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Praise be to the Lord God, the Almighty, who has granted us opportunities to glimpse at some of the wonders of the creation.

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ABSTRACT

Three insecticides (fenitrothion, carbaryl and lindane) were tested for their interference with normal Xenopus laevis development. At 10 mg/l, both fenitrothion and carbaryl were teratogenic whereas lindane was not. It was proposed that the malformations caused by the two insecticides could be related to a disruption of the cholinergic system leading to abnormal development of structures derived primarily from the mesoderm such as the heart, and the circulatory and skeletal systems. The study showed that although fenitrothion and carbaryl have short residual lives, both pose developmental hazards for embryonic and early juvenile frogs.

All three insecticides were embryotoxic at concentrations of 10 mg/l and higher. It was proposed that further study should investigate the interaction of different insecticides on the development of non-target organisms to determine the possibility of teratogenic effects.

INTRODUCTION

Outline of Study

An increasing number of xenobiotics in the aquatic environment have caused a growing concern about the effects of these chemicals on the survival, reproduction and development of non-target fauna.

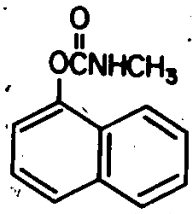
This study has concentrated on some of the effects of three different insecticides (Fig. 1): lindane (an organochlorine), carbaryl (a carbamate), and fenitrothion (an organophosphate), on the embryonic development and larval survival of Xenopus laevis, the South African clawed frog (Fig. 2). These compounds were chosen as representative of three classes of unrelated insecticides that are extensively used in North America. All three are reputed to be less toxic than many others in their respective groups. Their general effects on adults are known but there is little information on their effects on the embryos and young of most species. Xenopus laevis were chosen because they are aquatic, do not need extensive terraria, do not require well aerated water and are hardy animals.

In this project, six aspects were studied. The first was to establish whether or not any or all of the insecticides tested were toxic to either embryonic or larval frogs. The second was to establish whether or not the chemicals altered the behavioural pattern and survival of the

Figure 1:

The chemical structures and a brief overview of the insecticides used in this study.

CARBARYL (1-naphtyl N-methylcarbamate)



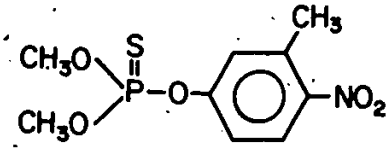
Molecular weight: 201

Solubility: 40 mg/1 (H_2O)

Stability: half-life in water is one to five days

Uses: crop protection, and pests affecting forests, range land, livestock, pets, poultry and buildings

FENITROTHION (O,O-dimethyl-O-(4-nitro-m-totyl) phosphorothioate)



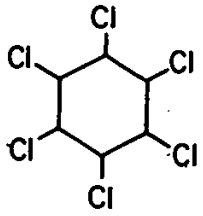
Molecular weight: 277

Solubility: 20 mg/1 (H_2O)

Stability: half-life varies depending on the acidity

Uses: crop protection, forest protection, control of disease-carrying insects

LINDANE (1,2,3,4,5,6-hexachlorocyclohexane, gamma isomer)



Molecular weight: 291

Solubility: very stable in the environment

Uses: crop and building protection, and in the treatment of some human parasites

juveniles. If the insecticide was found to be toxic, the question was then asked as to whether it only caused illness or interfered with the basic developmental pattern of the animal, thus classifying it as a teratogen.[1] The fourth aspect was to examine morphological changes in the embryos treated at different developmental stages to establish which range of stages was the most sensitive. The fifth area examined was the actual survival through metamorphosis. The final aspect was to discern whether or not the metamorphosing individuals treated with different insecticides were normal. All three insecticides were tested in a similar way with varying emphasis.

This study was designed to verify or to refute the hypothesis that the chemicals in question are safe for non-target aquatic vertebrates, such as the anurans. Unlike most toxicological studies, the emphasis has been on the developing young and not the adults. This was primarily because the embryonic period is the most sensitive stage of an animal's life.

Different Test Systems

A wide variety of different experimental approaches have been utilized in studying the effects of xenobiotics on vertebrate embryonic development.

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[1] The term teratogen was used to describe a xenobiotic that caused gross morphological abnormalities.

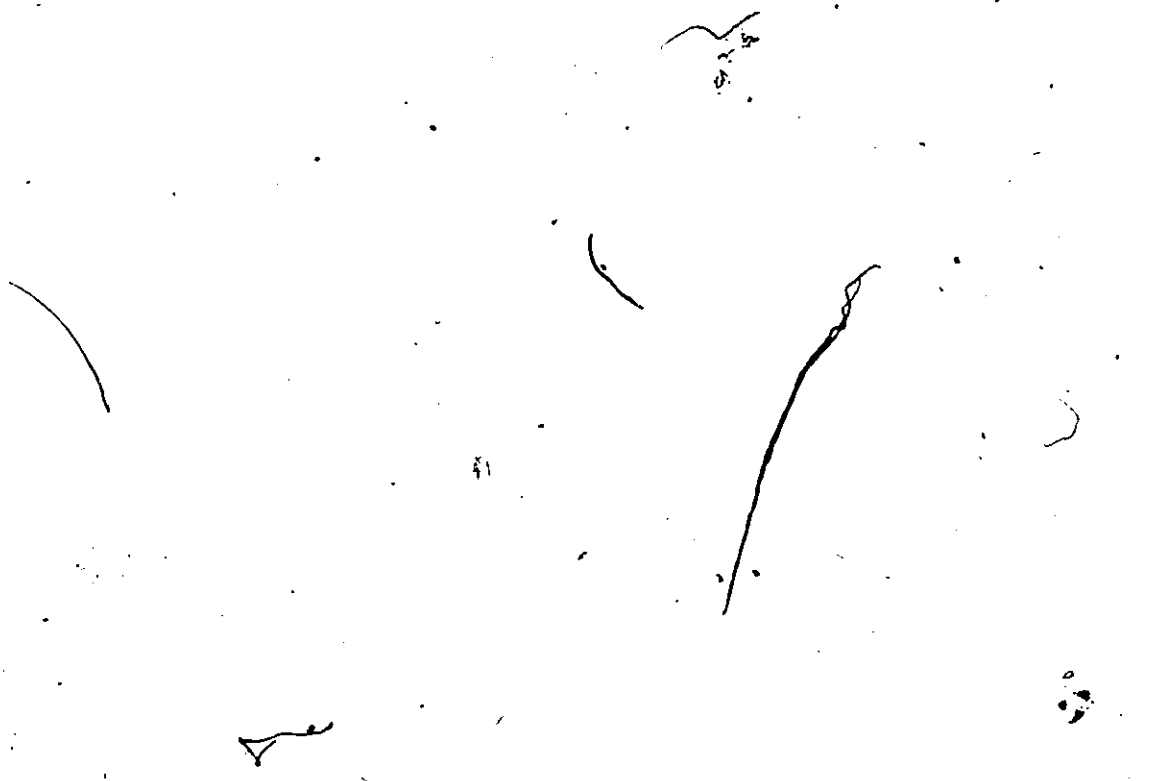

Figure 2:

Adult Xenopus laevis in amplexus. Note the relative size between the male and female.

In mammalian studies, basically two methods are used. The toxin is either injected or administered orally to the mother. The oral route is preferred because it more closely simulates a natural situation. Defined doses can be given to the mother for a specified period which correlates with specific embryonic stages. The stability of a toxin within the maternal system can be determined by chemical analysis of tissue samples, blood and waste products.

Injecting the mother with a given dose avoids the problems of detoxification by the maternal digestive system. This second method can be better employed to define the critical stages of development affected by the toxin. It could also be used to indirectly define LD_{50} and ED_{50} levels.

The mammalian test system, however, is plagued by several problems. It is limited to either a small sample size or great genetic diversity. In addition, the extra-embryonic milieu cannot be controlled, although it can be monitored by using several analytical techniques. For this reason, there is difficulty in determining both the form and quantity of the toxin reaching the embryo and the exact stages that are being affected.

In birds, either the toxin is injected into a specific region of the embryo or the eggs are soaked in a solution containing the toxin. In this way, the toxic effects on the embryo are not affected by the maternal system as in mammals. In addition to these short term studies,

generation studies have been done.[1]

Several factors such as seasonal and dietary variation, genetic constitution of the laying stock (Landaurer, 1954), length of toxin storage before injection (Clegg, 1964), concentration of the toxin, type of solvent used, specific gravity of the inoculum relative to the yolk (Walker, 1967), and developmental age at the time of injection may exert critical influences on the response of the embryos.

Very few studies have been done on reptiles (Mitchell and Yntema, 1973). However, the approach is similar to that used in birds. In both systems, the experimenter can closely regulate the quantity of toxin entering the embryo. There is usually one injection, unlike the prolonged exposures used in mammalian studies. The single injection is useful in determining the embryonic stages most sensitive to a given chemical and can be used to directly determine LD_{50} and ED_{50} levels. However, residues of the toxin and/or its breakdown products are retained until hatching. Although this method is experimentally very consistent, it does not duplicate the conditions of exposure in a natural environment.

Aquatic or semi-aquatic animals, such as fish and amphibians, can be examined in two ways. Either a generation study is done, or the embryos are placed in a given concentration of the pesticide. In the latter case, the pulse may be

[1] In the generation studies, the progeny of treated individuals are examined. These individuals may have been treated during the developmental stages or later, as adults, being exposed to a specific quantity of the pesticide either in food or in a contaminated milieu.

latter case, the pulse may be as short as six hours or as long as the developmental period. The two test systems complement each other. The first gives information on both the effects of the toxin on gametogenesis and possible mutagenic action, while the second yields information on teratogenesis and metabolic disruptions within the embryo. The external development exhibited by many aquatic vertebrate genera makes these types of studies much easier.

Regardless of the species used, the effects of a given insecticide on vertebrate development can be studied by placing the embryo in a contaminated aqueous milieu. This can be done either directly, as with fish and amphibians, indirectly as in the mammalian studies, or artificially as in avian and reptilian studies. With fish and amphibians, staging and xenobiotic concentration can be more closely monitored than in the other cases.

Role of Cholinesterases in Development

Landauer (1975) proposed that the cholinomimetic teratogens interfere with normal chick development either by displacing acetylcholine (Ach) from its receptors or by forming complexes with it. All of the cholinomimetic teratogens used in his experiments have nicotinic activity and inhibit cholinesterase activity as do the organophosphorus and carbamate insecticides. However, chemically closely related compounds lacking these two characteristics were non-teratogenic.

Cholinomimetic teratogens appear to be producing their effects on distinct metabolic pathways. Landauer has suggested that the origin of the malformations may be traced to either a displacement of acetylcholine from the receptors by the cholinomimetic compounds, to complex formation between the latter and Ach or to an attachment of the cholinomimetic compound to "spare" Ach receptors, leading to competitive interference in metabolic developmental events. If competition or a malfunctioning of the acetylcholine-acetylcholinesterase complex are implicated in deviations from normal development then one is led to question the conclusion that Ach receptors play no role in the early events of myogenesis (Fambrough and Rash, 1971).

Extensive work has been done to discover the role of both acetylcholine and cholinesterases on developing chick embryos. Wake (1976) found that intracellular cholinesterase activity in the chick muscle increased during the formation of the myoneural junctions and then decreased rapidly. Microscopically, he demonstrated the presence of cholinesterases in the nuclear envelope, sarcoplasmic reticulum, Golgi complex and in large granules which appeared to be derived from the Golgi complex. Wilson and Linkhart (1973) showed that levels of plasma cholinesterases were higher during embryonic development and decreased, in normal chicks, after hatching. Other studies have been done to establish a correlation between morphological differentiation (i.e. myogenesis, synaptogenesis, formation of the myoneural junctions) and biochemical differentiation (Butros, 1972; Oh and Johnson, 1972 ;

Kim and Oh, 1974; Vogel and Daniel, 1974). All except Butros strongly implicated Ach in the chick developmental processes. Most of the studies were done using cultured cells and not whole embryos.

Torand-Allerand (1974) also used tissue culture (from the cerebellum of mouse neo-nates) to establish a relationship between the Ach-AchE system and myelinogenesis. His work supports the concept that the neuron may play an active role in the myelinogenetic process. Few other mammalian studies have been done.

Little information is available on the role of cholinesterases in developing amphibians and fish. However, the studies of Anderson and Cohen (1977) and Fluck (1977) indicate that the cholinesterases have a similar function in fish and amphibians as that found in mice.

Fenitrothion

Uses

Fenitrothion is an organophoshorus insecticide which contains at least 95% of the active ingredient O,O-dimethyl-O-(3-methyl-4-nitrophenyl) phosphorothioate. It is used extensively around the world for pest control on rice, wheat, barley, pome, citrus, and stone fruits, grapes, vegetables, coffee, tea, cotton and grasses. Fenitrothion is used for forest protection in Canada and in Japan for the control of locust and grasshoppers. It is also being used to control insects of medical importance. (Sumitomo publication, 1978).

Fenitrothion goes by the following trade names: Sumithion((Sumitomo Chemical Co.), Folithion (Farbenfabriken Bayer-AG), Accothion (American Cyanamid), Agrothion, Novathion, Nuvanol, Metathion (Czechoslovakia), Methylnitrophos (Eastern Europe), and Danathion (Denmark).

Environmental Stability

Hydrolysis of fenitrothion will produce p-nitrocresol and various polar derivatives (Kovacicova et. al., 1973). Fenitrothion does not hydrolyze at an appreciable rate at 20°C in neutral to slightly alkaline purified water (pH $\leq$ 9). Zitko and Cunningham (1974) found fenitrothion to be stable for 45 days in tap water (pH $\leq$ 7). As the water becomes more alkaline, the rate of hydrolysis increases. Loss of fenitrothion

from natural waters also involves volatilization, adsorption, photolysis and microbial degradation. Fenitrothion appears to be readily adsorbed to the surface of soil particles and is then decomposed within 32 to 64 days (Muramoto, 1967: Yule and Duffy, 1972). The major breakdown products of fenitrothion are amino fenitrothion, nitrocresol, dimethyl fenitrothion, dimethyl phosphorothioic acid, fenitrooxon and s-methyl fenitrothion.

Gross Effects on Adult Vertebrates

Stewart (1952) proposed that the accumulation of excess acetyl choline at certain nerve endings throughout the body was responsible for the external effects of poisoning by organophosphorus compounds. Large doses of fenitrothion cause the following major symptoms in experimental mammals: salivation, dyspnoea, twitch, clonic convulsions, bloody tears, exothalmos, urinary incontinence and piloerection (Miyamoto et al., 1963; Namba, 1971; Yamamoto, 1972). Death in the experimental animals was closely connected with acetylcholinesterase inhibition. All of the above symptoms are considered typical of acetylcholine poisoning. Kohli et al. (1974) found that fenitrothion was readily absorbed through the skin of rats and mice, into the blood stream. Although fenitrothion was less toxic than methyl parathion, repeated exposures increased the hazard. In addition to these findings, latent behavioural changes have also been noted. Lehotzky and Ungvary (1976) found that fenitrothion caused alterations in the conditioned avoidance

reflex of rats. The number of conditioned responses diminished, the latency period lengthened and the tendency towards extinction was slowere in trained rats treated with 10 and 100 mg/kg fenitrothion. Latent forms of behavioural abnormalities, in humans, associated with organophosphorus poisoning include the following: impaired vigilance and reduced concentration; slowing of information processing and psychomotor speed; memory deficit; linguistic disturbance; depression; anxiety and irritability (Levin and Rodnitzky, 1976). There are no conclusive studies on the long term effects of fenitrothion on human behaviour.

Forest studies indicate that fenitrothion causes effects, in some birds and amphibians, ranging from mild intoxication to death within 48 hours from the time of spraying (Pearce, 1968; Morgan, 1968; Pearce and Teeple, 1969).

Coppage and Braidech (1976) measured levels of brain AchE to determine the combined poisoning potential of different compounds in freshwater fish. Atlantic salmon treated with 1 ppm fenitrothion for 16 hours showed a decrease in brain AchE levels and a 50% decrease in the number of territorial holdings six days after treatment. Severely affected fish swam stiffly and ceased feeding. Treatment with 0.1 ppm fenitrothion caused a 20% reduction in territorial holdings. It appears that the ereduction in AchE activity could have subsequent deleterious effects on the wild population (Hatfield and Riche, 1970; Symons, 1973).

Effects on Embryos

Little information is available on the effects of fenitrothion on developing vertebrates. The Sumitomo Company (1978) reports that this pesticide is neither teratogenic nor mutagenic in several mammalian species (not specified). No effect on reproduction was observed in their 3-generation study. Additional information on toxicity in fetal rats was added by Kanoh (1978). Neither study showed any teratological effects. No information was available on the effects on development in the remaining vertebrate classes.

No direct teratological effects were seen in insect or decapod studies (McLeese, 1974; Kapoor et. al., 1975; McLeese, 1976; Tanton and Khan, 1978). However, these studies showed a higher mortality among the younger larvae, growth retardation (thought to be caused by poor food utilization) and adverse effects on longevity, fecundity and fertility of the adults reared from treated larvae.

Carbaryl

Uses

Carbaryl is the common name for the active ingredient (1-naphthyl N-methylcarbamate) of the insecticide Sevin, a trademark of Union Carbide Corporation. It is a low hazard, moderately residual contact and stomach poison available as a 50% wettable powder, 80% or 85%

sprayable powder, 5% or 10% granule, 2% to 10% dust, 5% bait and several other formulations (Kuhr and Dorough, 1976). Carbaryl is the most widely used of the carbamate insectidides (Sabharwal and Lockard, 1978). In Canada, carbaryl is used to control leafhopper nymphs on grapes, strawberry weevils, aphids and leafhoppers on many vegetable crops, the European corn borer in sweet corn and peppers and leaf-miners on white birch (Morley, 1978). Elsewhere, it is used to control insect infestations in over one hundred different food, fiber and forage crops. In addition, carbaryl is applied to forests, range land, livestock, pets, poultry and buildings. Carbaryl is thought to present a minimum hazard to many non-target organisms such as farm animals, bird, fish and pets with one of the main exceptions being honey bees (Kuhr and Dorough, 1976).

Environmental Stability and Mode of Action

Carbaryl has an average half-life of 3 to 4 days on foliage, 7 to 9 days in soil and 1 to 5 days in water. Like most carbamates, carbaryl is rapidly degraded under alkaline conditions (Kuhr and Dorough, 1976).

Like the organophosphorus insecticides, carbamates inhibit acetylcholinesterase by acting as analogs to the normal substrate. This inhibitory effect is thought to be caused by the relatively long life of the carbamylated enzyme compared with that of the acetylated enzyme. of the physiological reaction. The reaction of acetylcholinesterase(EH)

with an inhibitor (AB), where A is the carbamylating group and B is The leaving group, may be written as follows (Corbett, 1974):

More information on the kinetics involved in the reaction between carbamates and acetylcholinesterase has been provided by Watts and Wilkinson (1977) and by Abdel-Aal (1977).

