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The University of Michigan, Ph.D., 1972
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THE UPTAKE, STORAGE, AND RELEASE OF DIELDRIN AND SOME EFFECTS OF ITS RELEASE IN THE FISH, *CICHLASOMA BIMACULATUM* (LINNAEUS)

by

Donald Lee Brockway

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Fisheries)
in The University of Michigan
1972

Doctoral Committee:

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Professor K. F. Lagler

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INTRODUCTION

The objective of this study was to determine the effects of the release of a persistent organochlorine insecticide from its storage in adipose tissue when a fish is forced to utilize fat stores. Based on the results of this study an attempt was made to evaluate the consequences of a period of stress imposed on a fish following environmental contamination with organochlorines.

The aquatic environment is contaminated by a large, variable burden of organic wastes--a complex chemical load, of which pesticides make up a considerable part. Before World War II, the principal pesticides used were inorganic compounds such as the arsenicals and naturally occurring poisons like pyrethrum, rotenone, and nicotine. Since 1945, however, synthetic chlorinated organic insecticides--such as DDT, dieldrin, and chlordane--have been used in increasing amounts. In 1965, 30% of the 437,000 tons of pesticides produced in the United States were organochlorines (USDA, 1966). By 1967, although the production of DDT was decreasing, the production of other organochlorines was increasing enough to cause an increase in the total production of organochlorines (USDA, 1967). Within the last three years another form of organochlorine, the polychlorinated

biphenyls (PCB's), has received considerable attention. Production figures are not available for these industrial chemicals, but they are used in great quantity and are considered to be widespread in the environment.

The stability of organochlorines has resulted in their persistence in the environment, creating world-wide environmental and health hazards. These compounds have been found in tissues of fishes from all over the United States, and DDT, in particular, has been found in the oil of fishes that live in mid-ocean and in fishes caught near the coasts of North America, South America, Europe, and Asia (President's Science Advisory Committee, 1963). Even Adelie penguins in Antarctica contain residues of DDT and its metabolites (Sladen, Menzie, and Reichel, 1966).

Acute and Chronic Toxicity

Initial investigations into the toxicity to fishes of organochlorines were acute toxicity bioassays, in which toxicity of the poisons over relatively short test periods was expressed in terms of the median tolerance limit (TL_m = concentration of a poison required to kill 50% of the test organisms in a given period of time). The most familiar and toxic of the persistent insecticides, in decreasing order of toxicity, are endrin, toxaphene, dieldrin, aldrin, and DDT. The 96-hour TL_m values for these poisons for the bluegill

(Lepomis macrochirus)[1] in soft water range from 0.6 parts per billion (ppb; micrograms per liter) for endrin to 16.0 ppb for DDT (Henderson et al., 1960). Katz and Chadwick (1961) studied the toxicity of endrin to some fishes of the Pacific Northwest and found the coho salmon (Oncorhynchus kisutch) to be the most susceptible with a 96-hour TL_m of 0.27 ppb. They found the tolerance of the marine three-spine stickleback (Gasterosteus aculeatus) to endrin did not vary much in waters with salinities between 5 and 27 parts per thousand. Mount (1962) investigated the chronic effects of endrin on the bluntnose minnow (Pimephales promelas) and the guppy (Lebistes reticulatus) and found toxic levels for chronic exposures to be not greatly different from acute concentrations.

Field Problems and Fish Kills

In addition to bioassay work, past research has included field investigations of fish kills and other population declines. Compilations of the number of fish killed by pollutants including pesticides have been made annually since 1961, first by the U. S. Public Health Service (1965), and later by the Federal Water Pollution Control Administration (1968). The number reported has varied from less than 100,000 in 1960 to more than 11.5 million in 1967.

---

[1]Insofar as practical, the common and scientific names in this paper follow Special Publication No. 6 of the American Fisheries Society, 1970.

Harrington and Bidlingmayer (1958) reported that 20 to 30 tons of fish were killed when dieldrin was sprayed on a salt marsh to eliminate sandfly larvae. An investigation into the cause of massive fish kills in the lower Mississippi River in 1963 clearly established endrin as the cause of the mortality (Mount and Putnicki, 1966).

Opportunity for a unique field study was created in New Brunswick in 1954 when large areas were sprayed with DDT to control a spruce budworm outbreak. The population of the Atlantic salmon (Salmo salar) of the Miramichi River had been studied for several years before the spraying commenced; thus, fortunately, there were baselines to which population density changes could be compared. After the spraying there was a substantial decrease in abundance of fry, but a lesser decline in older age-groups. The spraying was repeated, however, and all year-classes in the population were repeatedly exposed. Keenleyside (1959) predicted that future runs of the salmon would probably be severely limited.

In an experimental field study in Montana, selected trout streams were sprayed with DDT. Stream concentrations were reported to be above the lethal level, but mortality was not observed for several months (Graham, 1960). The following summer the population had decreased only slightly; this decrease could have been normal population fluctuation.

Concentrations Built Up in Food Chains

When it was realized how widely spread persistent pesticides had become in aquatic organisms, investigations were initiated to determine the mechanisms of pesticide distribution. Perhaps the best known study of the transfer of an insecticide through a food chain was made in Clear Lake, California, following sprayings with DDD for gnat control (Hunt and Bischoff, 1960). Thirteen months after the spraying, the concentration of DDD in lake plankton was 10 mg/kg (ppm); in fat of plankton-eating fishes, 903 mg/kg; in fat of carnivorous fishes, 2690 mg/kg; and in fat from fish-eating birds 2134 mg/kg (Hunt, 1966). Subsequent to the sprayings there was a die-off of the western grebe (Aechmophorus occidentalis) at the lake, ostensibly due to the build-up of high levels of DDD via food-chain transfer (Hunt & Bischoff, 1960).

Similarly, there was an unusually high mortality of piscivorous birds between 1960 and 1962 reported at the Tule Lake National Wildlife Refuge in California which was attributed to food-chain transfer of toxaphene (Keith, 1966).

In a Long Island salt marsh various organisms showed concentrations of DDT increasing with trophic level from 0.04 ppm in plankton to 75 ppm in a ring-billed gull (Larus delawarensis) (Woodwell, Wurster, and Isaacson, 1967). In general, the highest concentrations were in scavenger and carnivorous fishes and birds, with birds having 10 to 100

times more than the fishes. This phenomenon of concentrations increasing with trophic level was here termed "biological magnification".

Insecticides may be taken up directly by living organisms, or they may adsorb onto, or be absorbed into lower forms of life or inert substances that are subsequently ingested by larger organisms. Thus, persistent insecticides which have been stored may be passed up the food chain and accumulated. Such passage may apply to a terrestrial organism (such as a piscivorous bird), which is the last link in an otherwise aquatic food chain, since this is the principal contact which the bird has with the aquatic medium. There is evidence now, however, that the higher concentrations as one goes up the food chain may be a result of direct uptake from water and not completely a result of passage up the chain.

Reinert (1967), using a simple food chain consisting of the alga (Scenedesmus obliguus), the water flea (Daphnia magna) and the guppy (Lebistes reticulatus), concluded that it was the dieldrin in the water rather than that in the algae that was responsible for the build-up of dieldrin in the daphnia, when algae and daphnia were exposed to the same initial concentrations. He also showed that guppies held in dieldrin-free water and fed daphnia raised in a dieldrin solution, accumulated about 90% less dieldrin in 32 days than did guppies held for 32 days in a dieldrin solution of the same concentration as that in which the above daphnia were

raised; the latter guppies were fed non-exposed daphnia. Algae, daphnia, and guppies, raised separately in water of the same dieldrin level, had concentration factors of 1,202, 13,954 and 49,307, respectively. Reinert believed that this increase in concentration factor from lower to higher levels in the food chain arising from direct uptake from the water had been confused by earlier workers with accumulation from food. As stated previously, this direct uptake can occur only in the aquatic portion of a food chain, and not in terrestrial organisms.

Internal Effects of Organochlorine Insecticides

Many investigations in recent years have attempted to determine how and by what mode of action internal functions of organisms are influenced by insecticides. For organochlorines the question of mode of action is still unanswered. However, it is believed that such action is on the nervous system, as suggested by the demonstration that DDT forms a charge-transfer complex with a component of the nerve axon, thus causing a disturbance of function (O'Brien and Matsumura, 1964). There is also some evidence that the action of organochlorines is by the inhibition of an ATPase (Koch, 1969).

Much of the work on tissue effects of organochlorines is inconclusive. The only results which show much consistency concern the liver. Lowe (1964) reported

degeneration of the liver and thickening of the gill lamellae after exposure of the spot (Leiostomus xanthurus) to toxaphene. Marked histopathological conditions attributed to DDT have also been found in the liver of the guppy and the brown trout (Salmo trutta) (King, 1962), and liver damage due to BHC (benzene hexachloride) has been reported (Rudd and Genelly, 1956). Allison et al. (1964), however, found no histopathological changes due to DDT.

A number of physiological parameters have been studied in insecticide-exposed fish. Increased hematocrit values in the bluegill were observed after exposure to heptachlor (Andrews et al., 1966); Allison et al. (1964) observed no changes in microhematocrits due to DDT; and Mount and Putnicki (1966) reported lower than normal hematocrit values in fishes from the 1963 Mississippi River fish kills.

An effect of endrin exposure on normal liver function of the northern puffer (Sphaeroides maculatus) was indicated when cations were transferred from hepatic tissue into serum (Eisler and Edmunds, 1966). It was shown that concentrations of sodium, potassium, calcium and cholesterol increased in the serum, and those of sodium, potassium, calcium, magnesium, and zinc decreased in the liver of exposed fish.

Extremely important are internal effects of organochlorines on reproduction. Burdick et al. (1964), studying the causes of hatchery losses of fry of the lake trout (Salvelinus namaycush), found that the loss started when the

fry were starting to feed. Large amounts of DDT had been used in the surrounding watershed and high concentrations of DDT were found in the lipids of eggs. These lipids are metabolized during the sac-fry stage. Fry from crosses between females from Lake George and males from other waters failed to survive, but normal survival occurred when males from Lake George were crossed with females from other lakes. It was concluded that fry mortality was caused by DDT from the yolk sacs. Similar results were found by Brockway (1963) when the egg-laying acara (Cichlasoma bimaculatum) was exposed to pentachlorophenol. There was no effect upon the frequency of reproduction, but sac-fry died as soon as the yolk was absorbed.

Similar results were also obtained by Allison et al. (1964) who fed food contaminated with DDT to the cutthroat trout (Salmo clarki). The number and volume of eggs produced were not reduced by DDT but mortality among sac-fry appeared to be highest in the high dosage lots. Macek (1968) also reported increased fry mortality at the time of most rapid utilization of yolk, with eggs from DDT-treated brook trout (Salvelinus fontinalis).

It has been shown that accumulated DDT or dieldrin is stored extensively in the fat depots of the body (Woodard, Ofner, and Montgomery, 1945; Holden, 1962; and Bäckström, Hansson, and Ullberg, 1965). In the literature there has been the implication that when a fish undergoes a period of stress, the organochlorines stored in body fats will be

released into the blood to cause delayed toxication and increased stress (Warner, 1967, and Woodwell, personal communication). The need for research in this area was recognized by Cope (1960: p. 74): "It is also significant to learn about storage and release from storage in relation to effects on growth, on reproduction, on behavior, and on genetics."

In experiments with warm-blooded animals, both birds and rats have been studied. From studies of the robin (Turdus migratorius) and the sparrow (Passer domesticus), Bernard (1963) concluded that DDT stored in fat tissue was released during starvation and transferred to "vital" organs (brain and liver). In rats, mobilization of body fat during starvation resulted in an increased concentration of DDT-derived material in the remaining fat tissue, a corresponding increase in other tissues, and an increased excretion of DDT metabolites (Dale, Gaines, and Hayes, 1962). Also, there was an increase in concentration of DDT in the brain, which was associated with starvation and was correlated with the occurrence of signs of poisoning. Brain accumulation during starvation has not been reported in fish.

The only study of the effects of release of stored pesticides in fish has been done with endrin, the most toxic organochlorine insecticide presently in use. Mount (personal communication) observed deaths in the channel catfish (Ictalurus punctatus) during a period of starvation

following exposure to endrin, whereas starved control fish survived.

Since the toxicological effects of release of stored organochlorines in fish had not been investigated, I decided to study the release effects of stored dieldrin on a cichlid fish (Cichlasoma bimaculatum). Since dieldrin is less toxic than endrin, I felt such release effects might not be lethal; thus, possible sublethal effects should also be investigated.

Measurement of Non-Lethal Effects

Whole-body concentrations as well as most individual tissue concentrations of insecticides generally show little or no correlation with the physiological state of the organism. However, the levels of insecticide in a particular tissue, blood, have been correlated with death. Mount and Putnicki (1966) showed that all fish that died when exposed to endrin had an endrin-blood level above 0.22 ppm and surviving fish always had a level below 0.28 ppm; very few living or dead fish had endrin-blood levels between the two values. Therefore, I hypothesized that the concentration of dieldrin in blood could be used to indicate increased release of dieldrin from storage in addition to its proven use as an indicator of cause of death.

A second measure of non-lethal effects was desired for this analysis--preferably one which would give a broad,

non-specific indication of the physiological condition of the fish. Recent work by McKim (1966) showed that urinary excretion of steroid hormone metabolites increased in the rainbow trout (Salmo gardneri) when the fish was stressed with alkyl-benzene sulfate detergent (ABS), low dissolved oxygen, or both. His work was based on the demonstration by Selye (1946) that non-specific stresses increase the activity of the adrenal gland.

It has been shown that levels of 17-hydroxycorticosteroids (17-OHCS) in the blood increase when the carp (Cyprinus carpio) is subjected to a forced swimming stress (Hatey, 1958). He also showed that carp carried out of water have higher levels of 17-OHCS in the blood than fish which have not been handled.

The production of 17-OHCS within the interrenal tissue of a cichlid (Tilapia mossambica) has been demonstrated with in vitro tissue cultures (Nandi and Bern, 1960). On the basis of all these studies, and with the assumption that dieldrin mobilization would cause stress, the level of urinary 17-OHCS was chosen as an indicator of non-lethal physiological effects of dieldrin.

The planned sequence of steps for my research follows:

1. Establish blood and brain levels of dieldrin in fish killed by dieldrin as a basis for comparison with levels in living fish.

2. Study the uptake and storage of dieldrin by measuring levels in blood, brain, and the whole body minus blood and brain.

