DISTRIBUTION AND ELIMINATION OF DIELDRIN BY GREEN SUNFISH (LEPOMIS CYANELLUS, RAFINESQUE) FOLLOWING ADMINISTRATION OF A SINGLE ORAL DOSE

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This is to certify that the

thesis entitled

Distribution and elimination of Dieldrin by Green Sunfish (Lepomis cyanellus, Rafinesque) following administration of a single dose.

presented by

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ABSTRACT

DISTRIBUTION AND ELIMINATION OF DIELDRIN BY GREEN SUNFISH (LEPOMIS CYANELLUS, RAFINESQUE) FOLLOWING ADMINISTRATION OF A SINGLE ORAL DOSE

By

Willard Louis Gross

Green sunfish were administered a single oral dose of approximately 95 µg of dieldrin and placed in individual flow-through chambers (flow rate approximately 80 ml/min) for 3-day intervals over a 15-day period. Dosed fish, awaiting placement in chambers, were held in flow-through aquaria (flow rate 400 ml/min). Two fish in the chambers were cannulated to collect urine; feces were collected from the four remaining fish in the chambers; and the dieldrin content of water passing over these fish was monitored. At the end of a three-day interval, these fish were sacrificed and selected tissues and organs analyzed for dieldrin residue levels.

Green sunfish demonstrated an 86.6% efficiency in absorbing dieldrin from the digestive tract.

The distribution of dieldrin in tissues and organs analyzed remained constant with only the liver and gonads exhibiting considerable variability. A difference was noted in dieldrin residue levels in the liver of males

and females which was probably associated with ovarian development. Dieldrin levels generally declined in tissues and organs. However, the combined intestine and pyloric caeca samples demonstrated a slight increase in residue levels.

Dieldrin was lost from the fish at an exponential rate; the biological half-life for total dieldrin was 25.8 days. Rates of dieldrin loss from individual tissues and organs varied considerably, with storage tissues such as adipose tissue and ovary demonstrating long dieldrin half-lives.

Dieldrin was not detected in mucus and only small quantities were detected in urine late in the experiment suggesting that very little, if any, dieldrin was eliminated via these pathways. Essentially all of the insecticide was eliminated via the intestine (in feces) and from the gill. The gill was the most important pathway with a minimum of 55% of the dieldrin lost via this route.

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INTRODUCTION

The history of synthetic organic insecticides, particularly the chlorinated hydrocarbon group (organochlorine insecticides), spans a relatively short period of time (approximately 30 years). Yet, in this short period, their impact upon man and the environment has been of such magnitude that it has resulted in intensive research on these compounds. Volumes of literature exist regarding how these substances affect man and his environment.

Chlorinated hydrocarbon insecticides are noted for three properties: a broad spectrum of high toxicity, a long residual life, and an ability to penetrate biological tissue on contact. These properties are highly desirable in an insecticide; and these compounds, particularly DDT, appeared to be a panacea for insect control. Consequently, they were widely used. However, it was not long before scientists recognized that their widespread, indiscriminate use had resulted in a wide variety of ecological problems.

One of the problems attributable to these properties is the indiscriminate destruction of beneficial insects, frequently weakening this link of the food chain. Often, other desirable components of the ecosystem have been seriously depleted or obliterated. A second problem is the

accumulation of several of these compounds in the natural environment. Most of our soils contain detectable amounts of DDT and its metabolites; in agricultural areas, dieldrin is also found. The soils and sediments appear to act as storage reservoirs for these pesticides.

The presence of pesticides in water is also widespread. Although chlorinated hydrocarbon insecticides are not very water soluble, DDT and its analogs are found in water samples from all major river basins in the United States. Dieldrin was also detected from many of these basins (Weaver, et al., 1965). In addition to contamination of the soil and water substrata, their long residual life and high solubility in fats, oils, and waxes has resulted in the incorporation of several chlorinated hydrocarbons, notably DDT and dieldrin, in practically all living biological organisms including man. This accumulation in biological organisms has produced a magnification of insecticide concentrations through the food chain posing a threat to the survival of animals occupying the higher trophic levels.

Rachel Carson's book, Silent Spring, focused public attention on the perils of insecticide use and initiated intensive research on the effects of pesticides in ecological systems. In addition, interest was aroused in the long-term effects of these compounds at sublethal concentrations. A mass of scientific data, plus more recent economic losses from the confiscation of insecticide-contaminated foodstuffs, has awakened the public to the

hazards of these compounds and resulted in the banning of DDT in several countries, as well as in several states within the United States. The banning of other highly toxic, persistent insecticides, including dieldrin is being attempted. Even if the decision to ban the use of persistent chlorinated hydrocarbon insecticides is accepted, we are still faced with the problem of accrued concentrations of pesticides--notably DDT, its metabolites, and dieldrin--in the environment. A recent article in Time Magazine (Anonymous, 1969) stated, "Some scientists estimate as much as two-thirds of the 1.5 million tons of DDT [and its metabolites] produced by man may still be adrift [in the environment]." Therefore, we should not abandon our research endeavors, but continue to probe the effects these compounds have on the natural environment, particularly the biota.

An important part of gaining a thorough understanding of the effects of insecticides involves a knowledge of their dynamics in living organisms. This includes knowledge of amounts and rates of insecticide uptake, distribution and translocation to tissues, metabolism, and elimination. The uptake of various insecticides from water by fish has been studied, and knowledge of the distribution of various insecticides in fish tissues also exists. However, relatively few studies have been concerned with the process of insecticide elimination in fish. The

knowledge that most chlorinated hydrocarbon insecticides accumulate and are stored in biological tissue for varying periods of time appears to occlude the concept of actual excretion of these compounds. A review of our present knowledge regarding the uptake, tissue distribution, and elimination of organochlorine compounds by fish follows.

Uptake of Insecticides by Fish

Insecticides may be taken up by fish directly from the water via the gills, by absorption across the skin, or across the gut from contaminated food. Large differences in DDT concentrations in the skin and mucus of brown trout as compared to water, led Holden (1962) to conclude that DDT may be absorbed across the integument. Similar conclusions were reached by Premas and Anderson (1963) in studies of DDT uptake by Atlantic salmon and also by Ferguson and his associates (1966) in studies of endrin uptake by mosquitofish. However, all three studies concluded that the primary route of uptake is via the gill rather than the integument. In fact, Premas and Anderson (1963) found appreciable quantities in salmon tissues after only five minutes of exposure which could only be accounted for by absorption across the gills. Recently, Fromm and Hunter (1969) demonstrated that dieldrin can be transferred from environmental water to the vascular system of isolated perfused gills of rainbow trout.

Relatively little information is available regarding uptake across the fish gut. However, Mount (1962) did observe high endrin levels in the digestive tract and low levels in the gills of bluntnose minnows. On the basis of these data, he concluded that endrin enters the body through the intestine as a result of the fish's drinking water. His work, however, has been largely disputed and the gill is now accepted as the primary route of uptake. Yet, the digestive tract should not be discounted as a site of absorption as evidenced by the food-chain concentration studies observed in aquatic systems (Hunt and Bischoff, 1960; Hickey, et al., 1966). Both routes are undoubtedly important, with the gut increasing in importance with the presence of contaminated food (Johnson, 1968).

A few studies have compared uptake via the gill and the intestine; Lenon (1968) compared whole body concentrations in bluntnose minnows exposed to dieldrin in water with groups fed dieldrin-contaminated daphnia, and noted higher body concentrations in fish exposed to dieldrin in water. Reinert (personal communication, 1968) reached a similar conclusion working with a daphnia-guppy system. Allison, et al. (1963) conducted chronic toxicity studies of DDT in cutthroat trout by exposing some lots of fish to DDT in water and periodically feeding DDT contaminated food to different lots. They drew no conclusions regarding

differences in body concentrations except to note that body concentrations increased more rapidly in fish fed DDT in the diet.

Insecticide Distribution in Fish Tissues

Insecticide residues in fish tissue vary widely from organ to organ and even within the same organ or tissue. This variability persists even when tissues from different studies are simply ranked on an ordinal scale and compared in this manner. A few generalizations, however, may be noted: adipose tissue invariably contains the highest insecticide concentrations in studies which examined this tissue, while muscle and/or blood usually contains the lowest concentrations. Holden (1962) observed intermediate concentrations in the muscle of brown trout indicating that dieldrin concentrations in this tissue may vary with its fat content.

Fish liver was found to contain considerable concentrations after exposure to several different insecticides (Premas and Anderson, 1963; Bridges, et al., 1963; Holden, 1962; Allison, et al., 1963; and Gakstatter, 1966). Only Cope (1960) failed to observe DDT or any of its metabolites in the liver of fish from the Yellowstone River after contamination from a spraying project. Gakstatter (1966) included analysis of the gall bladder and observed high concentrations in this tissue as well. His results were

highly variable, but demonstrated a gradual increase in concentration during a three-day recovery from exposure to a sublethal concentration of dieldrin.

The digestive tract also accumulates relatively large amounts of chlorinated hydrocarbon insecticides, although studies of various portions of the gut provide conflicting results. Holden (1962) observed higher concentrations in the stomach than either the pyloric caecae or the intestine of brown trout exposed to lethal concentrations of DDT in water. Gakstatter (1966), working with DDT and dieldrin, found the reverse situation--low concentrations in the stomach and higher in the other two regions.

Most other body tissues contain intermediate, but variable concentrations of insecticides. Holden (1962) indicates that residues in such tissues as spleen, kidney, heart and, to some extent, gill, can be attributed to the blood volume of these organs. Tissues such as the brain and gonad appear to have intermediate to high insecticide concentrations with results reported in the literature being quite variable.

Brain dieldrin concentrations appear highly dependent upon degree of exposure and the amount of time elapsed after exposure. Gakstatter (1966) observed rapid declines in dieldren and DDT concentrations in brain tissue after exposure to a sublethal level. Concentrations of insecticide in the gonads appear dependent upon the maturity of

the fish and the seasonal condition of the gonads. In most studies, the ovaries of mature females acculumate large amounts of insecticide. Variable levels of dieldrin have been reported in testes; some studies report low concentrations, whereas others report very high concentrations. The reason for these conflicting results for the testes is apparently unknown.

Many factors influence pesticide concentrations in fish tissue. Some of these factors are size, sex, age and condition of the fish. Also, the concentration to which fish are exposed, the length of time that they are exposed, and the time lapse between exposure and sampling appear to influence concentrations in tissues. Consequently, only general statements as cited above are possible.

Insecticide Elimination by Fish

Although excretion of pesticides has been noted in mammals for some time, very little research has been conducted on elimination of these compounds by fish. Several references to possible elimination of insecticides by fish can be found in the literature and were the primary motivation for initiation of this project.

Recently, however, Ferguson, et al., (1966) provided definitive proof of insecticide excretion by fish in his studies on the resistance mechanisms of mosquitofish to endrin. One of their experiments involved placing a resistant female mosquitofish (previously exposed to

1,000 ppb endrin solution for 36 hours and rinsed four times) in 10 liters of tap water with five mosquitofish of a susceptible strain. Within 38.5 hours all susceptible fish had died. They did not measure the concentration of the water, but based upon bioassay tests of the susceptible strain, the endrin concentration in the water was approximately 2 to 3 ppb. This means that the resistant female had to excrete 20 to 30 micrograms of endrin in the 38.5 hours. Additional studies by this group demonstrated that both live and dead fish, exposed to varying concentrations of endrin for 7.5 hours, eliminated sufficient quantities of endrin in two liters of tap water in 8.25 hours to kill susceptible fish when they were placed in the jars of contaminated water after the exposed fish had been removed.

After initiation of the present research project, a study of the elimination of several chlorinated hydrocarbon insecticides by fish was reported by Gakstatter and Weiss (1967). Their studies with labeled DDT, dieldrin, and lindane showed that lindane was rapidly eliminated from goldfish and bluegills within two days and that 90% of the initial concentration of dieldrin was eliminated in the first two weeks of recovery. However, less than 50% of the labeled DDT was eliminated during the 32-day recovery period. The authors observed that both uptake and elimination of these insecticides were related to their water solubility, i.e., the least soluble compound (DDT) being

eliminated at the slowest rate. Recently Gakstatter (1968) obtained similar evidence on elimination of the aldrin-dieldrin complex from goldfish.

Although the above references afford evidence that chlorinated hydrocarbon insecticides are eliminated by fish, no one has determined the pathway or route of elimination. Most of the published information regarding excretion routes of various insecticides and their metabolites deals with mammals. This literature has been reviewed by O'Brien (1967) and Menzie (1967). In mammals, chlorinated hydrocarbon insecticides are primarily eliminated in feces with lesser amounts lost in urine. The findings are quite variable and, in a few instances contradictory. Whether different routes exist for different insecticides could not be ascertained from the literature.

One proposed pathway for dieldrin elimination in rats (Heath and Vandekar, 1964) involves removal from blood by the liver and transfer to the gall bladder, from there it is transported to the intestine in the bile and eventually eliminated in the feces. Gakstatter (1966) proposed a similar mechanism for elimination of DDT, dieldrin, and lindane in goldfish and bluegills based upon insecticide concentrations observed in the stomach, pyloric caeca, and intestine. Other investigators have observed comparable concentrations of different insecticides in the fish gut and support this proposed route of elimination by fish.

Other elimination pathways in fish include the kidney, the skin, and lastly, the branchial region. Mount (1962) observed high endrin concentrations in the kidney of bluntnose minnows and suggested that endrin might be eliminated via this organ. However, Weiss (1967) has also observed high levels of dieldrin in the kidney and believed this might indicate an inability to excrete dieldrin. Up to this time, no one has analyzed fish urine to resolve this matter.

No individuals have considered the integument or gill as possible elimination routes. However insecticides could feasibly be eliminated from the skin with the mucus from mucus-secreting glands as the mucus is sloughed from the fish. The gill is also a plausible excretory route since it is known to function as a secondary excretory organ in the elimination of various ionic compounds and nitrogenous wastes (Prosser and Brown, 1961). As mentioned previously, the gill is the primary route of insecticide uptake when fish are exposed to insecticide-contaminated water. Although the chlorinated hydrocarbon insecticides are relatively insoluble in water, the large surface area of the gill plus the close proximity of the blood to the external medium could permit a passive transport across the gill down a concentration gradient. The continuous exchange of water at the gill surface would aid in maintaining such a concentration gradient.

No studies of insecticide elimination by fish have included all possible elimination pathways, or the analysis of excretion products. Except for Gakstatter's estimates of the time necessary for elimination of DDT, dieldrin, and lindane, no information is available on the dynamics of insecticide elimination in fish.

The present research was undertaken to conduct a detailed study of the elimination of dieldrin in green sunfish. Dieldrin elimination was observed from several viewpoints: analysis of excretory products or the receiving medium (in the case of gill), analysis of organs and tissues associated with possible elimination pathways, and lastly, an analysis based on actual body losses of administered dieldrin. Oral administration of dieldrin provided information on uptake efficiency of dieldrin from the fish gut.

