EVALUATION OF A STATIC LC50, PARTIAL CHRONIC, AND CHRONIC BIOASSAY TOXICITY TEST ON *TANYTARSUS DISSIMILIS*

BY

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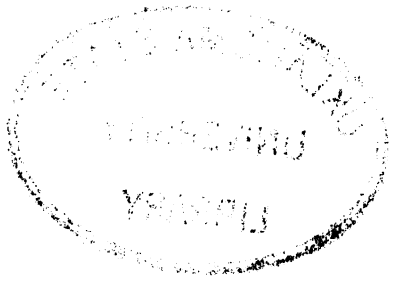

PREFACE

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CHAPTER I

INTRODUCTION

The Toxic Substances Control Act of 1976 states that our environment contains many chemical substances and mixtures, and that some of these present unreasonable risks to the environment and human health (Brungs and Mount 1978). Of the man-made chemicals in our environment, it is estimated that 90% may be hazardous to man (Wilson and Fraser 1977). Many of these potentially toxic chemicals enter the aquatic environment. Aquatic organisms become contaminated by ingestion or uptake via respiration or through the skin (Leegangh 1978). These chemicals originate primarily from industry and agriculture.

Contamination of aquatic systems necessitates judgements and decisions concerning the risk in manufacture, transport, use, and disposal of chemicals. Recently, numerous laboratories and research facilities have begun to document chemical toxicity. Field testing of toxicity is difficult to interpret because environmental conditions also influence responses of organisms to toxicants (Maciorowski and Clarke 1980). It is not possible to reproduce all the environmental conditions of an organism during testing, but results of laboratory tests can be used in assessing the toxicity of a substance (Cairns, Dickson, and Maki 1978). Interpretation of sublethal data from laboratory testing can be difficult because not all changes are adverse or beyond reversal. Results of laboratory testing must therefore be examined carefully

before being used for judgements or regulatory decisions.

Many methods for laboratory toxicity bioassays exist. One of the most widely used is the LC50. It is a measure of the concentration of chemical required to kill one-half of the test organisms within a given time period (Sprague 1969). Another measure of toxicity is the chronic bioassay. Chronic effects are usually induced by lower levels of a toxicant and occur over an extended time period. This type of bioassay enables measurement of effects of toxicants on parameters such as growth, reproduction, metabolism, and tissue damage. Partial life cycle tests involve studying the affects over a portion of the life cycle of an organism. Results of such tests could be used as components of a standard, multifaceted study to determine toxicity of chemicals.

Since aquatic invertebrates possess considerable physiological and ecological diversity, a variety of responses to toxicity are possible (Maciorowski and Clarke 1980). Chironomids are a major component of the benthic macroinvertebrate assemblage of many freshwater ecosystems (Walshe 1951) and have been used commonly in aquatic toxicity bioassays (Derr and Zabik 1972, Thornton and Wilhm 1974, Wegner and Hamilton 1976, Wentsel et al. 1977, Anderson 1980). One species that has considerable promise for use in bioassays is Tanytarsus dissimilis. This species reproduces parthenogenetically, which increases the ease of rearing (Anderson 1980). Acute effects of toxicants may be determined with this species as well as chronic effects on hatching, molting, pupation, and oviposition. Although the organism appears promising as a laboratory animal, few evaluations of various tests have been conducted.

One group of chemicals known to cause adverse effects on organisms are the phenols. Chlorinated phenols are used in manufacturing

antiseptics and disinfectants and have been found in raw domestic sewage (EPA 1980). The unpleasant taste or odor of tap water may be caused by halogenated, specifically chlorinated phenols (Campbell et al. 1958). Chlorinated phenols in the aquatic environment are concentrated by organisms. Exposure to man results from consumption of contaminated shellfish and drinking water (EPA 1980). One chlorinated phenol, 2,4,6-trichlorophenol, is a suspected carcinogen (Sittig 1981). Further toxicity testing for this compound is needed to assess the hazard to aquatic systems and to man.

Another compound needing further study is acenaphthene, a polynuclear aromatic hydrocarbon (PAH). Both trichlorophenol and acenaphthene are listed by the U.S. Environmental Protection Agency (EPA) as Priority Pollutants (1976) indicating a need for evaluation of toxicity. Low molecular weight (Anderson et al. 1974) and volatile aromatic hydrocarbons (Moore and Dwyer 1975, Lee et al. 1978, Lee and Nicol 1978) are considered to be the most toxic components of petroleum. Acenaphthene is relatively volatile and has a low molecular weight of 154. It is found in coal tar as a result of the high temperature carbonization of coal. It has also been detected in the effluent from petrochemical, pesticide, and wood preservative industries. Most of the toxicity data on this compound is for saltwater organisms. No information concerning bioconcentration in aquatic organisms is available (EPA 1980). Guidelines and standards for this compound are nonexistent. More toxicity data is needed before guidelines can be provided.

The objective of this study is to determine the effects on Tanytarsus dissimilis of 2,4,6-trichlorophenol and acenaphthene on the

following:

1. the LC50 as determined by a static bioassay;
2. hatching and larval survival as determined by a partial chronic bioassay; and
3. hatching, larval survival, and reproduction as determined in a continuous flow system.

Based on these studies, an evaluation of Tanytarsus dissimilis as an indicator species for toxicity studies will be made.

CHAPTER II

REVIEW OF LITERATURE

Chironomids

Taxonomy

The chironomid Tanytarsus dissimilis Joh. belongs to the Order Diptera, the true flies (Johannsen 1937). Currently, the species is also called Paratanytarsus sp. and the taxonomic status is unclear (EPA 1981). Adults of the family Chironomidae are characterized by a single pair of membranous mesothoracic wings. The hind wings are reduced to clublike balancing organs called halteres (Atkins 1978). The head is usually well defined and a pair of antennae are present. Larvae, like adults, vary in form, but generally are cylindrical, and soft-bodied. No thoracic legs with true segmentation are present (Barnes 1980).

Structure and Function

Members of the subfamily Chironominae possess two life forms. The larvae of Tanytarsus dissimilis have four instars (Anderson 1980). Beginning with first-instar larvae, the lengths are 0.87, 1.5, 2.4, and 3.75 mm, respectively (Nebeker 1973). The body is composed of a head, thorax, and abdomen (Simpson and Bode 1980). Pairs of prolegs exist on the first thoracic and last abdominal segments (Pennak 1978). Claws of the posterior prolegs are arranged in the form of a horseshoe (Johannsen

1937, Simpson and Bode 1980). Labial plates, used for feeding (Cavanaugh and Tilden 1930), and a mandible with a pre-apical comb (Johannsen 1937) occur on the head region. Adults are about 1.5 mm in length. The abdomen and thorax are light green with a darker shade along the sides of the abdomen (Cavanaugh and Tilden 1930).

Tanytarsus dissimilis is herbivorous (Pennak 1978) and appears to feed continuously. They construct fragile tubes composed of algae, fine silt, or sand grains cemented together with a salivary secretion. These tubes are open at both ends and the larvae produce a current of water by rapid undulations of the body. Almost four-fifths of the body may protrude from the end of the case when feeding. By pressing the posterior prolegs against the dorsal surface of the tube, they can remain within the rim. The larva sways from side to side as if selecting its food. The head is stretched outward and then withdrawn with a downward raking motion. Anterior prolegs assist in bringing food to the case (Cavanaugh and Tilden 1930). Adults do not feed.

