959): aldrin, chlordane, endrin, heptachlor, isodrin, and toxaphene. Thus, by studying dieldrin, much of the information revealed is also pertinent to these related compounds.

Difficulties associated with some of the chlorinated hydrocarbon insecticides stem from their persistence of long residue life in the environment. Alexander (1965) states that dieldrin is known to persist at least ten years in some soils. Westlake and San Antonio (1960) show dieldrin diminishing from 110 to 45 $\mu\text{g/g}$ (ppm) in six years. This is consistent with the results of Wheatley et al. (1960) who suggests that the half-life of dieldrin is approximately four years in mineral soil and five to seven years in organic soil. Nash and Woolson (1967) show that 31 per cent of technical dieldrin remained after 15 years. In general, the whole group is quite chemically stable and extremely resistant to physical or microbial degradation. Chacko et al. (1966), working with eight actinomycetes and eight fungi, found the first soil microbes that could degrade DDT to DDD. However, none would alter dieldrin. Matsumura and Boush (1967) screened more than 500 isolated soil organisms and finally found a few that were active in degrading dieldrin but the metabolites were unidentified. This is one of the very few thus far, indicating any degradation of this compound by

soil microbes. Likewise, only two studies have been found suggesting biological decomposition of dieldrin in the animal body: One showed a possible metabolite in bile (Morsdorf et al., 1963) and one demonstrated two metabolites in human urine (Cueto and Hayes, 1962). If we are going to continue to use such long-lasting nonbiodegradable chemicals so widely, it is absolutely essential that we understand their complete behavior in our environment.

Due to its widespread use and persistence, dieldrin residues, like those of DDT, are found in most components of our environment. Man, himself, cannot escape it. Duggan et al. (1966), sampled 82 foods collected from three different geographical areas and eighteen markets. They found dieldrin ranging from 0.003 to 0.142 $\mu\text{g/g}$ when these foods were prepared and ready to eat. It has often been found in raw milk samples (Clifford et al., 1959). The mean concentration found to exist in the body fat of the general population of the United States for 1961-1962 was reported to be $0.15 \pm 0.02 \mu\text{g/g}$ (Dale and Quimby, 1963). We, therefore, have reason to be concerned about the use of dieldrin, particularly when we do not understand its effects within an ecosystem.

It is clearly recognized that chlorinated hydrocarbons, including dieldrin, do not remain on the land (Hickey et al., 1966). Rosen and Middleton (1959) point out that some of these insecticides find their way to surface waters by runoff from treated cropland or forest areas. Lichenstein et al. (1968) have shown that C^{14} -labelled dieldrin volatilizes readily and can move through the air to fat rich surfaces and there be absorbed. The percentage that volatilized and was recovered on oil soaked paper strips under laboratory conditions was

large. Little is known about this process under field conditions but it seems reasonable that volatilization would occur and that it would ultimately be scrubbed from the air by precipitation and other means. Some of this would find its way to water bodies. Thus, even though pesticides are applied to land, considerable amounts eventually end up in surface waters, making aquatic systems especially vulnerable to these chemicals. In the past they were also frequently applied directly to bodies of waters, as in mosquito control and in the control of trash fish. Today they still get in by unintentional settling onto surface waters during aerial spraying of watersheds. Industrial discharge of waste waters is another major source of insecticide pollution (Nicholson, 1967). Perhaps the first reported large scale kill of aquatic animals, thought to be a result of the agricultural use of insecticides, was by Young and Nicholson (1951) in the Tennessee River Valley of Alabama. Fish kills resulted soon after heavy rains on cotton fields which had received heavy concentrations of several insecticides. Since then fish kills of this type are frequently reported. Another major one was the 1963 Mississippi River fish kill resulting from endrin pollution (Mount and Putnicki, 1966).

Breidenbach and Lichtenberg (1963) found dieldrin or DDT in 38 samples from 10 different rivers between May and December of 1962. Weaver et al. (1965), sampling 96 stations in 12 river basins, found a widespread occurrence of dieldrin which dominated all other chlorinated hydrocarbons present. It varied in concentration from 0.016 to 0.118 $\mu\text{g/L}$ (ppb). Dieldrin and endrin were recovered by Lauer et al. (1966) from surface waters where they had entered

through runoff from sugar cane fields in Louisiana. These residues were also found persisting in the soil from one agricultural season to the next, contributing small quantities to the water each time runoff occurred.

It should be remembered that, like most of the chlorinated hydrocarbons, dieldrin is quite insoluble in water, although about 100 times more soluble than DDT. According to Robeck et al. (1965), its solubility in distilled water is between 140 and 180 $\mu\text{g/L}$. Richardson and Miller (1960) report the water solubility at 25°C to be 250 $\mu\text{g/L}$. Consequently, when dieldrin is found as a pollutant of water the concentration would be expected to be low. Much of the insecticide in a stream is adsorbed on the suspended solids and silt common to some rivers. This may effectively remove pesticide from water (Beck, 1953). Hoffmann (1960) believes that water turbidity and high organic content can nullify the effect of DDT on fish. It is further suggested that a large proportion of DDT is biologically immobilized soon after its entrance into the ecosystem (Butler, 1966a). However, Mount and Putnicki (1966) who studied clay particles suspended in water point out that toxicity of endrin to catfish in turbid water is not reduced.

Toxicity Characteristics

Numerous toxicity studies with dieldrin have been performed by many different investigators using all forms of life. Because my laboratory studies deal primarily with bluntnose minnows, Pimephales notatus, and daphnia, Daphnia spp., I have considered only those reports dealing with closely related animals. A few other workers have used bluntnose minnows for their investigations, for example,

Mount (1962) working with endrin. However, toxicity studies involving dieldrin have not used this species. Several workers have used a closely related species, the fathead minnow, Pimephales promelas. The 96-hour TLM (the median concentration producing 50% mortality of the population in 96 hours) for dieldrin using fathead minnows has been reported to be 16 µg/L by Henderson et al. (1959) and again by Katz (1961). Using this same species of fish for bioassay, Tarzwell and Henderson (1957) demonstrated the toxicity of dieldrin from runoff. With the first and third rainfalls on a large treated area, the runoff waters were toxic to 50% of the fish in 96 hours. Runoff from the fourth rain still contained dieldrin but was not as toxic within this time period.

Anderson (1960) found the relative toxicity of dieldrin to daphnia to be low; 330 µg/L for immobilization of Daphnia magna in 50 hours. Bringmann and Kuhn (1960) report an LD₅₀ (lethal dose to 50% of the population) of 230 µg/L for this same species. This is not much different from the toxicity of 250 µg/L for Daphnia pulex found as the 48-hour EC₅₀ (concentration which produces a certain effect to 50% of the population in 48 hours) by Sanders and Cope (1966).

One interesting field study demonstrating the acute toxic results of spraying over an aquatic ecosystem was that of Harrington and Bidlingmayer (1958). After an aerial application of dieldrin to 2,000 acres of salt marsh in Florida at a rate of one pound active dieldrin per acre, they observed almost complete fish kill. This included 20 to 30 tons of fish of about 30 different species. The larger game and food fishes died first, the herbivorous fishes next,

and gobioid fishes last. Mollusks appeared to be unharmed but crustaceans were virtually exterminated.

Several factors tend to affect toxicity of insecticides to fish. Toxicity increases with temperature (Schoenthal, 1963). Iyatomi et al. (1958) demonstrated with carp that a decrease from 27°C to 7°C caused an increase in tolerance to endrin by a factor of 28. Butler (1966b) noted that for the chlorinated hydrocarbons, toxicity seemed to be greater at summer water temperatures than in winter. Age is another factor which affects toxicity, with older fish generally having a higher concentration and tolerance than younger ones (King, 1962). This is largely accounted for by the difference in the amount of fat in the different age groups, since insecticides are sequestered primarily in adipose tissue (Butler, 1966a). It is also suggested that only on starvation are they mobilized from the fat and lost. Anderson and Everhart (1966), found that older Salmon had higher concentrations than younger ones. However, they suggest that the build-up of DDT in the older fish may result in death, loss of vitality, and slowing of growth until fewer and fewer of the larger salmon are available to the sport fishery. There is also individual variability in the amount of insecticide stored. This is roughly related to size with the largest fish having the highest concentrations (Woodwell et al., 1967) and probably the highest fat content. Henderson et al. (1960) concluded that natural variations in pH, alkalinity, and hardness generally have no major affect on the toxicity of chlorinated hydrocarbons.

The chlorinated hydrocarbons are known to produce effects other than acute mortality. In Lake George, New York, it was shown that

female lake trout passed DDT along in their eggs. Fry hatched from these and survived until the yolk sac was nearly all absorbed. Death occurred at this time, thus interfering with natural reproduction (Burdick et al., 1964). Losses of Michigan Coho Salmon fry have recently been reported in Michigan hatcheries (MacMullan, 1968) and the symptoms, timing of death and confirmed presence of high DDT levels in the tissues led to a pesticide poisoning diagnosis. Widespread destruction of salmon runs by DDT in the rivers and streams of New Brunswick is well documented (Elson, 1967). Indirectly, populations may be affected through the diet. Fish may be forced to feed on substitute foods when an insecticide eliminates their usual supply (Warner and Fenderson, 1962; Keenleyside, 1967). Dieldrin has been shown to cause a shift in the age structure of guppy populations (Cairns and Loos, 1966). It is apparent from literature reports that persistent insecticides may affect a fishery in many ways, and the acute mortality may not be the most significant. Long term changes in a population may be more far reaching.

Biological Magnification

Biological magnification is the term that is frequently used when referring to the build-up of pesticides in plants, animals, and the environment. Perhaps the first major study suggesting this increase in concentration with higher trophic levels was that by Rudd (1958). Clear Lake, California was treated repeatedly with TDE (or DDD) for control of a gnat. Samples taken 13 months later showed the following levels of DDD (Hunt, 1966): 10 $\mu\text{g/g}$ in plankton, 903 $\mu\text{g/g}$ in fat of plankton-eating fish, 2,690 $\mu\text{g/g}$ in fat of carnivorous fish, and 2,134 $\mu\text{g/g}$ in fat of fish-eating grebes which

died. These residues represent a 500-fold increase in plankton over that in the water, and a 100,000-fold increase in the fish-eating birds. Many similar studies have since been reported (Woodwell et al., 1967; Hickey et al., 1966). It has generally been assumed in all of these studies that the accumulated concentrations resulted from translocation of the insecticide through the food. This is evidenced by Hunt (1966) who states that magnification results from ingestion of food and drink, and the storage of pesticides in body tissues, with its subsequent transfer from animal to animal. He further states: "In general the level of pesticides accumulating in various animals in the food chain is influenced by the amount of pesticide ingested, the time period over which the pesticides are ingested, the pesticide storage capabilities of the animal, and rate of detoxification and excretion of pesticides." This also suggests that an equilibrium is reached between ingestion and excretion. Mount (1962) provides evidence that endrin enters the carp through the intestines and is carried by the blood to other parts of the body.

Due to the high solubilities of chlorinated hydrocarbons in oil and fat, the greatest body storage is in fat tissues. Contrast the solubility of DDT in olive oil, 100 g/L, to its solubility in water, 1.2 $\mu\text{g/L}$ (Holden, 1962). In addition to this fat storage certain other tissues frequently have high concentrations. This does not seem to be a function of the lipid content (Duffy and O'Connell, 1968). Mount (1962) reported the highest concentrations of endrin to be in the liver, intestine, spleen, and kidney, with very low concentrations in the muscle. Grzenda (1967) working with

goldfish found the highest concentrations of DDT and dieldrin to be in nerve, followed by liver, gall bladder, and brain tissues.

Uptake and the Aquatic Ecosystem

Today, it is known that many of the chlorinated hydrocarbons can enter fish directly through the gills as suggested by Holden (1962). He exposed trout to radio-active DDT and in a short time, the concentration in the gills was about 300 times that in the water, suggesting direct uptake by the gills. He later showed that dieldrin, among others, could be taken in by this mechanism (Holden, 1965). Ferguson et al. (1966) indicated that endrin seemed to enter the mosquito fish primarily via the gills. In addition, many other studies which show high storage concentrations in a very short time following exposure to one of the insecticides, tend to suggest a mechanism of direct uptake. For example, Mount and Putnicki (1966) demonstrated that in just two hours, the blood of catfish can attain an endrin concentration of 1,000 or more times greater than that of the water in which it was exposed. They further point out that endrin is not stored in the blood but is merely carried by it to the various tissues. Crosby and Tucker (1966), commenting on pesticides in general, suggest that probably very little enters either fish or Daphnia magna by the usual oral route and that their gill contact is undoubtedly far more important.

In a terrestrial ecosystem organisms above the soil may be subjected to high concentrations immediately after treatment, followed by a rapid decline. Subsequently, the only mechanism for further uptake of an insecticide is through contamination of the food. In contrast, the aquatic ecosystem generally contains greater average

levels of contamination. There is a continuous influx of insecticide into the system with runoff, long after treatment, until most ends up there. Also, since aquatic organisms are in direct contact with the insecticides in the water, and since they are capable of taking them up through the gills, they are particularly vulnerable. From this standpoint, residues in water may present a more serious hazard to its animal life and food chains than would be the case on land. Contrast the potentials of the two possible mechanisms of uptake operating in the aquatic ecosystem. With direct uptake, the dose is small due to the insolubility of chlorinated hydrocarbons in water, but the mechanism is constantly functioning as long as there is contamination. In food chain uptake, the dose may be large but the mechanism acts only at certain times in the day, that is, only with feeding activity. Consequently, it is extremely important to find out which of these mechanisms is responsible for the problems encountered when using insecticides. Mount (1967) recently stated: "There is insufficient information to determine whether or not a given concentration of an insecticide in water is more adverse to a fish if the fish also is receiving clearly sublethal doses from the food as well." Woodwell and Martin (1964) also noted that since there is abundant evidence that food chains are contaminated there is ample reason to examine the cycling of these substances through ecological systems.

Objectives

The objectives of this research study were: 1.) to sample representative aquatic systems in a large area intensively treated

with dieldrin in order to observe the concentrations in various trophic levels over a three year period following the final treatment; 2.) to determine at what levels dieldrin persists in the water of this area during the same time period; 3.) to determine, from laboratory studies, the extent of dieldrin uptake by fish through a food chain; 4.) to determine for minnows the relative importance of direct and food chain uptake by evaluating the different rates of uptake and the storage equilibrium levels obtained with each; and 5.) to determine the interaction of these mechanisms when both are operating as in the natural environment.