METHODOLOGY

Experimental Animals and Apparatus

The green sunfish (Lepomis cyanellus, Rafinesque) utilized for this study were obtained from the Michigan State University Lake City Experiment Station. Their size range was 10.3 to 17.2 cm in total length (average 14.7 cm) and 20 to 106 g in weight (average 65.9 g). Fish used for different phases of the research varied in average size. Smaller fish (average length 10.0 cm and average weight 38.78 g) were utilized as unexposed aquarium controls, and the larger fish (average length 16.2 cm and average weight 83.4 g) were selected for cannulation to maximize urine collection. The treated fish and their controls which were employed for the main part of the study averaged 14.6 cm in total length and 64.57 g in weight. Analyses of the fish prior to use revealed only small amounts of DDT and its metabolites, particularly DDE. Dieldrin was not detected in any fish.

In pilot studies, dieldrin was administered by dissolving it in corn oil and placing 0.05 or 0.1 milliliters in a gelatin capsule which was then force fed to the fish. This procedure was found to be unsatisfactory because the fish could not assimilate this volume of oil and some of it

passed through the digestive tract to contaminate the water. Therefore, subsequent dieldrin administration involved force feeding the fish a dieldrin-treated dry food pellet. The treated pellets were prepared by applying five micro-liters of a dieldrin/acetone solution to the pellet surface with a 10 microliter syringe. The dieldrin (Shell Chemical Corporation--99% pure) was obtained from Dr. R. E. Monroe of the Entomology Department, Michigan State University. The intended dosage was 100 micrograms per pellet, but analysis of the treated pellets indicated an average dose per pellet of 94.93 ± 1.37 micrograms of dieldrin. Therefore, the average dose received by a fish was 1.46 mg/kg (based on the average weight of the fish). This value is well within dosages reported as sublethal for fish in other studies (Macek, 1968).

The experimental design (Figure 1) was to dose all fish at one time, and then divide them into groups which were placed in the experimental chambers for successive three-day periods. Each three-day group of fish consisted of two treated fish which were cannulated for urine collection, another three treated fish for studying other routes of elimination, and one treated fish fed a pellet dosed with acetone to serve as a control (referred to as chamber control). Thus, data regarding elimination was obtained for a 15-day experimental period from five different groups of fish placed in the experimental chambers

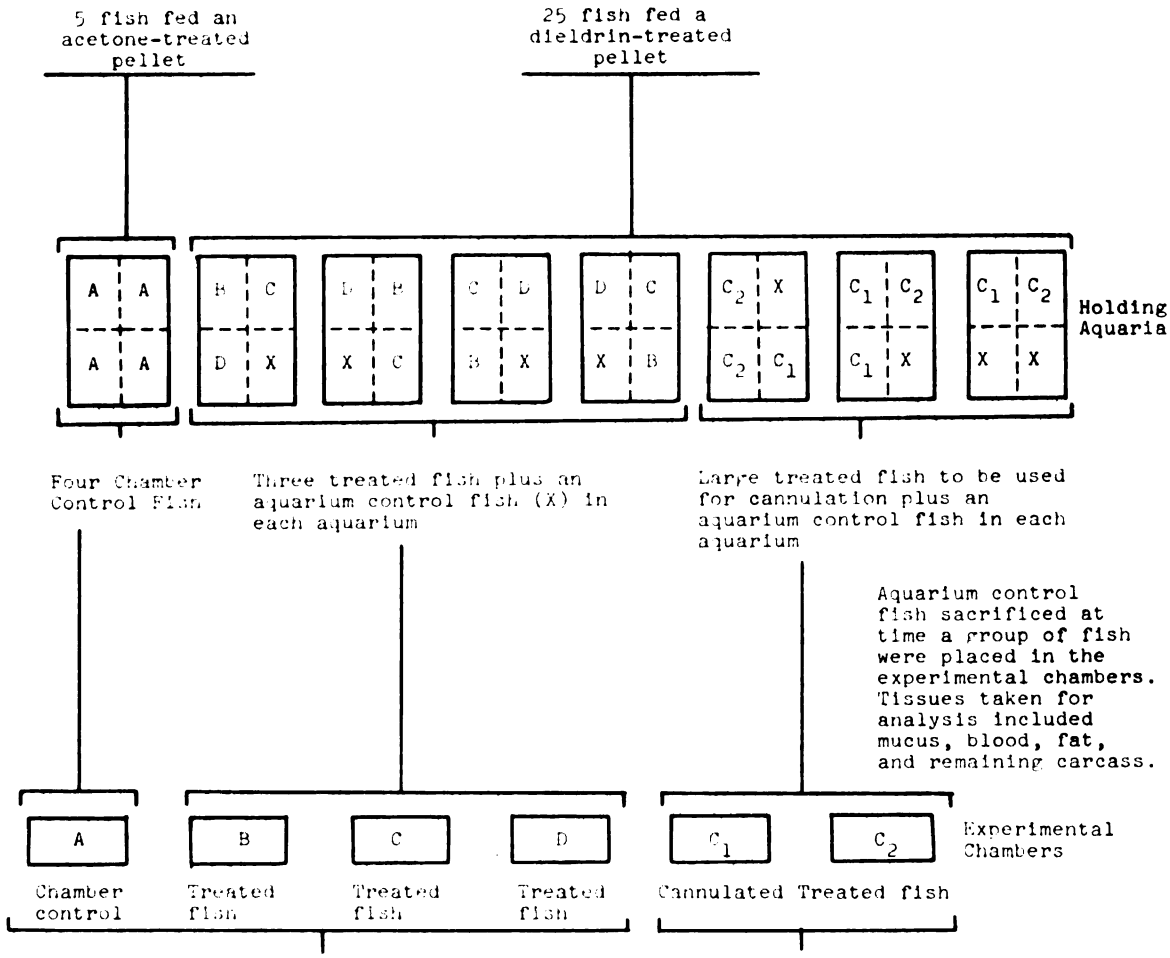


Figure 1.--Diagram depicting the general design of the experiment.

for successive three-day periods. A total of thirty experimental fish was employed.

After dosing the fish, they were assigned to individual compartments of 20-gallon aquaria having a flow rate of approximately 400 ml/min. The fish to be cannulated and the acetone-treated controls for the experimental chambers were placed in pre-selected aquaria. The other dieldrin-treated fish were randomly assigned to any of three compartments in each of the remaining aquaria. In each of these latter aquaria, a fourth compartment contained a small untreated fish (referred to as aquarium control fish) to serve as a monitor of resorption of excreted dieldrin while the exposed fish were held in the aquaria. Whenever a particular aquarium of treated fish was selected for placement in the experimental chambers, this untreated fish was sacrificed and tissues preserved for residue analysis.

Not all treated fish of a three-day group were cannulated for urine collection since previous cannulation attempts always imposed a stress condition which impaired the fish's ability to survive in the chambers. Therefore, data on possible dieldrin excretion via the kidney was obtained from two previously selected, large sunfish placed in experimental chambers on the same three-day schedule as other fish.

The only mortality experienced during the experiment was among the cannulated fish. A fish from the third sample

period (7-9 days) died of anoxia on the eighth day when the water siphon to the experimental chamber air-locked and stopped the flow. A fish in the fifth sample period was in very poor condition at the time it was sacrificed.

The experimental apparatus is shown in Figure 2. A 150-gallon continuous flow reservoir served as a common water source for both the experimental chambers and the holding aquaria. Water from the reservoir was siphoned to the holding aquaria and the flow regulated by a nylon valve. Water to the experimental chambers was pumped from the reservoir to a constant head tank from which it was siphoned to the chambers. The water supply was university tap water which was passed through a glass wool filter to trap iron oxide and other particulate matter. In preliminary studies, a charcoal filter was employed but was found to be unsatisfactory because during its use, unknown compounds were added to the water which caused interference on the chromatographic traces for water samples. The water temperature was maintained at 20° ± 0.5° C. with use of a refrigeration and mixing unit.

The experimental chambers were glass cylinders 20.3 cm long and 5.7 cm in diameter, closed at each end with rubber stoppers fitted with glass ports. In the posterior half of the chamber, glass tubing was glued to the inside to maintain the fish in an upright position. The experimental chamber emptied into the bottom of a 4-oz. food jar employed

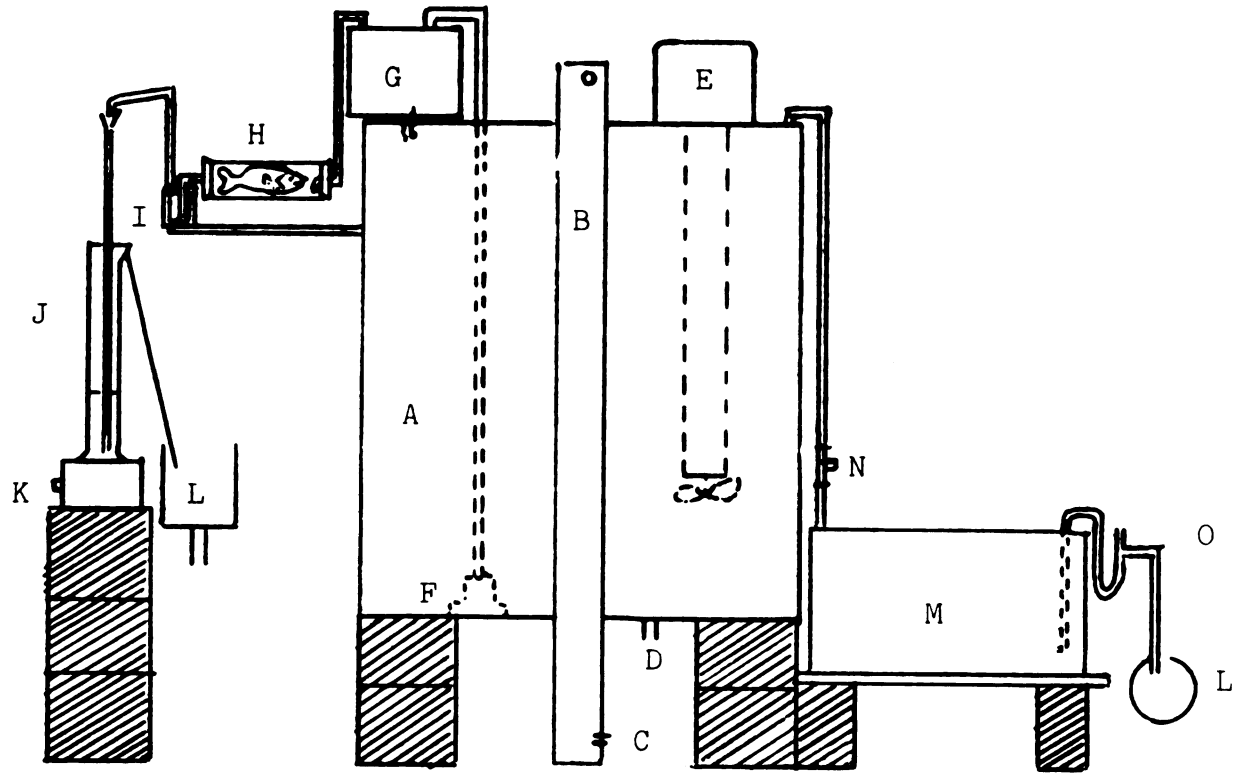


Figure 2.--Diagram of the experimental apparatus.

A.	Reservoir	F.	Submersible Pump	K.	Mag-mix
B.	Filter	G.	Constant Head Tank	L.	Water Drain
C.	Reservoir Inlet	H.	Experimental Chamber	M.	Holding Aquarium
D.	Reservoir Outlet	I.	Feces Trap	N.	Inlet Flow Regulator
E.	Refrigeration Unit	J.	Graduate Cylinder	O.	Outlet Flow Regulator

as a feces trap. The outlet from the trap was a U-shaped glass tube which conducted the surface water to the water extraction apparatus, but allowed the feces to remain in the bottom of the trap.

The flow rate through the chamber and trap was regulated by the height and degree of bend in this outlet tube. The flow rate varied slightly between chambers, but was never less than 77 ml/min nor more than 85 ml/min. Flow rates were measured daily and adjusted as close to 80 ml/min as possible. Daily differences in rate never exceeded 5 ml/min and the maximum differences between chambers was only 3 ml/min over the three-day period any one group was in the chambers. Each experimental chamber had a volume of 355 ml without a fish; so that, at an average flow rate of 80 ml/min, a chamber had a maximum turnover time of 4.4 minutes or turned over a minimum of 328 times each 24 hours. The experimental chambers utilized for cannulation were modified by placing a 22 gauge syringe needle through the rubber stopper so that the cannula could be conducted to the exterior and the urine collected in graduated centrifuge tubes. Except for the rubber stoppers, the entire system, from chamber through the water extraction apparatus was constructed of glass.

Collection Procedures

Dieldrin in the water passing over the fish was measured daily. Urine samples were also collected daily unless the volume was insufficient, in which case, two-day samples were collected. Fecal material was allowed to accumulate in the trap until the fish were removed. At the end of a three-day period, the fish were anesthetized, removed from the chambers, measured and weighed, and various tissues and organs removed for residue analysis.

All visceral fat and connective tissue around the gastro-intestinal tract was removed. The fat about the intestine, pyloric caeca and in the omentum was saved for residue analysis. In addition, the stomach and intestine were opened and the contents (mainly a yellow viscous fluid) discarded. Gill samples consisted of the entire branchial apparatus (including the gill arches) from one side of the fish. Muscle samples were taken from the expaxial or hypaxial masses on the right side of the fish, and blood samples were obtained from the caudal artery by severing the caudal peduncle. The gall bladder was removed intact so that analysis of this organ included the bladder plus bile. All samples taken were placed in tared screw-capped vials, reweighed to obtain tissue weights, sealed, and stored at -10° C. The remaining fish carcasses were individually weighed, placed in plastic bags which were sealed to exclude air, and then stored at -10° C. Later

the carcasses were homogenized with water and aliquots taken for residue analysis.

Cannulated fish were also sacrificed at the end of a three-day period and the various tissues and organs removed for analysis. Feces and water samples were not collected for these fish.

The cannula consisted of No. 20 polyethylene tubing heat-sealed at one end to form a smooth rounded surface, with numerous small openings made about the end with a heated needle. About 0.6 cm from the sealed end, below the openings, a small flange was formed around the tubing with a medical adhesive. The cannula was inserted into the urogenital opening, and sutured to the abdominal wall with surgical thread. The adhesive flange formed an excellent area for attaching the thread to the cannula, permitted a tight fit about the urogenital opening, and lastly, prevented further insertion of the cannula during movements of the fish in the chamber, thereby avoiding internal injuries. This type of cannula appeared to have several advantages over the flared-end type: easier insertion, less apparent irritation, no tendency to become blocked, and lastly, facilitated collection of increased urine volumes.

A continuous extraction apparatus was designed for monitoring the dieldrin content of the water passing over the fish in the experimental chamber. Water from the trap outlet (Figure 2) was conducted to the bottom of a 500-ml

graduated cylinder containing 150 ml of carbon tetrachloride. The graduate was positioned on a Magmix and thus, the water was continually mixed with the carbon tetrachloride. Since carbon tetrachloride is denser than water, it remained in the bottom of the cylinder while the water moved to the top and spilled over into a drain.

Extraction efficiency was not especially high. Six 4-hour and two 24-hour tests, utilizing two different dieldrin concentrations (0.1 and 0.5 micrograms/liter), had an average extraction efficiency of 46.82 ± 5.27%. Differences in extraction efficiency between length of tests or between dieldrin concentrations were not significantly different. In calculating the extraction efficiency, the data had to be corrected for carbon tetrachloride losses due to its slight solubility in water (average loss per 24 hours was 25 ± 4.2 ml). Although the efficiency was low and quite variable, the system appeared the best method in view of the volume of water utilized over a 24-hour period (average 114.6 liters/day; range 110-112 liters).