3. Study the effects of various post-exposure conditions on the release of dieldrin from storage.

4. Measure changes in dieldrin levels in blood and brain as indicators of release of dieldrin.

5. Measure urinary excretion of 17-OHCS hormone metabolites as an indicator of dieldrin effects.

MATERIALS, APPARATUS, AND METHODS

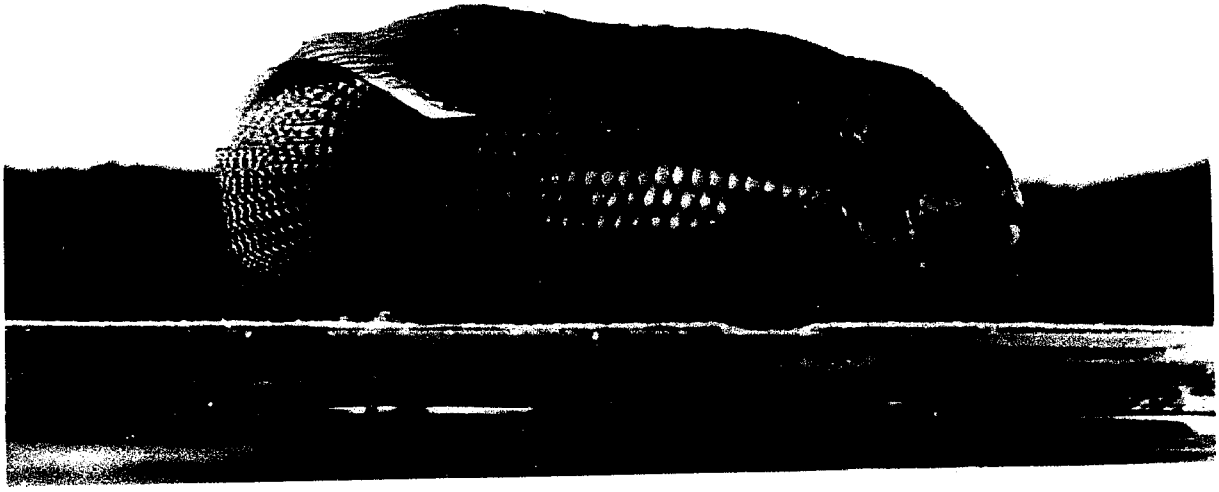
Experimental Animals

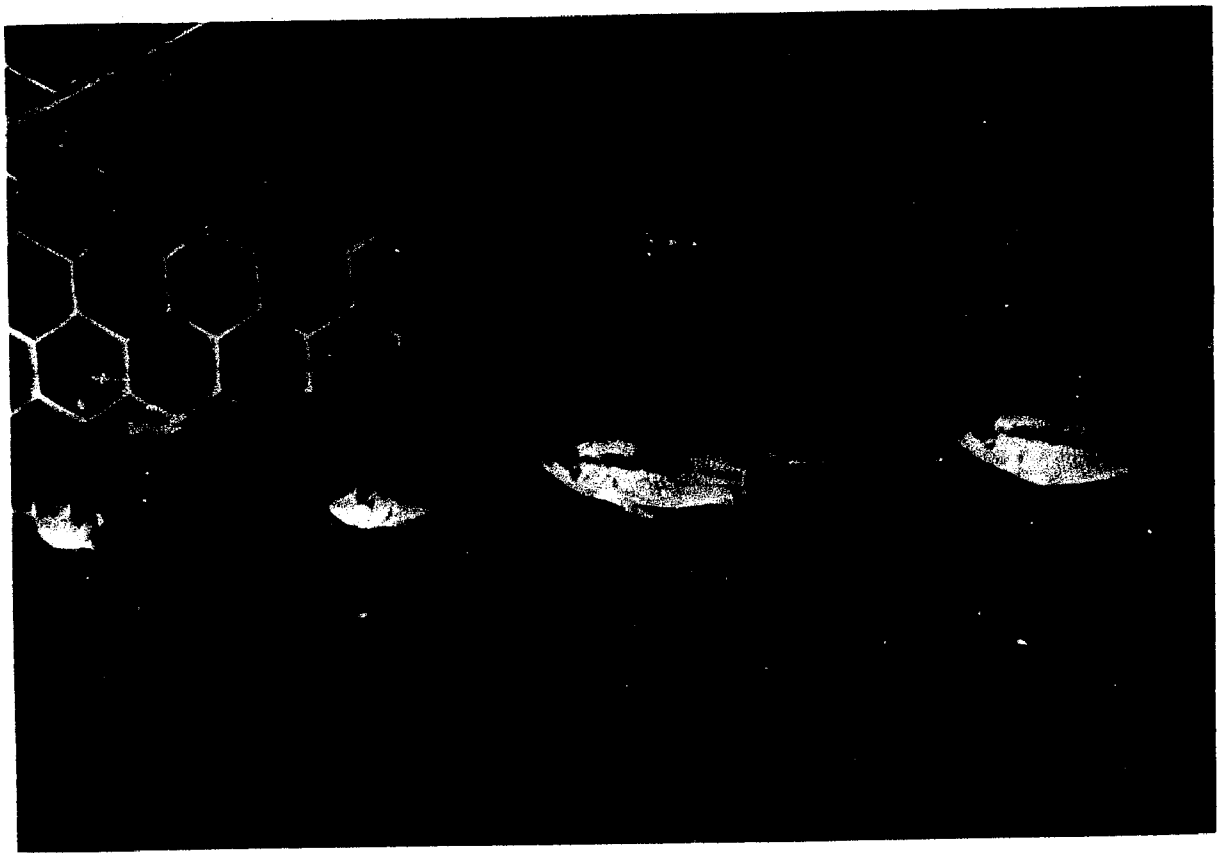
The test animal used in this research was the tropical aquarium fish, Cichlasoma bimaculatum (Linnaeus), which is native to South America (Figure 1). This fish was selected because of its proven utility as a test animal (Brockway, 1963). Since it spawns in aquaria throughout the year and produces large numbers of offspring, fish of a desired size with a known environmental history can be made available at all times. The resistance of this species to disease under crowded conditions in laboratory tanks and its ability to withstand handling are other desirable characteristics. An unfavorable characteristic is its aggressive behavior; when confined in a small tank, less aggressive individuals are frequently killed.

In December, 1965, fifty immature fish were obtained from the Department of Fisheries and Wildlife, Oregon State University. These fish and their progeny supplied the test animals used in all experiments. For an unknown reason, good reproductive success was never attained, so desired numbers of individuals of a consistent size were not always available.

Figure 1a.--An adult male cichlid (*Cichlasoma bimaculatum*) Linnaeus.

Figure 1b.--Immature cichlids (*C. bimaculatum*) used in experiments.





Young fish between 6 and 19 g were held in 200-gallon, circular, insulated, fiberglass tanks with flowing water. All other fish were held in 15- or 50-gallon glass aquaria equipped with air-lift filters. Fry up to 15 mm were fed newly-hatched brine shrimp. Larger fish were fed dry tropical fish food and trout-food pellets. This diet was supplemented by feedings of live daphnia about twice a week. All fish were held at a temperature of 24°C.

Experimental Apparatus

Water Supply

Water used in all experiments was water from the Ann Arbor city water supply which was modified by the following laboratory treatment. The water was first passed through a Permutit dechlorinator having a capacity of nine gallons per minute. At the outlet of the dechlorinator, the pH of the water was about 10 and the temperature fell as low as 6°C in the winter. Water was then passed through a Powers Foto-Guard mixer where the temperature was preheated to 20°C by mixing cold dechlorinated water with very hot tap water. The heated water then flowed through a float valve into a 250-gallon wooden tank. In this tank pH was regulated, dissolved gases were brought into equilibrium with the atmosphere by aeration, and the temperature was raised to 25°C. When the rate of withdrawal from the head tank was about one gallon per minute, the pH was adjusted to the

range of 7.2 to 8.7 by dripping a 50% solution of concentrated sulfuric acid into the tank from a Mariotte bottle at a rate of about 0.2 ml per minute. Water was heated by two 1500-watt electric heaters, each with a thermostat. From the head tank the water flowed by gravity to the distribution jars of the experimental apparatus.

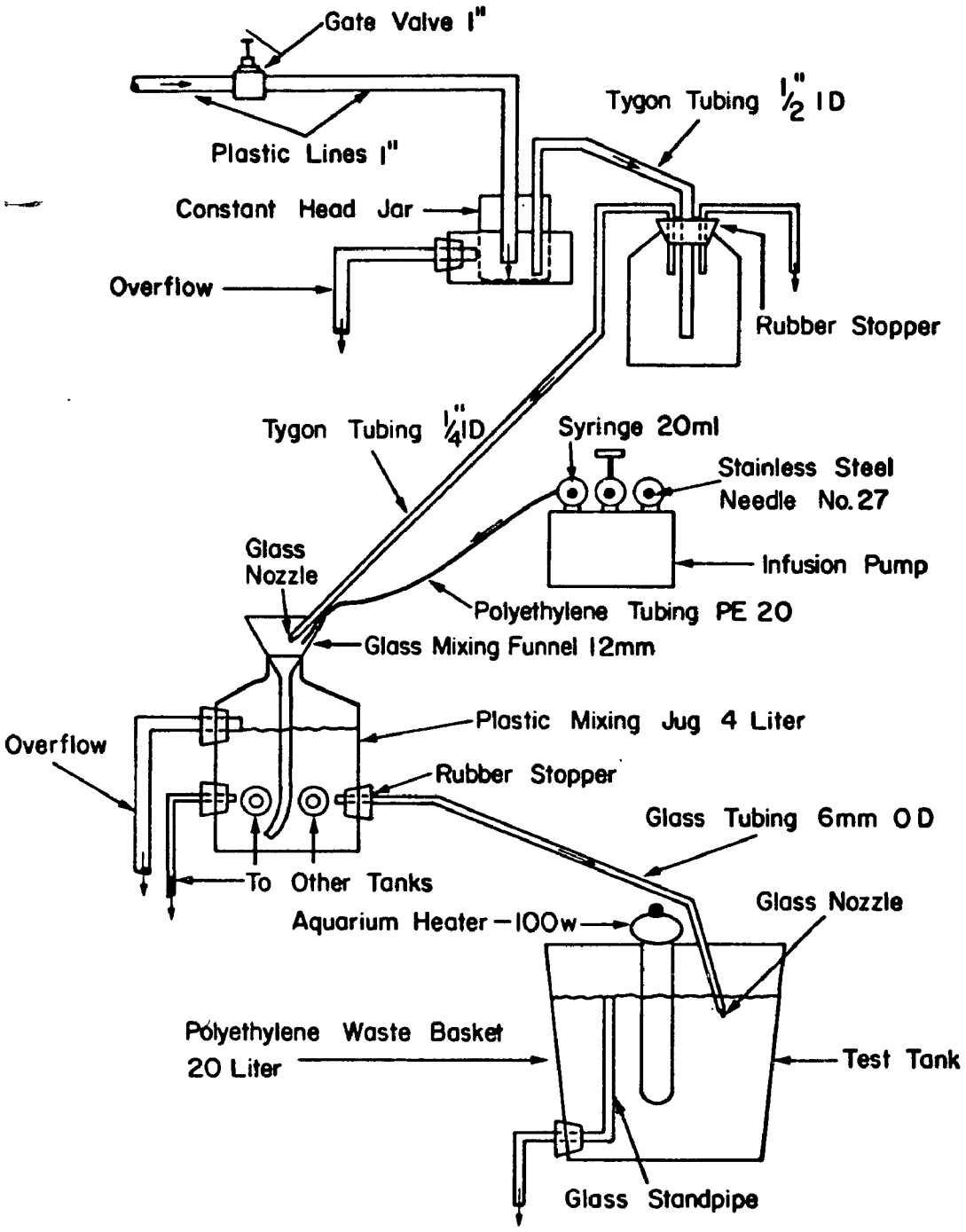
The quality of water in the experimental apparatus was monitored periodically and varied as follows: 1) temperature (24 to 25°C except as noted below); 2) dissolved oxygen (above 5.5 mg per liter, except in one experiment as noted); 3) pH (7.2 to 8.7); 4) chlorine (none detectable by the orthotolidine method); 5) M.O. alkalinity (50 to 65 ppm as $CaCO_3$).

Continuous-Flow Apparatus (Figure 2)

Experimental tanks used in this apparatus were inexpensive polyethylene waste baskets. Water level was set at 15 liters with 8-mm OD glass standpipes. The test tanks were covered with coarse mesh, neoprene-coated matting.

Dilution water from the head tank was delivered through polyethylene pipes and valves to an overflowing constant-head jar. Water then flowed into a distribution jar and out to the mixing jugs. A constant flow into each mixing jug was maintained by a drawn-glass nozzle. Mixing jugs were one-gallon polyethylene bottles with a 5-inch, long-stem funnel inserted in the mouth. A piece of Tygon tubing extended from the funnel to the bottom of the bottle.

Figure 2.--Diagram of constant-flow exposure apparatus.


Gate Valve 1"
Tygon Tubing 1/2" ID
Plastic Lines 1"
Constant Head Jar
Overflow
Rubber Stopper
Tygon Tubing 1/4" ID
Syringe 20ml
Stainless Steel
Needle No. 27
Glass
Nozzle
Infusion Pump
Polyethylene Tubing PE 20
Glass Mixing Funnel 12mm
Plastic Mixing Jug 4 Liter
Overflow
Rubber Stopper
Glass Tubing 6mm OD
To Other Tanks
Aquarium Heater - 100w
Glass Nozzle
Polyethylene Waste Basket
20 Liter
Test Tank
Glass Standpipe

Glass tubes with short Tygon connections and terminal glass nozzles conveyed the toxicant solution to the test tanks. Test and control solutions were introduced into each tank at a rate of about 110 ml per minute.

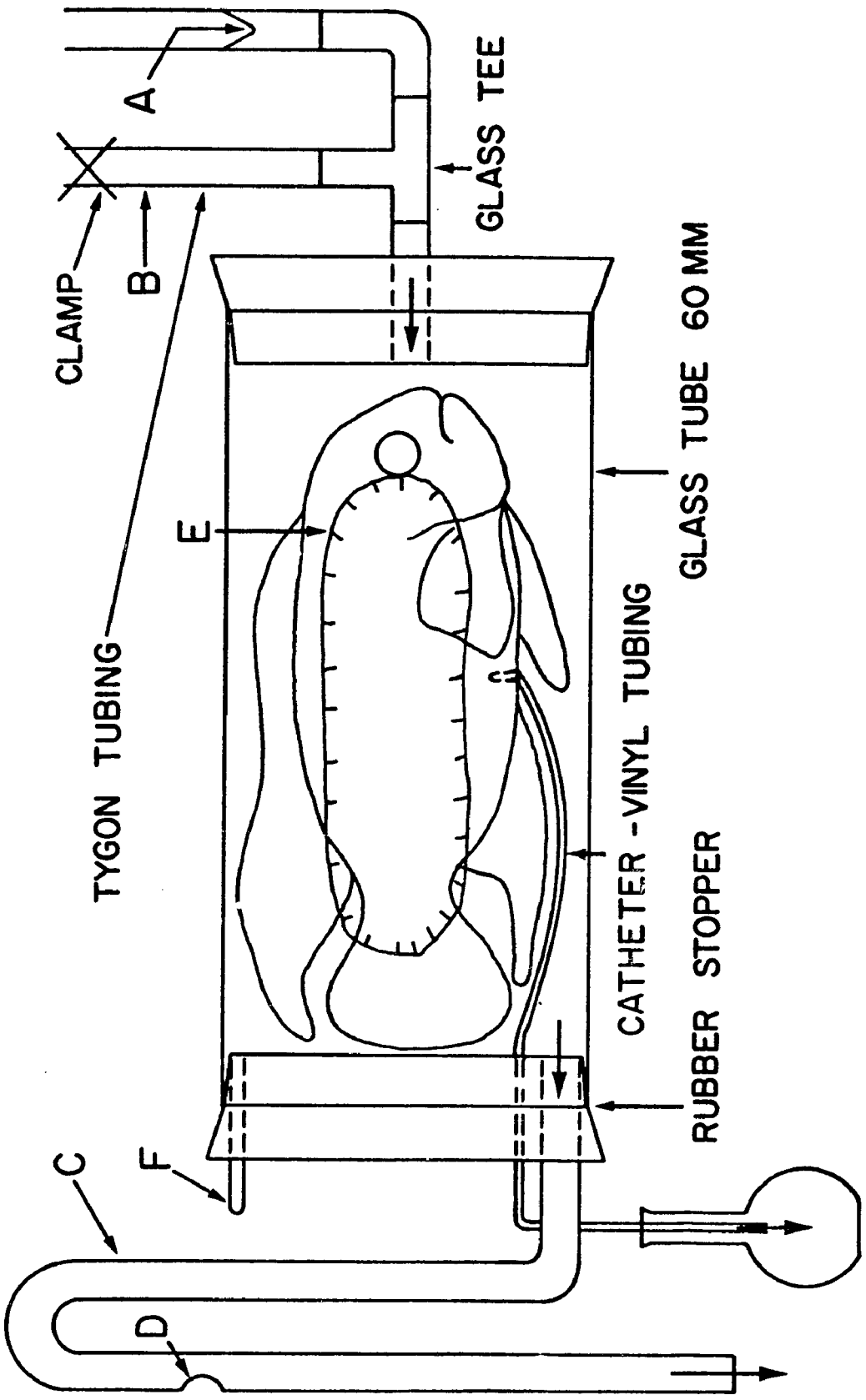
An infusion pump delivered solutions of dieldrin dissolved in acetone to the test mixing jugs. Acetone alone was delivered to the control mixing jug. The infusion pump was equipped with 20-ml glass syringes and No. 27 stainless steel needles. Intramedic polyethylene tubing (PE-20) connected the needles with the mixing jugs.

Urine-Collection Apparatus (Figure 3)

The continuous-flow apparatus used for urine collection resembles McKim's (1966) modification of that of Fromm (1963). Some additional modifications were made.

Water was delivered to fish-holding chambers via a system of a constant-head jar, a distribution jar, and a mixing jug, identical to the system for the continuous-flow apparatus (Figure 2). Water flowed by gravity from the mixing jug, through an air trap (Figure 3, B), which assured that flow would not be stopped, and then to the fish-holding chamber. Flow rate to each chamber was regulated by a glass nozzle inserted into the Tygon tubing connecting each chamber with the mixing jug (Figure 3, A). The water-flow rate through each chamber was about 110 ml per minute, which assured there would be no appreciable reduction in dissolved oxygen due to respiration of the fish. Outflow water passed

Figure 3.--Fish urine-collection apparatus. (A) Glass nozzle, (B) Air trap, (C) Latex tube outflow, (D) Hole in tube above chamber, (E) Constriction in each side of glass tube, (F) Glass rod in air release hole.