In most chironomids, respiration is by simple diffusion through the general integument (Borradille and Potts 1961). The skin is supplied with a rich network of fine tracheae. Spiracles are absent, but anal gills are present on the penultimate abdominal segment (Pennak 1978). These anal papillae are outgrowths of the tracheal gills and were formerly called gills. Water (Barrington 1967) and ions (Prosser and Brown 1950, Barrington 1967) can pass through these organs. Salt absorption by the papillae is inhibited by anticholinesterases. Isolated anal papillae swell in tap water and constrict in Ringer solution (Prosser and Brown 1950). Larvae of Chironomus can take up chloride from dilute solutions, but are unable to do this if the anal

papillae are destroyed (Barrington 1967).

Six to nine forms of hemoglobin have been found in aquatic larvae of the genus Chironomus (Hoar 1975). The hemoglobin appears to be necessary at low oxygen concentrations, but not at high. Chironomids alternate respiratory and feeding activities and the hemoglobin store may provide oxygen during the interruption of respiration for feeding. Under low oxygen conditions (7.5-9.0% saturation), feeding may be suspended and the respiratory movements continuous. Oxygen consumption of Tanytarsus is unaffected by CO down to about one-quarter to one-half air saturation. At air saturation, CO causes no alteration in respiratory movements (Prosser and Brown 1950). Pupae of Tanytarsus characteristically have simple, slender, thoracic respiratory organs (Johannsen 1937).

Sensory cells called scolopidia are the basis of the sensing system for dipterans. They are similar to hair cells, but do not protrude from the body surface. Clusters of scolopidia form chordotonal organs pm the legs or Johnston's organ on the second antennal segment. The leg organ picks up vibrations from the ground or substratum while Johnston's organ is used to register antennal movements or positions which act to maintain flight patterns or normal swimming positions. Johnston's organ is most highly developed in the Chironomidae where it contains several thousand closely crowded sensilla (Hoar 1975). The brain which coordinates input from such organs is located in the thoracic region of the larvae (Johannsen 1937).

In the fourth instar larvae, specific chromosomal "puffs" appear at the time of molting in response to ecdysone. These "puffs" are considered to be the sites of specific mRNA formation, a key link in

protein synthesis during metamorphosis (Hoar 1975).

Life Cycle

Most chironomids reproduce sexually, but parthenogenesis has been reported in the subfamily Tanytarsini and T. dissimilis (Edwards 1963). The egg mass of T. dissimilis is cylindrical, up to 5 mm long, and with a single row of eggs placed diagonally through the center. The eggs, which are deposited in two separate masses usually 1 day apart, are protected by a sticky covering. The second egg mass is the smaller of the two. Only a few masses have less than 30 or more than 200 eggs. Egg development is completed in about 69 h at 18° C. The whole mass usually hatches in 4 h with a hatch success of greater than 99%.

The newly hatched larvae are planktonic for a day before settling to a surface and building a tube (Anderson 1980). As the larvae grows, the tube is elongated by adding material to the end nearest the food source. After development through four instars has been completed, a pupa is formed in the larval case. When pupation is almost over, the pupa swims freely to the surface of the water. At this time it resembles a newly hatched tadpoles in appearance and movement. The pupal skin splits length wise and the adult emerges (Cavanaugh and Tilden 1930). Eggs are usually laid within 48 h of emergence. At 15-18° C, the time from egg hatch to pupation is about 18 days. At 22-28° C, the development is completed in 12-14 days (Anderson 1980).

Culturing

Until 1965, no efficient techniques existed for rearing midges because of low survival from egg hatch to the adult stage (Branch 1923,

Sadler 1935). Biever (1965) developed a successful method for rearing some chironomid species. He used 950 ml clear styrene plastic containers covered with mesh plastic screen to prevent the escape of emerging adults. Fine sand and water were placed in the containers and continuous aeration was supplied. Newly hatched larvae were placed in a smaller rearing unit. Newly emerged adults were transferred to a screened cage where mating occurred and a container of water in the cage provided a place for the females to oviposit eggs. Biever achieved an average survival rate of 82% with this method. Derr and Zabik (1972) used a mixture of chicken feed and paper towels to provide both food and substrate. Other containers have included coated, galvanized metal trays (Thornton and Wilhm 1974) and glass aquaria (Anderson 1980).

Powdered milk, chicken manure, yeast plus Purina laboratory chow, and several commercial dog foods have been used as food. Biever (1965) selected chocolate dog kisses by Hartz Mountain Products. This method was successful for Chironomus attenuatus and showed promise for many other midge species. Algae, powdered cereal leaves, and flake fish food have also been used successfully (Biesinger and Christiansen 1972, Credland 1973, Nebeker 1973, Anderson 1980).

Development from egg to adult varies with temperature. Development requires 16 days at 21° C, 13 days at 25° C, and 12 days at 28° C. Higher temperatures do not increase the rate of development. No adult emergence occurs at 33 or 36° C, and no embryonic development will occur in the eggs at 36° C. Midge emergence is good in constant light conditions from 23-26° C (Nebeker 1973). A photoperiod of 16 h daylight has been used successfully with T. dissimilis. Decrease in photoperiod has been used in some studies to reduce algal growth in cultures

(Thornton and Wilhm 1975). Other factors also influence development. Experiments with Tanytarsus dissimilis show that the species cannot survive below a pH of 5.5 (Bell 1970) and can tolerate dissolved oxygen levels as low as 0.6 mg/l at 18 C (Nebeker 1973).

Use as a Biological Indicator

Chironomids are an important item in the diet of fish and other predatory animals (Walshe 1951). Aquatic invertebrates, including the chironomids, are morphologically, physiologically, and ecologically diverse. They provide a wide range of responses to toxicants which can be used for toxicity testing (Hart and Fuller 1974). Easily identifiable life stages, a short laboratory life cycle, and a fairly large data base enhance the usefulness of chironomids for toxicity testing. In general, most facilities for aquatic invertebrate bioassays can be housed in less than 50% of the space required for similar fish toxicity testing (Anderson 1980).

Toxicity Testing

Aquatic invertebrates have been used in toxicity testing for about 50 years (Anderson 1980), but they have not been used as frequently as fish in laboratory toxicity tests (Maciorowski and Clarke 1980). The 1975 edition of Standard Methods for the examination of Water and Wastewater contains tentative bioassay procedures for chironomids. Eight species of chironomids have been commonly used. Numerous protocols have been used and it is recommended that test methods are developed based on the species involved (Anderson 1980). The U.S. Environmental Protection Agency has summarized acceptable methods for acute toxicity tests with

invertebrates (1975). Toxicity tests using chironomids can be short exposure, partial life cycle, or complete life cycle or chronic (Anderson 1980).

Short Term Tests

Various species of the genus Chironomus have been used in short term bioassays. Hilsenhoff used Chironomus plumosus fourth instars to determine toxic effect concentrations of 16 pesticides. Larvae were allowed to burrow in mud in slate-bottom aquaria before adding the insecticide. After 5-7 days the mud was strained and live larvae counted. Survival of adult Chironomus attenuatus, exposed to phenol, decreased substantially as pH increased (Thornton and Wilhm 1974). EC_{50} values were determined for fourth instar Chironomus tentans exposed to 3-trifluoromethyl-4-nitrophenol (TFM) and 2', 5-dichloro-4'-nitrosalicylanilide (Bayer 73) (Kawatski et al. 1975). The EC_{50} was defined as the concentration of toxicant, based on total formulation weight that immobilized 50% of the test organisms within the prescribed period of exposure. Calculated values for TFM in hard water were 8 h -6.47, 24 h -4.54, 48 h -2.53, 72 h -1.19, and 96 h -0.998 mg/l. Exposure of larvae to Bayer 73 in hard water produced EC_{50} values of 8 h -1.01, 24 h -0.640, 48 h -0.457, 72 h -0.360, and 96 h -0.295 mg/l. Mulla and Khasawinah (1969) exposed fourth instar larvae of Goeldichironomus holoprassinus (Rempel), Tanypus grodhausi (Sublette), and Chironomus sp. in waxed paper cups with a sand substrate and tap water. A pesticide was added to the water and mortality assessed after 24 h. This method was similar to one recommended by the World Health Organization in 1957 for evaluating mosquito larvicides.

