# THE TOXIC EFFECTS OF THE PESTICIDE LINDANE ON THE EARLY DEVELOPMENTAL STAGES OF THE FATHEAD MINNOW, *Pimephales promelas*

by

Anabel G. Tatis

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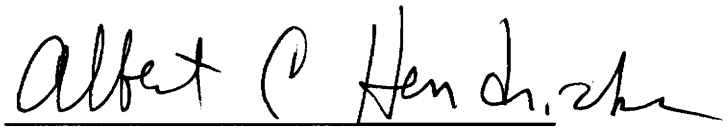

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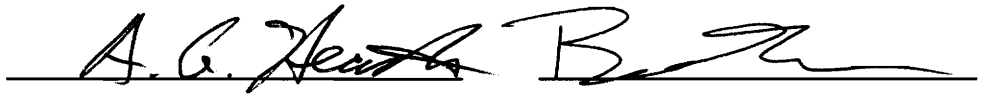

A. G. Heath B. J. Turner

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**(ABSTRACT)**

Toxic effects of the pesticide Lindane on the eggs, yolk-sac fry and larvae of the fathead minnow (*Pimephales promelas*) were investigated over 14 days at 25 and 18°C. The general objective was to understand the effects of the pesticide on the early life stages of the fish, and to evaluate the suitability of these stages as indicators of contaminated environments.

Egg, yolk-sac and larval stages that had been exposed to 300, 120, 90, 60, 30, 10, 5, and 0 µg/L of Lindane at 25°C exhibited a 14d-$LC_{50}$ of 44 µg/L. Similarly, the same stages exposed to 300, 120, 90, 60, 30, 10, and 0 µg/L of Lindane at

18°C exhibited a 14d-$LC_{50}$ of 37 μg/L. Lower temperature thus seemed to increase the susceptibility of the fish to the pesticide.

Mortality data were also used to determine the sensitivity of the early developmental stages of fish to the pesticide. At 25°C, the larval stage was the most sensitive stage. At 18°C, on the other hand, the most sensitive stage was the yolk-sac. Results obtained from experiments in which Lindane doses were applied at different intervals within the 14d-period supported these observations.

Low temperatures had a marked effect on the sensitivity of the fathead minnow to Lindane. Embryos exposed to Lindane at 18°C showed different temporal distribution of mortality than those at 25°C. A delay in hatching and emergence of smaller larvae were also noted at 18°C.

Lordoscoliosis, edemas and hemorrhages were the most common morphological changes induced by the pesticide during the larval stage. The frequency of these abnormalities was linearly related to doses of Lindane. Impaired swimming behavior was also noted, but it was not related to dosages.

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# INTRODUCTION

Pesticides, because of their design and application, induce a broad spectrum of selective and nonselective biocidal effects influencing all taxonomic groups of organisms (Matsumura et al., 1972; Brown, 1978). There is considerable variability in species sensitivity to a particular pesticide as well as variation in the toxicity of different pesticides to a particular species.

Until recently, however, fish toxicity studies have been almost entirely concerned with the reaction of adult fish to acute exposures. Increasing interest is now being shown in the reactions of the early developmental stages of fish to long-term exposures. Mass mortality of fish due to pesticide exposure is rare, and results only from accidents or direct spraying of bodies of water. More commonly, because of their mobility, adult fish can avoid or emigrate from polluted areas. Such is not the case, however, for embryonic and larval stages which are either planktonic or demersal. These stages are subject to long term exposures that, in the long run, may prove to be as deleterious as lethal exposures, because subtle and small effects on the fish eggs may alter their hatchability, growth, position in the school, reproductive success, behavior, feeding habits, etc.

The developing embryo or larva is generally considered the most sensitive stage in the life cycle of a fish. Certain stages in the life cycle of marine and freshwater fishes are most susceptible to environmental and pollutional stress than others (Rosenthal and Alderdice, 1976). Differences in susceptibility are particularly known to exist between the early developmental stages, that is, embryos, yolk-fry and larvae (Pickering and Vigor, 1965; Skidmore, 1965; Malone and Blaylock, 1970; Danil'chenko, 1978). Most of the studies related to this topic and published up to 1987 have been summarized by Weis and Weis (1989).

For many freshwater and marine fishes, the tolerances and sensitivities to individual pesticides have been investigated by bioassay procedures (Danil'chenko, 1978; Sharp et al., 1979; Niimi, 1983). Most of these investigations have involved the determination of the $LC_{50}$; i.e. the concentration which will kill 50% of a group of fish in a given period of time (Skidmore, 1964; Schimmel et al., 1974; Hansen et al., 1977; De Foe et al., 1978; Buckler et al., 1981; Holcombe et al., 1982; Pickering and Gilliam, 1982; Spehar et al., 1985; and others). Johnson (1968) provides a good review of the effects of pesticides on aquatic life. More recent, studies on the toxicity of chemicals to fish and aquatic invertebrates are reviewed by Johnson and Finley (1980).

On the other hand, studies of long-term exposure to pesticides are more difficult to perform, but in recent years a number of such investigations have been reported (McCann and Jasper, 1972; Wies and Weis, 1974, 1976; Kaur and Toor, 1977; Klaverkamp et al., 1977; Palawski et al. 1983). Much of the available information refers to the organochlorine pesticides (Schreiman and Rugh, 1949; Merhle and Mayer, 1975; Weis and Weis, 1976; Couch et al., 1977; Dethlefsen, 1977; Hansen et al. 1977; Venugopalan and Sasibhushana Rao, 1979; Goodman et al., 1982; Holdway and Dixon, 1986). In evaluating toxicity of pesticides to fish, consideration of sublethal effects should be investigated since they may indirectly lead to reduce survival and reproduction in natural populations (Rosenthal and Alderdice, 1976).

Factors such as water temperature and the stage of development are known to affect the tolerance and sensitivity of fish to pesticides. Differences or changes in temperature, can affect the distribution, growth, reproduction, metabolism and behavior of fish in ways which expose them to a greater or less degree to toxicants present in the water. Several reports suggest that temperature has a marked effect on the susceptibility of fish to pesticides (Cairns et al., 1975, Iyatomi et al., 1958; Bridges, 1965; Cope, 1964, 1965a, 1965b, 1968).

The purpose of the present study was to determine: (1) The 14d-$LC_{50}$ values of Lindane on the fathead minnow (Pimephales promelas) embryos at 18° and 25°C, (2) The distribution of mortality among developmental stages, and the sensitivity of fathead minnow embryos exposed to different concentrations of Lindane at 18° and 25°C, and (3) The more common morphological malformations observed in the larval stage at 25°C. I also determined $LC_{50}$ values for different concentration-interval treatments, and compared the survival distributions generated among treatments.

## LITERATURE REVIEW

### FATHEAD MINNOW

#### 1. Taxonomy, distribution and life history

PHYLUM Chordata

SUBPHYLUM Vertebrata

CLASS Osteichthyes

SUPER-ORDER Ostariophysi

ORDER Cypriniformes

FAMILY Cyprinidae

*Pimephales promelas* Rafinesque

Note: The specific name *Pimephales promelas* may be incorrectly applied to the species now known as fathead minnow. The latter does not fit the description originally given by Rafinesque in 1820 (Lee et al., 1980). Common names include "northern fathead minnow", and "blackhead minnow" in addition to fathead minnow.

The fathead minnow is widely distributed in North America. This species is most abundant in small lakes, ponds, small streams, brooks and creeks, and uncommon in large and deep impoundments, and in streams of high gradients (Trautman,

1957; Scott and Crossman, 1973).

The fathead minnow is primarily omnivorous. Adults feed on aquatic insects, worms, small crustaceans and other animals. Young fry have been reported to feed on organic detritus from bottom deposits and unicellular and filamentous algae (Klemm, 1985).

The life history and spawning behavior of the fathead minnow are very well known because of the early interest in this species as a highly desirable forage and bait fish (Markus, 1934; Flickinger, 1973; Andrews and Flickinger, 1974; Gale and Buynak, 1982). In the wild, fathead minnows spawn in spring and summer when the water temperature reaches 16°C to 18°C. The minimum temperature required for spawning is 16°C (Carlander, 1969). The ovaries of the females contain eggs in all stages of development, and they spawn repeatedly as the eggs mature. Immediately after the eggs are laid, they are fertilized and guarded by the male. The average number of eggs spawned per female is generally 100 to 150, though big females may lay 400 to 500 eggs per spawn.

The hatching time depends on temperature with an average time of 4.5 to 6 days at 25°C. Newly hatched larvae are about 4mm long, white to transparent, with prominent black eyes. In warm, food-rich waters, young grow fast, and are generally

ready to spawn in their first year.

Fathead minnows are short lived; individuals rarely survive to the third year. Nevertheless, Scott and Crossman (1973) reported that the age of this species seems to vary throughout its geographic range. While several authors have indicated that post-hatching mortality in the fathead minnow is often great in the wild, Gale and Buynak (1982) reported that post-hatching mortality is almost absent under laboratory controlled conditions.

## 2. Toxic effects in the early developmental stages of the fathead minnow.

A great deal of attention has been paid in experimental embryology to the question of the periodization of sensitivity to stressors, mainly in the embryonic and to a lesser extent the post-embryonic stages of development, in different groups of animals, including fish (Vladimirov, 1975). Many authors call the periods of high sensitivity "critical periods of development", following Stockard (1921) who was the first to introduce this term into experimental embryology, when studying the induction of deformities in Fundulus embryos by means of chemical substances. But a number of authors prefer to use the term "sensitive periods of development" which more

accurately reflects the sense of the matter (Vladimirov, 1975).

In aquatic toxicology, sensitivity is usually regarded as a concept that is the opposite of resistance. Organisms that survive at high concentrations of a substance are regarded as resistant. Organisms that die at relatively low concentrations of the same compound are classified as sensitive (Danil'chenko, 1978).

Investigators hold different opinions concerning the sensitivity and resistance of fishes to the effect of toxicants in different periods of their life. Some consider that resistance of fishes to toxicants declines in the course of ontogenetic development and is lowest in sexually mature fish (Katz and Chadwick, 1961; Malone and Blaylock, 1970; Pomazouskaya et al., 1970). Others single out pro-larvae, considering that eggs are more resistant, and newly hatched sac-fry least resistance, and that resistance increases in the course of subsequent development (Pickering and Vigor, 1965; Skidmore, 1965; Bahls et al., 1969).

Many studies have evaluated the sensitivity of the reproductive and early developmental stages of fishes to the effect of toxicants (Strogonov and Pozhitkov, 1941; Mironov, 1973, and others). The studies reviewed in the following

paragraphs are those mainly related to the exposure of fathead minnow eggs to different pesticides and metals (for more information about classification and chemistry of the cited pesticides and metals refer to Appendix 1).

Buckler et al. (1981) studied the effects of Kepone and Mirex on the fathead minnow. When fertilized eggs were exposed to concentrations of Kepone 0.31 μg/L or above, growth and survival were reduced compared to controls. Hatchability of fathead minnow eggs was also decreased at those concentrations. On the other hand, eggs exposed to Mirex showed increased hatchability success at 2, 3, and 7 μg/L and no significant effect at the two highest exposures of 13 and 34 μg/L. The study also investigated the effects of chlordecone on minnows. Within 24 to 48h after exposure, doses of 10 to 73 μg/L caused hemorrhages and dislocation of the vertebral column.

The effects of kelthane, Dursban$^{R}$, disulfoton and other pesticides on embryo, larval and early juvenile fathead minnows were studied by Holcombe et al. (1982a). Within 48h, Dursban$^{R}$ (47 to 383 μg/L) exposures caused spinal deformities and stiff vertebral columns. Fathead minnows exposed to even one tenth of the 96h-$LC_{50}$ of disulfoton for 24h developed hemorrhages in the dorsal fin.

Pentachlorophenol (PCP) was found to cause a significant decrease in hatching success of fathead minnow eggs when exposed to concentrations of 233 µg/L and above (Holcombe et al., 1982b). All larvae that hatched and survived at this concentration were lethargic, had severe pericardial edema, and would not respond to vibration or probing stimulus. PCP concentrations of 73 µg/L and above significantly reduced larval growth while those of 128 and 228 µg/L caused low survival.

Pickering and Gilliam (1982) found that concentrations of up to 340 µg/L of Aldicarb and 66 µg/L of Fonofos did not affect hatchability of fathead minnow embryos. However, 156 µg/L of Aldicarb and 33 µg/L of Fonofos were lethal to larvae exposed for 30 days post-hatch.

Cairns and Nebeker (1982) conducted early life stage toxicity tests with acenaphthene and isophrone, using the fathead minnow as a test animal. Tests indicated that survival was affected by 682 µg acenaphthene/L and higher, but not by 509 µg/L and less, and that growth was reduced by 495 µg/L and higher, but not by 345 µg/L and less. The results for isophrone indicated that survival was affected by 112 mg/L but not by 56 mg/L and lower.

Chronic effects on the larvae of the fathead minnow

exposed to Aroclor 1248 and 1260 were determined by De Foe et al. (1978). Newly hatched larvae (i.e. <8h old) were the most sensitive; the calculated 30d-$LC_{50}$ was 4.7 µg/L for Aroclor 1248 and 3.3 µg/L for Aroclor 1260.

Evaluation of zinc on the early life stages of the fathead minnow was reported by Benoit and Holcombe (1978). The most sensitive indicators of zinc toxicity were egg adhesiveness and fragility, which were significantly affected at 145 µg/L and above, but were not affected at 78 µg/L and below. Hatchability and survival of larvae were significantly reduced, and skeletal deformities at hatching increased at 295 µg/L and above.

LeBlanc and Dean (1984) perfomed tests involving the exposure of embryos and early larval stages to antimony and thallium. Hatchability, survival and growth of the fathead minnow was unaffected from exposure to antimony concentrations as high as 7.5 µg/L. In thallium tests, successful hatch was significantly reduced at 350 µg/L but unaffected at 200 µg/L. Larval survival, however, was very low at 40 µg/L.

Results of a long-term testing revealed lower survival and declining growth of the fathead minnow with an increase of lime-neutralized iron hydroxide concentrations (Smith et al., 1973). Hatchability was appreciably reduced in the

lowest insoluble iron concentration tested, 1.5 mg/L.

3. Toxic effects in the early developmental stages of other fish species

Insecticides cause developmental abnormalities if embryos are exposed at critical times. Symptomatology and possible mechanisms of the teratogenic effects have been described by Rosenthal and Alderdice, 1976; Niimi, 1983; and Weis and Weis, 1989). In this section, morphological and physiological effects of different pesticides and metals on other fish species are reviewed and summarized.

DDT was initially studied by Shreiman and Rugh (1949) on embryos of killifish (Fundulus heteroclitus). Embryos exposed to 1 mg/L hatched with bent tails. Only mild cases of lordosis were observed when larvae were treated. Spasmodic contractions of the body, and poor or total lack of swimming ability were also manifested. When embryos were placed in clean water before the 16th day of development, effects were reversed.

Mc Cann and Jasper (1972) found extensive hemorrhaging and damage of the vertebral region on bluegills exposed to a variety of pesticides. Optic malformations and skeletal

abnormalities such as scoliosis and lordosis were reported to be found in embryos of silversides (Menidia menidia) exposed to 25 μg/L of DDT (Weis and Weis, 1976).

Cod embryos (G. morhua) exposed to DDT concentrations of 0.025 mg/L and more reacted with irregular proliferations at the yolk surface, and developed a bent or zigzag-growing spinal column (Dethlefsen, 1977). Sheephead minnow (Cyprinodon variegatus) eggs, when subjected to DDT and malathion, carbaryl or parathion at 10 mg/L, displayed developmental arrest prior to the initiation of heart beat, and malformed spine (Weis and Weis, 1974, 1976).

Experiments conducted by Kaur and Toor (1977) with carp eggs (Cyprinus carpio) and the insecticides diazinon, fenitrotion, carbaryl, malathion, and phosphamidon. Upon exposure to concentrations of 0.008 diazinon, 0.25 fenitrothion, 1.0 carbaryl, 2.5 malathion, and 112 mg/L phosphamidon, the embryos showed stunted growth, curving of the tail, deformed head regions, enlargement of the pericardial sac, circulatory failure, deformed vertebral column, and poorly developed eye pigment.