MATERIALS AND METHODS

Instrumentation

All samples were analyzed by a gas-liquid chromatography using an Aerograph Hy-Fi Gas Chromatograph (Model 600-C) combined to an Isothermal Temperature Controller (Model 328) and equipped with an Aerograph Concentric Tube Electron Capture Detector. Two basic types of columns were packed and used according to Bonelli (1965): 5% DC-11 (silicone fluid) coated on 60/80 mesh Chromosorb W and 5% QF-1 (Fluorosilicone fluid) on 60/80 mesh Chromosorb W. The samples were generally run first on the former, a nonpolar type column, and then on the latter, a polar column. Utilizing the two different columns in this way served to verify both the qualitative and quantitative determinations. Quantitative values were generally taken from the QF-1 columns since this separated dieldrin from DDE, the compound most likely to interfere with dieldrin. Chromatographic responses were recorded on a Sargent recorder (Model SR) equipped with a Disc Chart Integrator (Model 204).

Operating conditions for the two columns included a gas flow of 40 ml of nitrogen per minute and oven temperatures of 182°C for DC-11 and 192°C for QF-1. The injection temperatures were maintained 2 to 3°C higher than the ovens. Sample injections were consistently within the size range of 2 to 7 μ l. Serial dilutions of standard dieldrin solutions were run periodically to yield linearity range curves when concentrations were plotted against disc units on log-log scales. Great care was then taken to use concentrations that yielded responses within this linear range of the detector. Also,

by observing a general rule (Bonelli, 1965) that one is within the linear range if the sample produces a signal equal to, or less than, 30% of the standing current, the problems of nonlinearity were reduced.

Reagents Used

n-Hexane, reagent grade, redistilled between 66 and 69°C.

Acetonitrile, practical grade, purified according to Mills et al. (1963).

Petroleum ether, reagent grade, purified by adding 10 g of drier-sodium per 3 L and distilling between 30 to 60°C.

Ethyl ether, reagent grade, redistilled at 34 to 37°C.

Acetone, reagent grade.

Activated Florisil, preactivated at 1,200°F, and reheated at 130°C for at least 5 hours immediately prior to using, according to Mills et al. (1963).

Anhydrous sodium sulfate, reagent grade.

Nuchar-Attacloy adsorbent, Wilkens Instrument and Research, Inc.

Celite 545, Johns-Manville Co.

Sea sand, reagent grade, granular.

Technical dieldrin, 85%, City Chemical Corp., N.Y.

Granular dieldrin, 5%.

Sampling, Extraction, and Clean-up Procedures

Water

Water samples were taken from field stations in 4 L quantities and sealed in containers. Care was taken to avoid any plant material, suspended silt or sediments. In the laboratory, these samples were all refrigerated until extraction could be accomplished within four

months time. Each 4 L water sample was filtered through three different sizes of Millipore filters: First, through 5 μ ; then 1.2 μ ; and finally, 0.45 μ filters. This procedure was intended to separate a stream water sample into the following components: Two size classes on nanoplankton (residues on the 5 μ and 1.2 μ filters), bacterial fraction (residue on 0.45 μ filter), and essentially cell-free water (final filtrate).

The residue samples were extracted like plant samples, as described below. Dieldrin was extracted from the filtered water by repeated liquid-liquid partitioning with purified n-hexane in a ratio of 1:20 to be consistent with earlier sampling from the same field stations by another investigator (Butcher, 1964 unpublished). No further clean-up was necessary and the combined n-hexane extracts were concentrated for gas chromatographic analysis.

Water samples were taken for laboratory studies by carefully siphoning water from the experimental aquaria through polyethylene (Tygon) tubing. About 500 ml of water were siphoned through the thoroughly cleaned tubing first before the sample was collected. This reduced the adsorption of dieldrin on the tubing during the actual collection of the sample. One liter samples were taken and extracted by repeated liquid-liquid partitioning with a total of 70 ml of purified petroleum ether. This was used in place of n-hexane to be more consistent with other extractions from laboratory samples. When concentrated these samples were ready for analysis.

Plants

Various types of plants were sampled from field stations by gross collecting of what was available. They were placed in plastic

bags, labeled, and frozen. Freezing was important, not only for slowing down changes in the pesticide, but also for breaking up the plant cells and facilitating extractions. When ready to extract, the plants were thawed and allowed to thoroughly air dry. They were then chopped into small pieces and samples of 1 to 10 g each were accurately weighed on an analytical balance, and carefully extracted according to the procedure of Mills et al. (1963). A mortar and pestle with a little sand and several portions of pure acetonitrile was used for grinding in place of a Waring Blender. A small amount of Nuchar-Attaclay adsorbent, as used by Cassil (1962) and by Demick and Hartman (1963), was added to take up the chlorophyll and waxes. This generally substituted for the Florisil clean-up, providing a satisfactory sample for gas chromatography. Occasionally, however, the florisil column was also required (Mills et al., 1963). This is described below as used routinely with the clean-up of fish extracts.

Residues filtered from the water samples were treated in the same manner as described for plant samples. Weights were taken of the filter paper before filtration and again afterwards, when completely dry, to obtain a weight for the actual residue. The filter paper with the residue was treated with acetonitrile. This completely dissolved the filter as well as the residue. When saturated sodium chloride was added later, the filter paper was precipitated. Thus, all of the dieldrin was removed from within and off the Millipore filter.

Invertebrates

Daphnia cultures were maintained in numerous 10 and 20 gallon aquaria on plankton by frequent additions of boiled lettuce and continuous light. Yeast solutions were occasionally added to supplement the plankton diet. With light but frequent cropping of these cultures, including a certain volume of water exchange, production was maintained at a high rate.

Daphnia cultures were sampled by siphoning from the aquaria, collecting the daphnia on cheesecloth in a large Buchner funnel and allowing the water to collect below in a three gallon glass jar. The daphnia were washed with tap water while on the cheesecloth and concentrated in a pocket. From this they could be removed easily with a knife blade or a thin spatula and placed on a pre-weighed cover glass slip. This was then immediately re-weighed with the difference being the wet weight of daphnia. Attempts were made to use dry weights in which the daphnia on glass cover slips were dried slowly in the oven. This appeared to give less consistent results with the dieldrin concentrations, probably due to its volatilization at the higher temperatures. Therefore, wet weights, taken as uniformly as possible, gave the best results and were used throughout the study.

Once the samples were taken extractions were run immediately. Since the sample sizes were quite small, generally between 50 and 400 mg, a fairly short and simple extraction procedure was developed which served adequately. The daphnia were ground in a mortar and pestle with a little sand and small portions of pure acetonitrile. Each small amount of extract was poured into a 250 ml separatory

funnel through a small amount of glass wool placed in the neck of the funnel. This was continued until a total of 20 ml of acetonitrile were used. Then, 10 ml of pure petroleum ether were added to the separatory funnel and the glass wool removed. After shaking, 60 ml of water were added and the mixture was again shaken for one minute. When completely separated the aqueous portion was drawn off and discarded. The petroleum ether fraction was washed with 10 ml more water. The water was discarded and the ether fraction was then transferred to a 125 ml Erlenmeyer flask, dried with anhydrous sodium sulfate and concentrated to a suitable volume for analysis. Little interfering material was ever observed when this procedure was carefully followed.

Fish

A few attempts were made to collect fish by seine and dip net from the field stations but with little success. This was partly due to the paucity of fish present at most of these stations and partly to the abundant emergent vegetation in other areas.

In the laboratory studies fish were sampled by netting at random from five gallon, or smaller, aquaria. Since the concentrations found in the minnows were small, it became necessary to combine several fish (usually five) per sample. This gave an analytical average concentration for the pooled sample. Ferguson et al. (1966) also used this procedure of pooling five fish per sample.

All fish sampled (laboratory and field) were frozen after first obtaining a live wet weight. Again, this freezing assisted in rupturing the cells. Whole body extraction and analyses were performed using the acetonitrile extraction procedure described by

Mills et al. (1963) with the following exception: The fish were chopped into small pieces and then ground in a large mortar and pestle with sand until evenly macerated. It was then allowed to soak for an hour in 25 ml of pure acetonitrile with further frequent periodic grindings. After filtering this extract, the macerated fish material was again ground with another 75 ml of acetonitrile in small portions. Sample sizes for the pooled minnows ranged between approximately 3 and 7 g wet weight.

The final extract was run through the Florisil clean-up recommended by Mills et al. (1963), as suitable for dieldrin analysis. Glass columns (25 mm X 300 mm o.d.) were used but best results were obtained, with my small sample sizes, when the amount of Florisil was reduced. Thus, I prepared columns with 40 mm of activated Florisil topped with 25 mm of anhydrous sodium sulfate. This was prewet and washed with 30 ml of pure petroleum ether, followed by transfer of the sample to the column with an additional 10 ml of ether in small portions. Elution was begun first, with 70 ml of 6% and then, with 50 ml of 15% ethyl ether in petroleum ether. The rate of flow was maintained at approximately 5 ml/minute. The column was not permitted to dry out at any time. The last eluant, containing the dieldrin fraction, was collected, concentrated and analyzed. Recovery of dieldrin from the Florisil ranged between 90 and 100% as determined by procedures noted below.

General Techniques

All extracted samples were concentrated in Erlenmeyer flasks on clay triangles with low heat from a hot plate. Great care was

essential here since the samples can not be allowed to go to dryness; however, when precautions were observed, this procedure worked very well. Once concentrated, the samples were transferred to a suitable volumetric flask and an accurate volume obtained with additional petroleum ether. The volume required was determined ultimately by the dieldrin concentration, shown by the gas chromatographic response. A large measurable response was desired while staying within the linear range of the instrument. When storage of the extracted samples was necessary it was done in a refrigerator to prevent great changes in the volume resulting from volatilization of the solvent. Minor volume adjustments were made before analysis after allowing the sample to return to room temperature.

Periodic recovery determinations were made on all extraction and clean-up procedures to check on my techniques while always striving for greatest repeatability. These were run by taking a known amount of standard dieldrin and running it through the complete procedure in question to determine the amount recovered. Consistently, all procedures produced recoveries ranging between 85 and 100%. Lisk (1966) noted that recoveries between 75 and 100% are normal.

In addition to procedure recovery determinations, frequent essential periodic checks were run on all glassware to guard against contamination. Sizable volumes of pure petroleum ether were run thoroughly through all glassware to be used in a given procedure. This was then greatly concentrated to small volumes and analyzed for dieldrin. The large concentration factor involved, being much greater than that used in the actual extraction and clean-up, detected trace amounts of dieldrin adsorbed on glass. It was found

that all contamination could be avoided if a good glassware cleaning routine was adhered to. This involved a thorough scrubbing of the glassware followed by at least one good liberal rinsing with acetone, in which dieldrin is very soluble (Frear, 1955). Also, it was necessary to avoid dieldrin coming in contact with anything other than glass (e.g., sponges) which might serve as a future source of contamination. Consequently, in addition to a rigorous glassware cleaning routine, constant checks for possible contaminations were performed.

Extraction and clean-up procedures were kept as simple as possible while striving for acceptable accuracy. As Lisk (1966) states, frequently only preliminary separation procedures are required and this is particularly true when the past pesticide history of a sample is known and representative control samples are available. This was the case in my work, both field and laboratory. He also notes that with gas chromatography, if the desired compound appears in an area of the chromatogram in which other peaks are absent, and if the accuracy of the procedure is acceptable as judged by recovery studies, the appearance of peaks at other times in the chromatogram is insignificant. This was what I was striving for and feel I accomplished.

Grzenda (1967) notes that insecticides, including DDT and dieldrin, are stored to some extent as tissue-bound insecticides and not recovered by the usual organic solvent extractions without boiling. Consequently, most studies to date have missed this part of stored insecticide. My studies are consistent with these earlier ones.

Quantitation

Quantitation of the unknown samples was performed with the use of the disc integrator, comparing the arbitrary disc units (D.U.) for a known standard with those for the unknown. Each unknown sample was run on the gas chromatograph at least twice or until fairly consistent peak areas (in terms of D.U.) were obtained. The standard solution was generally run before and after each new unknown. Calculations were by direct proportion between the unknown and standard.

FIELD STUDIES

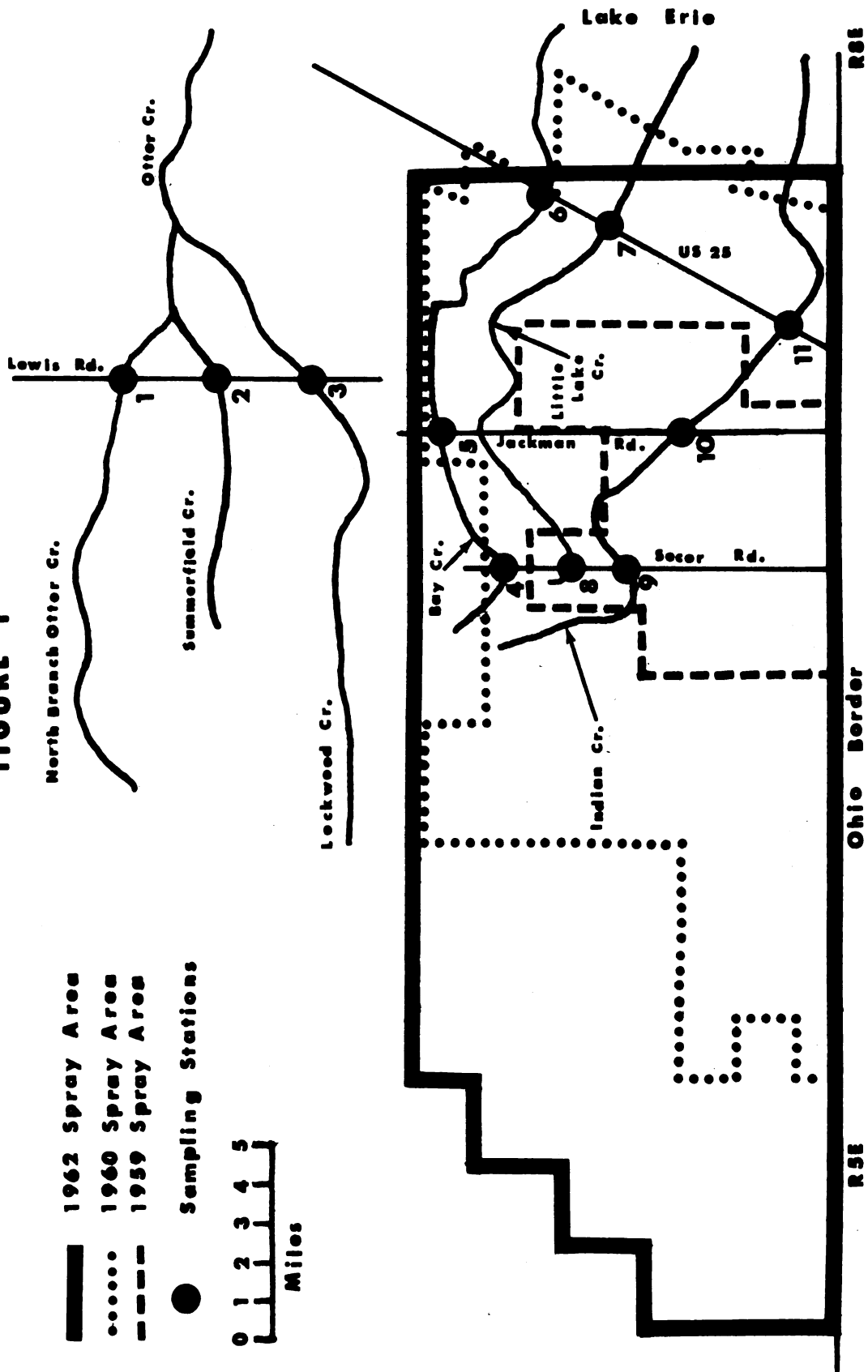
Description of the Study Area

The first phase of my study involved the analysis of samples taken from an area in Monroe County in the southeastern corner of Michigan. This is an area about six miles wide which extends from the west shore of Lake Erie along the southern boundary of Michigan for about 20 miles and encompassing approximately 80,000 acres. It has been intensively treated at two pounds per acre with 5% granular dieldrin or aldrin for control of the Japanese Beetle. Aldrin readily converts to the more stable dieldrin (Lichtenstein et al., 1960). Treatment was for three different years, as shown in Figure 1: In 1959, 1,200 acres were treated with granular aldrin; in 1960, 1,800 acres surrounding the 1959 area were treated with granular dieldrin; and in 1962, 80,000 acres, including both of the previously treated areas plus the remaining surrounding area, were treated with granular dieldrin. Consequently, this area presented a unique field situation in which the translocation and storage of an insecticide by various trophic levels could be observed. This is especially true since dieldrin is highly stable and is known to remain in the environment for a long period of time.