Tanytarsus dissimilis Johann. was used to test the acute toxicity of silver (Lima et al. 1982). Third instar larvae were allowed to acclimate for 48 h. Five exposure concentrations of silver were slowly added until 500 ml had flushed through each chamber. The remainder of the test (24 h) was static. Silver exposure concentrations were 371, 842, 1870, 3350, and 7190 µg/l. Mortality was calculated; death was defined as lack of movement after gentle prodding. The resulting LC_{50} concentrations and 95% confidence intervals were 24 h - 5030 µg/l (4470-5650) and 48 h - 3160 µg/l (2490-4010).

The sensitivity of aquatic invertebrates to toxicants is partially dependent on the test conditions. Temperature is the most important environmental factor controlling rates of biological processes and therefore would be expected to influence toxicity tolerance (MacLeod and Pessah 1973). Acclimated salmon (Salmo salar) were about 50% more tolerant of zinc at 19° C than at 3° C (Hodson and Sprague 1975). In another study, the 96 h TL_m values for mercuric chloride demonstrated a decrease with increasing temperature (MacLeod and Pessah 1973).

Sensitivity of aquatic organisms to toxicants can be affected by ambient dissolved oxygen concentrations. Periodic low dissolved oxygen concentrations (2 ppm for 2 h/day) decreased the tolerance of bluegill to zinc chloride, naphthenic acid, and potassium chloride (Cairns and Scheier 1957) in a 96 h TL_m. No change was recorded for potassium dichromate. Snails were also tested for tolerance to periods of low dissolved oxygen and exhibited a similar decrease for naphthenic acid and potassium cyanide (Ibid.).

Silver is one of the most toxic metals to freshwater aquatic organisms (Lima et al. 1982). Acute toxicity of silver to rainbow

trout, fathead minnows, and Daphnia has been shown to decrease with increasing water hardness (Davies et al. 1978). In one study the 96 h LC_{50} of total dissolved copper varied from 20 µg/l in soft acidic water to 520 µg/l in hard alkaline water (Howarth and Sprague 1978).

Partial Life Cycle Tests

Partial life cycle tests combine the advantages of the acute and chronic tests. These intermediate tests expose the organism through at least one developmental event such as hatching or molting. Because of the great variability in methods, a general description is difficult.

Partial life cycle testing enables use of many parameters for evaluating toxicity. Cole and Wilhm (1973) exposed eggs of Chironomus attenuatus to five concentrations of phenol for 21 days and measured the effect on fourth instar larvae oxygen consumption. Increase in the phenol level caused an increase in oxygen uptake and a decrease in the ash-free weight of the larvae. Eggs of Chironomus riparius Meigen were exposed to graduated aqueous solutions of calcium sulfide ranging from 1.0 -100 mg/l (Wegner and Hamilton 1976). Test solutions were replaced every 24 h. After 72 h the median lethal tolerance level (TL_m) was established at 9.15 mg/l. Little or no effect was observed in the embryos at the three lowest dilutions. Development of the fertilized eggs at 5.6 and 10.0 mg/l was normal to the point of hatching, but the numbers of larvae that hatched successfully were much lower than the controls. Second instar chironomid larvae were used to determine the effect of heavy-metal-contaminated sediment on larval survival or adult emergence (Wentsel et al. 1977). Larval survival was compared with data from a control sediment.

The toxicity of four heavy metals was evaluated using a partial life cycle test on T. dissimilis (Anderson et al. 1980). The LC_{50} concentrations for cadmium, copper, zinc, and lead were 3.8, 16.3, 36.8, and 258 µg/l, respectively. Growth was not reduced at concentrations below the LC_{50}. The LC_{50} values were as much as 1600 times lower than other similar exposures reported in the literature. The lower values may have resulted because Anderson exposed the organism during important life cycle events.

Life Cycle Tests

In life cycle tests an organism is exposed through all its life stages. The usual method begins with exposure of eggs and continues through the adult stage, egg deposition, and hatching. Chironomus tentans was exposed to a highly organic sediment from egg to adult stage (Derr and Zabik 1972a). Larval development was observed during the test and adults were collected on the last 5 days of the 30 day test period. Laboratory microcosms were used to measure the effects of pH, phenol, and sodium chloride on the survival of the life stages of Chironomus attenuatus (Thornton and Wilhm 1974). Other parameters measured were lipid and protein content of fourth instar larvae and caloric content of third and fourth instar larvae and adults. A continuous-flow system was used to maintain constant treatment levels. The system consisted of 24 polypropylene head tanks which supplied two sub-units each by constant gravity flow. The volume of water in the sub-units was replaced every 0.66 days. Each head tank was cleaned and replenished every 2 days to ensure a constant flow. Many chronic tests are completed using a similar type of continuous-flow system, often known as a dilutor. The

midge, Chironomus tentans, was exposed from egg through adult to varying concentrations of DDE (Derr and Zabik 1972b). At any concentration of DDE in the water, accumulation of DDE increased with exposure time to a level approximately 20,000 times over that in the water.

In 1967, Mount and Brungs introduced a simplified dilutor system for maintaining a series of constant concentrations of a material in flowing water. Their application of the system was in fish toxicology studies. Simultaneous dilution of one toxicant concentration provided an accurate way to achieve dilutions of 50% or less. Most continuous-flow systems used since the introduction of this proportional dilutor have been modifications of this idea. Garton (1980) modified a serial dilutor system in which one source of toxicant is diluted by successive additions of water to produce a range of toxicant concentrations. The unit consists of a dilution apparatus where toxicant is added to the first chamber of a multi-chambered dilutor box. This toxicant is mixed with fresh water from a headbox to produce the highest concentration desired. Excess solution from the first chamber spills through a notch into the next dilution chamber and becomes the toxicant source for this chamber. Similar dilution and overflow occur in this and each succeeding chamber. A small amount of toxicant is lost to the overflow at the end of the dilutor box.

Trichlorophenol

Background

Phenol was first isolated in 1834 by F. Runge from coal tar. Its manufacture from this natural source began in 1860. Phenol is a poisonous substance which mainly effects the central nervous system.

Chlorination of phenol in an aqueous solution rapidly yields large quantities of 2,4,6-trichlorophenol (TCP) (Hawley 1981). The main property of the chlorophenols is a germicidal activity greater than that of the parent compound. Chlorophenols have strong persistent odors, are irritating, and have toxic effects. The acidic character of phenol is enhanced by chlorination (deBussy and Longman 1972). The 2,4,6-TCP isomer is listed as a priority pollutant by the U.S. Environmental Protection Agency (1976).

Physical Properties

In pure form at room temperature, 2,4,6-trichlorophenol exists as a yellowish, needle-shaped crystal. Other properties include a mol. wt. of 197.46, m.p. 68° C, b.p. 244.5° C, and specific gravity of 1.490 at 75/4° C (Hawley 1981). The vapor pressure of 2,4,6-TCP is 1mm Hg at 76 (Weast 1978). Solubility at 25° C is 800 mg/l and considerably higher at 96° C, 2430 mg/l (EPA 1980). Higher solubility also occurs in organic solvents such as ethanol and dimethylformamide. Solubility in water is listed as slight (Weast 1978).