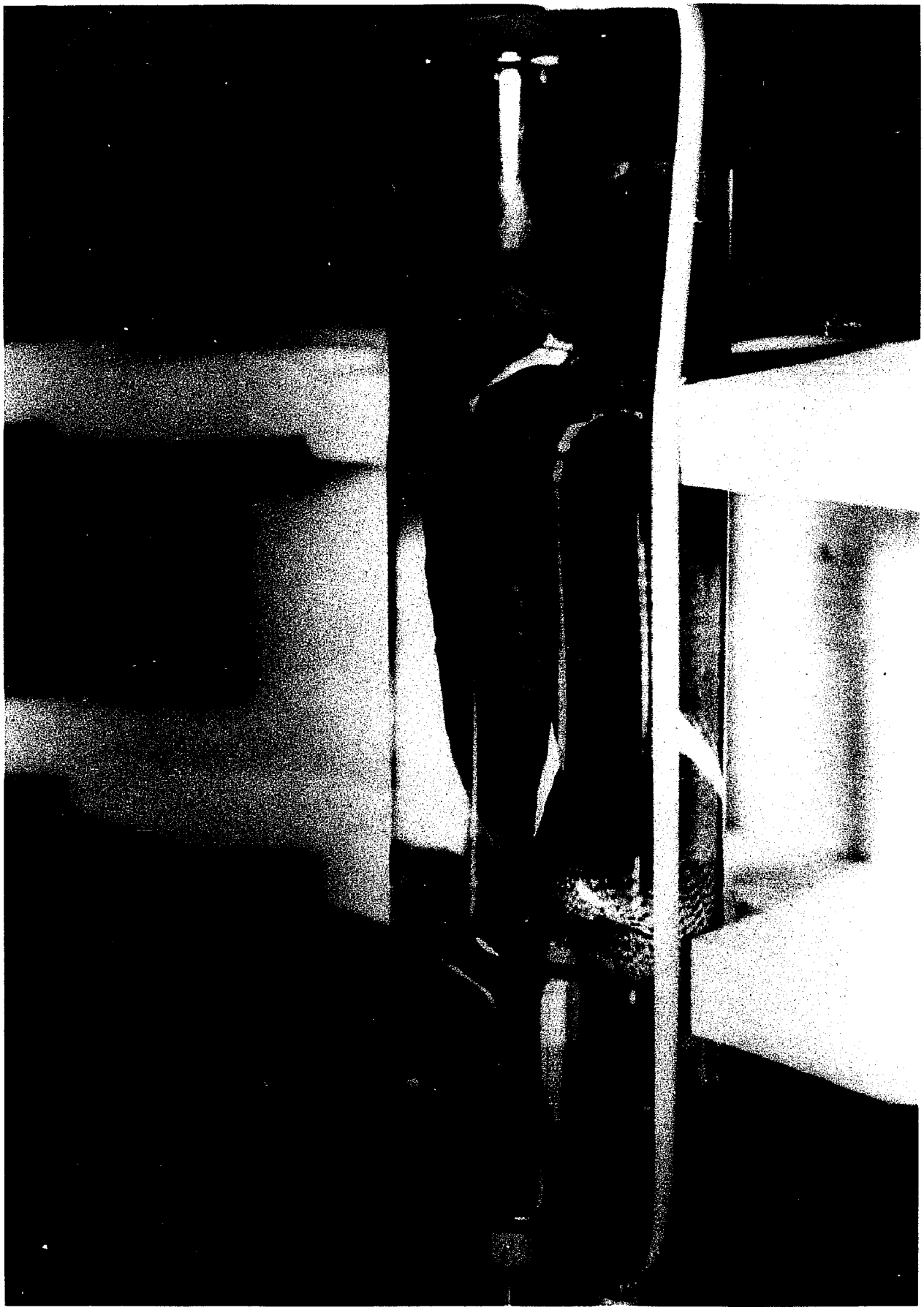

A
GLASS TEE
CLAMP
B
GLASS TUBE 60 MM
E
TYGON TUBING
CATHETER - VINYL TUBING
RUBBER STOPPER
C
F
D

into a latex tube which looped upward (Figure 3, C) about 20 cm above the chamber before going downward into the drain. This loop maintained a slight positive pressure inside the chamber, which allowed urine to flow out the catheter. A hole cut in the latex tube in the descending side of the loop (Figure 3, D) served to break any siphon from the tube.

Fish chambers were 57, 60, or 64 mm OD glass tubing (depending on fish size). Constrictions (Figure 3, E) molded into each side prevented the short, moderately compressed test animals from turning around. Chambers were closed at both ends with large rubber stoppers. Glass tubes inserted through the stoppers allowed passage of water. The tube at the outflow was located at the bottom of the stoppers. This allowed settled solids to pass out of the chamber. A small hole at the top of the outflow stopper (Figure 3, F) permitted release of gas. A glass rod was inserted into this hole as a plug. This air release and an air trap in the line were required to remove gases arising from the heated water. To obtain a temperature of 30°C, aquarium heaters were put into the constant-head jar. Figure 4 shows a cichlid confined within a fish chamber.

If desired, the apparatus could be modified for exposure of fish in the chambers to toxicants. Solutions could be introduced with an infusion pump into the mixing jug and control water could then be taken by additional tubes from the distribution jar.

Figure 4.--Test animal confined within the urine-collection apparatus. Catheter is shown above and behind fish (not in place).



Experimental Methods

Threshold Blood and Brain Concentrations in Fish Exposed to Acutely Toxic Water Concentrations of Dieldrin

Fish were exposed to lethal concentrations of dieldrin to determine if there is a threshold blood or brain concentration above which fish would die. Fish thus exposed survived for one to nine days depending on the concentration used. Concentrations between 3.5 and 9.0 ppb were used. To determine the blood concentration at time of death, fish were used only if they were under observation at the time of death or were known to have died a few minutes before the blood sample was taken. Fish dead an unknown period of time were discarded.

Blood and brain concentrations of dead fish were compared with blood and brain concentrations of live fish exposed to high sublethal levels of dieldrin. Seven of the 15 values for blood of live fish were from those fish which had been exposed to 1.8 to 2.0 ppb (a level which is lethal to some fish after 28 days) for 25 to 29 days, and then sacrificed. The remaining eight values were those of fish which had been exposed to 2.0 to 2.1 ppb for 25 to 29 days and then removed from dieldrin for 4 to 16 days, but whose blood values had not yet started to decrease. A better control would have been fish surviving in 3.5 to 9 ppb dieldrin, but because of a shortage of fish of the proper size, those available were used in other experiments.

Values for dieldrin in brains of live fish were obtained in a similar manner. The brains of all of the fish mentioned above were used, plus six others exposed to 2.0 to 2.1 ppb and one from a fish exposed to 1.1 ppb dieldrin, because it was the highest concentration measured in the brain from any live fish.

Blood samples were taken from the ventral aorta with a blood pipette previously treated with a 15% heparin solution. All possible blood was extracted, which for this species amounted to about 1% of the body weight. The brain was then removed and the brain and remaining whole fish were wrapped in aluminum foil, labeled, and frozen.

Sublethal Uptake and Release Experiments

To study the uptake, storage, and release of dieldrin, fish were exposed to sublethal levels in aqueous solution (Table 1). Exposure was continued until blood and whole-body concentrations leveled off as determined by analysis of tissues in Experiment I. At the start of each experiment, fish were marked by fin-clips so that gains or losses in weight of individuals could be followed. The number of test fish in each sample of each experiment is given in the respective figure in the results section. Control fish were carried through all experimental procedures except that dieldrin was absent.

These experiments consisted of two phases: 1) a dieldrin-exposure (uptake) phase; 2) a post-exposure

Table 1.--Sublethal dieldrin uptake and release experiments

Experiment Number	Dieldrin Concentration (ppb)	Days of Exposure to Dieldrin	Treatment of Fish After Dieldrin Exposure	Dissolved Oxygen (mg/l)	Size Range of Fish (grams)
I	1.1	33	Fasting	above 5.5	5.0-12.8
II	1.1	33	Fasting plus 5°C temp. elevation	down to 0.5	6.9-13.3
III	2.0	25	Fasting	above 5.5	7.0-13.6
IV	1.2	34	Fasting plus 5°C temp. elevation	above 5.5	6.6-13.3
V	2.1	29	Fasting plus 5°C temp. elevation	above 5.5	6.4-11.2
VI	1.2	29	5°C temp. elevation	above 5.5	9.6-19.8
VII	2.0	29	5°C temp. elevation	above 5.5	9.3-15.3

(release) phase in clean water. Samples taken during both stages were treated as given above for fish that received acute dosages, and consisted of blood (taken and analyzed immediately) and the brain and whole fish (frozen for later analysis).

Sample sizes and times varied in all experiments. These factors were dependent upon the number of fish available and the data being sought as determined by results of previous experiments. During the dieldrin-exposure phase of the tests the fish were fed daily, as previously stated, and excess food and debris were siphoned from the tanks every two days.

When the exposure period ended, the introduction of dieldrin into the inflowing water was stopped. Within 24 hours the dieldrin concentration in the tanks decreased to less than 5% of the exposure concentration. The animals were then exposed only to water and whatever dieldrin might slowly desorb from the walls of the chamber. At this time, one or two of the following treatments were imposed on the test animals: fasting, a 5°C temperature elevation, or both fasting and the temperature elevation (Table 1). During this release period, samples were taken at various intervals as shown in the respective figures in the results section. Fish not previously exposed to dieldrin, which were held in water without dieldrin in the above apparatus and treated similarly, served as controls.

Some of the samples taken during the experiments were from fish which appeared to be very emaciated or fatigued. Upon analysis, the parameters analyzed were much lower than in other fish at the same time. Since these fish were very low in fat, an arbitrary decision was made to discard fish with a fat content of 1.9% or less. The emaciation was most likely the result of aggression by other fish.

Urine-Collection Experiments

The dieldrin-exposure portion of the urine-collection experiments was run in the same continuous-flow apparatus as the other sublethal experiments. Adult males (apparently healthy) of the same cichlid species, weighing between 33 and 95 g, were exposed to 2.1 ppb dieldrin for 33 days. Each fish was then removed, weighed, and placed in a trough-shaped rack on a moist towel, the corners of which were draped over the eyes of the fish. A section of very flexible surgical vinyl tubing was fitted snugly into the urinary bladder. The inserted end of this catheter had a 90° bend 1 cm from the end. The end was tapered and sealed using heated forceps, and the terminal 6 mm was made porous by burning holes in it with a hot needle. The catheter was held in place by a suture first tied tightly around the tubing and then tied through the musculature anterior to the anal fin. A second suture fastened the catheter to the second anal spine.

Next, the fish was placed in the holding chamber of the urine-collection apparatus. The free end of the catheter, which had been passed through the rubber stopper of the chamber prior to catheterization, was then inserted into a 15-ml graduated centrifuge tube for urine collection. All urine was kept frozen from the time of daily collection until the time of analysis.

Analytical Methods

The insecticide used in this research was technical grade dieldrin obtained from the Shell Chemical Company. This compound has a minimum of 85% hexachloroepoxy-octahydro-endo, exo-dimethanonaphthalene (HEOD) as the principal constituent and not more than 15% of insecticidally active, related compounds. The molecular weight of HEOD is 380.93 and the empirical formula is $C_{12}H_8Cl_6O$. The solubility of dieldrin in water is less than 100 ppb, but dieldrin is highly soluble in most organic solvents.

Stock solutions of dieldrin were prepared in acetone solution. Further dilution with acetone produced the solutions that were used in experimental work. Final dilutions of analytical standards were made with glass distilled, n-hexane. Standards of 25, 50, 100 and 200 ppb were prepared which covered the linearity range of the gas chromatograph used.

The gas chromatograph used for both quantitative and qualitative analysis of dieldrin was a model 400 F & M with a tritium foil electron capture detector. The carrier gas was 95% argon and 5% methane, and the flow rate was 60 ml per minute. A flow of 40 ml per minute of the same gas mixture was used to purge the detector. Operating temperatures were: column oven, 175°C; detector, 200°C; flash heater, 240°C.

The "U"-shaped chromatograph column was made from a 1.3-m section of 4-mm OD glass tubing. Each column contained two different packings: the 65% of the column on the inlet side was packed with 5% QF-1 on 80/100 mesh Gaschrom Q; the remaining 35% of the column was packed with 5% DC-11 on 80/100 mesh Gaschrom Q.

Dieldrin Extraction from Water and Blood

The procedure used for the extraction of dieldrin from water is similar to that of Reinert (1967) (Table 2). Fifty microliter (µl) samples of heparinized fish blood were analyzed for dieldrin. Since dieldrin is stable in alkali, it was possible to use the relatively rapid saponification method of Schafer, Busch, and Campbell (1963) in the cleanup procedure for blood samples (Table 3).

Dieldrin Extraction from Brain

The most critical part of the extraction procedure for brain tissue is obtaining a consistent wet-weight value

Table 2.--Flow sheet for extraction of dieldrin from water

1) Pour 100 ml of the water to be tested and 10 ml of hexane into a 125-ml separatory funnel and shake vigorously for two minutes.

2) Allow contents to stand until hexane and water layers are completely separated.

3) If an emulsion forms in the hexane layer, add 2 ml absolute ethanol and shake for 10 seconds; rinse hexane layer with 2 ml water and allow to stand 15 minutes.

4) Discard water layer and collect hexane layer in a 15-ml graduated centrifuge tube.

5) Inject 5 µl of the hexane extract into the gas chromatograph. If the peak height is either above or below the linear range of the detector, dilute or concentrate (under an airstream) an aliquot of the extract to bring a 5-µl sample within the range of the detector.

6) Compare sample peak height to standard peak heights.

Table 3.--Flow sheet for extraction of dieldrin from fish blood (Schafer et al., 1963)

1) Transfer 50 µl of fish blood to a 50-ml Erlenmeyer flask.

2) Add 20 ml of 15% v/v, freshly prepared, aqueous solution of potassium hydroxide (KOH) in absolute ethanol. KOH solution is made up with 5 g KOH per 3 ml distilled water.

3) Heat over a steam bath for 15 minutes with occasional stirring.

4) Cool to room temperature and quantitatively transfer to a 125-ml separatory funnel; two rinses of the flask with 10 ml of distilled water each are added to the funnel.

5) Add 5 ml hexane, shake vigorously 2 minutes, and allow to stand until layers separate.

6) Proceed from step 3 in Table 2.

before the cleanup is attempted. As the brain thaws, it dries very quickly. Hence it was necessary to use a fixed, short thawing time (10 minutes) before recording the weight of the brain. The brains weighed between 25 and 55 mg. The extraction procedure was identical to that used for blood beginning with step 2 (Table 3).

Dieldrin Extraction from Whole Fish

To obtain a dry weight and to dehydrate the tissue for dieldrin and fat extraction, frozen whole fish were first thawed and then dried for four days at 45°C. Dried fish were stored in a desiccator and then pulverized by mortar and pestle before extraction. Any piece of skin and scales intact after the pulverization treatment was cut by scissors into tiny fragments. Each fish thus treated was stored in a sealed glass vial pending hexane extraction.

Saponification with alcoholic potassium hydroxide was also used to clean up the pulverized fish before extraction. A 0.5-g sample of pulverized fish was run through the procedures outlined in Table 3 with two minor changes. In step 2, 30 ml of alcoholic potassium hydroxide were used for each sample. In step 5, 20 ml of hexane were used to extract the dieldrin from the aqueous solution.

Fat Extraction from Whole Fish

The fat content of each fish was used as a measure of the condition of the animals. Simple ether extraction was

utilized since only relative values were needed to measure differences among individuals. One-gram portions of the pulverized fish were extracted with ether for five hours in a Soxhlet extractor. The extract and the pre-weighed collection flask were dried to constant weight in a desiccator and then weighed again.

Measurement of Total 17-Hydroxycorticosteroids in Fish Urine

Urine samples were taken every 24 hours from each fish and analyzed for 17-OHCS. The analytical method used here for urine (Table 4) was McKim's (1966) slight modification of a method developed to measure small amounts of steroids in plasma (Peterson et al., 1957). Since most of the steroids in fish urine are excreted as conjugated metabolites (McKim, 1966), they had to be separated before they could be analyzed. The enzyme Helix (Calbiochem) which contains both beta-glucuronidase and aryl sulfatase, was used to split both the glucuronic acid conjugates and the sulfate conjugates. A urine sample of 5 ml was used, unless less than this amount was excreted in a 24-hour period. When smaller amounts were excreted, the entire sample was used and diluted to 5 ml with distilled water.

Table 4.--Flow sheet for the Peterson modification of the Porter-Silber technique for measurements of total 17-OHCS in fish urine, as adapted by McKim (1966).

1) Take 5 ml of fish urine.

2) Incubate for 24 hours with Helix (10 µl Helix per 5 ml of urine) at pH 4.4 (0.5 M acetate buffer).

3) Add 25 ml of dichloromethane; shake gently for 2 minutes in 50-ml conical tubes with glass stoppers.

4) Aspirate off urine layer; save dichloromethane layer.

5) Add 2 ml of 0.1 N NaOH; shake gently 20 seconds.

6) Aspirate off the NaOH layer; save dichloromethane layer.

