EFFECTS OF DIELDRIN ON THE GROWTH AND DEVELOPMENT OF STEELHEAD TROUT

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Abstract: Experiments were conducted to determine survival, growth, and accumulation of HEOD (major component of dieldrin) in the tissues of steelhead trout (*Salmo gairdneri*) embryos, alevins, and fry exposed to dieldrin concentrations ranging from 0.012 to 52 ppb. The fish were held in specially designed exposure chambers provided with continuously renewed water for periods as long as 130 days. Eggs (embryos) exposed to dieldrin concentrations as high as 52 ppb from time of fertilization survived as well as controls through hatching; however, the mean weight of the newly-hatched alevins (minus yolk material) was reduced by high dieldrin concentration in the water. Alevins were much more sensitive to dieldrin than were embryos; their survival was reduced at all concentrations above 0.39 ppb. Trout fry, whose survival was unaffected at dieldrin levels of 0.12 ppb and lower, quickly succumbed at 0.39 ppb and higher test concentrations. HEOD accumulated in the yolk material and the embryo during development. The intensity of these accumulations was found to be directly related to the dieldrin level in the water. HEOD continued to accumulate in the fry throughout the test period, and the residue levels remained dependent on the exposure concentration. Growth rates of the test fish were reduced at dieldrin concentrations above 0.12 ppb.

Introduction

When the aquatic community becomes contaminated with insecticides it is difficult to evaluate changes that may take place. As yet, biologists do not fully understand how persistent pesticides such as dieldrin are cycled through the food web, nor do we understand the relationships between accumulated pesticide residues and the survival, growth, reproduction, and population densities of the various resident organisms. But still we are faced with the problem of providing information on which regulations can be based that will provide protection for the inhabitants of the aquatic environment and yet will not unnecessarily restrict the use of these highly effective pest control agents.

The uptake of dieldrin from food and water by fish and fish-food organisms has been studied by Chadwick and Brocksen (1969), Gakstatter and Weiss (1967), Reinert (1967, 1969), Hansen (1966), and Holden (1966). From these and other studies, it is apparent that the rate of uptake of dieldrin from the water is very rapid and that concentrations in the tissue of fish can reach levels that are several thousand times those present in the water.

The significance of high levels of dieldrin in the tissue of fish is uncertain. We know of no studies that have clearly shown that stored dieldrin, even at relatively high levels, has a detrimental influence on adult fish. But accumulated dieldrin in adult fish may affect future generations. The results from several studies have shown that quantities of stored DDT and dieldrin present in eggs at spawning time may ultimately affect the survival of the young.

Burdick et al. (1964) reported that DDT levels of 2.93 ppm and above in the eggs of lake trout (*Salvelinus namaycush*) reduced the survival of the alevins. Johnson and Pecor (1969) reported that eggs from Lake Michigan coho salmon (*Oncorhynchus kisutch*) contained from 1.1 to 2.8 ppm of DDT and that mortality through the eighth week after hatching was from 15 to 70 percent. Higher losses of fry during this period were generally associated with higher residual levels of DDT in the eggs. Unfortunately, neither Burdick et al. (1964) nor Johnson and Pecor (1969) reported dieldrin levels in the eggs. However, Johnson (personal communication) stated that dieldrin concentrations of 0.05 to 0.10 ppm were found in the eggs. Reinert (1969) reported substantial quantities of dieldrin in the tissue of adult fish from Lake Michigan, but residue levels in the eggs from these fish were not reported. From Reinert's study with adult fish and Johnson's work with coho salmon eggs, it seems likely that substantial quantities of dieldrin were present in the eggs of fish in Lake Michigan. Such accumulation may have contributed to the fry mortalities observed by Johnson and Pecor.