Analytical Procedures

Different methods of extraction and cleanup for recovery of dieldrin had to be employed for the different types of samples; details of the specific procedures used are given in Appendix A. All dieldrin concentrations reported are corrected for extraction efficiencies. All reagents were redistilled before used and some were refined,

if necessary, according to methods described by Hamelink (1969).

Provided all reagents were clean, good chromatographic traces for quantitating the dieldrin in water were obtained by evaporation of the carbon tetrachloride and transfer of the dieldrin to petroleum ether. (Overall recovery efficiency for the continuous water extraction procedure was 46.82 ± 5.27%.)

General methods described for extraction and cleanup of mammalian excretory products were not applicable to the small samples obtained from fish. A suitable extraction procedure was designed for urine which involved a 1:1 dilution with distilled water and extraction with petroleum ether. Chromatographic traces of samples extracted in this manner showed few extraneous peaks and no interference, so that additional cleanup steps were unnecessary. Efficiency tests, employing addition of dieldrin in acetone to the samples and evaporation of the acetone under partial vacuum, demonstrated an average recovery of 82.18 ± 7.95%.

Extraction and cleanup of fecal material was particularly difficult. The technique developed involved extraction of dried material with acetonitrile, partitioning the acetonitrile with a small amount of petroleum ether as a cleanup step, and finally, partitioning diluted acetonitrile with petroleum ether. The method was not entirely satisfactory as chromatographic traces frequently

contained artifacts and at times, the dieldrin in a sample was eluted above a broad sloping shoulder of some unknown compound. Additional cleanup steps such as saponification, adsorption with Nu-Char Attaclay, or elution from a micro-Florisil column did not improve the traces and always resulted in decreased recovery efficiencies. Although the chromatographic traces were not "perfect," sufficient resolution existed for quantifying the samples. Recovery efficiency averaged 45.82 ± 2.48%.

Dieldrin was extracted from fish tissue by a modification of the alcoholic-KOH procedure described by Schafer, et al. (1963). Twelve efficiency tests, involving addition of dieldrin in acetone to alcoholic-KOH solutions containing at least one of each of the tissues analyzed, gave an average recovery efficiency of 86.17 ± 2.18%.

Dieldrin levels in all samples were determined on Wilkens-Aerograph gas chromatographs (Models 600-C, 665, and 550-B) equipped with a tritium foil (activity 250 mc) electron-capture detector. The basic operating conditions of the chromatographs were as follows: Pyrex column 5' x 1/8" packed with approximately 3% QF-1 Gas Chrom Q; oven temperature 180° C.; detector temperature 200° C.; nitrogen carrier gas having a flow rate of 40 ml/min. Actual operating conditions varied slightly with different columns and samples to facilitate resolution of dieldrin

on the traces. A Micro-Tek (Model MT-220) gas chromatograph with electron capture or microcoulometric detector was employed on occasion to check for metabolites of dieldrin.

Samples were quantified by comparing the peak height of samples with heights obtained for known standards. Each sample was replicated three times on the gas chromatograph with three dieldrin-in-benzene standards (either 0.1 or 0.05 ppm) interspersed between sample injections. The average response of the chromatograph to the standards was employed to quantify each sample replicate and the average of the three replicates considered as the concentration in the sample. Preliminary studies of quantitative methods indicated that peak height was the least variable, hence, the most reliable of the methods.

To avoid errors due to photo-oxidation or evaporation in the dieldrin standards, these were made up in benzene and stored frozen at -10° C. when not in use. New standards were prepared every three or four weeks from a 1.0 ppm dieldrin/benzene stock solution.

RESULTS AND DISCUSSION

Dieldrin Uptake

Although the primary intent of this project was to evaluate the elimination of dieldrin by green sunfish, some information regarding uptake and absorption efficiency across the gut was obtained. The total amount of dieldrin recovered from the tissues and carcass of each treated fish was calculated and the results graphed as in Figure 3. The total dieldrin content decreased during the experimental period at an exponential rate. The equation for the line has the following form:

$$Y = Y_o e^{-2.3(b)t}$$

Where Y_o = amount of dieldrin present at time zero

b = slope of the line

t = time

The slope multiplied by 2.303 is a constant usually designated as "k" and represents the fractional rate of change in dieldrin. The calculated regression equation for the amount of dieldrin lost was

$$Y = 82.23\ e^{-0.0.269t}$$

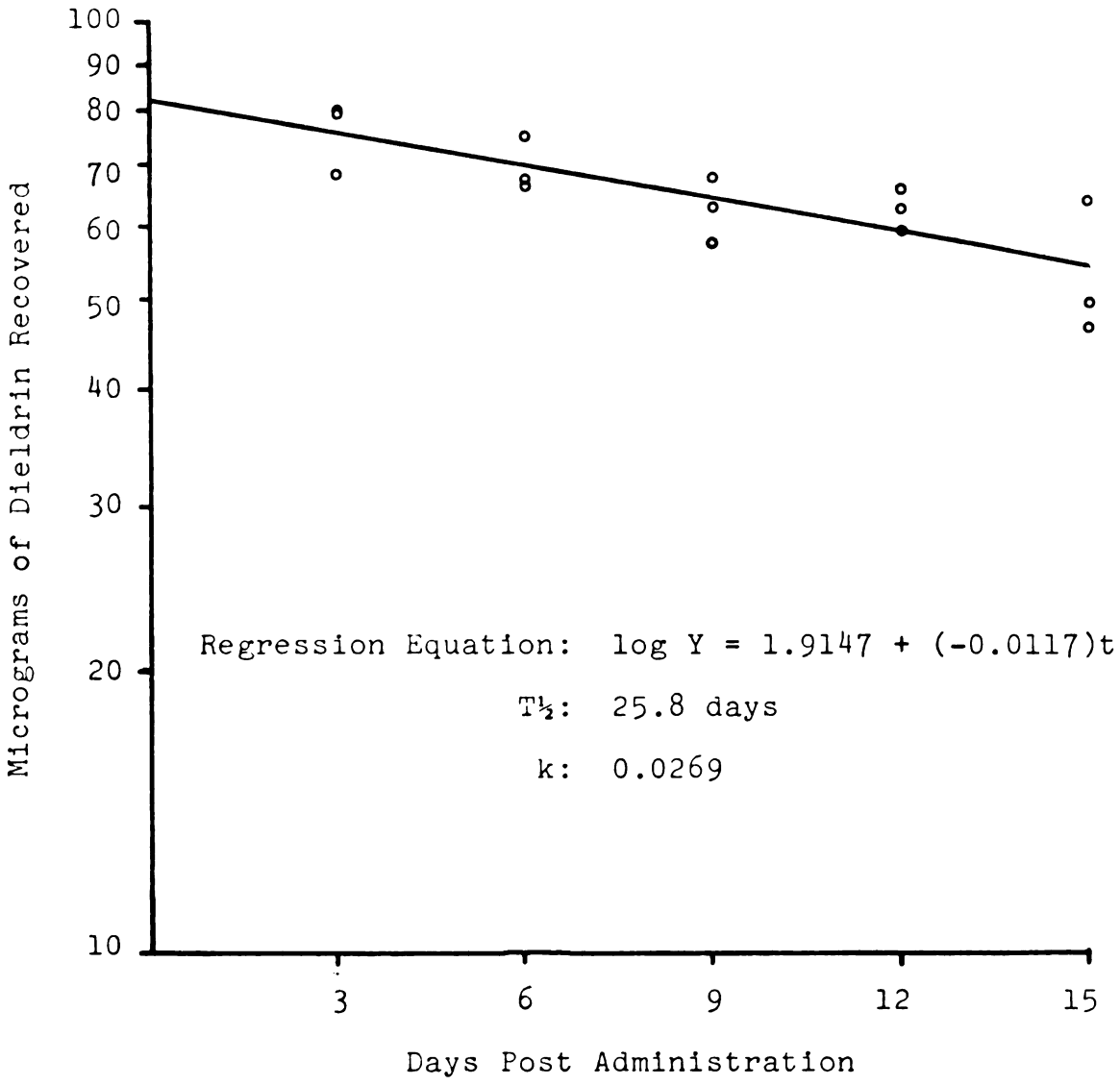


Figure 3.--Total dieldrin recovered from fish at various times during the experiment. Each point represents one fish.

A statistical test to determine if a true regression did exist--i.e., test whether the slope (b) is significantly different from zero--indicated that the regression was highly significant ($F = 22.2 > F_{.01} = 9.07$). Consequently, an estimate of the amount of dieldrin initially taken up by the fish is given by the point where the regression line crosses the ordinate (Y_o), which in this instance equaled 82.23 micrograms.

The rate of dieldrin uptake across the fish gut is rapid. The rate at which the food pellet passed through the digestive tract was not determined in this experiment. However, estimates of the rate of food passage through the gastrointestinal tract of green sunfish, at comparable temperatures, were obtained in preliminary studies where dieldrin was administered with oil in gelatin capsules. Since the dye in the capsule tainted the fecal material a pinkish color, the time at which the capsule was eliminated from the body could be easily ascertained. Nine observations showed that the average time for passage of the capsule through the digestive tract was 34.7 hours, with a range of 26 to 52 hours. The passage rate of the food pellet was probably comparable.

The data also permitted determination of the efficiency of dieldrin absorption from the gastrointestinal tract. The average amount of dieldrin taken up by a fish was 82.23 micrograms. As previously mentioned, the average

amount of dieldrin per food pellet was 94.93 micrograms. Therefore, the green sunfish gut demonstrated an efficiency of 86.62% for absorbing dieldrin. This is the first known measurement of the efficiency of insecticide uptake from the digestive tract. Other fish studies in which insecticides were administered orally (Allison, et al., 1963; Buhler, et al., 1969) involved feeding over a long time period rather than administering a single dose and thus could not readily be used to determine absorption efficiency.

Observations of the dieldrin concentrations in various portions of the digestive tract (stomach, pyloric caeca, and intestine) indicate that the intestine and pyloric caeca are primary areas of absorption. This agrees with areas of primary fat absorption (anterior intestine) observed in Tilapia (Sivadas, 1965).

The fact that green sunfish are capable of absorbing approximately 86% of an orally ingested dose of dieldrin has important implications in aquatic ecosystems. Even in the absence of dieldrin in the water (so that the gills are not a route of uptake) this degree of efficiency would easily permit a rapid accumulation by fish through feeding upon contaminated organisms. This high efficiency value certainly affords evidence of the importance of food-chain concentration in aquatic systems. Generally, insecticides do not persist in the water for long periods

of time. In streams and other lotic environments, contaminated water passes through the area and continues downstream. Under lentic conditions, photo-oxidation, microbial decomposition, absorption, and adsorption by the living and non-living organic components result in a relatively rapid decline of insecticides to non-detectable levels in the water. However, because of the high affinity of many of the chlorinated hydrocarbon insecticides for organic matter, large amounts of these insecticides remain tied up in the ecosystem. Further movement and concentration then occurs via the food chain which become important in understanding the fate and effect of pesticides in the environment.

Whether the efficiency of dieldrin absorption across the fish gut is as high under natural conditions as it was in this study (86%) is not definitely known. I believe the value observed in this experiment would represent a maximal value under field conditions. Much of the dieldrin was probably adsorbed onto the pellet rather than absorbed into the actual components. Consequently, the dieldrin was probably more readily available to the fish than dieldrin would be in natural food (insects, fish, etc.).

Dieldrin Distribution and Translocation in Tissues

In selecting tissues and organs for residue analysis, emphasis was placed on those associated with possible insecticide elimination routes. Tissues such as blood (transport vehicle for insecticides), visceral fat (chief insecticide-storage site), and muscle (most prevalent tissue) were analyzed with an interest in detecting translocation of dieldrin within the body. The dieldrin concentrations observed in the various tissues and organs of each fish are given in Appendix B.

Aquarium control fish indicated that little, if any, resorption of eliminated dieldrin occurred while the fish were in the holding aquaria. Analysis of mucus samples and the carcass of these fish showed no dieldrin, while the visceral adipose tissue from three of four control fish showed only trace amounts. The continuous water flow through the aquarium was apparently effective in diluting and removing the dieldrin. Therefore, it can be assumed that dieldrin concentrations, translocation, and rates of elimination were not affected by resorption from the water.

Distribution in Tissues

In order to evaluate dieldrin distribution in the various tissues, they were ranked (highest to lowest) according to the amount of dieldrin present per gram of tissue (Table 1). This ordinal ranking permitted comparing

TABLE 1.--Dieldrin distribution in fish tissues ranked ordinally according to their average dieldrin concentrations within each sample period.

3-days	6-days	9-days	12-days	15-days
Adipose T.	Adipose T.	Adopose T.	Adipose T.	Adipose T.
Intestine + P. Caeca	Intestine + P. Caeca	Intestine + P. Caeca	Intestine + P. Caeca	Intestine + P. Caeca
Liver-fem.	Gall Bladder + bile	Liver-fem.	----------[a]	----------
Gall Bladder + bile	Gill	Gill	Gill	Gill
Gill	Liver-fem.	Ovary	----------	----------
Ovary	Ovary	Gall Bladder + bile	Carcass	Carcass
Carcass	Carcass	Carcass	Gall Bladder + bile	Gall Bladder + bile
Kidney	Kidney	Kidney	Liver-male	Kidney
Stomach	Liver-male	----------	Kidney	Stomach
Testes	Testes	----------	Testes	Blood
Blood	Stomach	Blood	Stomach	Testes
Liver-male	Blood	Stomach	Blood	Liver-male
Muscle	Muscle	Muscle	Muscle	Muscle

[a]Data not available for tissue at this rank in preceding period. Space retained to facilitate comparisons of other tissues.

the tissues without becoming confused by variations in actual dieldrin concentrations in the tissues at different sampling periods. Visceral adipose tissue, combined intestine and pyloric caeca, and muscle maintained the same ranks throughout the experiment. The dieldrin distribution ranks for the remaining tissues varied considerably. The two most variable tissues were liver and gonad. As indicated in Table 1, large differences in residue levels were noted in male and female liver. The carcass samples were not homogeneous; in some instances, not all the visceral fat was taken for separate analysis and in other cases, not enough fat was available for a sample and therefore not removed. If the carcass, liver, and gonads are omitted from the table, the remaining tissues maintain a relatively constant rank throughout the experiment (Table 2).