7) Wash once with 2 ml H_2O; aspirate off H_2O; transfer two 10-ml aliquots into 15-ml conical test tubes with caps--one is for blank and one for sample.

8)[a] Add 0.5 mg of blank reagent; shake hard 20 seconds, then let stand 30 minutes.	8)[b] Add 0.5 ml of P-S reagent; shake hard 20 seconds, then let stand 30 minutes.

9) Remove dichloromethane phase.

10) Let stand ten hours or more in the dark at room temperature.

11) Measure the absorbency of the colored product at 410 mµ in a spectrophotometer using microcuvettes and a water blank.

12) Five ml water run through the entire procedure serves as reagent blank, and 5-ml aliquots of water containing 1 to 5 µg of hydrocortisone serve as standards.

[a]Blank Reagent. Two parts of 64% sulfuric acid with one part of ethanol. This reagent keeps indefinitely.

[b]Phenylhydrazine--Sulfuric Acid--Ethyl Alcohol. Fifty mg phenylhydrazine hydrochloride are dissolved in 50 ml of the blank reagent. This reagent is prepared fresh daily.

Statistical Methods

The data from the experiments to determine the critical dieldrin concentrations in blood and brain did not appear to follow a normal distribution. A chi-square "goodness of fit" was run and showed no significant deviation from normal, but this was rejected because of the small sample sizes. Because of the apparent non-normality of the data, the percentile test was used for data analysis (Snedecor and Cochran, 1967).

The differences between the mean blood- or brain-dieldrin levels of dead and surviving fish were compared by the Mann-Whitney test for non-normal data and also by the "T"-test for normal data (Snedecor and Cochran, 1967).

Linear regression analysis was used for the data obtained in the dieldrin-release experiments. The regression intercept, regression coefficient, and the sample standard deviation of the regression coefficient were calculated according to Snedecor and Cochran (1967). The logarithmic rates of loss of dieldrin were compared using the "T"-test as discussed in Steel and Torrie (1960).

RESULTS

Critical Dieldrin Concentrations in Blood and Brain

Blood

A comparison of fish killed by dieldrin with fish surviving in dieldrin showed a difference in dieldrin concentration in the blood of the two groups of fish. The lowest observed blood-dieldrin concentration for fish killed by dieldrin was 3.92 milligrams per liter (mg/l or ppm), whereas in fish surviving in dieldrin, the highest level recorded was 4.40 ppm (Table 5). Four of the 14 fish killed by dieldrin were in the range of overlapping concentrations and had less than 4.40 ppm, the maximum value in living fish.

For statistical analysis, 90 percentiles were calculated for the blood levels of the two groups of fish. The overlap of the lowest values for dead fish and the highest values for living fish was about 30% either way. The mean blood-dieldrin concentration of fish killed by dieldrin was 4.90 ppm and the mean of the living fish was 3.60 ppm. These values were judged to be significantly different at the 99% level by the Mann-Whitney test for non-normal data and also by the "T"-test for normal data.

Table 5.--Concentrations of dieldrin in blood and brain of living and dead cichlid fish

Number, Tissue and Condition of Fish	Range of Tissue Dieldrin Concentrations (ppm)	Mean Tissue Dieldrin Concentration (ppm)	Number of Fish with Less Than 4.35 ppm in Blood or 11.11 ppm in Brain[a]	Number of Fish with More Than 4.08 ppm in Blood or 10.57 ppm in Brain[b]
14 blood dead	3.9 - 8.0	4.9	4	11
15 blood living	2.1 - 4.4	3.6	14	4
10 brain dead	10.0 -15.1	12.6	2	8
22 brain living	5.7 -12.7[c]	8.3	21	3

[a]The values below which 90% of the levels occur in living fish as calculated by the percentile method (Snedecor and Cochran, 1967).

[b]The values above which 90% of the levels occur in dead fish.

[c]The second highest value was 11.1 ppm. See discussion in the text.

Brain

The relationship between concentration of dieldrin in the brain and lethality is comparable to that shown by blood levels. No fish survived a brain concentration exceeding 12.7 micrograms per gram (ppm), and only one exceeded 11.1 ppm (Table 5). No fish died with less than 10.0 ppm in the brain.

When the upper 90 percentile of the levels in living fish is compared with the lower 90 percentile of the levels in dead fish, there is an overlap of about 20% of the samples in both directions. However, the means of the brain-dieldrin concentrations of fish killed by dieldrin (12.6 ppm) and of the fish living in dieldrin (8.3 ppm) were again significantly different at the 99% level.

Dieldrin Uptake and Release Experiments

There were seven complete experiments for this study, each with an uptake portion and a release portion. For each consecutive experiment, at least one variable was changed (Table 1). However, for the sake of clarity, the significant changes will be presented as needed to identify results. The only variation in uptake portions was dieldrin-exposure concentrations, and the main purpose for running uptake was as a prerequisite to the release portions. Therefore, all uptake data will be considered

together briefly, before each set of release data is presented separately.

The curves drawn in Figures 5 to 11 for tissue-dieldrin levels represent (with a few exceptions) the best mathematical fit to least squares regressions of the empirical data. One exception is the curve for the brain residues in experiment I which was fitted by eye. The other exceptions are the uptake curves for experiments VI and VII, for which there are not enough points to fit a curve mathematically. The other uptake curves were fitted by computer with a quadratic equation and the release curves by computer with an exponential equation.

Uptake of Dieldrin

The pattern of dieldrin uptake was similar for all tissues for all exposure tests. Tissue residues rose sharply for about ten days and then gradually approached a plateau.

Since dieldrin is highly fat soluble, the amount of fat in the animals is important to uptake and storage. The range of fat content was from 6 to 20% on a dry-weight basis for all dieldrin-exposed fish at the time of sacrifice in the uptake portion of the experiments. From beginning to end of dieldrin exposure the fish in each experiment decreased in fat by up to about 10%, except for experiment II where about a 20% decrease was observed (Table 6).

Figure 5.--Experiment I - The uptake and release of dieldrin by fish exposed to 1.1 ppb dieldrin in water and then fasted. The size of each sample is three. The means and ranges are shown.

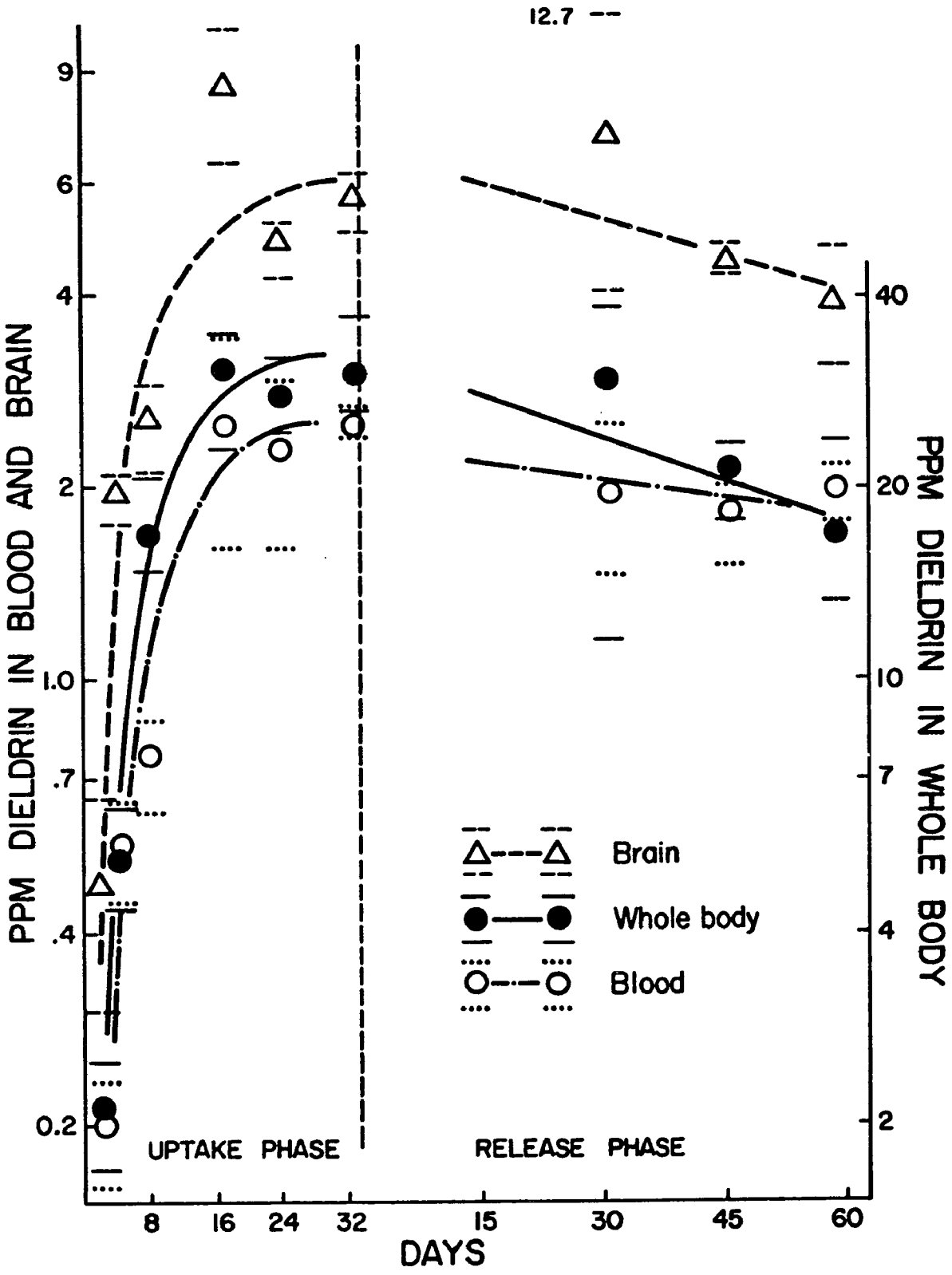

12.7
9
6
4
2
1.0
.7
.4
0.2
40
20
10
7
4
2
PPM DIELDRIN IN BLOOD AND BRAIN
PPM DIELDRIN IN WHOLE BODY
Brain
Whole body
Blood
UPTAKE PHASE
RELEASE PHASE
8
16
24
32
15
30
45
60
DAYS

Figure 6.--Experiment II - The uptake and release of dieldrin by fish exposed to 1.1 ppb dieldrin. The fish were subjected to low dissolved oxygen during exposure and then to a 5°C temperature elevation and fasting during the post-exposure period. The means and ranges are shown. The subscript by the blood mean is the sample size for that sampling time for all tissues.

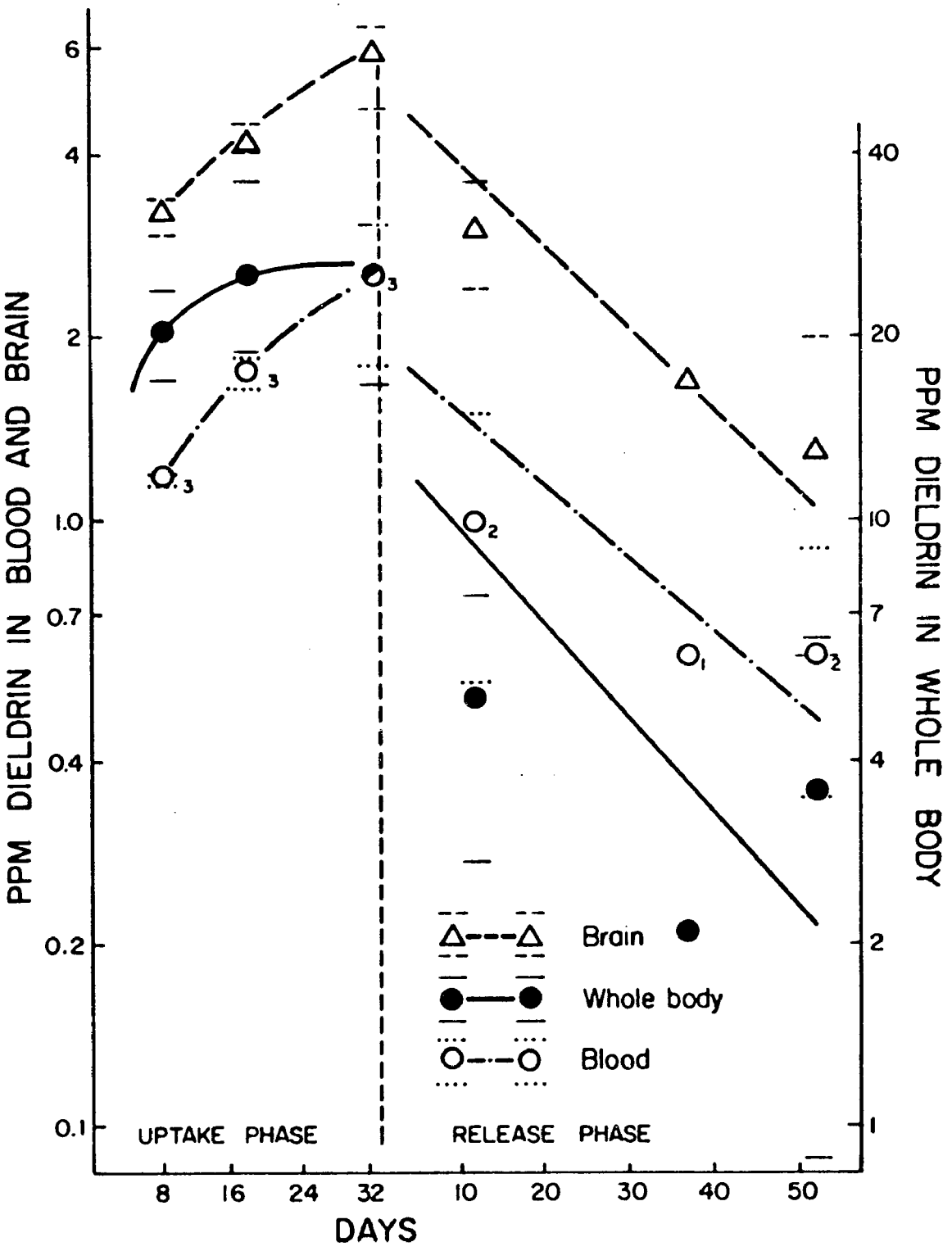

PPM DIELDRIN IN BLOOD AND BRAIN
PPM DIELDRIN IN WHOLE BODY
6
4
2
1.0
0.7
0.4
0.2
0.1
40
20
10
7
4
2
1
UPTAKE PHASE
RELEASE PHASE
8
16
24
32
10
20
30
40
50
DAYS
Brain
Whole body
Blood
O3
O2
O1

Figure 7.--Experiment III - The uptake and release of dieldrin by fish exposed to 2.0 ppb dieldrin and then fasted. The means and ranges are shown. The subscript by the blood mean is the sample size for that sampling time for all tissues.