Studies on rats have shown that carbaryl marginally inhibits monocyte esterase activity and has a non-specific hydrophobic bonding to macromolecules in the blood (Lee and Waters, 1977; Skalsky and Guthrie, 1977). It is possible that xenobiotics attached to lipoproteins could enter a cell by pinocytosis and then dissociate from the carrier molecule. A further study by Pipy et. al. (1978) indicates a definite relationship between the phagocytic inhibition of the reticuloendothelial system and the anticholinesterase activity of carbaryl. In addition, carbaryl has been shown to bind to heptatic microsomes and is thought to interfere with NAD biosyntheses (Moscioni et. al., 1977; Miller et. a. al. 1979).

Gross Effects on Adult Vertebrates

The external symptoms of carbaryl poisoning in vertebrates are the same as those following poisoning with other anti-acetylcholinesterases.

These are salivation, dyspnoea, twitch, convulsions, exothalmos, paralysis, and death. In addition, carbaryl tends to reduce the water drive (Sideroff and Santolucito, 1972). In quail, carbaryl caused a reduction in fecundity but not fertility. There was also a reversal of the female-male behaviour patterns with females displaying more agonistic behaviour following carbaryl injestion(DeRosa, 1976). Bursian and Edens (1977) found that carbaryl fed to Japanese quail over a period of 14 weeks resulted in decreased body weight, and increased relative brain, liver and kidney weights in one or both sexes at levels of 900 ppm or higher. However, no significant differences were found in the progeny. A study on mice has shown a correlation between cholinesterase inhibition and temperature decreases in animals treated with carbaryl. This loss of thermoregulation in carbaryl treated mice could be reduced by atropine at both high and low temperatures (Ahdaya et. al., 1976). Rats and gerbils fed excessive amounts of carbaryl showed decreases in litter size, number of liveborn progeny and the numbers weaned (Collins et. al., 1971).

Effect on Vertebrate Embryos

Congenital malformations in duck and chicken embryos treated with carbaryl have been reported by Marliac (1964), Marliac et. al. (1965), and Ghadire and Greenwood (1966). Further study by Lillie (1973) showed that carbaryl suppressed growth in leghorn chicks, irrespective of the maternal diet.

Mammalian studies show a wide variety of effects. Carbaryl appeared

to be non-teratogenic in pigs, rabbits, or hamsters but was teratogenic in dogs and guinea pigs (Robens et. al., 1969; Smalley et. al., 1969; Smalley et. al., 1968).

In fish, studies have been done using the Atlantic silverside, killifish and medaka. Circulatory problems arose in all three, but skeletal malformations were rare. In addition, several of the Atlantic silverside embryos showed a partial convergence of the eye cups, with the optic cups being directed ventrally rather than laterally. True cyclopia was rare. (Weis and Weis, 1974; Weis and Weis, 1976; Solomon and Weis, 1979).

There appears to be no information available on the effects of carbaryl on either the developing reptiles or amphibians.

Lindane

Uses and Chemical Properties

Lindane is the common name approved by the International Standards Organization for formulations containing at least 99% of the γ-isomer of 1,2,3,4,5,6-hexachlorocyclohexane (BHC). It is also known as Aficide, Agrisol, G-20, Agrocide, Aparasin, Aphtiria, Ben-Hex, Bexol, Detox 25, ENT 7,796, gamma BHC, gammahexa, gamma-hexane, gamma lindane, Gexane, Hexachloran, Hexachlorane, Jacutin, Kwell, Lorexane, Streunes, and Tri-6. (IARC, 1974).

Lindane is a colourless crystalline solid having a melting point of 112.5°C, a molecular weight of 290.9. It is very soluble in chloroform, ethanol, acetone, ether and benzene, and has a solubility of 10 mg/l in water. The γ-isomer of BHC can be dechlorinated by alkalis to form 1,2,4-trichlorohexane as the main breakdown product (IARC, 1974). Like other organochlorines, lindane is very stable in the environment (Oloffs et. al., 1972). Chadwick and Freal (1972) found the following breakdown products in the urine of rats fed 400 ppm γ-BHC; 3,4 dichlorophenol, 2,4,6-trichlorophenol, 2,3,4,5, and 2,3,4,6-tetrachlorophenol and 2,3,4,5,6 pentachloro-2-cyclohexen-1-ol.

Lindane is presently being used in Canada in the following ways: pre-treatment of vegetable and wheat seeds, control of the flea beetle, and as a prevention of tick paralysis in cattle (Morley, 1978).

Effect on Vertebrates

Lindane poisoning is characterized by ataxia, convulsions and eventual paralysis (Beaument et. al., 1971). Joy (1976) found that lindane is directly toxic to the mammalian central nervous system and does not need to be metabolized to an active form. It has a slightly greater ihhibitory effect on the Na - K ATPases than on the Mg - ATPases, an effect opposite to that of DDT, chlordane, aldrin and dieldrin. This inhibition of the ATPases could account, in part, for the observed toxic effects because of interference with cation transport (Kaah, 1969).

In addition, lindane has been shown to cause an increase in levels of ammonia and glutamine in the brains of rats and cockerels. In both cases, there was a direct correlation between the intensity of the c convulsions and the increased levels of brain ammonia (St. Omer, 1971). Further study has also shown that lindane caused destruction of rat brain cell processes which included vacuolization and swelling of the cell body (Offner et. al.,1973).

Few studies are available on the effects of lindane on other vertebrate classes. Schwarz and Kaschowitz (1968) showed that lindane had a slowing effect on the process of irritation in the frog retina. However, there is a dirth of information on the remaining areas.

Xenopus - an experimental tool

Xenopus (figure 2) has been used for laboratory research for over thirty-five years (Deuchar, 1975, page v). Coming from a semi-tropical region, it has a more adaptable breeding season than frogs of temperate zones and responds well to injections of gonadotropic hormones at most times of the year. The females are able to produce several hundred eggs during a single mating. Being totally aquatic, Xenopus can be maintained in a simple aquarium. The frogs have a large temperature range, having been successfully reared to the end of metamorphosis at both 15°C and 30°C without the water being aerated. This could,,in part, be accounted for because the lungs develop very early in la[illegible]

in larval life (stage 32)[1] and thus the larvae are not dependent on their gills for respiration, as are fish. Unlike many freshwater organisms, Xenopus can withstand salinities ranging from freshwater to forty percent seawater (Munsey, 1972).

The stages in Xenopus development have been studied in detail (Nieukoop and Faber, 1956). Xenopus have been used quite widely in both developmental and neurological studies. This large amount of information available on both the normal physiology and development of Xenopus makes it a good choice for toxicological studies. Normal deviations can be observed as such and the actual effects of the toxin noted.

Anuran Pesticide Studies

Although frogs have been used extensively in biological research, there have been relatively few studies done on the effects of insecticides on their behaviour, physiology or biochemistry. Such aspects should be considered to determine the desirability of a particular pesticide, especially in areas where anurans play an important agricultural role.

The majority of the studies that have been done on frogs have concentrated on the organochlorines, especially DDT. As early as 1952, it was reported that DDT caused mortality among both frogs and tadpoles (CWS - report, 1952). This group of insecticides has been found to

[1] a paired lung anlage is clearly visible by this stage.

cause egg spoilage in Rana temporaria (Fryer, 1973) and hyperemia of the internal organs, cytolysis, abnormal pigmentation and behavioural changes in tadpoles of the same species (Jordan et. al., 1977). Younger tadpoles appeared to be more resistant to poisoning than older ones, suggesting a definite stage sensitivity. Both the common frog and toad tadpoles (genera not specified) appeared to be the most susceptible either just before, or just after the hind limbs developed (Cooke, 1972; Jordan et. al., 1977). DDT simultaneously increased the level of activity in tadpoles, and caused a lack of response to external stimuli, leading to a higher predation rate by smooth newts (Cooke, 1971). Licht (1976) found that DDT was stored in the tissues of the tadpoles, then released during metamorphosis, causing the same behavioural abnormalities as those observed by Cooke. DDT had a negative temperature coefficient of activity in the sense organs of Xenopus laevis (Bercken, 1973). This could be a direct result of the changes in permeability properties that DDT causes in mylinated nerve fibers of both Xenopus and Rana (Bercken et. al., 1973a; Arhem et. al., 1974). Both DDT and dieldrin or their metabolites affect the sodium channels of the membranes of myelinated fibers in the nodes of Ranvier (Bercken, 1969; Akkermans et. al., 1975). This causes an increase in the duration of the action potential which may be responsible for some of the effects of DDT and dieldrin on the mechanical response of skeletal muscles after treatment (Bercken and Akkermans, 1971).

When the organophosphates and carbamates began to replace the organochlorines, the concern about their effect on non-target organisms

increased. Kaplan and Glazenski (1965) found that several organophosphates caused a reduction of lymphocytes and neutrophiles in Rana pipiens. They also caused motor paralysis resulting in a loss of posture and righting reflexes. Johnson and Price (1976) found that organophosphates not only reduced activity but also lowered the thermal tolerance of Bufo boreas when concentrations fifty percent less than those used in the field were applied. However, Pearce and Teeple (1969) stated that Sumithion (fenitrothion) had no detrimental effects on adult toads and frogs (genera not specified).

Of the carbamates studied, all caused some effect in anurans. Carbaryl was found to inhibit acetylcholinesterase in tadpole brain tissuee at concentrations as low as 1×10^{-6} moles (Perevoschenko, 1975). Rick and Price (1976) reported a high mortality among tadpoles after their test pond was sprayed with either ramatacil or zectran. In addition, Prahlad and Anderson (1975) reported that diquat and nabam (1 - 2 ppm) caused stunting, wavy notochords, disrupted myomeres and myocommata in Xenopus laevis embryos.

In addition, many pesticide studies have been done by both the Canadian and American goverments. Sampling was done immediately after an area was sprayed with an insecticide. In these reports, dead frogs or toads were collected and classified according to species. The tissues were sampled to determine the pesticide level in the animal. These studies did not deal with sub-lethal effects or the effects of a prolonged exposure of the organism to the insecticide oreits metabolites in the environment.

MATERIALS AND METHODS

Care and Maintenance of Adults

Mature Xenopus were obtained from C.W. Fletcher (Hempstead, Maryland), the Amphibian Facility, University of Michigan (Ann Arbor, Michigan) and Nasco (Guelph, Ontario). Adults from the latter two sources had been reared in the laboratiories, whereas those from C. W. Fletcher had been imported from South Africa. All were maintained in accordance with the principles of "Care of Experimental Animals - A Guide for Canada" (published by the Canadian Council on Animal Care). Embryos were obtained by injecting the adults with human chorionic gonadotropin (Gurdon, 1967)). The adults were mated no more than once every two months.

Source of Supplies

Lindane (25% gamma hexachlorecyclohexane [BHC], FMC of Canada, Ltds, Agriculture Chemical Division, Burlington, Ontario) and sevin (a liquid suspension of 4.2 lb. carbaryl per gallon, Chapman Chemicals Ltd., Stoney Creek, Ontario) were obtained from a local feed store. Fenitrothion (97.1% pure sumithion, technical grade, Sumitomo Chemical Company, Osaka, Japan) was obtained from Dr. Pearl Weinberger. Technical grade carbaryl was obtained from the Deparment of Agriculture, Ottawa, Ontario.

Treatment of Juveniles

Newly hatched juveniles were treated for 24 hours with concentrations of one of the pesticides ranging from 0.01 to 100 milligrams per liter. Animals were kept in the dark at either 18°C or 25°C. All were maintained at a pH of 6.8 - 7.2. After treatment, they were washed for 10 to 15 minutes in continuously flowing dechlorinated tapwater. Afterwards, they were examined for physical and behavioural abnormalities and placed in clean dechlorinated tap water.

Treatment of Embryos

Embryos[1] were sorted into groups of approximately the same age (staging according to Nieukoop and Faber, 1956) and treated the same as the juveniles, but at different stages. In addition, some embryos were treated for 12 and 36 hours with 10 mg/l fenitrothion and for 36 hours with 10 mg/l carbaryl.

Care After Treatment

Both treated and control groups were raised at either 18°C or at 25°C depending on their treatment. The rearing bowls contained three liters each of 25% modified Holtfreter's solution. The modified solution contained 3.46 g NaCl, 200 mg $MgSO_4$, 200mg $NaHCO_3$, 100 mg $CaCl_2$, and

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[1] One hundred embryos have an approximate wet weight of 400 milligrams.

50 mg KCl per liter of dechlorinated tap water. They were fed a commercially prepared Xenopus food. (Nasco). Numbers differed from bowl to bowl due to differential die-off. At the onset of metamorphosis, they were divided into smaller groups, if necessary, and fully metamorphosed individuals were removed, examined and abnormalities, if any, were noted.

Description of Embryos

External

Embryos were raised to stage 37 (hatching) and then examined. They were divided into six types according to the degree of visible abnormality. These were as follows:

1 - treated individuals that looked identical to the controls

2 - small localized edema(s) and/or the tail was bent ventrally. All other features appeared normal.

3 - edematous regions appeared larger, yolk was sometimes reduced, backs and/or tails were slightly curved or bent ventrally. All other features appeared normal.

4 - the entire body appeared mildly edematous, the backs had a marked curvature and outgrouths of tissue werepresent on the ventral surface. The head was distinct, of normal size, but often irregular in shape.

5 - the main body region was cylindrical and not flattened laterally as in the controls. Dorsal and ventral regions

were difficult to distinguish. The head was either reduced and irregular or absent. There were numerous irregular tissue outgrowths.

6 - animals that died before stage 37.

From each of these classes, a group was randomly selected and the following external parameters were measured (in mm): total length, head-cloaca length, body width (dorsal-ventral), head size, edema type, and pigmentation type. The lengths were used to determine ratios for the overall body proportions. This in turn would indicate whether a given embryos was small for it's stage or if it did not have a normal body shape.[1] The head size ratio dorsal-ventral:anterior-posterior was consistently near 1:1 in the controls. Noticeable variations in this ratio would indicate gross structural changes in the head.

Edemas were classified into five types as follows: 1-no edema, 2-one or two small localized edemas, 3-more than two localized edemas, 4-mild general edema, 5-gross general edema. This was used to indicate possible circulatory failure and/or illness.

Pigmentation was divided into four types as follows: 1-almost solid black on the dorsal surface, 2-predominantly black but with a few light patches, 3-very little pigmentation on the dorsal surface,

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[1] normal body proportions were, head-cloaca:overall length 1:2
head-cloaca:body width 2:1

4-no pigmentation except in the eyes. It was hoped that the amount of pigmentation could be used to give a rapid indication of hormonal changes such as alterations in MSH levels.

Internal

After sectioning and staining, the embryos were examined for the following: Brain shape, eye proportions, otic vesicle proportions, the number of cranial nerves present, shape of the spinal cord, shape of the notochord, heart size, pronephros size, internal edema type and the degree of lysing of the yolk cells. The first six parameters were used as indicators of alterations in the nervous system. The last four yielded information on the circulatory system, water regulation and the availability of food to the embryo.

The spinal cord and notochord were divided into five types as follows: 1-straight with no occulsions, 2-straight with small occulsions, 3-wavy with no occulsions, 4-wavy with some occulsions, 5-totally occulded.

The internal edemas were classified into six types as follows: 1-absent, 2-one localized edema, 3-two or more small, clear edematous regions, 4-large clear edematous regions, 5-mild general edema containing a few cells, 6-severe general edema.

The yolk was divided into three types according to the degree of

abnormal lysing present: 1-normal yolk, 2-small scattered regions of lysing, 3-large regions of abnormal lysing.

The heart was classified into five types: 1-normal, 2-normal but reduced in size, 3-regions of the heart not clearly differentiated, 4-a mass of tissue contained within the pericardium, 5-absent.

Description of Juveniles

Behaviour

During the test period the juveniles were examined every three hours and changes in their swimming patterns were noted. These were divided into six types as follows: 0-dead, 1-moribund but with the heart still beating, 2-individuals are not swimming but are wiggling at the bottom of the bowl, 3-individuals do not respond to the glass being tapped and swim in an uncoordinated pattern for four to five millimeters, 4-individuals are capable of swimming across the bowl, in a very jerky pattern, and they swim away from any point where the glass is being tapped, 5-normal behaviour with no signs of intoxication.

External Changes

Changes in pigmentation were classified according to the types listed in the embryo section. Other changes, such as bent tails, were also noted.

Histology and Staining

Embryos were fixed with Smith's solution and preserved in 4% formalin. Juveniles were preserved in Bouin's fixative for histological purposes, or in 10% formalin for cartilage and bone staining (Wassersug, 1976). All histological sections were stained with hematoxylin and phenol orange.

Live specimens were anesthetized with tricaine methane sulfonate (MS-222, Sigma Chemical Co., St. Louis, Missouri) for photography.

RESULTS

Fenitrothion

Embryo Treatment

Embryos treated with fenitrothion for 24 hours after gastrulation were more sensitive to low concentrations at higher incubation temperatures (Fig. 3). However, the embryos also develop more rapidly at the higher temperatures, and are consequently exposed to the fenitrothion over a wider range of developmental stages. When embryos raised at 25°C and 30°C[1] were exposed for the same developmental period (stages 14-25) as those raised at 18°C, the sensitivity at different concentrations of fenitrothion was found to be essentially the same.

At low concentrations of fenitrothion, abnormalities, if any, were minor (types 1 and 2). However, as the concentration was increased, a point was reached where most of the embryos still survived to hatching, but a majority were moderately to severly abnormal (Fig. 4). At still higher concentrations, most did not survive.

When embryos were pulsed for six hours (3 to 5 stages), they were found to be most sensitive around gastrulation (Fig. 5). Overall

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[1]Embryos were successfully reared at 30°C from the early stages of development through to the end of metamorphosis.

survival was lowest in the stage 11 to 15 group but more severe abnormalities were seen in the stage 8 to 11 group. Most of the severely abnormal embryos, obtained after treatment during the early embryonic stages, did not survive long after hatching (Fig. 6).

An examination of the external features of the different types (Fig. 8) showed a minimum decrease of 20% in total length compared to the controls (Fig. 7). The body width was similar to that of the controls in all groups (table 1). Abnormal head shapes were observed only in types 4 and 5. Severity of the edematous regions generally increased with the severity of the abnormality.

The internal structures (tables 2a and 2b)[1] of embryos that appeared normal (type 1) were the normal shape for the stage but smaller than the controls. For the other types, the degree of size variation from the control varied with the structure being examined. The brain was reduced in types 2 to 4 and absent in many of type 5. The shape of the brain was normal in types 1 to 3 and grossly distorted in type 4. The number of cranial nerves remained constant except in types 4 and 5. The otic vesicle was regularly shaped when present. Eye shape was relatively constant in types 1 to 3. Both the notochord and the

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[1] The data in both tables was obtained from animals treated before gastrulation. However, similar trends appeared in individuals examined that had been treated after gastrulation. Type descriptions remained constant regardless of the stage(s) during treatment.

spinal cord were wavy in many embryos in types 3 to 5 and the spinal cord was totally occluded in some regions of severely affected embryos. Heart size was reduced in all types and the overall heart structure was altered in types 2 to 5.[1] In contrast, the size of the prohephros increased in types 2 to 5 but fluid accumulation was a major problem in types 3 to 5.