The investigation reported in this paper was undertaken to determine for steelhead trout (*Salmo gairdneri*): (1) levels of dieldrin in the water that are lethal to eggs, alevins, and fry during short- and long-term exposures; (2) rate of dieldrin uptake by eggs, alevins, and fry, held at various concentrations; and (3) effect of dieldrin concentrations in water on the growth of the embryos, alevins, and fry.

Materials, Apparatus and Methods

One hundred percent technical grade dieldrin was used throughout this investigation. No carrier, dispersing agent, or other additive was used to assist the introduction of this material into solution. As chemical analyses were made only for the major component of dieldrin (1,2,3,4,10,10-hexachloro-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene, abbreviated HEOD) and not for whole dieldrin, the term HEOD will be used whenever reference is made to analytical measurements. Otherwise the term dieldrin will be used, since it is more familiar.

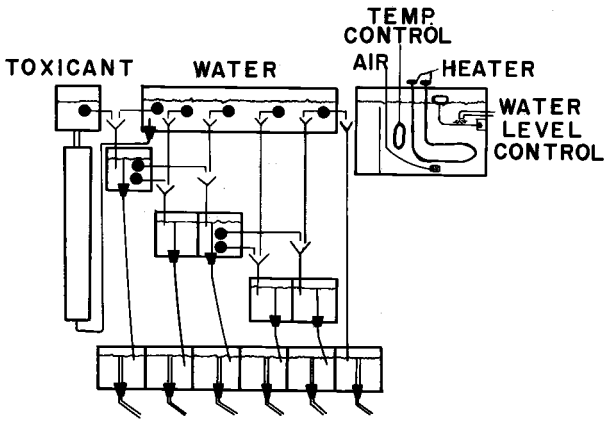

FIGURE 1. Schematic diagram of the dilution apparatus used in this study.

A dilution system similar to that described by Chadwick and Brocksen (1969) was used to maintain the desired dieldrin concentrations in the exposure chambers (Fig. 1). In this system control water was mixed with water discharged from a column of sand coated with dieldrin. As the water flowed through the column of sand, dieldrin was dissolved by the water (Chadwick and Kiigemagi, 1968). The concentration of dieldrin in the effluent from the column was periodically determined by gas-liquid chromatography and the ratio of control water to water from the column was adjusted to obtain the desired experimental concentrations. Water or dieldrin solutions from the dilution apparatus entered small acrylic plastic distribution chambers (Fig. 1). In Experiment 1 eggs were placed directly in these chambers and no other apparatus was used. In Experiment 2 water from the distribution chambers was distributed to egg-rearing chambers (Fig. 2) and to small aquaria.

The egg-rearing chambers used in the second experiment are described in detail by Chapman (1969) (Fig. 2). These chambers provided eggs and alevins with water at a controlled velocity and allowed the use of larger numbers of eggs and alevins than was possible in the plastic chambers used in Experiment 1. Each egg-rearing chamber was constructed from a 4-l. erlenmeyer flask with the base removed. Water from the dilution apparatus entered at the bottom of the inverted flask (See Fig. 2), and was dispersed upward through marbles and glass beads before flowing past the eggs. The chambers were painted black and fitted with lids to prevent excessive light from entering.

The aquaria were used in Experiment 2 once the fish began feeding. These aquaria were constructed from 1-gal. wide-mouth plastic containers. One-eighth inch holes were drilled 2 in. from the top of each aquarium for overflow. A galvanized pan collected the overflow and acted as a constant-temperature water bath. Water and dieldrin solutions from the diluter were distributed to each of 6 polyethylene manifolds through vinyl tubing. From each manifold small, plastic, leader tubes distributed the water to 12 aquaria.