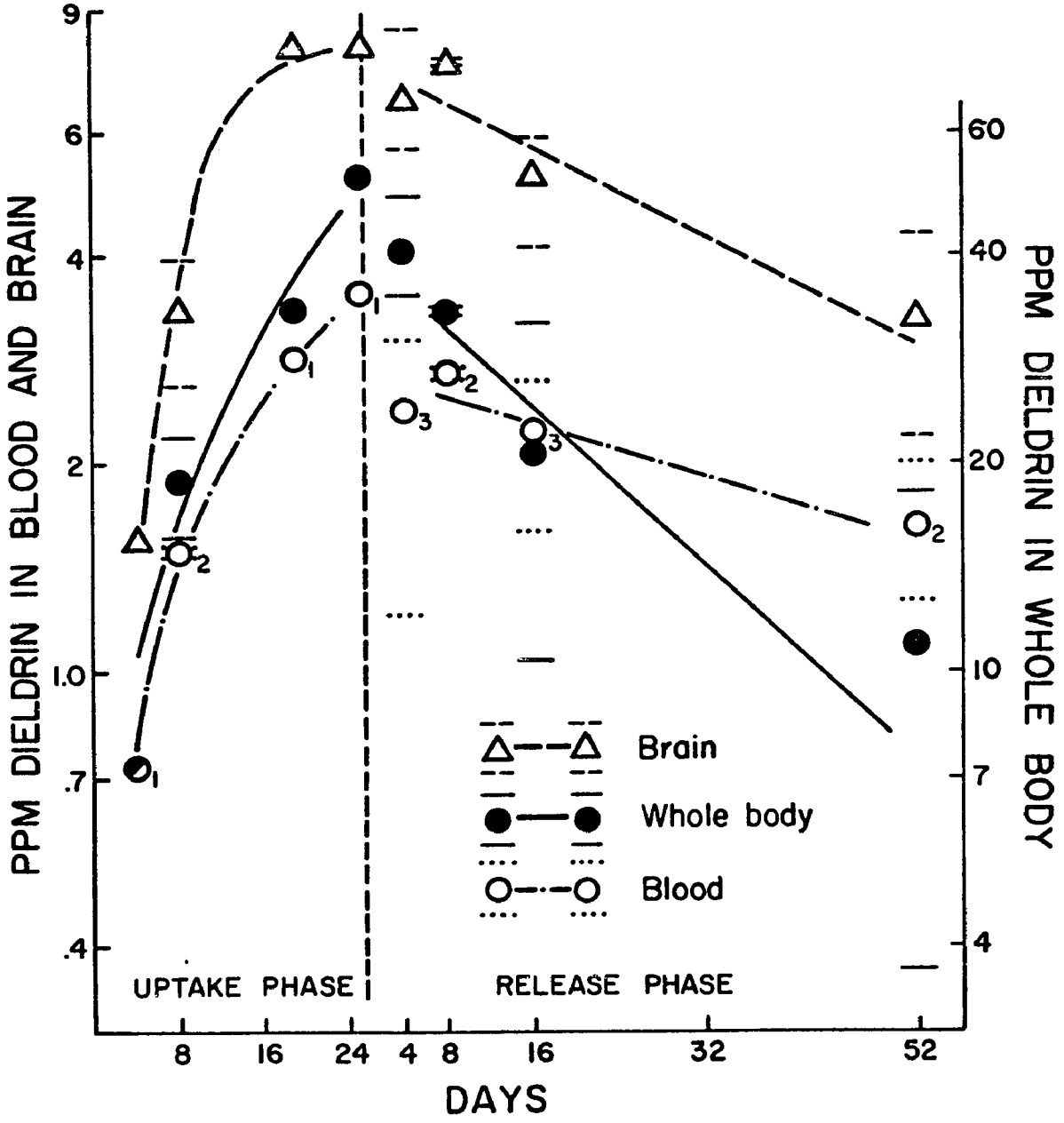

PPM DIELDRIN IN BLOOD AND BRAIN
PPM DIELDRIN IN WHOLE BODY
9
6
4
2
1.0
.7
.4
60
40
20
10
7
4
Brain
Whole body
Blood
UPTAKE PHASE
RELEASE PHASE
8
16
24
4
8
16
32
52
DAYS

Figure 8.--Experiment IV - The uptake and release of dieldrin by fish exposed to 1.2 ppb dieldrin. The fish were subjected to a 5°C temperature elevation and fasted during the post-exposure period. The means and ranges are shown. The subscript by the blood mean is the sample size for that sampling time.

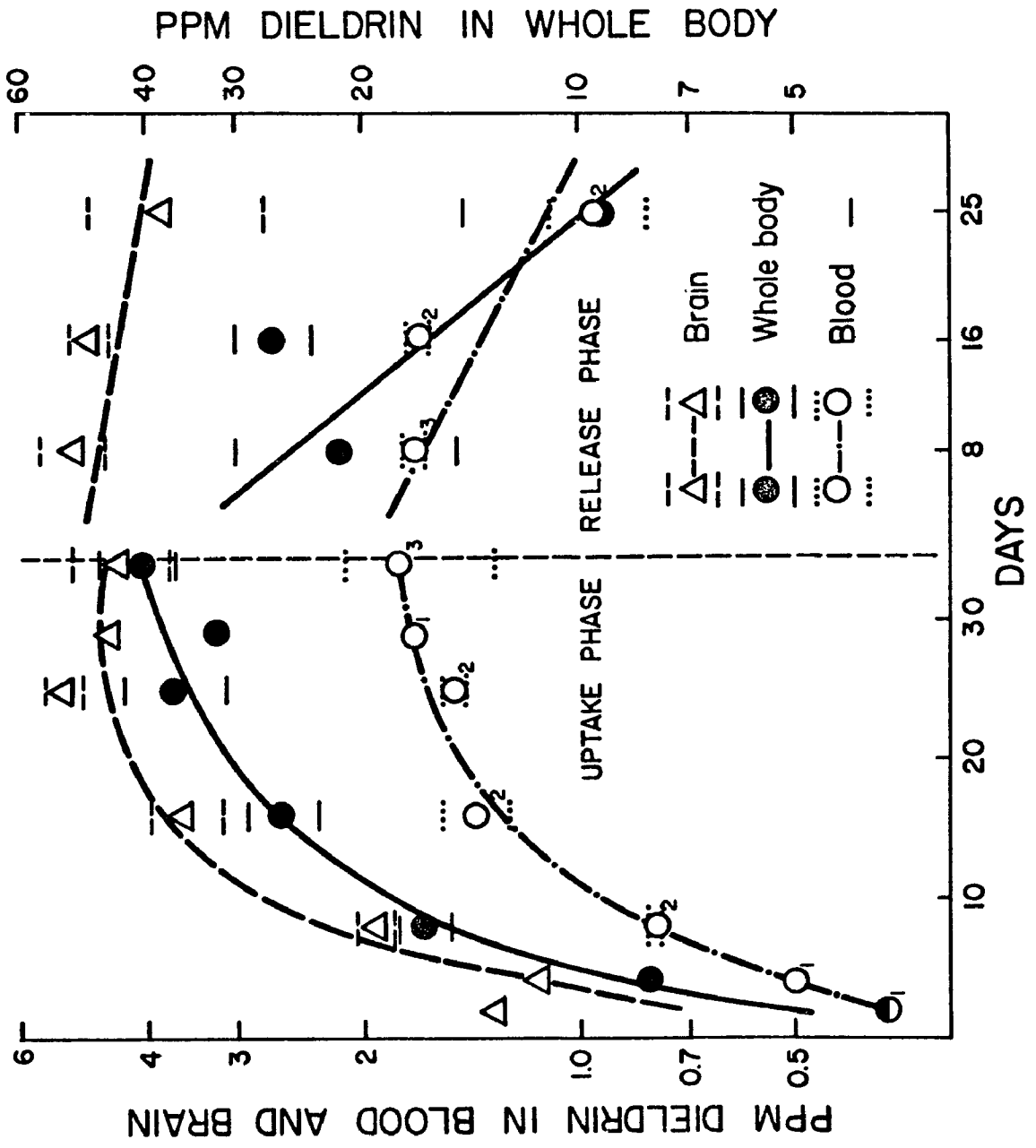

PPM DIELDRIN IN WHOLE BODY
60
40
30
20
10
7
5
PPM DIELDRIN IN BLOOD AND BRAIN
6
4
3
2
1.0
0.7
0.5
UPTAKE PHASE
RELEASE PHASE
Brain
Whole body
Blood
10
20
30
8
16
25
DAYS

Figure 9.--Experiment V - The uptake and release of dieldrin by fish exposed to 2.1 ppb dieldrin. The fish were subjected to a 5°C temperature elevation and fasted during the post-exposure period. The means and ranges are shown. The subscript by the blood mean is the sample size for that sampling time for all tissues.

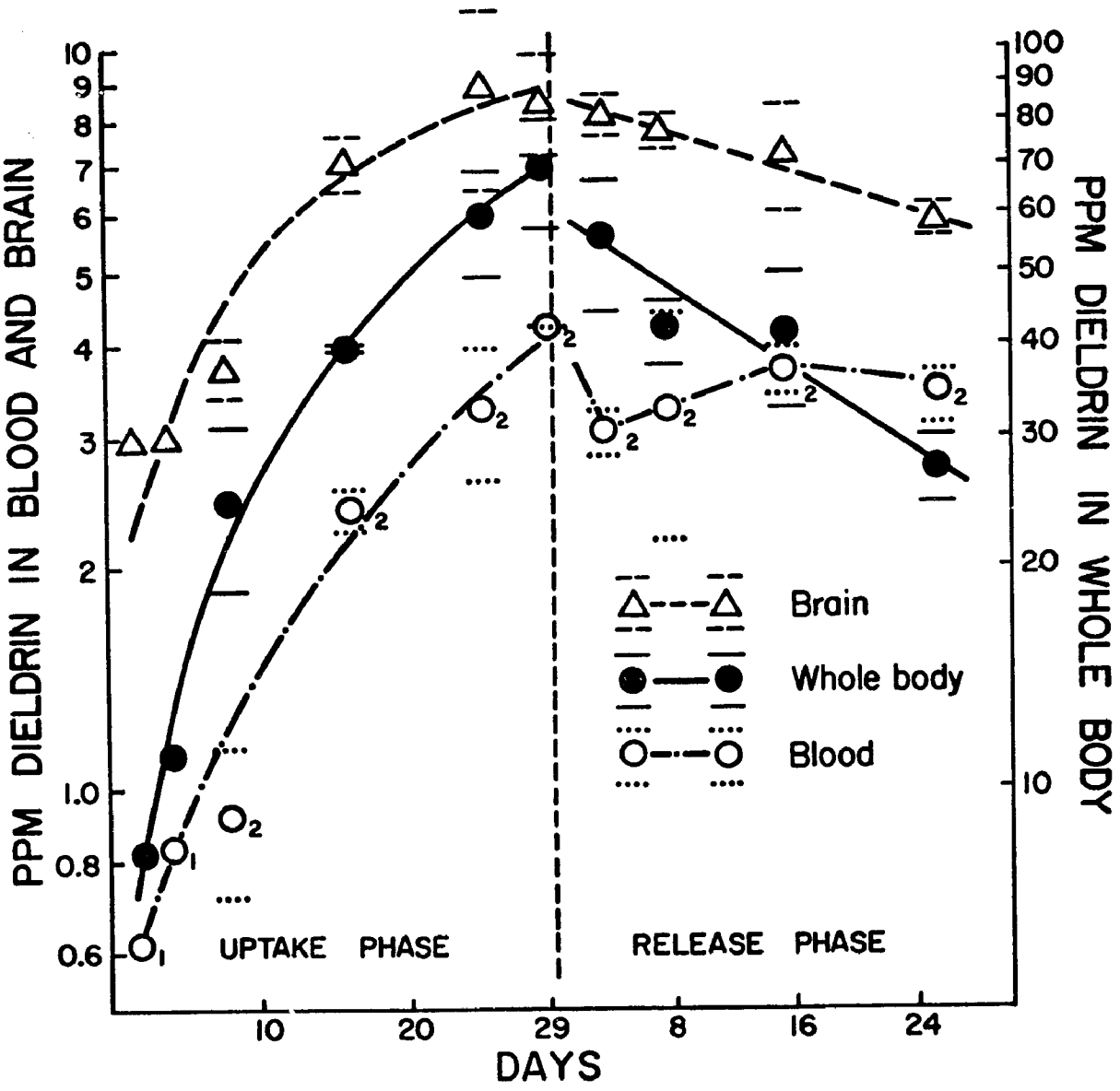

PPM DIELDRIN IN BLOOD AND BRAIN
PPM DIELDRIN IN WHOLE BODY
DAYS
UPTAKE PHASE
RELEASE PHASE
Brain
Whole body
Blood
10
20
29
8
16
24

Figure 10.--Experiment VI - The uptake and release of dieldrin by fish exposed to 1.2 ppb dieldrin. The fish were subjected to a 5°C temperature elevation and fed during the post-exposure period. The means and ranges are shown. The subscript by the blood mean is the sample size for that sampling time for all tissues.

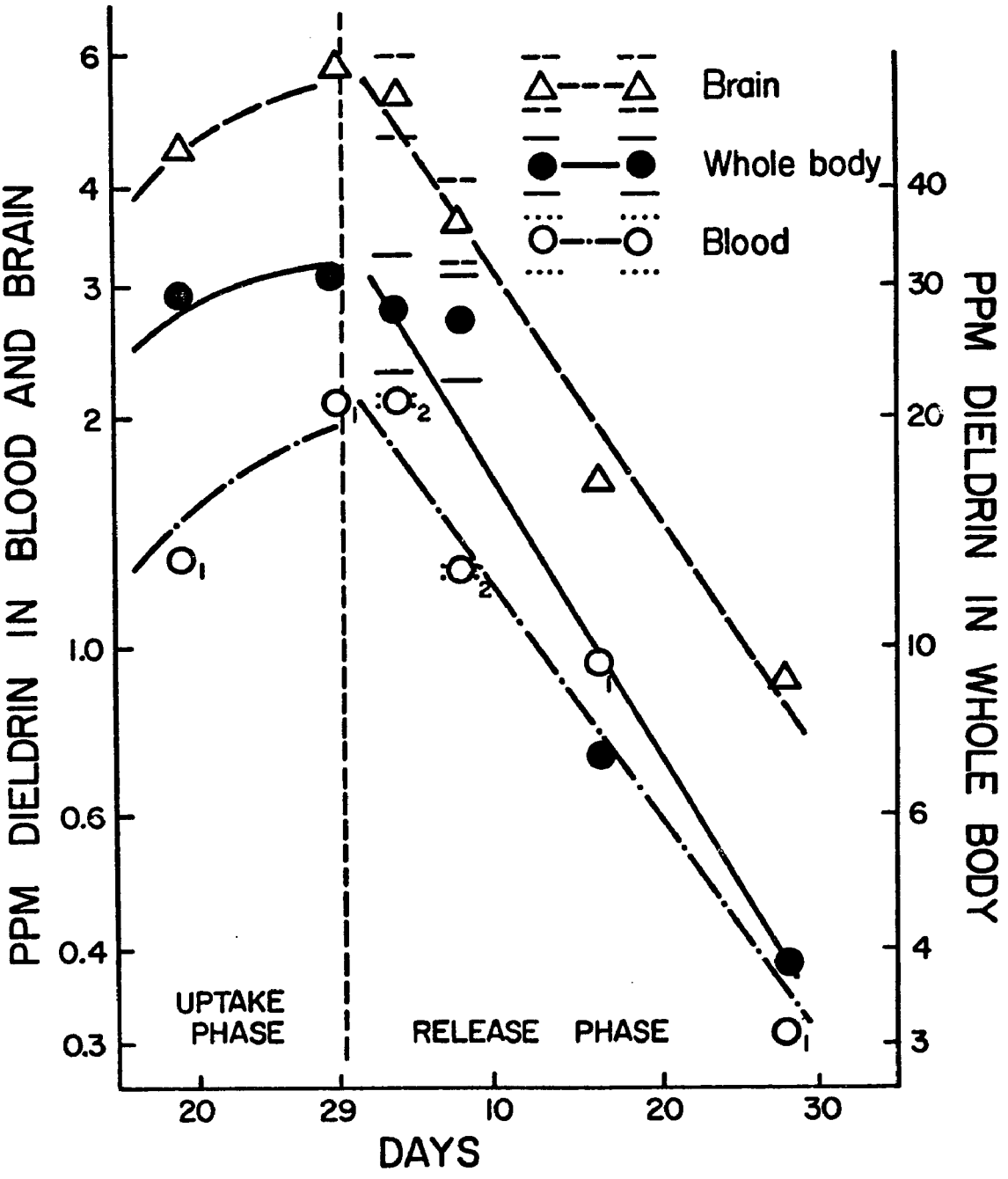

PPM DIELDRIN IN BLOOD AND BRAIN
PPM DIELDRIN IN WHOLE BODY
Brain
Whole body
Blood
UPTAKE PHASE
RELEASE PHASE
DAYS
20
29
10
20
30
6
4
3
2
1.0
0.6
0.4
0.3
40
30
20
10
6
4
3

Figure 11.--Experiment VII - The uptake and release of dieldrin by fish exposed to 2.0 ppb dieldrin. The fish were subjected to a 5°C temperature elevation and fed during the post-exposure period. The means and ranges are shown. The subscript by the blood mean is the sample size for that sampling time for all tissues.

