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Toxicity of Selected Pesticides to the Bay Mussel(*Mytilus Edulis*)

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TOXICITY OF SELECTED PESTICIDES TO THE BAY MUSSEL (*MYTILUS EDULIS*)

By

David H. W. Liu

Jean M. Lee

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Project Officer

C. S. Hegre
National Marine Water Quality Laboratory
National Environmental Research Center
South Ferry Road
Narragansett, Rhode Island 02882

NATIONAL ENVIRONMENTAL RESEARCH CENTER
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
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ABSTRACT

The toxicity of the insecticides Sevin, methoxychlor, and malathion and of the herbicides Treflan and 2,4-D to the bay mussel (Mytilus edulis) was investigated. Toxic effects were measured in terms of survival of and byssus-thread attachment by adults, embryo shell development, and larval growth and metamorphosis.

The results indicated that growth was the most sensitive measure of toxicity. All the pesticides produced statistically significant ($p = 0.05$) reductions in larval shell length after 10 to 20 days of exposure. Relative to potency, methoxychlor was the most toxic, and 2,4-D was the least toxic.

The 96-hour TL50 values for each pesticide, based on adult survival and attachment data, were estimated, as were the 48-hour EC50 values based on data from embryo bioassays.

The effects on embryo development of delaying the time of fertilization and of using seawater larval culture media of various ages also were studied, and substrate preference by metamorphosing larvae was investigated.

A critical evaluation of the experimental approach and procedures is presented.

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SECTION I

CONCLUSIONS

1. The insecticide Sevin is toxic to the bay mussel at concentrations lower than its estimated solubility in seawater. Toxic effects were observed in adults as well as in larvae.

2. Although adult mussels can tolerate exposure to a saturated solution of Treflan, they appear to be able to detect extremely low levels of this herbicide. Embryo shell development is not affected by Treflan at a concentration half that of its estimated solubility in seawater; however, larval growth and metamorphosis are reduced.

3. Adult mussels can tolerate and do not appear to detect methoxychlor concentrations approaching twice the estimated solubility of this insecticide in seawater. Mussel eggs incubated in a saturated solution of methoxychlor develop normally; however, larval growth is depressed, and metamorphosis is inhibited.

4. All the life history stages of the bay mussel can be affected adversely by exposure to 2,4-D at levels lower than 25% of its estimated solubility in seawater.

5. Malathion is toxic to the bay mussel at all life history stages, producing abnormal shell development in embryos, depression of growth, and inhibition of metamorphosis at concentrations lower than 20% of its estimated solubility in seawater. The response of adult mussels to this insecticide was inconsistent, the same concentration being lethal at times and nonlethal at others.

6. Larval growth is probably the most consistently sensitive indicator of toxicity, followed in order of decreasing sensitivity

by shell development in mussel embryos, byssus-thread attachment to a substrate by adults, and adult survival.

7. A delay of more than two hours in fertilization of mussel eggs after they have been shed can affect embryo development markedly.

8. Use of aged natural seawater for culturing of mussel embryos from eggs appears to be beneficial for larval development.

9. Given a choice of rigid, opaque PVC, Plexiglas, frosted glass, or stretched silk thread for setting, a higher percentage of the mussel larvae select PVC. The percentage increases with larval density.

SECTION II

RECOMMENDATIONS

1. The bay mussel (Mytilus edulis L.), particularly the embryos and larvae, should be used more frequently as a test organism for evaluating marine and estuarine water quality.

2. A continuous-flow bioassay technique should be developed for the bay mussel, expecially one that can be used effectively with the larval forms.

3. Additional studies should be performed on the substrate preference of metamorphosing larvae, and the information should be used to aid in the development of efficient, biologically meaningful methods for investigating the effect of potential water pollutants on larval metamorphosis.

4. Because of seasonal and geographical variations in natural seawater, a standard synthetic seawater formulation suitable for culturing of all life history stages of the bay mussel should be adopted.

SECTION III

INTRODUCTION

Pesticides are chemical compounds formulated specifically to kill or control undesirable life forms, be they protists, plants, or animals. Although the chemical industry has been endeavoring to develop selective pesticides, most currently in use are broad-spectrum formulations that may adversely affect nontarget life forms. In addition, the methods for applying pesticides, particularly those used in agriculture, provide considerable opportunity for pesticides to enter nontarget areas and thus become a hazard to many organisms.

This report describes the results of an investigation of the effects of five commonly used pesticides on Mytilus edulis, an estuarine mussel. This mussel inhabits many bays and estuaries throughout the world, and in some regions is esteemed as a food for human consumption. The pesticides selected for study were malathion, methoxychlor, and Sevin, which are broad-spectrum insecticides; Treflan, a selective preemergence weed killer; and 2,4-D, a selective herbicide used particularly in the control of broad-leafed weeds. These pesticides were studied with respect to their effects on the survival of mature mussels, on shell development in 48-hour-old larvae, and on shell growth and metamorphosis in developing larvae.

SECTION IV

MATERIALS AND METHODS

PESTICIDES

The pesticide formulations evaluated, their source, and their purity as specified by the manufacturer were the following:

- 2,4-D, 2,4-dichlorophenoxyacetic acid; The Dow Chemical Company; production lot No. 092440; purity, 94.8%.
- Sevin®, 1-naphthyl N-methylcarbamate; Union Carbide Corporation; production lot No. 72055; purity, 99.7%.
- Malathion, O,O-dimethyl phosphorodithioate of diethyl mercaptosuccinate; American Cyanamid Co.; production lot No. IV 90715; purity, 95%.
- Treflan®, α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine; Eli Lilly and Co.; production lot No. X-14788; purity, 99%.
- Methoxychlor, 2,2-Bis(p-methoxyphenyl)-1,1,1-trichloroethane; E. I. du Pont de Nemours & Co., production lot number not indicated; purity, minimum of 88% p,p' isomer.