After examination, the juveniles were raised to metamorphosis. The time between hatching and completion of metamorphosis ranged from one to three months depending on the temperature. There were individual differences in developmental rates but no overall differences between the rates of the control and treated groups. Feeding began during the first week post-hatching. Those individuals incapable of feeding died during this period. Individuals that survived usually completed metamorphosis. There was a great diversity of individual size in the newly metamorphosed froglets. Those raised at 18°C were generally larger than those raised at 30°C. There was no difference between the control and treated groups at a given temperature. The treated groups did not appear more susceptible to bacterial and fungal infections than the control group.

On two occassions, some of the juveniles that had been treated near hatching failed to develop limbs or parts of limbs (Fig. 9). As metamorphosis progressed, all of these individuals bloated and died.

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[1] See Materials and Methods section for descriptions of heart types.

The digestive tract contained no food but the stomachs were fluid filled and distended. This phenomena did not appear in the control groups at these times nor did it appear in other treatment groups. However, it has appeared once in an untreated group in our lab.

Juvenile Treatment

Juveniles were treated at 25°C for 24 hours (stage 43 to 47) at 10, 1.0 and 0.1 mg/l. After three hours, the behaviour of all three groups was affected to some extent (Fig. 10). After 18 hours, all the juveniles in the 10 mg/l solution had died. In the other two groups, the behavioural pattern stabilized between nine and fifteen hours and normal swimming patterns returned within a few hours after they were removed from the fenitrothion solution. At the end of the treatment period, those treated with 1.0 mg/l appeared less pigmented than those treated with 0.1 mg/l. However, normal pigmentation returned within 24 hours. Both groups were examined again at stage 53. Ninty-four percent of those treated at 0.1 mg/l and 92% of those treated with 1.0 mg/l survived and developed normally to this point. Some individuals developed severe edema and died within a few days. Those remaining survived and completed metamorphosis normally.

All the juveniles treated with either 1.0 mg/l or 0.1 mg/l for 36 and 48 hours survived the treatment period and appeared to return to normal after they were removed from the fenitrothion solution. However, all died within a two week period.

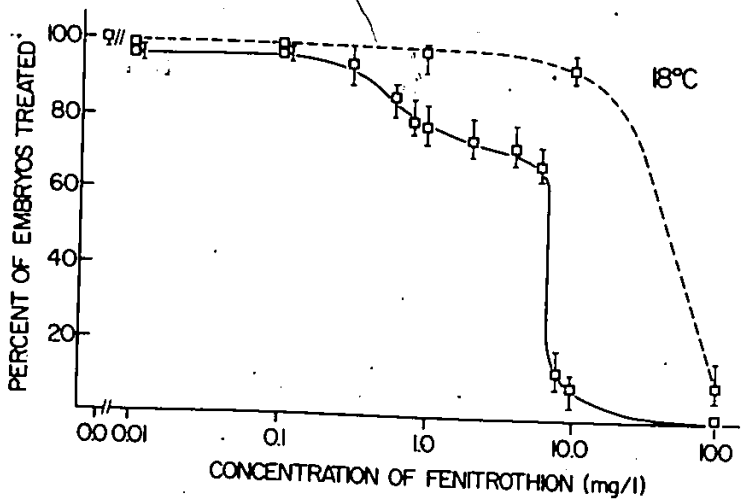
Figure 3:

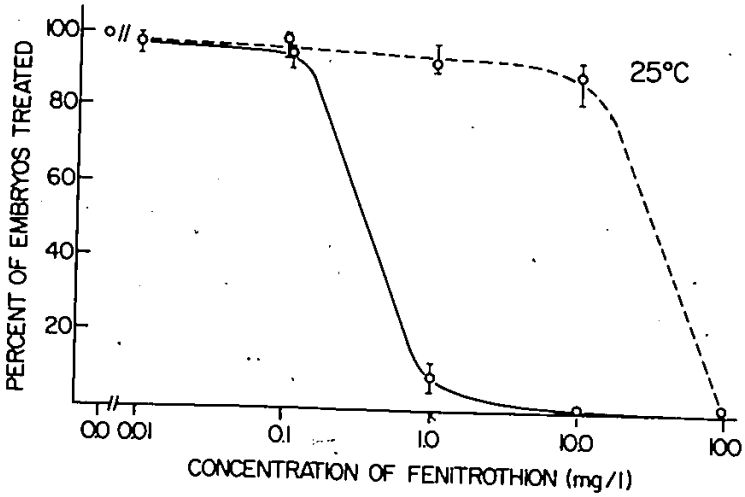
The effect of different concentrations of fenitrothion on Xenopus embryos treated for 24 hours post-gastrulation (Stage 14). At 18°C (□) the embryos had reached stage 25 by the end of the treatment period. At 25°C (●) and 30°C (▲) they had reached stages 30 and 36 respectively. The number of normal embryos (——) and the number of surviving (- - - -) were assessed at hatching (stage 37) for all the groups.

EC_{50} at 18°C was 7.1 mg/l
25°C was 0.33 mg/l
30°C was 0.23 mg/l

(1 mg/l = 3.6μM fenitrothion)

I are 95% confidence intervals.


18°C
PERCENT OF EMBRYOS TREATED
100
80
60
40
20
0.0 0.01
0.1
1.0
10.0
100
CONCENTRATION OF FENITROTHION (mg/l)


25°C
PERCENT OF EMBRYOS TREATED
100
80
60
40
20
0.0 0.01
0.1
1.0
10.0
100
CONCENTRATION OF FENITROTHION (mg/l)

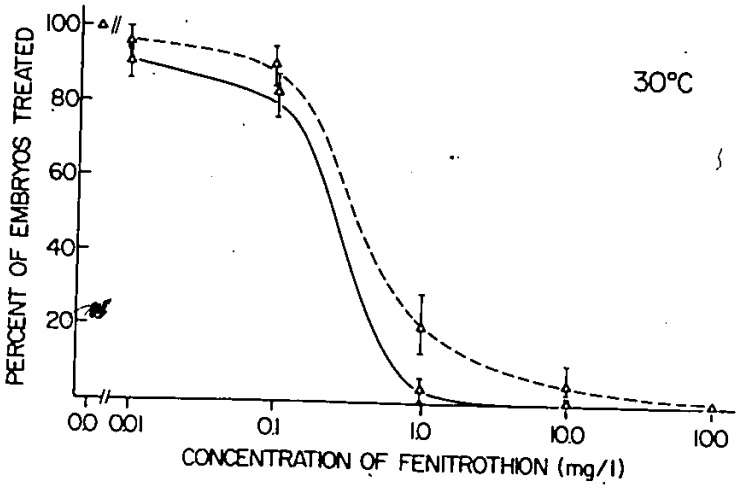
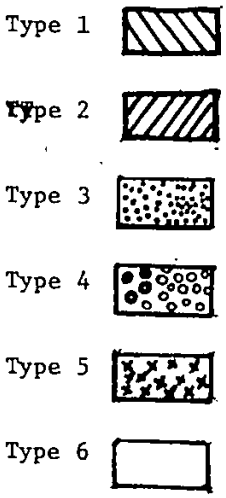

30°C
PERCENT OF EMBRYOS TREATED
100
80
60
40
20
0.0 0.01
0.1
1.0
10.0
100
CONCENTRATION OF FENITROTHION (mg/l)

Figure 4:

The effect of various concentrations of fenitrothion on Xenopus embryos treated from stage 14 to 25 at 18°C. Similar results were obtained when embryos were treated over the same range at 25°C and 30°C.



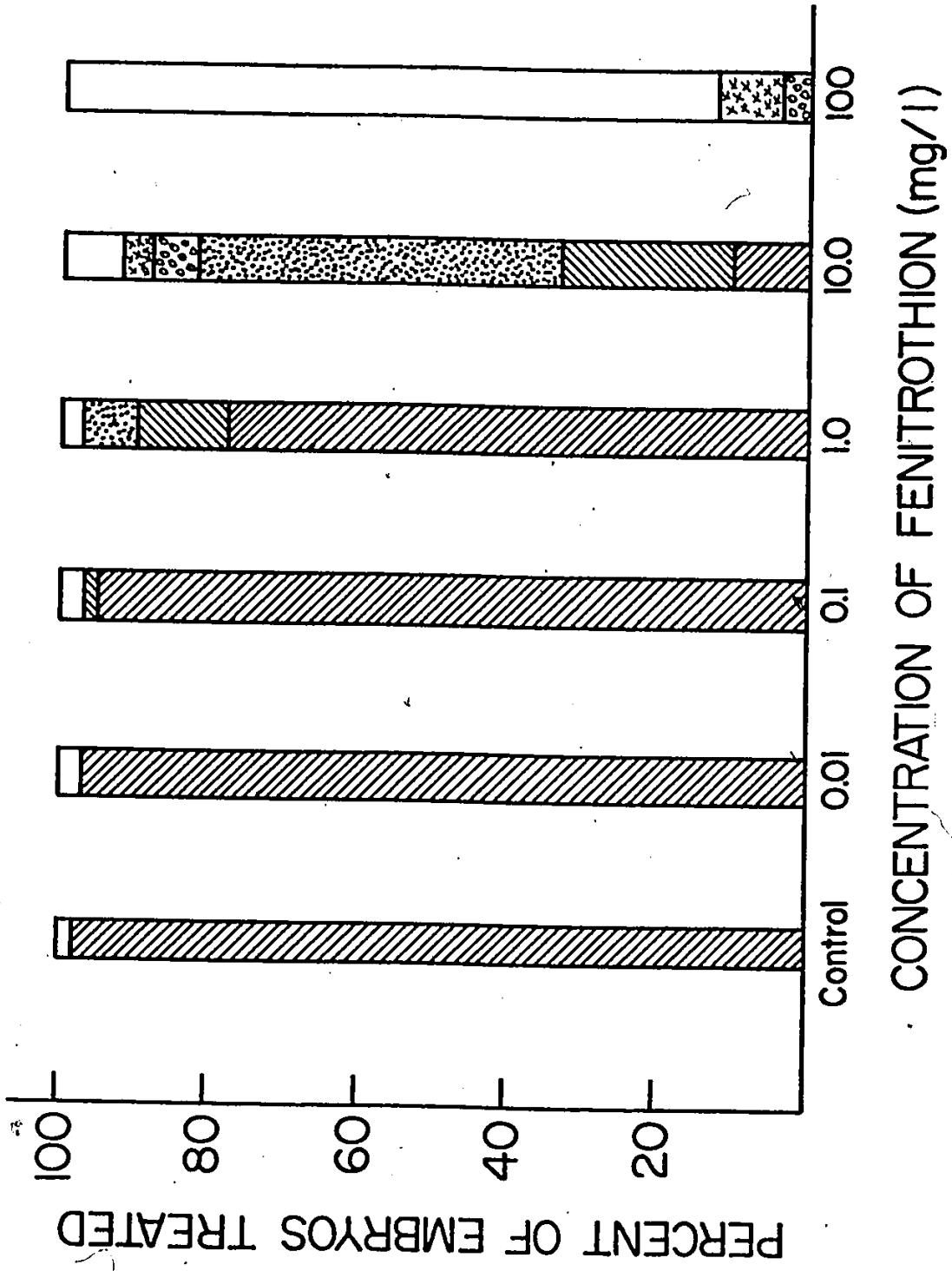
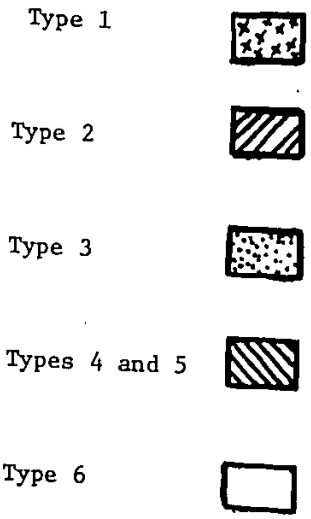
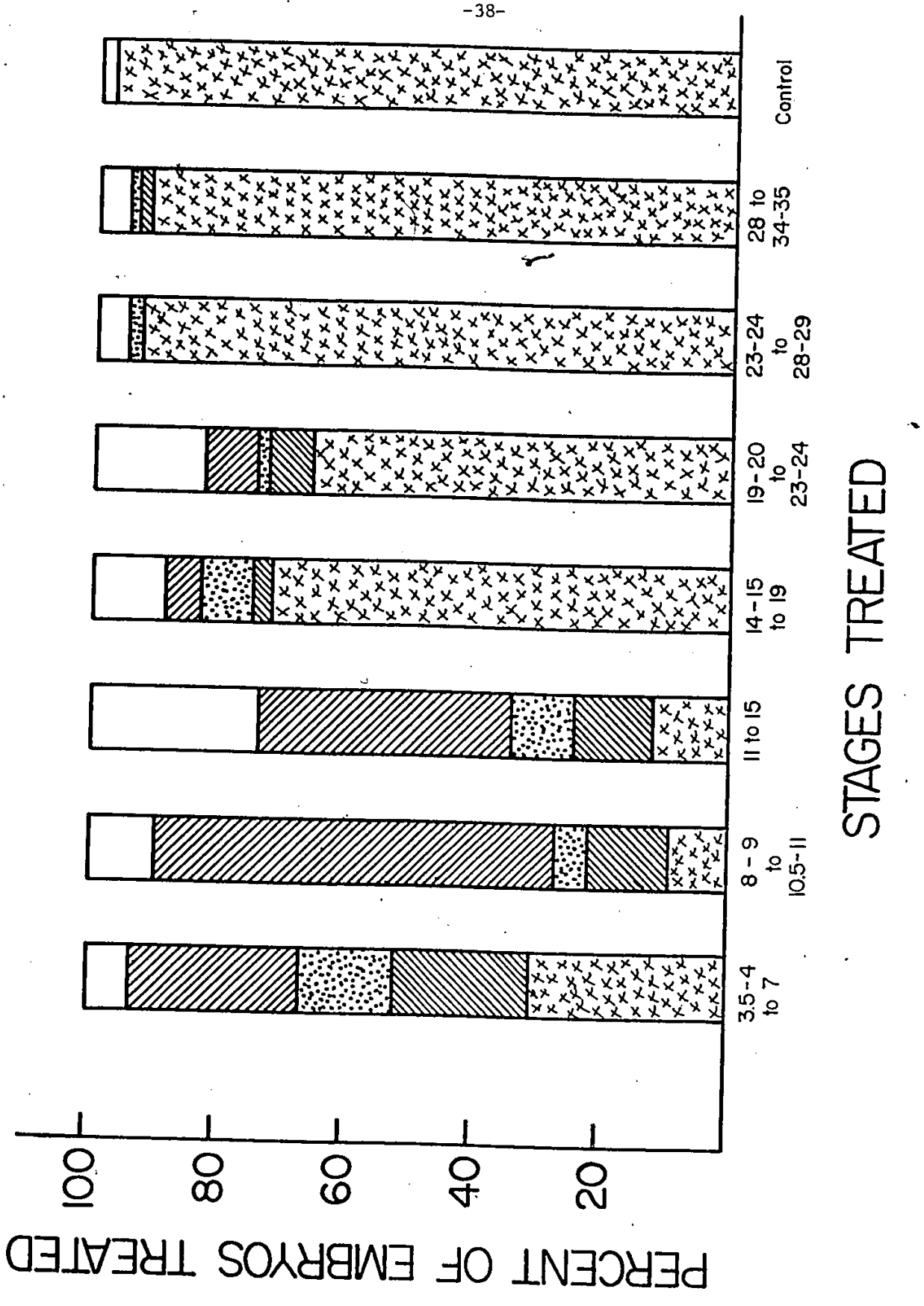

PERCENT OF EMBRYOS TREATED
100
80
60
40
20
Control
0.01
0.1
1.0
10.0
100
CONCENTRATION OF FENITROTHION (mg/l)

Figure 5:

Occurrance of different types of abnormalities in embryos treated with 10 mg/l fenitrothion at 25°C. (Data was pooled from three different experiments and examined at hatching [stage 37-38]. Types four and five were combined.)




PERCENT OF EMBRYOS TREATED
100
80
60
40
20
3.5-4 to 7
8-9 to 10.5-11
11 to 15
14-15 to 19
19-20 to 23-24
23-24 to 28-29
28 to 34-35
Control
STAGES TREATED

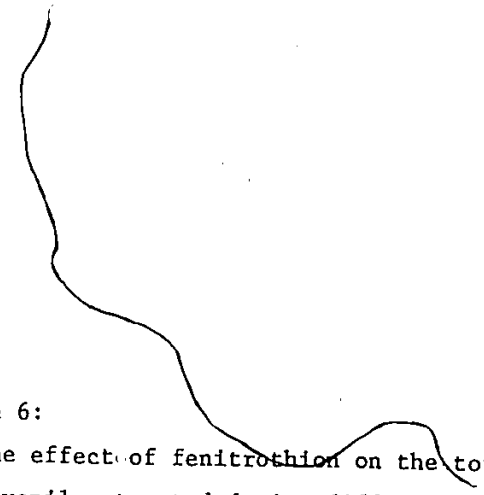


Figure 6:

The effect of fenitrothion on the total survival of juveniles treated during different stages of embryonic development. All embryos were treated in a 10 mg/l solution. Survival (as a percentage of the original number of embryos treated)) was evaluated one week after hatching.