Hansen et al. (1977) completed an entire life cycle study on the effects of endrin on the sheephead minnow (C. variegatus). Embryos exposed to 0.31 μg/L showed stunting

and some mortality, but no teratogenic effects were noted at concentrations of up to 0.72 µg/L. Larvae exposed to 0.31 µg/L exhibited scoliosis, darkened color and mortality.

Toxaphene has been reported to produce skeletal fragility and fractured vertebrae in the brook trout, Salvelinus fontinalis (Mehrle and Mayer, 1975), and in three other species of fish (Anonymous, 1975). In all the cases, the damage was suggested as attributable to a vitamin C deficiency. All vitamin C that was naturally in the diet of the fish appeared to be used for the detoxification of toxaphene and other toxicants, so there was little left over for bone development.

Exposure of eight-day old larvae of Jordanella floridae to 0.25 mg/L of methoxychlor for two hours resulted in decreased egg production and hatchability in the adult stages. The offspring of these adult fish also had increased incidence of abnormalities compared with controls (Holdway and Dixon, 1986).

There is a single report on the effects of lindane on the early developmental stages of fish. Venugopalan and Sasibhushana-Rao (1979) reported premature hatching in Caranx sp. embryos exposed to lindane. The authors suggested that this effect was due to impaired yolk utilization and

absorption. Scoliosis, fin deformity and malformation of the auditory vesicles were noted in yolk-sac stages.

Other literature related to teratogenic effects of different pesticides on fish embryos can be summarized as follows. Couch et al. (1977) reported scoliosis in sheephead minnows (C. variegatus) following exposure to chlordecone. All fish that were exposed to 4 μg/L for 10 days showed spinal injury, loss of equilibrium and tetanic convulsions. In a similar study, exposure of fathead minnows to as low as 0.78 μg/L chlordecone caused vertebral damage (Goodman et al., 1982). Distended abdomens and bent tails were observed in rainbow trout exposed fenitrothion (Klaverkamp et al., 1977), and methylparathion (Palawski et al., 1983).

In a long-term experiment with minnows (Phoxinus phoxinus), 0.2 to 6.8 mg/L Zn produced hemorrhaging, disturbances to pigmentation in the caudal region, and vertebral fractures (Bengtsson, 1974). In a later study (Bengtsson, 1975b), the same kind of vertebral damage was found in minnows exposed to sublethal concentrations of cadmium.

TEMPERATURE

Temperature has considerable influence on development, growth, metabolism and reproduction of fish. For embryonic and larval development, temperature seems to be one of the most important external factors (Penàz, 1974; Balon, 1975; Guilodov and Popova, 1981; Herzig and Winkler, 1986). In addition, water temperature can have a marked effect on the susceptibility of fish to pesticides (Walker, 1963; Bridges, 1965; Mahdi, 1966). Therefore, consideration of temperature effects should constitute an important part in the evaluation of toxicity to fish by pesticides.

The effect of temperature on the toxicity of a large number of chemicals to fish has been well documented. Among the studies made on pesticides, Iyatomi et al. (1958) found that endrin was 84 times more toxic to carp (Cyprinus carpio) at 27-28°C than at 7-8°C. Bridges (1965) examined toxicity of the insecticides heptachlor and chlordecone to sunfish (Lepomis microlophus). Both pesticides were tested at a series of times from 6 to 96h, and at five temperatures from 45 to 85°F, using static tests. The increase in toxicity of chlordecone from 45-85°F was about five-fold for periods from 24-96h. For heptachlor, the 24h-$LC_{50}$ was 0.092 mg/L at 45°F and 0.022 mg/L at 85°F.

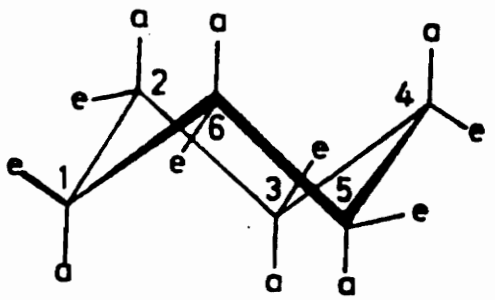
Toxicity of 15 selected pesticides at different temperatures was studied also by Macek et al. (1969) using bluegill and rainbow trout, again in static tests. In general, tolerance of fish to most pesticides decreased as temperature increased. However, the reverse was observed for methoxychlor, and was apparently related to chemical structure. Cope (1965a) found that with bluegills the effect of temperature on toxicity of trifluralin was considerable, although less in 96h tests than in 24h tests. At 7.2°C, the 24h and 96h-$LC_{50}$ values were 1.300 mg/L, and 0.280 mg/L, but at 29.4°C the corresponding values were 0.010 mg/L and 0.0084 mg/L. Such a wide range in $LC_{50}$ values causes considerable difficulty in predicting effects under field conditions.

Toxicity has, in some instances, been found to increase with decreasing temperature. The 48h-$LC_{50}$ of DDT for bluegills at 45°F was 0.0024 mg/L and at 85°F was 0.0064 mg/L (Cope, 1964). At 96h, the values were 0.0016 and 0.0056 mg/L respectively. A similar relationship was found for DDT with rainbow trout (Cope, 1965b), but endrin, lindane, dieldrin and aldrin tested against bluegills, all showed an increase in toxicity with temperature. Cope (1968) also reported that methoxychlor was more toxic to bluegills at low temperatures than at high temperatures.

LINDANE

## 1. Identification

Lindane is the gamma ($\gamma$) isomer of 1, 2, 3, 4, 5, 6-hexachlorocyclohexane (HCH). Worldwide, HCH is better known by its older name, benzene hexachlorine (BHC). The BHC molecule has a chair form where carbon atoms 1, 3, and 5 lie in one plane and carbon atoms 2, 4, and 6 in another parallel plane (See diagram below). The bonds of the carbon atoms with the other atoms and groups may be of two types. The bonds of the first type lie perpendicular to the planes mentioned, and are termed axial (a). The bonds of the second type are projected toward the periphery of the molecule, and are called equatorial (e). Based on the position of the bonds of the chlorine atoms, eight different forms of BHC can exist. Of these isomers, gamma-BHC is the most toxic to insects (Melnikov, 1971; Hassall, 1982).




BENZENE HEXACHLORINE MOLECULE

Commercial lindane, produced by selective crystallization of crude HCH, is required to contain not less than 99% of the gamma-isomer, and to have a melting point of at least 112°C. Lindane is an almost odorless, white solid. It is stable to light, heat, air and concentrated acids but is dechlorinated by alkali (EPA, 1979; Worthing, 1979; Hassall, 1982). Lindane is volatile (v.p. at 20°C, 9.4 X 10 mm of Hg); therefore, it requires the addition of some other compounds such as polychlorodiphenyls and polychloronaphtalenes to increase the duration of its effect (Melnikov, 1971).

Lindane acts as a stomach poison by contact, and has some fumigating action. It is used to control a wide range of soil-dwelling and phytophagous insects considered hazardous to public health, other pests and animal ectoparasites. However, its use in farming is decreasing because of possible accumulation in the tissues of animals. It is employed in various forms: dusts, wettable powders, emulsive concentrates, smokes and baits. The partition coefficients and the solubility in membrane components are considered useful parameters for elucidating mechanisms involved in penetration and translocation of the insecticide to sites of action. Log octanol/water and log benzene/water partition coefficients of lindane at 25°C are 3.72 and 4.22, respectively (Kurihara et al., 1973). Solubility of gamma-BHC in water ranges from 6.60 ppm to 8.70 at 25°C (Weil et al., 1974; Kanazawa, 1971; Biggar

et al., 1966; Robeck et al., 1965; Lipke and Kearns, 1960), and 7.40 ppm at 28°C (Kurihara et al., 1973).

## 2. Biotransformation and biodegradation

Biodegradation and biotransformation appear to be the primary fate of lindane, and this occurs most rapidly in anaerobic environments. When lindane is introduced into biologically rich aquatic environments, transformations occur with half-lives on the order of several days to more than a year (Benezet and Matsumura, 1973; Metcalf et al., 1973; Oloffs and Albright, 1974; Sanborn, 1974; Haider and Jagnow, 1975; Mathur and Saha, 1975; Beland et al., 1976; Matsumura et al., 1976; Steinwandter, 1976; Steinwandter and Schlutter, 1978).

Lindane is only slightly bioaccumulated in organisms. Goldfish (Carassius auratus L.) and bluegills (Lepomis macrochirus) exposed to lindane reached equilibrium within a few hours, and the lindane was eliminated in less than 2 days (i.e. more rapidly than dieldrin and DDT) after fish were transferred to clean water (Gakstatter and Weiss, 1967).

Lindane accumulation was rapid in both rainbow trout (Salmo gairdnerii) and roach (Rutilus rutilus linnaeus), and

when they were exposed to a lethal concentration, the residues found after a quarter of the median survival time fell within the lethal range. When surviving fish were placed into clean water the residues were rapidly eliminated from each of the tissues studied, reaching the level found in the control fish after 30 to 50 days (Tobby and Durbin, 1975).

Hamelink et al. (1976, 1977) noted that zooplankton and fish in a flooded quarry rapidly accumulated lindane, apparently reaching equilibrium after 5 days. The lindane content then declined as the concentration in the water declined.

Using $^{14}C$-radiolabelled lindane in a terrestrial-aquatic microcosm, Metcalf et al. (1973) reported recoveries of 92% for mosquito fish and 20% for snails after 33 days. Sanborn (1974) found that lindane concentration ratios slightly increased when Aroclor 5460 was added. These authors also reported concentrations factors of as much as 810 for daphnia, 125 for mosquito larvae, and 233 for mosquito fish exposed to lindane in small microcosms.

# MATERIALS AND METHODS

## TEST TOXICANT

The toxicant employed in the experiment was the organochlorine, Lindane. Lindane was chosen because it is widely used, relatively soluble, and persistent in water. Technical grade Lindane (99.5% α-BHC, 0.5% inert ingredients) used in the toxicity tests was supplied by Rhône-Poulenc Inc.

A stock solution was prepared by dissolving a weighed quantity of Lindane powder in a known volume of acetone in a small flask. To obtain the final desired concentration, an aliquot of known volume (100 µl) was taken from the stock solution and diluted in 1000 ml of dilution water. For example, a test-solution of 300 µg/L was prepared in the following manner:

(1) Stock solution: 30 mg of Lindane powder were weighed, and diluted in 10 ml of acetone.

(2) Final test-solution: An aliquot of 100 µl was taken from the stock solution and diluted to 1000 ml of dechlorinated tap water.

For all concentrations used at 25° and 18°C, test-

solutions were prepared following the same procedure.

Tap water was dechlorinated with technical sodium thiosulfate, 5 ml per each ten gallons of tap water. Every time that fresh dechlorinated tap water was prepared, pH and hardness were measured. Mean hardness and mean pH were 68.4 mg/L (range 67.0-69.3) and 7.2 (range 7.1-7.5) respectively.

## TEST ORGANISM

Eggs of fathead minnow (Pimephales promelas) were used as test organisms in the toxicity tests. To avoid problems associated with using fish embryos of unknown condition and age, the spawning unit at the Environmental Simulation Laboratory at Virginia Tech provided a continuous supply of eggs for all the experiments. The unit was designed to simulate natural spawning conditions. Breeding tanks of 20 gallons each were held at a temperature of 25±2°C, and a photoperiod of 16h light and 8h dark. All aquaria were equipped with continuous filtering units and spawning substrates. Spawning substrates consisted of inverted 15cm sections of 10cm diameter PVC pipes cut in half longitudinally. Five to six females and two males were placed in each breeding tank with three to four pipe sections.

Six to eight hours after spawning, eggs attached to two or three pipes were brought into the laboratory. Eggs were removed immediately from the substrates using a small spatula or the fingernail, and examined under a dissecting microscope to determine their viability and embryonic stage. Embryos that had reached blastula stage were separated from the rest, placed in a 1L beaker and aerated with sufficient air pressure to keep them in suspension.

## TEST CONDITIONS

Since available literature on the fathead minnow life history establishes 25°C as the optimal temperature for the rearing of embryos, toxicity tests were performed at 25°C initially. However, it was later found that most of the toxicity studies reported by the EPA are performed at 18°C instead of 25°C. This prompted tests at both temperatures (25° and 18°C) to explore the effects of temperature in the sensitivity of these fish to Lindane. An environmental chamber and a water bath were used to achieve appropriate test-water temperatures in all the tests. To carry out the toxicity tests, the environmental chamber was set at 18°C. Test-vessels were either exposed to ambient temperature (18°C) or temperature of a water bath (25°C), installed inside of the chamber. For the concentration-interval experiments, only the

environmental chamber was used. The chamber was first set at 18°C for three weeks, and then at 25°C for another three weeks. Temperatures in the test-vessels were constantly checked with a thermometer, and did not fluctuate more than one or two degrees from their assigned temperature.

Screening tests were performed with and without aeration to detect any possible problems related to oxygen content in the test-vessels. Because no differences in growth or development were found, test-vessels were not aerated during the final toxicity tests. Fungal growth was prevented for the entire incubation period (i.e. the first four days of development) by adding 1 drop of 1% methylene blue stock solution per test-vessel (EPA, 1985).

Quality and intensity of light in the environmental chamber and laboratory during working hours was adequate for the optimal development and growth of embryos. The photoperiod was set at 8h dark and 16h light.

Feeding was not required until reabsorption of the yolk-sac in newly-hatched larvae occurred (i.e. 48 to 72h after hatching). After this period, fathead minnow larvae were fed with freshly-hatched brine shrimp (Artemia) nauplii once daily until the end of the test (EPA, 1985). Summary of the main test conditions are provided in Table 1.

Table 1. General test conditions for fathead minnow eggs (*Pimephales promelas*).

| | | |
|---|---|---|
| 1. | TEMPERATURE: | 25 and 18 ± 1°C |
| 2. | LIGHT QUALITY: | Ambient lab illumination |
| 3. | PHOTOPERIOD: | 8-16h light/24h |
| 4. | SIZE TEST VESSELS: | 100ml |
| 5. | VOLUME TEST SOLUTION: | 100ml |
| 6. | STAGE OF FISH: | Late blastula/gastrula |
| 7. | NO. OF EGGS: | Variable (10 or 12) |
| 8. | NO. OF REPLICATES: | 4 |
| 9. | TOTAL NO. ORG./DOSE: | Variable |
| 10. | FEEDING REGIME: | Feeding not require until larvae hatch. Larvae were then fed with brine shrimp. |
| 11. | AERATION: | None |
| 12. | DILUTION WATER: | Dechlorinated tap water |
| 13. | TOXICANT TESTED: | Lindane |
| 14. | TEST DURATION: | 14 days (egg, yolk-sac and larva) |
| 15. | EFFECTS MEASURED: | Mortality<br>Morphological malformations |

GENERAL REQUIREMENTS

All toxicity tests were carried out in an environmental chamber. Daily renewal, data collection, glassware cleaning, test-solution preparation and other related activities were performed in a laboratory provided with all the necessary material and equipment.

Dilution water used for testing was dechlorinated tap water. The test chambers employed in static tests for fish eggs were 100ml beakers (Corning 1000).

Small and inexpensive plasticware that came in contact with the test solutions was discarded after each procedure. Equipment (large tanks, beakers, pumps, valves, etc) which could not be discarded after each use because of cost, was decontaminated in the following manner:

1. Washed in an automatic dishwasher.
2. Rinsed once with dilute (20%, V:V) nitric acid to remove scale, metals, and bases.
3. Rinsed twice with tap water.
4. Rinsed once with full-strength acetone to remove organic compounds.

5. Rinsed well with dilution water (dechlorinated tap water).

GAS CHROMATOGRAPHY ANALYSIS

In order to determine the decline of pesticide concentration in the test-solutions and test-vessels throughout the static tests, two different experiments were designed.