ANALYTICAL METHODS

Test concentrations of the pesticides were monitored routinely throughout each test. All analyses were performed on a Microtek Model 220 gas chromatograph equipped with an electrolytic conductivity detector in the oxidative mode. The column, 2 mm ID by 120 cm, was packed with 6% SE 30 on 100/200-mesh Gas Chrom Q.

All pesticides, except 2,4-D, were extracted directly from the water sample with an organic solvent. Methoxychlor and Treflan were extracted with hexane, and Sevin and malathion were extracted with chloroform and benzene, respectively. After the extract was dried in the presence of nitrogen gas, the residue was dissolved in acetone and analyzed on the gas chromatograph. Samples of the herbicide 2,4-D were acidified with HCl before extraction with diethyl ether. Analysis for 2,4-D was performed after the pesticide was esterified with diazomethane.

STABILITY AND SOLUBILITY DETERMINATIONS

To determine the behavior of the pesticides in seawater, and to estimate the highest concentration for use in the toxicity tests, the solubility and chemical half-life of each pesticide in seawater were assessed.

Solubility was determined by continuously shaking an excess amount of pesticide in natural seawater having an adjusted salinity of 25 ‰, a pH of 8 ± 2°C. The filtered (Nuclepore®, 0.8 μ) samples, taken at intervals, were analyzed for the pesticide. Concentration-time curves were constructed, and solubility was estimated from the curves.

The stability of each pesticide was determined by shaking a soluble amount of pesticide in seawater and analyzing samples of the solution taken at intervals over several days. The half-life was estimated from the resulting concentration-time curves.

PREPARATION OF TEST SOLUTIONS

All pesticide concentrations were prepared by dilution of the highest level tested with natural seawater. A measured amount of malathion, Sevin, and 2,4-D was added to a known volume of seawater, sonified, and then power stirred into a larger volume to obtain the highest concentration to be tested. Treflan and methoxychlor first were dissolved in acetone and then were added slowly to a constantly stirred vat of seawater. The pesticide-acetone solution was prepared so that the saturated stock

solution would contain no more than 200 µl/liter of acetone. Solutions of Treflan and methoxychlor were filtered before use.

SOURCES OF MYTILUS EDULIS L.

Mussels used in the adult survival studies were collected from floating docks located on Treasure Island, which lies in the central region of San Francisco Bay. Gametes used in the study of 48-hour embryo shell formation were obtained from mussels collected at Treasure Island and Marconi's Cove, located along the southeastern shore of Tomales Bay. The effects of the pesticides on larval shell growth and metamorphosis, and on metamorphosis alone, were determined using larvae derived from mussels collected at Marconi's Cove. Tomales Bay mussels were used in all nontoxicological studies, except those designed to determine the influence of artificial and aged natural seawater on larval shell formation. For these studies, we collected mussels from Tomales Bay, Treasure Island, and St. Francis Yacht Harbor (a small boat-docking area along northern San Francisco).

PRETEST TREATMENT OF THE MUSSELS AND SPAWNING TECHNIQUE

Adult mussels used in 96-hour survival experiments were transported from the collection site moist in plastic bags. Upon arrival at the laboratory, the shells were scraped of extraneous material and then transferred to 45-gal. aquaria containing recirculating seawater maintained at 20° C. The mussels were used within two days after collection. Mussels collected as a source of gametes were stored moist at about 5° C after being scraped free of debris. They were induced to spawn within 24 to 48 hours after collection.

Several methods of inducing spawn were tried. However, the only consistent method involved placing the chilled mussels in individual glass bowls containing enough 20° C natural seawater to cover them. The mussels usually began to produce gametes within an hour.

DILUENT WATER

The natural seawater used in all pesticide toxicity studies was obtained from the Steinhart Aquarium in San Francisco, California. The aquarium facility draws the water from a point approximately one-half mile offshore in the Pacific Ocean through a Raney collecting system. The intake is buried by about 20 ft of sand. The suitability of this water for maintaining marine life has been proven by Steinhart personnel's consistent success in rearing a myriad of marine organisms.

The water was transported weekly from the aquarium to SRI's testing laboratory in 400-gal., black polyethylene containers. Before use, the water was passed through an ultraviolet irradiator (Ultradynamics Model 500). When necessary, the salinity was reduced to $25^{o}/_{oo}$ by adding deionized tapwater (1 megohm), and the pH was adjusted to 8 ± 0.2 by the addition of HCl or NaOH.

ALGAE CULTURE

Cultures of Monochrysis lutheri Droop (1953) were maintained to feed the larvae used in the growth and metamorphosis study. Starting cultures were obtained from the Department of Botany, Indiana University, Bloomington, Indiana. The culture medium of Matthiessen and Toner (1966) was used.

Sterile cultures were maintained in 250- and 2000-ml Erlenmeyer flasks set on a rotary shaker and were illuminated with Sylvania Gro-lux fluorescent lamps. The temperature of the culture room was maintained at 18 ± 1°C. Algae in the 250-ml flasks were used to inoculate the 2000-ml flasks, which in turn were used to inoculate the 5-gal. carboys from which we took the cells used to feed the mussel larvae.

Cultures maintained in the 5-gal. carboys were not kept sterile. Agitation was accomplished by vigorous aeration. Population growth was monitored daily in cultures set up early in the project until sufficient data were accumulated to serve as a guide for predicting population growth rate. Algae used to feed the larvae were removed from the carboy cultures starting at

approximately the fourth day after the medium was inoculated and continuing until about the seventh day. The culture then was discarded, and the carboy was "sterilized" by filling it with a 10% solution of hypochlorite, which was allowed to stand for two to three days.

Algae used as food were separated from the culture medium in a Sorvall continuous-flow centrifuge (Model KSB3), using an SS-34 head at 1500 rpm. The algae pellets were resuspended in uv-treated seawater with an adjusted salinity and pH, and the cell density was determined with a Coulter particle counter using the procedures described by Maloney and co-workers (1962).