I represents 95% confidence intervals

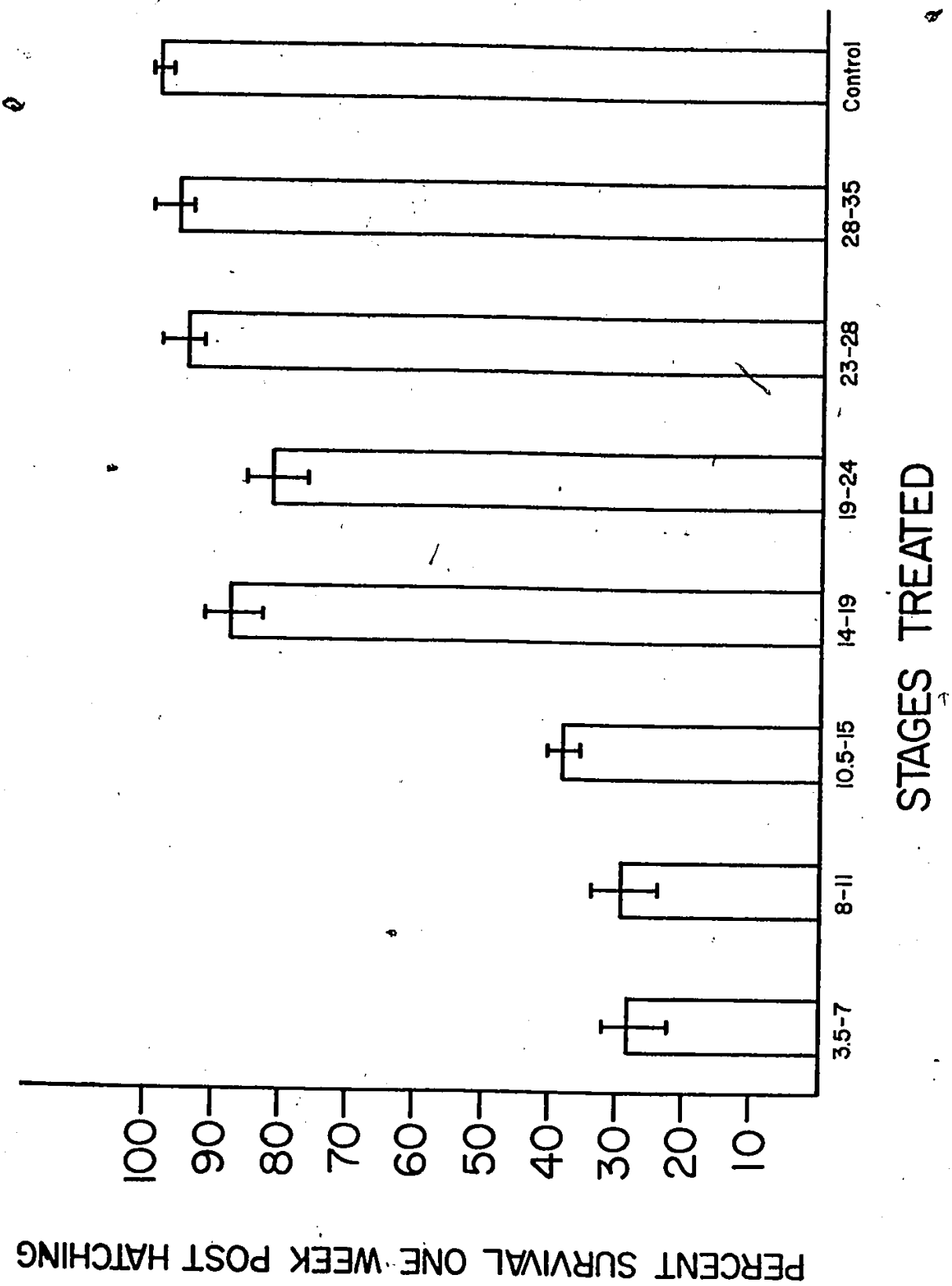
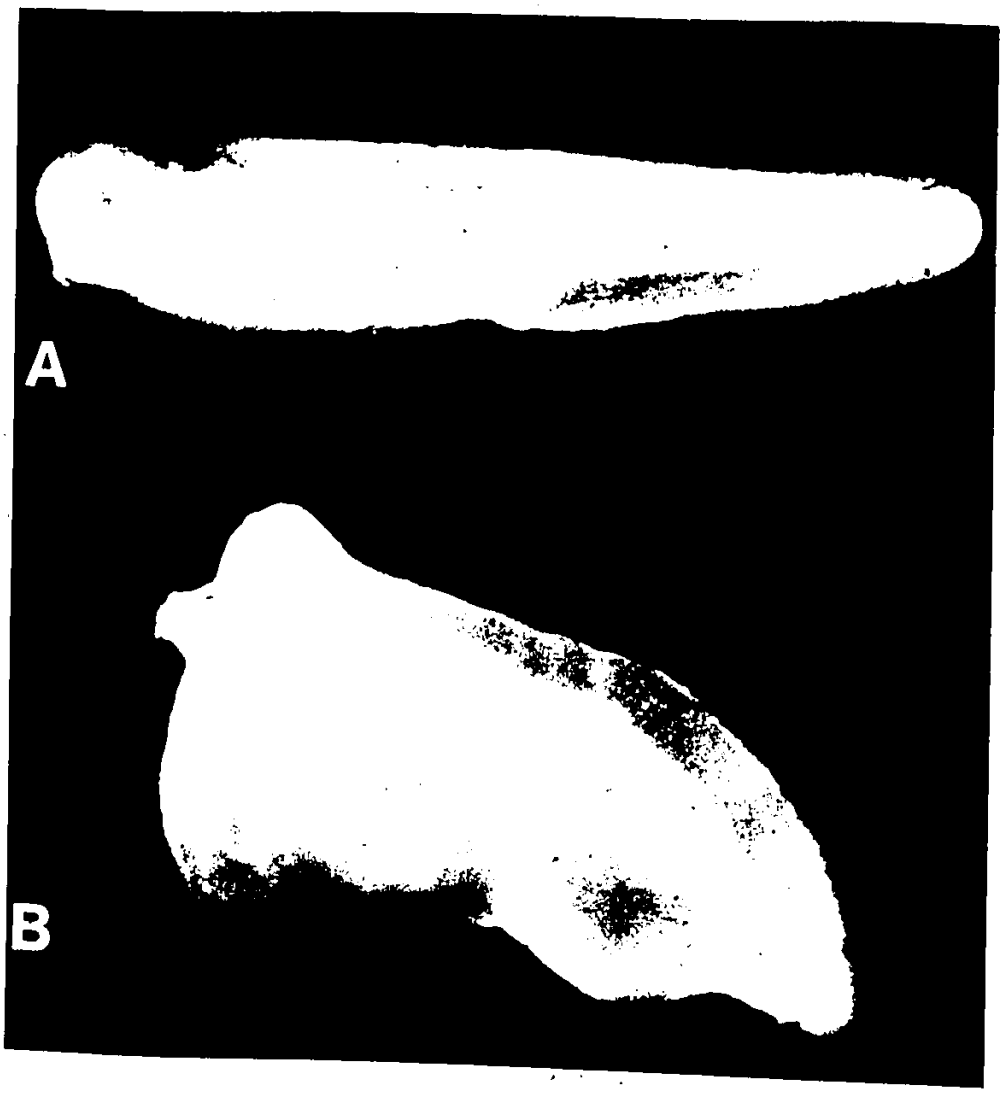

PERCENT SURVIVAL ONE WEEK POST HATCHING
100
90
80
70
60
50
40
30
20
10
3.5-7
8-11
10.5-15
14-19
19-24
23-28
28-35
Control
STAGES TREATED

Figure 7:

Embryos from the control group at stage 39. A represents a normal control. B represents a natural abnormality which occurred infrequently. When present, they formed only 3% of the total number of hatched juveniles. However, the "normal" siblings in these spawnings were not as hardy as juveniles from spawnings where these did not occur.


A
B

Key to photographs of hatching (stage 37-38) embryos.

a - bent tail
b - localized edemas
c - curved spine
d - reduced yolk
e - ventral tissue outgrowths
f - normal head
g - reduced abnormal head
h - head absent

1 OF

Outline of Different T

External Features

Type 1 - app

Type 2 - has
iis

Type 3 - lar
tai

Type 4 - gen
irr
of

Type 5 - gro
or

Type 6 - dea

Edemas were classified :
two small localized eder
edema, 5-gross general e

Internal Features

The spinal co
types: 1-strai
small occulsic
with occulsior

The internal e
as follows: 1-
or more small
5-mild general

The yolk was d
the degree of
scattered regi

The heart was
2-normal but r
not clearly di
diac region, 5

ıt Types

appears identical to the normal controls

has small localized edemas and/or the tail is bent ventrally

larger edematous regions: backs and/or tails curved or bent ventrally

general mild edema, back curved, head irregular in shape, ventral outgrowths of tissue

grossly distorted bodies, heads irregular or absent, irregular tissue outgrouths.

dead

ed into five types as follows: 1-no edema, 2-one or edemas, 3-more than two localized edemas, 4-general al edemas

cord and notochord were divided into five traight with no occulsions, 2-straight with sions, 3-wavy with no occulsions, 4-wavy ions, 5-totally occulded.

l edemas were classified into six types 1-absent, 2-one localized edema, 3-two ll edemas, 4-large edematous regions, ral edema, 6-severe general edema.

s divided into three types according to of lysing present: 1-normal yolk, 2-small egions of lysing, 3-large regions of lysing.

as classified into five types: 1-normal, t reduced in sixe, 3-regions of the heart differentiated, 4-mass of tissue in car-, 5-absent.

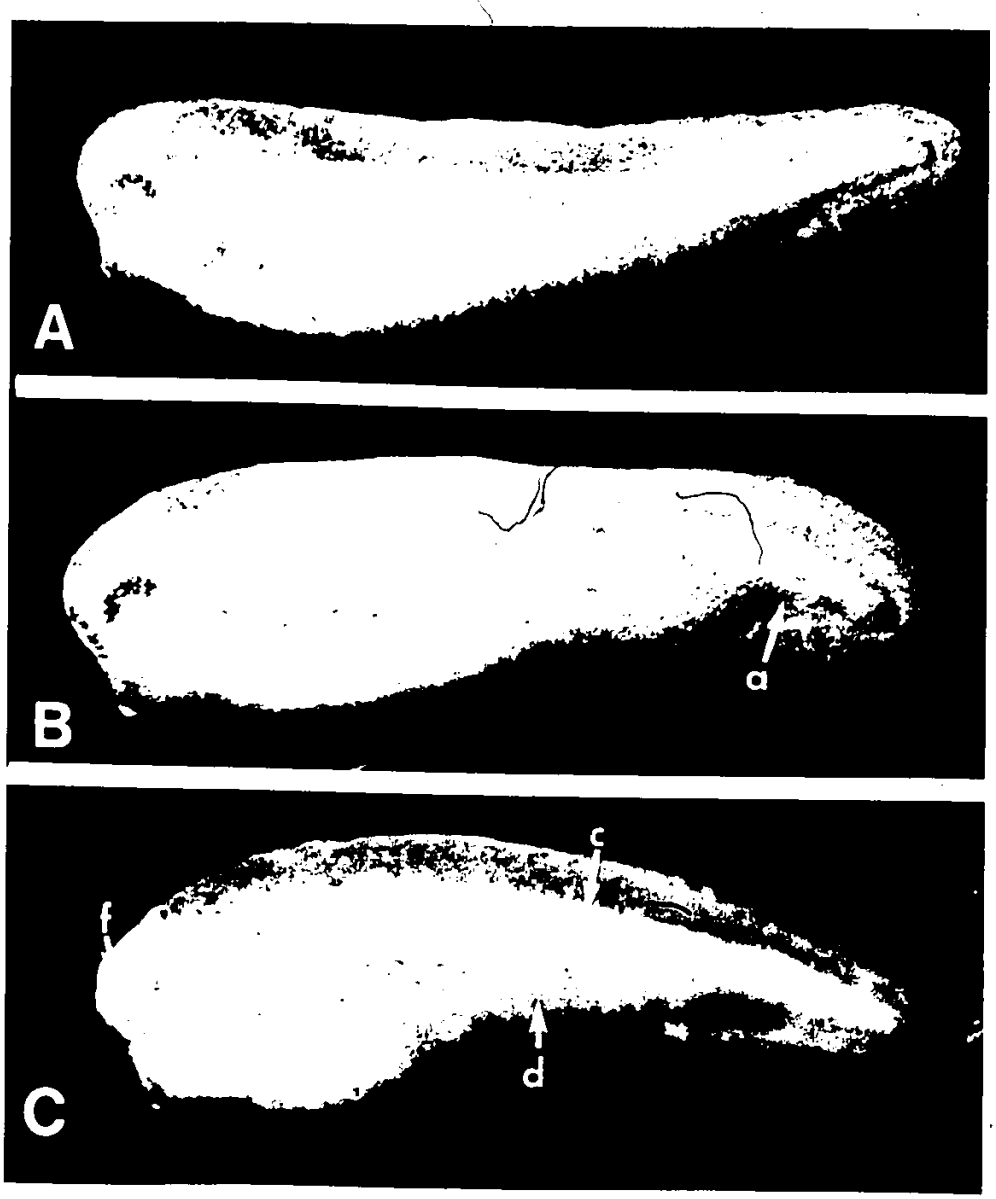
Figure 8:

Embryos from groups expos
sents type 1, B type 2,
and F type 5. Type descr
less of the stage that th

2 OF

Figure 8:

Embryos from groups exposed to fenitrothion. A represents type 1, B type 2, C type 3, D type 4, and E and F type 5. Type descriptions were constant regardless of the stage that the embryos were treated at.


A
B
a
C
c
f
d

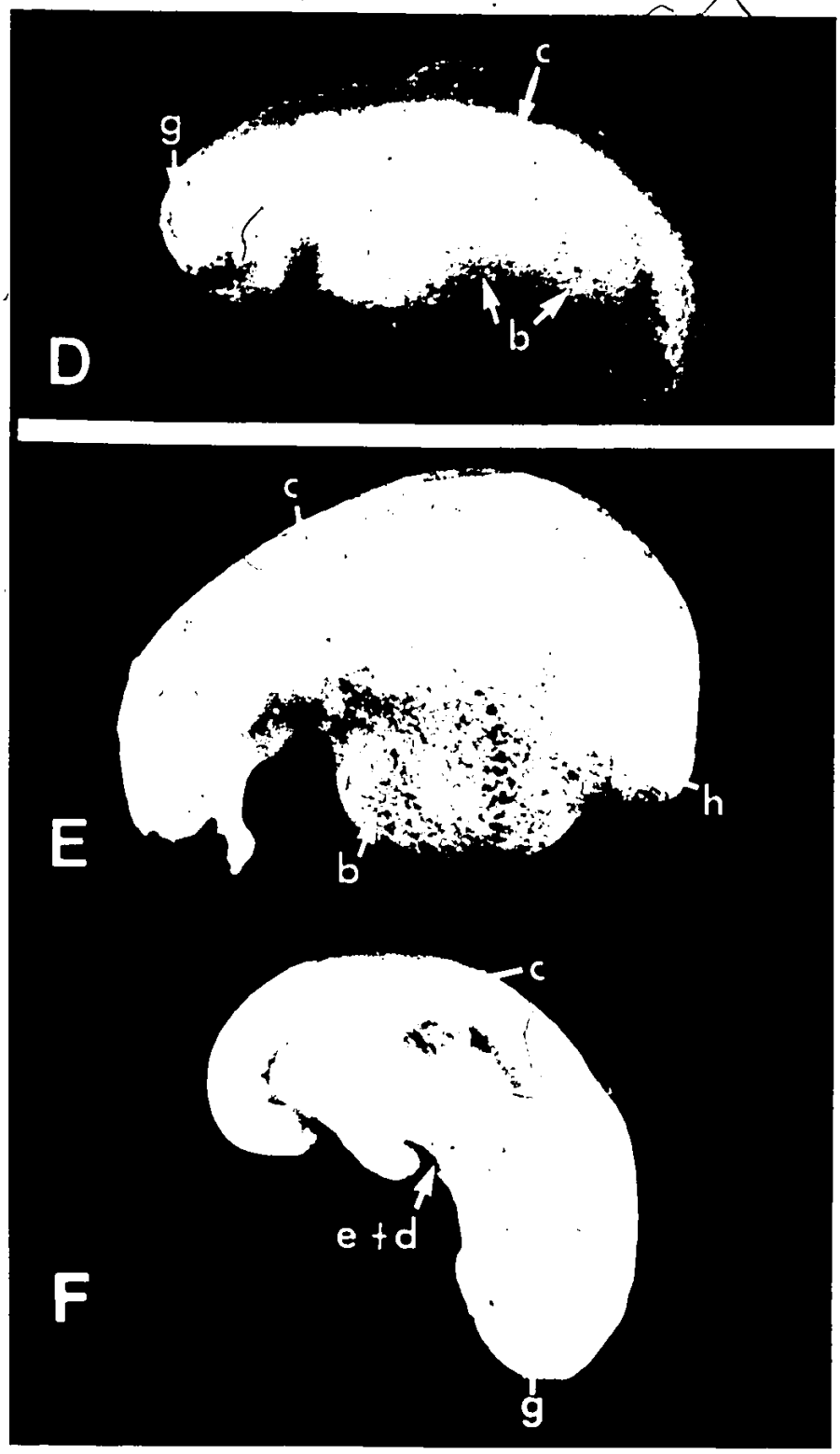

c
g
b
D
c
h
b
E
c
e + d
g
F

Table 1

External characteristics of embryos treated with 10 mg/l fenitrothion at 25°C

CHARACTERISTICS	TYPES					
	C	1	2	3	4	5
n	10	14	15	16	16	13
STAGE	39	39	39	39	39	-[1]
MORPHOLOGY						
Head-tail length (mm)	6.1±0.1[a][2]	5.0±0.1[b]	4.2±0.3[c]	4.3±0.6[c]	3.5±0.6[d]	2.7±0.3[e]
Head-cloaca length (mm)	2.9±0.1[a,b]	3.0±0.2[a]	2.8±0.3[b,c]	2.7±0.4[b,c,d]	2.4±0.5[d]	1.6±0.3[e]
Body width (mm)	1.5±0.1[a]	1.5±0.1[a]	1.5±0.1[a]	1.8±0.3[b]	1.7±0.3[b]	1.6±0.3[a,b]
Head size (mm)						
(dorsal-ventral)	1.0±0.0[a]	1.0±0.1[a]	1.1±0.1[a]	1.1±0.1[a]	1.1±0.1[a]	1.0±0.2[a][3]
(anterior-posterior)	1.0±0.0[a]	1.0±0.0[a]	1.0±0.0[a]	1.0±0.1[a]	0.9±0.2[a]	0.6±0.2[b]
MAJOR EDEMA TYPE[4]	1	1	2	3	3	4

[1] All embryos were at the same stage at the beginning of the treatment but at the end of the treatment period, the stage of type 5 embryos could not be determined.

[2] Values followed by the same letter are not significantly different at the 5% level (t-test). Those underlined are significantly different from the same characteristic in carbaryl treated embryos. Standard deviations are given after the mean values.

[3] the heads could not be measured in four specimens.

[4] See outline attached to Fig. 8.

Table 2a

Internal characteristics of animals treated with 10 mg/l fenitrothion during stages 3-11 and examined at stage 37.