Initially, the gall bladder + bile and gill ranked third and fourth in residue levels, with the two organs reversing this order between the sixth and ninth days. However, the dieldrin concentrations in both organs were very similar for the first six days (Table 4, p. 46) so that assignment of a definite rank was somewhat arbitrary. Due to a more rapid decline in dieldrin levels in the gall bladder than in the gill after the sixth day, the residue levels were markedly different and thus the organs could be properly ranked. In general, stomach ranked sixth

TABLE 2.--Dieldrin distribution in selected fish tissues at various periods of the experiment.*

3-days	6-days	9-days	12-days	15-days
Adipose T.	Adipose T.	Adipose T.	Adipose T.	Adipose T.
Intestine + P. Caeca	Intestine + P. Caeca	Intestine + P. Caeca	Intestine + P. Caeca	Intestine + P. Caeca
Gall Bladder + bile	Gall Bladder + bile	Gill	Gill	Gill
Gill	Gill	Gall Bladder + bile	Gall Bladder + bile	Gall Bladder + bile
Kidney	Kidney	Kidney	Kidney	Kidney
Stomach	Stomach	Blood	Stomach	Stomach
Blood	Blood	Stomach	Blood	Blood
Muscle	Muscle	Muscle	Muscle	Muscle

*Tissues are ordinally ranked as in Table 1.

and blood ranked seventh in dieldrin residues; although in one instance (at nine days), blood showed a higher dieldrin concentration than did the stomach. This may have resulted from sample variation, since both tissues had similar concentrations, never differing by more than 0.1 ppm at any sample period.

The tissues and organs analyzed in the experiment can be grouped according to dieldrin residue levels with visceral adipose tissue in a group by itself--containing dieldrin levels approximately ten-fold higher than any other tissues or organs. A second group of tissues possessing 1-4 ppm of dieldrin includes intestine and caeca, gill, ovary, gall bladder + bile, and female liver. A third group showing low dieldrin concentrations (0.4-0.8 ppm) includes kidney, male liver, testes, blood, and stomach. Muscle tissue could be included in this group or actually represent a fourth group containing 0.1 ppm dieldrin or less.

It was not possible to determine the sex of green sunfish except by dissection and as a result the two sexes were not equally represented in the experiment. Of the 15 fish for which data are reported, only five were females. They were represented in only the first three sample periods (through nine days), while males were present in all sample periods except at nine days. This disparity prevented comparing the two sexes throughout the experiment.

In general, however, dieldrin levels in the ovaries (1.55 ppm) were approximately three-fold higher than in the testes (0.55 ppm). All fish examined were mature and the gonads appeared to be at the stage of rapid development which precedes spawning in the spring. When comparing dieldrin residues in the gonads with residue levels in other tissues (Table 1), ovaries would rank high (comparable to gills), whereas dieldrin levels in the testes would rank low (comparable to concentrations in the stomach and blood).

Male and female fish also demonstrated substantial differences in dieldrin residues in the liver. Although individual dieldrin measurements within each sex were quite variable; the average dieldrin levels in the female liver exceeded levels in the male liver several fold and the ranges for the two never overlapped. Comparing dieldrin levels in the liver of each sex with levels in other tissues, the females would again contain relatively high dieldrin concentrations with the male liver exhibiting much lower levels, comparable to the kidney (Table 1).

In other insecticide studies (Gakstatter, 1966; Holden, 1962; Premas and Anderson, 1963), actual dieldrin concentrations in tissues and organs vary considerably and the assignment of ranks becomes quite arbitrary, often influenced by the type and number of tissues analyzed. Consequently, comparing the results of this experiment

with others reported in the literature could be misleading. Despite these difficulties, the rank of the tissues and organs according to residue levels in this study are comparable with other investigations--where exposure was to dieldrin in water (Gakstatter, 1966; Holden, 1962; Mount, 1962). These authors noted that visceral fat accumulated dieldrin in much higher concentrations than observed in any other tissue or organs; the gill and digestive tract also ranked high in dieldrin residues, while muscle and blood were generally much lower in rank. Consequently, the method of administering the insecticide does not appear to affect the pattern of insecticide accumulation in tissues. However, absolute dieldrin levels in this study were lower than levels observed in the above studies.

Generally, those organs associated with possible elimination routes possessed higher dieldrin levels than other tissues. The high residue levels in the combined intestine and pyloric caeca sample were expected initially since this was the route that the dieldrin was administered. However, oral administration would not necessarily account for the continuously high levels throughout the experiment. The proposed pathway of elimination via the intestine (Gakstatter, 1966) appears plausible based upon dieldrin concentrations observed in these organs. The initial high concentrations of dieldrin in the gall bladder + bile

provide additional evidence of possible elimination via this route.

The fish gill also contained considerable amounts of dieldrin when compared with other tissues, ranking third among the tissues analyzed. Both Gakstatter (1966) and Holden (1962) also noted high concentrations in this organ. However, both were short-term studies in which fish were exposed to a lethal level of DDT (Holden) or a chronic level of DDT or dieldrin in water (Gakstatter). In view of this method of exposure, one would expect reasonably high dieldrin concentrations in the gill. Gakstatter demonstrated that uptake from water was correlated with the solubility of the insecticide in water. The less soluble the insecticide, the more it will adsorb to the mucus surfaces of the fish--particularly the gills. Holden did not measure blood dieldrin levels, but he believed that DDT concentrations observed in the gill were present largely in the blood rather than in the gill tissue itself.

In this study, fish gills received little, if any, exposure to dieldrin in the water. The most probably instance of exposure was while the fish were in the holding aquaria, prior to placement in the experimental chambers. However, analyses of aquarium control fish carcass, adipose tissue, and mucus revealed no dieldrin accumulation in these fish. The water supply showed no traces of dieldrin, and gill samples from chamber control fish also contained no

dieldrin. The possibility exists that some contamination of the gills may have taken place while fish were in the experimental chamber, but this exposure was certainly minimal since the chamber water turned over rapidly and the water flow minimized mixing. Therefore, in this study, dieldrin concentrations in the gill cannot be attributed to adsorption of dieldrin from water into the mucus or gills.

The dieldrin residues in the gill do not appear to be the result of the blood present (as postulated by Holden) because the dieldrin levels in the gill were always five to ten-fold higher than in blood samples. If dieldrin in the gill were due solely to the blood volume, the additional weight of gill tissue would depress the concentrations below that observed in the blood and this was not the case. Consequently, dieldrin accumulation in the gill appears to represent insecticide actually absorbed by the tissue and not attributable to the tissue's vascularity. Therefore, the data suggest that the gill serves as either a storage site or a site for dieldrin elimination.

The fish kidney exhibited low dieldrin levels relative to other organs possibly associated with dieldrin elimination (gills - intestine), but high levels relative to other tissues such as stomach, muscle, and testes. The size of the blood component of the sunfish kidney compared to the fluid component of the forementioned tissues is not

definitely known. Hoffert (1966) observed a higher blood volume in the kidney than in the liver, muscle, spleen and swimbladder of lake trout. Consequently, it appears plausible that the high insecticide levels observed in the kidney in this as well as other studies (Mount, 1962; Cope, 1960; Weiss, 1967) may be partially due to the size of the fluid (blood) component. However, some dieldrin must be stored in kidney tissue since dieldrin levels there were approximately two-fold higher than observed in blood.

The difference noted in dieldrin levels for male and female liver were unexpected. To my knowledge, no such difference has been previously reported. A comparison of dieldrin levels in the male liver for the first six days with levels in the female liver for nine days indicated that dieldrin levels in the liver of the two sexes were significantly different ($t = 3.64 > t_{.95} = 2.365$). Only the first six-day data were included for the male liver because no data were available at nine days and inclusion of any later sample period data would have induced a bias caused by dieldrin elimination from male liver in the later periods.

The high dieldrin levels observed in the female liver may be associated with ovarian development. The ovary is noted for the accumulation of fats and oils which are deposited in the eggs to serve as nourishment for the

embryo in the event of fertilization. The liver is acknowledged as the site of lipid synthesis and consequently would be expected to contain high fat concentrations as a result of ovarian activity. Since dieldrin is primarily deposited in lipid, one would expect higher residue levels in the female liver than in the liver of males.

In conjunction with this hypothesis, one might expect elevated dieldrin levels in the blood of females resulting from a rapid turnover of body lipids to provide energy for increased metabolism and also to provide fatty acids for the synthesis of egg lipids. For the period for which females were included in the experiment, their blood possessed higher residue levels than the blood of male fish (Table 3). However, the dieldrin levels between the two sexes were not significantly different. Presumably, if data were available for males at the ninth-day sample period, their average blood concentration would have been lower because of dieldrin excretion and differences in the blood residues levels would have been more apparent. This statement is substantiated by the average dieldrin concentration for three male fish after 12 days (0.27 ppm).

Since differences in dieldrin residues in the liver appear to be associated with ovarian development, this phenomena is probably a seasonal event. To what extent this sex-linked factor applies to residue levels in other

TABLE 3.--Blood dieldrin concentrations in male and female fish during the first nine days of the experiment.

Days after Dieldrin Administration	Blood Dieldrin Concentrations (ppm)	
	Males	Females
3	0.48	0.41
	0.40	
6	0.33	0.55
	0.49	
9		0.33
		0.30
		0.75
	Average 0.425	Average 0.468

tissues and organs is unknown. However, it appeared that differences in residue levels may also exist in the blood.

Translocation in Tissues

Except for the fact that blood serves as the transport medium for insecticides in the body, little is known about translocation of dieldrin or other insecticides within the body, such as movement from the vascularized tissue to principle storage sites or to organs associated with elimination. In the present study, movement within the fish was evaluated by observing changes in dieldrin residues in tissues at successive sample periods (Table 4).

TABLE 4.--Average dieldrin concentrations (expressed as ppm) in fish tissues at various sample periods. Each value represents the average of three fish except when noted.

Tissue	Days After Administration of Oral Dose of Dieldrin				
	3-days	6-days	9-days	12-days	15-days
Blood	0.430	0.454	0.403	0.271	0.237
Stomach	0.528	0.466	0.325	0.286	0.242
Intestine	-----[a]	-----	1.915	2.332	1.948
Pyloric caeca	-----	-----	0.901	1.469	1.802
Combined intestine + caeca	3.505	3.966	2.816	3.801	3.750
Adipose tissue	23.492 (2)[b]	25.570	18.642	24.478	16.629(2)
Gill	2.685	2.294	1.937	2.031	1.534
Testis	0.504 (2)	0.504 (2)	-----	0.275	0.176
Ovary	1.503 (1)	1.554 (1)	1.376	-----	-----
Muscle	0.157	0.175	0.129	0.094	0.107
Kidney	0.616	0.827	0.514	0.536	0.414
Liver-male	0.415 (2)	0.794 (2)	-----	0.544	0.174
Liver-female	3.540 (1)	2.012 (1)	2.266	-----	-----
Gall bladder	2.731 (2)	2.347	1.148	0.883 (2)	0.797
Carcass	1.380	1.338	0.999	0.899	0.805

[a]Data for intestine and caeca combined.

[b]Number in parenthesis represents number of fish in sample if less than three.

Individual tissues and organs displayed increases in dieldrin concentrations at two different times--between the 3-day and 6-day sample periods and again between the 9-day and 12-day sample periods. The change in residue levels between the latter sample periods reflects a recovery from the low tissue dieldrin levels in the three female fish sampled at nine days rather than evidence of dieldrin translocation.

Increased dieldrin levels in tissues between the 3-day and 6-day sample periods may reflect one or more of the following conditions:

1. redistribution of dieldrin among the various organs and tissues.
2. continued dieldrin absorption from the intestine, despite previous voidance of the food pellet.
3. random sample variation.

The increased dieldrin levels in blood, adipose tissue, muscle, kidney, and male liver suggest further absorption from the intestine. However, the changes in dieldrin concentrations observed were never statistically significant, so that in terms of probability, these changes represent sample variation. As a consequence, relatively few inferences regarding translocation of dieldrin in fish are possible.

Except for the slow increase in dieldrin levels in the combined intestine and pyloric caeca sample, the tissues and organs showed a general decline with time suggesting that translocation was mainly from sites of storage to sites of elimination. This general decline in dieldrin residue levels also suggests that elimination rates were probably equal to rates of mobilization from fish tissues. In fact, the rate of elimination may be limited by the rate at which dieldrin was transported to elimination sites. Heath and Vandekar (1964) noted that disposal of dieldrin in rats was not controlled by the capacity of the rat to metabolize dieldrin, but rather by the flow of dieldrin to the place where it was metabolized (liver).

The differences in dieldrin levels observed in portions of the digestive tract were also of interest. The combined intestine and pyloric caeca was the only tissue sample which demonstrated a slight total increase in dieldrin levels during the experiment. When the two organs were analyzed separately, later in the experiment, the intestine appeared to maintain a relatively constant dieldrin level whereas the pyloric caeca increased in residue levels. In contrast to these two organs, dieldrin levels in the stomach declined continuously throughout the experiment.

Holden (1962) considered the high DDT residues in brown trout caeca and intestine as an indication that these

organs served as storage sites, while Gakstatter (1966) associated the DDT and dieldrin levels in these two organs with dieldrin elimination via the intestine (in the bile).

Neither statement appears entirely satisfactory for explaining the residue levels in intestine and caeca observed in this study. Since dieldrin declined in all other tissues, including visceral adipose tissue, one would expect a similar decrease in dieldrin stored in these organs. In addition, the removal of the digestive tract contents (except in pyloric caeca) at the time of sampling, should have removed any dieldrin being eliminated.

An alternate explanation is that some of the dieldrin transported to the lower digestive tract with bile was resorbed by the intestine and pyloric caeca. The addition of resorbed dieldrin to amounts already stored in these organs would account for the constant dieldrin concentrations observed during the experiment. If resorption exceeded losses of stored dieldrin, the levels could increase as observed in the caeca.

Exactly where the bile duct enters the intestine in green sunfish is unknown, but Lagler, et al., (1962) indicates that the bile duct enters at the beginning of the intestine in the pyloric region. Other evidence suggesting that dieldrin may be reabsorbed in the intestine include knowledge that bile salts function as fat emulsifiers which facilitate the hydrolysis of fats, and also that fat

absorption is intensified in the pyloric caeca of some fish such as the genus Salmo (op. cit.). Additional data are needed to verify this hypothesis; however, it seems probable that dieldrin may be recycled internally between the digestive tract and other parts of the fish body.

Dieldrin Losses from Individual Tissues

In the previous section, the distribution and translocation of dieldrin among tissues were considered. The application of regression analyses to these data provided information on dieldrin losses from individual tissues.

Tests of the linearity of the calculated least-square regression lines indicate that dieldrin was lost from fish tissues and organs in an exponential manner (Figures 4, 5, 6 and 7). However, all of the regression lines may not represent accurate descriptions of the dynamics of dieldrin in the tissues. For several of the tissues, the slopes of the regression lines were not significantly different from random sample variation. For these particular tissues--liver, visceral adipose tissue, ovary, and the combined intestine + pyloric caeca sample--the regression line could have been drawn as a horizontal line indicating no change in dieldrin concentrations throughout the experiment (slope equal to zero). Consequently, any inferences regarding changes in dieldrin concentrations in these tissues must be interpreted with caution.

The slopes of the regression lines for the remaining tissues were statistically significant from zero (Table 5) and reflect dieldrin losses from these tissues under the conditions of this experiment. Dieldrin appeared to be eliminated from this second group of tissues at varying rates. However, an analysis of covariance demonstrated that the slopes of the regression lines for these tissues were not significantly different, i.e., that there was no significant departure from parallelism. Consequently, definite statements regarding differences in rates of dieldrin loss from individual fish tissues must await further study.