SECTION V

EXPERIMENTAL PROCEDURES

EXPLORATORY STUDIES

Time of Fertilization and Larval Shell Development

Approximately 6000 eggs, obtained from a single Tomales Bay female, were placed in each of six 250-ml beakers containing 200 ml of natural seawater. Sperm from a single Tomales Bay male was introduced into two beakers at a time 45, 110, 130, 165, and 260 min after the first eggs were shed. About 47 hours after the sperm were introduced, a few drops of neutral red stain--enough to impart a light orange color to the water--were added to each beaker. One hour later, the contents of each beaker were transferred to 250-ml plastic Falcon culture flasks containing 50 ml of 10% buffered formalin. Two-hundred consecutive larvae in each flask were examined through a dissecting microscope, and the number with straight-hinged shells was recorded.

Seawater Type and Larval Shell Development

The effect of artificial seawater (Kester et al., 1967) and of 2-, 48-, and 168-hour-old natural seawater on shell formation of larvae was investigated using the gametes of mussels collected in July 1973 from two areas in San Francisco Bay (St. Francis Yacht Harbor and Treasure Island) and from Marconi's Cove in Tomales Bay.

The artificial seawater was prepared with reagent-grade chemicals and deionized water (1 megohm) and stored overnight. The natural seawater, obtained from the Steinhart Aquarium, was allowed to age in polyethylene containers at room temperature. The salinity and pH were adjusted, and the adjusted water was exposed to ultraviolet light before use.

Each seawater type was tested using eggs from four females from each collection site. The eggs from different females were tested separately and in duplicate. The number of normal-shelled larvae was determined after 48 hours, as described in the section on time of fertilization and larval shell development.

Substrate Preference

The following substrates were evaluated: frosted glass; smooth rigid, opaque polyvinyl chloride (PVC) sheet; smooth, clear acrylic plastic sheet; and white silk thread. Each substrate was constructed in the shape of an "L," each leg having approximately the same dimensions. The horizontal and vertical sections of the rigid substrates were attached to each other with Dow-Corning silicone adhesive. Excess adhesive was removed from the joints. The silk thread was wound vertically over a frame made of a 1/8-inch-diameter glass rod. Each frame contained the same number of strands. The approximate areas exposed to the larvae were 4.6, 3.9, 4.5, and 4.8 cm^2, respectively, for the acrylic sheet, PVC sheet, frosted glass plate, and silk thread substrates. The surface area of the silk thread was obtained by measuring the diameter of a length of wetted thread at ten different points and averaging the diameters. Fine filaments extending from the main body of the thread were not included.

Mussels used in the study were obtained from Tomales Bay in September 1973. The eggs from several females were incubated in separate groups according to female, and two samples from each group were examined after 48 hours. All groups in which less than 85% of the larvae in both samples had straight-hinged shells were discarded. The remaining groups were reared for at least 30 more days in 1.25-gal. Pyrex® animal jars, each containing 3 liters of natural seawater and 3000 larvae. During this period, the water was renewed every other day. M. lutheri was provided daily throughout the experiment at a rate of 20,000 cells/ml. Daily algae cell counts indicated that a higher feeding rate was not necessary.

Beginning on day 30, the animal jars were examined daily for attached larvae. On day 35, when the attached larvae were first noticed, 200 swimming larvae from each of three females were divided equally between two 75 X 150 mm Pyrex crystallizing dishes, and one of each substrate was placed in each dish. The volume of seawater was 600 ml. For five days, the substrates were inspected daily for larvae attached by byssus threads. All those so attached were counted and discarded. On the fifth day, the number of larvae attached by byssus threads to the test container also was determined.

The water was not renewed during the test. The larvae were fed 20,000 algal cells/ml of water every other day.

Larval Density and Larval Attachment

The four substrates used in the previously described test were reevaluated. The procedures used in this study were the same as those for the substrate preference study, except that the numbers of larvae per test were 50, 100, 200, 300, and 600. Each density was tested in duplicate. All the larvae were hatched from eggs of one Tomales Bay female.

TOXICITY STUDIES

96-Hour Adult Survival

The survival of adult mussels exposed to various pesticide concentrations for 96 hours was determined, and, where possible, the 96-hour TL_{50} concentration and 95% confidence limits were estimated by the method of Litchfield and Wilcoxon (1949).

The tests were conducted in 5-gal., wide-mouth glass jars. Three to five pesticide concentrations plus a seawater control were used in each test. When applicable, a second control was used to determine the effect of the organic solvent. To minimize the need to aerate the test solutions, only two mussels were placed in each jar; however, ten mussels simultaneously were exposed to each level of pesticide.

The mussels were inspected daily, and the number dead or attached to the jars was recorded. Mussels were considered dead if, upon being prodded with a glass rod, those with gaping valves showed no valvular or body movement.

Larval Shell Development

The procedures described by Dimick and Breese (1965) were used to determine the effect of the pesticides on larval shell development. The mussels from which the gametes were obtained were collected from Treasure Island and Tomales Bay. Treasure Island mussels were used in tests on Sevin. In tests on Treflan, methoxychlor, 2,4-D, and malathion, the mussels were collected from Tomales Bay.

The tests were conducted in 250-ml Pyrex beakers containing about 6000 eggs in a volume of 200 ml. The larvae were cultured for 48 hours after fertilization of the eggs and then killed. The number of normal larvae (straight-hinged shells) in a group of 200 consecutive larvae was determined and expressed in relative percentage units, which were calculated by dividing the percentage of normal larvae in a pesticide-exposed group by the percentage of normal larvae in the control group and multiplying by 100. Only data obtained from tests in which 85% of the control larvae were normal were considered acceptable.

Four to seven different pesticide levels were tested. In tests on methoxychlor and Treflan, two control groups were used. One group was exposed to seawater alone, and the other was exposed to seawater containing 50 µl/liter of acetone. All treatment levels, including the controls, were tested in duplicate.

Upon initiation of a series of tests on a given pesticide, a sufficient volume of each test level of the pesticide was prepared to fill four extra beakers. These beakers did not contain larvae. The contents of two beakers were analyzed for pesticide at the beginning of the test, and the contents of the other two were analyzed at the end of the test. Water temperature, pH, dissolved oxygen, and salinity also were measured in samples taken from the same four beakers initially and after 48 hours.