Uses and Distribution

Trichlorophenol is used in the manufacture of antiseptics, bactericides, fungicides, and germicides. Its use in the formulation of wood and glue preservatives and as an anti-mildew agent for textiles is also common (Sittig 1981). This chlorinated phenol is found in industrial and petrochemical effluent. It is also responsible for the unpleasant odor and taste sometimes present in tap water (Campbell et al. 1958, EPA 1980). Phenols are common in raw domestic sewage at 70 to

100 µg/l. Complex phenols are partially released by bacterial action in sewage treatment trickling filters. Human exposure to trichlorophenol can result from ingestion of animals that have grazed in areas exposed to chlorophenoxy acid herbicides 2,4,5-T or Silvex. Residues of these herbicides can be between 100-300 ppm (EPA 1980). Exposure to other animals can occur by metabolic degradation of other chemicals (Kohli et al. 1976, Foster and Saha 1978). Even plants can metabolize another chemical to form a trichlorophenol metabolite. Corn and pea plants can metabolize pentachlorocyclohexane to the 2,4,6-, 2,3,5-, and 3,4,6-trichlorophenol isomers.

Toxicity Studies on Trichlorophenol

Acute toxicity of the 2,4,6-isomer was determined to be 276 mg/kg in rats (intraperitoneal LD_{50}) (Farquharson et al. 1958). Injection intraperitoneally produced convulsions. Using the Ames test, 2,4,6-trichlorophenol was found to be non-mutagenic (Rasanen et al. 1977). It is a suspected carcinogen and therefore the recommended concentration in water is zero. Since zero levels are realistically unattainable, the criterion levels are based on prevention of undesirable organoleptic qualities. The level recommended for the 2,4,6-trichlorophenol isomer is 2.0 µg/l (EPA 1980).

Acenaphthene

Background

This compound is also known as 1,8-ethylenenaphthalene and 1,2-dihydroacenaphthalene (Sittig 1981). The chemical formula is $C_{12}H_{10}$. Oxidation yields acenaphthenequinone and naphthalic acid (Hey 1966).

Acenaphthene is listed as one of the priority pollutants by the EPA (1976).

Physical Properties

Acenaphthene is a white, crystalline solid at room temperature. This compound melts at 95-97° C (Sittig 1981). It is a polynuclear aromatic hydrocarbon with mol. wt. of 154 and b.p. of 178° C. Vapor pressure is less than 0.02 mm Hg (Lidner 1931). This compound is listed as soluble in water, but solubility is enhanced by organic solvents such as ethanol, toluene, and chloroform. The solubility is low in water but its presence may be significant due to possible adsorption on particulates (EPA 1980).

Uses and Distribution

Acenaphthene occurs in coal tar produced during the high-temperature carbonization of coal. Petroleum refining and shale oil processing are additional sources of acenaphthene (Vershueren 1977). It is used as a dye intermediate, an insecticide, fungicide, and in the manufacture of some plastics. Cigarette smoke and gasoline exhaust condensates also contain acenaphthene (Sittig 1981). Natural sources include water and air (Verschueren 1977). Organoleptic effects in water have been attributed to acenaphthene at concentrations of 0.02 to 0.22 mg/1 (EPA 1980). Incidence in food has only been indicated in one study (Onuska et al. 1976).

Toxicity Studies on Acenaphthene

Little is known about the toxicity of this compound to humans. It causes irritation to skin and mucous membranes and may induce vomiting if swallowed in large quantities (Sax 1975). Information concerning aquatic toxicity of acenaphthene is also sparse. The freshwater acute value for bluegill sunfish is 1700 µg/l or an adjusted LC_{50} (96 h) of 929 µg/l. Saltwater acute toxicity for the sheepshead minnow was 2230 µg/l. The adjusted EC_{50} (48 h) for Daphnia magna is 34,900 µg/l and calculated value for invertebrates is 1700 µg/l (EPA 1980). Data on toxic effects to mammals is also limited. A study with rats and mice produced an LD50 of 10 g/kg and 2.1 g/kg, respectively (Knoblock et al. 1969). It was noted in this study that more damage to the kidney and liver resulted from subacute dosage than acute.

The most studied aspect of acenaphthene effects is its ability to produce change in nuclear and cytoplasmic material in plants and microbes (EPA 1980). No teratogenicity data is available and further study is needed to confirm carcinogenicity (Sittig 1981). With the current lack of data on acenaphthene effects, criterion for setting water quality standards cannot be established (EPA 1980).

CHAPTER III

MATERIALS AND METHODS

Cultures

The original laboratory population of T. dissimilis used in the present study was established from eggs obtained from the Environmental Protection Agency at Duluth, Minnesota (1981). The stock culture was raised in 19 liter glass aquaria supplied with 6 liters of aged tap water and light aeration. A 16-h light photoperiod was provided by two fluorescent lamps. Tap water for culture was aged 48 h in a Nalgene carboy before use. All aquaria had glass covers to retain adults. After approximately 3 months, tanks were cleaned and restarted. During the length of the experiment, 60 different rearing units were maintained.

Cultures were fed a mixture of Purina trout-fry granules and Cerophyl brand rye grass (Biesinger and Christensen 1972). Food consisted of 10 g trout chow and 0.5 g cerophyl in 300 ml distilled deionized water. Rate of feeding was about 2 ml/liter every other day (Anderson 1980).

Eggs were obtained by isolating adults from the stock cultures 1 day prior to beginning an experiment. Each adult was transferred from the aquarium with an aspirator to vials containing 10 ml of aged tap water and plugged with cotton. The aspirator was made from a glass tube, a small piece of screen, and a flexible tube. The screen was

placed over one end of the glass tube and that end was fitted into a flexible tube. A gentle inhalation on the free end of the flexible tube sucked the insects into the glass tube. Generally, eggs were found at the bottom of the vials the next morning. For chronic and partial chronic experiments, groups of 20 eggs were separated from the string of eggs with forceps and a dissecting scope. Larvae for acute experiments were obtained from the stock aquaria with pipet.

Cleaning

All new equipment was washed with detergent and then rinsed with water, acetone, water, 10% hydrochloric acid, and then twice with deionized water. At the end of every test, chambers and equipment were emptied, soaked and washed with detergent, rinsed with hot water, cleaned with acids or solvents, and then rinsed twice with distilled water.

Acute Exposures

Short term experiments were conducted to determine LC50 values for T. dissimilis exposed to trichlorophenol and acenaphthene. Second and third instar larvae were used. The acute tests were conducted in standard crystallizing dishes (150 x 75 mm). Crystallizing dishes were used because their flat bottom and low sides permit easy handling and viewing under microscopes. Six concentrations and a control were replicated in each experiment. Nominal concentrations ranged from 3.1-100 mg/l for trichlorophenol amd 1.18-2.93 mg/l for acenaphthene. Dimethylformamide (DMF) was used to increase solubility of trichlorophenol. DMF has no effect on T. dissimilis at 1%

concentrations Ethanol was used as a carrier with acenaphthene because DMF interfered with the chemical analysis. Two LC50 tests of each chemical were conducted.

The acute experiments were conducted over a 4 day period. On day 1, 200 ml of aged tap water, food, and a sand substrate was added to each test container. The test containers were undisturbed for several hours to allow settling of the food and substrate. Then, ten second or third instar larvae from the rearing colony were transferred with eye droppers to each test chamber. Containers were then randomized and labelled for each toxicant level. On the second day, the toxicant was prepared in a 1 liter volumetric flask. The number of larvae in each experimental unit was counted. Dead organisms were removed and replaced with larvae from the stock tanks. Then, the overlying water was removed by carefully siphoning to a depth which just covered the larvae. Concentrations of the chemical to be tested were prepared by serial dilution and added slowly from separatory funnels. After each container had been filled with the toxicant, the larvae were again counted and a sample of exposure water from each test chamber was acquired for chemical analysis. On day 4, 48 h after the addition of the toxicant, live larvae were counted. The LC50 was determined by a computer program using probit analysis (Finney 1971).