Three streams run through a major part of the treated area and lie entirely within the 80,000 acres for their total course. These streams made convenient aquatic systems for my study. Eight sampling stations, numbered 4 through 11 (Figure 1), were set up on the three streams. These streams are small and sluggish, particularly during the summer, and in drought periods portions

Figure 1. Field Study Area, Monroe County, Michigan.

FIGURE 1



of some may become essentially dry. Their waters range in pH from 7.5 to 8.5, with a hardness from 275 to 342 ppm calcium carbonate. Emergent aquatic vegetation almost completely fills stream beds during the summer months. Just north of the treatment area are three tributary streams to the Otter Creek (Figure 1) which are very similar to those in the treated area. One sampling station was set up on each, numbered 1 through 3, to be used as controls. Since the farming practices and the general landscape are basically the same throughout both adjacent areas, these streams provided a good comparison of a treated area with a nontreated one.

The first set of samples were taken in 1963 by Dr. James Butcher, Michigan State University, one year after the last and most extensive treatment. I began collecting in 1964 and continued in 1965. Thus, samples were obtained from the area for three succeeding years following the final treatment with dieldrin. Samples included primarily water and aquatic plants, with a very limited sampling of snails and one usable sample of minnows.

Results

Analytical values obtained for the concentration of dieldrin in all field samples are summarized in Tables 1 through 4. Blanks in the table represent lack of data, generally as a result of the stream becoming dry, absence of suitable plants at a particular station, or inability to locate or catch fish.

Water (Table 1)

In 1963 water samples were collected by Dr. Butcher on May 21 from the same sampling stations and the same analytical procedures

TABLE 1. Field Collected Water Samples (ug/L Dieldrin).

Sampling Stations

Sample Dates	Control Area			Treated Area							
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
May 21, 1963*	trace	-----	-----	0.6	1.1	1.6	2.0	5.0	3.6	1.9	1.0
July 8, 1964	no	no	no								
	trace	trace	trace	0.09	-----	0.13	0.31	0.04	-----	-----	0.11
October 17, 1964	no	no	no								
	trace	trace	trace	0.03	0.04	-----	0.14	0.12	0.09	0.13	0.09
June 15, 1965	no										
	trace	-----	trace	0.08	-----	-----	0.22	0.11	0.17	0.09	0.15

* Data gathered by Dr. James Butcher, Michigan State University.

were used. The data indicates a wide range in dieldrin concentrations in the treated area, 0.6 to 5.0 $\mu\text{g/L}$, with the average for all stations being 2.1 $\mu\text{g/L}$ in this first year following treatment.

Two sets of samples were collected in 1964, one in the summer (July 8) and one in the fall (October 17). These water samples were all separated into four fractions by Millipore filtration as described above. This was an attempt to more closely determine the exact location of the dieldrin storage in the water samples. Was it in the nanoplankton, the bacteria, or dissolved in the water? After fractioning the samples and analyzing each separate fraction, an inherent problem in this procedure was discovered. It greatly exaggerated the values obtained for all residues on the Millipore filters and diminished those for the final filtrate, or organismic-free water. The problem was this: While filtering the water, the Millipore filters adsorbed dieldrin, removing it from the water and adding it to the residue fractions. There was no way of removing this adsorbed dieldrin without also interfering with the concentration associated with the residue. Consequently, this procedure had to be abandoned and the analyses corrected for each sample as though the water was unfiltered. It is felt that no significant dieldrin loss occurred as a result of this step and that the recalculated values for the samples are correct. Using these readjusted values for the two sets of samples in 1964, it can be seen that the concentrations contained in the water are sharply lower than those of 1963. The average value for 1964, including both sampling dates and all stations is 0.11 $\mu\text{g/L}$.

One set of samples was collected in 1965 (June 15) and these were not filtered but extracted in total. The average concentration is $0.14 \mu\text{g/L}$. Thus, it appears that the dieldrin concentration in the water decreased quite rapidly after the final dieldrin application (1962), through the following year (1963) and until it reached a low level where it appeared to plateau (1964 and 1965).

Samples taken from the streams in the untreated area showed the absence of dieldrin in the water except for two samples which showed slight traces too low to measure. Therefore, the dieldrin found in samples from the treated area was the result of dieldrin remaining from the previous aerial applications to the area and not from recent local domestic uses.

In 1963, the highest dieldrin concentrations were in the upper half of the stream while in 1965 they were in the lower half of the stream. This is particularly evident in Little Lake Creek and in Indian Creek. It is possible that this reflects a slow but gradual purging of the stream system and perhaps the whole watershed, however data is not complete enough to be conclusive.

Green Filamentous Algae (Table 2)

No data was available for 1963. However, algal samples were collected twice in 1964 and once in 1965 along with water. The high dieldrin values make it apparent that algae were taking up the dieldrin from the water and storing it at levels much higher than those found in the water. All values show a decrease in concentration with time except for stations 8 and 9. The average values for all samples in 1964 is $247.7 \mu\text{g/Kg}$, and for 1965, $316.0 \mu\text{g/Kg}$. Storage ability, either by uptake or adsorption, is suggested by dieldrin

TABLE 2. Field Collected Samples of Green Filamentous Algae
($\mu\text{g/Kg}$ dieldrin, dry weight).

Sampling Stations

Sample Dates	Control Area			Treated Area							
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11
July 8, 1964	no trace	-----	no trace	262.1	-----	-----	620.3	-----	-----	-----	-----
October 17, 1964	no trace	-----	no trace	154.4	-----	-----	-----	93.5	108.2	-----	-----
June 15, 1965	15.8	-----	42.4	68.4	-----	-----	272.2	409.1	431.4	398.9	-----

concentrations up to 5,000 times for the algal samples over the level in their habitat. The average concentration factor, relative to the water, was approximately 2,500.

No trace of dieldrin was found in plants from the control streams in 1964 but a small amount was found in those collected in 1965. However, in the latter year the average for the untreated samples was more than an order of magnitude lower in concentration than those for the same time from the treated area. Thus, most of the dieldrin stored in the algae from the treated area represents dieldrin persisting from the major insect control program last applied three years earlier.

Higher Aquatic Plants (Table 3)

Higher aquatic plants sampled were all Lemna minor except in station number 8 where Potamogeton spp. were collected. Vascular plants were not collected until the fall of 1964 (October 17) and again in 1965, corresponding to the dates of the water samples. There is considerable variability among the values obtained but it is obvious that Lemna minor is capable of very great dieldrin storage - note especially the value for station number 10 in 1964. This one sample represents a concentration 20,230 times larger than that in the water for the same site. The average concentration for all samples in 1964 was 745.7 $\mu\text{g/Kg}$, and for 1965, 437.5 $\mu\text{g/Kg}$. Again, no trace of dieldrin was found in the vascular plant samples from the control streams in 1964 but small amounts were detected in June of 1965.

TABLE 3. Field Collected Samples of Higher Aquatic Plants
(Lemna minor and Potamogeton spp.)
($\mu\text{g/Kg}$ dieldrin, dry weight).

Sampling Stations

Sample Dates	Control Area			Treated Area						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10 No. 11
October 17, 1964	no trace	-----	no trace	158.5	-----	-----	-----	86.2	108.2	2630.0 -----
June 15, 1965	-----	-----	51.1	150.0	-----	-----	-----	457.9	704.6	----- -----

Animals (Table 4)

Three samples of snails were obtained in 1965 and the average overall dieldrin concentration was 332.9 $\mu\text{g/Kg}$. These animals concentrated dieldrin 2,400 to 3,500 times over that in the water. One sample of fish (common shiners, Notropis cornutus) was obtained and found to have a concentration of 98.1 $\mu\text{g/Kg}$. This is a concentration factor, relative to water, of 1,226. No control samples were obtained for either fish or snails.

Discussion

Data obtained from this field study is very difficult to evaluate and interpret except in very general terms. Nevertheless, it does re-emphasize a few important points concerning the intensive and widespread use of a stable insecticide such as dieldrin. First, I found that dieldrin was present in the aquatic ecosystems for at least three years following a heavy and extensive aerial application for insect control. It may be at very low levels, as in the water, two and three years after treatment, but it was in every water sample from the area. It was found in much higher concentrations in plants and animals sampled and, again, present to some extent in every sample from the area. This reaffirms the fact that dieldrin, like many other insecticides of the same cyclodiene group, has a very long persistence in the environment once it is introduced. It is concentrated and stored in varying degrees by the different trophic levels of these ecosystems and is readily available to be passed along through a food chain at rather high dosage levels. Also, it appears from my results that this insecticide is continually

TABLE 4. Field Collected Samples of Animals
($\mu\text{g/Kg}$ dieldrin, live wet weight).

Sampling Stations

Sample Dates	Control Area			Treated Area						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10 No. 11
June 15, 1965:										
Snails	-----	-----	-----	201.4	-----	-----	-----	387.2	410.1	-----
Fish	-----	-----	-----	98.1	-----	-----	-----	-----	-----	-----

moving off the terrestrial surfaces of the watersheds and into the aquatic ecosystems. The exact route by which this is accomplished is not elucidated by the present study, but other works (Rosen and Middleton, 1959; Hickey et al., 1966; Breidenbach and Lichtenberg, 1963; Lichenstein et al., 1968) would lead me to suspect two primary routes: Adsorption on soil particles or dissolution in fatty constituents of dead organisms or parts thereof washing into the stream, and volatilization into the air and subsequent washing, settling or direct adsorption from the air. In any case, the aquatic environment is particularly subjected to the affects of such long-lived insecticides since, sooner or later, much of it could reach the water. This places aquatic organisms in a position of higher probability and duration of exposure at generally higher levels than is true with terrestrial organisms subsequent to the initial high dose period.

LABORATORY STUDIES

General

A series of separate individual experiments were run in the laboratory to help explain the mechanisms operating in an aquatic ecosystem when an insecticide like dieldrin is introduced. How important is food chain translocation compared to direct uptake of the insecticide? Are the rates of accumulation different for these two mechanisms of uptake, and if so, which has the greater rate? What is the rate of accumulation when both mechanisms are operating at the same time? Are they additive or is there interaction? When the equilibrium between uptake and elimination is obtained, is it at the same level for both mechanisms and for the combined effects? These are the major questions which I set out to answer by working through the following experiments.

The same 5% granular dieldrin that was used for the field treatment of the Monroe County area was used in all experiments. It was prepared in acetone on the basis of active dieldrin and in concentrated form so that only a small amount of acetone solution was necessary for a given dieldrin treatment. Whenever dieldrin was added to a culture or an aquarium it was done so as to give a desired concentration assuming that it all goes into solution and stays in solution. However, it is realized that this is never the situation for immediately after being added it begins to become adsorbed slowly to various substrates, including the glass. Nevertheless, treatments can be made with this understanding. So when a treatment is made giving a certain concentration to the total

aquarium under static conditions, it must be kept in mind that the actual concentration in the water is something less and that it is gradually decreasing with time.

As previously mentioned, the principle organisms used were: *Daphnia*, *Daphnia* spp., the invertebrate serving primarily as the food organism; and bluntnose minnows, *Pimephales notatus*, the fish used for the basic part of the study. The daphnia cultures were started from vernal ponds and rapidly built up to very large numbers. Before any tests were run, the cultures were carried through several generations so as to rid the organisms of any insecticide burden that they might have possessed. These stock cultures were periodically sampled, analyzed for dieldrin and were maintained at 0.00 µg/g. This was quite easy as long as a careful routine was adhered to. The minnows were all obtained, by seining, from one small lake in Barry County where this species was found in relatively large numbers. Approximately 1000 were collected at one time and held in a very large tank in the laboratory, constantly maintained with care. They were held several months before being used to become well-adapted to laboratory conditions. Also, the sick and weaker individuals were removed by this time and only the healthy and active were used. There was no particular problem with these fish as they fed readily on dry pellet food while being held and they took readily to the daphnia when used in the food chain. In one experiment small mouth bass, *Micropterus dolomieu*, were used and these were obtained from the Wolf Lake Fish Hatchery, Michigan. They ranged from approximately 6 to 10 inches and had been held in a holding pond, feeding only on natural foods.

Experiment 1: Dieldrin Treatment of Daphnia

Before using daphnia for a food organism, dieldrin treatment studies were necessary. How much dieldrin could be added to a culture and how long an exposure was necessary to bring the daphnia to a maximum level of dieldrin without too great a mortality? From some preliminary work using low levels of dieldrin it was observed that the daphnia seemed to reach their maximum dieldrin concentration at approximately three days. After this the concentration tended to decrease, probably the combined result of dieldrin leaving solution by adsorption and the continual turnover of individuals in the culture.

On this basis, one 10 gallon aquarium was started with daphnia. Once the population had built up, dieldrin was directly added to the culture to give an initial concentration of 1.5 $\mu\text{g/L}$. At the end of three days, three replicate samples of daphnia (Table 5) and three of the water (Table 6) were taken.

TABLE 5. Dieldrin Concentration in Daphnia at the End of Three Day Exposure, Using 1.5 $\mu\text{g/L}$ Initial Dieldrin.

Replicate	Wet Weight Of Daphnia (g)	Dieldrin Concentration ($\mu\text{g/g}$)
1	0.3167	1.08
2	0.2795	1.25
3	0.3281	1.31
		Average = 1.21 $\mu\text{g/g}$

TABLE 6. Dieldrin Concentration in the Daphnia Culture Water at the End of Three Day Exposure, Using 1.5 $\mu\text{g/L}$ Initial Dieldrin.