In the first experiment, water samples were taken daily during three days from each of the 1L-bottles containing the different test-solutions (i.e. 300, 120, 90, 60, 30 and 10 ppb). The analysis of water samples was carried out according to the extraction method established by the EPA (1980). Samples of 150 ml were extracted immediately in separatory funnels with 100 ml of methylene chloride. Extracts were cleaned up through a sodium sulfate column, and then dissolved and concentrated in hexane for later gas chromatography (GC) analysis.

In the second experiment, water samples were taken daily during nine days from the test-vessels containing fish embryos exposed to the different concentrations of Lindane (i.e. 300, 120, 90, 60, 30, and 10 ppb). Since the test-solutions kept

in the 1L-bottles were freshly prepared every three days, samples from days one, four and seven were averaged together and considered 24h old; from days two, five and eight, 48h old; and from days three, six and nine, 72h old. The extraction and the analysis of water samples was accomplished by using the procedure previously explained.

For the analysis of purified extracts, a Pye 104 chromatograph was used with a $Ni^{63}$ electron capture detector. A 6'¼'' glass column was employed, packed with 1.5%/1.95% OV-17/QF-1 on 80/100 mesh Chromosorb G at 210°C. The system was set at a detector temperature of 190°C.

Quantification of Lindane levels was determined by comparing between the size (height or area) of the peak in the sample and the size of the peak from a standard mixture injected under the same GC conditions just before and after the unknown sample. This standard mixture contained Lindane amounts known to fall between the linearity range of the detector and also to produce peaks comparable in size to those obtained from test-samples.

The equation for any GC analysis where an unknown peak is calculated against a peak resulting from injection of an standard of known concentration is given below:

$$R = \frac{abe}{cd}$$

where R = residue concentration (ppm or ppb)

a = ng of pesticide represented by the standard peak

b = height (or area) of sample peak

c = height (or area) of standard peak

d = grams (or ml) of original sample

$$e = \frac{\text{volume of final extract } (\mu l \text{ or ml})}{\mu l \text{ injected}}$$

Finally, to obtain the percent recovery, the residue concentration was divided by the expected concentration, and multiplied by 100.

DETERMINING $LC_{50}$ VALUES AND MORTALITY DISTRIBUTIONS.

In the first static test, conducted at 25°C, a total of 320 embryos were exposed during 14 days to the following concentrations of Lindane: 300, 120, 90, 60, 30, 10, 5, and 0 µg/L. For each concentration, 40 embryos at blastula stage were randomly distributed in four beakers (10 eggs per test-vessel) containing 100ml of test-solution. Each concentration thus had four replicates. Embryos were checked every 24 hours under a dissecting microscope to detect and record possible malformations and mortalities.

For the second static test, conducted at 18°C, a total

of 288 embryos were exposed to 300, 120, 60, 30, 10, and 0 µg/L during a 14d-period. Each concentration had 48 embryos at blastula stage which were randomly distributed in four beakers (10 eggs per test-vessel) containing 100ml of test-solution. Each concentration also had four replicates. Embryos were examined daily with a dissecting microscope and mortality counts were made.

In all tests, test-solutions were renewed daily. After two days, when test solutions were almost used up, residues were discarded, and fresh solutions prepared from the stocks. During the egg stage, renewals were made by decanting approximately 90 ml of the old test-solution, and then replacing the amount with fresh solution. In the yolk-sac and larval stages, the same amount was renewed, but old test-solutions and build-up debris were discarded by siphoning off with a pipette instead of decanting. Every 4 days, test-vessels were replaced with clean ones.

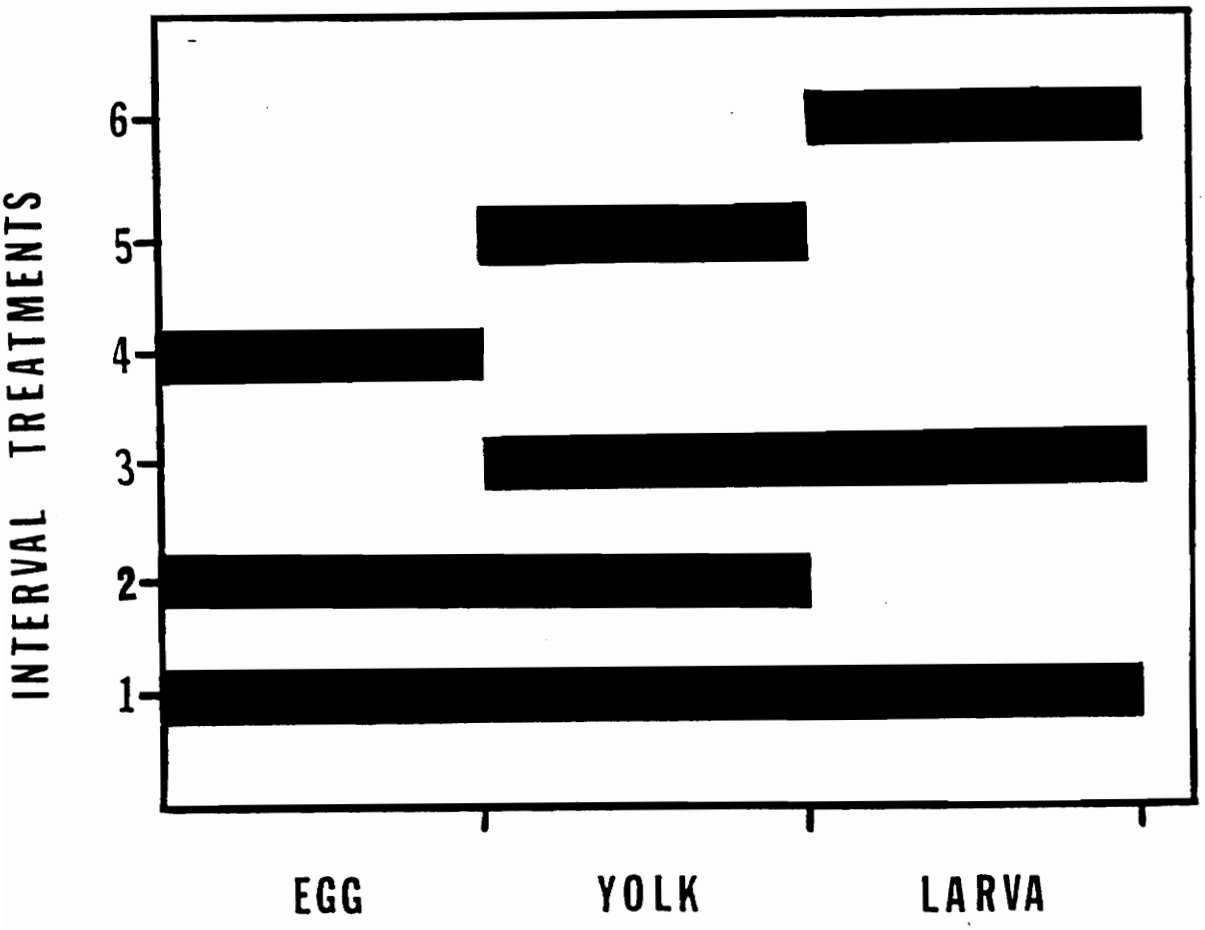
The susceptibility of fathead minnow embryos to Lindane was measured in terms of $LC_{50}$, which was defined as the concentration of the pesticide in water which caused 50% mortality among the test fish under the test conditions. To determine the 14d-$LC_{50}$ values, the percent dying at each dose level was recorded daily until the end of the 14d-period, and then analyzed using Probit Analysis (Finney, 1971).

The Probit Model assumes that the log dose is related to the percent mortality as the cumulative normal distribution. That is, the log doses may be used as variables to read the percent dying from the cumulative normal. Using the normal distribution, rather than other probability distributions, influences the predicted response rate at the high and low ends of possible doses, but has little influence near the middle. Hence, much of the comparison of different toxicants and drugs is done using response rates of fifty percent.

The Number Cruncher Statistical System (Hitnze, 1988) provided an integrated set of computer programs for the probit and survival analyses. The NCSS Survival Analysis program was used to analyze data in which the response variable represented the time from the beginning of the test until death was observed.

## THE CONCENTRATION-INTERVAL EXPERIMENT

Each of the Lindane concentrations was applied at 6 different intervals throughout the 14d-period (Fig. 1). Each treatment started with 20 embryos at blastula stage randomly distributed in two beakers containing 100ml of test-solution. A total of 620 embryos were used in the 36 concentration-




(1) 14 days exposure

(2) Egg-yolk exposure

(3) Yolk-larva exposure

(4) Egg exposure

(5) Yolk exposure

(6) Larval exposure

Figure 1. Diagram of the concentration-interval treatments applied to the early life stages of the fathead minnow.

interval treatments.

The experiment was performed at 25 and 18°C, under the same conditions and requirements established above for the toxicity tests (see TEST CONDITIONS section, and table 1). Embryos were checked under a dissecting microscope to record mortalities each day before test-solutions renewals were made. The NCSS Probit and Survival Analyses were used to examine the data. Survival distributions for each concentration-interval treatment were compared using the non-parametric test of Peto/Wilcoxon. This test has more power than any other non-parametric tests when the hazard ratio is nonconstant across time and the data are from the Weibull distribution (Lee, 1984).

MORPHOLOGICAL ANALYSIS

The 14d-static test at 25°C also provided quantitative insight into teratogenic effects of Lindane. Larvae were checked daily during the larval stage using a dissecting microscope, and the frequency of the more commonly observed abnormalities was recorded. These included: lordoscoliosis (bilateral and dorso-ventral spinal flexures), edemas of the pericardial cavity, hemorrhages and erratic swimming. Pictures of the distinct malformations were taken immediately after their appearance using a Kodak Color Snap 35 Camera,

Model 2 hooked up to a Bausch and Lomb dissecting microscope.

All the observations were made during the larval stage (i.e. from day 10 to 14) for two main reasons: (1) the recognition of abnormalities in the egg stage was difficult because the whole embryo is encapsulated in the egg cover, and (2) the recognition of edemas in the yolk-sac stage might be confusing due to the presence of the yolk-sac in the ventral area of the larval.

The functional relationship between observed frequencies of abnormalities (the dependent variable) and different doses of Lindane (the independent variable) was determined using simple linear regression analysis (Zar, 1984). The significance of the regression was tested through two different procedures: analysis of variance and Student's t statistic.

# RESULTS

## GAS CHROMATOGRAPHY ANALYSIS

For the first recovery experiment, water samples from the test-solutions gave recoveries greater than 75% after 48h and 65% after 72h (Table 2). According to EPA standards (Sherma, 1979), the percent recoveries obtained after 48h were within the acceptable ranges for toxicity testing.

For the second recovery experiment, water samples from test-vessels also gave recovery values within the acceptable range (i.e. between 79 and 98%) after 48 hours (Table 3). Based on GC results of both experiments, fresh test-solutions were prepared every 48h instead of every 72h as had been originally planned.

## 14d-$LC_{50}$ VALUES

Total percent mortalities were tabulated at the end of the 14d-period for bioassays performed at 25 and 18°C (Tables 4 and 5). The 14d-$LC_{50}$ values were calculated with probit analyses. First, percent mortalities were plotted against log concentrations at 25° and 18°C to obtain characteristic normal

TABLE 2. Recovery percentages of Lindane for the first experiment.

| CONCENTRATION (ppm) | % OF RECOVERY | | |
|---|---|---|---|
| | 24h | 48h | 72h |
| 300 | 91.6 | 75.6 | 67.8 |
| 120 | 100.0 | 98.0 | 68.2 |
| 90 | 91.8 | 84.4 | 67.5 |
| 60 | 99.8 | 86.8 | 69.7 |
| 30 | 92.7 | 87.6 | 78.8 |
| 10 | - | 91.6 | 78.1 |

TABLE 3. Recovery percentages of Lindane for the second experiment.

| CONCENTRATION (ppb) | % OF RECOVERY | | |
|---|---|---|---|
| | 24h | 48h | 72h |
| 300 | 94.9 | 93.7 | 89.5 |
| 120 | 99.6 | 88.8 | 88.0 |
| 90 | 98.1 | 98.1 | 91.1 |
| 60 | 95.5 | 91.8 | 83.9 |
| 30 | 96.4 | 74.6 | 68.8 |
| 10 | 79.7 | 68.9 | 64.0 |

TABLE 4. Number of deaths and % mortality of fathead minnow embryos exposed to Lindane doses for 14 days at 25°C.

| DOSE(μg/L) | # OF DEATHS | % MORTALITY |
|---|---|---|
| 300 | 39 | 97.5 |
| 120 | 28 | 70.0 |
| 90 | 20 | 50.0 |
| 60 | 19 | 47.5 |
| 30 | 16 | 40.0 |
| 10 | 11 | 27.5 |
| 5 | 7 | 17.5 |
| 0 | 4 | 10.0 |

TABLE 5. Number of deaths and % mortality of fathead minnow embryos exposed to Lindane doses for 14 days at 18°C.

| DOSE(μg/L) | # OF DEATHS | % MORTALITY |
|---|---|---|
| 300 | 44 | 91.67 |
| 120 | 35 | 70.83 |
| 60 | 27 | 56.26 |
| 30 | 13 | 39.58 |
| 10 | 15 | 31.25 |
| 0 | 3 | 14.58 |

sigmoidal curves (Fig. 2 and 3). Finally, to show the linear relationship of the probit regression model and calculate $LC_{50}$ values, the percentages were converted to probits and plotted again against the log doses (Fig. 4, 5). The NCSS Probit Analysis program estimated $LC_{50}$ values of 44 (c.i. 60-32) µg/L for fathead minnow embryos exposed at 25°C, and 37 (c.i. 52-26) µg/L at 18°C.

## DISTRIBUTION OF MORTALITY AMONG DEVELOPMENTAL STAGES

In order to determine the sensitivity of fathead minnow in the early developmental stages to different concentrations of Lindane, the tabulation of the frequency of deaths throughout the 14d-period was categorized into three main stages: egg (1-5d), yolk-sac (6-9d) and larva (10-14d). For each concentration of Lindane, the number of deaths at each stage was recorded at 18° and 25°C. When embryos were exposed to different concentrations of Lindane (i.e. 120, 90, 60, 30, 10, and 5 ppb) at 25°C, high mortality was always found in the egg and larval stages. The distribution of the total percent mortality among the three stages (Fig. 6) revealed the egg and larval stages as the sensitive stages, and the yolk-sac as the most resistant one. At the lethal concentration of 300 ppb, all the stages appeared to be highly affected in relation to the controls.

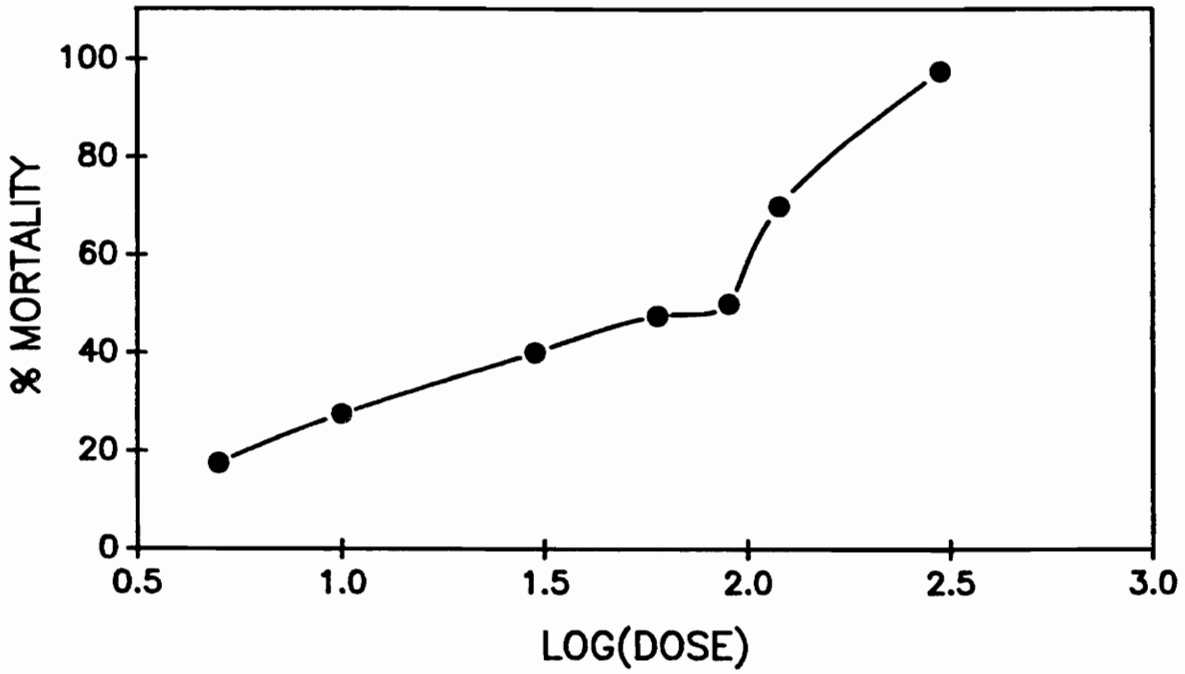



FIG. 2. Percent mortality as a function of Lindane dose at 25°C.