The rates of dieldrin loss from various tissues can provide additional information on the dynamics of dieldrin in fish. Both visceral adipose tissue and the ovary--which are known sites of dieldrin storage--exhibited very slow rates of loss. In fact, the biological half-life for dieldrin loss from adipose tissue ($T_{½}$ = 24.3 days) was nearly the same as the half-life observed for the entire fish ($T_{½}$ = 25.8 days). This similarity reflects the importance of lipid deposits as storage sites and further demonstrates that elimination from fish may be strongly influenced by the rate of mobilization from sites of storage. In addition, fish blood had an intermediate rate of loss ($T_{½}$ = 12.1 days), attesting to its role as a transport medium for dieldrin in the fish body. It was

TABLE 5.--Summary of pertinent statistics for dieldrin losses from selected tissues whose slopes demonstrated a significant difference from zero.

Tissue	Regression Equation	Biological Half-life Days	Constant "k"
Kidney	log y = $\bar{1}$.9042 + (-0.0175)t[a]	17.3	-0.0401[b]
Gill	log y = 0.4711 + (-0.0182)t	16.5	-0.0419
Muscle	log y = $\bar{1}$.2832 + (-0.0205)t	14.8	-0.0470
Blood	log y = $\bar{1}$.7545 + (-0.0246)t	12.1	-0.0573
Stomach	log y = $\bar{1}$.7914 + (-0.0283)t	10.4	-0.0652
Testes	log y = $\bar{1}$.8499 + (-0.0385)t	7.8	-0.0887
Gall bladder	log y = 0.5280 + (-0.0466)t	6.5	-0.1074

[a]Equation presented in form of an equation for a straight line

$$y = a + bx$$

where: y = log of concentration at any time (t)
a = log of the y intercept
b = slope of the line
x = any time period

[b]"k" (constant) = slope times 2.303. This represents the fractional rate of change in dieldrin concentration in the exponential equation:

$$Y_t = Y_o e^{-kt}$$

also interesting to note that despite the great differences in dieldrin residue levels in the liver of male and female fish, the rate of dieldrin loss was similar ($T_{½}$ = 8.4 and 8.1 days for male and female, respectively).

Attempts to resolve the issue of whether fish tissues eliminate dieldrin at different rates by comparing the present data with previous fish studies was not possible because, to my knowledge, calculation of rates of dieldrin loss from individual fish tissues has not been previously attempted. However, one study (Robinson, et al., 1967) determined the rates of dieldrin loss from muscle, fat, brain and liver of pigeons. They observed that the rate of loss for these tissues was approximately the same ($T_{½}$ = 40 to 57 days). Since statistical significance was lacking in the present experiment, it cannot be definitely stated that the situation is different in fish. However, the fish tissues demonstrated much greater differences in rates of dieldrin loss (Table 5) than was observed in the pigeon. Therefore, although the results of this study were statistically inconclusive, it appears that fish tissues may exhibit different rates of dieldrin loss and that further research is warranted. Additional research in this area could be particularly beneficial in evaluating the dynamics, especially translocation, of dieldrin within the fish body.

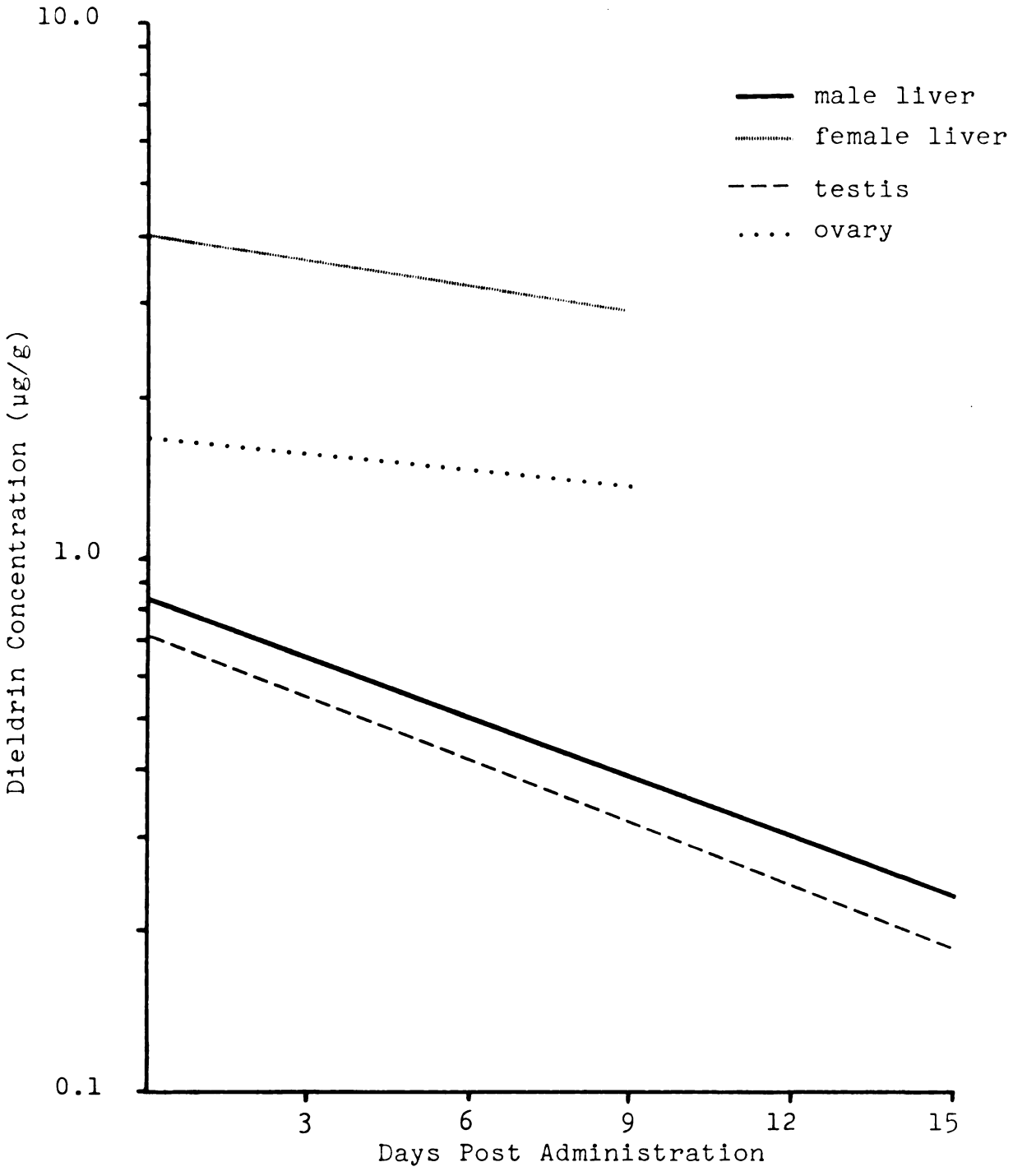


Figure 4.--Semi-log plot of calculated least-squares regression lines for changes in dieldrin concentrations in the liver and gonad of a fish after administration of a single oral dose (94.93 µg). Data for females is based upon five fish present during the first nine days. Data for males is based upon nine fish.

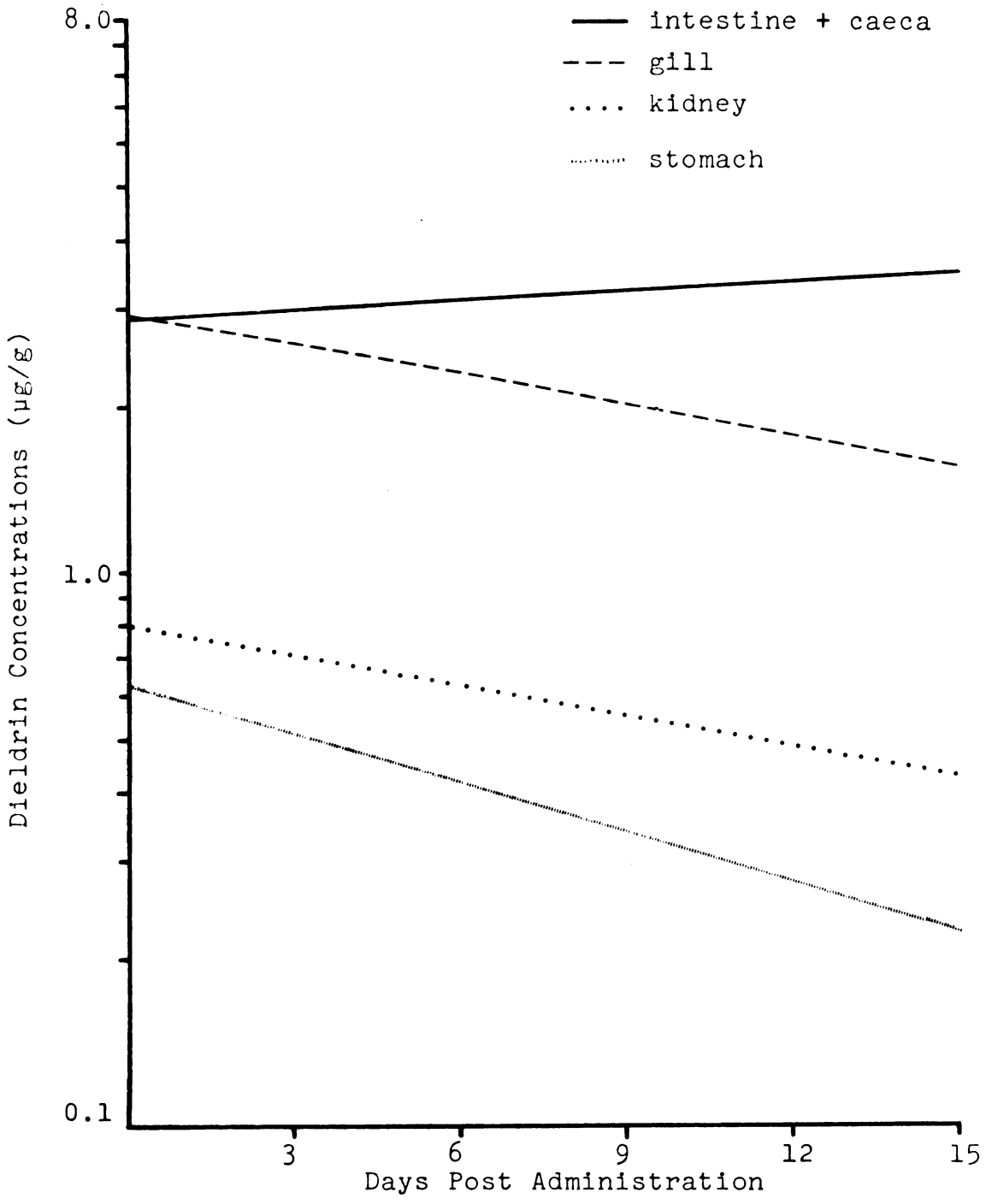


Figure 5.--Semi-log plot of calculated least-squares regression lines for changes in dieldrin concentrations in the intestine + caeca, stomach, gill and kidney of a fish after oral administration of a single oral dose (94.93 µg). Data are based upon 15 fish for each tissue.

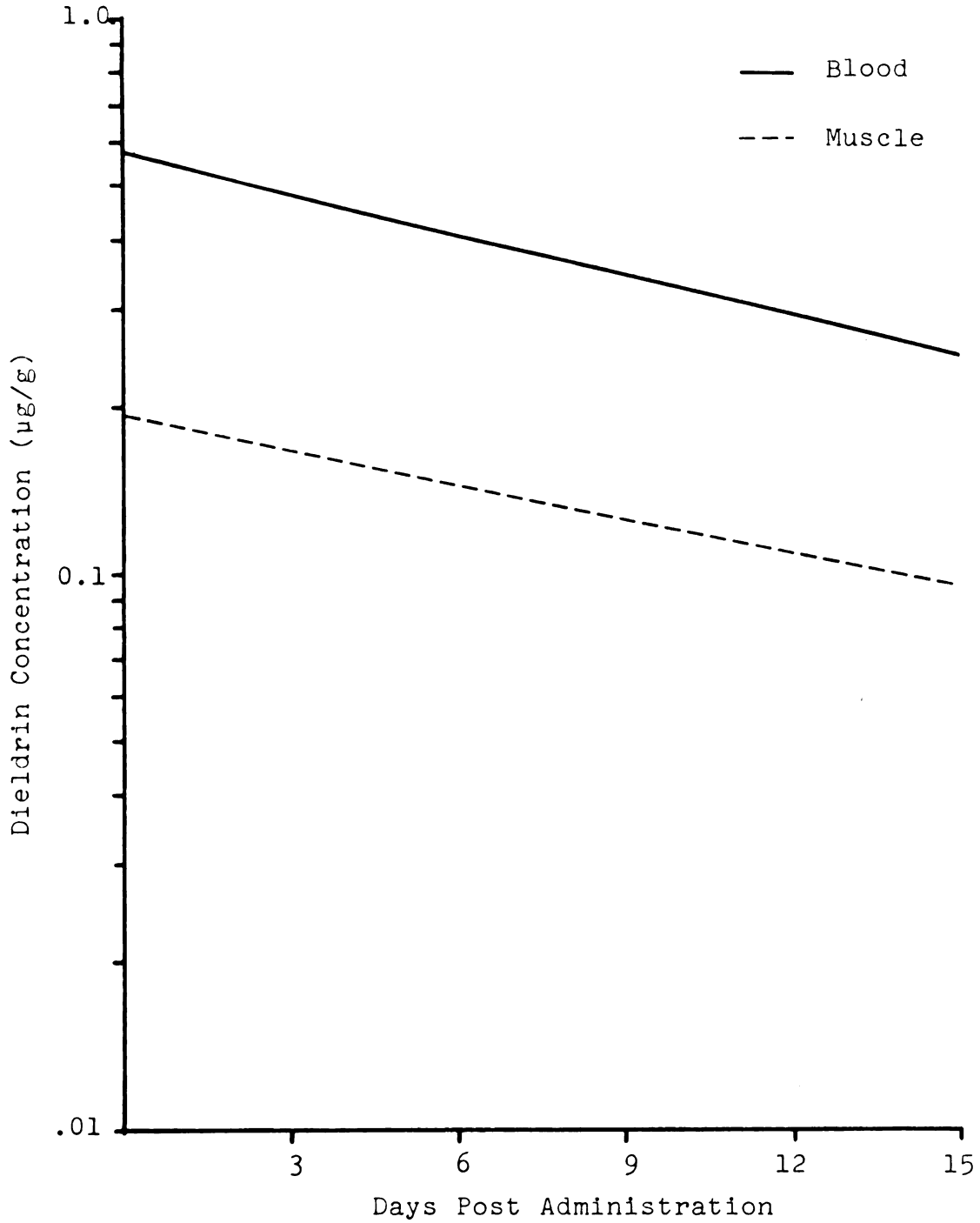


Figure 6.--Semi-log plot of calculated least-squares regression lines for changes in dieldrin concentrations in the blood and muscle of fish after oral administration of a single oral dose (94.93 µg). Data are based upon 15 fish for each tissue.