Partial Life Cycle Tests

Partial experiments were performed to accumulate data on hatch success and larval survival of Tanytarsus dissimilis exposed to acenaphthene and trichlorophenol. These tests were conducted over a 6-7 day period. All experiments were started with eggs. Crystallizing

dishes (150 x 75 mm) served as test chambers. The carriers used in acute experiments were used in the partial tests.

On day 1, adults were isolated for egg deposition. On the second day, the toxicant was prepared in a 1 liter volumetric flask and the concentrations prepared by serial dilution. The given toxicant concentrations, sand, and food were added to each test chamber. After the food and sand had settled, water samples were collected for analysis. Groups of 20 eggs were randomly placed in the test chambers. On the remaining days of the test, each unit was observed for hatching. Number of surviving larvae was recorded on the last day of the experiment. Food was added during the experiment on day 4.

Nominal concentrations used were 9.08-70.0 and 4.3-55.0 mg/1 for trichlorophenol and dilutions of 12.96, 21.6, 36, 60, and 100% for acenaphthene. Each concentration was replicated and tests with each chemical were duplicated. Data on hatch success and larval survival were recorded. The dilution water was measured for pH (Corning Model 610 A), conductivity (Yellow Springs Instrument Model 33 Conductivity Meter), dissolved oxygen (Yellow Springs Instrument Model 54A Oxygen Meter), total hardness, alkalinity (Lind 1979), and temperature.

Life Cycle Experiments

Chronic testing was conducted using a proportional dilutor system constructed by Enviro Tox Co.; Easley, South Carolina. The system operated by gravity flow. Toxicant and dilution water were supplied from separate reservoirs. These solutions entered the system, were mixed in a chamber, and transported to the toxicant chamber which divided the mixture into a sequence of volumes. Solenoid valves

released the solutions into stainless steel mixing cups. The valves were controlled by a timing system. Simultaneously, dilution water was released into each mixing cup attaining the final concentrations of toxicant. The contents of each mixing cup then emptied through stainless steel 1 mm tubing into replicated test chambers. On the Enviro Tox dilutor system, cup 7 serves as the control while cup 6 contains the maximum concentration of chemical. The first cup contains the second highest concentration and the level of toxicant decreases through cup 5 which contains the lowest level. For the trichlorophenol testing, a plastic carboy for dilution water and a glass toxicant container were located above the dilution system. For acenaphthene, the toxicant container was replaced with a glass column. The test units were crystallizing dishes (150 x 75 mm) with a glass overflow tube attached. The dishes were covered with plexiglass lids.

Before each test, the dilutor system was operated for at least 3 days. During this pre-test period, samples were analyzed to establish chemical consistency. Physical characteristics such as temperature, flow, light intensity, dissolved oxygen, pH, hardness, and alkalinity were measured.

On the evening before beginning the test, eggs were obtained by collecting and transferring adults from the rearing colony to vials for egg laying. The next morning after an adequate number of egg masses were deposited, they were separated with forceps and a stereomicroscope into groups. Food was added and allowed to settle. Then, groups of 20 eggs were randomly placed in the test chambers. Procedure for the chronic test is shown in Table 1. The test was completed when all larvae had pupated or emerged. Life cycle tests for each chemical were

Table 1. Daily activities during life cycle tests with Tanytarsus dissimilis.

Day	Activity
1	Isolate adults from rearing colony
2	Add food (2 ml/l) to test containers, allow to settle
3	Observe for hatching, sample water
4	Same as day 3
5	Feed if tubes are visible, sample water
6	Feed
7	Count larvae, sample water
8	Feed
9	Same as day 7
10	Feed
11	Same as day 7
12	Observe for pupae and feed
13	Check for adults and remove if present, transfer all adults to vials for oviposition, sample water
14+	Check all test chambers daily until all adults have emerged or pupae or larvae are dead, count all eggs

duplicated and data on hatch success, larval survival, and reproduction were recorded. Samples of the exposure water were analyzed on alternate days to determine actual chemical concentrations.

Analytical Procedures

The concentration of trichlorophenol was measured on either a Hewlett-Packard Research Chromatograph Model 5750 with an electron capture detector, a strip chart recorder, and an H-P Integrator or a Tracor 560 Gas Chromatograph with a Hewlett-Packard 3390A Integrator. Injections were made with the oven at 240°C, the carrier gas was methane argon, and the regulator was set on "20". Extraction of trichlorophenol was a modification of a method by Giam et al. (1980). The sample volume of 1 ml was acidified with 6 M sulphuric aid and extracted with hexane. Actual concentrations were achieved using the following equations:

1. (Peak Area X m) + b = ng injected

2. $$\frac{\text{ng/µl X volume-hexane (µl)}}{\text{Sample volume (ml)}} = \text{ng/ml}$$

3. $$\frac{\text{Convert ng/ml to µg/ml; ng/ml} \times 10^{-3}}{\text{µg/ml = mg/l}}$$

Ether was used as a solvent in the original method. Methylene chloride was tried as a solvent instead of ether, due to safety. Since the solvent layer separated below the water layer, removal of the solvent necessitated passing through the water layer. This resulted in water being removed with the solvent which made quantification difficult. After raising the temperature to evaporate the water, toxicant was lost. Thus, hexane which is less dense than water was used as the solvent instead of methylene chloride. This method saved

considerable time, involved less glassware and solvent, and little chance existed of water mixing with the solvent.

Acenaphthene was measured on an Aminco-Bowman Spectrophotofluorometer with wave lengths of 291 (excitation) and 336 nm (emission) (Sawicki 1960). Although the analysis was quick and efficient, considerable problems were involved in preparing a saturated solution of acenaphthene. Solutions could be obtained by mixing the chemical and carrier in concentrated amounts, but the acenaphthene fell out of solution when diluted. A glass column, 85 cm in length and 6 cm in diameter, packed with glass wool to absorb the acenaphthene, was used to obtain a saturated solution. Five-ten g acenaphthene dissolved in 750 ml of acetone were added to the column. The acetone was aspirated and then aged tap water containing 1% ETOH was passed through the column at a flow of 100 ml/min. Samples were collected and analyzed on the spectro photofluorometer. Sufficient concentrations were achieved using this technique to perform the acute, partial chronic, and life cycle experiments with acenaphthene.

CHAPTER IV

RESULTS

Acute Experiments

Trichlorophenol

Nominal concentrations of trichlorophenol ranged from 7.78 to 100 mg/l and analyzed concentrations from 3.26 to 67.36 mg/l in the first successful experiment (Table 2). Mortality in the highest concentration was 85% after 24 h and less than 20% at the other concentrations. After 48 h, mortality was at least 30% in the four highest concentrations of trichlorophenol. No larvae survived at 67.36 mg/l. Tanytarsus dissimilis exposed to trichlorophenol for 48 h had LC50 values of 23.6 and 27.2 mg/l for the two replicates. The combined value was 25.4 mg/l (Table 3).

In a duplicate experiment the same nominal concentrations were used, but the analyzed concentrations ranged from 6.33 to 82.55 mg/l (Table 2). After 24 h, mortality was relatively low at all concentrations. After 48 h, mortality was 65% at 43.41 mg/l trichlorophenol and 100% at 82.55 mg/l. No mortality existed in the unit with the lowest concentration. LC50 values were 27.3 and 26.3 mg/l for the replicates and 26.8 for the combined data (Table 3).

Table 2. Nominal and analyzed concentrations of trichlorophenol and percent mortality of Tanytarsus dissimilis after exposures of 24 and 48 h.