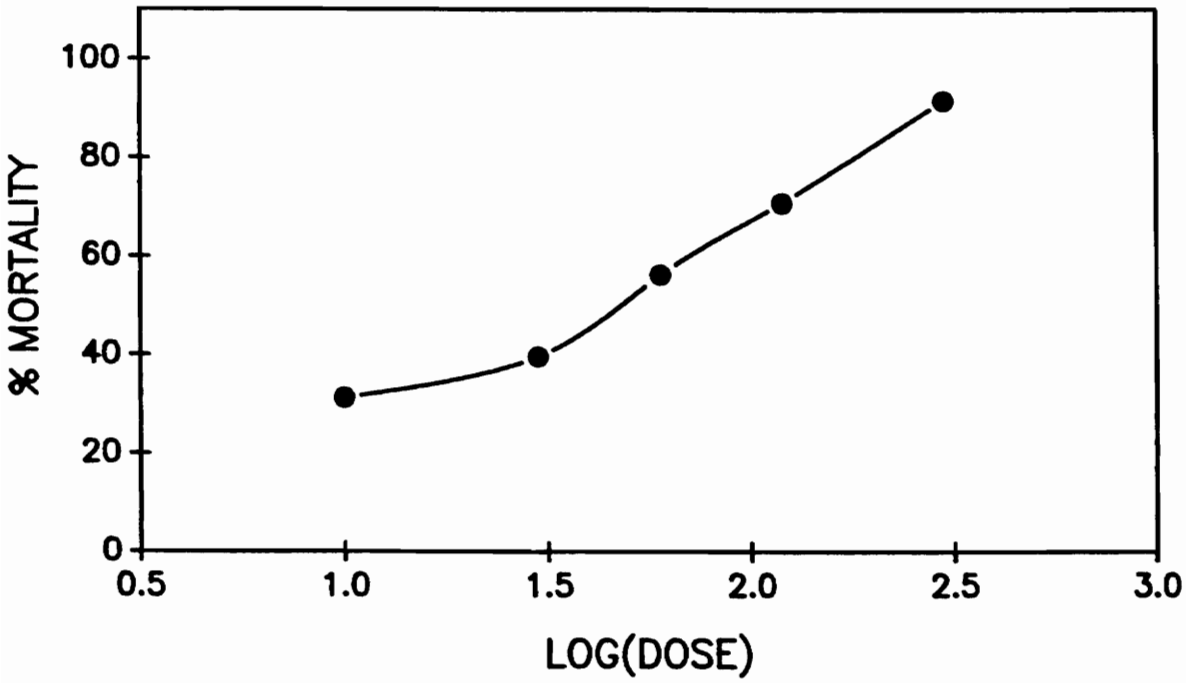



FIG. 3. Percent mortality as a function of Lindane dose at 18°C.

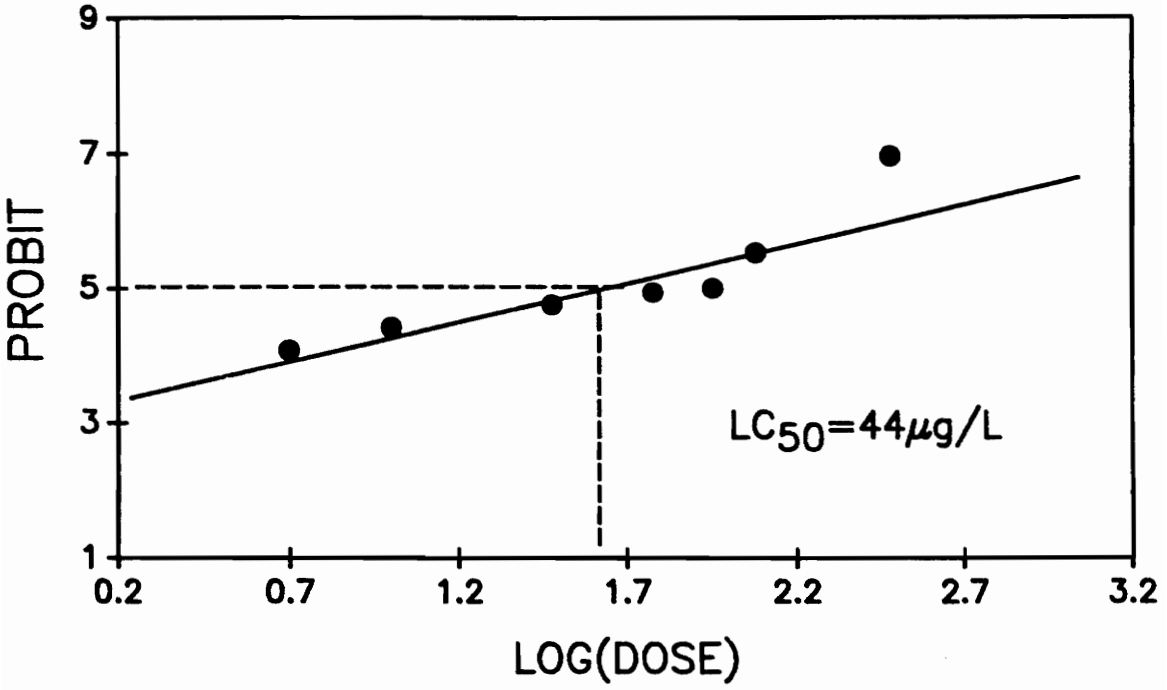



Fig. 4. Probit regression to determine $LC_{50}$ at 25°C.

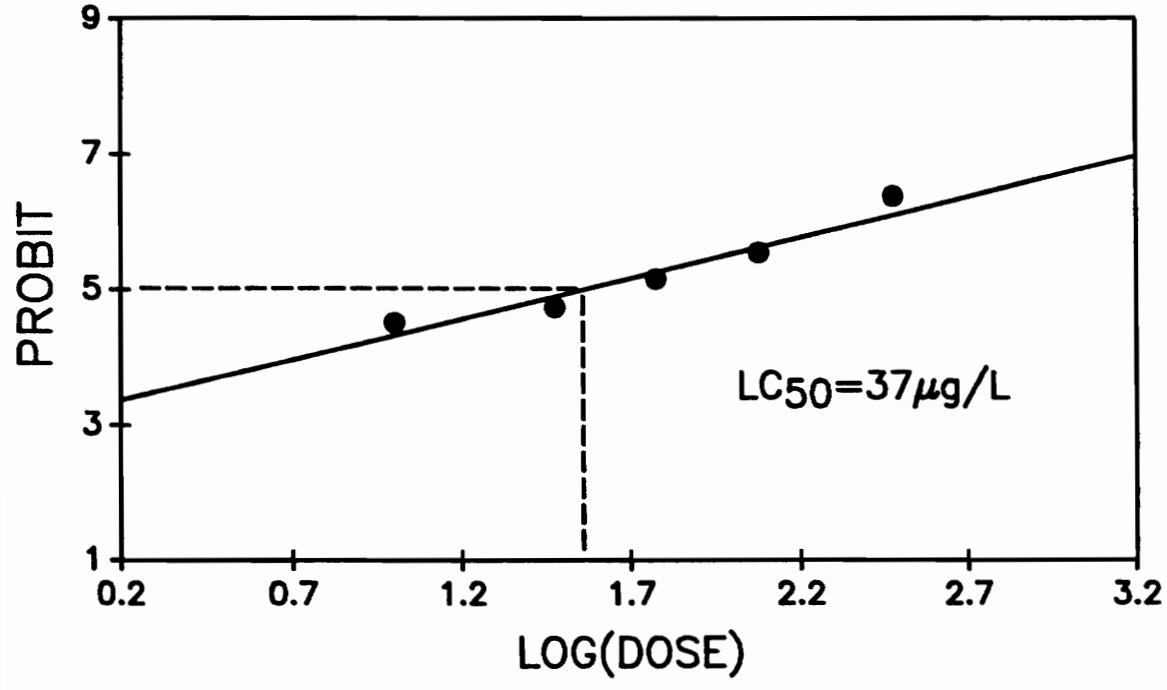



Fig. 5. Probit regression to determine $LC_{50}$ at 18°C.

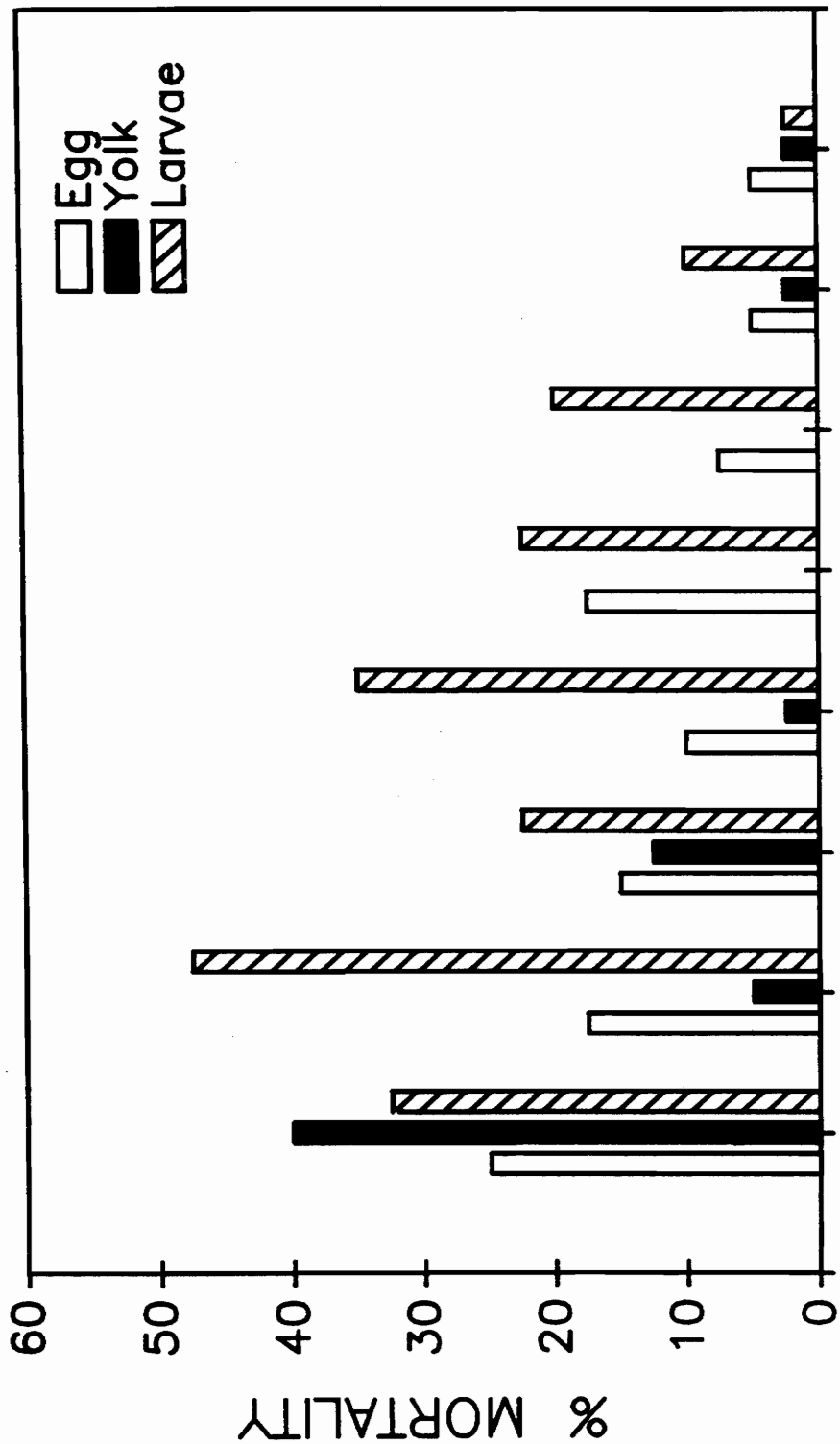



Fig. 6. Distribution of mortality at 25°C.

At 18°C, the distribution of mortality among the three stages (Fig. 7) showed a different pattern. The lower temperature affected the development of fathead minnow embryos by delaying the hatching of the yolk-sac larvae one to two days, so categorization of the 14d-period had to be adjusted as follows: egg (1-7d), yolk-sac (8-11d), and larva (12-14d). For all the concentrations tested at 18°C (i.e. 300, 120, 60, 30, and 10 ppb), the most sensitive stage was the yolk-sac stage, while the egg and larval stages showed about equal resistance. In addition to the delay in hatching, larvae emerging at 18°C was found to be smaller in size and weight when compared with their contemporaries at 25°C.

## THE CONCENTRATION-INTERVAL EXPERIMENT

Total percent mortalities for each concentration-interval treatment performed at 25° and 18°C were compiled in Tables 6 and 7. The data was then used to calculate the $LC_{50}$ values of each treatment at 25° and 18°C (Table 8).