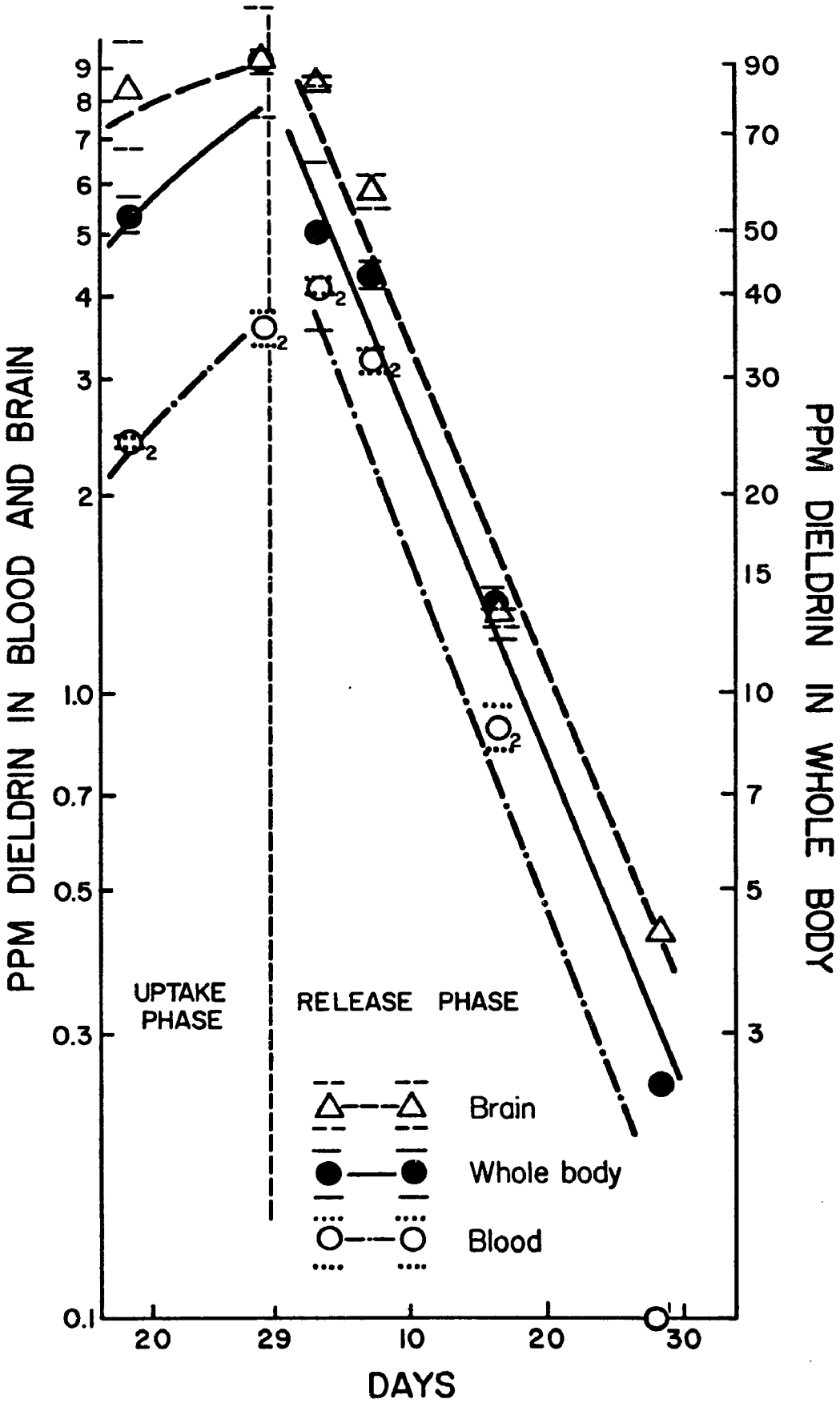

PPM DIELDRIN IN BLOOD AND BRAIN
PPM DIELDRIN IN WHOLE BODY
9
8
7
6
5
4
3
2
1.0
0.7
0.5
0.3
0.1
90
70
50
40
30
20
15
10
7
5
3
UPTAKE PHASE
RELEASE PHASE
Brain
Whole body
Blood
20
29
10
20
30
DAYS

Table 6.--Fat content of fish at time of sacrifice

Experiment I			Experiment II			Experiment III		
			Uptake Phase					
Day	Fish	% Fat	Day	Fish	% Fat	Day	Fish	% Fat
2	1	14.7						
	2	20.5						
	3	11.4						
	c*	8.1						
4	1	12.9				4	1	10.1
	2	14.4						
	3	8.1						
	c	7.5						
8	1	19.4	8	1	10.4	8	1	8.6
	2	17.9		2	10.1		2	10.4
	3	14.5		3	12.2		c	1.2
	c	14.5		c	12.7			
17	1	13.0	18	1	8.6	19	1	5.8
	2	7.9		2	13.3		2	1.3
	3	7.6		3	7.4		c	1.9
	c	16.8		c	7.6			
24	1	11.6				25	1	1.8
	2	12.7					2	8.2
	3	15.7					c	8.1
	c	4.1						
33	1	14.6	33	1	4.9			
	2	7.7		2	12.8			
	3	7.5		3	7.9			
	c	12.0		c	5.8			

*Control will be designated as "c" throughout Table 6.

Table 6.--Fat content of fish at time of sacrifice (cont.)

Experiment I			Experiment II			Experiment III		
			Release Phase					
Day	Fish	% Fat	Day	Fish	% Fat	Day	Fish	% Fat
						4	1	5.5
							2	12.3
							3	6.7
							c	8.8
						8	1	5.9
							2	6.3
			12	1	3.3		3	1.1
				2	2.9		c	4.6
				3	1.8			
				c	4.7	16	1	6.7
							2	3.4
							3	3.5
							c	1.2
31	1	12.8						
	2	11.4						
	3	5.4						
	c	8.1	37	1	2.5			
				2	1.3			
				c	1.5			
46	1	6.3						
	2	11.0						
	3	8.7	52	1	4.1	52	1	6.6
	c	6.3		2	2.3		2	2.3
				3	1.0		3	1.2
59	1	2.8		c	3.1		c	1.3
	2	5.9						
	3	10.1						

Table 6.--Fat content of fish at time of sacrifice (cont.)

Experiment IV			Experiment V		
Uptake Phase					
Day	Fish	% Fat	Day	Fish	% Fat
2	1	7.2	2	1	6.2
4	1	14.6	4	1	9.6
8	1	14.4	8	1	14.8
	2	13.8		2	12.9
16	1	13.4	16	1	10.8
	2	12.0		2	11.0
25	1	10.7	25	1	8.0
	2	11.8		2	15.8
29	1	10.7	29	1	8.0
				2	9.9
34	1	13.0		c	5.4
	2	11.5			
	3	12.8			
Release Phase					
			4	1	11.6
				2	8.3
				c	11.5
8	1	8.7	8	1	8.3
	2	5.8		2	6.3
	3	4.5		c	6.9
	c	4.6			
16	1	8.4	16	1	9.2
	2	7.0		2	7.0
	3	8.0		c	9.0
	c	1.8			
25	1	2.8	26	1	4.3
	2	4.8		2	6.3
	c	1.9		c	2.4

Table 6.--Fat content of fish at time of sacrifice (cont.)

Experiment VI			Experiment VII		
Uptake Phase					
Day	Fish	% Fat	Day	Fish	% Fat
19	1	13.3	19	1	11.1
				2	6.3
29	1	6.1	29	1	10.4
				2	14.7
Release Phase					
4	1	9.4	4	1	5.6
	2	6.7		2	8.6
8	1	8.8	8	1	7.0
	2	10.8		2	8.3
17	1	6.9	17	1	10.6
				2	8.6
29	1	7.8	29	1	8.3
				2	6.5

Exposure to 1.1 ppm dieldrin. Four different experiments were run at the low exposure level: I, II, IV and VI (Figures 5, 6, 8 and 10, respectively). After 29 to 34 days exposure to dieldrin, the mean levels in the blood in the four experiments reached 1.8 to 2.5 ppm. The blood concentration apparently leveled off in experiments I and IV, but had not yet leveled off in experiments II and VI when exposure was terminated.

The concentration in the brain, by the end of the exposure period, reached mean levels of from 4.6 to 6.0 ppm. The brain levels apparently leveled off in experiment IV, but not in experiments I, II, and VI.

Whole-body residues reached mean levels of from 26 to 40 ppm during the exposure period. The residues in the whole fish appeared to have leveled off in the 33-day exposure period in experiments I and II, and were close to it in IV and VI.

Exposure to 2.1 ppm dieldrin. Three experiments were run at the high exposure level: III, V, and VII (Figures 7, 9 and 11, respectively). None of the tissues reached a saturation or leveling off point by the end of the exposure period (25 to 29 days), although brain tissue was close to it in all three experiments.

In the three experiments, blood reached mean levels of 3.5 to 4.2 ppm and brain concentrations reached mean levels of 8.0 to 9.2 ppm. Whole-body residues reached mean levels

of 52 ppm dieldrin in experiment III, 69 ppm in experiment V, and 91 ppm in experiment VII.

When comparing uptake at the two exposure levels, the percentage increase in tissue level is similar to the increase in exposure concentration. The high exposure concentration was roughly 90% higher than the low exposure concentration, and the blood level increase varied from 70 to 95%. In brain tissue the increase varied from 53 to 74%, and in the whole fish the residue increase was between 100 and 125%.

Release of Dieldrin

The dieldrin-residue data for each tissue, when plotted against time on semi-logarithmic paper, were linear with a negative slope (Figures 5-11). The equation for this relationship is as follows:

$$(1) \quad Y = (A)(B)^X$$

where Y = concentration of dieldrin in the tissue (ppm)

A = the regression intercept (ppm)

X = days following the cessation of dieldrin in the water

B = the relative rate of change of residue

or for the rectified data:

$$(2) \quad \log Y = \log A + (\log B)X$$

Linear regression analysis was run on the rectified data.

The regression coefficient or regression slope (log B) estimates the logarithmic rate of residue gain or loss. In this case the value (log B) is negative so the residue is decreasing. The residue half-life values (Table 7) computed from equation (2) are a measure of the time required for the loss of 50% of the residue from a tissue.

Fasting after cessation of dieldrin exposure. The release of dieldrin from storage was very slow in the first experiment. The first samples were taken 31 days after dieldrin exposure and the residues had not significantly decreased from their starting level. The rate of loss continued to be low and the half-life values of 69.8, 151, and 107 days for whole body, blood, and brain, respectively, were the longest observed for the respective tissues except for brain in experiment IV and blood in experiment V.

In the other experiment, III, with the same post-exposure conditions, the half-lives were much shorter. The initial tissue residues were approximately twice as great, and the rates of loss more than twice as great as in experiment I. The half-life values for whole body, blood, and brain were 20.8, 67.4, and 39.2 days, respectively.

The total lipid levels of fish in experiment I were higher throughout than during any other experiment. The rate of lipid loss (25% in 40 days) was slightly less than during other fasting runs. During the post-exposure period of experiment III, the lipid levels decreased by about 40%.

Table 7.--Half-lives and rates of loss of dieldrin residues from whole-body, blood, and brain tissues after transfer of fish to dieldrin-free water

Experiment	Tissue	Half-life (Days)	Logarithmic Rate of Change (B) $\pm S_b$	Correlation Coefficient
	whole body	69.8	-0.00431 ± 0.00200	.588
I	blood	151.0	-0.00200 ± 0.00098	.631
	brain	107.0	-0.00282 ± 0.00189	.537
	whole body	16.1	-0.01870 ± 0.01690	.413
II	blood	25.5	-0.01180 ± 0.00392	.790
	brain	22.3	-0.01350 ± 0.00293	.889
	whole body	20.8	-0.01450 ± 0.00375	.796
III	blood	67.4	-0.00447 ± 0.00248	.548
	brain	37.2	-0.00767 ± 0.00174	.839

$\pm S_b$ (Sample standard deviation of the regression coefficient)

Table 7 (Continued)

Experiment	Tissue	Half-life (Days)	Logarithmic Rate of Change (B) $\pm S_b$	Correlation Coefficient
	whole body	12.2	-0.02460 ± 0.00718	.770
IV	blood	31.2	-0.00963 ± 0.00324	.831
	brain	109.0	-0.00277 ± 0.00314	.431
	whole body	22.3	-0.01350 ± 0.00268	.872
V	blood	419.0	-0.00072 ± 0.00346	.042
	brain	51.5	-0.00585 ± 0.00186	.860
	whole body	8.5	-0.03540 ± 0.00506	.951
VI	blood	10.2	-0.02950 ± 0.00295	.975
	brain	9.6	-0.03150 ± 0.00255	.982
	whole body	5.9	-0.05100 ± 0.00372	.982
VII	blood	5.6	-0.05350 ± 0.00650	.958
	brain	6.0	-0.05010 ± 0.00402	.981

Fasting plus a 5°C temperature rise after cessation of dieldrin exposure. Very different results were produced in the fish from the two different exposure levels when they were exposed to a 5°C temperature rise. At the low exposure level, fish exposed to a 5°C temperature rise (experiment IV, Figure 8) showed a much faster loss of dieldrin from whole body and blood than those not exposed to a temperature rise (experiment I, Figure 5). The loss from the brain was almost identical in both I and IV. The half-lives were 12.2, 31.2, and 109 days for whole body, blood and brain, respectively.

When fish exposed to the high dieldrin level were subjected to a temperature rise, no differences were obvious when compared to III. The half-lives in experiment V were 22.3 and 51.5 days for whole body and brain, respectively, which are very similar to those in experiment III. There was too much variation in the blood values to be able to give a precise half-life measurement, but it appeared to be very long.

The mean lipid levels during fasting decreased by about 50% in experiment IV and about 40% in experiment V.

Experiment II had the same post-exposure variables as IV and V, but during the last half of the exposure period, the animals were under severe low oxygen stress. Thus, they were in poor condition and had the lowest mean post-exposure lipid levels of all the fish, about 3%. The total body

burdens of dieldrin were also the lowest as a whole during the post-exposure period, of all the tests run. The tissue half-lives were 16.1, 25.5, and 22.3 days for whole body, blood, and brain, respectively.

Fish fed and exposed to a 5°C temperature rise after cessation of dieldrin exposure. The animals in experiments VI and VII showed the lowest loss of fat of all the tests. They also showed the fastest rate of loss of dieldrin from the tissues. The low-exposure fish (experiment VI) had tissue half-lives of 8.5, 10.2 and 9.6 days for whole body, blood, and brain, respectively.

The half-lives for the high-exposure fish (experiment VII) were 5.9, 5.6 and 6.0 days for whole body, blood, and brain, respectively.

The only variable which produced an obvious difference in tissue residue release in both low- and high-exposure groups was feeding as opposed to fasting. Since the whole-body residue is the measure of the total dieldrin remaining in the animal (minus blood and brain dieldrin), these values were compared statistically between IV and VI, and between V and VII. The logarithmic rates of loss (log B in equation (2)) between the fasted and fed fish (experiments V and VII) after high exposure were significantly different at the 99% level. The rates of loss in experiments IV and VI were not significantly different at the 95% level. However, they probably would have been so with

slightly larger sample sizes. No statistical tests were run on the differences between the other tissues, but the rates of loss were obviously significantly greater in experiments VI and VII.

The total lipid content showed no major loss in either VI or VII during the post-exposure period, but matched the approximately 10% decrease observed in the exposure period.

Other observations on dieldrin-release experiments. One of the most important results of these experiments is that the dieldrin levels in the tissues did not rise once the post-exposure period was begun except in three instances. One exception was in blood in experiment VII where the 4-day value was higher than the final exposure value, but was no higher than the final exposure value of experiment V. The other exceptions were with brain in runs IV and I. In IV the 8- and 16-day post-exposure values were as high or slightly higher than the exposure period values, but still not as high as exposure levels in other experiments. The final exception was the 32-day brain level in I, which was the highest value recorded in a living fish and was about three times the value normally recorded for a comparable time period.

One other result observed throughout the release experiments is the difference in rate of loss among the tissues. In almost all instances rate of dieldrin loss from the tissues was in the decreasing order of whole body,

brain, then blood. The major exception was test IV where the loss was slowest from the brain, followed by blood and whole body. In experiment VII, the rate of loss was nearly equal for the three tissues.