Replicate	Dieldrin Concentration in Water ($\mu\text{g/L}$)
1	0.775
2	0.695
3	0.723

Average = 0.731 $\mu\text{g/L}$

The average dieldrin concentration in the daphnia was 1.21 $\mu\text{g/g}$ and the concentration in the water had decreased to an average of 0.731 $\mu\text{g/L}$. There was little mortality of daphnia during this time period and with this treatment concentration.

Another similar experiment was set up, starting with three 10 gallon aquaria of daphnia. This time they were treated initially with 3.0 $\mu\text{g/L}$ dieldrin. One sample was taken from each, by siphoning concentrated masses of daphnia, on days numbered 1, 2, 3, 4, and 6 following the treatment. No water was put back so the dieldrin concentrations in the water were not altered by sampling. Consequently, three replicates were obtained for each day and from three different cultures. Results (Table 7) show that the maximum dieldrin concentration did occur in the daphnia between the third and fourth days under the static conditions of this test, with the maximum being 1.9 $\mu\text{g/g}$. After this time, the concentration declines quite rapidly. This is not quite double the maximum concentration obtained with half the treatment dose used in the first phase of the

TABLE 7. Dieldrin Concentration in Daphnia at the End of Three Day Exposure, Using 3.0 $\mu\text{g/L}$ Initial Dieldrin.

Number of Days After Treatment	Replicate Number	Wet Weight Of Daphnia (g)	Dieldrin Concentration ($\mu\text{g/g}$)	Average Dieldrin Concentration ($\mu\text{g/g}$)
1	1	0.1432	0.72	0.74
	2	0.1028	0.74	
	3	0.1755	0.77	
2	1	0.1479	1.21	1.28
	2	0.2134	1.12	
	3	0.1568	1.51	
3	1	0.1938	2.10	1.93
	2	0.2711	1.98	
	3	0.1436	1.71	
4	1	0.1123	1.99	1.91
	2	0.1781	1.79	
	3	0.2063	1.95	
6	1	0.3356	1.31	1.47
	2	0.2322	1.48	
	3	0.1837	1.61	

experiment. However, it is close enough to postulate that the concentration acquired by daphnia is directly proportional to the concentration in the water. The slight difference here could be quite easily explained, perhaps, by the fact that the greater the treatment dose added to the culture, the greater the initial adsorption. Thus, more goes out of solution with the higher initial dose so the resulting solution would not be exactly double that from the lower dose. The way in which the cultures were treated permitted uptake of dieldrin by both the food chain and directly from the water.

Some mortality, estimated at less than 25%, occurred with the 3.0 $\mu\text{g/L}$ treatment by the third day. So this appeared to be about

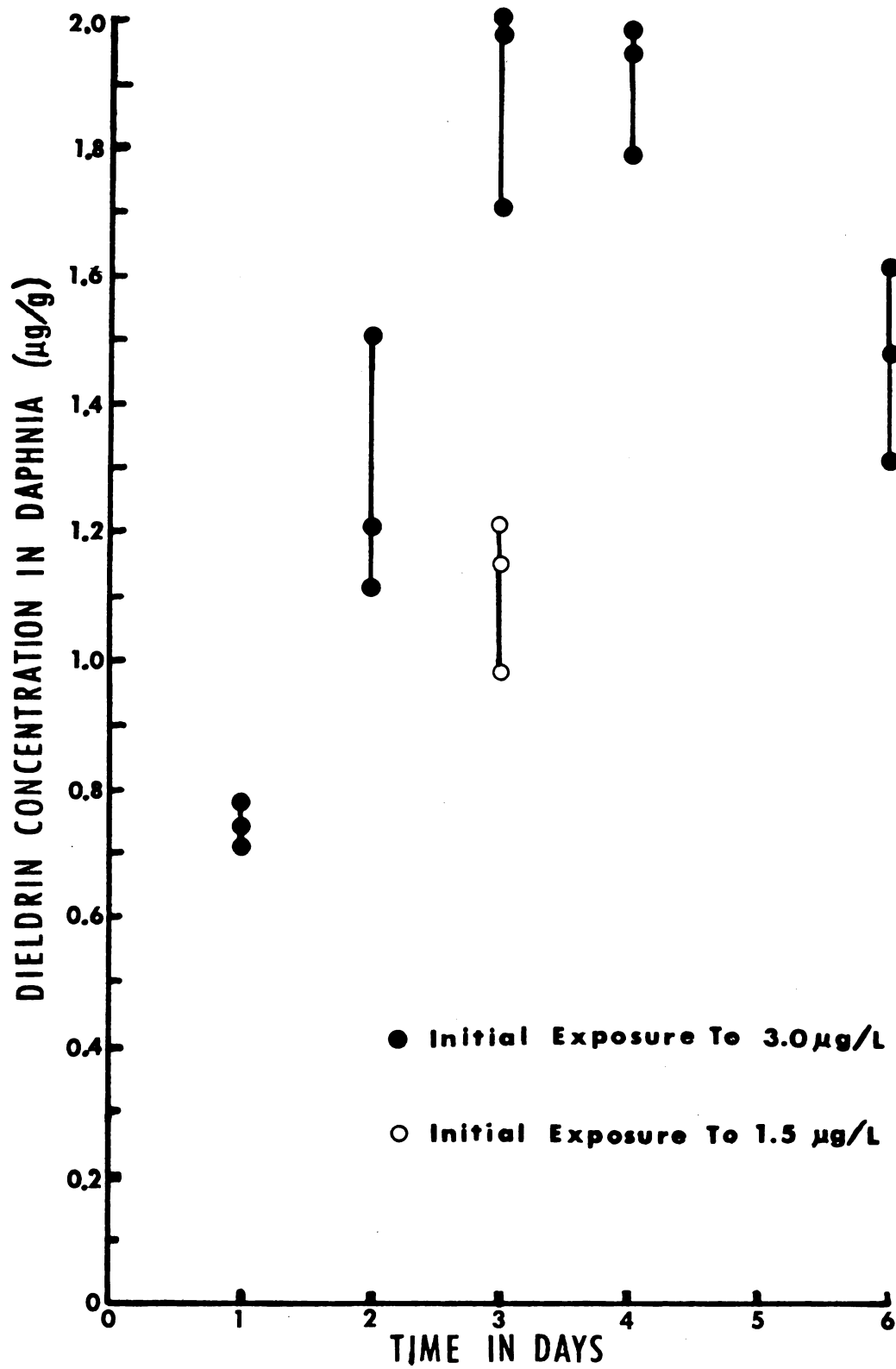
the highest treatment level that could be used without excessive mortality, yielding $1.9 \mu\text{g/g}$ as the highest approximate storage concentration which could be obtained by daphnia. It was reached with a three day exposure. These treatment levels and procedures were used in the following experiments. The results of both treatment levels are shown graphically in Figure 2.

Experiment 2: Food Chain Study

It was next possible to study the amount of dieldrin translocation via a food chain. Basically, treated daphnia were fed to bluntnose minnows. This was the first step to the more important questions to be investigated later. Five 5 gallon aquaria were set up with six bluntnose minnows in each. These tanks were well filtered continuously with an excess of activated charcoal so that any dieldrin excreted by fish or from the food source direct would be taken out of the water in a very short time. Also, three 1 gallon daphnia cultures were set up for each tank of six fish. Each set of three cultures could then be rotated through the dieldrin treatment with three day exposures, using one culture for food for one tank of six minnows each day. After each culture was used for food the jar was washed thoroughly, rinsed with acetone, new daphnia started from a large stock culture, and treated for another three days. Daphnia treatment was with $1.5 \mu\text{g/L}$. One set of three cultures was left untreated but rotated the same for the control feedings. There was also one additional culture which was treated and recycled to be used for analysis of the dieldrin concentration in the daphnia every three days. When the fish were sampled, one whole

Figure 2. Dieldrin Treatment of Daphnia Cultures.

FIGURE 2



aquarium was terminated and the six fish were randomly divided into three replicates of two fish each for analysis. The feeding and sampling schedule was as follows: One tank each was fed treated daphnia for 3, 6, 9, and 12 days; and one was fed untreated daphnia (control) for 12 days.

A wet weight of the daphnia fed to each tank of minnows was obtained and is shown (Table 8) as accumulated amounts. Also, from the four analyses of daphnia the average dieldrin concentration in this food source was $1.3 \mu\text{g/g}$ (Table 9). On this basis, the accumulated amount of dieldrin fed to each group of six fish via the daphnia was calculated (Table 8). When daphnia were put into the aquaria with fish they were readily consumed. This was carefully watched so that the fish were not overfed, allowing for possible contamination of the water by unused food. However, the feeding was set up to give near the maximum amount of food that could be readily consumed by six fish. Obviously, not all six fish ate the same amount of food and the splitting of the six into three replicates of two each was intended to offset this. However, the variation which resulted among the replicates is probably still largely the reflection of this feeding variability. Hence, the calculated average for each of the three replicates is representative of the dieldrin concentration. All fish were allowed to remain 24 hours after the final feeding to permit the elimination of dieldrin and food which would still be moving through the intestinal tract.

From the results (Table 8) it can be seen that the amount of dieldrin accumulated in the bluntnose minnows is, up to a certain point, a function of the amount consumed through its food over a

TABLE 8. Results of Feeding Treated Daphnia to Bluntnose Minnows.

Treatment Replications (two fish / replicate)	Accumulated Amount Of Daphnia Eaten Per Six Fish (g Wet Wt.)	Accumulated Amount Of Dieldrin Fed Per Six Fish Via Daphnia (μ g)	Live Weight Of Fish Sample (two fish each) (g)	Dieldrin Conc. Of Fish (μ g/g)	Average Dieldrin Conc. Of Fish (μ g/g)
12-Day Control:	7.279	0.0			0.022
1			2.295	0.014	
2			3.014	0.020	
3			3.488	0.032	
3-Day Treated:	2.392	3.171			0.079
1			2.956	0.109	
2			3.102	0.058	
3			2.161	0.073	
6-Day Treated:	3.985	5.280			0.132
1			2.254	0.121	
2			4.947	0.203	
3			2.161	0.073	
9-Day Treated:	5.141	6.813			0.165
1			3.471	0.170	
2			1.788	0.199	
3			1.924	0.125	
12-Day Treated:	8.734	11.571			0.169
1			5.838	0.177	
2			2.675	0.142	
3			2.852	0.187	

TABLE 9. Daphnia Analyses for Food Chain Experiment,
Sampled Every Three Days

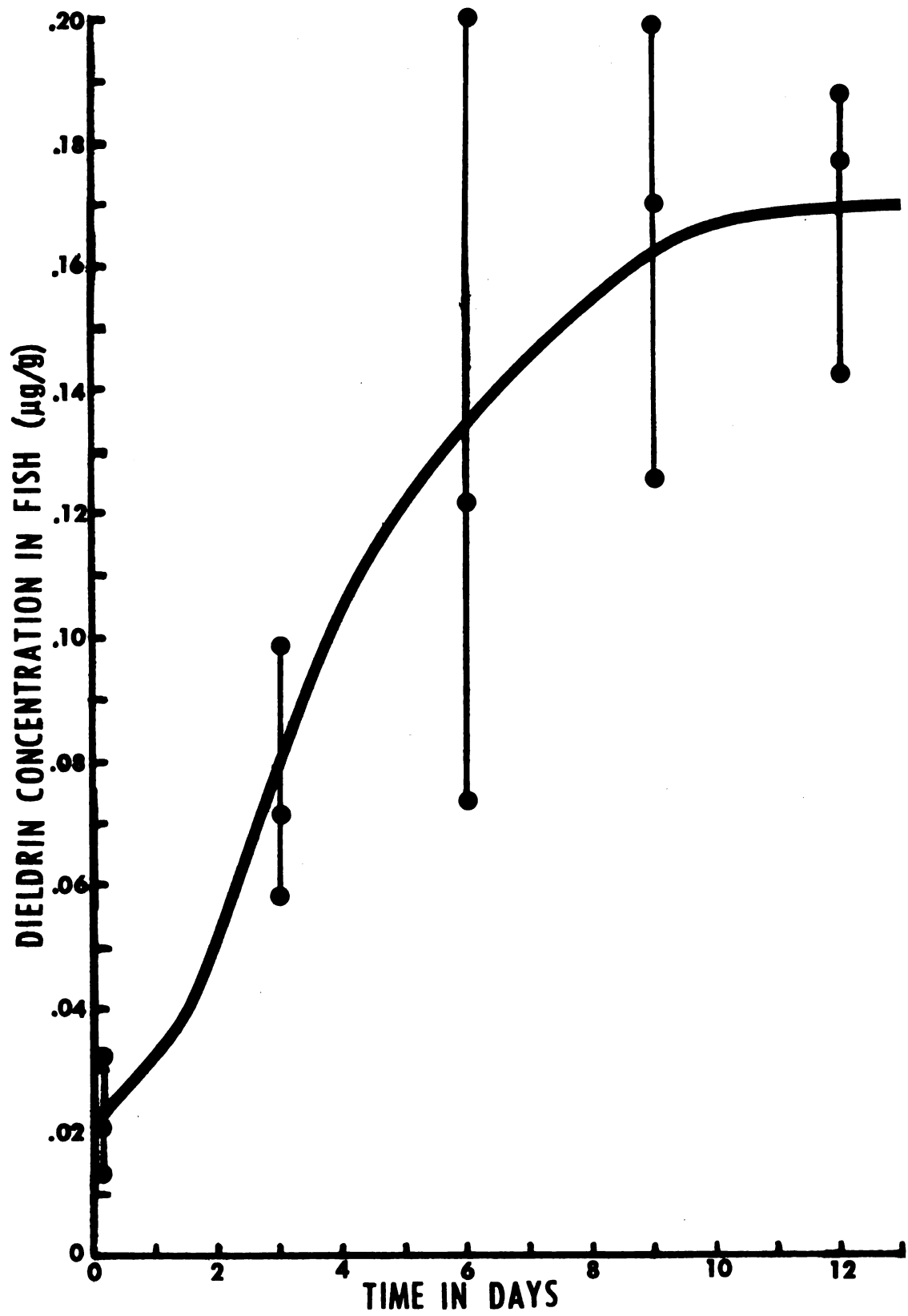
Daphnia Sample Size (g Wet Weight)	Dieldrin Concentration in Daphnia ($\mu\text{g/g}$ Wet Weight)
0.2732	1.771
0.1958	1.161
0.3608	1.152
0.4894	1.216
Average = 1.325 $\mu\text{g/g}$	

period of time. Equilibrium was reached between 9 and 12 days and at a concentration of 0.17 $\mu\text{g/g}$ for this particular feeding schedule (Figure 3). The control fish showed a slight amount of dieldrin present and this served as the starting point for dieldrin uptake. Consequently, I demonstrated the small amount of dieldrin that could be passed along this food chain with the level of food contamination and feeding rate used. I also illustrated the short time required for equilibrium to be reached, that is, when elimination of dieldrin equals ingestion. Further feeding on beyond this time, at a more or less constant rate, does not result in any greater storage of dieldrin.