Larval Growth and Metamorphosis

Effects of the pesticides on larval growth and metamorphosis were investigated by exposing the larvae continuously to various consentrations of each pesticide for periods of up to 40 days. During the exposure period, the shell length of the larvae was measured every other day, and the number of larvae undergoing metamorphosis was recorded.

The gametes used in the tests were obtained from mussels collected at Tomales Bay during August and September 1973. Before exposure to the pesticides, eggs were placed in 5-gal. glass jars containing 15 liters of natural seawater and fertilized. Eggs were kept in separate groups according to female at a concentration of 30,000 per liter.

Forty-eight hours after fertilization, the contents of each jar were mixed thoroughly by a stream of air. During mixing, a 20-ml aliquot was removed from each jar, and the number of normal-shelled larvae was determined from a group of 200. The contents of all jars containing less than 85% normal-shelled larvae were discarded Larvae from three females then were pooled. All larvae in a 1-ml sample were counted so equal numbers from the three females could be combined. After thoroughly mixing the larvae, we determined the number again.

Exposure to the pesticides was initiated within approximately 52 hours after fertilization of the eggs. The experiments were conducted in 1.25-gal. Pyrex animal jars, each containing 3 liters of test solution and approximately 3000 normal-shelled larvae. Four levels of each pesticide were used. Control groups were reared in natural seawater.

In tests on Treflan and methoxychlor, seawater and solvent groups were included. The solvent control groups were reared in natural seawater containing 50 µl/liter of acetone--the same level present in all chambers containing pesticide. Each pesticide was tested twice, and each treatment level was run in duplicate.

The test solutions were renewed every other day, and the pesticide solutions were prepared from freshly mixed stock. The larvae were separated from the test solutions by collecting them on 53-μ Nitex® screens.

Before the larvae were returned to them, and before they were refilled, the jars were scrubbed and rinsed with deionized fresh water; no detergent was used. During renewal of the test solutions, 20 to 40 larvae were removed from the submerged screens, fixed in neutral buffered formalin (10%), and measured under a compound microscope equipped with a calibrated ocular micrometer. As soon as the larvae approached 250 μ in size, the jars were inspected for metamorphosed larvae on the days of solution renewal. All larvae with a dissoconch shell were counted, measured, and discarded. Metamorphosed larvae were processed in addition to those removed for growth measurements. Each test was terminated when none of the jars contained enough larvae to proceed further.

During the tests, the larvae were fed M. lutheri daily at a rate of 20,000 cells/ml of test solution per day. Algal cell counts were made on aliquots removed from each jar on the days between solution renewal, and algal cells were added to maintain the nominal number. However, if less than 20% of the cells had been consumed, additional rations were not provided. On the days of solution renewal, the full nominal number of algal cells was provided to each test jar.

Each level of pesticide was prepared by serial dilution. The highest nominal test concentration was equal to, or slightly higher than, the EC_{50} determined in the 48-hour embryo shell formation tests; if the EC_{50} could not be determined, the nominal concentration was equivalent to the maximum solubility of the pesticide in the diluent seawater. The highest test concentration was determined analytically each time the test solutions were renewed. Salinity, pH, temperature, and dissolved oxygen were measured in each jar immediately after and just before solution renewal.

Metamorphosis

The effect of exposure to the five pesticides on metamorphosis was investigated by exposing 29- to 30-day-old larvae to four different

concentrations of each pesticide. The four levels of pesticide were tested in duplicate, and duplicate control groups also were used. Because acetone was required to aid dispersion of Treflan and methoxychlor, solvent controls also were used in tests on these pesticides.

The tests were conducted in 75 X 150 mm Pyrex crystallizing dishes. The volume of test solution was 600 ml, and the initial number of larvae per dish was 100. A saturated solution of each pesticide was prepared every other day, and the desired test concentrations were prepared by serial dilution. The test solutions in the dishes were changed every other day.

To renew the test solution, a 3-in. length of 1/2-in. diameter PVC pipe, with one end sealed by a piece of 53-μ Nitex screen, was placed into the dish. The fluid in each dish was siphoned out through the screened pipe. Fresh solution was poured into the test dish, and the screened pipe was sprayed with a mist of seawater.

The larvae were reared in natural seawater for 29 or 30 days before being exposed to a pesticide. The larvae population used in the tests was composed of larvae that developed from eggs of three females collected at Tomales Bay. We selected for study only those larval groups in which at least 85% of the larvae developed normal shells during the first 48 hours after egg fertilization. During the pretest rearing period, the larvae were fed M. lutheri daily at a rate of 20,000 algal cells/ml of seawater. The seawater was renewed every two days.

The pesticide exposure period was 40 days. During this time, all the dishes were examined under a dissecting microscope every other day. Larvae attached to the dish by byssus threads were removed gently and reexamined under a compound microscope for presence of a dissoconch shell. Those having a dissoconch shell were measured, counted, and discarded. Those without new shell growth were placed back in the test dish. Dead larvae, as well as those that were accidentally crushed during renewal of the test solutions, were counted and discarded. M. lutheri was fed daily at a rate of 20,000 cells/ml of test solution throughout the exposure period.

Water temperature, pH, salinity, and dissolved oxygen content were measured daily. Two samples of the highest test level were analyzed for pesticide every other day. The samples were collected immediately after the solution was prepared.

SECTION VI

RESULTS AND DISCUSSION

PESTICIDE SOLUBILITY AND STABILITY IN SEAWATER

Table 1 presents the solubility and half-life of each pesticide used in this study. The salinity, pH, and temperature of the natural seawater were 25 $^{o}/_{oo}$, 8 ± 0.2, and 20 ± 2°C, respectively. The half-life of malathion was not determined.