Replicate	Trichlorophenol concentration (mg/l)		Mortality (%)*	
	Nominal	Analyzed	24 h	48 h
1	0	0	5	5
2	0	0	0	5
1	7.78	3.26	0	5
2	8.00	6.33	0	0
1	12.96	6.78	0	5
2	13.00	8.86	5	10
1	21.60	11.34	5	30
2	22.00	14.24	5	25
1	36.00	25.32	10	40
2	36.00	26.90	10	45
1	60.00	37.64	20	45
2	60.00	43.41	15	65
1	100.00	67.36	85	100
2	100.00	82.55	25	100

*n = 20

Table 3. 48 h LC50 (mg/l) and 95% confidence intervals for Tanytarsus dissimilis exposed to trichlorophenol and acenaphthene.

Date	Variable	Duplicates 1	Duplicates 2	Combined
		Trichlorophenol		
9-12 Dec 81	LC50	23.6	27.2	25.4
	95% CI	(15.2-41.5)	(6.9-0)	(10.0-204.4)
16-19 Dec 81	LC50	27.3	26.3	26.8
	95% CI	(20.2-38.7)	(19.2-37.9)	(21.7-33.8)
		Acenapthene		
19-22 Feb 82	LC50	1.62	1.65	1.63
	95%	(1.45-1.81)	(1.49-1.86)	(1.53-1.75)
23-26 Feb 82	LC50	2.46	2.14	2.35
	95%	(2.17-2.86)	(2.21-2.48)	(2.66-2.96)

Acenaphthene

A column apparatus was used to achieve saturated solutions of acenaphthene. The concentration of acenaphthene in the tube was 5 g in 800 ml of acetone and ethanol was used as the carrier. Analyzed concentrations in the first experiment ranged from 1.23 to 1.93 mg/l (Table 4). Mortality in the two highest concentrations was 75% after 48 h. Of the twenty organisms used, 17 survived in the most dilute solution. Larvae exposed to acenaphthene for 48 h had LC50 values of 1.62 and 1.65 mg/l and 1.63 for the combined data (Table 3).

In a second experiment, 5 g of acenaphthene was added to the column and the same dilutions were used. The analyzed concentrations ranged from 1.63 to 2.93 mg/l (Table 4). Mortality was similar to values measured in the first experiment. The lowest concentration produced only 20% mortality. The LC50 values were 2.46 and 2.14 mg/l and the combined LC50 was 2.35 mg/l (Table 3).

Partial Chronic Tests

Physicochemical Conditions

Physicochemical conditions during the duplicate experiments were stable (Table 5). In the trichlorophenol experiments, dissolved oxygen did not drop below 7.0 mg/l. Alkalinity and hardness varied by only 5 and 10 mg/l, respectively. Other parameters including pH and conductivity were higher than those measured during the acenaphthene partial chronic tests.

Physicochemical conditions measured during the acenaphthene

Table 4. Percent dilution and analyzed concentrations of acenaphthene and percent mortality of Tanytarsus dissimilis after a 48 h exposure.

Replicate	Acenaphthene concentration		Mortality (%)*
	Dilution (%)	Analyzed (mg/l)	48 h
1	0	0	0
2	0	0	0
1	3.75	1.23	15
2		1.63	20
1	50.00	1.50	35
2		2.08	45
1	62.50	1.58	45
2		2.29	45
1	75.00	1.66	40
2		2.56	55
1	87.50	1.80	75
2		2.80	60
1	100.00	1.93	75
2		2.93	75

*n = 20

Table 5. Physicochemical conditions in the partial chronic tests of the effects of trichlorophenol and acenaphthene.

Test	Temp (C)	DO (mg/l)	pH	Alk. (mg/l)	Hardness (mg/l $CaCO_3$)	Cond. (µmhos/cm)
			Trichlorophenol			
1	23.0	7.5	8.0	145	172	422
2	23.5	7.0	7.8	130	162	415
			Acenaphthene			
1	23.0	7.0	7.2	142	152	400
2	23.0	7.4	7.4	145	172	399

experiments were extremely consistent (Table 5) and were well within the range of tolerance for Tanytarsus dissimilis.

Trichlorophenol

Nominal concentrations in the first partial chronic test ranged from 5.44 to 70.0 mg/l and analyzed concentrations from 10.78 to 72.52 mg/l (Table 6). Analyzed concentrations were erratic at the three lower concentrations. No eggs hatched in the two highest concentrations of trichlorophenol (Table 6). Although variation among replicates was high at 15.12 and 25.20 mg/l, the means of the percent hatch of the two concentrations were similar. Hatch rate exceeded 77% at the two lowest concentrations. After 7 days, mortality was at least 85% in all units except 5.44 mg/l. Three concentrations had 100% mortality.

In a second experiment with trichlorophenol, nominal concentrations ranged from 4.30 to 55.00 mg/l. Analyzed values were somewhat higher than the nominal concentrations (Table 6). In the two highest concentrations, only one of 80 eggs hatched. In the two lowest concentrations, hatch success was considerably lower than in the first experiment. After 6 days, mortality exceeded 90% at trichlorophenol concentrations of 15.50 mg/l and above. The replicate chambers with the concentration of 4.07 mg/l had mortality of 60% and 85% for a mean mortality of 72.5%.

Acenaphthene

The procedure used to achieve saturated solutions of acenaphthene in the acute tests was also used in the partial chronic tests. Twenty grams of acenaphthene in 1000 ml of acetone was used in the column to

Table 6. Nominal and analyzed concentrations of trichlorophenol, percent hatch, and percent mortality of Tanytarsus dissimilis after partial chronic exposure.

Replicate	Trichlorophenol concentration (mg/l) Nominal	Analyzed	Hatch (%)*	Mortality (%)*
1	0	0	82.5	25.0
2	0	0	82.5	25.0
1	5.44	10.78	92.5	35.0
2	4.30	4.07	35.0	72.5
1	9.08	20.67	77.5	85.0
2	7.16	10.28	37.5	70.0
1	15.12	45.06	45.0	100.0
2	11.94	15.50	67.5	92.5
1	25.20	22.05	40.0	97.5
2	19.20	23.28	47.5	97.5
1	42.00	40.88	0.0	100.0
2	33.00	39.00	2.5	100.0
1	70.00	72.52	0.0	100.0
2	55.00	63.38	0.0	100.0

*n = 40

achieve adequate concentrations for testing. Ethanol was the carrier. Analyzed concentrations in the first experiment ranged from 0.72 to 2.04 mg/l (Table 7). Hatching success was 95% or above in all units except the highest concentration. No mortality was observed in the 0.72 mg/l solution. In the 1.16 and 1.72 mg/l solutions, mortality was less than 8%. No organisms survived in the highest concentration.

In a duplicate experiment, 10 g of acenaphthene in 500 ml acetone was added to the column. Analyzed concentrations ranged from 0.75 to 2.94 mg/l (Table 7). Hatching was at least 88% successful in all units except the two highest concentrations. No eggs hatched in the 2.68 and 2.94 mg/l solutions. Mortality was greater than 50% in all units above 0.75 mg/l. No larvae survived in the two highest concentrations.

Chronic Experiments

Dilutor Calibration

Concentration factors in the cups on the dilutor system were calculated by measuring the volume of outflow from the dilution chamber, effluent chamber, and cups. Standpipes were adjusted to decrease the volume of water necessary for the test. This adjustment resulted in a concentration factor of 9% at the lowest concentration to 100% at the highest.