CHARACTERISTICS	TYPES C	1	2	3	4
n	10	18	13	15	16
MORPHOLOGY					
TELENCEPHALON width (L)	302.9±32.8[a1]	271.7±31.9[b3]	249.0±23.3[c]	244.4±44.4[c,d]	215.1±37.8[d]
height (DV)	361.7±29.9[a]	309.2±38.4[b]	290.0±43.5[b]	280.0±50.1[b,c]	255.2±35.3[c]
MYLENCEPHALON overall (L)	302.5±12.8[a]	286.9±28.7[a,c]	259.4±25.1[b]	290.0±35.4[a,c]	266.2±34.2[c,b]
(DV)	263.3±18.0[a]	207.7±27.7[b]	196.2±23.9[b,c]	207.7±30.7[b]	178.5±26.7[c]
ventricle (L)	172.9±29.5[a]	144.6±35.5[b]	104.8±44.5[c]	127.3±30.7[b,c]	95.5±18.0[c]
(DV)	205.4±15.1[a]	176.2±29.3[b]	166.5±22.6[b]	160.9±21.9[b]	136.7±23.8[c]
EYE cornea-retina distance	242.6±9.7[a]	196.4±16.5[b]	173.3±36.3[c,d]	190.0±18.4[b,c]	168.2±31.3[d]
lens thickness	122.9±12.8[a]	85.0±12.6[b]	72.0±14.0[c]	78.2±11.7[b,c]	77.9±16.2[b]
retina thickness	88.7±3.3[a]	84.3± 9.4[a,b]	89.8±14.0[a]	87.0±11.9[a]	77.6±16.2[b]
height	317.7±18.7[a]	267.1±23.7[b]	273.3±26.2[b]	272.0±27.1[b]	232.8±32.8[c]
NERVE BUNDLES	A[2]	A	A	A	C
OTIC VESICLES (L)	149.0± 5.8[a]	120.7± 8	112.2±14.0[c]	111.2±17.6[c]	106.7±20.8[c]
(DV)	171.4± 7.8[a]	134.3±12.2[b]	125.6±17.7[b,c]	136.6±17.2[b]	116.0±20.8[c]
SPINAL CORD DIAMETER	140.4±22.7[a]	112.1±14.2[b]	106.2±15.8[b]	106.9±17.7[b]	105.0±20.1[b]
major type[4]	1	1	1	4,1,2	4,2
NOTOCHORD DIAMETER	107.4±4.3[a]	123.6±10.1[b]	133.3±14.4[c]	128.4±17.0[b,c]	125.6±13.2[b,c]
major type	1	1	3,1	2,3,4	4,2

all footnotes are at the bottom of Table 2b

Table 2b

CHARACTERISTICS	TYPES				
	C	1	2	3	4
HEART					
Size of pericardium (DV)	378.4±43.6[a]	282.0±27.0[b]	245.8±47.2[c]	-	-
(L)	412.3±27.3[a]	403.6±39.5[a]	362.5±54.2[b]	-	-
Size of ventricle (DV)	235.8±16.8[a]	158.6±16.1[b]	167.5±24.9[b]	-	-
(L)	332.4±20.6[a]	231.4±30.1[b]	215.8±35.4[b]	-	-
Size of atrium (DV)	151.0±22.0[a]	103.8± 7.7[b]	102.9±15.8[b]	-	-
(L)	201.4±22.9[a]	159.3±31.0[b]	138.6±30.4[b]	-	-
Major type	1	1	1,2	3	4,3
PRONEPHROS					
Width of tubule	44.5± 4.2[a]	46.0± 4.9[a,b]	47.5± 6.2[a,b]	50.4±10.9[b]	-[5]
lumen	20.4± 1.3[a]	13.4± 3.4[b]	32.9±12.8[c]	18.7±10.9[a,b]	-
cells	12.8± 3.1[a]	16.6± 2.1[b]	42.1±11.4[c]	17.2± 5.1[b]	
MAJOR EDEMA TYPE	1	2,1	2,3	2,3	3,5
MAJOR YOLK TYPE	1	1	1,2	2,1	2,3

[1] standard deviations all measurements are in micrometers, values followed by the same letter were not significantly different (t-test).

[2] A represents nerve bundles 1,2,5,7,8,9 and 10 are easily distinguishable, B is one or two of the nerve bundles listed in A is missing, C means that three or more nerve bundles are missing.

[3] Underlined values are significantly different (t-test) from the same characteristic in carbaryl treated embryos.

[4] Major types include only those observed in more than 30% of the embryos and are listed with the most frequent type first. (Refer to legend for figure 7 for description of the types)

[5] Pronephros was missing in two thirds of the specimens.

Figure 9:

Froglets that had been treated with fenitrothion near hatching and then developed edema and failed to develop all their limbs during metamorphosis. Arrows indicate the location of missing limbs.

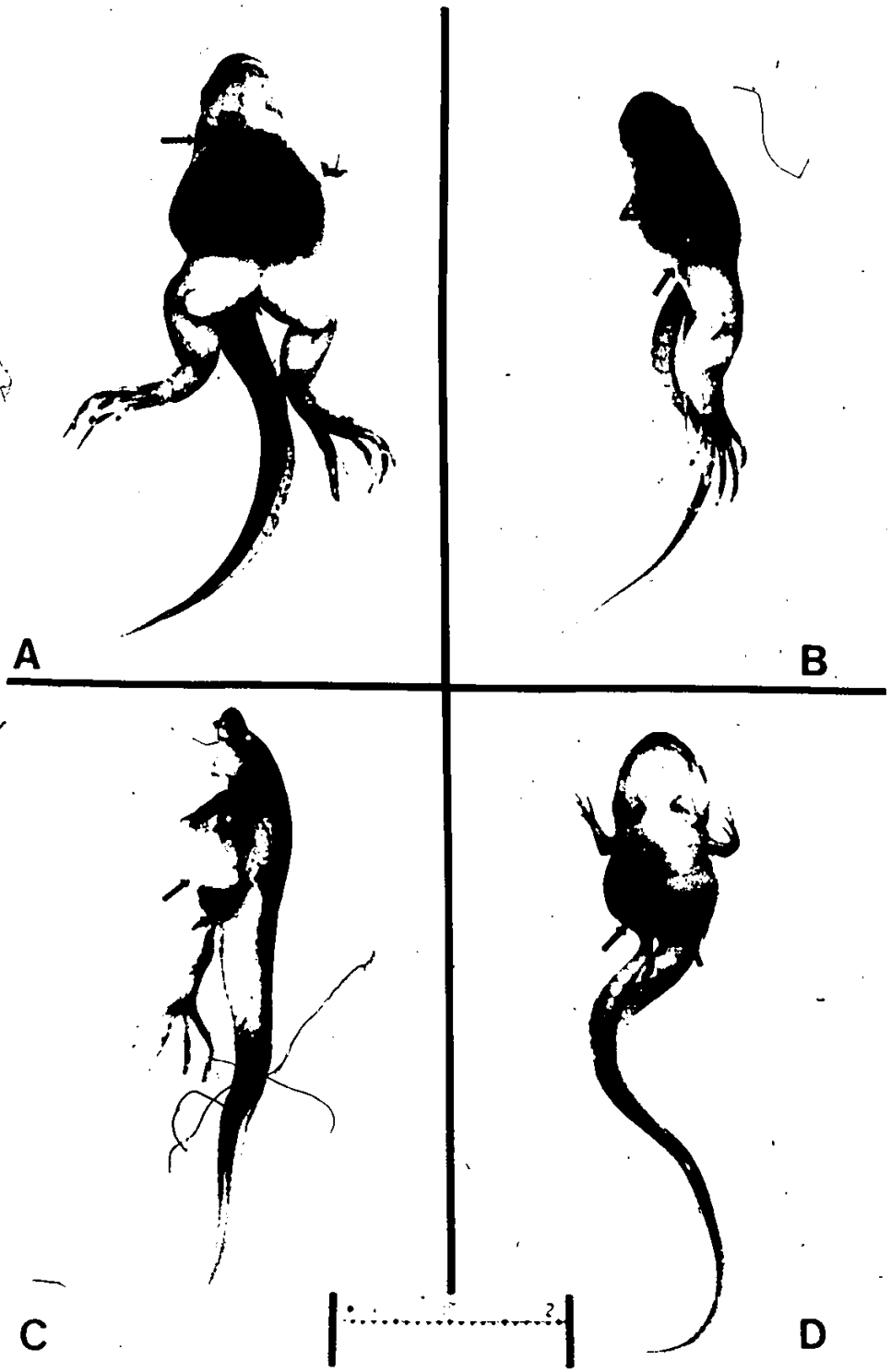
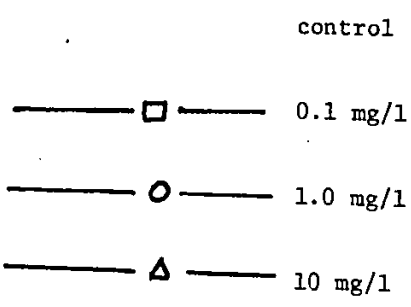
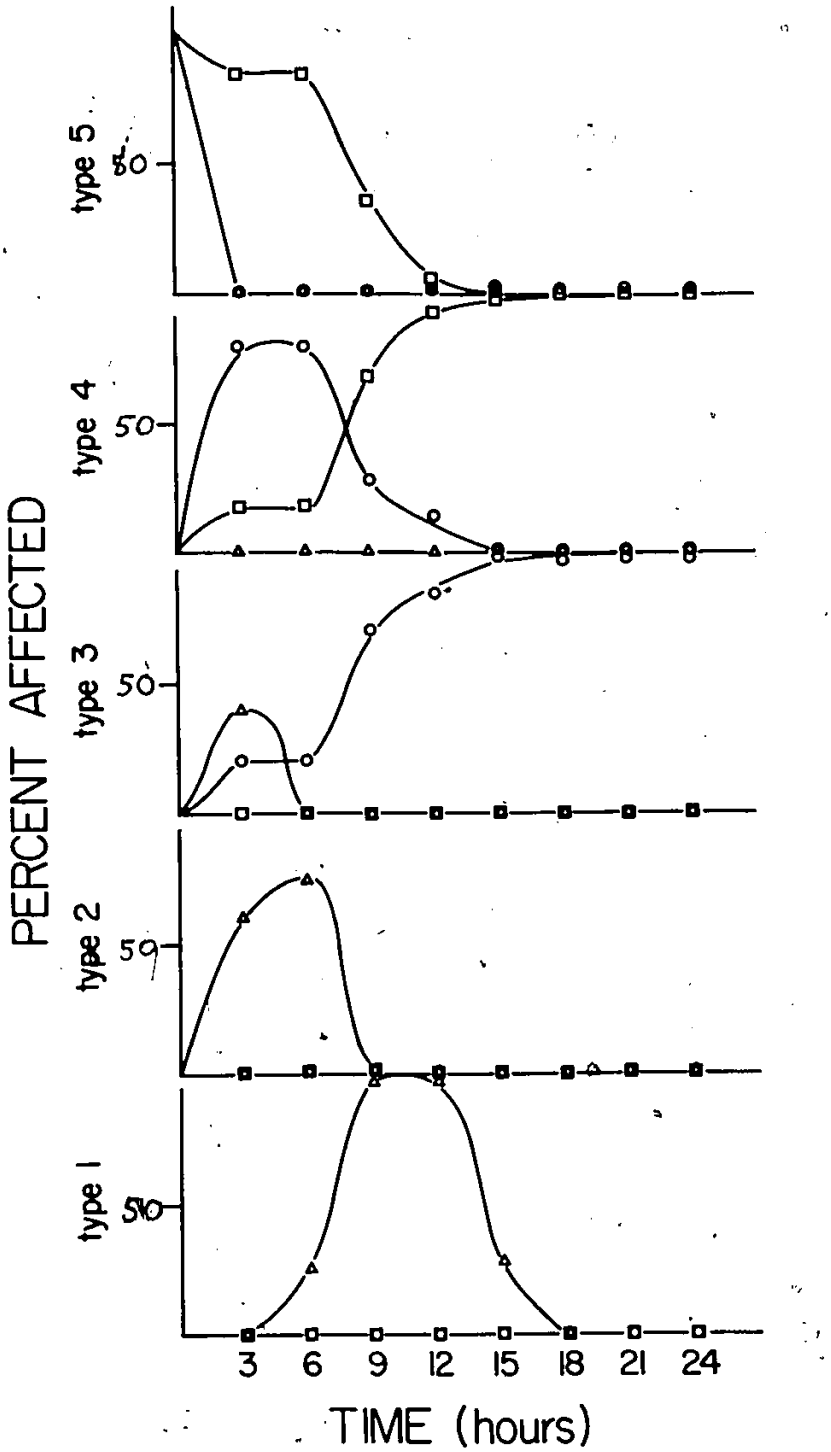

A
B
C
D

Figure 10:

The response of juveniles treated for 24 hours at 25°C with different concentrations of fenitrothion. Treatment began at stage 43 and ended at stage 46. Degrees of activity of response are outlined in the Materials and Methods.




PERCENT AFFECTED
type 5
50
type 4
50
type 3
50
type 2
50
type 1
50
3
6
9
12
15
18
21
24
TIME (hours)

Carbaryl

Embryos Treatment

Embryos were treated with carbaryl for 24 hours post gastrulation (stages 16 to 37) at 18°C. The EC_{50} was approximately 0.1 mg/l (Fig. 11) compared to 7.1 mg/l for fenitrothion. However, only minor abnormalities (type 2) occurred in embryos treated with 1.0 mg/l or less. At 10mg/l, those embryos that survived were grossly malformed (types 4 and 5).

Embryos treated at 25°C and 30°C over a constant developmental period (stages 14 to 30) showed little difference in the types of abnormalities present (table 3). Like fenitrothion, carbaryl does not appear to have a direct temperature related effect.

An examination of the external features of embryos treated at 25°C with 10 mg/l carbaryl (Fig. 12) showed that abnormal embryos were significantly shorter than the controls.[1] The degree of shortening was not as severe as that produced by fenitrothion. Like fenitrothion treated embryos, all the abnormalities produced by carbaryl, except type 5, had a relatively constant body width, head shape and pigmentation (table 4).

---

[1] t-test

The internal structures (tables 5a and 5b) were similar to those in embryos treated with fenitrothion for the corresponding types, with the following exception. Many type 1 carbaryl characteristics were significantly larger than the corresponding embryos treated with fenitrothion, however, they were qualitatively the same. In type 2, the eyes of the carbaryl treated embryos were significantly larger than those treated with fenitrothion. In addition, the optic cups in a few embryos treated with carbaryl were fused. This was seen in none of the fenitrothion treated embryos. The hearts in type 2 embryos were not significantly different, overall. However, the pronephros was significantly different in size.

The size of the notochord and the degree of heart malformation varied significantly between type 3 embryos treated with carbaryl and those treated with fenitrothion. The hearts in type 3 carbaryl treated embryos were not significantly different from the hearts found in type 2 fenitrothion-treated embryos. All other characteristics were similar.

In type 4, the mylencephlon height was significantly larger in the carbaryl-treated embryos, as were the otic vesicles, but the notochord was significantly smaller. The heart abnormalities were more severe in the embryos treated with fenitrothion. The hearts in type 4 carbaryl treated embryos were not significantly different from the hearts in type 2 fenitrothion-treated embryos except in the height of the ventricle and the width of the pericardium. In addition, the size of

the pericardium and ventricle was significantly smaller than those found in type 2 fenitrothion-treated embryos.

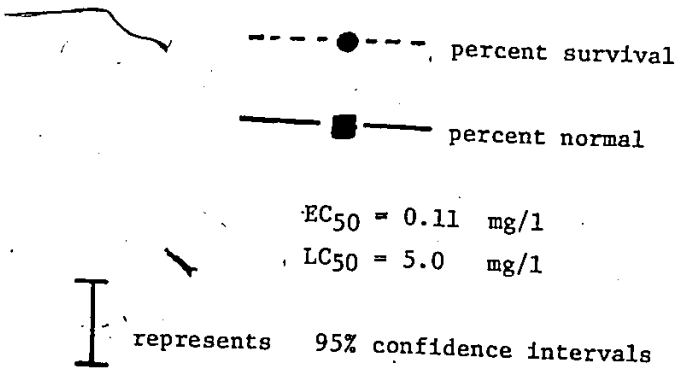
Juveniles usually developed normally to the end of metamorphosis. However, on one occassion approximately 80% of the individuals treated with doses higher than 10 mg/l carbaryl developed either extra limbs or the normal number of limbs with at least two that were non-functional (Fig. 13). Unlike fenitrothion-treated individuals that failed to develop limbs, these froglets were viable and grew to maturity.

Juvenile Treatment

Juveniles were treated at 25°C for 24 hours (stages 43 to 47) at 10, 1.0 and 0.1 mg/l carbaryl. There was a decrease in activity in all three groups during the first twelve hours (Fig. 14). Carbaryl had less effect on the juvenile behaviour than the corresponding concentration of fenitrothion. Pigmentation was not affected in any group. All three groups were examined at stage 53. Ninety-six percent of those treated at 0.1 mg/l, 92% of those treated at 1.0 mg/l and 94% of those treated at10 mg/l carbaryl survived normally to this stage.

Figure 11:

Preliminary EC_{50} and LC_{50} curves for embryos treated with different concentrations of carbaryl from stage 16 to 37 at 18°C.



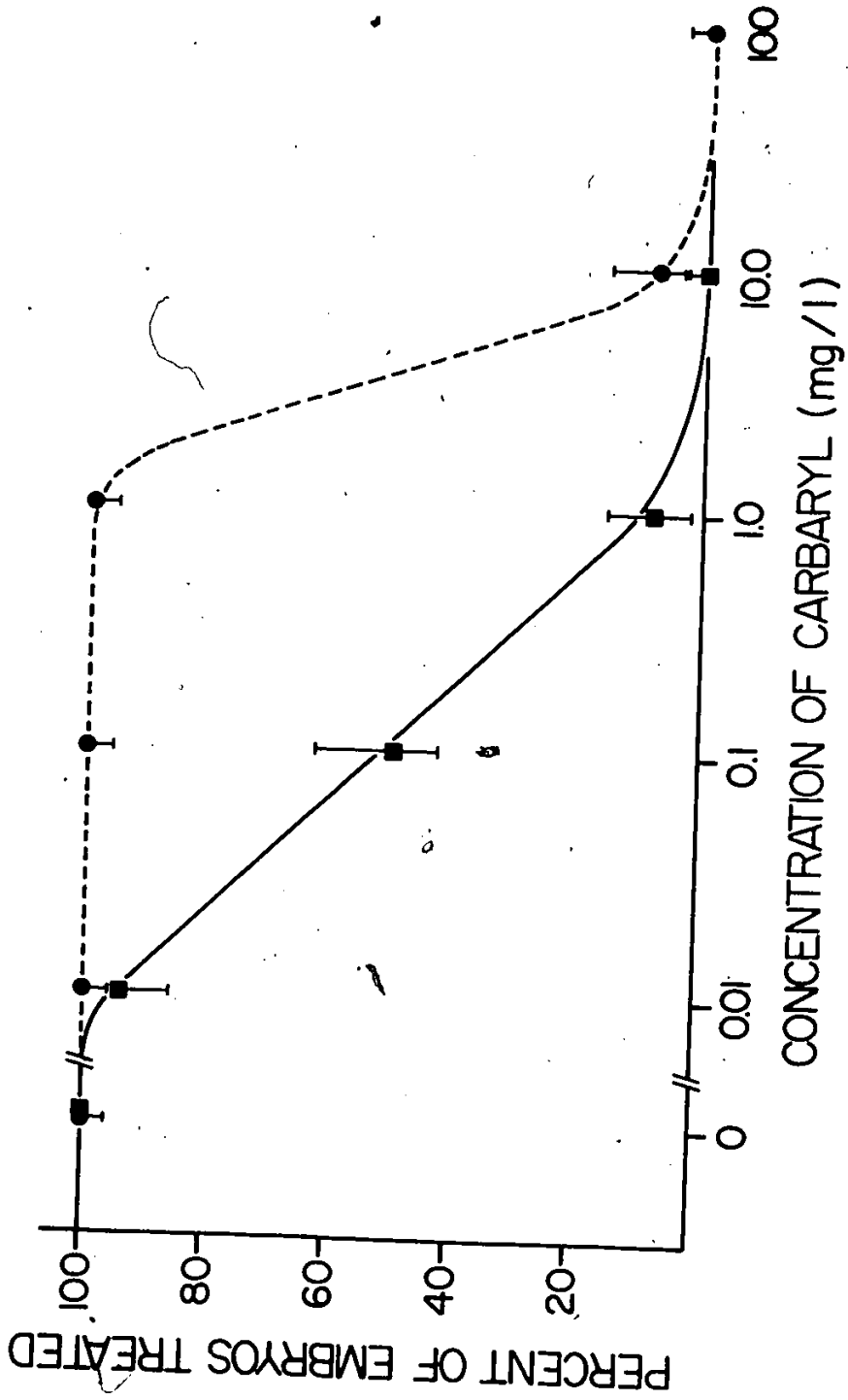

PERCENT OF EMBRYOS TREATED
100
80
60
40
20
0
0.01
0.1
1.0
10.0
100
CONCENTRATION OF CARBARYL (mg/l)

TABLE 3

A comparison of animals treated with carbaryl from stages 14-30 at two different temperatures.

