Insecticide Effects on Normal Development and Hatch of Embryos of *Paratanytarsus Parthenogeneticus* (Diptera: Chironomidae)

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INSECTICIDE EFFECTS ON NORMAL DEVELOPMENT AND HATCH OF EMBRYOS OF *PARATANYTARSUS PARTHENOGENETICUS* (DIPTERA: CHIRONOMIDAE)

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ABSTRACT

Simple, low cost methods are needed to determine the effect of pesticides on non-target aquatic organisms. In this report, embryos of *Paratanytarsus parthenogeneticus* were exposed from deposition to hatch to five pesticides. Four of the five pesticides affected development or hatch only at concentrations which exceeded 96-h LC50 values of other non-target invertebrates. One pesticide, fenitrothion, affected hatch at 13 μg/l which is similar to 96-h LC50 values for other aquatic invertebrates. Because of the low sensitivity of the embryo to pesticides, this method may not be a useful pesticide screening test for non-target invertebrates.

The effect of pesticides on non-target aquatic macroinvertebrates has been documented in many reports. Generally, the reports describe exposures of immature insects or immature and adult crustaceans for 2, 4, and up to 28 days of continual exposure to the chemical. The animals used in these tests are often collected from natural habitats and only a few, such as chironomids or amphipods are routinely reared in the laboratory. The need for and expense of collecting and holding the animals in the laboratory, coupled with seasonal availability, are a major restraint of research which determines the toxicity of pesticides to non-target animals. Also, emphasis on a single portion of the life cycle does not provide a complete picture of the potential for unwanted effects.

The effect of an insecticide on the embryonic developmental stage has not been extensively studied. Friesen (1979) used the eggs of the burrowing mayfly *Hexagenia rigida* McDunnough in exposures to methoxychlor and found that the eggs were sensitive to methoxychlor and could be a useful toxicity test material. This study showed a potential for using embryological material for toxicity testing. However, this type of test is limited by the seasonal availability of eggs and the extended developmental times for many species.

Chironomids provide life-cycle characteristics which remove the short-comings of other insect orders and many may be candidates for a rapid screening method for determining toxicity. This report describes an egg exposure system and the results of exposures of the eggs of the chironomid *Paratanytarsus parthenogeneticus* to five pesticides.

MATERIALS AND METHODS

A colony of *Paratanytarsus parthenogeneticus* (Freeman) has been maintained at this laboratory for over 10 years. The laboratory life cycle of this insect has been recently described by Anderson (1980). A unique feature of the life cycle is the parthenogenetic mode of reproduction which is reflected in the specific name. A detailed description of the insect is given in Sasa (1979). Routine rearing procedures are described in Anderson (1980). At temperatures of 20–22°C a life cycle is completed in about two weeks.

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Eggs are deposited on the surface of the water and immediately begin absorbing water which results in full sized eggs surrounded by a sticky sheath. The mass is cylindrical, contains up to 250 eggs, and can be as long as 5 mm. The eggs are arranged in a linear manner through the center of the sheath. This arrangement allows, with the use of a stereomicroscope, easy counting and monitoring of the embryo development.

Exposure Procedure.   One goal of these tests was to expose the eggs from deposition to hatch to simulate the extreme exposure situation. To accomplish this goal, freshly-emerged adults were collected from the rearing colony with a mouth aspirator and transferred to a 250 ml Erlenmeyer flask which contained about 75–100 ml of a pre-selected concentration of pesticide prepared in Lake Superior water. The flasks were checked at no greater than 60-min intervals. At each check, egg masses deposited in that time period were removed and individually transferred to 150 x 20 mm test tubes which contained about 10 ml of the same water. The test tubes were placed in a water bath at 22°C for incubation.

At 22°C, normal development is completed and hatching has begun within 52 h after deposition. In the exposures, control and exposed egg masses were usually assessed for development at both 24 and 48 h. The extent of development at each check was compared to the control and to a set of previously prepared normal development photographs taken at 1-h intervals. Delayed development and hatch success were used as end-points. All assessments were accomplished using either a Zeiss Universal photomicroscope or a Bausch and Lomb stereomicroscope.