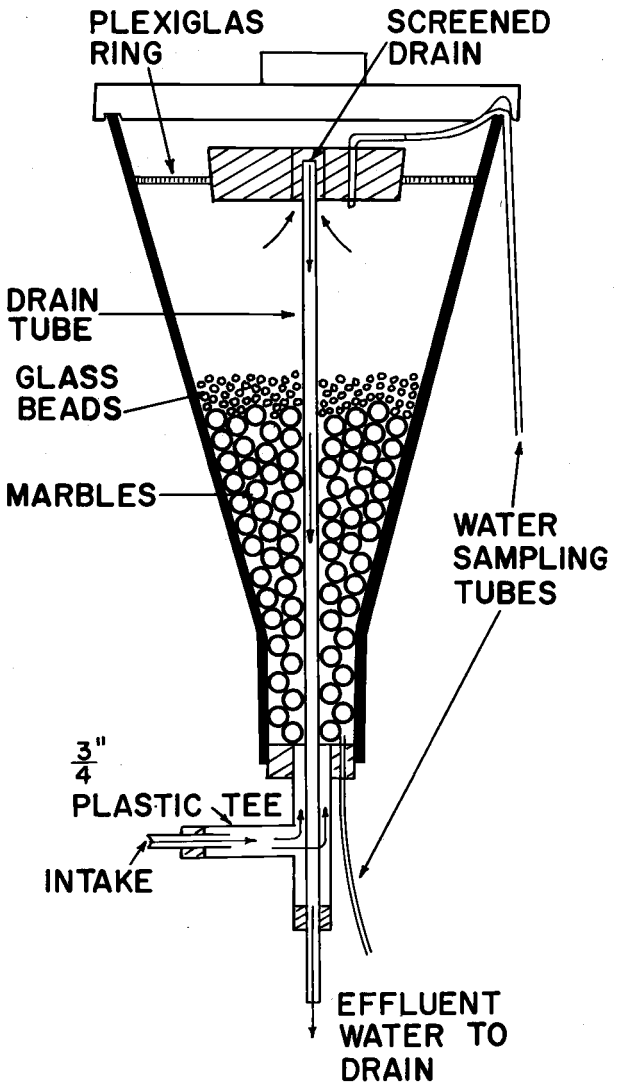

FIGURE 2. One of six experimental chambers used to expose steelhead trout eggs and alevins to dieldrin in Experiment 2. The eggs were placed in a single layer directly on the glass beads.

Water and animal samples were analyzed for dieldrin by personnel of the Department of Agricultural Chemistry, Oregon State University, using gas-liquid chromatography. Water samples were collected in 1-l. brown glass bottles and analyzed within 1 week. All animal samples were wrapped in aluminum foil and stored frozen until analyzed for dieldrin.

Freshly-fertilized steelhead trout eggs from the Oregon Game Commission's hatchery on the Alsea River were transferred to the Oak Creek Laboratory at the start of each experiment and placed in the apparatus within 3 hrs after fertilization.

In Experiment 1, approximately 60 eggs were placed directly in each distribution chamber at the bottom of the dilution apparatus (Fig. 1). The concentrations of dieldrin used were 0.17, 0.52, 1.7, 5.2, 17, and 52 ppb. Continuous renewal of the test solution