Table 1. SOLUBILITY AND HALF-LIFE OF FIVE PESTICIDES IN NATURAL SEAWATER AT 20°C

Pesticide	Solubility, mg/liter	Half-life, hours
Methoxychlor	0.05	Stable
Treflan	0.20	82
Sevin	60	82
Malathion	110	--
2,4-D (acid)	1100	Stable

The true equilibrium solubility of most "insoluble" pesticides in water is essentially unknown. Although the values in Table 1 are approximate, they provide an estimate for maximum test concentrations that should be employed in toxicological experiments in which the presence of undissolved pesticide is undesirable.

Our estimate for the solubility of methoxychlor in seawater is the same as that reported by Millemann and Caldwell (1973) of Oregon State University. Our estimate of the solubility of Treflan,

however, is 40% higher than theirs, perhaps because of the difference in method and temperature. The Oregon State University values were obtained at 13°C; and, instead of analyzing a filtered sample of a saturated pesticide solution prepared by mixing an excess of the pesticide in a known volume of water, the investigators analyzed samples of seawater that had been allowed to trickle through a bed of pesticide-laden sand prepared by filling a column of sand with a pesticide-acetone solution and evaporating off the acetone.

FACTORS AFFECTING THE DEVELOPMENT OF NORMAL LARVAE

Although the bay mussel embryo bioassay (Dimick and Breese, 1965) appears to be easy to perform and should be within the capability of many laboratories having access to a natural population of mussels and seawater, problems can arise. Our major difficulty was in defining the conditions under which acceptable percentages of the control larvae could develop normally. Seldom are 100% of the larvae in a sample normal; but, under the proper conditions, a large percentage do develop normal shells. In the bay mussel embryo bioassay, 85% normal larvae in a sample is considered minimum for acceptable test results. This criterion was difficult to meet during our early performance of the bioassay.

Measures undertaken to solve this problem culminated in several experiments, one of which was to determine the extent to which a delay in the time between shedding of eggs and introduction of sperm to fertilize them affected the number of larvae developing normal straight-hinged shells. The decision to investigate this factor was based on our observation that different females seldom shed their eggs simultaneously, and that there often is a considerable delay between the time a mussel begins to shed eggs and the time that it has shed a sufficient number for use in the experiment. Since several series of tests on a given pesticide usually were conducted on the same day, each with eggs from a different mussel, it was most convenient to initiate all the tests at approximately the same time. Because of this, some egg groups were not fertilized until several hours after they had been shed. Breese (1972) found that a four-hour delay did not seriously affect the development of normal larvae, but he advised limiting the delay to no more than three hours.

In our investigation of this factor, we discovered that the percentage of normal larvae decreased markedly when fertilization was delayed for more than two hours (Figure 1). The percentages shown in Figure 1 are relative, calculated by dividing the percentage of normal larvae observed in egg groups fertilized at different times into the highest observed percentage (110 min) and multiplying by 100. There was a 45-min lapse between the time a female began to shed eggs and the time when a sufficient number of eggs for the experiment were produced.

The experiment was not designed for determining whether the effects of delaying fertilization were due to the age of the eggs or to the age of the sperm. All egg groups were fertilized by sperm from a single male. Microscopic examination of the sperm showed that they were active at all times.

The second factor investigated was the age of the diluent seawater. During this study, we also evaluated Kester's artificial seawater formulation (Kester et al., 1967) as a mussel embryo culture medium. Personnel from the National Marine Water Quality Laboratory in West Kingston, Rhode Island, use this formulation for culturing of marine plankton, and they suggested we use it. The formulation was not suitable for culturing of mussel larvae, however. The mean number of normal larvae found in 15 duplicate cultures (30 tests), started originally from fertilized eggs taken from mussels collected in four different geographical areas, was equivalent to 4.5% of the larvae inspected. The range was 0.25 to 41.5%.

The unsuitability of Kester's synthetic seawater for mussel larvae culture is probably due not to the presence of toxic components but more likely to the absence of one or more substances essential for larval development. Although Kester and his associates recommend the use of reagent-grade chemicals, we substituted Leslie rock salt for reagent-grade NaCl. Because this substitution could have influenced the results, we cannot unconditionally reject the formulation. The substitution was made in an effort to include trace substances that might be beneficial to the larvae. Use of nonreagent-grade chemicals to supply trace substances was the intent of LaRoche and coworkers (1970) in their modification of the synthetic seawater formulation of Zaroogian and associates (1969).