Toxicant Preparation and Analysis.   Because the sensitivity of chironomid eggs to pesticides was unknown, each chemical exposure series started with a saturated or near saturated water solution. The saturated solutions were diluted with Lake Superior water and the dilutions were tested until a concentration was found that did not affect development or hatch. The saturated solutions were prepared using either a modified saturator (Veith and Comstock 1975) system or by mixing in lake water with the aid of a stirrer. No solvents were used in either method.

Samples for analysis were taken for each pesticide and analyzed according to procedures described by Thompson (1974). To determine the accuracy of the extraction procedures, known amounts of each pesticide were added to a control water sample before extraction. All reported values are based on measured concentrations not corrected for recovery.

RESULTS AND DISCUSSION

Five pesticides, representing three classes, were tested: a synthetic pyrethroid. AC 222 705; two chlorinated, pentachlorophenol and endrin; and two organophosphate, fenitrothion and malathion. The data from these exposures show a wide range in sensitivity of the hatching process and a low sensitivity of the development process unless the embryos were exposed to unnaturally high concentrations.

Fenitrothion was the only pesticide which showed deleterious effects at concentrations which approximate traditionally obtained 4-day LC50 values (Fig. 1). The highest exposure concentration that resulted in > 95% hatch was 13 µg/l. The next concentration tested. 20 µg/l, resulted in about 85% hatch. Concentrations greater than 20 µg/l resulted in a disruption of the hatch process and none of the fully developed larva survived.

Concentrations of fenitrothion substantially higher than 20 µg/l were tested and apparently normal development occurred until concentrations greater than 7400 µg/l were used. Within the limits established by the concentrations tested, no partial effects were seen. At 7400 µg/l, or less, 100% of the embryos developed normally but did not hatch. At 10,000 µg/l, none of the eggs developed. Effects seen at the 24-h check included a drawing in of the vitelline membrane from the chorion, a loss of cellular integrity in the embryo, and a swelling of the chorion.

The other organophosphate pesticide tested, malathion, did not show toxic effect until the concentration exceeded 350 µg/l (Fig. 1). No embryos hatched at 3500 µg/l. Because of the wide difference between 350 and 3500 µg/l it is not possible to define the exact
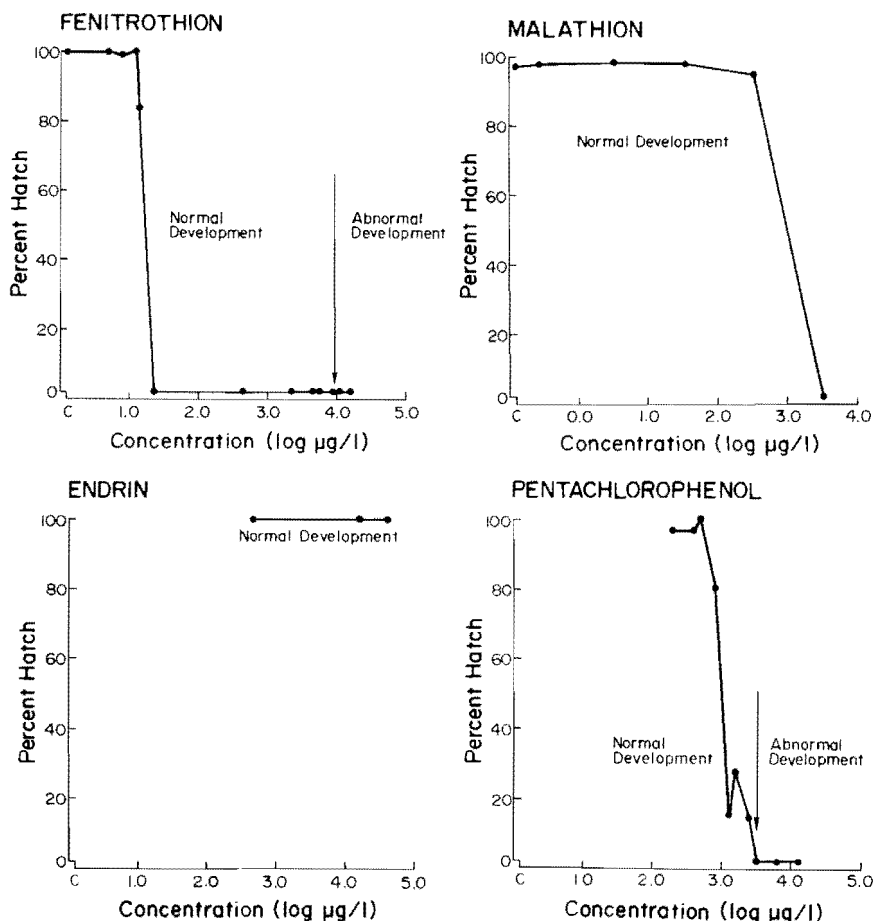
Fig. 1. Effect of fenitrothion, malathion, endrin and pentachlorophenol on hatch and development of eggs of *P. parthenogeneticus*.