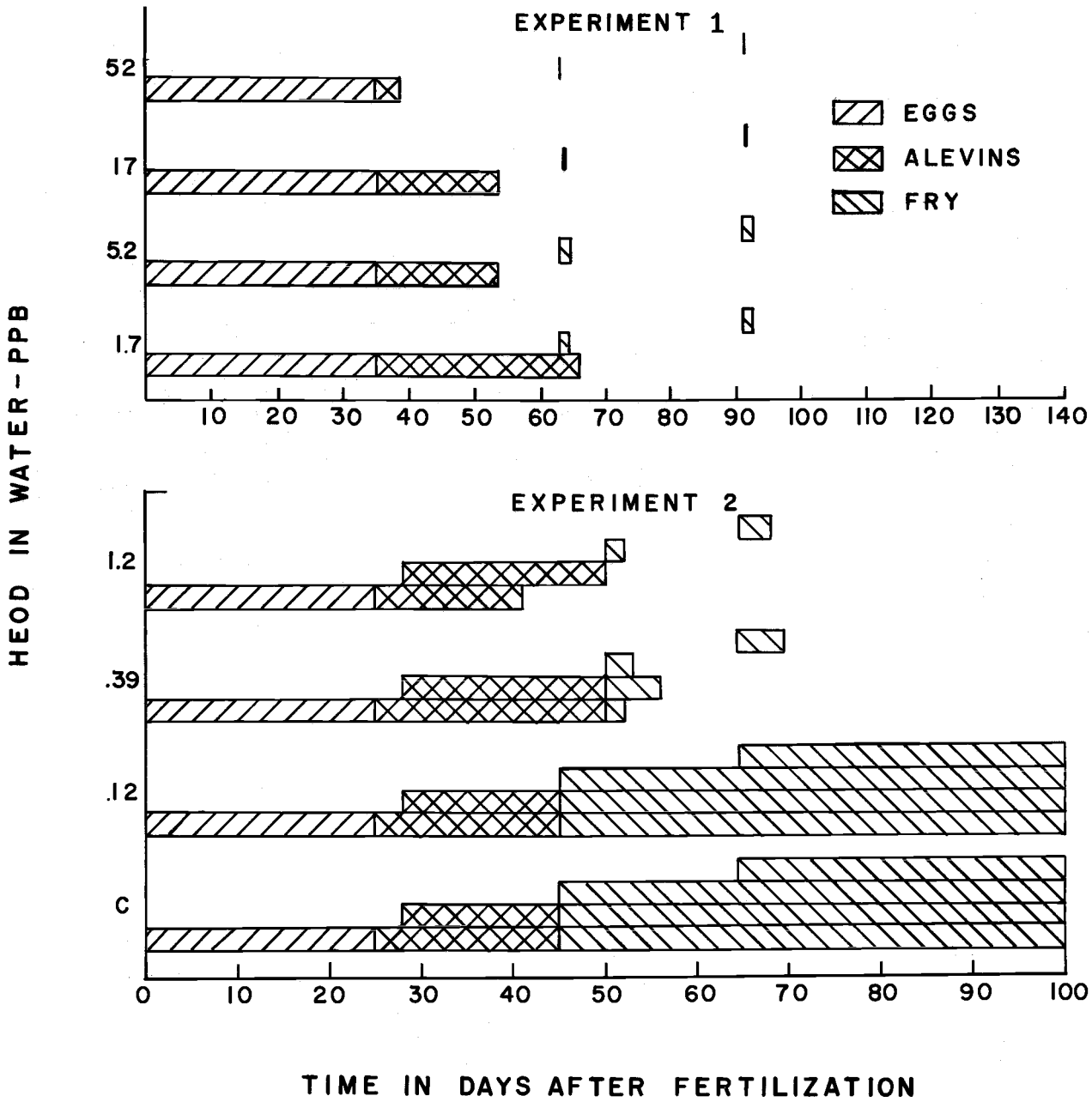

FIGURE 3. The bars indicate survival times of steelhead trout eggs (embryos), alevins, and fry exposed to various dieldrin concentrations in Experiments 1 and 2. Each bar starts with the age of the fish (days after fertilization) when they were first exposed to dieldrin and ends with the age of the fish (days after fertilization) when 50 percent of the fish died, or with the termination of the experiment.

at approximately 50 ml per min prevented a buildup of metabolic wastes or significant changes in dieldrin concentrations during the exposure periods. Temperature of the experimental water fluctuated with natural stream conditions. The average experimental temperature was about 9° C.

One day after hatching, all but 5 alevins in each exposure chamber were removed and analyzed for HEOD. The remaining alevins were removed for HEOD analysis when those in the control chamber reached the "buttoned-up" stage. Throughout the experiment, records were kept of embryo, alevin, and fry mortalities.