When treatments were carried out at 25°C, the yolk-sac stage revealed a high $LC_{50}$ value (366 μg/L) compared with the values of the egg (39 μg/L) and larval (40 μg/L) stages. Certainly, these values supported the results previously obtained from the distribution of mortality (Fig. 6). Both

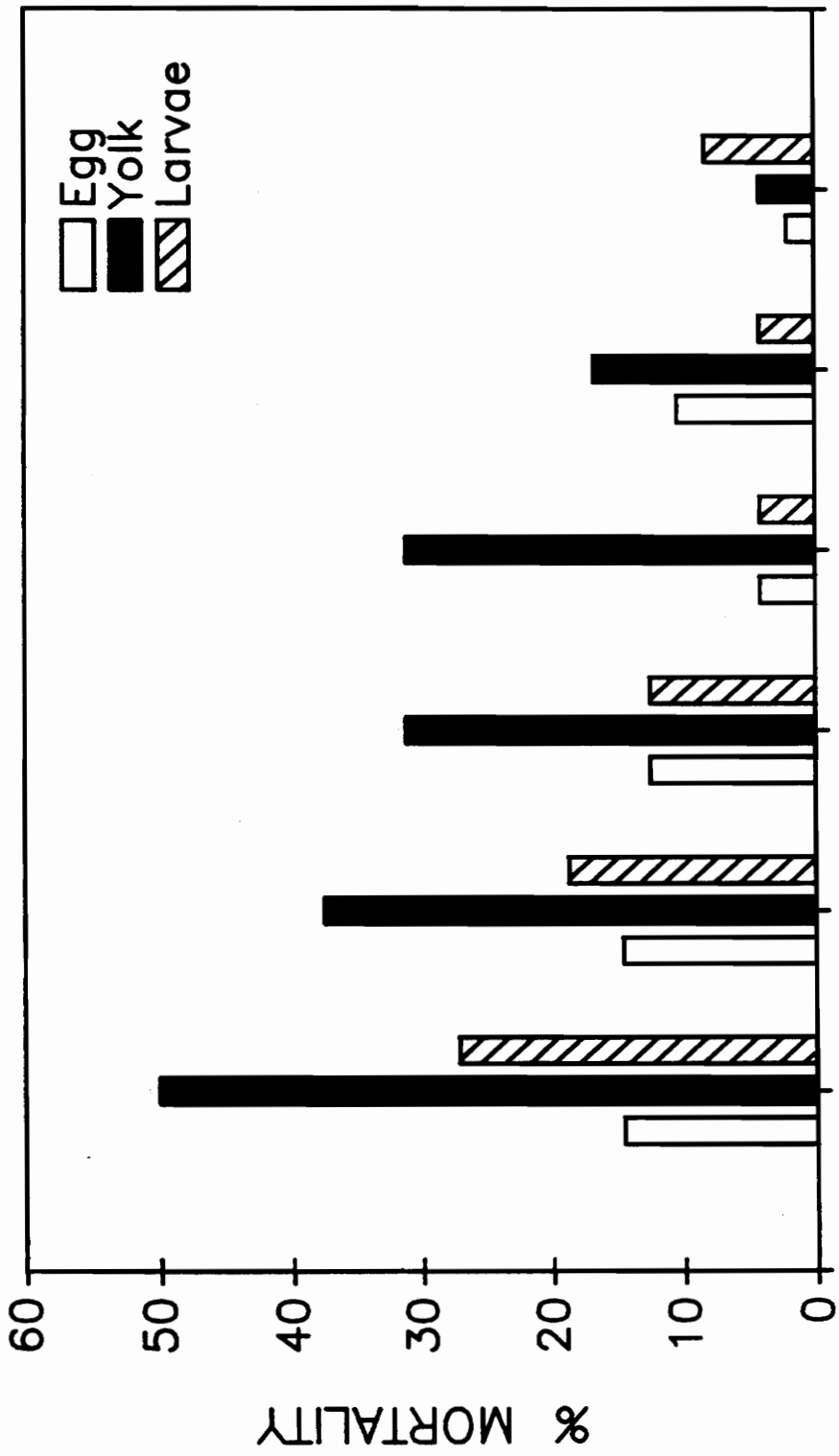



Fig. 7. Distribution of mortality at 18°C.

TABLE 6. Percent mortalities for fathead minnow embryos exposed to the different concentration-interval treatments at 25°C.

| TRTM. / CONC. | 14 DAYS | EGG-YOLK | YOLK-LARVA | EGG | YOLK-SAC | LARVA |
|---|---|---|---|---|---|---|
| 300 | 95.8 | 83.3 | 87.5 | 62.5 | 70.8 | 33.3 |
| 120 | 75.0 | 66.7 | 79.2 | 50.0 | 58.3 | 29.2 |
| 90 | 62.5 | 58.3 | 62.5 | 45.8 | 58.3 | 29.2 |
| 60 | 58.3 | 54.2 | 54.2 | 37.5 | 54.2 | 25.0 |
| 30 | 41.7 | 37.5 | 45.8 | 29.2 | 37.5 | 16.7 |
| 10 | 29.2 | 25.0 | 20.8 | 12.5 | 33.3 | 16.7 |
| 0 | 12.5 | 16.7 | 4.2 | 0.0 | 4.2 | 8.3 |

TABLE 7. Percent mortalities for fathead minnow embryos exposed to the different concentration-interval treatments at 18°C.

| TRTM. / CONC. | 14 DAYS | EGG-YOLK | YOLK-LARVA | EGG | YOLK-SAC | LARVA |
|---|---|---|---|---|---|---|
| 300 | 100.0 | 95.0 | 100.0 | 70.0 | 55.0 | 100.0 |
| 120 | 85.0 | 75.0 | 85.0 | 65.0 | 30.0 | 65.0 |
| 90 | 55.0 | 60.0 | 65.0 | 50.0 | 30.0 | 60.0 |
| 60 | 45.0 | 45.0 | 60.0 | 30.0 | 20.0 | 55.0 |
| 30 | 35.0 | 40.0 | 60.0 | 20.0 | 15.0 | 45.0 |
| 10 | 20.0 | 15.0 | 40.0 | 15.0 | 15.0 | 30.0 |
| 0 | 10.0 | 10.0 | 5.0 | 5.0 | 10.0 | 0.0 |

sets of data established that the yolk-sac stage was the resistant stage, and that the egg and larval stages were the sensitive ones.

At 18°C, on the other hand, the egg (125 μg/L) and larval (2700 μg/L) stages had much higher $LC_{50}$s compared with the one obtained for the yolk-sac stage (52μg/L). Once again, the figures seemed to support the results obtained previously from the distribution of mortality at 18°C (Fig. 7). Thus, it was confirmed that the larval stage was the most resistant stage, and the yolk-sac the most sensitive one at 18°C.

Survival distributions of each concentration-interval treatment were compared using the non-parametric test of Peto/Wilcoxon. Tables 9 and 10 group the "p" values obtained from the Peto-Wilcoxon test at 25° and 18°C respectively. No pattern could be derived from the analysis. No significant differences between survival distributions were detected for most of the comparisons. In fact, very few comparisons were significantly different.

## MORPHOLOGICAL CHANGES

Exposure of fathead minnow embryos to the different doses of Lindane at 25°C for 14 days resulted in the appearance

TABLE 8. $LC_{50}$ values for the six concentration-interval treatments at 25 and 18°C.

| TREATMENT | 25°C-$LC_{50}$ (µg/L) | 18°C-$LC_{50}$ (µg/L) |
|---|---|---|
| 1. 14 days | 48 | 36 |
| 2. Egg-yolk | 46 | 49 |
| 3. Yolk-larva | 22 | 41 |
| 4. Egg | 39 | 125 |
| 5. Yolk | 366 | 52 |
| 6. Larva | 40 | 2700 |

**TABLE 9.** Probability values obtained from comparison of survival distributions at 25°C.

| | 14d-300 | 14d-120 | 14d-90 | 14d-60 | 14d-30 | 14d-10 |
|---|---|---|---|---|---|---|
| Egg | 0.000* | 0.475 | 0.330 | 0.174 | 0.495 | 0.540 |
| Yolk-sac | 0.000* | 0.002* | 0.011* | 0.043* | 0.149 | 0.540 |
| Larva | 0.000* | 0.085 | 0.079 | 0.531 | 0.623 | 0.623 |
| Egg-yolk | 0.000* | 0.034* | 0.636 | 0.341 | 0.554 | 0.600 |
| Yolk-larva | 0.000* | 0.399 | 0.542 | 0.621 | 0.214 | 0.393 |
| | E-300 | E-120 | E-90 | E-60 | E-30 | E-10 |
| Yolk | 0.007* | 0.005* | 0.118 | 0.515 | 0.513 | 0.973 |
| Larva | 0.363 | 0.103 | 0.629 | 0.108 | 0.177 | 0.260 |
| Egg-yolk | 0.000* | 0.585 | 0.334 | 0.130 | 0.223 | 0.270 |
| Yolk-larva | 0.189 | 0.438 | 0.559 | 0.033* | 0.051* | 0.104 |
| | Y-300 | Y-120 | Y-90 | Y-60 | Y-30 | Y-10 |
| Larva | 0.000* | 0.084 | 0.100 | 0.046* | 0.045* | 0.291 |
| Egg-yolk | 0.000* | 0.000* | 0.013* | 0.026* | 0.033* | 0.271 |
| Yolk-larva | 0.000* | 0.003* | 0.058* | 0.014* | 0.004* | 0.108 |
| | L-300 | L-120 | L-90 | L-60 | L-30 | L-10 |
| Egg-yolk | 0.000* | 0.005* | 0.044* | 0.391 | 0.673 | 0.664 |
| Yolk-larva | 0.000* | 0.011* | 0.812 | 0.229 | 0.186 | 0.358 |
| | Y-L300 | Y-L120 | Y-L90 | Y-L60 | Y-L30 | Y-L10 |
| Yolk-larva | 0.000* | 0.016* | 0.283 | 0.490 | 0.522 | 0.852 |

**TABLE 10.** Probability values obtained from comparison of survival distributions at 18°C.

| | 14d-300 | 14d-120 | 14d-90 | 14d-60 | 14d-30 | 14d-10 |
|---|---|---|---|---|---|---|
| Egg | 0.049* | 0.070 | 0.183 | 0.149 | 0.391 | 0.150 |
| Yolk-sac | 0.026* | 0.052* | 0.193 | 0.364 | 0.406 | 0.861 |
| Larva | 0.000* | 0.002* | 0.013* | 0.068 | 0.065 | 0.438 |
| Egg-yolk | 0.560 | 0.751 | 0.643 | 0.642 | 0.838 | 0.771 |
| Yolk-larva | 0.141 | 0.746 | 0.354 | 0.450 | 0.785 | 0.363 |
| | E-300 | E-120 | E-90 | E-60 | E-30 | E-10 |
| Yolk | 0.547 | 0.814 | 0.618 | 0.355 | 0.710 | 0.103 |
| Larva | 0.026* | 0.168 | 0.208 | 0.596 | 0.337 | 0.514 |
| Egg-yolk | 0.145 | 0.190 | 0.287 | 0.282 | 0.514 | 0.256 |
| Yolk-larva | 0.398 | 0.059 | 0.339 | 0.320 | 0.356 | 0.477 |
| | Y-300 | Y-120 | Y-90 | Y-60 | Y-30 | Y-10 |
| Larva | 0.007* | 0.067 | 0.059* | 0.134 | 0.156 | 0.314 |
| Egg-yolk | 0.021* | 0.034* | 0.258 | 0.594 | 0.529 | 0.672 |
| Yolk-larva | 0.244 | 0.062 | 0.397 | 0.983 | 0.577 | 0.315 |
| | L-300 | L-120 | L-90 | L-60 | L-30 | L-10 |
| Egg-yolk | 0.000* | 0.006* | 0.022* | 0.119 | 0.111 | 0.630 |
| Yolk-larva | 0.000* | 0.000* | 0.023* | 0.119 | 0.047* | 0.947 |
| | Y-L300 | Y-L120 | Y-L90 | Y-L60 | Y-L30 | Y-L10 |
| Yolk-larva | 0.048* | 0.529 | 0.584 | -- | 0.817 | 0.528 |

malformations. The most common and distinct abnormalities were: edemas, lordoscoliosis, hemorrhages and erratic swimming.

At the end of the 14d-period, the total number and percentage of the malformations for each concentration of Lindane were tabulated (Table 11), and analyzed. Results of simple linear regression analyses revealed a strong correlation between observed percent abnormalities and different doses of Lindane (Fig. 8). Except for erratic swimming, the other three malformations (i.e. edemas, hemorrhages and lordoscoliosis) showed high significance in the regression analysis (Table 12).

TABLE 11. Total number and percentage of malformations for each concentration of Lindane.

| CONCENTRATION | NO. OF REPLICATES | NO. OF ORGANISMS | LORDOSCOLIOSIS | | EDEMAS | | ERRATIC SWIMMING | | HEMORHAGES | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | % | No. | % | No. | % | No. | % |
| 300 | 4 | 40 | 32 | 80.0 | 33 | 82.5 | 6 | 15.0 | 23 | 57.5 |
| 120 | 4 | 40 | 21 | 52.5 | 18 | 45.0 | 13 | 32.5 | 11 | 27.5 |
| 90 | 4 | 40 | 19 | 47.5 | 17 | 42.5 | 5 | 12.5 | 9 | 22.5 |
| 60 | 4 | 40 | 16 | 40.0 | 14 | 35.0 | 5 | 12.5 | 6 | 15.0 |
| 30 | 4 | 40 | 14 | 35.0 | 13 | 32.5 | 4 | 10.0 | 5 | 12.5 |
| 10 | 4 | 40 | 8 | 20.0 | 9 | 22.5 | 2 | 5.0 | 1 | 2.5 |
| 5 | 4 | 40 | 7 | 17.5 | 9 | 22.5 | 0 | 0.0 | 0 | 0.0 |

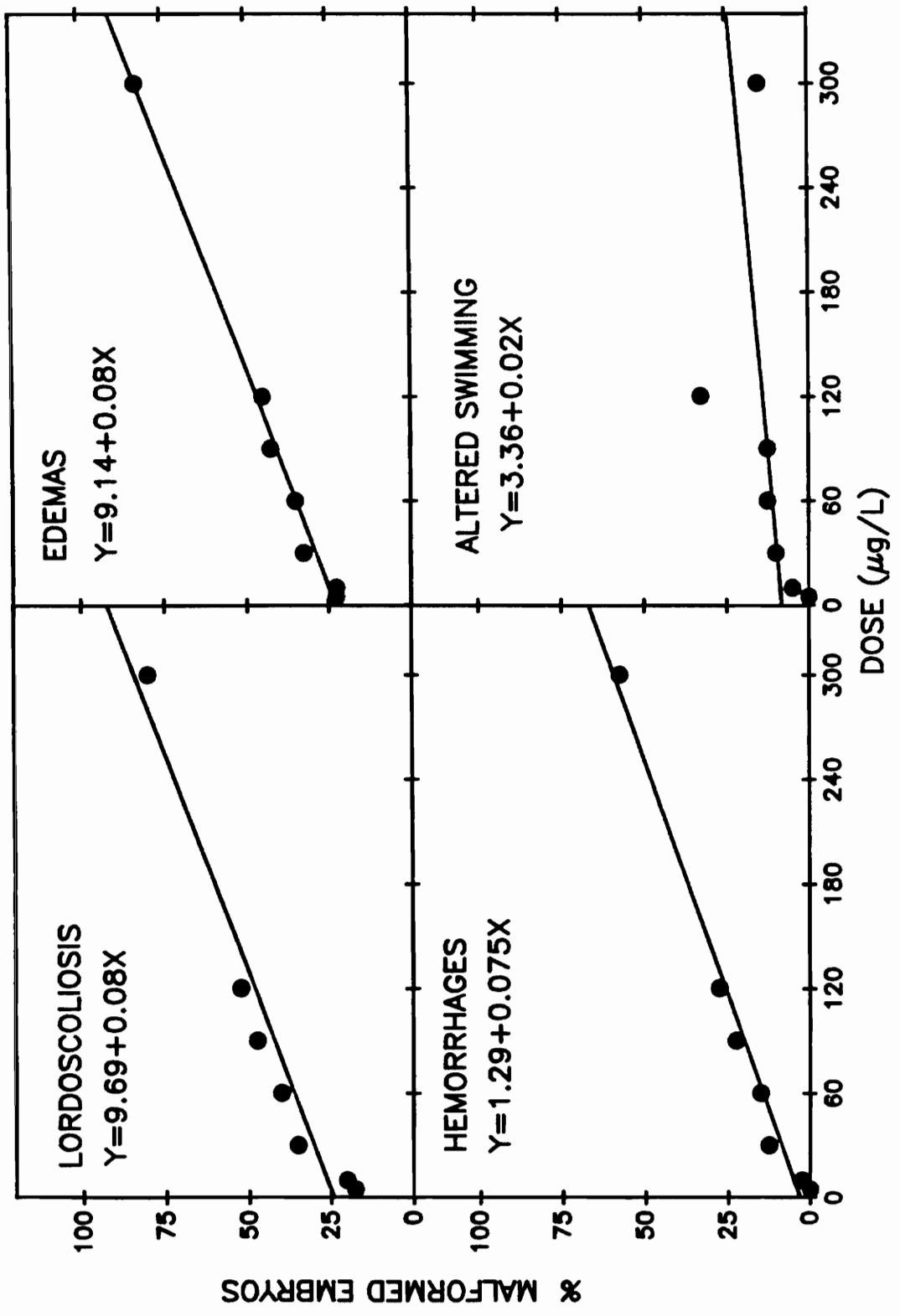



Fig. 8. Percent malformations as a function of Lindane dose at 25°C.