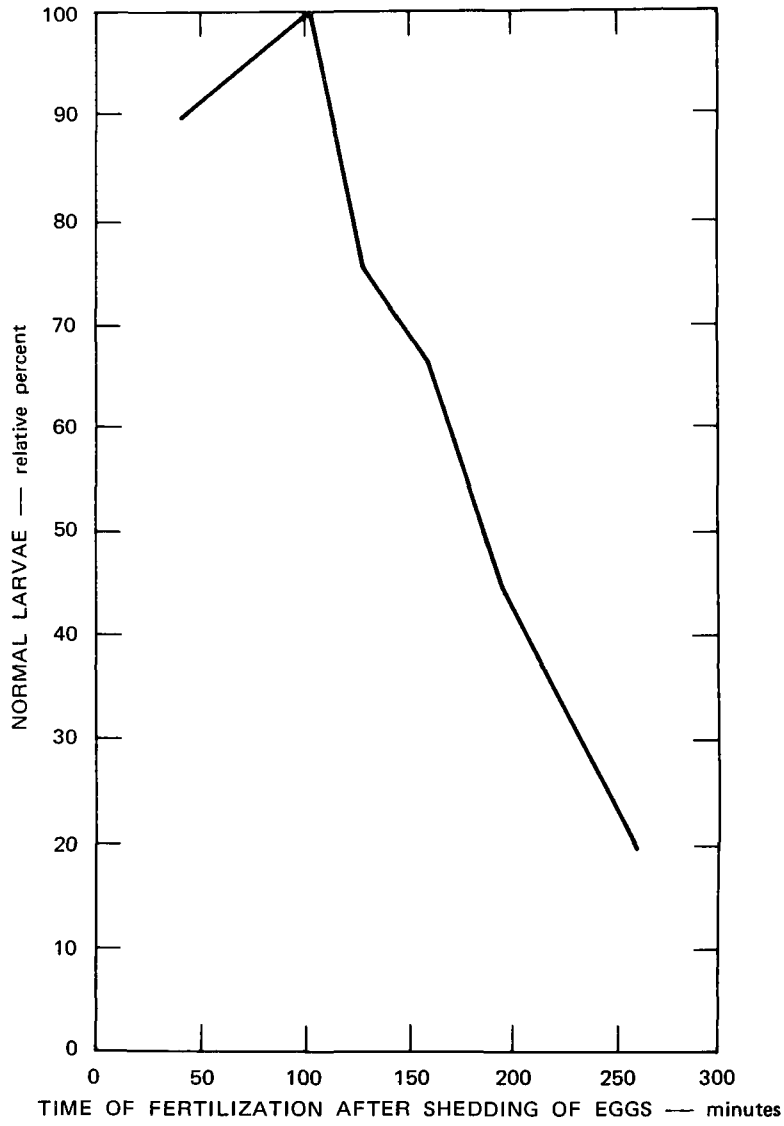

FIGURE 1 EFFECT OF TIME OF FERTILIZATION ON NUMBER OF NORMAL LARVAE DEVELOPING FROM EGGS OF THE BAY MUSSEL

In their evaluation of various synthetic seawater formulations, Courtright and associates (1971) found that none of the commercial formulations tested were suitable for mussel larvae culture. They developed the BioSea formulation, one of the essential components of which is an L factor from Leslie coarse hide salt.

Although the percentage of normal larvae did not approach 85 in any of the cultures used in the study of the effect of aged natural seawater, allowing the water to stand at room temperature for two or more days before use as a culture medium appeared to be beneficial (Figure 2). The percentages of normal larvae developing from eggs taken from mussels collected in different geographical areas and reared in seawater of a given age varied considerably; however, the response of all larvae to seawater of different ages was about the same, regardless of their source. On the average, only 24.7% of the larvae reared in unaged (two-hour-old) seawater were normal. Of those reared in water left standing for two days, 40.8% were normal. Cultures reared in seven-day-old water contained an average of 46.8% normal larvae. It appears that allowing the water to age for more than two days does not increase appreciably the percentage of normal larvae.

These findings are contrary to those of Woelke (1972), who reported that seawater aged for 16 and 24 hours had a statistically significant adverse effect on development of larvae from fertilized eggs of the Pacific oyster Crassotrea gigas. We know of no report in which aged seawater is recommended for the culture of marine organisms. To the contrary, fresh seawater is recommended, and its frequent renewal is emphasized (Hauenschild, 1972; Spotte, 1970). Aging may allow heavy metals or other toxic materials to precipitate or adsorb on the polyethylene container. Chemical changes in the water were not determined.

In working with the larvae, we observed other factors that influenced the development of normal larvae. Mussels induced to spawn may release eggs individually or in groups attached to form a cluster or a string. Eggs released in attached groups produce abnormal larvae. The size and shape of the mussel eggs are usually irregular immediately after release but become uniform after the eggs become turgid; however, eggs produced by some females remain irregular

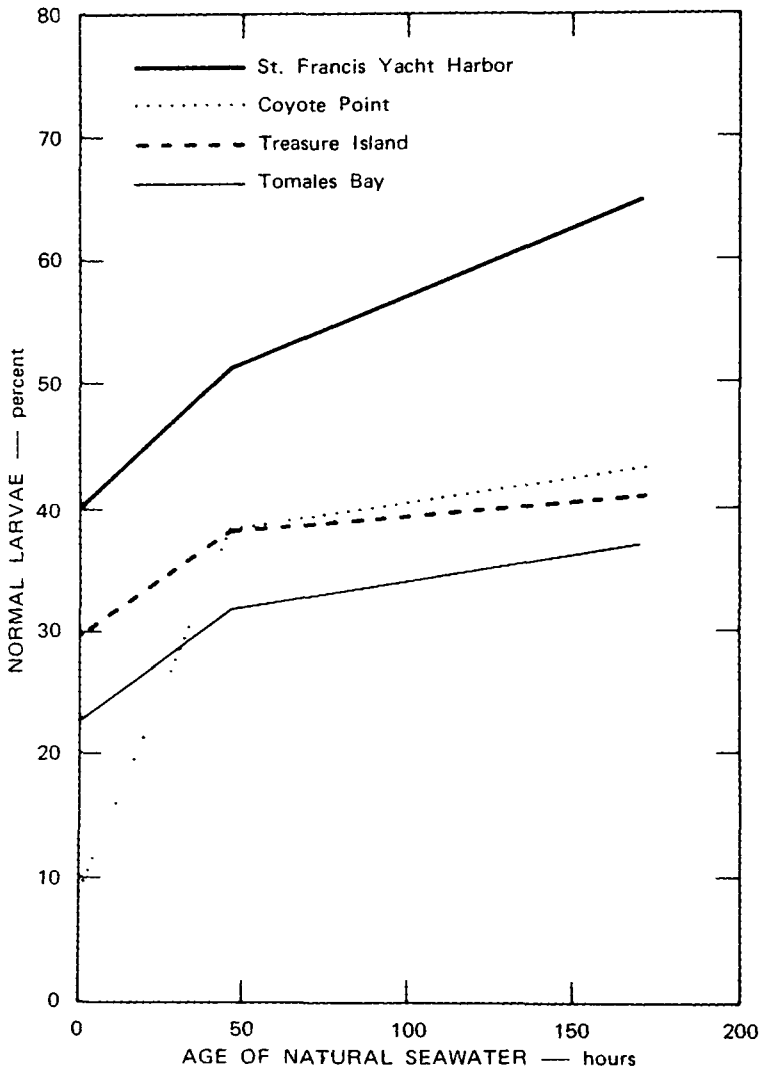

FIGURE 2 EFFECT OF AGE OF NATURAL SEAWATER ON THE NUMBER OF NORMAL LARVAE DEVELOPING FROM EGGS OF THE BAY MUSSEL

in size and shape even when turgid, and these eggs also result in abnormal larvae.