At the start of Experiment 2, 450 eggs were placed in each of the egg-rearing chambers and held at about 12° C. These eggs remained in the chambers through hatching and until the alevins were ready to feed. At that time they were moved to 1-gal. aquaria.

Tubificid worms (*Tubifex sp.*) were used as food for the fry while they were held in the aquaria. These worms were periodically collected from the gravel

bottoms of raceways at the Oregon Game Commission's Roaring River Hatchery, Linn County, Oregon, and transferred to holding troughs at the Oak Creek Laboratory.

An initial sample of eggs was taken for dieldrin analysis, but because steelhead eggs are easily killed if disturbed during the early developmental stages, no additional samples were taken until after the eggs were well-eyed at 19 days. At that time a sample of eggs was removed from each concentration and divided into 2 equal subsamples. The chorions were removed from the eggs in 1 subsample from each concentration and both samples were weighed and analyzed for dieldrin.

After hatching, the alevins were sampled at weekly intervals. These samples were also divided into 2 equal subsamples. The alevins in 1 subsample were counted, weighed, and analyzed intact, while the yolk was removed from the alevins in the other subsample before they were weighed and analyzed for dieldrin. After the yolk appeared to be completely absorbed, samples of the remaining fish were taken at 1- to 2-week intervals for weights and dieldrin analyses.

Results and Interpretation

Acute toxicity of dieldrin to steelhead. Although this study was conducted to determine sublethal effects of dieldrin on steelhead trout, some data on lethal levels were obtained also. Figure 3 presents survival time to 50 percent mortality for several groups of steelhead trout embryos, alevins, and fry exposed to a wide range of dieldrin concentrations (0 to 52 ppb). Each bar in Fig. 3 indicates the age of the fish in days after fertilization when exposure commenced and the age after fertilization when 50 percent of the exposed fish had died. Tests with fry held at 0.12 ppb of dieldrin and under control conditions in Experiment 2, were terminated 100 days after fertilization without reaching the 50 percent mortality level. The fish in Experiments 1 and 2 were held at about 9° C and 12° C, respectively.

Egg, embryo, and alevin deaths felt to be unrelated to dieldrin exposure were excluded in determining time to 50 percent mortality. Approximately 20 percent of the eggs at each concentration were not viable. The only other significant loss occurred during the second week of incubation when approximately 20 percent of the viable eggs in 0.12 ppb of dieldrin died as a result of a failure in the exchange water system.

Survival of developing embryos was not reduced measurably by exposure to dieldrin concentrations as high as 52 ppb. Although substantial quantities of HEOD accumulated in the eggs prior to hatching at all test concentrations, the number of days required for hatching remained unchanged by dieldrin exposure. Some effect of dieldrin on the embryos was apparent, however, since alevins from the higher concentrations were smaller at time of hatching. Accumulation of HEOD by the eggs will be discussed later in the text.

Alevins were found to be more susceptible to dieldrin than were embryos (Fig. 3). More than 50 percent of the alevins exposed to dieldrin concentrations of 0.39 ppb and below, regardless of whether or not they were exposed to dieldrin during embryonic development (pre-hatch period), survived to reach the fry stage. However, at this level, appreciable delay occurred in reaching the fry stage and some increase in the mortality rate was noted. At 1.2 ppb and above, 50 percent mortality occurred during the alevin stage.

Steelhead trout fry were found to be more susceptible to dieldrin than were earlier stages, regardless of whether or not they had been exposed previously to dieldrin under test conditions. As may be seen in Fig. 3, more than 50 percent of the fry held at 0.12 ppb of dieldrin survived to the end of the 100-day experiment. Fry exposed to dieldrin concentrations of 0.39 ppb and above reached the 50 percent mortality level within a few days regardless of their previous exposure history.

The concentration of HEOD in newly-hatched steelhead trout alevins appeared to be directly related to the exposure concentration (Fig. 4). In Experiment 1, exposure of eggs to dieldrin concentrations of 0.174 to 55 ppb resulted in HEOD concentrations in newly-hatched alevins (tissue plus yolk material) from 0.69 to 146 ppm.