This study does not confirm the concept of biological magnification in the aquatic system if such magnification is taken to mean that the dieldrin concentration becomes greater with each higher trophic level as it is passed along with the food through a food chain. Under the conditions of my experiment, where only the food chain mechanism was operating in the translocation of dieldrin, the daphnia had a fairly high average concentration (1.325 $\mu\text{g/g}$) and

Figure 3. Results of Feeding Treated Daphnia to Bluntnose Minnows. Each point represents two fish pooled for analysis. The three points for any one day sampled represent the six fish in one tank and fed together. Equilibrium is reached at 0.17 $\mu\text{g/g}$.

FIGURE 3



were fed in substantial amounts to the minnows (averaging 0.121 g/fish/day on the basis of the 12-day accumulative total). Thus, the average amount of dieldrin passed along through the food was also quite large, averaging 0.161 $\mu\text{g}/\text{fish}/\text{day}$ (on the basis of the 12-day accumulative total). Yet, the highest concentration reached in the fish at the equilibrium level was only 0.17 $\mu\text{g}/\text{g}$, or approximately 1/8 that in the daphnia. If the dieldrin treatment of daphnia had been 3.0 $\mu\text{g}/\text{L}$ and the amount of daphnia fed to the minnows each day had been more constant and nearer the maximum amount the fish could consume, then the maximum extent of sublethal food chain dieldrin accumulation for this daphnia-minnow food transfer would have been established.

Experiment 3: Extended Food Chain Study

The previous experiment was essentially repeated but with an additional link to the food chain. One smallmouth bass was used as a top carnivore. All procedures were the same and the daphnia was again exposed to an initial dieldrin concentration of 1.5 $\mu\text{g}/\text{L}$. This time, however, three 5 gallon aquaria were used with five bluntnose minnows in each. The minnows were fed treated daphnia for three days and then sacrificed with a randomly chosen one saved for analysis and the remaining four fed to the bass. The minnow aquaria were recycled in the same way the daphnia cultures were to provide continuous daily food for the bass. This experiment was conducted for a 14-day feeding period for the smallmouth bass. Four other bass were held in separate tanks and fed minnows from the same stock but untreated, serving as controls. After 14 days of feeding, the bass was held 24 hours before sacrificing. Separate

tissue analyses were made instead of a whole body analyses.

TABLE 10. Feedings for the Extended Food Chain Study

No. Of Day	Total Amount Of Daphnia Fed to Each Group of Five Minnows For Three Days (g wet wt.)	Amount Of Dieldrin Contained In This Amount Of Daphnia ¹ (µg)	Live Weight Of Four Minnows Fed to Bass (g)	Amount Of Dieldrin Contained In This Amount Of Minnows ² (µg)
1	0.8241	0.924	7.5	0.600
2	0.9529	1.068	6.3	0.504
3	1.1330	1.270	4.2	0.336
4	1.4320	1.605	6.7	0.536
5	1.4647	1.642	5.7	0.456
6	1.5513	1.739	5.0	0.400
7	1.8205	2.041	7.3	0.584
8	0.7963	0.893	6.7	0.536
9	1.2113	1.358	4.7	0.376
10	1.6068	1.801	6.6	0.528
11	2.4872	2.788	6.5	0.520
12	1.7783	1.994	5.6	0.448
13	2.1332	2.391	4.4	0.352
14	1.5788	1.770	5.8	0.464
Average	1.4835 g	1.663 µg	5.9 g	0.472 µg

¹ Calculations based on the average dieldrin concentration of daphnia (Table 2).

² Calculations based on the average dieldrin concentration of minnows (Table 2).

The feeding schedule is presented in Table 10 for both the feeding of treated daphnia to minnows and the feeding of these minnows to the bass. Each group of five minnows received in three days, an average of 1.4835 g of treated daphnia (with an average of 1.121 $\mu\text{g/g}$ dieldrin as shown in Table 11) or an average total of 1.663 μg of dieldrin in three days.

TABLE 11. Daphnia and Minnow Analyses for the Extended Food Chain Study

Daphnia Sample Size ¹ (g wet wt.)	Dieldrin Concentration In Daphnia ($\mu\text{g/g}$ wet wt.)	Live Weight Of Pooled Minnow Samples ² (g)	Dieldrin Concentration In Minnows ³ ($\mu\text{g/g}$)
0.1531	0.447		
0.3608	1.482	5.26	0.081
0.4894	1.226	4.16	0.063
0.5542	1.223	3.96	0.113
0.4230	1.227	3.92	0.062
Average	1.121 $\mu\text{g/gm}$		0.080 $\mu\text{g/gm}$

¹ Samples from a culture treated three days and recycled, providing one sample every three days.

² One fish was taken from a tank of 5 minnows each day for this analysis while the remaining 4 were fed to the bass. The first two samples each represent 3 fish pooled, one from each of three days, and the last two samples represent 4 fish each, one from each of four days.

³ Variation here is due largely to the fact that each of the five minnows per tank did not eat the same amount of daphnia, and hence, dieldrin. The average of these figures offsets this variation.

The smallmouth bass received an average of 5.9 g of minnows per day containing an average total of 0.472 μg of dieldrin. Results of the extent of accumulation in the bass is shown in Table 12. The

TABLE 12. Smallmouth Bass Tissue Analysis After 14 Days of Feeding.

Tissue Analyzed	Tissue Weight (g)	Dieldrin Amount (μg)	Dieldrin Concentration ($\mu\text{g/g}$)
Heart	0.8614	trace	trace
Liver and Gall Bladder	4.2148	0.219	0.052
Kidney	0.8081	0.040	0.049
Ovary (non-ripe)	1.3874	0.021	0.015
Brain	0.2766	0.014	0.051
G.I. Tract (empty)	22.3848	0.584	0.026
Remainder	401.4	0.108	trace
Total		0.986	

Total Length = 31.1 cm

Total Live Weight = 435.1 gm

Calculated Whole Body Concentration = 0.002 $\mu\text{g/g}$

liver, kidneys, and brain were about equal and contained the highest concentrations. The gastro-intestinal tract, which was empty, was about half this amount and the heart contained only a trace. This agrees with the results of Mount (1962) using endrin, and with Grzenda (1967) using DDT and dieldrin. The liver and brain of the four control bass were analyzed and only trace amounts of dieldrin

were found in any of these.

When the tissue concentrations are combined, the whole body concentration is only approximately $0.002 \mu\text{g/g}$. Consequently, this experiment further emphasizes that whole body accumulations cannot be built up very high with fairly constant ingestion of an insecticide like dieldrin when sublethal levels occur two steps lower in a food chain if this is the only mechanism involved. For the food chain in this experiment, biological magnification seems quite unlikely if explained on the basis of food chain translocation alone. Using average concentrations, the following sequence was revealed with this three step food chain: $1.121 \mu\text{g/g}$ in the daphnia, $0.08 \mu\text{g/g}$ in the minnows, and $0.002 \mu\text{g/g}$ in the bass. This represents considerable exclusion of dieldrin from the tissues at each higher level even with extended continuous feedings. It does not seem likely in this system that one trophic level can build up a higher concentration than a lower trophic level by means of a food chain.

This experiment had major weaknesses as a model for food chain behavior in the natural setting. First, the three day feeding of treated daphnia to the minnows was really not long enough. Referring back to Figure 3, it can be seen that six, or preferably nine, days of feeding would have been closer to their equilibrium and, hence, closer to their maximum accumulation. However, this increased feeding tremendously increased the requirement for laboratory facilities and work involved, and this became limiting. Second, the smallmouth bass used were so large that four minnows a day were insufficient to provide their complete food requirement. Third, since the water concentrations of dieldrin for both the minnow and

the bass were essentially nil, loss via the gills of dieldrin ingested with the food would be maximal. Hence, these weaknesses tended to reduce the amounts of dieldrin translocated and thus, maximum accumulations were not realized at any of the three trophic levels. The weaknesses are not serious as long as they are recognized as present.

Experiment 4: Food Chain Uptake Versus Direct Uptake

With the previous preliminary studies completed, a more critical evaluation of the food chain as a mechanism of insecticide uptake was undertaken. This involved a direct comparison of the food chain mechanism with the direct uptake mechanism and also with the action of both of these working together. Consequently, I sought to answer the questions which I set forth at the beginning. Not only were direct comparisons designed but the study was extended to 30 days, to more accurately be considered a long term study. Also, the initial treatment levels were increased to just below lethal levels so that near maximum long term effects for sublethal levels could be observed.

Basically, four systems were run simultaneously on a carefully planned comparative basis. They were as follows (and hereafter referred to by their series designations):

SERIES A: Measured Food Chain Uptake -

Bluntnose	Dieldrin-Treated	Untreated
Minnows	fed Daphnia	in Water

SERIES B: Measured Direct Uptake -

Bluntnose		Untreated		Dieldrin-Treated
Minnows	fed	Daphnia	in	Water

SERIES C: Measured Effects Of Combined Mechanisms -

Bluntnose		Dieldrin-Treated		Dieldrin-Treated
Minnows	fed	Daphnia	in	Water

SERIES D: Control -

Bluntnose		Untreated		Untreated
Minnows	fed	Daphnia	in	Water

Aquaria ($4\frac{1}{2}$ gallon size) were started with five minnows in each. Water was adjusted to 184 ppm calcium carbonate hardness by diluting tap water with distilled water. This hardness was considered about average for Michigan waters and gave a total alkalinity of 205 ppm and a pH of 7.4. The temperature was fairly constant at a room temperature of 20°C. Whenever water was changed, as described later, it was first adjusted to these conditions.

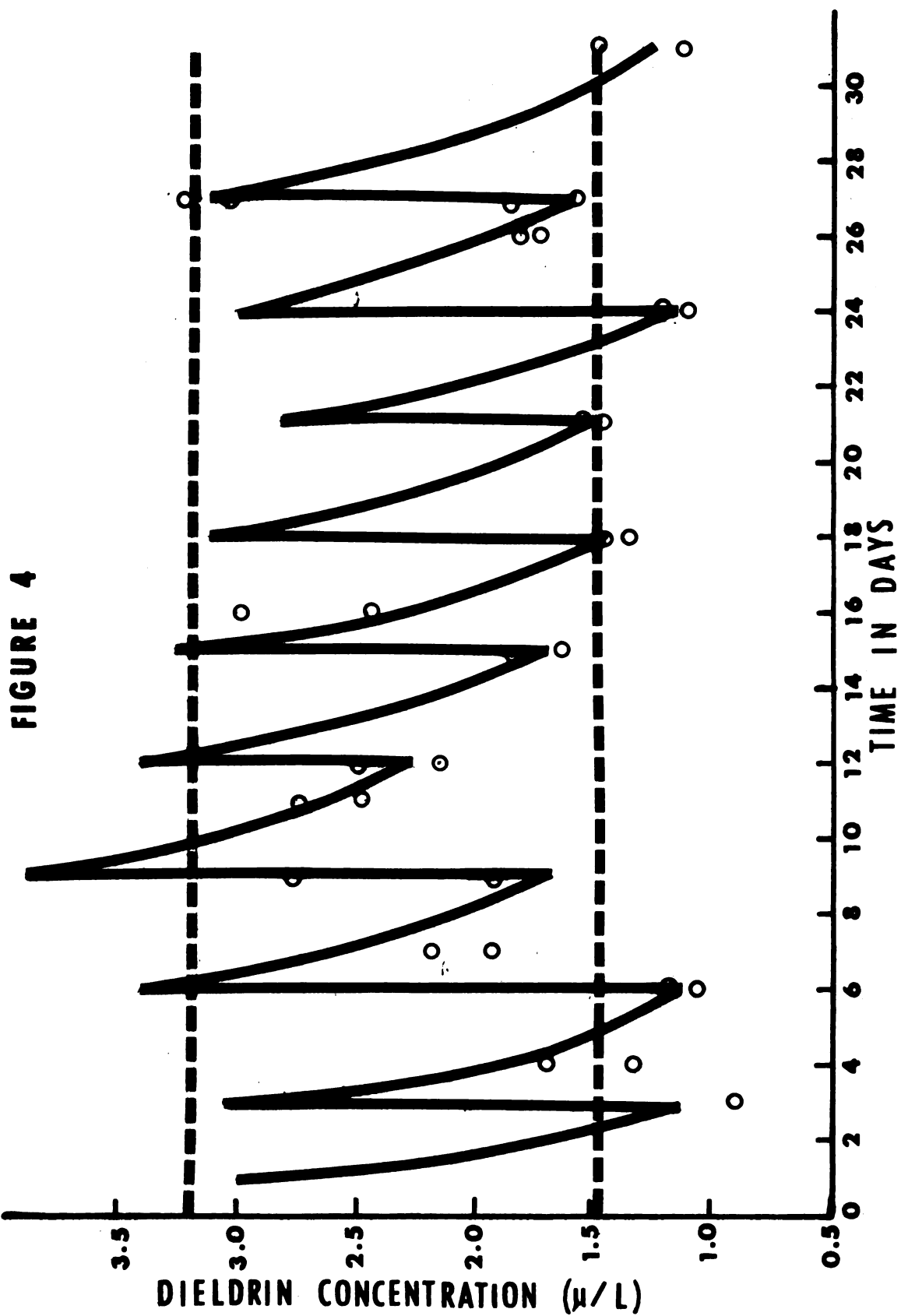
Samples for series A through C were taken on the following days: Day 3, 6, 10, 15, 20, 25, and 30. Thus, seven samples were taken for each of these series over the 30-day period. Each sample consisted of one tank of five minnows terminated and pooled for analysis. Consequently, this required 21 aquaria. For series D (control), three samples were taken on days 3, 15, and 30. So a total of 24 aquaria were started at the same time, each with five bluntnose minnows.

Where the fish were exposed to dieldrin in the water (series B and C) no filters were used in the aquaria, only slow aeration. They were used, however, in all others to prevent dieldrin contamination

of the water. Since the former two series were not filtered the water would begin to foul by the third day. Therefore, it was necessary to change the water in these tanks every three days. The fish were removed and the water exchanged after thorough cleaning and rinsing with acetone to remove all dieldrin. With the clean water and the fish replaced, the tanks were immediately retreated with dieldrin. Because static water conditions were used, the dieldrin concentration of the water would decrease after the time of treatment. To monitor the concentrations in the water, two tanks were randomly sampled (1 L each) from among those of series B and C, each day that water was changed and when they were terminated. The aquaria to be sampled on these days were all predetermined by a table of random numbers. A graphic representation of the water concentrations is shown in Figure 4. With each change of water, retreatment was intended to give initially 3.0 $\mu\text{g/L}$, after which time the concentration decreases. It was found that the concentration tended to build up with each exchange of water, thus, the initial dose was reduced to compensate. This resulted in a high point at 9 days and a subsequent low at 21 days. The average high concentration was 3.20 $\mu\text{g/L}$ and the average low was 1.48 $\mu\text{g/L}$. Each time a tank was terminated in series A, a 1 L water sample was also taken to check for contamination. Dieldrin was never found in these samples indicating that the filters were effective.