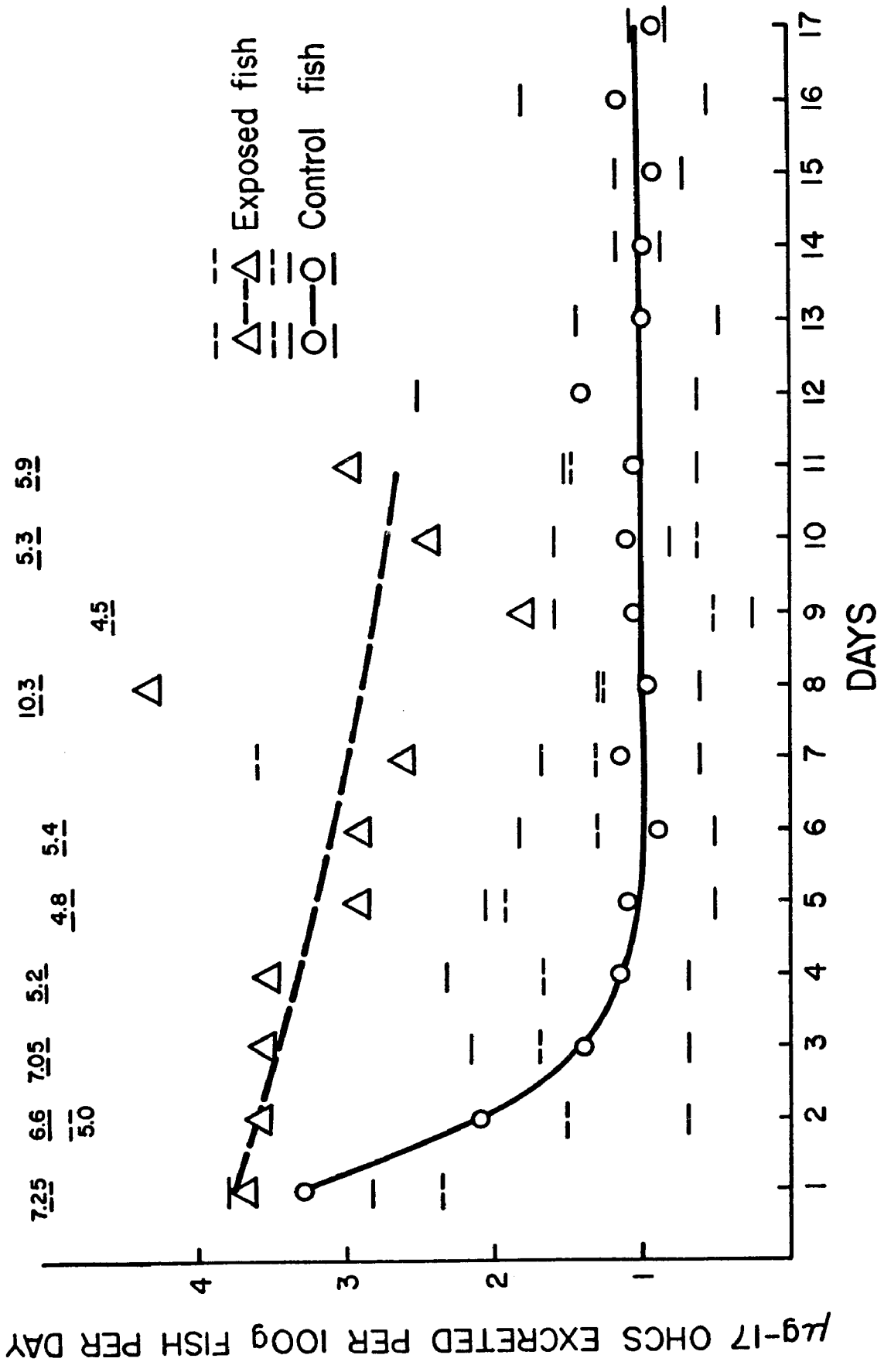
Total 17-OHCS in Urine

The quantity of 17-hydroxycorticosteroid hormones excreted during a 24-hour period by each fish was based on an adjusted weight of 100 g (Figure 12). This calculation was made because of the wide range in the size of fish used (33 to 95 g). The curves plotted in Figure 12 are based on the mean values for up to six fish for the controls and up to five fish for the dieldrin-stressed fish.

In the 24-hour period after catheterization, both the controls and the dieldrin-exposed fish excreted approximately 3.4 µg 17-OHCS per 100 g fish. This was the beginning of the period of post-operative stress. The steroid excretion level for control fish rapidly decreased for the next three days and then leveled off. By the sixth day after catheterization the daily hormone excretion was approximately 1.0 µg 17-OHCS and held steady for the remaining ten days that urine was collected.

In the fish which had been exposed to dieldrin the excretion of 17-OHCS's was slightly higher than in controls during the initial 24-hour period. But as the control values dropped markedly, the exposed fish excretions

Figure 12.--Means and ranges of daily urinary excretion rates of 17-OHCS for fish removed from 2.0 ppb dieldrin, and their controls. The curve for exposed fish was fitted by computer.


Exposed fish
Control fish
DAYS
µg-17 OHCS EXCRETED PER 100g FISH PER DAY
7.25
6.6
5.0
7.05
5.2
4.8
5.4
10.3
4.5
5.3
5.9

decreased very slowly but steadily until urine was no longer collected. The mathematical plot of the means of the hormone excretion data for exposed fish stayed at least 2.5 times as high as the curve for the controls, except for the first two days of collection when the animals were recovering from the operational stress.

The ranges of values for the exposed fish were much greater than the ranges for the control fish (Figure 12). Nevertheless, the minimum values for exposed fish were higher than the minimum values for control fish for all but two days, and the maximum values for exposed fish were much higher than for control fish except for one value (day two). The extremely high maximum values for exposed fish were not all for the same fish, but represented several animals, the steroid excretions of which fluctuated from day to day.

DISCUSSION

This research was performed with five distinct types of experiments and objectives. The first of these was designed to establish critical blood- and/or brain-dieldrin levels with which to compare blood- and brain-dieldrin levels of sample fish.

Critical Dieldrin Concentrations in Blood and Brain

The purpose of investigating blood- and brain-dieldrin level differences between surviving and dieldrin-killed fish, was not simply to validate the merit of this test as an autopsy technique for fish kills. The intent was to test the hypothesis that, under a given set of conditions, in order for dieldrin to exert a toxic effect it would have to be present in the blood and brain at levels higher than found in blood and/or brain of living exposed fish. No other tissues have ever been shown to have pesticide levels consistently higher in dead than in living fish. The results obtained here tend to verify the above stated blood- and brain-level hypothesis, because the blood-dieldrin levels were higher in dead fish than in surviving fish. The

critical blood level for this fish under these conditions was 4.14 mg/l dieldrin, and the critical brain level was 11.1 µg/g.

Blood

Because of the lack of fish and the method of sampling, all the values for living fish were from individuals exposed to sublethal or chronic doses of dieldrin, whereas most of the values for dead fish were from acutely exposed fish. Nevertheless, there were two fish under chronic exposure which did show chlorinated hydrocarbon toxicity symptoms and died after 27 and 30 days. Their blood levels were 5.25 and 4.70 mg/l, which fall near the middle of the values for acutely exposed fish, so the hypothesis remains valid.

There are only two studies of this nature in the literature, both done with endrin. One is the work of Mount and Putnicki (1966), cited in the introduction, where a critical level of 0.28 µg/g was found in the blood of the channel catfish. Other work (Brungs and Mount, 1967) was done on field and laboratory-exposed individuals of the gizzard shad (Dorosoma cepedianum), which showed that the critical level of endrin in blood (0.10 µg/g) obtained in in the laboratory is also applicable to naturally exposed gizzard shad. The authors also concluded that the critical level of endrin in the blood (the level above which few fish can survive) could be used for the detection of endrin-caused mortality. Taking into consideration the

experimental data and the reports in the literature, I conclude that the blood-dieldrin concentration can be used as an indication of whether the chances an animal has of being adversely affected by a dieldrin source are increasing or decreasing.

Brain

The correlation of clinical signs of poisoning with concentration of DDT in the brain has been shown in rats (Dale et al., 1963) and humans (Brown, Hunter and Richardson, 1964). It has not been definitively demonstrated in fish, but Allison et al. (1964) presented some evidence of brain-level-death correlation and suggested the subject shows promise for future study.

I found good correlation between concentration of dieldrin in the brain and the death of the animal. The critical concentration was higher in brain (11.1 µg/g) than in blood, but the degree of correlation was very similar.

There was one brain value for a live fish which was much above any other values for that sampling time or experiment and was the highest level recorded for a living brain. The value was 12.7 µg/g which is 15% higher than the next highest level for a living fish. The other two fish taken at that sampling time had brain levels of 4.03 and 4.45 µg/g. It does not seem likely that biological variation would produce a value so far above any other values recorded for the brain of a fish from the low exposure level.

It is possible that there was an error in tissue analysis, but the most likely cause is contaminated glassware somewhere in the procedure. One other possibility is that the fish was dying, but it showed no symptoms and the blood level was well below the critical level.

Since there was correlation between blood level and death, and brain level and death, it was judged that the two parameters together would be a good indicator of whether or not the animal was being exposed to increased stress due to release of dieldrin from lipid storage.

Dieldrin Uptake

Tissue Residues

There have been many analyses of pesticide residues in tissues and organisms, but few studies have dealt with the uptake rate or change in concentration of residues with time. The pattern of uptake shown in the present experiments is similar to that shown by Grzenda, Paris and Taylor (1970), whose work is probably the most definitive to date on the uptake of pesticides over a long period of time. Their study covered 192 days of ingestion by the goldfish (Carassius auratus) of food dosed with DDT and showed two phases of accumulation: 1) an initial 32-day phase of rapid then decreasing uptake; and 2) a long, constant, slow rate of uptake. The phase two or slow accumulation rate was not

significantly different from zero. The results were similar in the 15 different tissues studied.

Other work of Grzenda (personal communication) with oral uptake of dieldrin by the goldfish showed the same relationship as that above. However, the phase-two rate of uptake, though slow, was significantly different from zero.

In my study, the total uptake of dieldrin in experiments I and II had apparently leveled off by 33 days, so exposure was terminated. As previously stated, the primary purpose of this part of the experiments was to get dieldrin into the animals; therefore, based on the curves obtained in I and II, exposure in experiment III was stopped after 25 days. Since the whole-body residues were not determined until after the experiments were run, it was not immediately known if the residues had leveled off when exposure was terminated.

These residue data indicate residue concentrations directly proportional to exposure concentration. Another way of expressing uptake data is the concentration factor, which is the ratio of the concentration of residue in an organism or tissue to the concentration of the surrounding medium. In this study the concentration factors for the whole fish at the 1.1 ppb exposure level varied from about 6,000 to 10,000 on a wet-weight basis, and, similarly, at the 2.1 ppb level the factors varied from about 6,000 to 11,000. These values again indicate that, at least for the concentrations tested, there is a direct positive correlation of exposure concentration to uptake.

Other workers have found similar concentration factors. Holden (1966) worked with rainbow trout which died within 17 to 23 days in 2.3 ppb dieldrin. The concentration factors were about 4,000 for muscle and 8,000 for liver. The whole-body factor would probably have been somewhere in between. Reinert (1967) found concentration factors of about 12,500 for guppies for three different concentrations of dieldrin. These values are highly comparable to my data and also indicate linearity of uptake from water over the range of concentrations studied.

Residue Distribution

The actual path of an insecticide into and within the animal is important. The hypothesis under which this work has been carried out is that the material moves from the water to the blood, which is the transport medium in the body, and then to other tissues and/or organs, which may include the site of action. It is now generally believed that organochlorine insecticides enter the body mainly through the gills (Holden, 1962).

In order for a material to move from the water into the blood, it must pass through cell membranes. One of the most important factors regulating the entry of a molecule into a cell is its lipoid solubility--the greater the lipoid solubility, the more readily it will enter a cell (Giese, 1962). Dieldrin has an extremely high lipoid solubility and is approximately 6,600,000 times more soluble in benzene than in water (Zweig, 1964).

Studies on rats have shown that in the blood, dieldrin is located mainly in the erythrocytes and the albumin fraction of the plasma (Moss and Hathaway, 1964). From the blood, the dieldrin is taken up by the tissues. The high fat solubility of dieldrin also explains its affinity for and storage in the fatty tissues of the body; organochlorines are particularly soluble in triglyceride fats (Holden, 1962), and are stored at least in part in those tissues (Burdick et al., 1964). If exposure is low, most of the pesticide can be stored by the fat deposits and by fatty tissues, but if the exposure is high enough the pesticide may also accumulate at a site of toxic action.

Equilibration in the uptake-transport-storage process is a rapid phenomenon. Both Gakstatter and Weiss (1967) and Holden (1962) have shown that fish will remove more than 50% of the DDT from a static tank in a few hours. In the flowing systems I used, fish continuously removed a significant fraction of dieldrin. The test tanks were allowed to stabilize at a dieldrin concentration just above the desired test concentration. When fish were introduced, the concentration decreased and by the following day was 20% below the initial concentration. The level then slowly increased, and within one week it could not be distinguished from the starting concentration. There were no controls run on this phase of the work, but the results were similar in all tests.

Some of the variation in final residue levels in uptake experiments can be explained on the basis of fat content. As previously stated the last half of the exposure stage of experiment II was run under a low oxygen condition. Low oxygen resulted from a change in the method of dieldrin introduction to the mixing jug which increased the acetone concentration in the test tanks, and increased BOD. During low oxygen exposure the fish did not feed and lost weight. The resulting loss of fat explains the early leveling off of dieldrin accumulation and the lowest total residue of all tests.

An even better way to relate the effect of fat content to residue concentration would be to measure the dieldrin actually occurring in the fat throughout the body. Unfortunately, however, this cannot be done. The fat alone cannot be extracted from the tissues without extracting the pesticides from all the tissues, not just that related to fat. Conversely, the pesticide cannot be extracted without also extracting fat which may or may not be associated with it. A solution would be to analyze adipose-tissue aggregates, but the small fish used in these experiments did not have fat deposits of useable size.

Since organochlorines are stored in fatty tissues, it was assumed for calculation and comparison purposes that the dieldrin throughout the body was found only in the fat. Relative values for dieldrin concentration on a fat basis

were then calculated by dividing the dieldrin residue by the percent fat. A more direct correlation between dieldrin and fat could be obtained by measuring fat content and dieldrin content of portions of homogenized individual organs; but, in this study the fish were of insufficient numbers and too small for such an approach. Other workers (Dale et al., 1963 and Holden, 1962) have also found more uniform correlations between residues and exposures by expressing concentrations on a per unit weight of lipid basis instead of per unit weight of tissue.

On the basis of the calculations I have made, the mean concentration of residues reached during the low exposure periods for experiments I, II, IV and VI were 321, 317, 328, and 508 ppm, respectively. The value for VI was based on one individual and it may not be typical. For the high exposure periods, III, V, and VII, residues were 634, 781, and 748 ppm, respectively. It is evident (with the exception of the single value for experiment VI) that the uniformity is greater on a fat-content basis than on a tissue-weight basis.

Release of Dieldrin

Fasting During Post-Exposure Period

The pattern of dieldrin release found in experiment I was not repeated in any of the later experiments. The half-life values for other fasting experiments were not as

long as in experiment I, and the fat contents of the fish were slightly higher for the release period of I than for the other experiments. For some reason the fish did not lose dieldrin or fat as rapidly in experiment I as in other experiments. Possibly they had greater carbohydrate stores and utilized these and proteins for a longer time before relying heavily on fat.

It is possible that experiment III was actually the one which was not consistent with the other experiments. There are two possible explanations here for the different results obtained in the two fasting-only experiments: 1) the results of experiment I are typical and a temperature rise in IV did indeed cause a more rapid release of fat and dieldrin; or 2) the results of experiment III are typical and a temperature rise in V caused no change in the fat utilization and dieldrin loss. Number one is the most likely alternative here since a temperature rise should increase metabolism.

The important result here is that the dieldrin released during fasting did not go to the site of action and cause the death of the organisms. When fat was utilized and dieldrin release increased, elimination of dieldrin apparently kept pace with release from storage so that there was no increase in the amount of dieldrin in the blood or at the sites of action.

The three-compartment hypothesis, of tissue-blood-action site movement, is important here, and there is double

evidence that released dieldrin did not produce or would not have soon produced acute effects in the fish: 1) the levels in the blood (transport medium) did not rise; and 2) the levels in the brain (possible site of action) did not rise. If acute effects were going to be expressed in these studies, they would have had to be evident in the blood and brain.

The dieldrin-release data of experiment II cannot be related to any other experiments because of the oxygen stress previously imposed on the animals. However, by themselves the results are interesting. The dieldrin residues and the fat contents of these fish were the lowest of all, and, perhaps more significant, the dieldrin on a per lipid weight basis was also lower throughout the post-exposure period than in the other experiments. This could indicate that if an animal survives a period of stress and dieldrin exposure, it may be better off when environmental conditions improve because of a lower body burden of the pesticide.

The stresses imposed on these fish did not produce the results hypothesized by other authors. For example, Holden (1966: p. 51) stated: "In times of physiological stress, such as starvation or spawning, these lipid reserves are metabolized, and the pesticide content of the lipids will be increased, perhaps to a level at which the pesticide circulating in the vascular system may become toxic for some target organ or nerve center." There was no evidence of

toxic effects, and the pesticide concentration of the remaining lipids did not increase; this will be discussed later.

Feeding During Post-Exposure Period

Some of the most significant and perhaps unexpected results in my work were obtained when the fish were fed after having been exposed to dieldrin. The data here did not fit the previously voiced concept of a stored pesticide being released more rapidly under fasting or stress conditions (Holden, 1966 and Warner, 1967). As already shown the more rapid loss of dieldrin when the animals are fed was highly significant in experiment VII. Although the rate of loss in experiment VI was higher than IV, the difference was not statistically significant.

If one looks at the data in terms of concentration on a per lipid basis, as discussed previously for uptake, the rates of loss of dieldrin in both feeding experiments were increased (at the 0.01 level of significance) over the comparable fasting experiments (Figures 13 and 14). The loss of lipid was lowest in experiments VI and VII, whereas the rate of loss of dieldrin was highest. In experiments IV and V there was a significant loss of dieldrin, but on a per lipid basis the values stayed almost constant. In VI and VII, however, on a per lipid basis the values decreased rapidly.

Figure 13.--Post-exposure loss of dieldrin residues on a per unit weight of lipid basis: fasting (Experiment IV: half-life, 48 days) versus feeding (Experiment VI: half-life, 8.4 days). Subscript numbers are sample size. Lines above and below data points are ranges. Half-lives were calculated graphically.