TABLE 12. Simple regression analysis report.

| Malformation | t-value | Prob.level | R-square |
|---|---|---|---|
| LORDOSCOLIOSIS | 7.95* | 0.0005 | 0.9267 |
| EDEMAS | 21.58* | 0.0000 | 0.9894 |
| HEMORRHAGES | 14.78* | 0.0000 | 0.9776 |
| ERRATIC SWIMMING | 1.48 | 0.2898 | 0.2188 |

# DISCUSSION

## 14d-$LC_{50}$ VALUES

The 14d-$LC_{50}$ values for fathead minnow embryos exposed to Lindane doses at 25 and 18°C were 44 (c.i. 60-32) and 37 (c.i. 52-26) μg/L, respectively. Whereas the effect of temperature was not noticeable on the susceptibility of the whole 14 day- developmental period, it was on the sensitivity of the different developmental stages within that period (discussed below).

Though several investigators have determined long-term $LC_{50}$ values for fish exposed to different pesticides (Skidmore, 1964; Schimmel et al., 1974; Hansen et al., 1977), there has been no report of Lindane $LC_{50}$s in the early developmental stages of fathead minnow. As noted in table 13, EPA reported Lindane 96h-$LC_{50}$s for fathead minnow juveniles that were twice higher than the ones reported here for the early life stages. The results of my study and that of EPA corroborate the fact that early developmental stages are often the most susceptible stages in an animal's life cycle, and that they should be used in toxicity studies to provide more accurate and realistic guidelines. Considerable 96h-data have been generated concerning the effect of pesticides on the juvenile and adult

TABLE 13. EPA reported $LC_{50}$ values for fathead minnow juveniles exposed to Lindane.

| STAGE | TEST TYPE | T°C | $LC_{50}$ |
|---|---|---|---|
| Juvenile | Static | 18 | 87 |
| Juvenile | Static | 18 | 67 |
| Juvenile | Flow through | 18 | 77 |
| Juvenile | Static | 12 | 86 |

stages of fish (Katz, 1961; Eisler, 1970; Korn and Earnest, 1975; Buckler et al., 1981), but very few studies have reported comparative $LC_{50}$ data with the early developmental stages. Tobby and Durbin (1975), for instance, reported lower 24, 48, and 96h-$LC_{50}$s for yolk-sac fry than for yearlings of rainbow trout exposed to Lindane. Similar results were generated by Schimmel et al. (1974) when exposing embryos, fry, juveniles and adults of sheepshead minnow to Aroclor 1254. They found that early life stages, especially the yolk-fry, were more susceptible than adults to the effect of Aroclor 1254. Later on, while evaluating the use of bioassays with early life stages of fish, McKim (1977) found that in 56 life cycle toxicity tests performed with 34 inorganic and organic compounds, and 4 species of freshwater fish, the embryo-larval and early juvenile stages were the most, or among the most, sensitive. The results of this study and the reported literature not only supported the view that numerous pesticides, especially the aromatic and chlorinated hydrocarbons, are highly toxic to the early developmental stages of fishes, but also that fish embryos and larvae are particularly suitable organisms for toxicity tests with organic and inorganic compounds because of their high sensitivity and simple culture requirements. Fertilized fish eggs as test subjects have their advantages. In the first place, from the embryonic development, the action of the pollutant on the different stages of embryogenesis can be

evaluated on a fine scale. Second, observing the development of prolarvae and larvae, it is possible to discover the after-effects of the pollutant and to show its action in small concentrations on the later stages of development.

## DISTRIBUTION OF MORTALITY AMONG DEVELOPMENTAL STAGES

The distribution of mortality at 25°C (Fig. 6) showed that the egg and larval stages are more sensitive to the effect of Lindane than is the yolk-sac stage. Egg mortality occurring either at 25 or 18°C within day 1 and 2 can be explained not so much by a sensitive period, as by the dying off of unfertilized and poor-quality eggs (Detlaf and Ginzburg, 1954; Vladimirov, 1975). It is natural then that poor quality eggs will be more sensitive to the action of pollutants.

The other period of heightened mortality, the larval stage (from day 10 to 14), corresponded with the end of the yolk-sac reabsorption. Larvae dying during this period usually had developed a variety of abnormalities. The most likely explanation for the occurrence of malformations as well as for deaths is that, at this time, larvae had finally changed over to exogenous feeding, making the intake of pesticide more direct and effective through the digestive

system.

Not all researchers agree about the periods of sensitivity and/or resistance during development of fishes exposed to toxicants. A number of authors maintain that yolk-sac larvae are more sensitive than eggs, but that resistance increases after yolk-sac reabsorption (Pickering and Vigor, 1965; Skidmore, 1965; Bahls et al., 1969). Other authors consider that the resistance of fishes to a toxicant declines in the course of early ontogeny. The results obtained here at 25°C verify that the yolk-sac stage is more resistant to the effect of Lindane than are the egg and larval stages, and support the assumption that resistance would then decline during subsequent development.

The concentration-interval experiments corroborated the previous findings. Embryos treated during the yolk-sac stages at 25°C, showed a higher $LC_{50}$, than embryos treated during either the egg or the larval stage (Table 8). In other words, at 25°C, very high concentrations were required to affect the susceptibility of the resistant yolk-fry. Resistance of the yolk-fry was explained by the fact that, at this stage, larvae were still depending on the yolk-sac reserves for its feeding, thus delaying the direct intake of the pesticide to its target.

The results of tests performed at 18°C were completely different than those conducted at 25°C. Although susceptibility (determined by the 14d-$LC_{50}$ value) of fish to the pesticide seemed to increase as temperature decreased, differences were not statistically significant. The distribution of mortality among the three stages, on the other hand, grouped the highest number of deaths in the yolk-sac stage (Table 7). Furthermore, in the concentration-interval experiment, embryos exposed to Lindane during the yolk-sac stage only, generated low $LC_{50}$ values compared with the egg and the larval treatments (Table 8). Macek (1968) observed a similar mortality in the sac fry hatching out of eggs of DDT treated brook trout. The observed sensitivity at this critical stage must be due to the release of accumulated pesticide from the yolk at the time of its utilization. This was referred to as latent toxicity effect by Cook (1970). The excessive accumulation of pesticide in the yolk-sac might be explained by the observed delay in hatching occurring at this temperature. As summarized by Blaxter (1969), there exists a close relationship between temperature and incubation time. Embryos that not only stay longer inside of their chorions but also are growing slower compared to 25°C embryos, will be more sensitive to this type of bioaccumulation. As for 25°C, eggs will also accumulated pesticide, but they will be developing normally, and fully activating their detoxification systems at the proper time. Although hatching is not a distinct

ontogenetic stage, incubation time from fertilization to hatching can be important to the survival potential of a species developing under different ecological circumstances (Rosenthal and Alderdice, 1976).

The observed pattern seemed unusual when compared with the results of another study in which most of the pesticides tested showed an increase in the susceptibility of fish as temperature increased (Macek et al., 1969). However, a couple of reports have reported similar phenomena. Metcalf (1955) and Cope (1965) referred to the increased mortalities at lower temperatures for insects treated with methoxychlor, and for rainbow trout exposed to DDT, respectively.

The lower temperature, evidently, had an effect on the sensitivity of the fish to the pesticide. During the course of the tests at 18°C, it was noted that hatching was lengthened by one or two days, and that size and weight were reduced. Delay in hatching was also noticed by Buckler et al. (1981) in fathead minnow eggs exposed to 0.31 µg/L of chlordecone. However, a good explanation for late hatching is yet to be found. Among all the environmental factors, the effect of temperature on the toxicity of pesticides is relatively well known ( Macek et al., 1969 Murty, 1986). But temperature also acts directly or indirectly through its influence on other factors such as enzymatic activity. Most

enzymes show an optimum temperature at which they reach maximum catalytic activity (Brett, 1971). Thus, Kaur and Toor (1977) cited the probable effect of pesticides on the inhibition of hatching enzymes suggesting a clue for the retention of the embryos within the eggs. For this, I do have to suggest a marked temperature effect in the activity of hatching enzymes elicited by the pesticide treatment as an explanation for the delayed embryonic condition.

The other observed response at 18°C on embryos of fathead minnow exposed to Lindane was the emergence of smaller larvae at hatching. It has been reported that incubation temperature has a marked influence on larval size at hatching. In Pacific herring (Clupea harengus pallasi) temperature influenced larval size at hatching (Alderdice and Velsen, 1971). Dethlefsen (1974), on the other hand, found that several organochlorine pesticides had a similar effect on eggs of Atlantic cod, flounder and plaice. Obviously both temperature and pesticide are involved in the reduced larval size at hatching. Smaller larvae with smaller yolk sacs could occur because the Lindane increased costs of maintenance metabolism during embryonic development, and consequently there would be a shorter period between hatching, initiation of feeding on exogenous food, and successful learning of prey-capture manoeuvres (Rosenthal and Alderdice, 1976). In this instance, smaller larvae at hatching would be exposed to predation for

a further period of growth, the period required for them to achieve the initial size of larger larvae incubated under more favorable conditions.

During early embryonic development, energy levels are small but almost completely required for full differentiation (Von Westernhagen, 1988). The presence of pollutants and/or changed levels of abiotic factors such as temperature, would affect the rate or extent of cell or tissue differentiation at that time. Those effects may not be directly lethal but lead to reduced survival potential at subsequent levels of embryonic, individual or population organization (Rosenthal and Alderdice, 1976).

## MORPHOLOGICAL CHANGES

Many morphological changes have been reported following the exposure of larval fish either to high concentrations for brief periods or to sublethal doses for extended periods. Changes may be as apparently innocuous as change in body coloration or may be as gross as scoliosis, distortion of vision, bicephalia, etc. Small or large, such changes can prevent the fish from functioning effectively and efficiently in the environment.

Lordoscoliosis (bilateral and dorso-ventral spinal flexures) was the first noticeable response of fathead minnow larvae to Lindane exposure (Figures 9, 10, 11). This was caused by vertebral fractures, usually in the post-anal region. The largest muscle mass of the vertebral column is at the base of the caudal region. If Lindane affects the neuro-muscular complex, muscular convulsions would overload vertebrae in this region (Bengtsson, 1974; Bengtsson, 1975a; Bengtsson et al., 1975b; Spencer, 1967). In general, organochlorine pesticides are well known to cause effects on the nervous system, including hypersensitivity to minor disturbances which can cause strong spasms (O'Brien, 1965; Johnson and Mayer, 1973). Experiments with fish and electric currents have shown that a strong stimulation of the neuro-muscular complex alone can produce immediate fractures of the vertebrae (Hauck, 1949; Spencer, 1967).

Exposure of killifish (Fundulus heteroclitus) and silverside (Menidia menidia) embryos to DDT resulted in skeletal abnormalities such as lordosis and scoliosis (Shreiman and Rugh, 1949; Weis and Weis, 1976, respectively). Dethlefsen (1977) also found that cod embryos exposed to DDT developed fractures in the spinal column.

Hansen et al. (1977) observed scoliosis and darkened color in sheephead minnow (Cyprinodon variegatus) larvae

Figure 9. Normal larva of fathead minnow developed at 25°C.

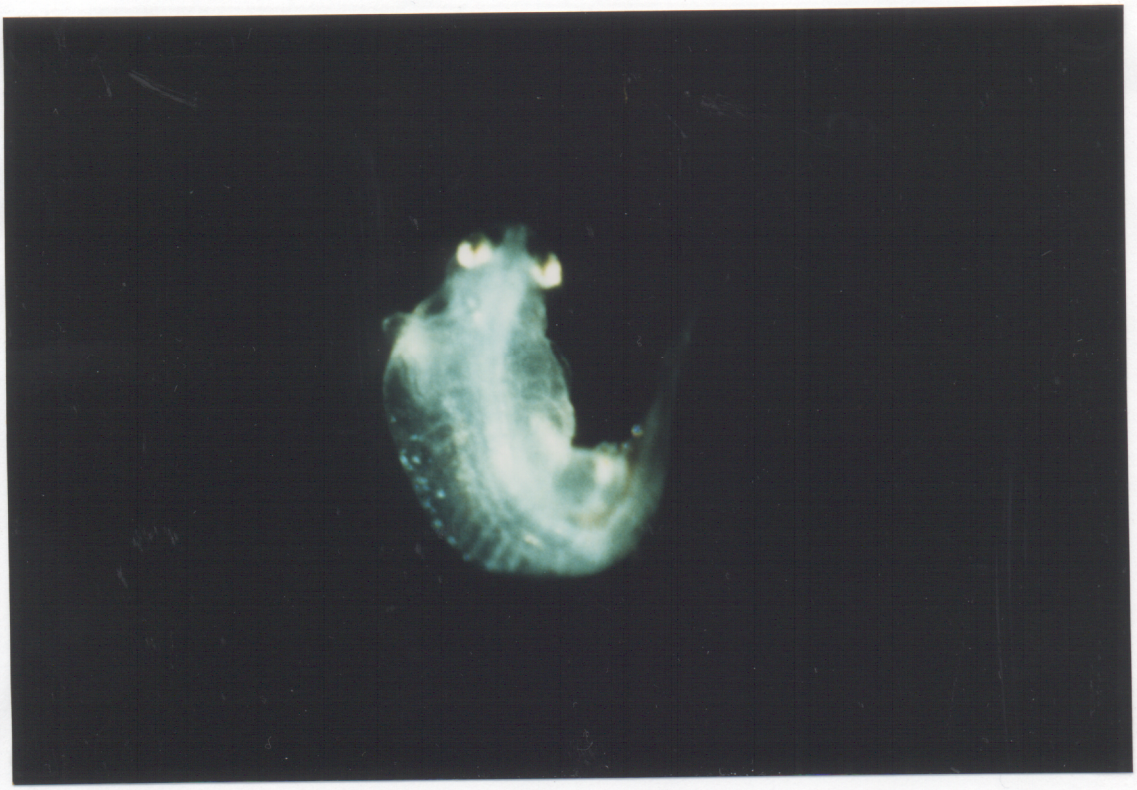


Figure 10. Lordosis in fathead minnow larva exposed to Lindane at 25°C.

Figure 11. Scoliosis in fathead minnow larva exposed to Lindane at 25°C.

exposed to endrin. A single study on Lindane reported scoliosis and fin deformity in the yolk-sac stages of Caranx sp. (Venugopalan and Sasibhushana-Rao, 1979).

The same effects have been reported for embryos of sheephead minnow exposed to DDT , malathion, carbaryl, parathion and chlordecone (Weis and Weis, 1974, 1976; Couch et al., 1977; Goodman et al., 1982); and of fathead minnow exposed to chlordecone and Dursban$^{R}$ (Buckler et al., 1981; Holcombe et al., 1982)

Toxaphene, another organochlorine, has been reported to develop scoliosis in fish (Mehrle and Mayer, 1975; Anonymous, 1975; Bengtsson, 1975a; Mayer et al., 1978). Wtih toxaphene and perhaps with other organochlorine compounds, contaminant-induced competition for vitamin C between collagen metabolism in bone and microsomal function oxidases (involved in the detoxification) has been proposed to cause the vertebral damage. The competition for vitamin C would decrease the vitamin C and the collagen content of the bone, with an increase in the ratio of bone minerals to collagen, resulting in an increased fragility of the bone.

It is rather difficult to state what actual mechanism(s) is(are) responsible for vertebral damage. It seems reasonable to suggest there could be both a neuro-muscular effect, and

a demineralization of the skeleton.

Hemorrhages in the region of the dorsal fin and the head were usually observed prior to the appearance of lordoscoliosis, between day 9 and 10 (Figure 12). Hemorrhaging in connection with vertebral damage seemed to be related to vertebral collapse, leading to damage of the surrounding tissue and blood vessels as well.

Other studies have reported similar findings. McCann and Jasper (1972) reported extensive hemorrhaging of the vertebral region in bluegills exposed to a variety of pesticides. After short exposure of fathead minnows to chlordacone, extensive hemorrhaging, associated with an apparent fracture of the vertebrae occurred (Buckler et al., 1981). Fathead minnows exposed to disulfoton developed hemorrhaged areas anterior and posterior to the dorsal fin (Holcombe et al., 1982). In all the cases, the condition was related to fractures of the vertebral column.