TEMPERATURE	n	TYPE					
		1	2	3	4	5	Dead
25°C	335[1]	54.9[2,3]	21.6	4.9	2.9	2.0	13.7
30°C	300	54.6	16.1	5.1	5.4	2.4	16.4

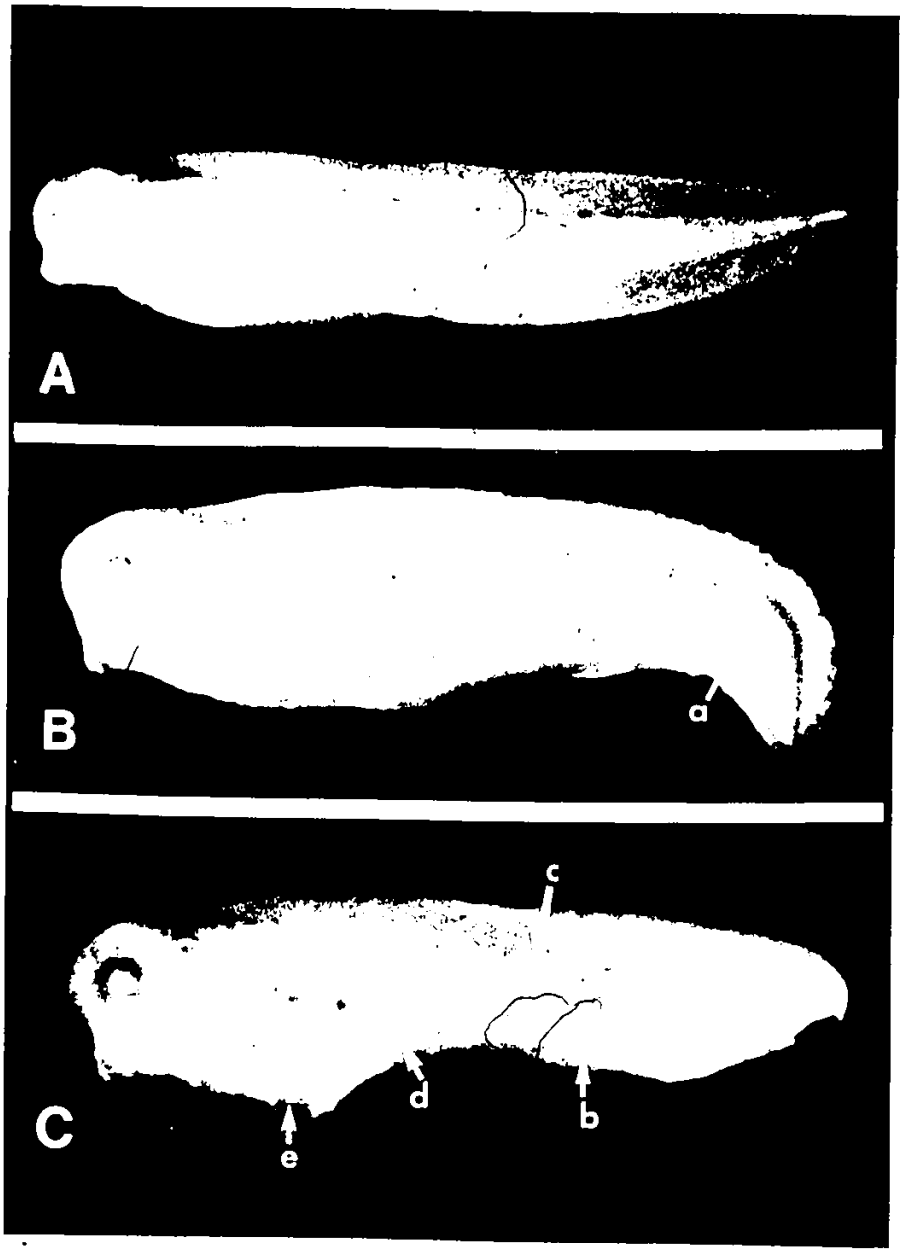
[1] information pooled from three samples

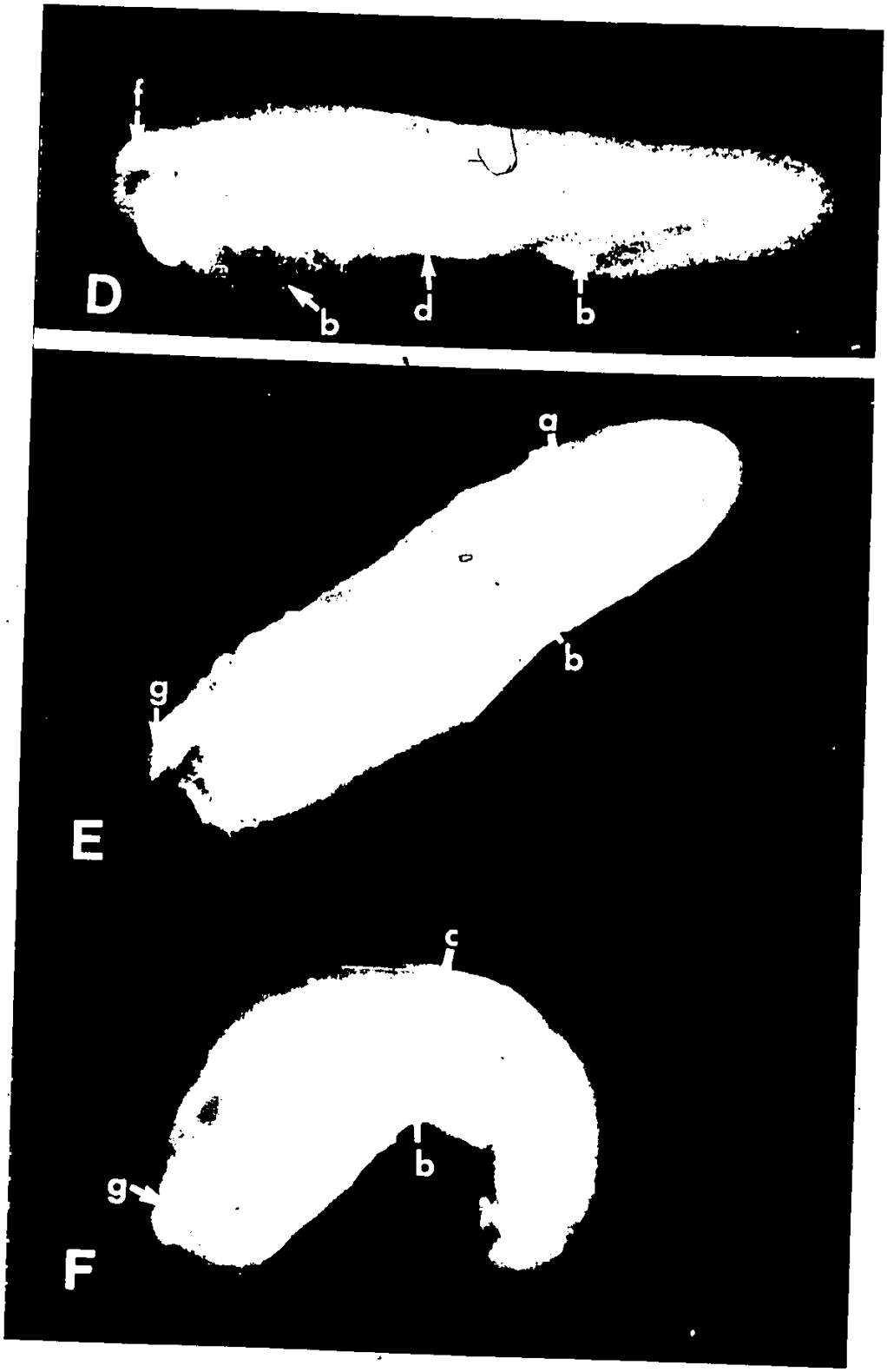
[2] numbers are expressed as percentages of the total sample.

[3] both groups were treated with 10 mg/l solutions of carbaryl

Figure 12:

Type representatives rfrom groups of embryos exposed to carbaryl. A represents type 1, B type 2, Cand D type 3, E and F type 4, and G and H type 5. Type descriptions were constant regardless of the stage that the embryos were treated at.


A
B
a
C
c
d
b
e


f
D
b
d
b
a
b
g
E
c
b
g
F

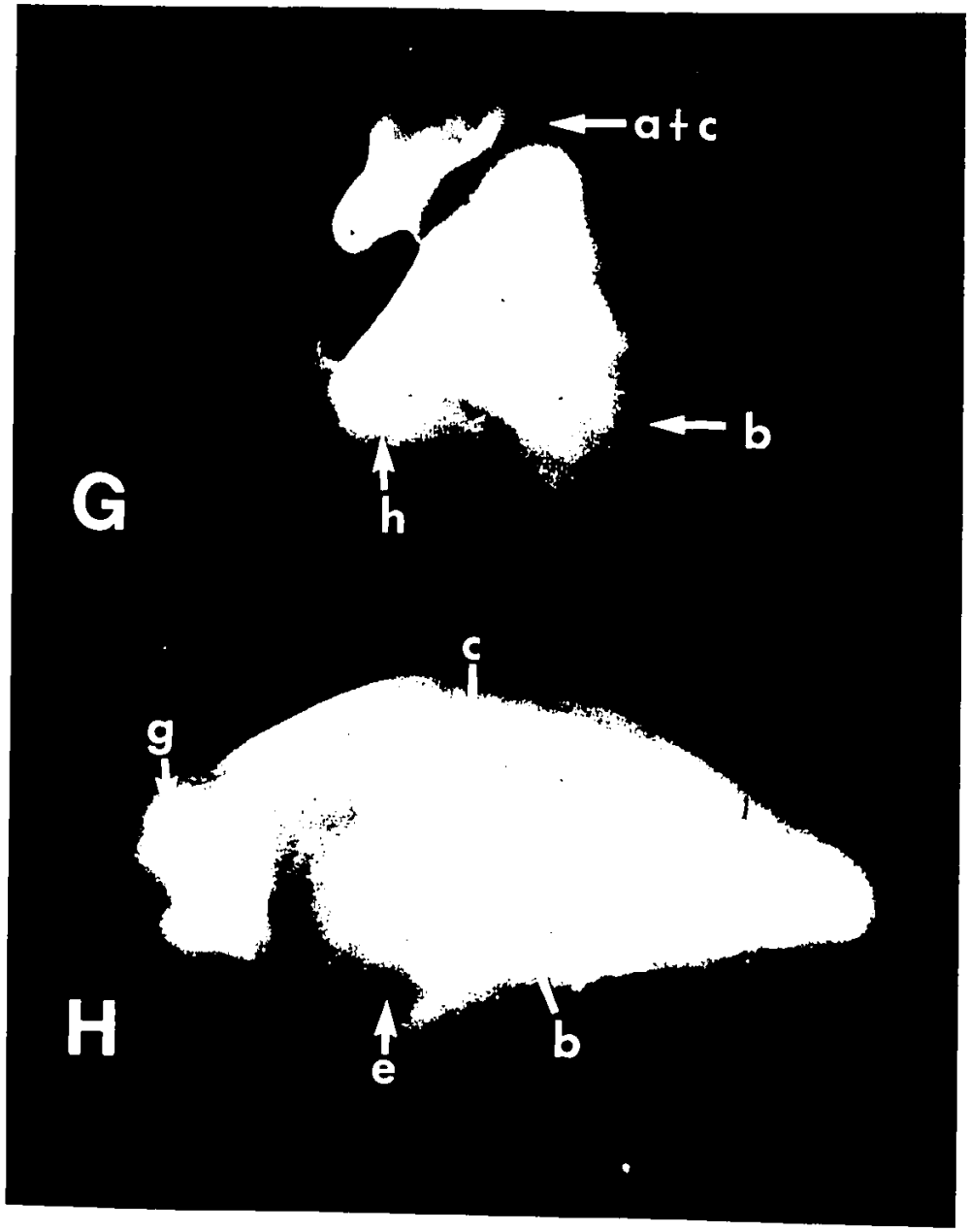

a+c
b
G
h
c
g
H
e
b

TABLE 4

External characteristics of embryos treated with 10 mg/l carbaryl at 25°C

CHARACTERISTICS	TYPES					
	C	1	2	3	4	5
n	10	11	12	10	10	10
STAGE	39	39	39	39	39	[1]
MORPHOLOGY						
Head-tail length (mm)	6.1±0.1[a]	5.7±0.3[b][2]	5.2±0.5[c]	5.1±0.5[c]	4.2±0.7[d]	2.8±0.7[e]
Head-cloaca length (mm)	2.9±0.1[a]	2.9±0.1[a]	2.8±0.3[a]	2.6±0.4[a,b]	2.4±0.5[b]	2.0±0.1[c]
Body width (mm)	1.5±0.1[a]	1.5±0.1[a]	1.8±0.2[b]	1.5±0.3[a,b]	1.7±0.2[b]	1.6±0.5[a,b]
Head size (mm) (dorsal-ventral)	1.0±0.0[a]	1.1±0.1[a]	1.1±0.1[a]	1.0±0.1[a]	1.0±0.1[a]	[3]
MAJOR[4] EDEMA TYPE	1	1	2	3	3	4

[1] All embryos were at the same stage at the beginning of the treatment but at the end of the treatment period, the stage of type 5 embryos could not be determined.

[2] Values followed by the same letter are not significantly different at the 5% level (t-test). Those underlined are significantly different from the same characteristic in fenitrothion treated embryos. Standard deviations are given after the mean values.

[3] Heads were either absent or could not be measured.

[4] Major types include only those observed in more than 30% of the embryos.

TABLE 5a

Internal characteristics of animals treated with 10 mg/l carbaryl during stages 3-11 and examined at stage 37.

CHARACTERISTICS	TYPES				
	C	1	2	3	4
n	10	12	12	10	8
MORPHOLOGY					
TELENCEPHALON width (L)	302.9±32.8[a1]	303.8±15.5[a3]	261.1±13.8[b]	246.7±27.5[b]	225.6±19.2[c]
height (DV)	361.7±29.9[a]	318.2±17.5[b]	290.0±16.5[c]	282.2±22.2[c]	242.8±50.9[c]
MYLENCEPHALON overall (L)	302.5±12.8[a]	292.8±26.7[a,b]	263.2±16.8[b]	264.6±37.4[b,c]	260.7±44.2[b]
(DV)	263.3±18.0[a]	244.4±18.3[b]	209.6±14.7[c]	205.6±21.1[c]	209.7±33.4[c]
ventrical (L)	172.9±29.5[a]	167.3±22.2[a]	133.5±15.5[b]	131.2±28.1[b]	175.0±35.4[a]
(DV)	205.4±15.1[a]	198.2±11.9[a]	161.5±23.0[b,c]	173.8±20.0[b]	137.7±55.8[c]
EYE cornea-retina distance	242.6± 9.7[a]	244.3±21.1[a]	210.0±17.9[b]	206.7±29.4[b]	185.8±32.0[c]
lens thickness	122.9±12.8[a]	117.3±16.0[a]	102.0±17.9[b]	84.4±18.3[c]	82.9±17.0[c]
retina thickness	88.7± 3.3[a]	83.7± 6.4[b]	79.9±10.0[b]	97.8±18.7[a]	78.3±6.4[b]
height	317.7±18.7[a]	258.9±14.9[b]	280.0±27.1[c]	248.9±35.7[b]	246.0±21.2[b]
NERVE BUNDLES	A[2]	A	A	B	C
OTIC VESICLE (L)	149.0± 5.8[a]	145.0±11.2[a,b]	134.0±12.0[b,c]	128.6±27.5[b,c]	130.0±14.1[c]
(DV)	171.4± 7.8[a]	160.9±14.4[a]	150.0±10.0[b]	140.8±25.5[b]	150.0±14.1[b]
SPINAL CORD diameter	140.4±22.7[a]	123.7±13.7[b]	115.5±10.1[b]	120.0±11.5[b]	110.6±20.8[b]
major type[4]	1	1	1	1,2	1,4
NOTOCHORD diameter	107.4± 4.3[a]	110.9± 6.7[a]	110.0± 4.5[a]	114.4± 4.9[b]	109.0±13.6[a]
major type	1	1	1	1,2	4

all footnotes and explanations are at the bottom of Table 5b

Table 5b

CHARACTERISTICS		TYPES				
		C	1	2	3	4
HEART						
Size of pericardium	(DV)	378.4±43.6[a]	359.7±51.2[a,d]	317.8±33.6[b]	278.6±41.2[c]	310.0±66.0[b,c,d]
	(L)	412.3±27.3[a]	437.3±40.2[a,d]	384.4±30.9[b]	374.6±71.4[b,c]	455.8±81.6[d]
Size of ventricle	(DV)	235.8±16.8[a]	191.8±24.4[b]	171.1±26.8[b]	171.4±33.1[b]	180.0±21.2[b]
	(L)	332.4±20.6[a]	296.3±20.9[b]	264.4±25.0[c]	212.8±49.7[d]	256.8±28.6[c]
Size of atrium	(DV)	151.0±22.0[a]	117.3±16.1[b]	94.4±18.3[c]	100.3±22.7[c,d]	110.0±14.1[b,d]
	(L)	201.4±22.9[a]	189.1±21.1[b]	156.7±12.5[c]	130.0±22.4[d]	136.8±22.7[d]
Major type		1	1,2	1,2	2	2,3
PRONEPHROS						
Width of tubule		44.5± 4.2[a]	44.0± 2.4[a]	44.8± 1.6[a]	43.6± 3.5[a]	53.4± 7.2[b]
lumen		20.4± 1.3[a]	20.0± 1.7[a]	17.3± 4.0[b,c]	14.6± 2.6[c]	21.3± 4.4[a,b]
cells		12.8± 3.1[a]	12.4 1.1[a]	13.1 6[a,b]	14.0± 2.7[a,b]	14.7± 3.0[b]
MAJOR EDEMA TYPE		1	2,1	3,2	3,2	2,3
MAJOR YOLK TYPE		1	1	1	2	2,3

[1] standard deviations, all measurements are in micrometers, values followed by the same letter are not significantly different, (t-test)

[2] A represents nerve bundles 1,2,5,7,8,9 and 10 are easily distinguishable, B is one or two of the nerve bundles listed in A are missing, C means that three or more nerve bundles are missing.

[3] Underlined values are significantly different (t-test) from the same characteristic in fenitrothion treated embryos.

[4] Major types include only those observed in more than 30% of the embryos and are listed with the most frequent type first. (Refer to legend for figure 7 for description of the types).

Figure 13:

Metamorphosing froglets treated with 10 mg/l carbaryl during the late embryonic stages and during hatching. Some developed non-functional limbs and others developed extra limbs or digits. A had functional front limbs but malformed hind limbs that caused it to swim in a spiral. B had an extra limb, one normal front limb and two malformed hindlegs. C had normally shaped, nonfunctioning front limbs and crippled hind legs. D had non-functional front limbs and normal hind legs. This froglet swam in a normal pattern.