Even though static conditions were used, here with uniform cyclic fluctuations, it is suspected that this as closely approximates true natural conditions as does a continuous flow system. Surely in the natural environment there are fluctuations, although they are less

Figure 4. Dieldrin Concentration in the Water of the Fish Tanks.
The average high and low concentrations (indicated by
broken lines) are 3.20 and 1.48 $\mu\text{g/L}$ respectively.



regular. They occur with high and low water levels and with runoff which recharges the stream with insecticide from the watershed. It is true, however, that a continuous flow system provides a better experimental system in regards to interpreting data.

Setting up the daphnia food source was basically the same as for the previous experiments except that the quantity was greatly enlarged to meet requirements. A total of 180 gallons, in 10 and 20 gallon aquaria, were started with daphnia cultures and maintained at a high rate of production. These were used as stock and for untreated daphnia feedings in series B and D. For the dieldrin treatment of daphnia used in series A and C, 16 gallons of cultures were needed for each day's supply. Since the treatment exposure was again three days, three of these cultures were established and rotated to provide the necessary daphnia each day. Initial treatment was, in this study, $3.0 \mu\text{g/L}$, the maximum sublethal dose. Consequently, both systems, the water containing fish (series B and C) and the daphnia cultures used as food, were treated at the same dieldrin concentration to simulate the situation to be expected within a single aquatic ecosystem.

For the feeding of minnows, a procedure was devised whereby the amounts of daphnia fed to all 24 aquaria were the same without having to weigh each and adjust it. Thus, treated daphnia were filtered onto cheesecloth in a Buchner funnel as before. They were washed and discharged into a 2 L separatory funnel, shaken and swirled vigorously to obtain uniform distribution, and the required number of aliquots (number of tanks to be fed plus one) quickly drawn off as equal volumes into graduated cylinders. Each aliquot was filtered

through new cheesecloth and the daphnia introduced into each tank by dipping the cheesecloth. The one additional aliquot drawn provided one wet weight estimate of the daphnia fed to each tank and then served as the sample for analysis which was carried out every day. Two other aliquots were also weighed so that an average of three weights gave the estimated amount fed.

Untreated daphnia for feedings were handled in the same way except that the total daphnia in the separatory funnel had to be adjusted so as to give equal aliquots with the same wet weight of daphnia as in those with treated daphnia. This was done by starting with excess daphnia in the funnel and determining the volume necessary to give the proper amount of daphnia per aliquot. Hence, in this way all 24 aquaria received equal amounts of daphnia on any one day.

This procedure was tested before the experiment was run. Ten aliquots were drawn and the variation measured. The mean was 0.1254 g wet weight and the standard deviation was 0.0212. Feedings of both treated and untreated daphnia were randomized at the beginning with a table of random numbers so that each aliquot was not always fed to the same aquarium. This tended to reduce the variation among the aliquots as received by each group of fish.

It was more difficult to make the feedings equal in amounts from day to day while trying to keep them near the maximum the fish could consume. This varied with the amounts available in the treated tanks, for the cultures did not always produce what I desired. The mean daily amount of daphnia fed was 0.480 g wet weight and the standard deviation was 0.263. Feedings for day 23 were purposely increased to absolute maximum (1.3446 g) to observe what effect this would have

in all systems.

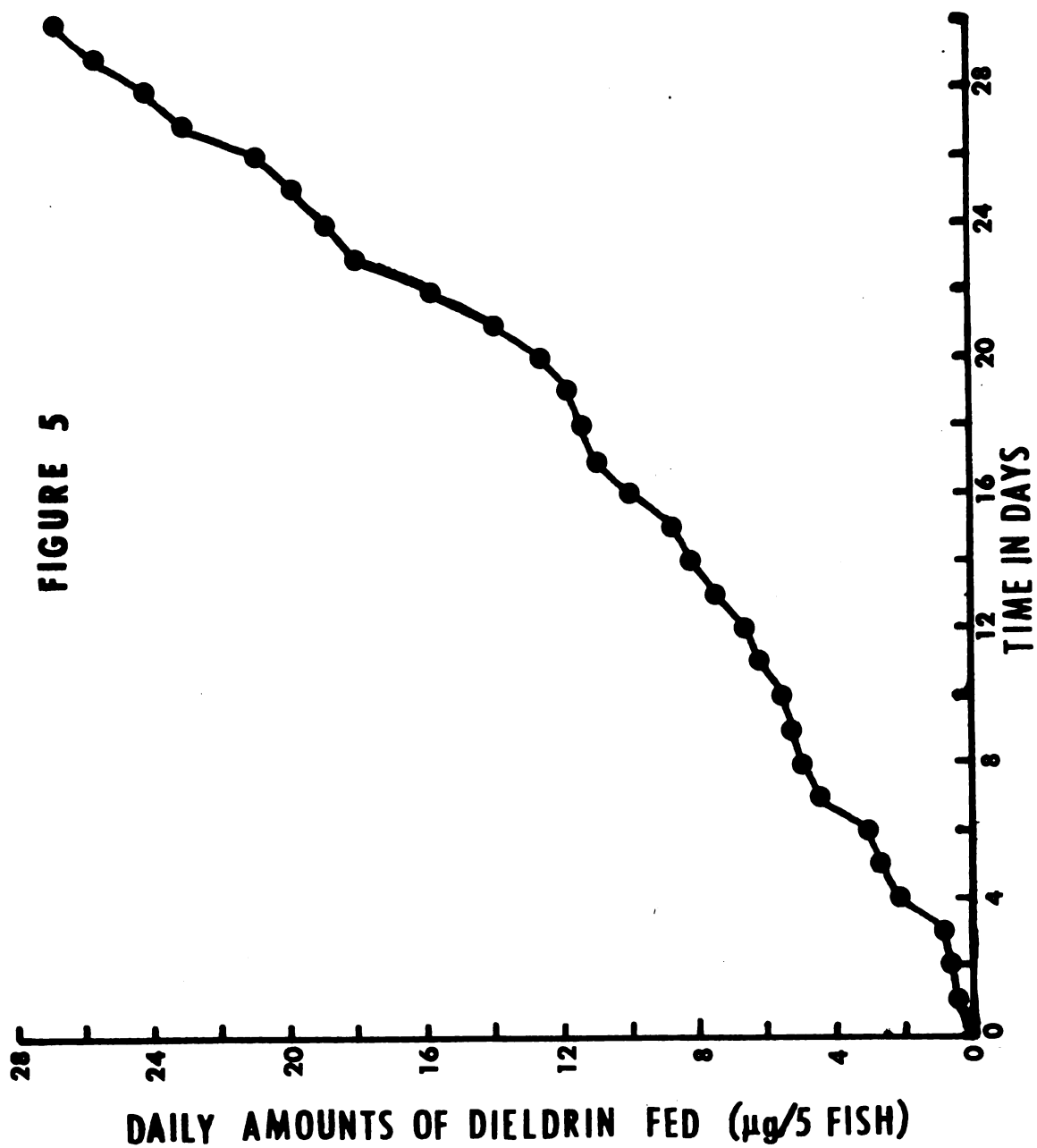
Weights of the five minnows in the 30-day control tank were taken before and after the experiment to determine whether the amount of daphnia fed served as a starvation, maintenance, or growth diet. These fish collectively gained approximately 1.0 g on this diet for the 30-day period. Thus, it represented a fairly good growth diet.

Treated daphnia were analyzed each day to monitor the dieldrin concentration in this food source. The mean dieldrin concentration was 1.89 $\mu\text{g/g}$ and the standard deviation was 0.49. This conforms with the first experiment using 3.0 $\mu\text{g/L}$ initial treatment. Four water samples were taken from the treated daphnia cultures at the end of the three day exposures to observe the concentration change within this time period. The results were 1.995, 2.308, 1.553, and 1.984 $\mu\text{g/L}$, giving an average of 1.960 $\mu\text{g/L}$. This is just slightly higher than the average low water concentration (1.48 $\mu\text{g/L}$) in the treated fish tanks after three days. Therefore, the concentrations and fluctuations for both food and fish treatments were very close. It approximates the condition where the components are all in one ecosystem as found naturally. The accumulative amount of dieldrin fed to the fish, as calculated from the average concentration in the daphnia, is shown in Figure 5. This shows the average rate of feeding to be 0.83 μg dieldrin/day/5 fish. Three control (untreated) daphnia samples were also analyzed to check for dieldrin. None was found except for a very slight trace by day 30.

On the designated days when the fish were to be sampled, feedings stopped for those tanks involved. The fish remained an additional 24

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Figure 5. Rate of Dieldrin Feeding to Minnows (based on the average dieldrin concentration of daphnia equal to $1.89 \mu\text{g/g}$). The calculated average rate of feeding is $0.83 \mu\text{g}$ dieldrin per day per five fish.

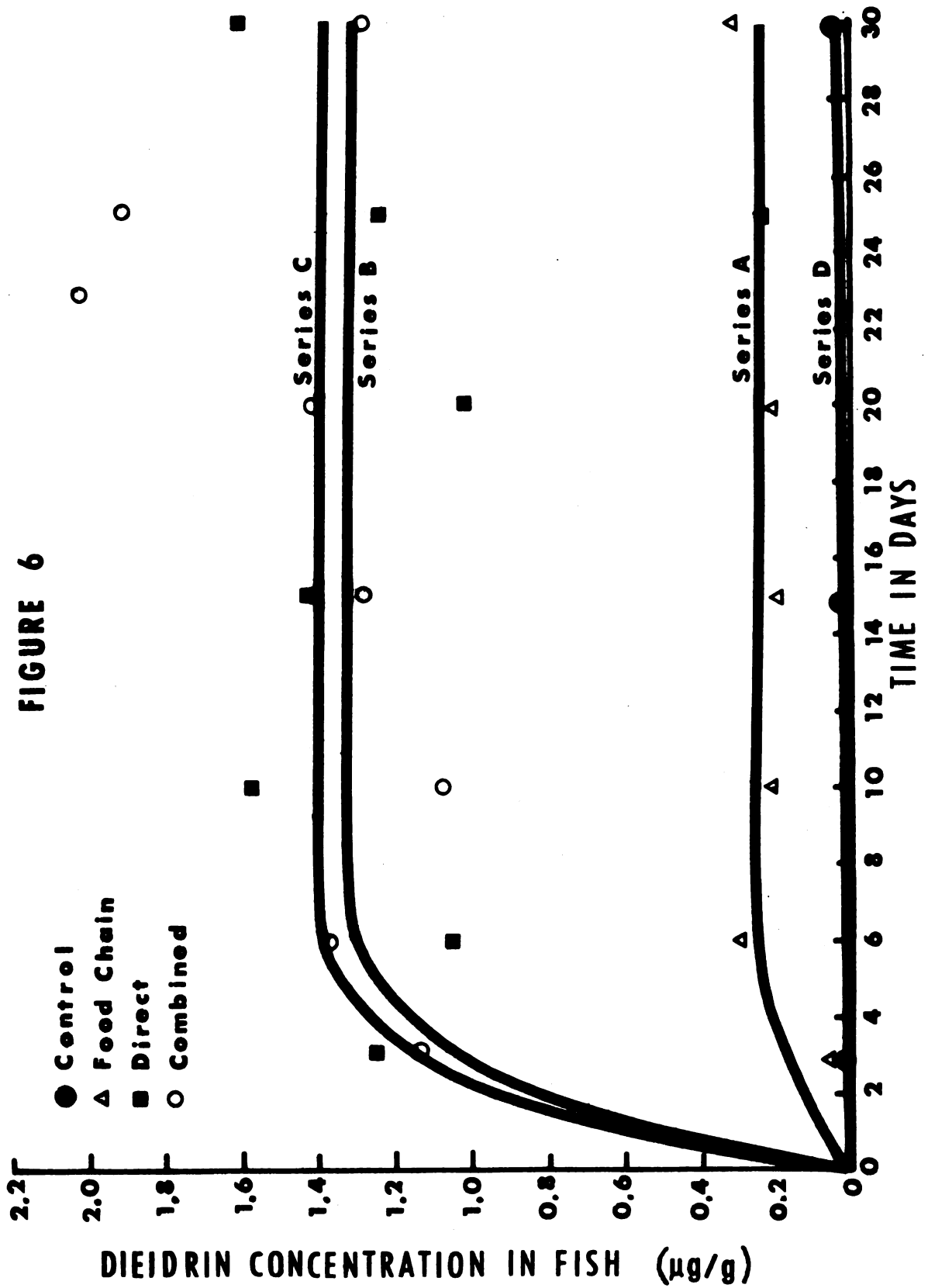


hours, weighed (live) and their complete gastro-intestinal tracts were removed. The contents were squeezed out by running a probe over it and flushing with a syringe. These contents for five fish combined, were analyzed for dieldrin and gave 0.011, 0.014 and 0.012 $\mu\text{g/g}$. The average was 0.012 $\mu\text{g/g}$ and this is an insignificant amount of dieldrin relative to whole body analyses. Consequently, the dieldrin found in the fish with sampling and that consumed by his predator is, for all practical purposes, that stored in the fish tissues, the undigested food in the gastro-intestinal tract being of minor importance in this food chain.

Results are shown graphically in Figure 6. It can be clearly seen that the equilibrium storage levels are widely different for direct uptake (mean concentration 1.30 $\mu\text{g/g}$) and food chain uptake (mean concentration 0.24 $\mu\text{g/g}$). This data also indicates that for this food chain the combination of these two mechanisms does not produce a strongly additive effect. There would obviously be no statistically significant difference between the storage by direct uptake alone and that by the combined mechanisms. Therefore, the former mechanism seems to be the critical one in determining the actual equilibrium (storage) level with these bluntnose minnows. The equilibrium levels appear to be reached by about the same time (six days) with all mechanisms. Consequently, to reach the higher levels by direct uptake, the rate is more rapid than for food chain uptake.

It is also apparent that there is very little variation where the food chain mechanism is operating alone, and considerable variation when direct uptake is involved. This is probably explained by

Figure 6. Dieldrin Uptake by Bluntnose Minnows. The mean equilibrium levels are as follows: 0.0 $\mu\text{g/g}$ for Series D, 0.24 $\mu\text{g/g}$ for Series A, 1.30 $\mu\text{g/g}$ for Series B, and 1.38 $\mu\text{g/g}$ for Series C.



the fact that changes occur much more slowly through the food chain so that variations in the amounts eaten from one day to the next are all balanced out over several days. However, because the direct uptake mechanism is so rapid, daily fluctuations in the water concentrations are reflected in the body concentrations. This is also apparent when the variations of the direct uptake curve is compared with the water concentrations (Figure 4). The highest and lowest fish samples (days 10 and 20) compare favorably with the high and low water concentrations. The variations in the combined mechanisms' curve suggest the interactions of the mechanisms.