Fathead minnow larvae exposed to Lindane often exhibited edemas in the ventral region of the body (Figure 13). Many pesticides, irrespective of the group to which they belong, induce edemas in different species of fish. As early as 1959, Henderson et al. reported that HCH-exposed fathead minnows developed swelling of the abdomens and bent bodies.

Figure 12. Hemorrhage in fathead minnow larva exposed to Lindane at 25°C.

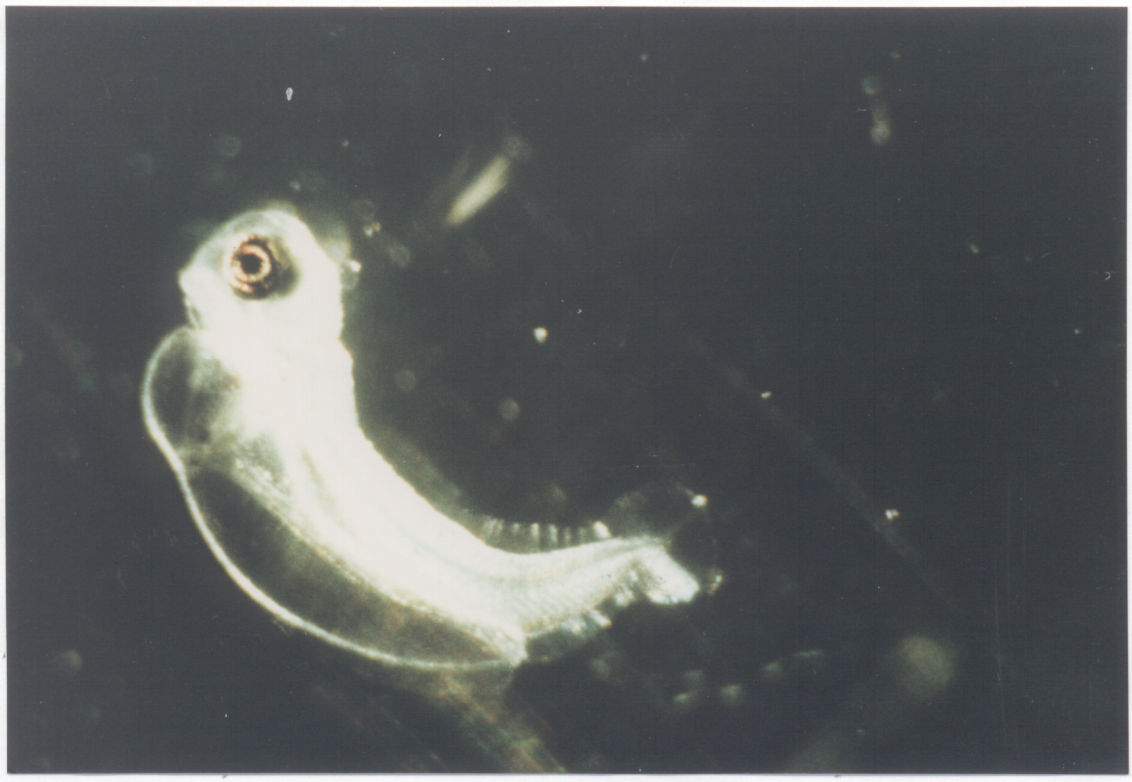


Figure 13. Edema en fathead minnow larva exposed to Lindane at 25°C.

Pericardial edemas were observed later in carp exposed to a variety of pesticides (Kaur and Toor, 1977), and in fingerling rainbow trout exposed to lethal concentrations of fenitrotion (Klaverkamp et al., 1977). More recently, Holocombe et al. (1982a, 1982b) found that permethrin and pentachlorophenol caused distention of the peritoneal cavity in fathead minnows. Similar effects were also documented for rainbow trout exposed to methylparathion (Palawski et al., 1983).

The appearance of edemas may reveal the incapacity of the liver to produce amounts of important proteins such as albumin. Albumin is the most important factor in the regulation of the colloidal osmotic pressure, which pushes extracellular fluids from interstitial spaces back into the capillary bed. When the levels of albumin and other proteins are reduced, body fluids tend to accumulate within interstitial areas where they produce swelling and distention.

Many fish demonstrated impairment of swimming capability. Larvae exhibited difficulty in maintaining position or equilibrium, a tendency to lay on the bottom, and tilted swimming and darting movements. These behavieors were always observed following the appearance of lordosoliosis and thus, could have resulted from the effect of lindane on the disruption of the normal nerve functions. The severity and duration of the response exhibited little relationship to dose

so it may be an all-or-none effect (Table 12).

Changes in swimming behavior like the ones reported here, have been observed in other fish larvae exposed to different pesticides (Bengtsson, 1974; Dill and Saunders, 1974; Aldeman et al., 1976). Thus, chronic exposure of pinfish to Aroclor$^{R}$ 1216, for example, induces erratic swimming and sluggish feeding (Hansen et al., 1971). In all the cases, a potential long term effect on larval feeding, and susceptibility to predation would depend on the severity and the duration of impaired swimming capabilities.

## CONCLUSIONS

1. The 14d-$LC_{50}$ values for fathead minnow embryos exposed to Lindane at 25 and 18°C was 44 (60-32) and 37 (52-26) µg/L, respectively.

2. At 25°C, egg and larval stages were the most sensitive to Lindane, and yolk-sac the most resistant. On the other hand, yolk-sac was the most sensitive stage at 18°C, while egg and larva showed about equal resistance. Retarded hatching and reduced larval size was noted at 18°C.

3. Morphological abnormalities (i.e. edemas, hemorrhages and lordoscoliosis) showed a strong positive correlation with Lindane doses, except for altered swimming.

## BIBLIOGRAPHY

Adelman, I. R., L. L. Smith, and G. D. Siesennop. 1976. Acute toxicity of sodium chlorine, pentachlorophenol, Guthion$^{R}$, and hexavalent chromium to fathead minnows (Pimephales promelas) and goldfish (Carassius auratus). J. Fish. Res. Board Can., 33, 203.

Alderdice, D.F. and F.P.J. Velsen. 1971. Some effects of salinity and temperature on early development of Pacific herring (Clupea pallasi). J. Fish. Res. Board Can., 15:229-249.

Andrews, A. and S. Flickinger. 1974. Spawning requirements and characteristics of the fathead minnow. Proc. Ann. Conf. Southeastern Assoc. Game Fish Comm., 27:759-766.

Anonymous. 1975. Some bad news about toxaphene. Science, 188, 343.

Bahls, L. L., R. A. Soltero and K. E. Tuinstra. 1969. The toxicity of organic wastes on eggs and fry of rainbow trout. Proc. Mont. Acad. Sci., pp. 29.

Balon, E. K. 1975. Terminology of intervals in fish

development. J. Fish. Res. Board Can., 32:1663-1670.

Beland, F. A., S. O. Farwell, A. E. Roboker, and R. D. Geer. 1976. Electrochemical reduction and anaerobic degradation of lindane. J. Agri. Food Chem., 24(4):753-756.

Benezet, H. I. and F. Matsumura. 1973. Isomerization of $\gamma$-BHC to $\alpha$-BHC in the environment. Nature, 243:480-481.

Bengtsson, B.-E. 1975a. Vertebral damage induced by pollutants. In J. H. Koeman, and J. J. T. W. A. Strik, Eds., Sublethal effects of toxic chemicals on aquatic animals, Elsevier, Amsterdam, 23.

Bengtsson, B.-E., C. H. Carlin, A. Larson, and D. Swanberg. 1975b. Vertebral damage in minnows, Phoxinus phoxinus L., exposed to cadmium. Ambio, 4:166-168.

Bengtsson, B.-E. 1974a. Vertebral damage in the minnow Phoxinus phoxinus exposed to zinc. Oikos, 25, 134.

Bengtsson, B.-E. 1974b. The effect of zinc on the ability of the minnow, Phoxinus phoxinus L., to compesate for torque in a rotating water-current. Bull. Environ. Cont. Toxicol., 12:654-658.

Benoit, D. A. and G. W. Holcombe. 1978. Toxic effects of zinc on fathead minnows, Pimephales promelas, in soft water. J. Fish. Biol., 13, 701.

Biggar, W.J. and R.L. Riggs. 1974. Apparent solubility of organochlorine insecticides in water at various temperatures. Hilgardia, 42(10):383-391.

Blaxer, J. H. S. 1969. Development: eggs and larvae, p. 177-252. In W.S. Hoar and D.J. Randall, ed. Fish physiology. Vol III, Academic Press, Inc., New York and London.

Brett, J. R. 1959. In C. M. Tarzwell, Biological problems in water pollution. Trans. of the 1959 Seminar, U. S. Dept. of Health, Education and Welfare, R. A. Taft San. Eng. Center, Cincinnati, Ohio, Section 3. Temperature-Fishes, pp. 110-117 (Tech. Rep. W60-3).

Bridges, W. R. 1965. Trans. 3rd Seminar on Biol. Problems in Wat. Poll. U.S. Publ. Health Serv., Publ. PHS-999-WP. 25, 247.

Brown, A. W. A. 1978. Ecology of Pesticides. John Wiley & Sons, New York.

Buckler, D., A. Witt, F. Mayer, J. Huckins. 1981. Acute and chronic effects of kepone and mirex on fathead minnow. Trans. Am. Fish. Soc., 110, 270.

Cairns, M. A., and A. V. Nebeker. 1982. Toxicity of acenaphthene and isophrone to early life stages of fathead minnows. Arch. Environm. Contam. Toxicol., 11:703-707.

Carlander, K. 1969. Handbook of freshwater fishery biology, Vol. 1. Ames, Iowa: Iowa State Univ. Press.

Cook, A. S. 1970. The effect of pp'DDT on tadpoles of common frogs. Environ. Pollut., 1:57-71.

Cope, O. B. 1964. Sport fisheries investigations. Laboratory studies and toxicology. U.S. Bur. Sport Fish. Wildl. Serv. Circ., Wash., No. 199, 29.

Cope, O. B. 1965a. Some responses of freshwater fish to herbicides. Proc. 18th Ann. Meet. Southern Weed Conf., Dallas, Texas, 439.

Cope, O. B. 1965b. Sport fisheries investigations. Laboratory studies and toxicology. U.S. Bur. Sport Fish Wildl. Serv. Circ., Wash., No. 226, 51.

Cope, O. B. 1968. U.S. Bur. Sport Fish. Wildl., Resourse Publ., No. 64, 125.

Couch, J. A., J. T. Winstead, L. R. Goodman. 1977. Kepone induced scoliosis and its hystological consequences in fish. Science, 197, 585.

Danil'chenko, O. P. 1978. The sensitivity of fish embryos to the effect of toxicants. J. Ichthyol., 17(3):445-463.

Danil'chenko, O. P. and N. S. Strogonov. 1975. Evaluation of toxicity to the early ontogeny of fishes of substances discharged into a body of water. J. Ichthyol., 15(2):311-319.

De Foe, D. L., G. D. Veith, and R. W. Carlson. 1978. Effects of Aroclor$^{R}$ 1248 and 1260 on the fathead minnow (Pimephales promelas). J. Fish. Res. Board Can., 35:997-1002.

Dethlefsen, V. 1974. Effects of DDT and DDE on embryos and larvae of cod, flounder and plaice. Int. Counc. Explor. Sea Counc. Meet., 1974/E:10. Fish. Improv. Comm., 17.

Dill, P. A., and R. C. Saunders. 1974. Retarded behavioral development and impaired balance in Atlantic salmon

(Salmo salar) alevins hatched from grastrulae exposed to DDT. J. Fish. Res. Board Can., 31, 1936.

EPA. 1979. Water-related environmental fate of 129 priority pollutants. Vol. 1. EPA-44014-79-029a. Introduction and technical background, metals and inorganics, pesticides and PBC's.

Finney, D. J. 1971. Probit Analysis. 3$^{d}$ ed. Cambridge University Press., Chap. 2, 3.

Flickinger, S. A. 1966. Determination of sexes in the fathead minnow. Trans. Amer. Fish. Soc., 98(3):526-527.

Flickinger, S. A. 1973. Investigation of pond spawning methods for fathead minnows. Proc. Ann. Conf. Southeastern Assoc. Game Fish Comm., 26:376-391.

Gakstatter, J.H. and C.M. Weis. 1967. The elimination of DDT-$^{14}$C, dieldrin-$^{14}$C, and lindane-$^{14}$C from fish following a single sublethal exposure on aquaria. Trans. Amer. Fish. Soc., 96(3):301-307.

Gale W. F. and G. L. Buynak. 1982. Fecundity and spawning frequency of the fathead minnow - A fractional spawner. Trans. Amer. Fish. Soc., 11: 35-40.

Goodman, L. R., D. J. Hansen, C. S. Manning, and L. F. Faas. 1982. Effects of kepone on the sheephead minnow in an entire life-cycle toxicity test. Arch. Envron. Contam. Toxicol., 11, 335.

Gulidov, M. V. and K. S. Popova. 1981. The hatching dynamics and morphological features of larvae of roach, Rutilis rutilus in relation to incubation temperature. J. Ichthyol., 19:87-92.

Haider, K.,and G. Jagnow. 1975. Degradation of $C^{14}$, tritium$^{14}$, and chlorine$^{36}$ labeled of hexachlorocyclohexane by anaerobic soil microorganisms. Arch. Microbiol., 104(2):113-121.

Hamelink, J.L. and R.C. Waybrant. 1976. DDE and lindane in a large-scale model lentic ecosystem. Trans. Amer. Fish. Soc., 105(1):124-134.

Hamelink, J.L., R.C. Waybrant, and P.R. Yant. 1977. Mechanisms of bioaccumulation of mercury and chlorinated hydrocarbon pesticides by fish in lentic ecosystems. In I. M. Suffet, Fate of pollutants in the air and water environment. Part II, pp. 261-268. John Wiley and Sons, New York, N.Y.

Hansen, D. J., P. R. Parrish, and J. Forester. 1971. Arochlor 1016: toxicity to and uptake by estuarine animals. Environ. Res., 7, 363.

Hansen, D. J., S. C. Schimmel, and J. Forester. 1977. Endrin: effects on the entire life cycle of saltwater fish, Cyprinodon variegatus. J. Toxic. Environ. Health, 3, 721.

Hansen, P-D. 1980. Uptake and transfer of the chlorinated hydrocarbon lindane ($\gamma$-BHC) in a laboratory freshwater food chain. Environ. Pollut. (Series A), 21:97-108.

Hassall, K.A. 1982. The chemistry of pesticides: Their metabolism, modes of action, and uses in crop protection. Section 6.6. Benzene hexachloride (HCH); pp. 133-135.

Hauck, F. R. 1949. Some harmful effects of the electric shocker on large rainbow trout. Trans. Amer. Fish. Soc., 77:61-64.

Henderson, C., Q. H. Pickering, and C. M. Tarzwell. 1959. Relative toxicity of ten chlorinated hydrocarbons insecticides to four species of fish. Trans. Am. Fish. Soc., 88, 23.

Herzig, A. and H. Winkler. 1986. The influence of temperature on the embryonic development of three cyprinid fishes, Abramis brama, Chalcalburnus chalcoides mento and Vimba vimba. The Fisheries Society of the British Isles, 171.

Hintze, J. L. 1988. Number Cruncher Statistical System - Survival Analysis, Version 5.5.

Holcombe, G. W., G. L. Phipps, and D. K. Tanner. 1982a. The acute toxicity of kelthane, Dursban$^{R}$, disulfoton, pydrin and permethrin to fathead minnows P. promelas and rainbow trout S. gairdneri. Environ. Pollut. No. 29, 167.

Holcombe, G. W., G. L. Phipps, and J. T. Fiandt. 1982b. Effects of phenol, 2,4-dimethylphenol, 2,4-dechlorophenol, and pentachlorophenol on embryo, larval and early juvenile fathead minnow (Pimephales promelas). Arch. Environm. Contam. Toxicol., 11, 73.