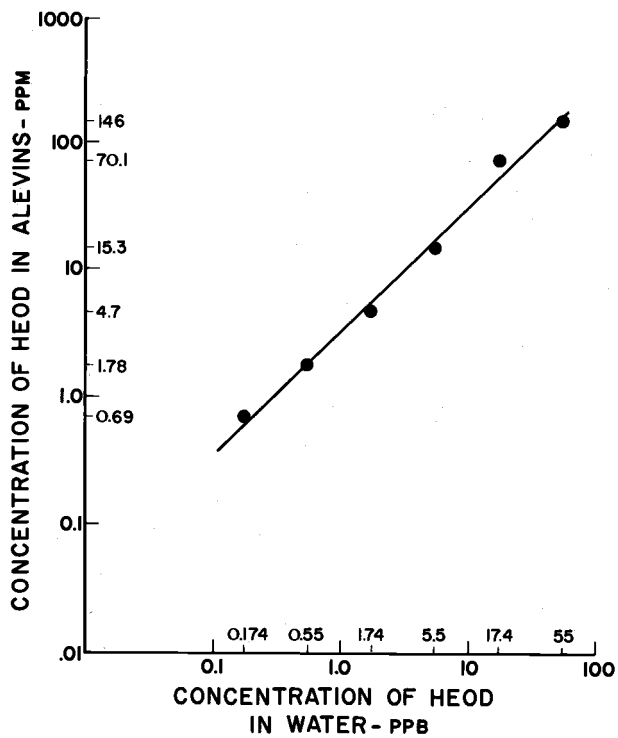

FIGURE 4. The concentration of HEOD in newly-hatched steelhead trout alevins exposed to various dieldrin concentrations for 35 days at about 9° C in Experiment 1. Samples of alevins analyzed for dieldrin were removed within 24 hours after time of hatching.

The HEOD concentration in the alevins increased with increasing exposure concentration and as a function of the level of pesticide in the water. The relationship between HEOD concentrated by the fish and the