effect concentration. However, a conservative comparison of the safe (350 µg/l) concentration with data from 4-day exposures of other invertebrates (Johnson and Finley 1980) show that for malathion, the embryo exposure would not be a sensitive screening test. In 96-h tests with 16 invertebrates, only two (*Atherix variegata* Walker and *Asellus* St. Hilaire) exceeded 350 µg/l. Eleven of the 16 species had 4-day LC50 values of 10 µg/l or less. In our exposures, only four out of the 1534 eggs exposed had abnormal development so malathion, at concentrations up to 3500 µg/l, did not affect embryo development.

Two chlorinated pesticides were tested and pentachlorophenol was toxic at a lower concentration than endrin (Fig. 1). This was a surprising result because endrin has been shown to be toxic to fish and invertebrates at concentrations of 1 µg/l or less in 4-day exposures (Anderson and DeFoe 1980, Grant 1976). The no-effect-on-hatching concentration for pentachlorophenol was between 500 µg/l which did not affect hatch and 1200 µg/l which resulted in only 19% hatch. Only 49% of the embryos developed normally at 2500 µg/l and > 93% developed normally at 1600 µg/l or less. These pentachlorophenol
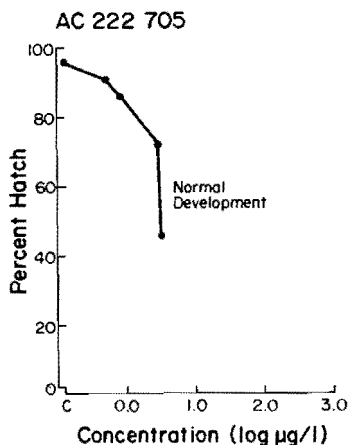
Fig. 2. Effect of AC 222–705 on hatch of *P. parthenogeneticus*.

concentrations are substantially higher than 4-day values for fish which range from 32 to 205 µg/l (Johnson and Finley 1980).

The synthetic pyrethroid AC 222 705 was the most toxic chemical tested (Fig. 2). At 3.1 µg/l, 46% of the embryo hatched. At 0.55 µg/l, the hatch was 90%. Although this pesticide was the most toxic, a comparison of our effect concentration with other fish and invertebrate 4-day exposure data show that the embryos were not particularly sensitive. For example, the fathead minnow *Pimephales promelas* Rafinesque has a 4-day LC50 value of 0.22 µg/l and a predicted chronic no-effect concentration between 0.03 and 0.07 µg/l (Spehar et al. 1983). No effect on development was seen at our exposure concentrations.

The results of this study show that only one of the pesticides, fenitrothion, affected a critical developmental event at a concentration which was similar to that affecting survival of other aquatic animals. Because of the low correlation between embryo-sensitivity and 4-day LC50 values of other aquatic animals, it is doubtful that this embryo exposure system would be useful as a surrogate test for determining the sensitivity of aquatic animals to pesticides.

This embryo exposure technique may not be a sensitive test for pesticides. However, the chironomid is easy to culture, the eggs are easy to count, and development can be easily followed from deposition to hatch with simple equipment. These attributes of the system point toward its application to other toxicity situations.

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