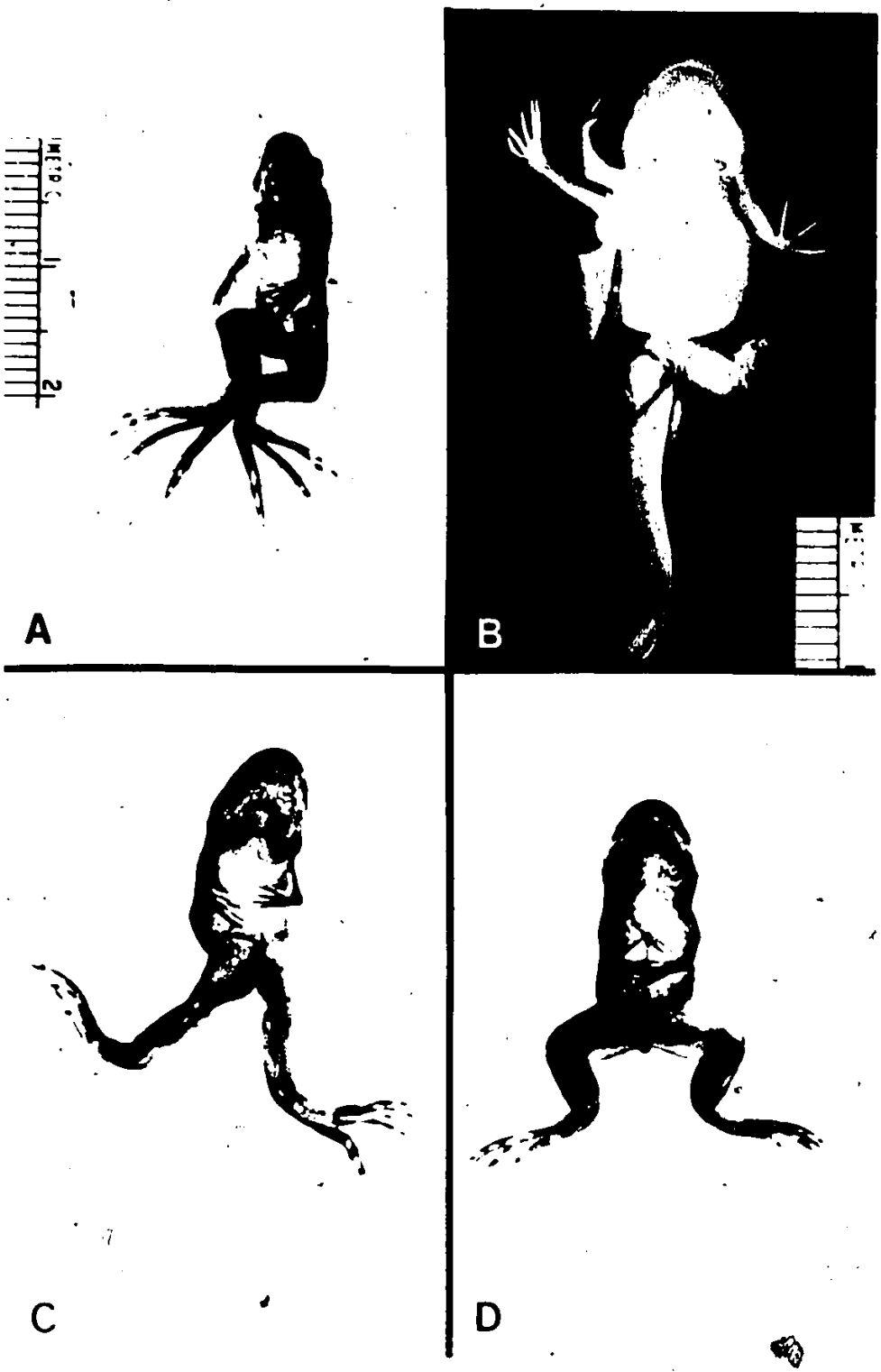
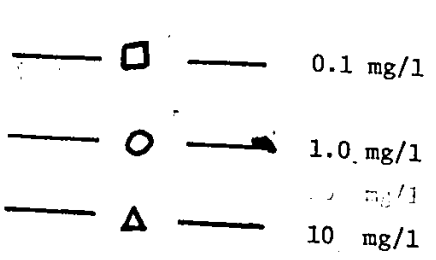
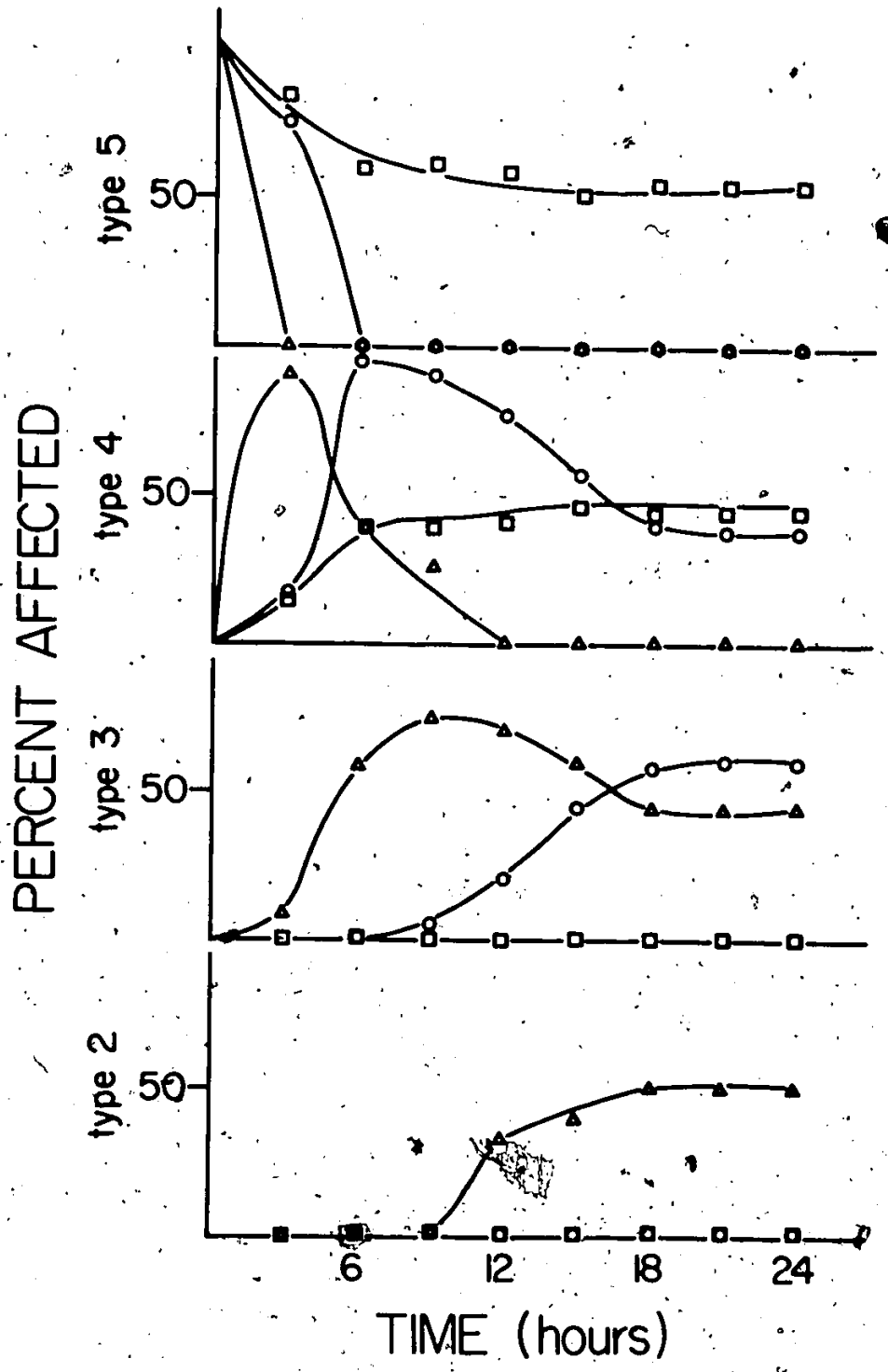

A
B
C
D

Figure 14:

The response of juveniles treated for 24 hours at 25°C with different concentrations of carbaryl (0.1, 1.0, and 10 mg/1). Treatment began at stage 43 and ended at stage 46. No individuals fell into types one or zero.




PERCENT AFFECTED
type 5
50
type 4
50
type 3
50
type 2
50
6
12
18
24
TIME (hours)

Lindane

Embryo Treatment

Unlike fenitrothion and carbaryl, lindane did not produce gross defects in embryos treated at 18°C and 25°C (Fig. 15) at concentrations up to 10 mg/l. the only effect at these concentrations was a mild localized edema (type 2 abnormality), which at 10 mg/l, was more prevalent in the 25°C group. Lindane was embryotoxic at concentrations higher than 10 mg/l but at lower concentrations only mildly affected the embryos and showed no adverse effects on either cleavage of gastrulation.

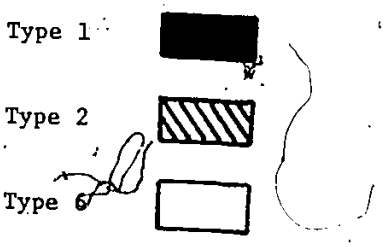
Although the juveniles appeared to develop normally, at metamorphosis many bloated and died (Fig. 16). There was no consistent pattern internally, indicating that death may have been caused by a greater susceptibility to infection. Treated animals reared at 18°C looked healthier than those reared at 25°C (Fig. 17) indicating a possible temperature stress. The tendancy to bloat was not observed in the controls.

Juvenile Treatment

Unlike juveniles treated with fenitrothion and carbaryl, those treated with lindane showed only mild intoxication (Fig. 18). Those treated with 10 mg/l lindane also exhibited a 50% decrease in pigmentation, but returned to normal within 24 hours after treatment. Ninety eight percent survived normally to stage 53.

Figure 15:

Effect of various concentrations of lindane on Xenopus embryos treated from stage 14 to 25 at 18°C (A) and 25°C (B).



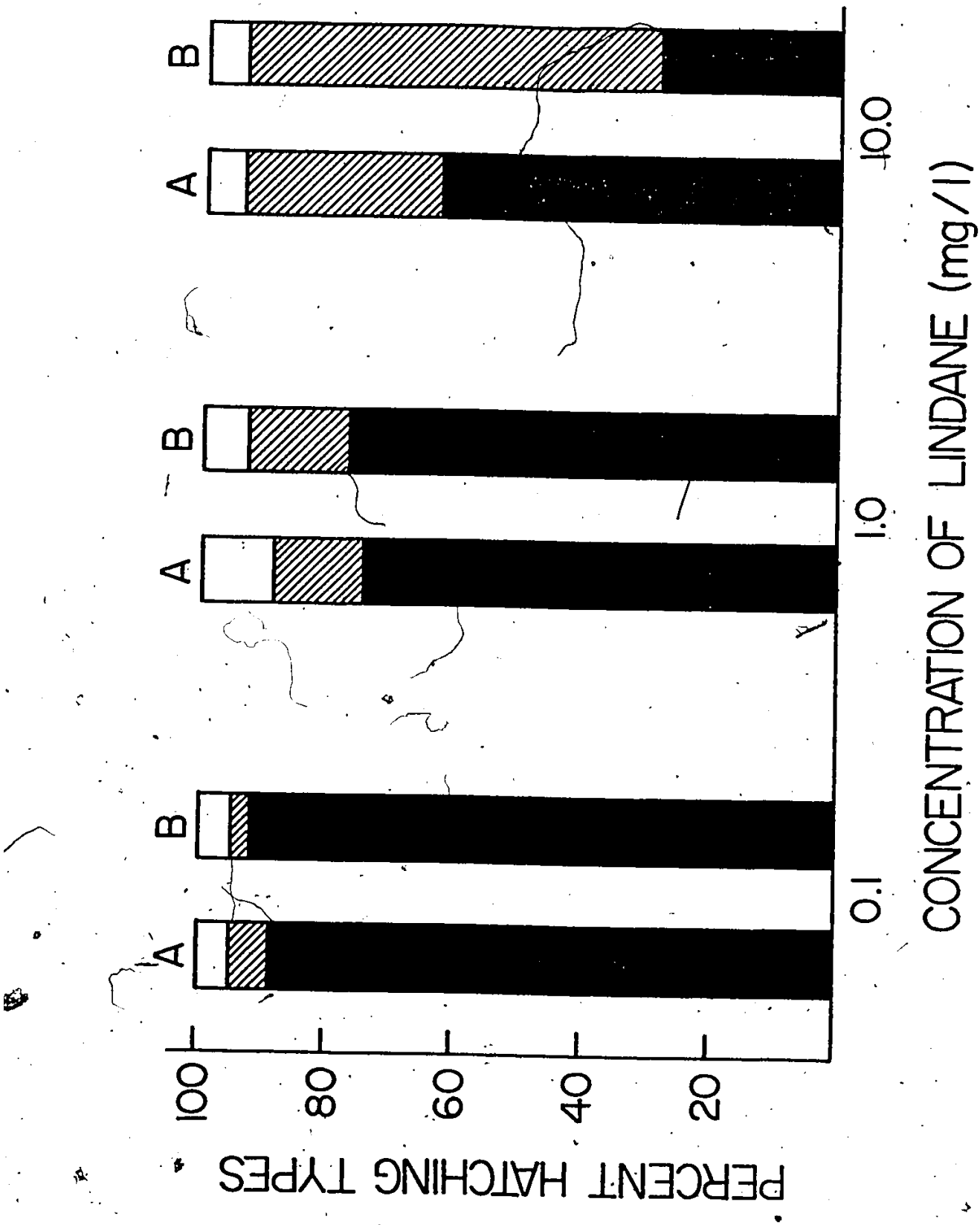
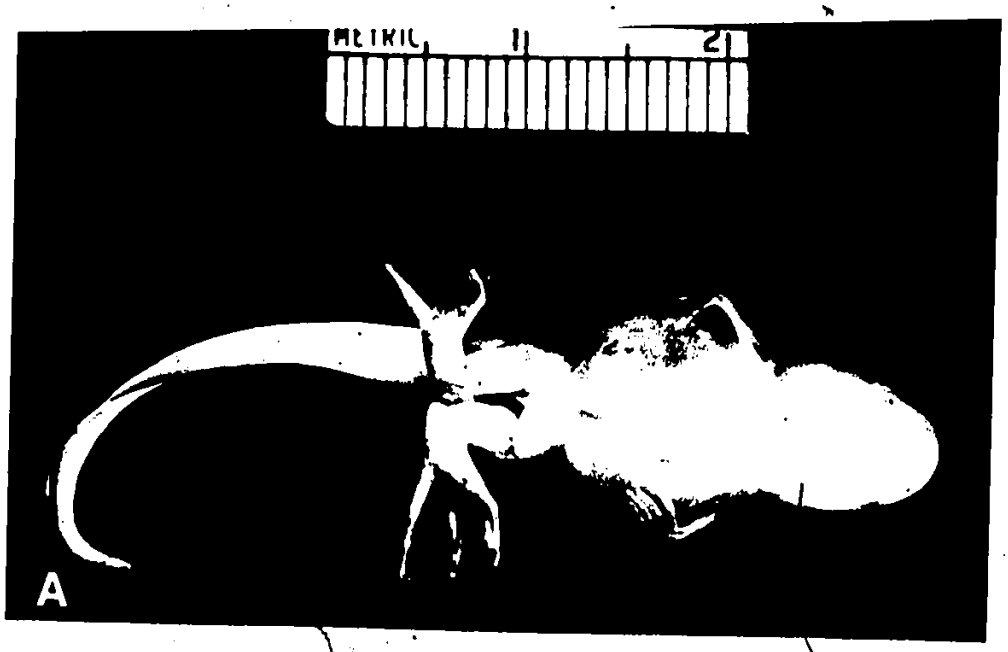

PERCENT HATCHING TYPES
100
80
60
40
20
A
B
0.1
1.0
10.0
CONCENTRATION OF LINDANE (mg/l)

Figure 16:

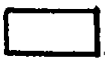
A comparison of a bloated metamorphosing froglet (A) which had been treated with lindane during embryonic development and a control froglet (B) of the same age.


A


B

Figure 17:

Comparative survival of Xenopus juveniles treated for at either 18°C or 25°C during the embryonic stages 14 to 25 and then raised at either temperature depending on the treatment.