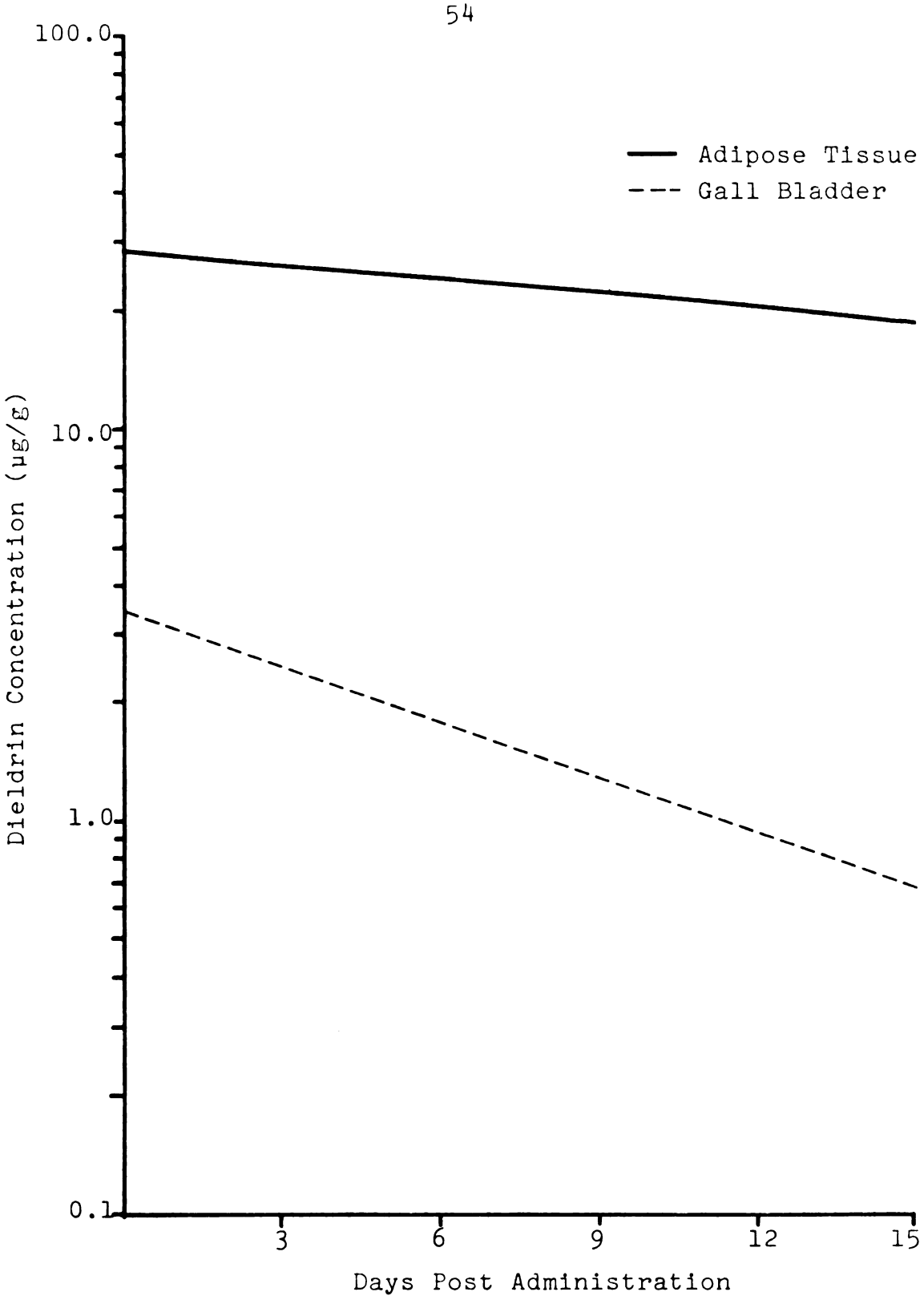


Figure 7.--Semi-log plot of calculated least-squares regression lines for changes in dieldrin concentrations in the adipose tissue and gall bladder + bile of a fish after oral administration of a single oral dose (94.93 µg). Data for adipose tissue based upon 13 fish. Data for gall bladder + bile based upon 12 fish.

Dieldrin Elimination

As previously indicated, dieldrin elimination from fish can be examined in two ways: dieldrin losses from the body (either on the basis of absolute quantity or body concentration) and secondly, by measurement of dieldrin in waste products. In the present study, dieldrin elimination was observed from both viewpoints thereby providing two independent estimates of dieldrin elimination. In addition, the analysis of waste products for dieldrin provided a means for evaluating the importance of the various possible routes as insecticide elimination pathways in fish.

Fish excretory organs are the liver, kidney, integument, and gill. The manner in which these various organs may function in insecticide elimination was discussed in the introduction. To evaluate dieldrin excretion via these organs, analyses were performed on feces, urine, mucus, and water passing over the gill respectively. The quantities and concentrations of dieldrin in the waste products as well as excretory organs are given in Appendix B.

The results obtained on dieldrin elimination from green sunfish are not conclusive because the two methods for determining dieldrin elimination were not in agreement. Only 63.3% of the total quantity of dieldrin lost from a fish during the experiment (27.38 µg) was recovered in the waste products (17.33 µg). This discrepancy prohibited definitive statements regarding the rate of dieldrin loss and routes of dieldrin elimination.

Since dieldrin is known to be metabolized in mammals, a possible explanation for the low recovery would be degradation of the dieldrin to undetected metabolites. Korte and Arnt (1965) found six dieldrin metabolites in the excretia of rabbits after oral administration of labelled dieldrin; but only one of these metabolites (6,7, trans-dihydroxy-dihydroaldrin) was of major importance, constituting 86% of the total metabolites found. Other investigators (Heath and Vandekar, 1964; Morsdorf, et al., 1963; Datta, et al., 1965) have observed a variable number of dieldrin metabolites in rats. All the studies agreed that the metabolites were more polar (hydrophilic) than dieldrin and hence, more soluble in water. Heath and Vandekar observed one metabolite, representing 78% of the total radioactivity in the bile, that they considered a probable glucuronide of a dieldrin derivative.

Although unknown peaks were obtained on chromatograms of most extracted materials in this study, no dieldrin metabolites were suspected. To verify the presence or absence of metabolites, all samples of a particular tissue or waste product were combined, concentrated, and then analyzed on a Micro-Tek gas chromatograph equipped with a microcoulemetric detector. The amount of dieldrin in the combined samples was small in relation to the sensitivity of the detector so that the sample produced only a small response (5-20% of the chart paper). However, in all

cases, only one peak, whose retention time matched that of the dieldrin standard was observed on the trace, demonstrating that only one halogenated compound--namely dieldrin--was present. Grzenda (personal communication, 1968) also found that neither gas-liquid chromatography nor radio-thin-layer chromatography indicated metabolism of dieldrin in goldfish. Consequently, metabolism of the dieldrin did not appear responsible for the low recovery of dieldrin in the waste products. An explanation of the low dieldrin recovery from several waste products will be discussed later in conjunction with elimination from the intestine (p. 62).

Dieldrin Elimination Via the Integument

As indicated previously, no one has considered the integument a possible pathway for insecticide elimination. However, Ferguson, et al. (1966) noted that dead mosquito fish exposed to 1.0 ppm of endrin for 11.5 hours, washed four times in tap water and once in acetone, and transferred to clean water, released small amounts of endrin into the water. The mechanism of this release is unknown, but since the fish were dead, one might suspect diffusion across the integument as a possible explanation. Since the sunfish in this study received little, if any, exposure to dieldrin in the external medium, its presence in mucus would indicate elimination across the integument.

Mucus samples were collected by scraping the sides of a fish with a spatula immediately after it was removed from the experimental chambers. The mucus was placed in vials and stored in the same manner as the tissue samples. Mucus samples from treated fish in the three-day and six-day sample periods contained no detectable dieldrin (limit of detectability was 0.1 ppm). Consequently, only two mucus samples from the three fish in each succeeding sample period were randomly selected for analysis. Again, dieldrin was not detected in these samples. Hence, on the basis of these findings, one can conclude that dieldrin was not eliminated across the integument in the green sunfish.

In addition, the mucus samples from chamber control fish and aquarium control fish did not contain detectable dieldrin. The absence of dieldrin in mucus from these fish was of interest because it afforded evidence of the minimal exposure of the experimental fish to dieldrin in the external medium. Absorption or adsorption of dieldrin to the gill from the water was definitely minimal.

Dieldrin Elimination Via the Kidney

A total of 21 urine samples were collected from the ten cannulated fish in the experiment (two fish for each of the five three-day sample periods). Eighteen samples were daily samples and three consisted of combined two-day

samples. Daily urine volumes ranged from 1.0 ml to 9.3 ml with an average volume of 3.7 ml/day. The average urine flow for green sunfish was 57.5 ml/kg/day.

Despite the significant amounts of dieldrin observed in the fish kidney, no dieldrin was detected in fish urine during the first nine days of the experiment. A tenth-day urine sample of one fish contained a small quantity of dieldrin (12 ng) for a concentration of 2.26 ppb. Urine from the same fish contained 79 ng in a combined 11-12 day sample of 1.3 ml of urine giving a dieldrin concentration of 45 ppb in the urine. Urine from the second cannulated fish contained no dieldrin at 10 or 11 days and no urine was collected on the last day.

Both cannulated fish in the last sample period (13-15 days) contained small quantities of dieldrin in the urine, although one fish was negative on the thirteenth day. But, again, the dieldrin amounts recovered were low and the maximum concentration was only 5.8 ppb. Most of the dieldrin levels observed in urine were close to the minimum detectable limits of the gas chromatograph (1-2 ppb).

The data indicate that some dieldrin may be eliminated from fish via the kidney after 10 or 12 days, but the quantities observed in urine were small compared with levels observed in feces and water as will be shown later. The rather short duration of the present experiment may have failed to reveal the nature of dieldrin elimination from

the kidney. Korte and Arnt (1965) and Heath and Vandekar (1964) both observed a slow increase in radioactive substances in urine after cessation of feeding labelled dieldrin or intravenous perfusion of labelled dieldrin in rats over a period of 4 to 26 weeks. Fish may exhibit a similar action and levels in urine may have increased with time.

The urine flow for sunfish observed in this study (57.5 ml/kg/day) may have been lower than normal, thereby depressing dieldrin levels observed in urine. Urine output for green sunfish or other centrarchids was not available from the literature. But, data on urine flow in rainbow trout show volumes of 101 ± 8 ml/kg/day (Fromm, 1963) and a maximal flow of 91.9 ml/kg/day at 24 hours post-catherization (Hunn, 1969). Therefore, sunfish urine volume was approximately one-half that recorded for rainbow trout.

The data demonstrate that dieldrin levels in the kidney are not indicative of dieldrin excretion by this organ. Dieldrin was not observed in the urine until levels had decreased in the kidney. Even if the urine output for green sunfish was low, one can infer that very little, if any, dieldrin is eliminated via the kidney. However, additional studies over a longer time period may produce slightly different results. Apparently, the kidney serves more as a low level storage site than as an excretory site.

Dieldrin Elimination Via the Intestine

This pathway involves removal of insecticides from the blood by the liver followed by transport to the intestine in the bile and elimination in the feces. Some dieldrin may be transported to the intestine by alternate pathways; Heath and Vandekar (1964) observed dieldrin in fecal material of rats when the bile duct was cannulated. They believed some dieldrin was eliminated across the intestinal mucosa.

The water passing over a fish was in contact with feces which accumulated in the trap over a three-day period. Little exchange of dieldrin was expected because of the high affinity of chlorinated hydrocarbon insecticides for organic material. However, to verify whether an interaction between water and feces existed, a short three-day experiment was conducted. Two samples of feces with known amounts of dieldrin were placed in the chambers and both feces and water monitored for their dieldrin content. An average of 34.2% of the total dieldrin recovered was found in the water (Table 6).

This amount of dieldrin lost to the water from feces was probably a maximal value since the dieldrin concentration employed for the test was higher than that normally encountered in fish feces and also because most of the dieldrin applied probably remained on the surface rather than being incorporated into fecal material. It was

TABLE 6.--Summary of the conditions and results of the water-feces interaction experiment.

	Sample 1	Sample 2
Weight of feces	11.2 mg	55.4 mg
Dieldrin concentration	428 µg/g	86 µg/g
Dieldrin recovered in feces at 72 hours	2.366 µg	2.542 µg
Dieldrin recovered in water	1.362 µg	1.188 µg
Per cent dieldrin recovered in water	36.54%	31.84%

assumed that a similar dieldrin loss from feces existed during the experiment and all feces values were corrected for this loss as well as recovery efficiency.

Dieldrin levels in the first three-day fecal sample were much higher than those observed in subsequent sample periods (Table 7) because the sample included dieldrin voided with the food pellet residue and not absorbed by

TABLE 7.--Average dieldrin quantities and concentrations in feces at each sample period.

	Days After Administering Dieldrin				
	3-days	6-days	9-days	12-days	15-days
Total dieldrin recovered (µg)	1.166	0.083	0.038	0.074	0.049
Dieldrin concentration (µg/g dry wt. of feces)	142.609	0.905	0.672	0.899	0.503

the fish. The pattern of dieldrin elimination in feces in the subsequent sample periods was difficult to determine. Excluding the first three-day period, the differences noted in feces dieldrin concentrations were not significantly different from one another and could, therefore, be attributed to sample variation. This would infer that the dieldrin in feces remained constant during the latter part of the experiment rather than declining. Consequently, dieldrin concentrations in feces was considered as constant with an average of 0.744 µg/g dry weight feces/3 days or 0.248 µg/g dry weight feces/day.

The amount of dieldrin a fish would excrete in the feces per day was estimated from the average dry weight of feces obtained from all fish in the study. The average weight amounted to 60.03 ± 12.01 mg feces/fish/3 days. Using this value in conjunction with the average dieldrin concentration in feces, the average quantity of dieldrin excreted by a fish was calculated as 0.0447 µg dieldrin/3 days or 0.015 µg dieldrin per day.

Assuming that all dieldrin in fish was eliminated by this route, it would require 5,482 days or 15 years to eliminate the average quantity of dieldrin taken up by a green sunfish (82.23 µg). This time period is inconsistent with losses observed from fish. The total quantity of dieldrin lost during the experiment was 27.33 µg and based upon the exponential decline observed

(Figure 3), the biological half-life was only 25.8 days. Consequently, other routes for dieldrin elimination must exist.

The dieldrin quantities eliminated in fish feces was much lower than is the case for mammals which excrete approximately 90% of incorporated dieldrin or its metabolites via this route (Heath and Vandekar, 1964; Ludwig, et al., 1964). In view of the importance of this route in mammals and the high dieldrin concentrations in the intestine, pyloric caeca, and the combined gall bladder-bile samples, one might question whether the dieldrin levels observed in feces accurately represent elimination via this route in fish. This is particularly true when one recalls the disagreement between dieldrin quantities lost from the fish (27.38 µg) and the amounts recovered in all waste products (17.33 µg).

Of the four possible elimination pathways, it appears most plausible that the missing dieldrin was eliminated via the intestine. The low recovery of dieldrin from feces may have resulted from not extracting and detecting dieldrin bound to proteinacious material in the feces. It has been shown that dieldrin and other organochlorine insecticides may be bound to proteinacious material (probably lipoproteins) and not completely extracted with standard solvent extraction methods. Witt, et al. (1966) demonstrated that only two-thirds of the DDT in cow's

blood was extracted by a simple ethyl-ether extraction. Grzenda (personal communication, 1968) encountered difficulty in extracting dieldrin from fish testes and had to resort to a formic acid digestion procedure to obtain adequate recoveries.