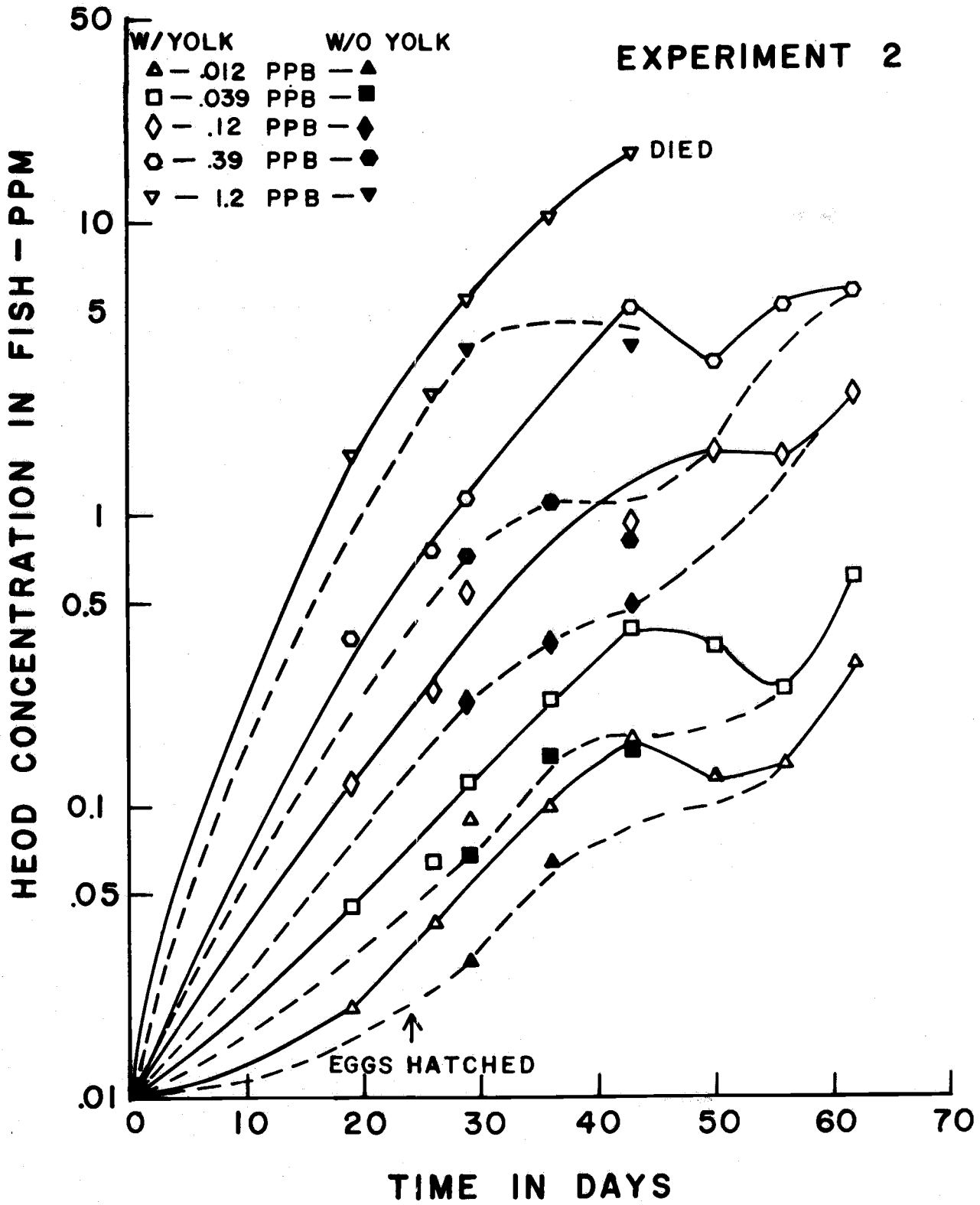

FIGURE 5. The relationship between exposure to dieldrin in days and the concentration of HEOD in steelhead trout in Experiment 2. The open plots represent tissue concentrations of HEOD in embryos and alevins with yolk. Solid plots represent alevins with the yolk removed. In pre-hatch samples the chorions were removed from the eggs prior to analysis.

concentration in the water is linear. This result is similar to that observed by Chadwick and Brocksen (1969) for adult sculpins. It is interesting to note that the degree of magnification is higher at the lower dieldrin concentrations tested (about 4,000 times) than at the highest concentration tested (about 2,600 times). The same general relationship was reported by Chadwick and Brocksen (1969), who also showed that the degree of magnification is time-related.

In Experiment 2, steelhead trout eggs were exposed to dieldrin concentrations of 0 to 1.2 ppb from time of fertilization to the termination of the experiment some 60 days later. Two curves are presented for each exposure concentration of dieldrin in Fig. 5. One curve (solid line) at each level expresses the relationship between the length of exposure and the HEOD level in the fish with the yolk material attached. The other curve (broken line) relates the length of exposure and the HEOD levels in the elaborated tissue minus the yolk material. It is apparent from these data presented in Fig. 5 that HEOD readily penetrates the chorion of the egg during development and concentrates in both the elaborated tissue and the yolk material. HEOD levels are somewhat higher in the yolk material than in the elaborated tissue. Presumably the higher lipid content in the yolk material accounts for the difference in the rate and level of HEOD accumulation.