18°C

25°C

indicate 95% confidence intervals

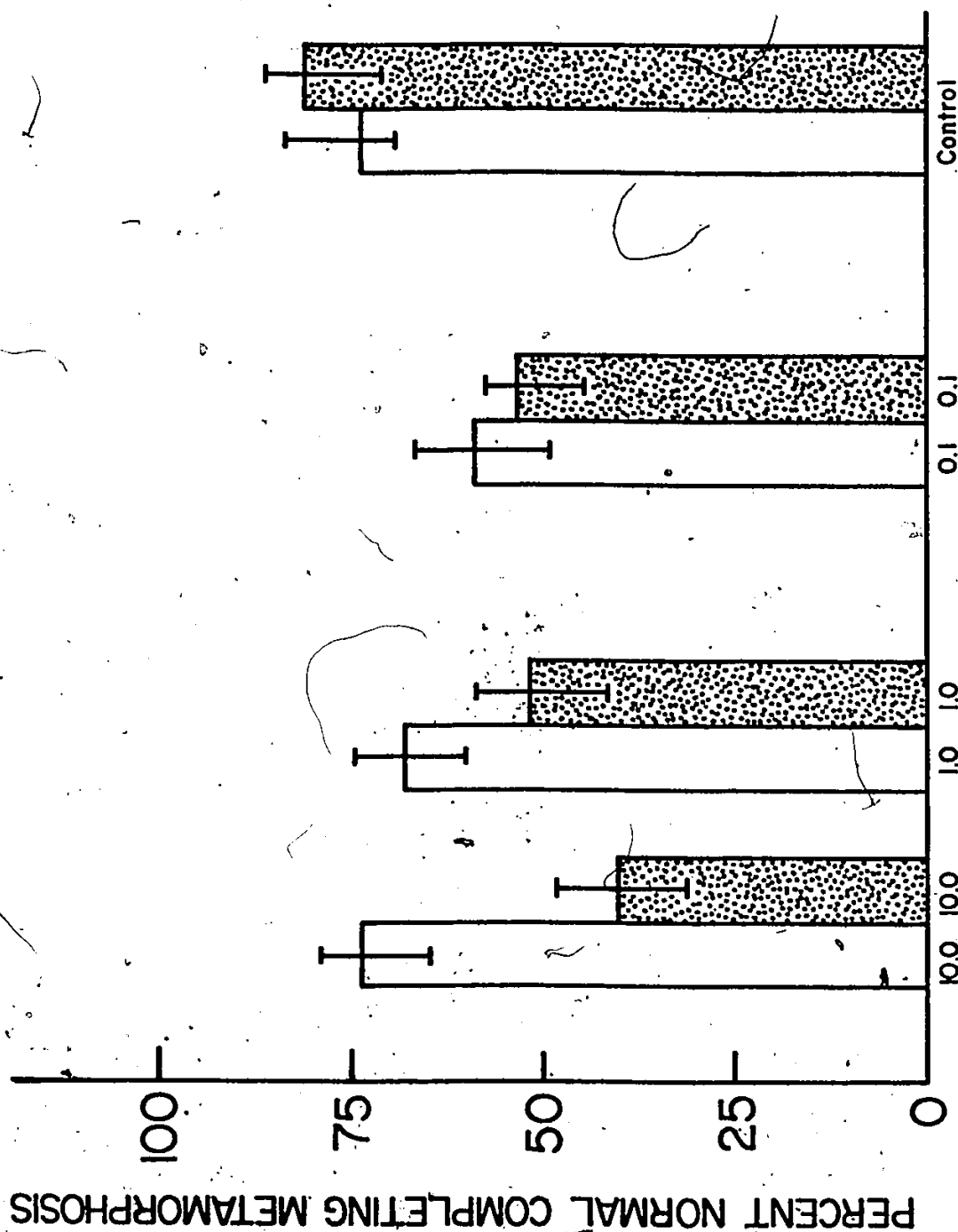
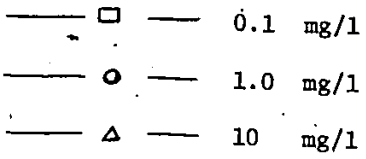
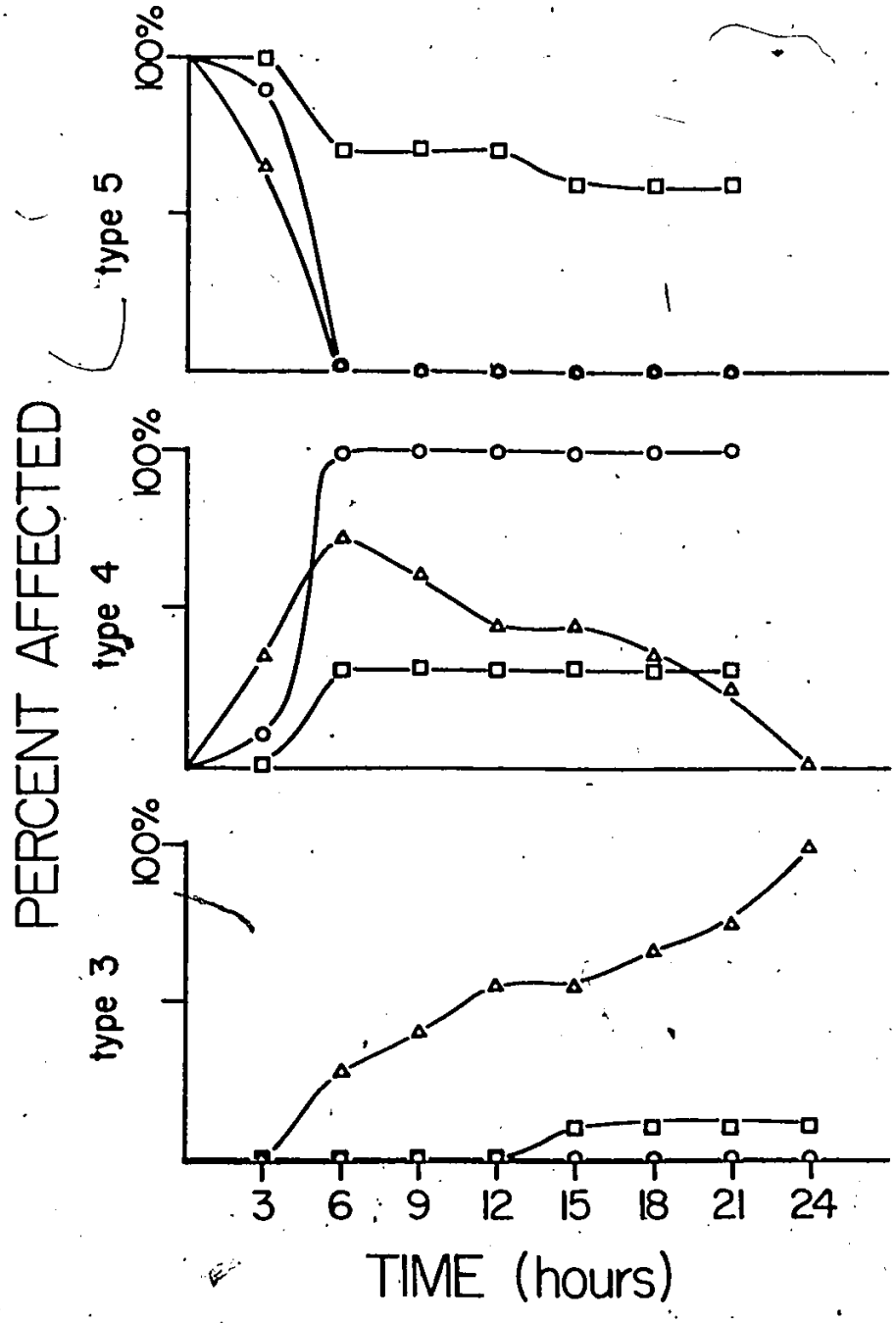

PERCENT NORMAL COMPLETING METAMORPHOSIS
100
75
50
25
0
Control
0.1
0.1
1.0
1.0
10.0
10.0
CONCENTRATION OF LINDANE (mg/l)

Figure 18:

The response of juveniles treated for 24 hours at 25°C with different concentrations of lindane. Treatment began at stage 43 and ended at stage 46. Degrees of activity of response are outlined in the Materials and Methods.




PERCENT AFFECTED
100%
type 5
100%
type 4
100%
type 3
3
6
9
12
15
18
21
24
TIME (hours)

DISCUSSION

Comparison of Fenitrothion and Carbaryl

The results of the present study support the idea that fenitrothion and carbaryl cause similar types of abnormalities. Both carbaryl and fenitrothion caused dose and stage related malformations. In both cases, the embryos were most sensitive to developmental disruptions during gastrulation and least sensitive during the later developmental stages, when massive tissue movements were not occurring. Externally, embryos treated with carbaryl could not be distinguished from those treated with fenitrothion except in overall length and in types 2 and 3, where the body width was significantly different. This may be attributed to larger, but similar edematous regions. Both test groups had shorter tails than the controls. This may be related to a slower developmental rate since tail growth is very rapid during the early post-hatching stages. The pronephros, when present, was fairly constant within a given type regardless of the insecticide used. However, tubule dilation and vacuole formation, similar to that observed by Lutz-Ostertag et. al. (1969, using parathion), was noted in types 3 and 4 and occassionally in type 2. The nervous systems of embryos of the same type were qualitatively the same, as was the severity of the edematous regions and lysing in the yolk.

An increase in temperature did not cause a significant increase in the teratogenic effects of either insecticide, when the developmental

stage was held constant. Thus, the degree of abnormality and the major structures affected were more closely linked to the stage(s) of exposure than to the temperature.

In spite of the numerous similarities in types of abnormalities, it is clear that a given concentration of fenitrothion produced more severe abnormalities than the same concentration of carbaryl.[1] Even though the percent survival and the percent normal at the same concentration at 25°C were very similar, the percent of severely abnormal embryos was higher in the fenitrothion treated groups than in those treated with carbaryl. This difference in severity can be seen by examining the internal structures. The most notable difference is the degree of heart malformation in embryos which, externally, appeared similar. The hearts found in types 3 and 4 of carbaryl treated embryos were not significantly different from the hearts found in type 2 fenitrothion treated embryos suggesting a difference in the degree of effect and not in the pattern of effect. The presence of deformed hearts could indicate other circulatory problems which would lead to an increase in fluid accumulation, as was seen in both groups, as the number of terata increased. In general, the notochord in embryos treated with carbaryl did not differ significantly from that of the control, however, the notochord was significantly larger in embryos treated with fenitrothion. This may indicate a problem in the regression of the notochord caused by the treatment but to a lesser extent than that observed by Greenhouse (1976).

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[1] The same concentration of carbaryl and fenitrothion, in mg/l, is within the same order of magnitude in terms of molar concentrations.

Limb abnormalities produced by carbaryl were also less severe than those produced by fenitrothion. Juveniles with limb abnormalities caused by carbaryl completed metamorphosis whilst those exposed to fenitrothion died before completing metamorphosis. The behaviour of the juveniles treated post-hatching was also more severly affected by fenitrothion than by carbaryl.

These differences can be directly related to the respective abilities of the insecticides to penetrate the protecting barriers of the embryo. O'Mellia (1972) tested the effects of over 70 different cholinergic and cholinolytic compounds on gastrulas of Arbacia punctulata. That study indicated that only the lipid soluble compounds caused effects. The more hydrophilic the compound, the less the effect. In this context, it should be noted that carbaryl is much more water soluble than fenitrothion. The relationship between lipid solubility and teratogenicity of a given compound is further substantiated by the work of Solomon and Weis (1979) who found that carbaryl was far more teratogenic when used with a solvent (acetone) than when it was used with only distilled water.

Mechanisms of Action

Recent studies have indicated that many cholinomimetic agents cause teratogenic effects by interrupting the development of structures derived primarily from the mesoderm, such as the circulatory and skeletal systems. For years, investigators have shown that insecticides such as parathion and malathion cause congenital malformations in

developing birds (Gill and LaHam, 1972; Jackson and Gibson, 1977; Laley and Gibson, 1977; Lillie, 1973; Lutz-Ostertag et al., 1969; Meiniel, 1970, 1976, 1977: Walker, 1971; and Wilson et al., 1973). Similar trends have been shown in studies using other cholinomimetic agents on dogs (Smalley et al., 1968), rodents (Budreau et al., 1973; Robens,1969), fish (Johnson and Prine, 1976; Kapur et al., 1978; Solomon and Weis, 1979; Weis and Weis, 1976a, 1976b, 1974) and turtles (Mitchell and Yntema, 1973). All of the above studies used high concentrations of given insecticides and raised many questions concerning the actual mechanism(s) involved. In addition to these studies, Prahlad and Anderson (1975) found ultrastructural changes in skeletal muscle of X. laevis juveniles after they had been treated with low doses of nabam and diquat (1-2 ppm) over a six day period.

Two predominant positions are put forth to explain the occurrances of terata in the above studies. The first proposes that cholinomimetic teratogens interfere with basic biochemical pathways causing hypoglycemia (Arsenault et.al., 1975), reduced growth by limiting the embryo's access to yolk hydrolysis products (Fish, 1966; Upshall et al., 1968; Budreau, 1973; Wilson et al., 1973) and by interfering with carbohydrate metabolism (Prahlad and Anderson, 1975) and altering the levels of NADPH (Upshall et al., 1968; Walker, 1971). These alterations then interfere with other pathways such as calcium regulation causing faulty chondrification resulting in weaker, shorter bones. This in turn results in the numerous skeletal abnormalities observed (Arsenault et al., 1975). This position is indirectly supported by

the work of Durham (1974) showing the importance of the calcium ions in cell movement necessary for morphogenetic events. These studies do not support the hypothesis that acetylcholine and the cholinergic system p play a major role in differentaiation processes. On the contrary, they propose that the terata could be eliminated by supplying the appropriate nutritional supplements. (Arsenault et al., 1975; Budreau, 1973; Upshall et al., 1968; Walker, 1971; and Wilson et. al., 1973).

The second position states that acetylcholine and/or the cholinergic system play a crucial role in the developmental processes. The histochemical results put forth by Drews (1975) show that in the early stages of development of every organ he studied, there was a phase of cholinesterase activity. This was outlined as follows; as the organ starts to differentiate, cholinesterase activity increased and then after the organ was established the cholinesterase activity diminished and disappeared. Activity reappears again when disintegration of a structure occurs. This pattern of cholinesterase activity was also demonstrated in the amphibian embryo [Triturus cristatus] during the formation of the primitive organs (Kother-Becker et al., 1970). In addition, Fambrough and Rash (1971) found that the acetylcholine receptors were easy to identify and differentiating muscle cells in mice embryos and that the myoblasts became sensitive to acetylcholine before innervation occurred and before the organization of myofilaments into myofibrils. The movement of developing muscle in the chick embryo is further outlined by Bellairs (1979). Both Drews (1975) and Fambrough and Rash (1971) correlate the increased sensitivity of the cell to acetylcholine

with cytoplasmic aspects of differentiation. Drews further illustrates that during embryonic development microfilament contraction powers both the pseudopodial movement of the cells and the conformation of the epithelial sheets. Contraction of the microfilaments appears to be regulated by calcium ions, the concentration of which appears to be regulated by potential changes in the membrane (Durham, 1974). The exact relationship between these steps is still controversial.

O'Mellia (1972) showed that cholinesterase activity was not present in the blastula of the sea urchin but appeared and increased during gastrulation. Kocher-Becker et al. (1976) further found that in the sea urchin gastrula, cholinesterase activity was found in primary and secondary mesenchyme cells and in the invaginating archenteron. As development progressed, cholinesterase activity disappeared from the primary mesenchyme cells. This rise and fall in cholinesterase activity correlated with the tissue movement and differentiation. Inhibition of the cholinesterases during this phase of development could explain the high percentage of severely abnormal and dead embryos that were found in groups tested with 10 mg/l or more of either fenitrothion or carbaryl.

Drews (1975) found that in the development of the chick heart, cholinesterase levels are low until after the fusion in the midline of the mesodermal palisades which rearrange to form the myocardial walls of the tubular heart. In the ensuing stages, the myocardial layer retains its strong cholinesterase activity until the formation of the

trabeculae and endothelial cushions. At that point, the myocardial layer looses its activity and the endothelial cells increase in cholinesterase activity. In the heart, cholinesterase activity and morphogenetic movements closely correlate with those found in the development of other organ anlagen with the exception, that in the heart embryonic cholinesterases appear to become involved in the control of the rhythmic contractions. Again, inhibition of these embryonic cholinesterases could be responsible for the failure of the folding of the embryonic heart during exposure to the higher concentrations of pesticides, as observed by Solomon and Weis (1979) and in our own study.

Three stages in chick limb formation were outlined (Drews, 1975). In the early limb bud cholinesterase activity resided in the ectodermal layer and then general activity appears in the mesodermal core; and finally, differentiation of cartilage and muscle is accompanied by high levels of activity. All three phases proceed from the proximal to the distal end and partially overlap each other. Bellairs (1979) has shown that the events of muscle development in the chick are very similar to those in Xenopus. In addition, Cameron and Fallon (1977) have shown the importance of ectodermal-mesodermal interactions in limb formation of Xenopus by rotation presupmtive tissue in the proximodistal axis.

These studies support the position that xenobiotic interruption of the cholinergic system during critical stages of embryonic development (either cell movement, differentiation or disintegration) can result in the appearance of terata such as were observed in the present study.

Environmental Implications

Both fenitrothion and carbaryl are considered non-persistent (i.e. they have a half-life of less than two weeks) in natural waters (McEwen and Stephenson, 1979). In the present study, concentrations of insecticides have been used which correlate with levels that may be present in the natural environment immediately following spraying (Roberts et. al., 1977). If a region was sprayed only once during the growing season, these pesticides could possibly be considered harmless to developing anurans, if application was made during a time when they would be least sensitive. Unfortunately, this is not the present situation. Spray programmes are conducted several times during the growing season.

The present study verifies previous work which showed that certain stages in both embryonic and juvenile development are more sensitive to xenobiotics than other stages. Studies by the CWS which indicated that fenitrothion was harmless to embryonic anurans, did not account for long term effects on the juveniles (they were not examined one week or even a month after spraying) nor the survival of the embryos after hatching. This apparent lack of effect could be correlated to a less sensitive stage at the time of spraying. However, our study illustrates that an embryo may appear to be unaffected externally but in fact may have suffered damage. For example, mild edemas were often found in type one embryos. In the lab, these posed no problem. However, in a natural setting, the additional physiological stress could affect both feeding and predation.

Any embryos that developed type three abnormalities or greater were found not to survive in a natural setting. If the young are exposed to low levels of pesticide during embryonic development, and again at different time during their juvenile growth, it will invariably weaken them, leading to a decrease in the overall population of the species. In addition to the problems encountered by the direct application of the pesticide to the embryos, there exist the problems of bioaccumulation of the pesticide in the food chain such as in algae and in the sediments where the juveniles burrow.

Alternative methods of insect control sould be sought which would pose less of a threat to the survival of the non-target fauna, such as the anurans, than the insecticides which are presently being used.

Lindane

Unlike fenitrothion and carbaryl, lindane did not produce any teratogenic effects. However, it was embryotoxic at the higher c concentrations (10 and 100 mg/l). Embryos treated with lindane were more susceptible to infection than the control. This could have been caused by lindane's ability to inhibit ATPases and microsomal enzymes thus weakening the animal. This inhibition of ATPases could indirectly interfere with the developmental processes by reducing the biosynthesis of enzymes such as acetylcholinesterase and other esterases. In addition, lindane, unlike fenitrothion or carbaryl, can be stored in the embryonic tissue (Licht, 1976) and released as the juvenile grows. This could account for the prolonged weakening of the treated groups in relation to the controls.

The present study indicates that embryos exposed to low levels of lindane for a short period of time are unaffected. However, long term exposure in the natural environment may cause a decrease in the anuran population similar to that caused by the other organochlorines such as DDT. For this reason, long term studies on the effects of lindane or other organochlorine pesticides on developing anurans should be conducted, as various pesticides of this type are still in use.

SUMMARY

Fenitrothion and carbaryl were toxic to embryos of Xenopus laevis at concentrations exceeding 1.0 mg/l. In addition, it was shown that both were teratogenic at specific stages of embryonic development. Embryos treated for a short period of time during gastrulation showed the most severe effects, when examined at hatching, followed closely by those treated during cleavage, blastula formation and neurulation. Embryos exposed after neurulation usually appeared normal at hatching. Neither insecticide affected the long term survival of embryos that survived for one week after treatment. In addition, juveniles with crooked spines as a result of treatment during their embryonic period, as well as juveniles that appeared normal, metamorphosed successfully. It was proposed that carbaryl and fenitrothion caused malformations by inhibiting cholinergic enzymes and by interfering with carbohydrate metabolism.

The effects of lindane contrasted with those of fenitrothion and carbaryl by a marked absence of terata. In addition, lindane was embryotoxic only at concentrations exceeding 10 mg/l. However, lindane-treated embryos showed a greater susceptibility to infection especially during metamorphosis. It was proposed that long-term exposure to lindane could alter normal embryonic development by inhibiting ATPases, thus interfering with normal metabolism.

Juveniles that had been treated with one of the three insecticides showed changes in behaviour. Fenitrothion caused the most severe effects ranging from loss of coordination and alertness to total paralysis and death depending upon the concentration((ranging from 0.1 to 10 mg/l). Carbaryl treated juveniles showed a loss of alertness and coordination at the lower concentrations and partial paralysis at 10 mg/l. Juveniles treated with 10 mg/l lindane showed only minor signs of intoxication. The difference in the degree of intoxication caused by the three insecticides in juveniles correlates with the results from the embryotoxicity tests.

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