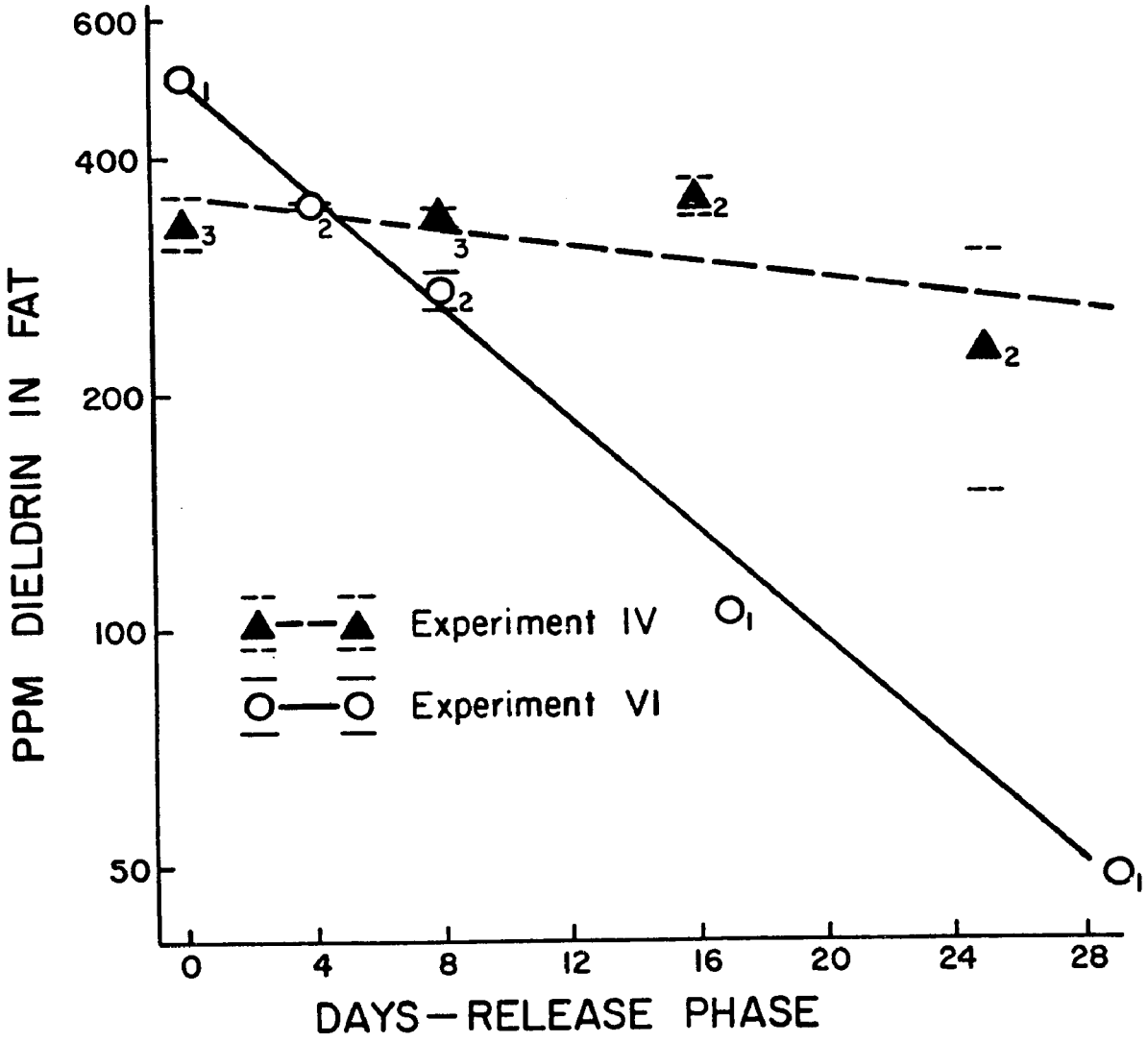
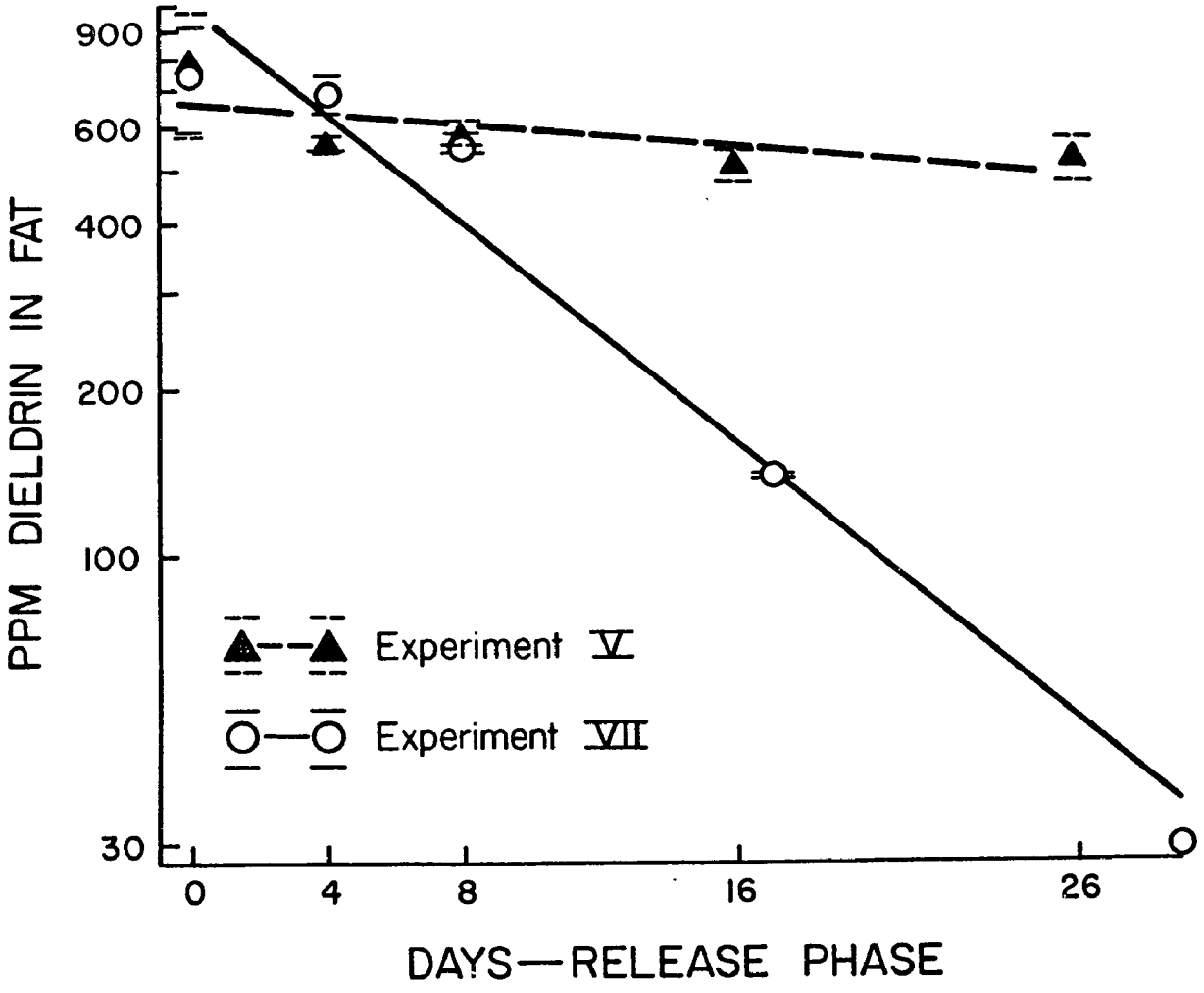

600
400
200
100
50
PPM DIELDRIN IN FAT
0
4
8
12
16
20
24
28
DAYS—RELEASE PHASE
Experiment IV
Experiment VI

Figure 14.--Post-exposure loss of dieldrin residues on a per unit weight of lipid basis: fasting (Experiment V: half-life, 60 days) versus feeding (Experiment VII: half-life, 6.0 days). Subscript numbers are sample size. Lines above and below data points are ranges. Half-lives were calculated graphically.


900
600
400
200
100
30
PPM DIELDRIN IN FAT
Experiment V
Experiment VII
0
4
8
16
26
DAYS—RELEASE PHASE

The data obtained here in a laboratory situation on the rate of loss of dieldrin when fasted or fed is contrary to the theories which have been advanced by most authors, but can be readily explained on the basis of metabolic phenomena. In rats and birds where fasting or starvation has been shown to produce a more rapid rate of release of organochlorines from lipid storage, a fairly constant metabolic rate must be maintained and lipids must be utilized rapidly to maintain the rate. In fish, however, it has been shown that the metabolic rate decreases markedly with time as the animal is fasted (Fry, 1957; Beamish, 1964; and Glass, 1968).

Dynamic equilibrium processes are constantly exchanging fatty acids between fat stores and blood, and insecticides may move with them. It is not known exactly how the insecticides enter the tissues and are stored. But, if they move with the lipids and there is constant intake of lipid in food and no intake or uptake of insecticide, the turnover would create a gradient favoring net movement of insecticide out of the tissues to the blood and it could then be degraded or eliminated. In fasting fish, the movement of fats between tissues would be much reduced, the ratio of fat in tissue to fat in blood would be higher, and the concomitant release of dieldrin would be slower.

One other aspect of dieldrin release which has been demonstrated in these experiments is that there was no

build-up of dieldrin in the fatty tissues as fat was used up. In contrast, Holden (1966) showed that in the rainbow trout, pesticide concentration increased 40 times if lipid levels were reduced by starvation and expenditure of energy during migration. At no time in my fasting experiments, however, was there an increase in concentration on a lipid basis. Data for experiments IV and VI, and V and VII are shown in Figures 13 and 14, respectively, and the results of the other experiments are similar. Even though dieldrin is not rapidly released during fasting, evidently it is released at a rate slightly faster than that at which the fat is utilized. Also, as discussed previously, the breakdown-elimination processes of the fish apparently remain active and are not decreased as much as the general metabolism under fasting conditions. Evidence to support this hypothesis was presented by Hart and Fouts (1965) who found that starvation of rats previously exposed to DDT could re-induce activation of drug metabolism by the liver. Dieldrin stored in fish may produce similar effects.

Urinary Excretion of 17-OHCS Hormones

One of the hypotheses under which this work was undertaken was that if the release of dieldrin were causing increased stress on the animal the effect would show up as an increase in excretion of "stress" hormone metabolites. Following the insertion of catheters and the placing of

the fish in their chambers, the post-operative stress induced approximately the same mean initial excretion of hormones in both groups of fish. Ideally, it would have been valuable to study hormone excretion through the entire uptake-release period of this study. However, urine collection in the tests that were run was successful only up to a maximum of 17 days, because of loosening or plugging of the catheter. Since the number of large fish available was limited and only one phase of the total picture could be studied, it was felt the release phase was much more important in this project.

It was obvious that the fish with dieldrin in them were under more stress than those without dieldrin. In this species of fish under the conditions of these experiments, the "normal" or base rate of excretion of 17-OHCS hormones by control fish was slightly more than 1 µg per 100 g fish per day. The test fish obviously excreted at a rate much above this level. Whether this increase in excretion was still an effect of previous dieldrin exposure or was due to the release of stored dieldrin going on at that time cannot be resolved from these data. Whatever the cause, for some reason the animals were not in as good condition after being removed from dieldrin exposure as were fish which had not been exposed to dieldrin.

The most important observation here is that for at least nine days of fasting after removal from dieldrin there was no increase in stress. If an increase had been found

it might indicate that stored dieldrin was affecting a critical tissue. After nine days there appears to be the start of an increase, but no further data could be collected and the variation was too great to tell if the increase was real.

The consistently high maximum values of the ranges for test fish were not for the same fish, but varied among fish with no pattern. Two minor differences between test and control fish were apparent: 1) the variation between fish on a given day was greater in the test fish; and 2) urine collection failed sooner in the test fish. These differences may be indicative of a general weakening of the test fish.

The foregoing would seem to be a good area for further research. If techniques could be improved with this fish (or another species) which would enable longer urine collection, the study of a continuous uptake and release period would be valuable. If that is not feasible, then the uptake portion should be studied alone. Also valuable, in light of the results obtained by the feeding of fish after exposure has ceased, would be the study of the stress imposed on the fish during this period of rapid release and loss of dieldrin.

General Comments

There was no direct evidence obtained that the release of dieldrin from fat depot storage was harmful to this species, although fish previously exposed to dieldrin were apparently under more stress than control fish. However, some conclusions can be drawn which are similar to those drawn by other investigators, and results have been obtained by others which support those found here.

Lowe (1965), after chronic exposure of the spot (Leiostomus xanthurus) followed by a period of starvation, concluded that endrin residues in the tissues of living fish do not appear to be detrimental during periods of starvation when fat is being mobilized. These observations support the results of the present study; but, the spot had only 67 ppb in whole-body residues. Conversely, Mount (personal communication) also used endrin when he observed the death of channel catfish during a period of fasting. A difference between dieldrin and endrin toxicity during fasting could possibly be explained on purely physical distribution factors because of the relatively small amount of endrin needed to cause toxicity. Much less endrin would have to be released to overbalance the blood-lipid equilibrium and allow a toxic amount to reach a critical site. However, this is probably not the explanation, since all concentrations would be lower and the tissue ratios should be similar to those for dieldrin. Most likely, Mount's observations

are the result of a different species and a different pesticide.

A very important factor affecting release from lipid depots is the amount of stress to which the fish is subjected. My experiments were done in the laboratory with fewer stresses than fish might be exposed to in the natural environment. Stresses such as escape from predators, swimming in a current, disease or parasitism, migration and spawning, or any combination of these could cause more insecticide release and toxic effects than were produced in the laboratory. The 40-fold concentration of pesticide in lipids found by Holden (1966) was in migrating fish.

The sex of an animal in relation to storage and release might be significant if adults were being studied, but in these experiments the gonads of the fish were just starting to develop and there was no detectable difference in the uptake, storage, or release between males and females.

A conclusion reached by Allison et al. (1964) that DDT increased mortality by reducing resistance to other, nonspecific stresses may also apply to fish with a high body burden of pesticide some time after actual exposure. Katz (1961: p. 267) stated: "Even if mortalities do not occur following insecticide applications, the depression of normal activity patterns (physiological functions, physiological defense mechanisms, etc.) may make some fish unable to carry on their functions with the competitive

efficiency required for survival in their natural environment." This statement also applies here and very nicely sums up the probable effects of the release of stored dieldrin on fish.

It is apparent from these experiments that even though there may be no obvious gross effects from the exposure of fish to dieldrin, the fish still has a body burden of a potentially toxic substance. If the exposure passes and the fish has an adequate diet, the dieldrin will be released quickly with probably no further effects. When the fish is subjected to fasting, however, a dieldrin burden would remain which could make the animal more susceptible to a further dieldrin exposure; residues could be passed via eggs to offspring and could exert an effect upon the next generation.

SUMMARY

1. Concentrations of dieldrin in blood and brain of the test fish (Cichlasoma bimaculatum) were higher in individuals killed by dieldrin than in those exposed to, but not killed by dieldrin.

2. When exposed to sublethal levels of dieldrin, fish showed a rapid initial uptake which slowly leveled off. By about 30 days, there was little or no further increase in net uptake.

3. When dieldrin exposure was terminated, the concentration factors in all experiments for whole-body residues were between 6,000 and 10,000 on a wet-weight basis.

4. During the post-exposure period, fasting alone caused a slow rate of loss of dieldrin from the tissues studied.

5. Fasting plus a 5°C temperature rise produced a more rapid loss of dieldrin from the tissues studied than did fasting alone.

6. When fed normally, but subjected to a 5°C temperature elevation, the fish showed a very rapid loss of dieldrin with very little loss of fat.

7. During the post-exposure period there was never a significant increase in blood- or brain-dieldrin concentration above the final concentration attained during the exposure phase of a given experiment.

8. Much greater linearity and less variation were observed in dieldrin residues when the data were expressed on the basis of per unit weight of lipid than when expressed on a per unit weight of total body tissue.

9. When dieldrin concentrations were calculated on a per unit lipid basis by using estimates of body fat, there was no increase in concentration of dieldrin in remaining lipids when stored energy reserves were mobilized.

10. Urinary excretion of 17-OHCS hormone metabolites was higher in fish previously exposed to dieldrin than in controls at all times after the first day of urine collection. The level in exposed fish continuously decreased thereafter, indicating the absence of any sudden increase in stress in the test animals during the period in which dieldrin mobilization was expected.

11. There was no evidence that dieldrin was released in toxic quantities when a fish was forced to utilize its fat stores under the test conditions utilized in these studies.

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