This study was designed also to determine whether the gametes obtained from mussels collected at one location were more viable than those from mussels collected at other locations. Although the percentage of normal larvae developing from eggs from all four locations was poor, eggs taken from mussels collected at St. Francis Yacht Harbor performed best. However, in subsequent toxicity tests, the 85% figure was seldom attained when eggs from this area were used. Eggs from Treasure Island were frequently irregular in size and shape, and a high percentage did not develop normally. The best results were observed with eggs from Tomales Bay.

SUBSTRATE PREFERENCE BY METAMORPHOSING LARVAE

Mussel larvae undergo morphological changes during maturation. After fertilization, the first stage of the larvae is called a trochophore (Field, 1922). It is free-swimming and does not have a shell. Next is the veliger stage. Veliger larvae are shelled, free-swimming, and posses a large, ciliated lobe or velum. As the larva increases in size, a foot develops, and the size of the velum diminishes. When the foot is large enough to enable the larva to crawl as well as swim, the larva is known as a pediveliger. This is the stage that settles or attaches to a substrate by means of byssus threads.

Primary attachment to a solid object is made by the ciliated foot, and "permanent" attachment occurs when the mussel secretes byssus threads. During the early stages of attachment, the larva may reposition itself by detaching old threads and forming new ones after moving to a new position with its foot, or by allowing itself to be transported to another location by the tide. We have on numerous occasions observed mussels break their byssus threads and move to another site. After initial attachment, the larva metamorphoses. Metamorphosis, the process by which the juvenile mussel is developed, is complete when the dissoconch shell (adult shell) appears (Bayne, 1965).

In this project, substrate preference experiments were conducted to provide information that would aid in the design of test procedures for determining the effects of the five pesticides on larval growth and settling. The substrates evaluated were polyvinyl chloride (PVC) sheet, frosted glass, acrylic plastic sheet (Plexiglas®), and silk embroidery thread. Table 2 presents the mean number of larvae attached to the various substrates in six tests. The differences between the means were analyzed statistically by analysis of variance followed by the Duncan multiple-range test.

Substrate preference in order of rank was:

PVC is greater than frosted glass and Plexiglas
Silk is greater than Plexiglas
PVC is equal to silk
Silk is equal to frosted glass
Frosted glass is equal to Plexiglas.

The number of larvae settling on the glass test chamber was significantly greater than that settling on any of the test substrates. Bayne (1965) found that although a few larvae attach to smooth glass when this is the only substrate provided, most do not and eventually die.

In our experiments, all four substrates and the glass chamber were simultaneously available to each group of 100 larvae. The average number of larvae settling on all available surfaces was 67 per 100 larvae. Of those that settled, about 27% settled on the smooth glass chamber, 25% settled on PVC, 22% settled on silk thread, 15% settled on frosted glass, and about 12% settled on acrylic plastic. Larval density was greatest on silk thread, followed in order by PVC, frosted glass, acrylic plastic, and the glass test chamber.

De Blok and Geelen (1959) evaluated 13 different substrates relative to selection by settling M. edulis larvae. They found that the larvae preferred filamentous substrates to solid substrates, silk embroidery thread being the most preferred Bayne (1965) also found silk embroidery thread to be superior to all the substrates he tested except for natural algae fibers.

Table 2. NUMBER OF M. EDULIS LARVAE ATTACHED TO SELECTED SUBSTRATES DURING A FIVE-DAY PERIOD

Substrate	Substrate surface area, cm^2	No. of tests performed	No. of larvae/ test	Mean no. of larvae attached	Mean no. of larvae/cm^2
Silk thread	4.79	6	100	13.5	2.82
Acrylic plastic	50.24	6	100	8.2	0.16
PVC plastic	45.16	6	100	16.8	0.37
Frosted glass	51.08	6	100	10.3	0.20
Glass chamber	275.60	6	100	18.2	0.07

The number of larvae in the test chambers had a considerable effect on substrate selection by the larvae. As the number of larvae per 600 ml of seawater was increased above 100, the number of larvae settling on PVC also increased, while the number selecting the other test substrates as well as the glass chamber decreased (Figure 3). At the highest larval density tested (600 per chamber), 50% of the larvae that settled were attached to the PVC substrate.

Although we cannot explain why preference for PVC increased with larval density, the negative phototaxic response of pediveliger larvae (Bayne, 1965) may explain their preference for PVC. The PVC substrate was the only one that did not transmit light. The experiments were conducted under well-illuminated conditions, and it is possible that the dark color of the PVC substrate as well as its opaqueness attracted the larvae.

TOXICITY STUDIES

General Considerations

In the following sections, the data obtained on each pesticide are reported and discussed separately. Except where specifically noted, all pesticide concentrations are measured concentrations, and the TL_{50} and EC_{50} values have been estimated on the basis of the measured levels. In the study of pesticide effects on growth and metamorphosis and on metamorphosis alone, only the highest test concentration was monitored throughout the exposure period. The lower concentrations were estimated on the basis of a 0.5 dilution factor. Actual measurements of the lower concentrations were performed once or twice during the test and were found to be very similar to the estimated values.