Considering the sample points for days 23 and 25 on the combined curve (Figure 6), an interesting observation is seen. An extra large amount of treated daphnia was fed on day 23 (1.3446 g). The following morning three of the five fish in the tank were dead and the other two were greatly stressed. Therefore, this sample was broken up, the three dead fish composing one sample for day 23 (plotted according to the day of the last feeding, but actually taken the following day), the two survivors making up the sample for day 25 as originally scheduled. Hence, from these data it appears that when fish are exposed to both mechanisms of uptake, they can reach a high equilibrium level as a result of direct uptake, and then with a little heavier ingestion of dieldrin through increased food consumption, mortality results. If this is true, then the food chain mechanism can become critical when direct uptake already has the fish at high body burdens. That is, only a little extra push is needed from the food intake mechanism. Unfortunately, this interpretation rests on only this one sample and would have to be confirmed

with a more intensive study before definite conclusions can be drawn. The food chain series (A) also received this same high amount of treated daphnia on day 23 but no effect is detected in the point on the curve averaging the fish sampled three days later. If the observed mortality was dieldrin induced it apparently required the interaction of both mechanisms. This was the only mortality encountered throughout the experiment.

The dieldrin in fish samples from the untreated controls were essentially zero indicating that the fish had little initial dieldrin and that the water and daphnia remained uncontaminated. The last sample taken on day 30 had a very slight trace of dieldrin but this was not measurable.

The preceding experiment prompted two further questions. First, would it be possible to increase the food chain dieldrin uptake rate by increasing the food supply to the minnows? At this higher injection rate would the equilibrium level more closely approach the direct uptake equilibrium level? Secondly, what rate of dieldrin elimination occurs when fish are taken from high levels of storage and placed in clean water? Is the rate of elimination similar to the rate of uptake? Experiments 5 and 6 were designed to investigate these questions.

Experiment 5: Food Chain Uptake Related to Feeding Rates

A feeding experiment similar to series A of the previous study but carried through only 15 days was set up. Samples were taken closer together than in series A: on days 3, 5, 7, 9, 12, and 15. Six aquaria received approximately twice the previous amount of

treated daphnia and six received three times this amount. Because the previous study indicated the fish could not consume much more at one time, these fish were fed twice a day (series E), or three times a day (series F).

The amounts of daphnia fed are shown in Table 13. Amounts for series A are from the previous experiment to which comparisons are made. The average amount fed series A in the first 15 days (0.323 g/day) is less than the average of 0.480 g/day for the entire 30 day experiment. Averages for the other two are 0.798 and 1.299 g/day and these represent, respectively, $2\frac{1}{2}$ and 4.0 times the previous lower rate of feeding. However, the great variations in the latter two can easily be seen. Note that the first seven days of series E is not really much different from those of series A.

Considering the accumulative amounts of dieldrin fed per day via the daphnia, the actual rates are depicted in Figure 7. It is evident that the best way to represent series E feedings is by two separate rates: One for the first seven days, 0.90 μg dieldrin/day, and one for the last eight days, 1.98 μg dieldrin/day. So, both the amount of daphnia and the rate of dieldrin fed in the first part of series E is not too different from that of series A. The last part is, however, quite different. For series F, the rate is perhaps uniform enough so that one rate is satisfactory, 2.40 μg dieldrin/day. Also, the actual amounts fed (Table 13) is quite different from both series E and A.

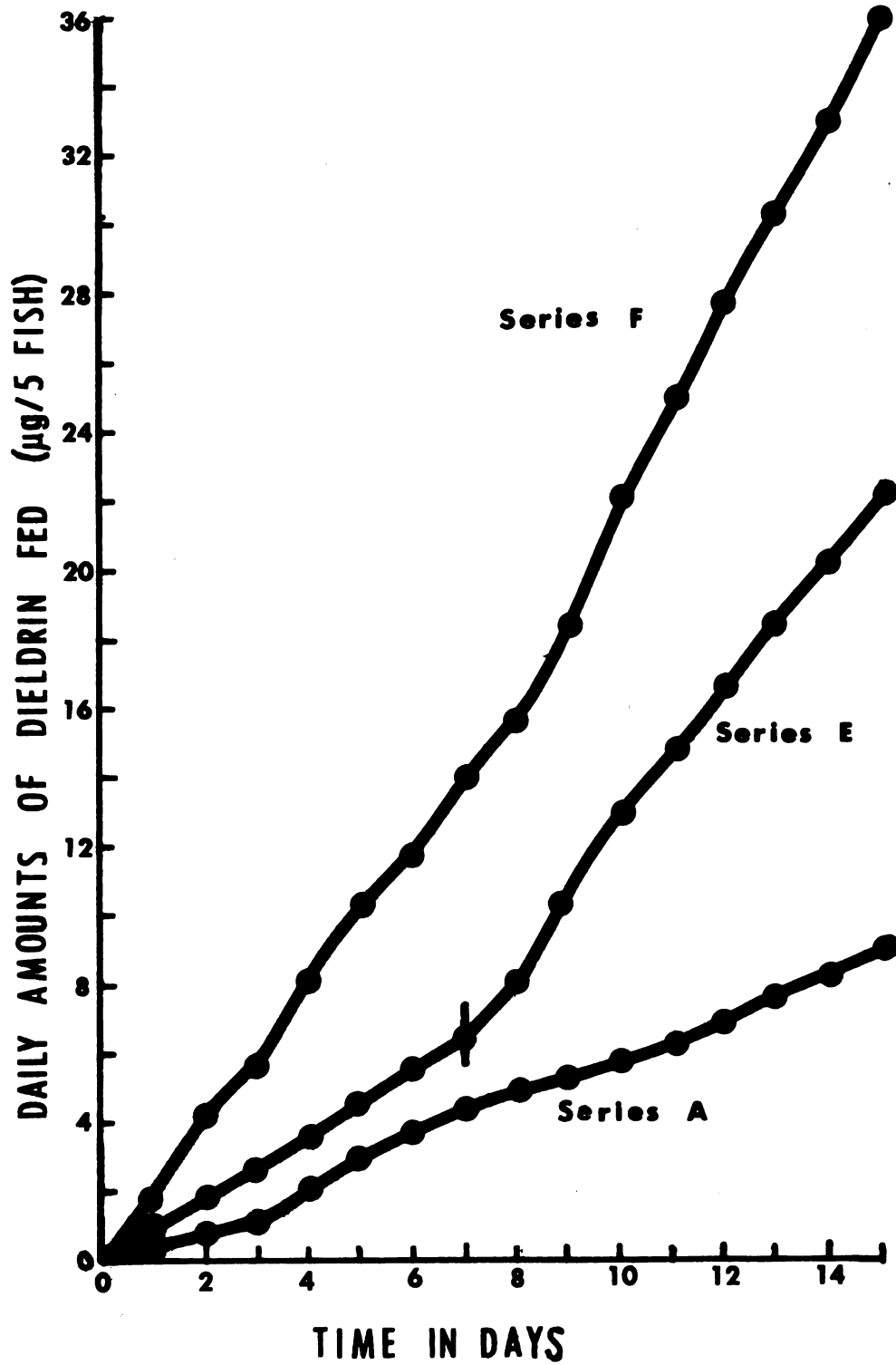
Water samples (1 L each) were taken from both series E and F on days 12 and 15 to check for contamination. The former series had no trace of dieldrin either days but the latter did show a trace, not

TABLE 13. Daily Amounts of Daphnia Fed Utilizing Three Feeding Rates

Days	Daily Amounts of Daphnia Fed (g Wet Weight)		
	Series A	Series E	Series F
1	0.2422	0.4695	0.9887
2	0.1676	0.5610	1.2312
3	0.1648	0.4373	0.7760
4	0.6338	0.4240	1.4482
5	0.3632	0.6203	1.1692
6	0.5015	0.5404	0.7442
7	0.3498	0.3552	0.7351
8	0.2548	1.0040	1.3103
9	0.1753	1.0759	1.4760
10	0.2575	1.4746	2.0746
11	0.2697	1.0007	1.5033
12	0.3262	1.0018	1.5022
13	0.4215	1.0026	1.5085
14	0.2976	1.0026	1.5041
15	0.4133	1.0075	1.5078
Average	0.323 g/day	0.798 g/day	1.299 g/day

Figure 7. Rates of Dieldrin Feeding to Minnows (based on the average dieldrin concentration of daphnia equal to 1.89 ug/g). In Series A (1 feeding/day) the calculated average rate is 0.60 ug/day/5 fish; in Series E (2 feedings/day) the first calculated average rate is 0.90 ug/day/5 fish, and the second rate is 1.98 ug/day/5 fish; and in Series F (3 feedings/day) the calculated average rate is 2.40 ug/day/5 fish.

FIGURE 7

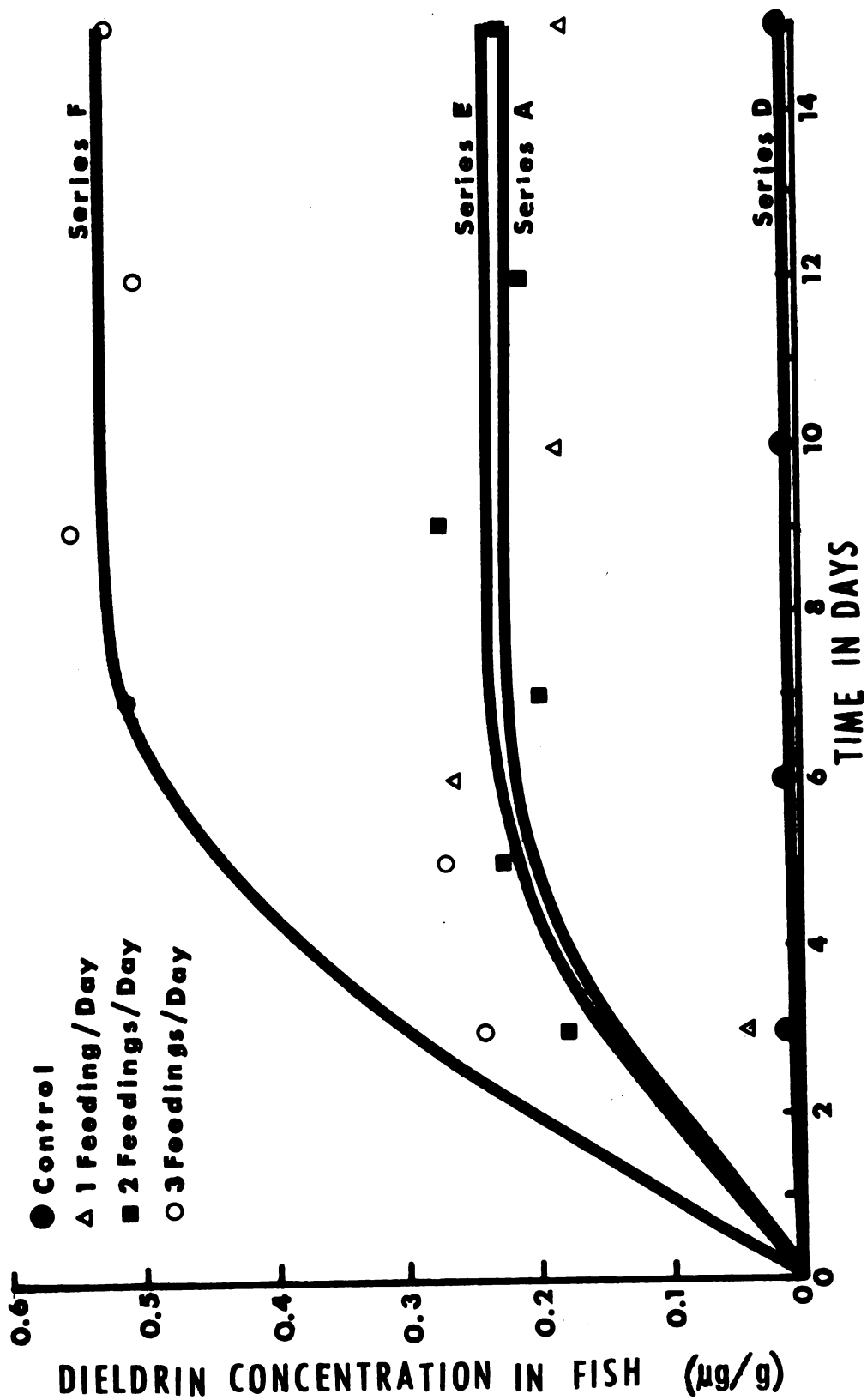


quite measurable, for both dates. Fish receiving two feedings a day appeared to be capable of consuming all the daphnia without much delay. Those receiving daphnia three times a day were pushed somewhat beyond their capacity and not all the daphnia would be consumed immediately, allowing for some contamination of the water. Also, feeding so heavily, these fish must have been excreting considerable amounts of dieldrin making contamination even more likely. The filters in all the tanks were changed midway during the experiment. They were apparently effective in preventing contamination in series E but not completely in series F.

Results of the experiment are inconclusive as shown in Figure 8. The rates of uptake and equilibrium levels are not significantly different for series A and E. This is especially true for the first seven days as would be expected from the nearly identical feeding rates (Table 13 and Figure 7). However, the final equilibrium level would be expected to be greater in series E as a result of a much greater feeding rate in the last eight days. Since this was not really true, two explanations are put forth. First, increased food (and dieldrin) consumption does not necessarily result in significantly increased storage of dieldrin. Second, due to the fact that the fish were receiving this increased amount of treated food in two separate feedings a day instead of one, the actual amount of dieldrin in the gastro-intestinal tract at any one time was not much different in the two situations. Therefore, the actual absorption from the intestine was no greater, and the storage level did not increase. I would tend to favor the latter explanation.

Figure 8. Food Chain Uptake With Three Feeding Rates. The mean equilibrium levels are as follows: 0.0 $\mu\text{g/g}$ for Series D, 0.22 $\mu\text{g/g}$ for Series A, 0.24 $\mu\text{g/g}$ for Series E, and 0.53 $\mu\text{g/g}$ for Series F.