Holdway, D. A., and D. G. Dixon. 1986. Impact of pulse exposure to methoxychlor on flagfish (Jordanella floridae) over one reproductive cycle. Can. J. Fish. Aquat. Sci., 43, 1410.

Iyatomi, K., T. Tamura, Y. Itazawa, I. Hanyu and S. Sugiura, 1958. Toxicity of endrin to fish. Progr. Fish-Cult.,

20:155-162.

Johnson, D. W. 1968. Pesticides and fishes: A review of selected literature. Trans. Am. Fish. Soc. 97:398-424.

Jonhson D. W. and M. T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U. S. Dept. of Interior, Wildlife Sec., Resource Publ. 137.

Katz, M. and G. Chadwick. 1961. Toxicity of endrin to some Pacific north west fishes. Trans. Amer. Fish. Soc., 90.

Kaur, K., and H. S. Toor. 1977. Toxicity of pesticides to embryonic stages of Cyprinus carpio communis Linn. Indian J. Exp. Biol., 15:193-196.

Klaverkamp, J. F., M. Duangswasdi, W. A. Macdonald, and H. S. Majewski. 1977. An evaluation of fenitrothion toxicity in four life stages of rainbow trout, S. gairdneri. In F. L. and J. L. Hamelink, Eds., Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, , ASTM, Philadelphia, 1977, 231.

Kurihara, N., M. Uchida, T. Fujita, and M. Nakajima. 1973. Studies on BHC isomers and related compounds: V. Some

physicochemical properties of BHC isomers (1). Pesticide Biochemistry and Physiology, 2:383-390.

LeBlanc, G. A. and M. L. Frank. 1984. Antimony and thallium toxicity of embryos and larvae of fathead minnows (Pimephales promelas). Bull. Environm. Contam. Toxicol., 32, 565.

Lee, D.S., C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and R. Stauffer, Jr. 1980. Atlas of North American freshwater fishes. Publ. 1980-12. Raleigh, N. Carolina: N. Carolina State Museum Natural History, pp. 341.

Lee, E. 1984. Sattistical methods for survival data analysis. Wadsworth. Belmont, California.

Lipke, H., and C. W. Kearns. 1960. DDT-dehydrochlorinase III, solubilization of insecticides by lipoprotein. J. Econ. Entomol., 53, 31.

Macek, K. J., C. Hutchinson, and O. B. Cope. 1969. The effects of temperature on the susceptibility of bluegill and rainbow trout to selected pestides. Bull. Environ. Contam. Toxicol., 4:174-183.

Macek, K. J. 1968. Reproduction of brook trout Salvelinus fontinalis fed sublethal concentrations of DDT. J. Fish. Res. Bd. Can., 25:1787-1796.

Malone, C. R. and B. G. Blaylock. 1970. Toxicity of insecticide formulations to carp embryos reared in vitro. J. Wildlife Manag., 34, No. 2.

Markus,H. 1934. Life history of the blackhead minnow (Pimephales promelas). Copeia, 1934:116-122.

Mathur, S. P., and J. G. Saha. 1975. Microbial degradation of $C^{14}$ labeled lindane in a flooded sandy loam soil. Soil Sci., 120(4):301-307.

Matsumura, F., G. M. Boush, and T. Misato (Eds). 1972. Environmental toxicology of pesticides. Academic Press, New York.

Matsumura, F., H. J. Benezet, and K. C. Patil. 1976. Factors affecting microbial metabolism of $\tau$-BHC. Nippon Woyaku Gakkaishi 1(1):3-8. In Water related environmetal fate of 129 priority pollutants, EPA, Vol. 1, EPA 44014-79-029a, 1979.

Mc Cann, J. A., and R. L. Jasper. 1972. Vertebral damage to

bluegills exposed to acutely toxic levels of pesticide. Trans. Amer. Fish. Soc., 101, 317.

McKim, J. M. 1977. Evaluation of tests with early life stages of fish for predicting long- term toxicity. J. Fish. Res. Bd. Can., 34:1148-1154.

Mellanby, K. 1970. Pesticides and pollution. The New Naturalist; pp. 133-135, 157.

Melnikov, N.N. 1971. Halogen derivatives of alicyclic hydrocarbons. In N.N. Melnikov (Ed.), Chemistry of pesticides; pp. 42-50.

Mehrle, P. M. and F. L. Mayer. 1975. Toxaphene effects on growth and development of brook trout (Salvelinus fontinalis). J. Fish. Res. Board Can., 32, 609.

Metcalf, R.L., I.P. Kapoor, P-Y. Lu, C.K. Shuth, and P. Sherman. 1973. Model ecosystem studies of the environmental fate of toxic organochlorine pesticides. Environm. Health Perspect., 4:35-44.

Mironov, O. G. 1973. Marine oil pollution. In O. P. Danil'chenko, The sensitivity of fish embryos to the effect of toxicants, J. Ichthyol., 1978, 17(3):445-463.

Murty, A. S. 1986. Toxicity of pesticides to fishes. Vol II. CRC Press, Inc., Boca Raton, Florida.

Niimi, A. J. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. Can. J. Fish. Aquat. Sci., 40:306-312.

Oloffs, P. C., and L. J. Albright. 1974. Transport of some organochlorines in B. C. water. Proc. Int. Conf. Transp. Persistent Chem. Aquat. Ecosyst., I:89-92.

Palawski, D., D. R. Buckler, and F. L. Mayer. 1983. Survival and condition of rainbow trout (S. gairdneri) after acute exposures to methylparathion, triphenyl phosphate, and DEF. Bull. Environ. Contam. Toxicol., 30, 614.

Peñáz, M. 1974. Influence of water temperature on incubation and hatching in Chonndrostoma nasus (L.). Zool. Listy, 23:53-59.

Pickering, Q. H. and W. T. Gilliam. 1982. Toxicity of aldicarb and fonofos to the early-life-stage of the fathead minnow. Arch. Environm. Contam. Toxicol., 11, 699.

Pickering, Q. H. and W. N. Vigor. 1965. The acute toxicity

of zinc to eggs and fry of the fathead minnow. Progress Fish Culturist, 27, No. 3.

Pomazovsskaya, I. V., Ryzhkov, A. F. Samylim and Ye. I. Remezova. 1970. The effect of pesticides used in the treatment of driftwood. In Problems of water toxicology. Moscow, Nauka Press.

Robeck, G. G., K. A. Dostal, J. M. Cohen, and J. F. Kreissl. 1965. Effectiveness of water treatment processes in pesticide removal. J. Amer. Water Work Ass., 181.

Rosenthal, H., and D. F. Alderdice. 1976. Sublethal effects of environmental stressors, natural and pollutional, on marine fish eggs and larvae. J. Fish. Res. Board Can., 33:2047-2065.

Sanborn, J.R. 1974. The fate of selected pesticides in the aquatic environment. U.S. Environ. Prot. Agency, Off. Res. Dev., [Rep] EPA, EPA-660/3-74-025, pp.83.

Sherma, J. 1979. Manual of analytical quality control for pesticides and related compounds in human and environmental samples. U.S. Environ. Prot. Agency, Off. Res. Dev., EPA-600/1-79-008.

Schreiman, E., and R. Rugh. 1949. Effect of DDT on functional development of larvae of Rana pipiens and Fundulus heteroclitus. Proc. Soc. Exp. Biol. Med., 70, 431.

Scott, W. and E. Crossman. 1973. Freshwater fishes of Canada. Fish. Res. Bd. Can. Bull., 184, pp. 966.

Skidmore, J. 1965. Resistance to zinc sulphate of the zebra fish (Brachydanio rerio Hemilton-Buchabab) at different phases of its life history. Ann. Appl. Biol., 15, No.3.

Smith, E. J., J. L. Sykora, and M. A. Shapiro. 1973. Effect of lime neutralized iron hydroxide suspensions on survival, growth and reproduction of fathead minnow (Pimephales promelas). J. Fish. Res. Board Can., 30, 1147.

Spencer, S. L. 1967. Internal injuries of largemputh bass and bluegills caused by electricity. Progr. Fish-Cult., 29, 168.

Stockard, Ch. 1921. Developmental rate and structural expression: an experimental study of twins "doble monsters" and single malformates, and the interaction among embryonic organs during their origin and development. Amer. J. Anatomy, 28, No. 2.

Strogonov, N. S. and A. G. Pozhitkov. 1941. The effects of industrial effluents on aquatic organisms. Uch. zap. Mosk. gos. un-ta, No. 60.

Sugira, K., N. Matsumoto, T. Washina, Y. Mihara, and M. Goto. 1979. Accumulation of organochlorine compunds in fishes: Distribution of 2, 4, 6, 2', 4', 6'-hexachlorobiphenyl in tissues. Chemosphere. No.6, pp. 365-368.

Tobby, T.E. and F.J. Durbin. 1975. Lindane residue accumulation and elimination in rainbow trout (Salmo gairdnerii Richardson) and roach (Rutilus rutilus Linnaeus). Environ. Pollut. 8:79- 89.

Trautman, M B. 1957. The fishes of Ohio. Columbus, Ohio: Ohio State Univ. Press; pp. 683.

Venugopalan, V. K. ,and P. Sasibhushana Rao. 1979. Pesticide induced impairment in incubation and post-embryonic development of planktonic eggs of estuarine fishes of Porto Novo (South India) waters. In Proc. Symp. Environ. Biol., Academy of Environmental Biology, Muzaffarnagar, India, pp. 397-408.

Vladimirov, V. I. 1975. Critical periods in the development of fishes. Journal of Ichthyology, 15(6):851-868.

Von Westernhagen, H. 1988. Sublethal effects of pollutants on fish eggs and larvae. In W. S. Hoar and D.J. Randall, Eds., Fish Physiology, Volume XI: The physiology of developing fish, Part A: Eggs and larvae, pp. 253-330, Academic Press, Inc.

Walker, C. R. 1963. Endothal derivatives as aquatic herbicides in fishery habitats. Weeds, 11:226-232.

Weis, J. S.,and P. Weis. 1989. Effects of environmental pollutants on early fish development. Review in Aquatic Sciences, 1:45-75.

Weis, J. S., and P. Weis. 1976. Optical malformations induced by insecticides in embryos of silversides, Menidia menidia. Fish. Bull., 74, 208.

Weis, P. and J. S. Weis. 1976. Abnormal locomotion associated with skeletal malformations in the sheephead minnow, Cyprinodon variegatus, exposed to malathion. Environ. Res., 12, 196-200.

Weis, P. and J. S. Weis. 1974. Cardiac malformations induced by insecticides in embryos of the killifish, Fundulus heteroclitus. Teratology, 10, 263-268.

Worthing, C.R. (Ed.). 1979. Pesticide manual: A world compendium. The British Crop Protection Council, 6th ed., pp. 290,295.

Zar, J. H. 1984. Biostatistical Analysis. 2d ed. Prentice-Hall, Inc., N.J., Chap. 17.

## APPENDIX

SYNTHETIC INSECTICIDES

1. Organochlorine compounds

| | | |
|---|---|---|
| Aldrin | $C_7H_{14}N_2O_2S$ | m.w. 190.3 |
| Dieldrin | $C_{12}H_8Cl_6O$ | m.w. 380.9 |
| DDT(pp'-DDT) | $C_{14}H_9Cl_5$ | m.w. 354.5 |
| Endrin | $C_{12}H_8Cl_6O$ | m.w. 380.9 |
| Heptachlor | $C_{10}H_5Cl_7$ | m.w. 373.3 |
| Kelthane(Dicofol) | $C_{14}H_9Cl_5O$ | m.w. 370.5 |
| Kepone(Chlordecone) | $C_{10}Cl_{10}O$ | |
| Methoxychlor | $C_{16}H_{15}Cl_3O_2$ | m.w. 345.7 |
| Toxaphene | $C_{10}H_{10}Cl_8$ | m.w. 413.8 |
| Lindane($\gamma$-HCH) | $C_6H_6Cl_6$ | m.w. 290.8 |

2. Organophosphorus compounds

| | | |
|---|---|---|
| Diazinon | $C_{12}H_{21}N_2O_3PS$ | m.w. 304.3 |
| Disulfoton | $C_8H_{19}O_2PS$ | m.w. 274.4 |
| Dursban$^R$ | $C_9H_{11}Cl_3NO_3PS$ | m.w. 350.6 |
| Fenitrothion | $C_9H_{12}NO_5PS$ | m.w. 277.2 |
| Fonofos | $C_{10}H_{15}OPS$ | m.w. 246.3 |
| Malathion | $C_{10}H_{19}O_6PS$ | m.w. 330.3 |
| Parathion | $C_{10}H_{14}NO_5PS$ | m.w. 291.3 |
| Parathion-methyl | $C_8H_{10}NO_5PS$ | m.w. 263.2 |
| Phosphamidon | $C_{10}H_{19}ClNO_5P$ | m.w. 299.7 |

3. Carbamates

| | | |
|---|---|---|
| Aldicarb | $C_7H_{14}N_2O_2S$ | m.w. 190.3 |
| Carbaryl | $C_{12}H_{11}NO_2$ | m.w. 201.2 |

4. Other pesticides

Aroclor 1248 - Polychlorinated biphenyl(PCB)

Aroclor 1260 - Polychlorinated biphenyl(PCB)

Pentachlorophenol(PCP)

Trifluralin - Dinitroaniline

METALS

| | | |
|---|---|---|
| Zinc | Zn | a.w. 65.37 |
| Antimony | Sb | a.w. 121.75 |
| Thallium | Tl | a.w. 204.37 |
| Iron | Fe | a.w. 55.84 |

# VITA

## ANABEL G. TATIS R.

Department of Biology
Virginia Polytechnic Institute
and State University
Blacksburg, Virginia 24061
(W) (703)231-9053

Apartado No.6
Penonome, Prov. de Cocle
Republica de Panama
America Central
(H) (507) 97-9284

## EDUCATION

**B.S. Biology,** December 1985
Pontificia Universidad Javeriana
Bogota, Colombia
**Thesis title:** "Toxic effects of the insecticide Aldrin in the embryonic development of Hyla labialis".

**M.S. Zoology,** September 1990
Virginia Polytechnic Institute and State University
Blacksburg, Virginia
**Thesis title:** "Toxic effects of the pesticide Lindane on the early developmental stages of Fathead minnow (Pimephales promelas)".

## SPECIAL TRAINING

Workshop in **Reproduction and Development** at Pontificia Universidad Javeriana, Bogota, Colombia, September 1983.

Practical training in **Reproduction Techniques** at Centro Colombiano de Fertilidad y Esterilidad, Bogota, Colombia, July 1984.

## PROFESSIONAL EXPERIENCE

**Teaching assistant** for General Biology Laboratory, Department of Biology, Virginia Polytechnic Institute and State University, Fall 1988 and Spring 1990.

**Field assistant** for the project "Experimental exploration of the causes of seasonal growth and reproduction in the

tropics: Egg mortality of the lizard Anolis limifrons", Smithsonian Tropical Research Institute, Barro Colorado Island, Panama, 1986.

**Laboratory assistant** for "in vitro" fertilization program, Centro Colombiano de Fertilidad y Esterilidad, Bogota, Colombia, 1984-85.

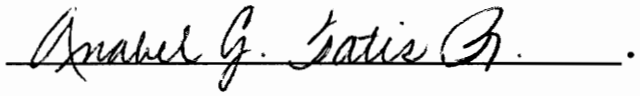
**Teaching assistant** for Comparative Animal Anatomy Laboratory, Department of Biology, Pontifica Universidad Javeriana, Bogota, Colombia, Fall 1984.

HONORS AND ACTIVITIES

- Fulbright scholarship to persue M.S. Zoology, January 1987 to December 1989.
- North American Benthological Society student membership, 1990-present.
- Society of Environmental Toxicology and Chemistry student membership, 1988-present.
- President of the Biology Student Association, Pontificia Universidad Javeriana, 1985.
- Secretary of the Biology Student Association, Pontificia Universidad Javeriana, 1984.

ADDITIONAL SKILLS

- Fluent and proficient in speaking and writing Spanish.
- Travel throughout South America and the United States.



Anabel G. Tatis R.