Some evidence exists that a similar situation may have existed in this study. A large quantity of the unabsorbed dieldrin which initially passed through the fish digestive tract with the treated food pellet, was not recovered in the fecal material during the first sample period. Only 1.166 µg of an estimated 12.7 µg of dieldrin not absorbed was actually recovered. Since dieldrin was not detected at this time, it appears plausible that additional dieldrin may have escaped detection during the rest of the experiment.

No data on dieldrin quantities in waste products of fish were available in the literature. However, Grzenda (personal communication, 1968) recovered substantial quantities of labelled dieldrin in the feces of goldfish. Consequently, although I cannot definitely state that the missing dieldrin lost from the fish was eliminated in the feces, the above discussion indicates that this was the most probable route.

In summary, it appears that the quantity of dieldrin observed in fish feces was possibly much less than is actually eliminated via this route. More adequate

extraction procedures such as alkaline or acid hydrolysis are probably necessary to accurately measure dieldrin quantities in feces.

Dieldrin Elimination via the Gill

As indicated previously, dieldrin elimination from the fish branchial region was determined from the corrected dieldrin content of water passing over the fish. The water could not be isolated from dieldrin contamination from other excretory routes, but dieldrin levels were corrected for contamination from urine and also amounts leached from feces as a result of the water-feces interaction in the feces trap.

The quantity of dieldrin present in water rather than concentration was emphasized in this case since it better reflects the dieldrin losses from the gill. Because of the large volume of water passing through the experimental apparatus each day, dieldrin concentrations were very small, rarely exceeding 10 pptr (parts per trillion).

Water showed a pattern of dieldrin content similar to that found for feces except that the total amounts of dieldrin were much greater in water. More dieldrin was present in the first three-day sample periods than in subsequent sample periods (Figure 8) with the highest dieldrin quantity observed on the second day (2.305 µg). Thereafter, the daily measurements demonstrated a rapid

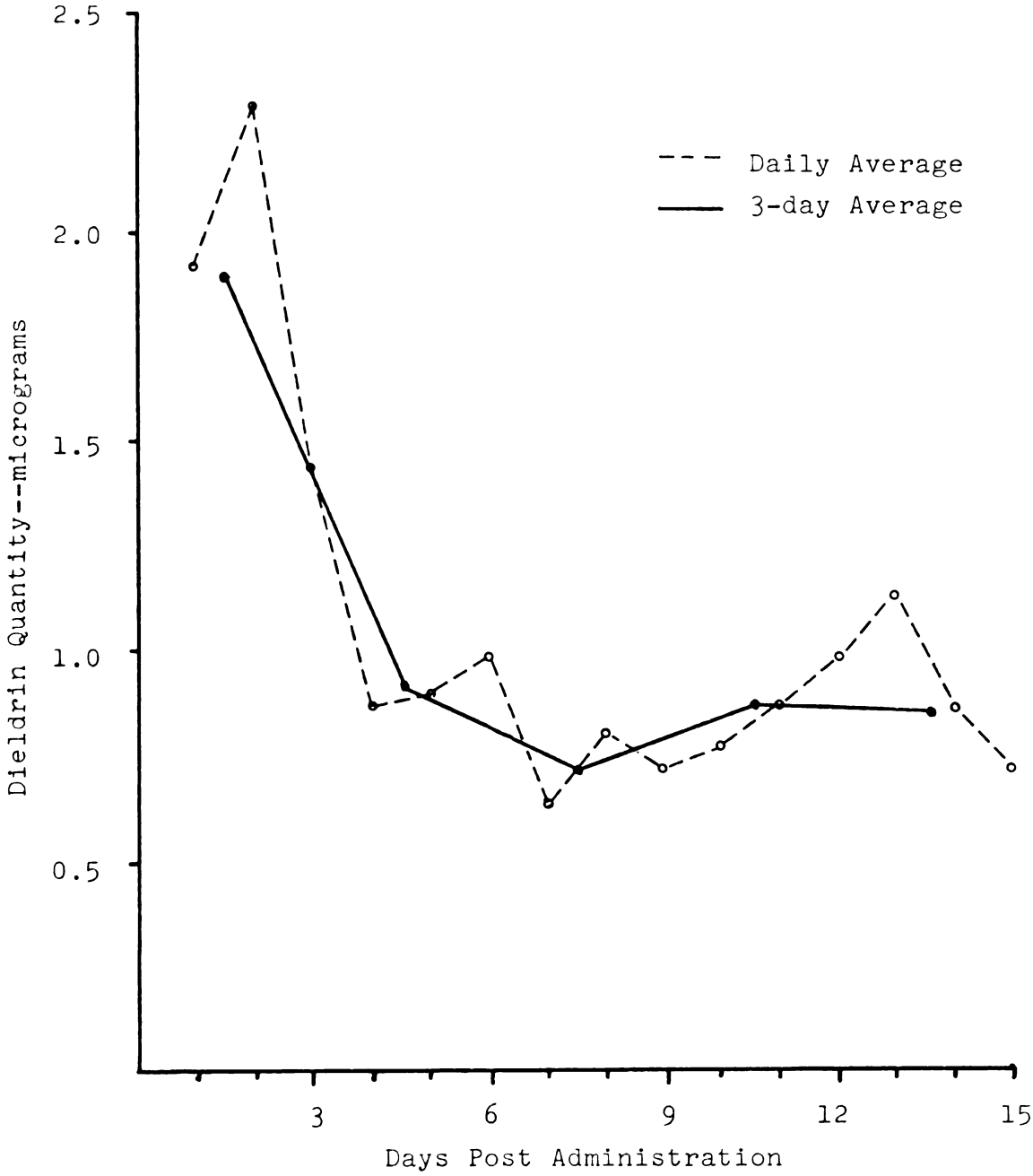


Figure 8.--The average quantity of dieldrin eliminated into water from the branchial region of a green sunfish after administration of a single oral dose of 94.93 micrograms of dieldrin.

decline to about the fourth day. Dieldrin in the water further declined slowly to the seventh day after which it slowly increased to a second peak at thirteen days and then began to decline once more at termination of the experiment. These fluctuations in daily measurements apparently were random sample variations since the average quantity of dieldrin during the last four sample periods were not significantly different in an analysis of variance test of the mean dieldrin levels in each period (Table 8).

TABLE 8.--Average dieldrin quantities and concentrations in water at each sample period.

	3-days	6-days	9-days	12-days	15-days
Total dieldrin recovered (µg)	1.879	0.924	0.722	0.868	0.853
Dieldrin concentration (ng/liter = pptr)	16.8	7.9	6.1	7.4	7.6

The high dieldrin levels in the first three-day period suggested contamination of water by dieldrin which passed through the digestive tract and was not absorbed by the fish. However, an average quantity of 1.912 µg of dieldrin was recovered from water on the first day--before the food pellet was voided from the digestive tract. Consequently, the high dieldrin levels were apparently not the result of fecal contamination.

Presumably, the dieldrin concentration in blood was much higher during the first two days than that observed on the third day, which thus resulted in increased elimination from the gill during this period. Using the blood and water data from subsequent sample periods, I calculated the probable levels in blood which would account for the dieldrin quantities observed in the water. The calculations showed that the dieldrin concentrations in blood necessary to account for the quantities observed in the water during the first and second days were 0.968 ppm and 1.170 ppm, respectively. These values are comparable to blood dieldrin levels observed in catfish (Gakstatter, 1966) and endrin levels in the blood of both catfish (Mount, 1966) and golden shiners (Ludke, et al., 1968) shortly after exposure to these insecticides in water. Therefore, the dieldrin quantities observed in water the first three days could be attributed to dieldrin being eliminated from the gill during this period.

The above explanation infers that the rate of dieldrin elimination from the gill is proportional to the dieldrin concentrations in the blood when the gill region is bathed in insecticide-free water. However, this was not the case throughout the experiment. The average quantity of dieldrin eliminated into the water appeared to level off after the fourth day to a constant rate of 0.852 µg/fish/day, despite continued decreases in blood dieldrin

levels. These results suggest that a threshold exists for blood dieldrin levels below which the relationship is no longer proportional. This value for green sunfish blood is near 0.43 ppm (the dieldrin concentration in blood at the third day).

Fromm and Hunter (1969) demonstrated that dieldrin uptake from water into blood plasma across isolated perfused trout gills could take place by passive transport. Dieldrin and other insecticides are probably lost in a similar manner when insecticide levels in the water bathing the gills are sufficiently below their solubility limit and below existing insecticide concentrations in the blood. In this study, the average dieldrin concentration in water after the fourth day was 0.0075 ppb which was far below the solubility limit of 140-180 ppb reported by Robeck, et al. (1965) and was at least 5000 fold less than concentrations observed in fish blood. In addition, the continuous flow of insecticide-free water across the gills would facilitate maintenance of a concentration gradient between insecticide levels in blood and the water.

The actual process of passive transport across the gill is unclear. Dieldrin was lost from the blood, gill, and other tissues at an exponential rate and, as a consequence, one would have expected the rate of excretion to also decline at an exponential rate. However, the dieldrin

recovered from water was constant during the last 12 days. During this same period, blood dieldrin levels declined 47% and dieldrin levels in the gill declined 34%.

The reason for this inconsistency in the manner in which dieldrin was lost from the fish and recovered in water is not definitely known. Possibly, the rate of dieldrin transport into the gill epithelial cells may differ from the rate of transport of dieldrin from these cells to the environment. If so, transport across the gill epithelium could occur at a constant rate with the exponential rate of loss from the gill representing an interaction between dieldrin in the gill and blood compartments. Unfortunately, analysis of the sunfish gill was not limited to gill tissue exclusively, but included dieldrin present in the vascular compartment (blood and lymph). In addition, other factors such as variations in blood flow patterns under different physiological conditions as shown by Richards and Fromm (1969) may play a role in dieldrin elimination from the gill. These results and speculations demonstrate that the dynamics of dieldrin elimination from the fish gill merits further study.

The presence of dieldrin in water passing over the fish and the high residues in the gill afford evidence of the gill's function as an insecticide excretory organ. After the initial dieldrin loss during the first three days, the average amount of dieldrin eliminated from the gill

was 0.852 µg/fish/day which represented the greatest recovery of dieldrin from any of the waste products. Assuming that the gill served as the only route of dieldrin elimination in fish, approximately 90 days would be required to completely eliminate the average amount of dieldrin taken up by a fish (82.23 µg). The 90 days compares favorably with the 25.8 day half-life calculated for loss of dieldrin from the entire fish.

Comparison and Discussion of the Various Elimination Routes

Of the four possible insecticide excretion systems in fish, dieldrin was detected in the waste products of three systems (Table 9). Only the integument (mucus) failed to show any evidence of dieldrin excretion. The presence of dieldrin in urine, late in the experiment, suggested that some dieldrin may be eliminated via the kidney, but the quantities were very small and it is doubtful that a significant amount (less than 1%) is actually eliminated via this route in fish.

Dieldrin quantities eliminated by way of the intestine and feces were several fold higher than those observed in urine, but much lower than quantities detected in the water (Table 9). Only 2 or 3% of the absorbed dieldrin recovered in waste products was collected from feces with 95-98% recovered in the water.

TABLE 9.--The average and total quantity of excreted dieldrin recovered in waste products in each sample period and the percentage of total dieldrin recovered in water.

Sample Period	Water (µg)	Feces (µg)	Urine (µg)	Total (µg)	Per Cent in Water
3-days	5.6349	1.1665	0.0000	6.8014	82.8
6-days	2.7708	0.0826	0.0000	2.8534	97.0
9-days	2.1649	0.0377	0.0000	2.2026	98.3
12-days	2.6053	0.0742	0.0459	2.7254	95.6
15-days	2.6812	0.0494	0.0177	2.7483	97.6

Based on the actual quantities of dieldrin which were observed in waste products, the most important pathway of dieldrin elimination in green sunfish was across the gill--the same structure which serves as the primary route of insecticide uptake. Excluding the first sample period, at least 95% of all dieldrin recovered was eliminated via this route. However, if the assumption that the dieldrin lost from the fish but not recovered in waste products was actually eliminated with the feces, about two micrograms of dieldrin could be added to the amount actually observed in feces in each sample period. The intestinal pathway would then be much more important than the data actually indicated. If this assumption is correct, approximately 55% of the dieldrin excreted by a fish was eliminated via the gill and the remainder (45%)

via the feces. Regardless of the actual amounts of dieldrin detected in feces, the data demonstrates that the gill serves as an important route for dieldrin elimination.

A comparison of these results with those of others shows that green sunfish exhibited a slower rate of dieldrin loss than other species studied. Gakstatter and Weiss (1967) found that 90% of the absorbed dieldrin was eliminated from goldfish and bluegills in 14 days. This is about six times faster than the 85 days determined for the green sunfish. Grzenda (personal communication, 1968) found that seven weeks (49 days) was required for complete turnover of dieldrin in goldfish, which was also substantially faster than the rate of loss from sunfish.

Attempts to explain these large differences in rates of dieldrin elimination from fish are difficult because the techniques and experimental conditions vary considerably. However, it appears that the rate of dieldrin elimination may be related to the levels of dieldrin stored in the fish. Gakstatter and Weiss (1967) exposed their fish to a toxic concentration of dieldrin in water until the fish began to exhibit signs of insecticide poisoning. Under these conditions, the fish probably received a large dose. In the studies by Grzenda, the goldfish were fed a small dose daily until dieldrin reached an equilibrium level in the fish tissues. The

fish were then placed on an insecticide-free diet and the rate of dieldrin loss determined. The total body accumulation was probably less than in the fish used by Gakstatter and Weiss but this cannot be definitely verified since Grzenda's work has not been published. In the present study with green sunfish, the fish received a single sublethal dose and would have taken up less dieldrin than fish in either of the other two studies. If one can assume that dieldrin is accumulated in fish as postulated, then the rate of dieldrin elimination followed an inverse order. Additional evidence that the rate of elimination may be inversely related to the levels stored in fish was demonstrated by the increased dieldrin levels observed in water the first two days of the experiment when blood levels were presumably higher.

The fact that dieldrin was eliminated from fish in the feces and particularly from the gill has important implications with reference to the cycling of dieldrin and other insecticides in aquatic ecosystems. Hamelink (1969) proposed that DDT in a free or unbound state was a primary factor controlling equilibrium relationships between the natural environment and animals of the various trophic levels. The dieldrin eliminated via the gill would probably exist in such a form and thus perpetuate the presence of unbound dieldrin in water. In this form, the eliminated dieldrin would contribute to the continued

cycling of dieldrin among the various trophic levels of the ecosystem. Gakstatter and Weiss (1967) observed that both dieldrin and DDT were readily transferred from insecticide-exposed fish to control fish maintained in the same aquarium during recovery periods.

Knowledge of the rates of insecticide elimination from fish may have some practical implications. If one were permitted a choice of insecticides for use in a particular situation, it would appear plausible to select an insecticide which was rapidly excreted, providing other factors were the same for the choices available. Another possible application of knowing elimination rates would be in situations in which repeated doses of insecticide were necessary. Calculating the rate at which the insecticide was lost from a natural population of fish would provide meaningful data as to when it would be permissible to make a second application to avoid excessive losses of wildlife. Although many other factors which exist in such situations would also have to be taken into consideration, elimination rates would definitely be one useful criterion.