FIGURE 8

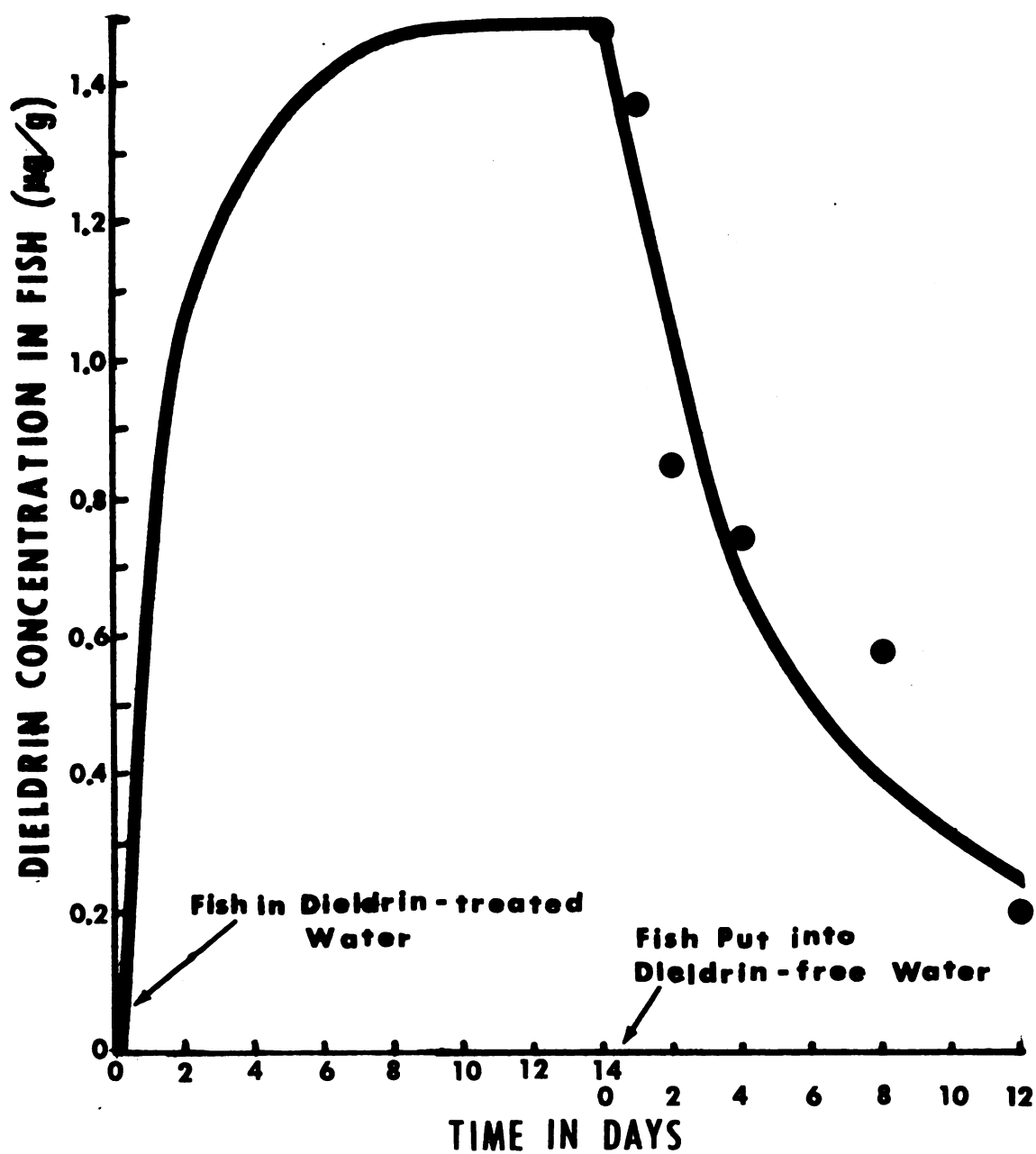


Forcing the minnows to their daily maximum consumption (series F) appears to have given a significantly higher equilibrium level. However, care must be taken in evaluating this result since there was a small amount of dieldrin present in the water. From what was observed in the previous experiment, a small amount of dieldrin in the water can result in considerable storage and this is very rapid. So, perhaps the major difference in the equilibria of series E and F is the result of limited direct uptake. Unfortunately, these results are inconclusive. Note, however, that the average equilibrium level for series F is $0.53 \mu\text{g/g}$ and that this is still considerably short of that for direct uptake alone (series B of Figure 6), $1.30 \mu\text{g/g}$. So, even if this equilibrium could be explained entirely on the basis of food chain uptake, the maximum consumption still would not approach the storage level possible by direct uptake.

In evaluating these data with some subjectivity, I personally feel that the curve for series F reaches too high a level to be due to the probable small amount of direct uptake, but suspect that even in the absence of direct uptake it would be somewhat higher than that for series E. If this is true then it would be correct to say that the amount of food ingested dieldrin does determine, through a direct relationship, the level of dieldrin storage at equilibrium. However, this is probably not a direct proportion, that is, doubling the amount of food consumed does not double the equilibrium level obtained. More work is required to clarify this matter.

Figure 9. Dieldrin Elimination Curve.

FIGURE 9



in the body concentrations of fish, as shown with bluntnose minnows.

GENERAL DISCUSSION

My results indicate that the mechanism involving direct uptake of dieldrin, presumably through the gills as suggested by Holden (1965) and Ferguson et al. (1966), is the major one determining the storage level in fish at equilibrium. This agrees with results of Ferguson et al. (1966) using endrin where they noted that the gills seem to be the primary pathway into the mosquitofish. Likewise, Reinert (1967, unpublished) working concurrently and independently at the University of Michigan, indicates he found almost identical results using dieldrin in direct uptake and food chain studies with algae, daphnia, and guppies. Different equilibrium levels are established for direct uptake and food chain uptake with the former always being much higher. When the two mechanisms are operating together and interacting as they are under natural conditions, the food chain contributes little or nothing to this storage level as long as sublethal levels are present and feeding activity is relatively constant.

It must be remembered that all of my studies deal with long term relationships using sublethal levels of dieldrin. Obviously, short term studies and toxic dosages present a different situation leading to rather sudden mortality. Mount (1967) stated that "many investigations while termed chronic, are really too short and are only survival or growth studies for thirty days or more". However, he also noted earlier (Mount, 1962) that generally fish which survived 30 days were likely to survive much longer. Thus, I considered this time period to be sufficient for considering survival using sublethal levels of exposure. Also, because equilibria were reached

and maintained with the exposures and feeding rates of the experiment, survival for 30 days does appear to be adequate for long term studies of this nature, provided one is not interested in the effects on reproduction or behavioral activity more widely spaced in time.

Throughout my investigations, static water testing conditions have been employed. It is well known that under such conditions, the insecticide concentration decreases with time by adsorption to the glass and waste materials and by organism uptake (King, 1962), particularly when the volume of the test solution is small (Ferguson et al., 1966). Holden (1962) and Mount and Putnicki (1966) have criticized the validity of static water testing in favor of a continuous flow system with a constant insecticide concentration maintained. The results of the two different procedures are not always the same. However, Ferguson et al. (1966) argues in defense of static tests emphasizing that results are reproducible when test conditions are specified. Burdick (1967) states that static assays simulate most closely a single application of chemical or pesticide to a lake or pond, and with modifications, additional applications. In my long term study a cyclic fluctuation occurred in the concentration so that a mean may be representative and yet the fluctuations more closely approximate natural fluctuations.

Storage equilibrium levels were reached in about the same time in both food chain uptake and direct uptake, as well as in the interaction of the two. This was between 7 and 10 days and it did not seem to vary much, if any, with the amount of dieldrin containing food that was consumed. In the second experiment where daphnia were exposed to a lower initial concentration the equilibrium was obtained

in approximately 11 days. Thus, with smaller dosages it may take slightly longer to reach this storage level, however, the variation is not very different in any case. Grzenda (1967) stated that equilibria were always obtained within two weeks with his goldfish, using C-¹⁴ labelled DDT and dieldrin. Although equilibria are reached at approximately the same time by the different mechanisms, direct uptake is represented by a much faster rate since it results in a higher level in the same time.

The direct uptake mechanism being most important and very rapid is consistent with field observations. Many investigators have reported mortality after a heavy spraying of a watershed and this always occurs in a relatively short time (a few days) after the treatment. Hoffman and Surber (1948) observed mortality of several fish species three to seven days after a 1 lb/acre aerial application using 50% wettable DDT powder. Using oil spray, mortality reached a peak within two days. Hence, it is apparent that the fish kills occurred soon after the treatment of the watershed and the actual time was dependent on the ease with which the insecticide entered the aquatic ecosystem. Bridges (1961) also noted the short time required for mortality to begin. Within the four days after beets were treated with endrin, substantial destruction to fish in a pond occurred. My results would suggest that if these food chains behave similarly with the various related insecticides that fish kills could not occur in these short time intervals if only food chain uptake was involved. However, direct uptake, and particularly the interaction of the two mechanisms might account very nicely for these mortalities following treatments. This tends to confirm the importance

of direct uptake. It would appear that after the peak of mortality the insecticide concentration in the water is reduced considerably to sublethal levels and the fish then remain at equilibrium with this concentration. Continued feeding is very unlikely to result in further build-up and eventual death of species in this type of food web. Occasionally, a second, or even a third smaller fish kill may result, but these will generally be following heavy rainfalls and runoffs carrying additional large loads of insecticides into the aquatic system.

Reinert (1967, unpublished), going one step further than I and exposing guppies to different concentrations of dieldrin, found that the concentration at equilibrium was directly proportional to the amount in the water. He also indicated that this was not true with food chain uptake, which agrees with my results. As I have indicated there seems to be an increase in the storage level with increased food and dieldrin but that this is not directly proportional. There is apparently a limit to how much dieldrin can be absorbed through the intestine, and forcing more food through or providing a greater concentration in the food does not seem to make a great difference. Instead, more appears to move through the intestinal tract without being absorbed. Grzenda (1967) noted this also, using C-14 labelled DDT and dieldrin. He found the feces to contain very high radioactivity and suggested that most insecticide taken in with the food passes straight through the gastro-intestinal tract.

I should suggest again, and this is pure speculation on the basis of only one sample, that perhaps the food chain could become important in an aquatic environment when it interacts with the direct

uptake mechanism. Rapid direct uptake unquestionably accounts for the quick high storage levels in fish. These high but sublethal levels might be elevated to lethal concentrations by an unusually high ingestion of pesticide laden prey such as could occur when the pesticide reduces escape success of the prey species. Mortality probably also results from direct uptake alone where the water concentration locally is very high, particularly if the relationship is a direct proportion. However, it seems quite unlikely from my results that the food chain alone could account directly for much mortality, in the type of aquatic food web represented by my species. This emphasizes again the extreme vulnerability of organisms living within the aquatic ecosystem, directly exposed, as opposed to a terrestrial system in which the food chain appears to be a more major avenue of pesticide buildup. A comparative study of aquatic and terrestrial food webs would be especially worthwhile.

My dieldrin elimination study demonstrated that fish which have a high storage equilibrium established, as by direct uptake, can largely rid themselves of this dieldrin rapidly when placed in clean water. As the concentration is reduced in the fish the rate of elimination slows down so that at least a couple weeks are required to return to near zero levels. Initially the amount stored is reduced very rapidly from high levels, as rapidly as it was taken up. Studies reported by Gakstatter and Weiss (1967) very closely parallel my data. Working with direct exposures of bluegills and goldfish to C-¹⁴ labelled DDT, dieldrin and lindane, they found that more than 90% of the dieldrin was eliminated in the first two weeks. The relationship was nearly identical to mine. However,

there appeared to be considerable variation among the different chlorinated hydrocarbons. Lindane was almost completely eliminated with two days, while less than 50% of the DDT was eliminated after 32 days. In the case of insecticides such as lindane and dieldrin, storage concentrations will closely reflect daily water concentration levels. This could only be true where a rapid method of uptake (e.g., gills) occurs and an equally rapid elimination is possible, which may also be at the gills.

As was found from the field samples, plants and animals accumulate and store dieldrin at levels one to several thousand fold that in the water. Also, as cited previously, many other investigators have discovered similar increases in the storage of insecticide in animals over that in the physical environment. This has led to the concept of biological magnification, which generally further suggests that the concentrations are increased with each higher trophic level. Sublethal levels become lethal to one or more species in the food chain as the result of ingestion of the toxic ingredient that was accumulated by the more resistant organisms in the food chain. Perhaps this is true in terrestrial systems and in certain situations involving aquatic food chains. However, under the conditions of my studies there is reason to question this interpretation of biological magnification for aquatic food webs such as mine. The fact that there is magnification of concentrations from the environment to the organisms cannot be disputed. But that the concentration will necessarily be greater at each higher trophic level and the result of contaminated food alone does not appear to be justified. Since in an aquatic ecosystem the entire medium, water, is contaminated and

since aquatic organisms must expose membranes to a great volume of contaminated medium in order to take up oxygen and release carbon dioxide the direct uptake is the most important mechanism in the aquatic ecosystem. It has been suggested that there is a direct proportional relationship between storage and water concentrations with this mechanism. Thus, it operates not only as an uptake but also as an elimination mechanism. Magnification by any trophic level merely represents the solubility differential between water and fat or oil of the animal tissues. This implies that individuals with the greatest fat content are capable of the greatest storage without adverse effects, and this has been noted and cited previously. Holden (1962) suggested that the high solubility of DDT in oil or fat probably accounts for the rapid direct uptake which he observed. Reinert (1967, unpublished) also indicated agreement with this idea and even suggests going further to include plants. The storage concentration at any trophic level in an aquatic system then would be essentially a function of the oil or fat content of it's organisms, the concentration in the water, and the solubility differential for the insecticide. The body burdens of the organisms go up as the water concentration or their fat contents go up and they would go down as these two variables go down. This still involves considerable speculation but seems reasonable from my data and those of others. More work along these lines is essential for a clearer understanding.

In conclusion, it was found that, following an extensive treatment program, dieldrin persisted in the streams of the area for as long as three years after treatment. The concentrations were not always very

great, especially in the water, but it was continually present. This indicates that conditions do exist for both mechanisms of uptake to operate following such a treatment. It is now apparent that when this is the case, direct uptake rapidly determines the storage concentration which exist for the fish at least, and perhaps many other organisms. Fluctuations in the water concentrations with runoffs, slow purging, and fixation will be reflected in the storage concentrations. Direct pesticide induced mortality probably occurs any time the water concentrations increase above a certain level, or perhaps when excessive feeding on pesticide contaminated food takes place in a water medium containing high but sublethal levels of the pesticide.

These laboratory and field studies have confirmed the results of others which indicate that aquatic organisms can concentrate chlorinated hydrocarbon pesticides from minute concentrations in the water to high levels in their tissues. Since lakes and streams continue to receive these materials from the terrestrial systems long after the pesticide is applied, the particular vulnerability of aquatic systems needs to be rigorously assessed as we work our way to responsible pest control strategies. The present study dealt only with quantifying pesticide levels in organisms occupying various trophic niches and in the water medium. No attempt was made to assess effects of sublethal loads on reproduction, vulnerability to predators, reduction of parasite loads or other indirect effects. It would be hazardous in the absence of such evidence to assume that lakes and other aquatic ecosystems could be permitted to reach pesticide contamination levels anywhere close to those at which direct uptake by its organisms

results in mortality. These studies have also confirmed others which question, at least for this kind of aquatic ecosystem, the food web route of pesticide concentration. This suggests we should rigorously test this pathway using other webs, aquatic, terrestrial, and combinations of these. The concentrations reported in some carnivorous birds would be difficult to account for by other routes, but it is important that postulated pathways be subjected to rigorous experimental corroboration.

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