A few days before the yolk material was completely utilized, a reduction in the HEOD level of the whole fish was observed at nearly all exposure levels (Fig. 5). This reduction in concentration can be explained by the loss of HEOD from the fish, possibly through direct excretion of either HEOD or fat containing the HEOD, although we do not have data to support this. Levels of HEOD in the alevins minus the yolk showed a reduction in the rate of HEOD accumulation at about the same time as the above reduction was noted, although the absolute tissue levels show a slight increase in most cases. Once the yolk material had been utilized by the fish, the rate of HEOD accumulation appeared to increase to rates equal to or greater than those noted earlier (Fig. 5).

Although the accumulation of HEOD by steelhead trout embryos, alevins, and fry was directly related to the levels of HEOD in the water, growth was unaffected at levels of 0.12 ppb and below (Fig. 6). At 0.39 ppb of dieldrin, both growth and survival were greatly reduced. Only about 3 percent of the fry reared at 0.39 ppb level survived the 130 day experiment. At 1.2 ppb of dieldrin, complete mortality occurred by 45 days of exposure. During the last 10 days of the experiment, those fish reared at the 0.39 ppb level appeared to grow as well as the control fish. The reason for this is uncertain. In any event, the remaining fish appear to have recovered from the earlier growth-depressing effects of dieldrin.

In our study the growth and survival of steelhead trout embryos, alevins, and fry appeared to be unaffected by dieldrin concentrations of 0.12 ppb and below. Higher concentrations (0.39 and above) can be expected to inhibit growth or alter the survival of steelhead trout. The influence of other factors such as temperature, consumption of dieldrin-contaminated food, or the presence of other toxicants, on growth and survival of steelhead trout should receive further attention before a definite safe-level for dieldrin can be predicted.

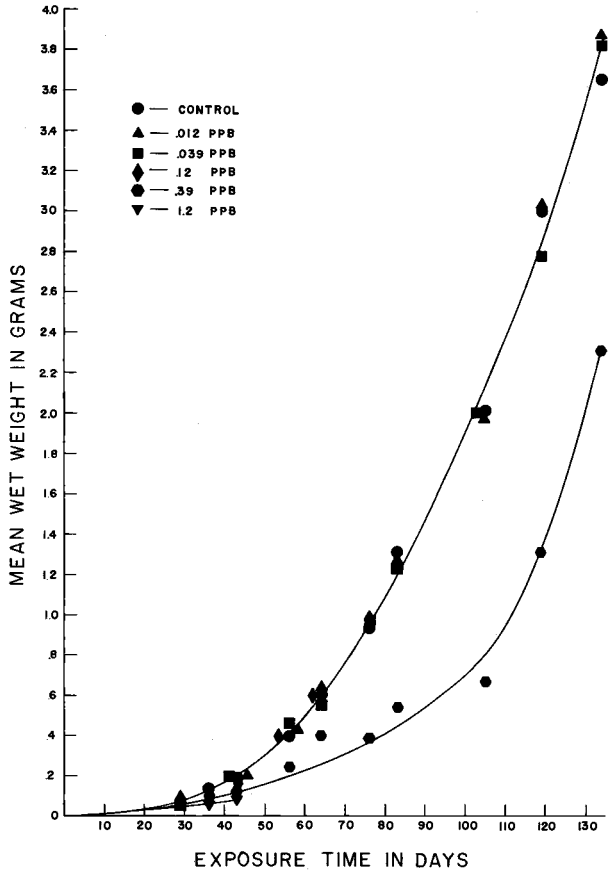

FIGURE 6. The relationship between mean wet weight of steelhead trout and length of exposure to different dieldrin concentrations in Experiment 2. The fish were exposed to the test concentrations from time of fertilization. Less than 3 percent of the fish held at the 0.39 ppb level survived to the end of the experiment. The upper curve was fitted by eye to the data obtained at 0.12, 0.039, and 0.012 ppb of dieldrin and the control data.

Acknowledgment

Technical Paper No. SR291, Oregon Agricultural Experiment Station. A contribution from the Pacific Cooperative Water Pollution Laboratories, Oregon State University. This investigation was supported by the U. S. Public Health Service Grant ES-00040.

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