SUMMARY

The uptake, distribution, and elimination of a single oral dose of approximately 95 micrograms of dieldrin administered to green sunfish was determined. All fish were dosed at the same time and separate lots placed in specially constructed flow-through chambers at three-day intervals over a 15 day experimental period. Dieldrin losses via various excretory routes were monitored while fish were held in these chambers. Dieldrin distribution was evaluated from measurements of residue levels in tissues and organs removed from fish when they were recovered from the chambers at the end of a three-day interval. Application of regression analyses to changes in residue levels of various tissues, organs, and remaining carcass permitted estimates of the rate of dieldrin loss from the entire fish and also from individual tissues and organs. Procedural problems and the lack of statistical significance in the data occasionally precluded making definite statements regarding dieldrin elimination from fish. However, I believe the data adequately demonstrated the following findings:

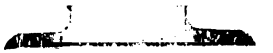
1. Green sunfish absorbed 82.23 µg of an average 94.93 µg of dieldrin administered demonstrating an 86.6%

efficiency for absorbing dieldrin across the intestinal tract.

2. Ranking the tissues and organs according to their dieldrin concentrations in each sample period demonstrated that dieldrin distribution remained relatively constant throughout the experiment, with the exception of the liver and gonad.

3. The tissues and organs analyzed could be grouped together according to their similarities in dieldrin residue levels. Visceral adipose tissue represented a level by itself containing dieldrin levels at least six-fold higher than in any other tissue. A group, possessing 1 to 4 ppm dieldrin, included gill, ovary, female liver, combined intestine and pyloric caeca sample, and lastly the gall bladder plus bile sample. Generally, those organs associated with insecticide elimination routes were in this latter group. A second group, having low dieldrin concentrations (less than 1.0 ppm), included kidney, male liver, testes, blood, stomach and muscle tissue.

4. In addition to noting previously observed differences in dieldrin residue levels in ovary and testis, differences in dieldrin concentrations in the liver of male and female fish were also noted. The higher dieldrin residue level observed in female livers was associated with ovarian development and probably represents a seasonal



phenomena since such a difference has not be previously observed.

5. Very little translocation among tissues was actually observed during the experiment. However, dieldrin levels declined in all tissues except the combined intestine and pyloric caeca sample which suggests that any translocation was mainly from sites of storage to sites of elimination. This general decline in residue levels in tissues plus the similarity of the biological half-lives for dieldrin loss from the fish body and from adipose tissue suggests that mobilization rates from dieldrin storage sites, particularly adipose tissue, may be the chief factor controlling dieldrin elimination rates.

6. The combined intestine and pyloric caeca sample demonstrated a slight increase in dieldrin levels during the experiment. Separate analyses of these two organs later in the experiment indicated that the residue levels in the intestine remained essentially constant, but that levels in the pyloric caeca increased with time. It is hypothesized that dieldrin transported to the intestine for elimination in the feces is resorbed from the digestive tract, particularly in the pyloric caeca.

7. Dieldrin was lost from fish at an exponential rate. The biological half-life for dieldrin in the entire fish was 25.3 days. The data suggest that dieldrin

was lost from individual tissues at different rates, but the results were not statistically significant.

8. Dieldrin was not detected in mucus and therefore, is probably not eliminated across the integument. Some dieldrin was eliminated in urine after the tenth day, but the quantities were small and it is doubtful that significant amounts of dieldrin are lost via the fish kidney.

9. The importance of the intestine and feces as an excretory pathway for insecticides was not definitely demonstrated. More dieldrin was lost from the fish than was actually recovered in waste products and it is thought that the missing dieldrin may have been eliminated, undetected, in the feces. Based upon the dieldrin amounts actually recovered in feces, less than 5% of the absorbed dieldrin was eliminated via this pathway. If, however, the missing dieldrin was lost via this route, the intestine may account for as much as 45% of the dieldrin eliminated from the fish.

10. The gill represented the major route for dieldrin elimination from fish. At least 95% of the dieldrin recovered was from the water passing over the fish. Even if the actual amount of dieldrin eliminated in feces was greater than that observed, the gill still accounted for slightly more than half (55%) of the dieldrin lost during the experiment.

A paradoxical condition existed in that, except for the first sample period, dieldrin levels in the water remained constant (average was 0.852 µg dieldrin/fish/day) whereas dieldrin levels in the gill and remaining fish declined at exponential rates.

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APPENDICES

APPENDIX A

PROCEDURES FOR EXTRACTION AND CLEANUP OF SAMPLES

APPENDIX A

Water

1. The carbon tetrachloride was separated from the water by pouring the contents of the graduate into a separatory funnel and draining off the carbon tetrachloride into a 250 ml erlenmeyer flask.
2. Approximately two grams of sodium sulfate (Na_2SO_4) was added to the flask and the carbon tetrachloride evaporated down until the Na_2SO_4 was just moist, by drawing an air current across the surface with an aspirator while the erlenmeyer was immersed in a warm-water bath.
3. Benzene (approximately 10 ml) was added, swirled with the Na_2SO_4 and evaporated down. This step was repeated two or three times with petroleum ether to remove traces of carbon tetrachloride.
4. The dieldrin was eluted off the Na_2SO_4 with more petroleum ether, decanted into glass-stoppered centrifuge tubes with several petroleum ether rinses and finally, brought to a constant volume (10 or 15 ml).

Urine

1. Urine was poured from a storage vial into a 125 ml separatory funnel, rinsing the vial once with distilled water and twice with petroleum ether.
2. The urine was then diluted with an equal volume of distilled water.
3. Petroleum ether was added (10 ml) and the mixture shaken vigorously for two minutes after which the two immiscible phases were allowed to separate. The mixture was then agitated a second time.
4. After partitioning, the aqueous phase was discarded.
5. The petroleum ether was dried with Na_2SO_4 and then decanted into glass-stoppered centrifuge tubes. The separatory funnel and Na_2SO_4 were rinsed with aliquots of ether and added to the centrifuge tube. The sample was then evaporated down to a constant volume (usually 4 ml).

Feces

1. Fecal material was separated from water in the feces trap by filtration (under vacuum) through a tared piece of glass wool.
2. The feces and glass wool were dried for at least 24 hours in an oven at 40° C. After removal from the oven, the material was cooled and weighed to obtain the dry weight of the feces.

3. The feces and glass wool were transferred to a mortar and ground with 4 or 5 ml of acetonitrile (CH_3CN). This step was repeated three times, transferring the acetonitrile (with filtration) to a 250 ml separatory funnel. Finally, the mortar, pestle, and filter were rinsed with CH_3CN.
4. Approximately 8 ml of petroleum ether was added and the mixture shaken for one minute (Clean-up step).
5. The acetonitrile was drawn off into a clean 1-liter separatory funnel and the ether shaken with another 5 ml of CH_3CN which was added to the original CH_3CN. (Total volume of CH_3CN was 30 ml.)
6. The acetonitrile was diluted 20 fold with 600 ml of a 1.0% sodium sulfate distilled water solution.
7. Sixty milliliters of petroleum ether were added and the mixture shaken for two minutes. The phases were allowed to separate and the aqueous portion was discarded.
8. The ether was dried with Na_2SO_4 and decanted into a 250 ml erlenmeyer flask, adding the ether from three rinses of the separatory funnel.
9. The petroleum ether was evaporated down in a current of air drawn across the solution surface with vacuum pressure, transferred to graduated centrifuge tubes and brought to a constant volume (usually 5 ml).

Tissues

1. Each sample was placed in a tared 100 ml beaker and weighed. Twenty milliliters of a 20% (w/v) KOH-methanol solution (prepared fresh daily) was then added.
2. The samples were heated on a hot plate with occasional stirring until all the tissue had digested (approximately 15 minutes). With blood, the alcoholic-KOH was added directly to the storage vial and heated until completely digested.
3. The sample was allowed to cool and then transferred to a 125 ml separatory funnel, rinsing the beaker once with a small amount of distilled water and twice with petroleum ether (total volume of ether was 20 ml).
4. The mixture was shaken vigorously for four minutes, the two phases allowed to separate, and the aqueous portion discarded.
5. The ether was shaken once more with 10 ml of distilled water to remove traces of the alcoholic-KOH. After separation, the water was discarded.
6. The petroleum ether was then decanted into an erlenmeyer, adding the ether from three separate rinses of the separatory funnel.
7. The ether was evaporated down to a few milliliters with a current of air while the erlenmeyer was immersed

in a warm-water bath. Using a disposable pipette, the ether was transferred to a graduated centrifuge tube, together with three ether rinses of the erlenmeyer, and brought to a constant volume (from 3 ml to 25 ml, depending upon the tissue).

APPENDIX B

DIELDRIN AMOUNTS AND CONCENTRATIONS FOUND IN TISSUES AND WASTE PRODUCTS OF EACH FISH FOR EACH SAMPLE PERIOD

3-DAY SAMPLE PERIOD (0-3 DAYS)

	Fish B	Fish C	Fish D	Cannulated Fish #1	Cannulated Fish #2
TISSUES Concentrations (µg/g = ppm)					
Blood	0.4836	0.3976	0.4079		
Stomach	0.7367	0.3782	0.4704		
Intestine and P. Caeca	6.5667	2.3278	1.6208		
Adipose Tissue	--	21.0837	25.9006		
Gill	2.9436	2.7270	2.3829		
Testis	0.6688	0.3393	--		
Ovary	--	--	1.5028		
Muscle	0.1684	0.1578	0.1450		
Kidney	0.7530	0.4569	0.6383		
Liver	0.5532	0.2768	3.5396		
Gall Bladder	4.3611	1.0999	--		
Remaining Carcass	1.3871	1.2978	1.4527		
WASTE PRODUCTS					
Feces					
Amount (µg)	1.1194	1.4984	0.8816		
Concentration (µg/g)	211.215	121.819	94.794		
Water					
Amount (µg)	2.1785	2.5262	0.9314		
Concentration (µg/liter)	0.019	0.0226	0.0086		
Urine					
Amount (µg)				0.0000	0.0000
Concentration (µg/liter)				0.0000	0.0000

6-DAY SAMPLE PERIOD (4-6 DAYS)

	Fish B	Fish C	Fish D	Cannulated Fish #1	Cannulated Fish #1
TISSUES Concentrations (µg/g = ppm)					
Blood	0.3264	0.4868	0.5502		
Stomach	0.3505	0.4140	0.6328		
Intestine and P. Caeca	2.0117	3.6302	6.2573		
Adipose Tissue	29.2196	31.3840	25.1050		
Gill	2.2005	2.3929	2.2897		
Testis	0.3540	0.6540	--		
Ovary	--	--	1.5545		
Muscle	0.1597	0.1944	0.1710		
Kidney	0.8250	0.8019	0.8525		
Liver	0.7688	0.8185	2.0124		
Gall Bladder	3.6759	1.2459	2.1200		
Remaining Carcass	1.2659	1.3230	1.4244		
WASTE PRODUCTS					
Feces					
Amount (µg)	0.0615	--	--		
Concentration (µg/g)	0.6741	--	--		
Water					
Amount (µg)	0.7134	1.3707	0.6899		
Concentration (µg/liter)	0.0060	0.0116	0.0060		
Urine					
Amount (µg)				0.0000	0.0000
Concentration (µg/liter)				0.0000	0.0000

9-DAY SAMPLE PERIOD (7-9 DAYS)

	Fish B	Fish C	Fish D	Cannulated Fish #1	Cannulated Fish #2
TISSUE Concentration (µg/g - ppm)					
Blood	0.3314	0.3001	0.5924		
Stomach	0.3203	0.2002	0.4545		
Intestine	1.1268	1.8084	2.8094		
Pyloric Caeca	0.5471	0.5745	1.5810		
Intestine and Caeca	1.6739	2.3829	4.3904		
Adipose Tissue	11.4441	18.3572	26.1252		
Gill	1.2094	1.7811	2.8210		
Testis	--	--	--		
Ovary	1.3606	1.8213	0.9473		
Muscle	0.0629	0.1259	0.1993		
Kidney	0.4406	0.4722	0.6294		
Liver	2.7353	2.9940	1.0019		
Gall Bladder	1.1250	1.1703	--		
Remaining Carcass	0.6261	0.9961	1.3749		
WASTE PRODUCTS					
Feces					
Amount (µg)	0.0360	0.0069	0.0414		
Concentration (µg/g)	0.5363	0.3078	0.6582		
Water					
Amount (µg)	0.5684	0.5143	1.0866		
Concentration (µg/liter)	0.0050	0.0043	0.0090		
Urine					
Amount (µg)				0.0000	0.0000
Concentration (µg/liter)				0.0000	0.0000

12-DAY SAMPLE PERIOD (10-12 DAYS)

	Fish B	Fish C	Fish D	Cannulated Fish #1	Cannulated Fish #2
TISSUES Concentrations (µg/g = ppm)					
Blood	0.3490	0.2592	0.2035		
Stomach	0.2711	0.3404	0.2452		
Intestine	1.2325	1.6230	4.1415		
Pyloric Caeca	2.4157	0.9656	1.0260		
Intestine and Caeca	3.6482	2.5886	5.1675		
Adipose Tissue	31.4977	22.0650	19.8699		
Gill	2.5373	1.7683	1.7863		
Testis	0.4096	0.1685	0.2453		
Ovary	--	--	--		
Muscle	0.1207	0.0773	0.0836		
Kidney	0.7034	0.4608	0.4434		
Liver	0.2067	0.8707	0.5531		
Gall Bladder	0.4659	--	1.2992		
Remaining Carcass	1.2493	0.7603	0.6862		
WASTE PRODUCTS					
Feces					
Amount (µg)	0.0550	0.0517	0.0592		
Concentration (µg/g)	0.9330	0.5429	0.5329		
Water					
Amount (µg)	0.8301	0.6125	1.0713		
Concentration (µg/liter)	0.0080	0.0053	0.0090		
Urine					
Amount (µg)				0.0000	0.0918
Concentration (µg/liter)				0.0000	0.0262

15-DAY SAMPLE PERIOD (13-15 DAYS)

	Fish B	Fish C	Fish D	Cannulated Fish #1	Cannulated Fish #2
TISSUES Concentration (µg/g = ppm)					
Blood	0.2422	0.2302	0.2395		
Stomach	0.2331	0.2293	0.2634		
Intestine	1.2586	2.8923	1.6915		
Pyloric Caeca	1.4984	2.6706	1.2381		
Intestine and Caeca	2.7570	5.5629	2.9296		
Adipose Tissue	18.3617	--	14.8970		
Gill	1.2954	1.5477	1.7603		
Testis	0.1610	0.1839	0.1822		
Ovary	--	--	--		
Muscle	0.0925	0.1060	0.1236		
Kidney	0.3853	0.4653	0.3908		
Liver	0.1547	0.0861	0.2821		
Gall Bladder	1.0098	0.9936	0.3860		
Remaining Carcass	0.8832	0.7262	0.8042		
WASTE PRODUCTS					
Feces					
Amount (µg)	0.0225	0.0372	0.0571		
Concentration (µg/g)	0.2734	0.3855	0.4261		
Water					
Amount (µg)	0.7242	1.4046	0.6924		
Concentration (µg/liter)	0.0063	0.0120	0.0060		
Urine					
Amount (µg)				0.0064	0.0113
Concentration (µg/liter)				0.0010	0.0033