Physicochemical Conditions

Physicochemical conditions during the chronic experiments were relatively stable (Table 8). In the two trichlorophenol experiments, temperature varied from 20-22C and 22-23 C. Dissolved oxygen did not drop below 7.9 mg/l in the first experiment. Values were generally high

Table 7. Percent dilution and analyzed concentrations of acenaphthene, percent hatch, and percent mortality of Tanytarsus dissimilis after partial chronic exposure.

Replicate	Acenaphthene concentration		Hatch (%)*	Mortality (%)*
	Dilution (%)	Analyzed (mg/l)		
1	0	0	100	0
2			98	30
1	12.96	0.72	100	0
2		0.75	100	30
1	21.60	1.16	100	7.5
2		1.53	88	52.0
1	36.00	1.72	95	5.0
2		2.19	88	82.0
1	60.00	1.90	100	75.0
2		2.68	0	100.0
1	100.00	2.04	30	100.0
2		2.94	0	100.0

*n = 40

Table 8. Ranges of physicochemical conditions in the chronic tests of the effects of trichlorophenol and acenaphthene.

Test	Temp (C)	DO (mg/l)	pH	Alk. (mg/l)	Hardness (mg/l)	Cond. (μmhos/cm)
			Trichlorophenol			
1	20-22	7.9-8.3	7.8-8.2	145-155	162-198	395-450
2	22-23	2.5-7.8	7.5-7.6	108-132	138-140	350-360
			Acenaphthene			
1	23-25	5.0-6.4	7.2-7.9	144-162	78-212	412-430
2	23-25	6.5-7.3	6.7-6.8	148-157	143-160	388-420
3	24	6.7	6.8	162	160	400
4	24	-	6.8-7.6	150-158	148-188	430-433

in the second experiment, but decreased to 2.5 mg/l on one occasion. All other parameters, including pH and conductivity were higher in the first test than in the second.

Physicochemical conditions measured during the four chronic acenaphthene tests were similar to those given for trichlorophenol (Table 8). Little temporal variation existed. The minimum dissolved oxygen and pH values measured were 5.0 mg/l and 6.7, respectively.

Trichlorophenol

Analyzed concentrations in the first life cycle experiment ranged from 0.90-0.97 mg/l to 4.27-4.73 mg/l in the lowest and highest concentrations, respectively (Table 9). Mortality equaled or exceeded 90% by day 9 in the three highest concentrations (Table 9). Mortality was less than 35% at the lowest concentration until day 15. Adults were isolated from three test units, two controls and 0.90-0.97 mg/l, by day 17 and eggs were observed on day 17. Seventeen masses were observed in the control units and six in the lowest concentration of trichlorophenol. Egg masses were not observed at the other concentrations. Egg masses generally appeared earlier in the control units. The number of egg/mass varied from 49-123 in the control and from 48-134 at 0.90-0.97 mg/l (Table 10). The number of eggs/mass was found to be highly variable and no clear difference between the control and treatment was observed.

In a second life cycle test with T. dissimilis concentrations of chemical ranged from 0.50-0.80 mg/l to 3.40-4.17 mg/l (Table 9). Mortality was generally greater in the second experiment than in the first except at the two highest concentrations. The first adults were

Table 9. Analyzed concentration of trichlorophenol and percent mortality in two life cycle experiments.

Test	Trichlorophenol concentration (mg/l)	Mortality* Day 7	9	11/12	13/14	15/16
1	0.0	17	17	17	17	24
2	0.0	88	90	88	88	88
1	0.90-0.97	34	34	34	34	57
2	0.50-0.80	88	100	82	92	72
1	1.08-1.18	87	93	97	97	97
2	0.72-0.86	58	58	55	68	68
1	1.21-1.37	47	47	47	50	93
2	0.78-1.37	65	70	82	100	92
1	1.75-1.96	83	90	90	90	100
2	1.62	75	100	85	100	92
1	2.76-3.22	93	97	100	100	100
2	2.38-2.68	88	90	55	62	88
1	4.27-4.73	80	100	100	100	100
2	3.40-4.17	80	82	85	98	98

*Test 1 - percent of 30 animals
Test 2 - percent of 40 animals

Table 10. Egg production of Tanytarsus dissimilis exposed to trichlorophenol.

Test	Day of test	No. of eggs/mass (0.9-0.97)	(Control)
1	17	48	
	18		96,112
	19		63,92,96,97
	20		49,81,83,91,97
	21	85,99,100,101,134	65,65,94,96,117,123

Test	Day of test	No. of eggs/mass Control	0.72-0.86	0.78-1.37	2.38-2.68
2	16	90,128			
	17	138		129	78
	18	98,125	97,107	101,137,140,165	
	19		115	39	
	20	9,56	19	101,129	
	21	18		34,108	
	22	27			
	23				

removed on day 17. Twenty egg masses were observed with the number of eggs/mass ranging from 9 to 165 (Table 10). Although nine egg masses were observed in the control, 10 existed at a concentration of 0.78-1.37 mg/l. Number of eggs/mass varied from 9-128 in the controls and from 19-165 in the units exposed to trichlorophenol. Although the first eggs were observed in the control, their occurrence in the treatments was not delayed, as in Test 1.

Acenaphthene

Life cycle tests using acenaphthene were unsuccessful. Four tests were conducted with this chemical. Duration, range of concentration, and observations for the four acenaphthene chronic tests attempted are shown in Table 11. In several pilot tests, it was difficult to attain sufficient concentrations of acenaphthene in the units. Concentration at various sites on the dilutor was measured and compared. Effluent from the column measured 2.87 mg/l, the mixing chamber contained 0.96 mg/l, and the effluent chamber contained 0.81 mg/l. The concentrations in the experimental units ranged from 0.02 to 0.36 mg/l.

Table 11. Duration, range of concentration of acenaphthene, and observations for four life cycle experiments with Tanytarsus dissimilis.

Test	Duration (days)	Acenaphthene (mg/l)	Observations
1	2	0.05-0.27	Low concentrations, few larvae
2	4	No analysis	Unsuccessful hatch
3	7	0.06-0.07	unsuccessful hatch
4	15	0.0-0.10	Low concentrations, few larvae

CHAPTER V

DISCUSSION

Solubility

In the acute tests, trichlorophenol was relatively easy to maintain in solution using dimethylformamide (DMF) as a carrier. Concentrations as high as 82.55 mg/l were maintained in the units containing the highest concentrations. This value is considerably lower than the solubility of 800 mg/l listed for trichlorophenol in water at 25° C, of 800 mg/l (EPA 1980). A maximum concentration of 82.55 mg/l was sufficient to obtain an LC50 value with T. dissimilis.

Acenaphthene was difficult to maintain in solution in the acute tests. Several solvents were tested for use as a carrier. Since acetone and DMF interfered with acenaphthene analysis, ethanol was used as the carrier. A glass tube filled with glass wool was used to absorb the acenaphthene and water with ethanol was passed over the glass wool. This technique achieved concentrations of 4.50 mg/l. This value is much lower than the solubility of 100 mg/l listed by the EPA (1980). However, the concentrations achieved in this study are in agreement with values of 3.93 mg/l stated by Mackay and Shiu (1977) and 3.9 mg/l by Bastian (1982).

Acute Tests

Considerable precision existed in measuring the trichlorophenol LC50 values for T. dissimilis. Mean values for the two tests varied by only 1.4 mg/l. The combined LC50 for the two tests was 26.1 mg/l. Tanytarsus dissimilis has also been used to test the acute toxicity of silver (Lima et al. 1982). The LC50 values for silver were considerably lower than those for trichlorophenol. The 48 h LC50 was 3.16 mg/l. Other comparable LC50 values for T. dissimilis were not found in the literature. Although studies have been conducted on the effects of trichlorophenol on rats, mice, cattle, and sheep, LC50 values on other aquatic organisms were not found in the literature.

The LC50 values generated in the two experiments for acenaphthene were similar. The two values differed by only 0.72 mg/l. The mean LC50 for the two experiments was 1.99 mg/l. Values obtained in this study are similar to those found in the literature. The acute value derived from a Daphnia magna 48 h exposure was 1.70 mg/l. Results of a fish bioassay were lower. The 96 h LC50 for bluegill (Lepomis machrochirus) exposed to acenaphthene was 0.24 mg/l (EPA 1980).

Partial Chronic Tests

In the partial tests, analyzed trichlorophenol values were higher than the nominal concentrations. Perhaps because of analytical errors. All samples were analyzed twice, first on a Hewlett-Packard Research Chromatograph and then on a Tracor Research Chromatograph. Variation existed between the two methods of analyses and results from the Tracor chromatograph were used.

Percent hatch was similar in the two experiments involving trichlorophenol except at the two lowest concentrations. At concentrations above 9.08 mg/l, hatch success was less than 50%. This indicates trichlorophenol has a toxic effect on hatching of Tanytarsus dissimilis despite the protective egg sheath.

Mortality was extremely high in the trichlorophenol experiments. Eight of the concentrations tested had mortality rates greater than 90%. The two lowest concentrations, 4.07 and 10.78 mg/l, had a mean mortality of 54%. Concentrations of trichlorophenol in water must be less than 4 mg/l to prevent significant mortality.

Analyzed concentrations of acenaphthene were higher in the second partial test than in the first. Hatch success was similar in both tests. Acenaphthene had little effect on hatching at concentrations below 2.0 mg/l.

Mortality was similar at the two highest levels of acenaphthene. Mortality was highly varied in the second and third lowest concentrations. Concentrations of acenaphthene below 1.0 mg/l have negligible effects on Tanytarsus dissimilis.

Although two relatively successful partial life cycle tests were conducted for each chemical, the test is not cost effective. Because of the low hatch success in the controls, three tests were conducted for trichlorophenol and five for acenaphthene in order to obtain the two usable tests.

Chronic Tests

In the chronic tests, trichlorophenol was relatively difficult to maintain in high concentration in the units. Although 15 mg/l was

placed in the toxicant chamber, the maximum concentration attained in the units was 6.49 mg/l.

Acenaphthene was extremely difficult to maintain at high concentra-tions in the experimental units. The decrease in concentration observed throughout the dilutor was probably caused by photodegradation and/or bacteria. Adsorption to the walls of the dilutor chambers was another possible reason for the low concentrations in the units. Thus, the units were covered with black plastic, washed with chlorox, and subjected to ultraviolet light. Significant levels of acenaphthene in the experimental units were still not attained.

Although 50 egg masses were observed in the two tests of the chronic effects of trichlorophenol, only 7 of the 28 units contained egg masses. A problem that existed during the chronic tests on the dilutor system was the lack of survival of the larvae in the experimental units. This probably resulted from either the quality of the dilution water, difficulty in hatching in running water, or difficulty in hatching in a small volume of water. Organisms were unable to survive in tests with lake water and the midges have been cultured for over a year in aquaria containing aged tap water. A successful egg hatch was accomplished in test units of the same size as used with the dilutor but without a flow of water. This suggests that current may inhibit egg hatch or survival of the young larvae.

The chronic tests did not clearly demonstrate the effects of trichlorophenol on T. dissimilis. Although more eggs were observed in the control units, the low numbers of eggs in the other units precluded meaningful comparisons. Furthermore, little difference existed in the number/mass or in the time in which the first egg masses appeared.

CHAPTER VI

SUMMARY

1. Acute, partial life cycle, and chronic toxicity tests were conducted on Tanytarsus dissimilis.

2. Acute toxicity tests were the most consistent and reproducible bioassay of the three types used in this study. The LC50 values for trichlorophenol were 25.4 and 26.8 mg/l. The combined LC50 was 26.1 mg/l. Acenaphthene had lower LC50 values than trichlorophenol. The values were 1.63 and 2.35 with a combined value of 1.99 mg/l.

3. Partial chronic tests were less successful than acute tests. Exposure of eggs to trichlorophenol resulted in an unsuccessful hatch at an analyzed concentration of 39.0 mg/l and above. Lower concentrations decreased survival. Concentrations of acenaphthene above 2.0 mg/l resulted in extremely high mortality rates.

4. Chronic life cycle tests were the most difficult of the three bioassay methods to complete successfully. Concentrations of both trichlorophenol and acenaphthene were difficult to maintain in the exposure chambers. Survival of larvae in the units was also a problem. The number of eggs/mass was not a good indicator of toxicity.

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APPENDIXES

APPENDIX A

SAS COMPUTER PROGRAM FOR LC50

```
 1      TITLE ACENAPHTHENE ACUTE TEST #3 COMBINED;
 2      DATA TOXICITY;
 3      INPUT DOSE 1-5   N 6-7   RES 8-9;
 4      CARDS;
 5      2.27      20      6*
 6      --        --      -
 7      --        --      -
 8      --        --      -
 9      --        --      -
10      34.6      20      13*
11      PROC PRINT DATA=TOXICITY;
12      PROC PROBIT LOG10;
13      VAR DOSE N RES;
```

This is a canned program in the Statistical Analysis System (SAS) titled "PROBIT."

"DOSE" = the analyzed chemical concentration

"N" = number of animals exposed

"RES" = number of animals expired

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*Sample data

APPENDIX B

RANGES OF PHYSICOCHEMICAL CONDITIONS IN THE NINE LIFE CYCLE TESTS OF THE EFFECTS OF TRICHLOROPHENOL

Table 12. Ranges of physicochemical conditions in the nine life cycle tests of the effects of trichlorophenol.

Test	Temp (C)	D.O. (mg/l)	pH	Alk (mg/l)	Hardness (mg/l)	Conductivity (mhos/cm)
1	20-24	5.8-5.9	8.0-8.1	150	192-196	410-420
2	23-24	5.3	8.0	145-147	180-184	445-450
3	20-22	7.9-8.3	7.8-8.2	145-155	162-198	395-450
4	19-24	7.8-7.9	7.8-7.9	147-152	162-186	405-445
5	23-25	7.6-7.7	7.8-8.0	146-151	172-176	400-470
6	23-25	8.3-9.3	8.0	150-154	170-184	435-455
7	19-21	2.6-6.2	8.0-8.2	148-164	148-170	380-428
8	21-24	-	7.9-8.0	131-157	132-156	355-405
9	22-23	2.5-7.8	7.5-7.6	108-132	138-140	350-360

APPENDIX C

RANGE OF CONCENTRATION OF TRICHLOROPHENOL AND DURATION OF THE STUDY FOR THE NINE LIFE CYCLE EXPERIMENTS

Table 13. Range of concentration of trichlorophenol and duration of the study for the nine life cycle experiments.

Test	Duration (days)	TCP range (mg/l)	Remarks
1	8	0.23-6.39	Few larvae or egg cases
2	4	0.87-9.27	Few larvae or egg cases
3*	15	0.75-5.58	Adults from 3 units
4	9	0.24-2.08	Few larvae
5	7	0.16-0.63	Few larvae, erratic concentration
6	7	0.14-0.59	Few larvae, erratic concentration
7	15	0.05-0.80	Larvae in 4 units, none in control
8	9	0.20-6.49	Few larvae
9*	23	0.04-9.20	Adults from 8 units

*Successful tests

VITA

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Master of Science

Thesis: EVALUATION OF A STATIC LC50, PARTIAL CHRONIC BIOASSAY TOXICITY TEST AND CHRONIC BIOASSAY ON TANYTARSUS DISSIMILIS

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