The 48-hour EC_{50} values estimated from the data obtained in the embryo bioassay experiments were estimated by the graphical method described in Standard Methods (1973). Although the line of best fit, drawn visually through the data points obtained in some of the tests, touched virtually all the points, the large number of larvae counted for each test concentration increased the power of Chi^2 sufficiently during application of the Litchfield-Wilcoxon

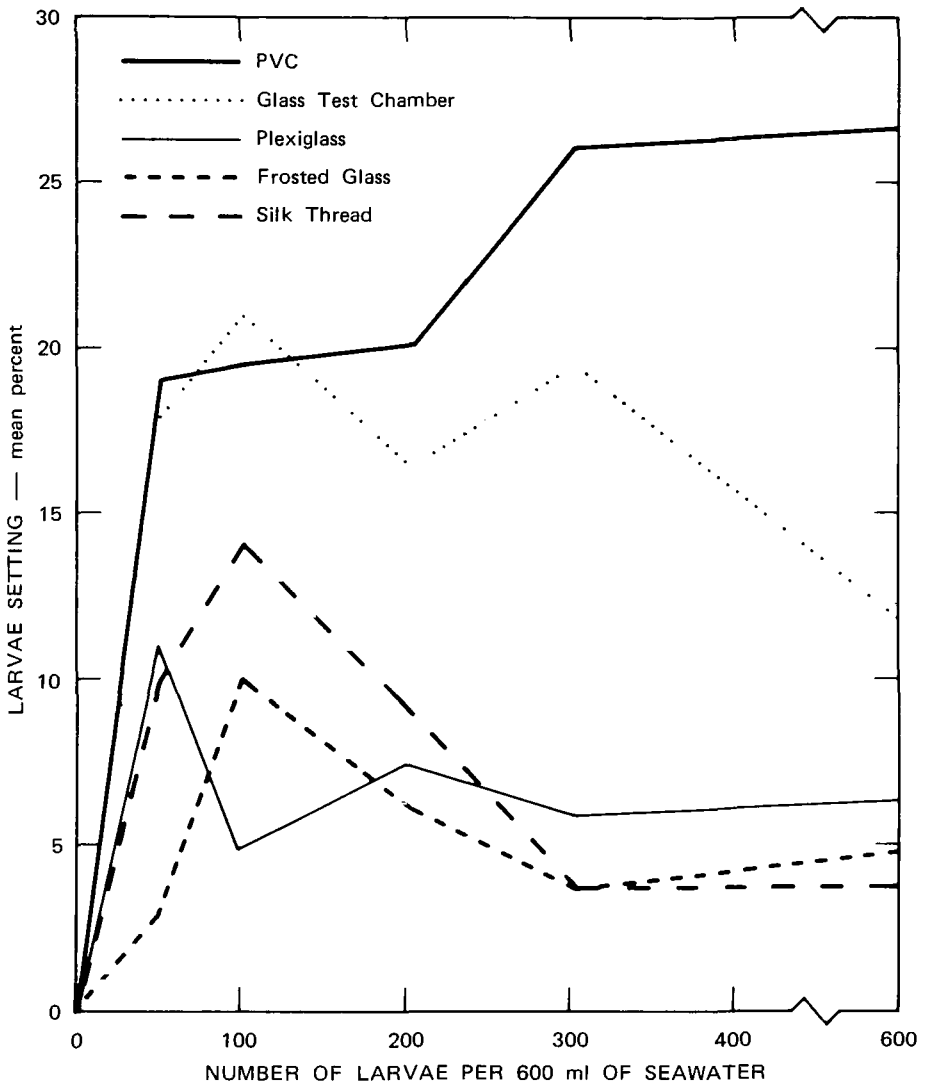

FIGURE 3 INFLUENCE OF LARVAL DENSITY ON LARVAL SELECTION OF SETTING SUBSTRATE

method that a poor fit was indicated. Consequently, the method was abandoned in favor of the one recommended by the APHA. The estimates are based on relative percentages calculated as described under "Material and Methods"; however, the percentages presented in the tables are the actual percentages.

Although 20 larvae were removed every other day and measured in the experiments on the effect of continuous exposure to the pesticides on larval growth and metamorphosis, statistical analysis of the data to determine the magnitude of effect was performed only on length measurements taken on the 10th and 20th day of exposure. Appendix B presents complete growth data. Appendix A contains the water quality data obtained during each phase of the study. Appendix C presents all data obtained in the metamorphosis experiments.

Sevin

Although five adult survival tests were performed on Sevin, only the data from one test (Test 53, Table 3) were suitable for estimating the 96-hour TL_{50}. The pesticide concentrations in two of the tests were not high enough to kill more than 50% of the test animals; and in another test, 30% of the control animals died. The 96-hour TL_{50} and 95% confidence limits, estimated by the method of Litchfield and Wilcoxon (1949), were 22.7 and 15.5 to 33.4 mg/liter, respectively.

Fewer Sevin-exposed mussles than controls attached themselves to the test chamber. In Test 53, 70% of the mussels exposed to 11.3 mg/liter of Sevin survived; however, only 50% attached. At the highest concentration of 30.9 mg/liter, 40% of the mussels survived, but only 10% attached. The estimated attachment EC_{50}, based on 96 hours of exposure, was 10.3 mg/liter.

Mussel embryos were considerably more sensitive than adults to Sevin. The 48-hour EC_{50} ranged from 1.21 to 1.80 mg/liter for three tests in which each concentration was tested in duplicate. The mean was 1.5 (Table 4).

Table 3. SURVIVAL AND ATTACHMENT DATA FOR ADULT MUSSELS EXPOSED TO SEVIN FOR 96 HOURS

Test no.	Test concentration		No. of animals	Survival, percent	Attached, percent
	Nominal	Measured			
45	Control	0	8	88	--
	2.5	2.3	8	88	--
	5.0	4.9	8	100	--
	10.0	10.0	8	100	--
	20.0	19.8	8	75	--
25	Control	--	9	100	80
	Control (solvent)	--	9	100	90
	5.0	--	9	100	80
	10.0	--	9	89	70
	20.0	--	9	78	70
	40.0	--	9	67	40
50	0	0	10	70	70
	20	9.4	10	70	60
	30	13.6	10	50	40
	50	34.2	10	30	20
	75	25.2	10	0	0
53	0	0	10	100	100
	20.6	11.3	10	70	50
	31.7	16.0	10	70	30
	48.8	20.3	10	50	20
	75.0	30.9	10	40	10

Table 4. PERCENTAGE OF NORMAL LARVAE DEVELOPING IN 48 HOURS FROM MUSSEL EGGS FERTILIZED IN VARIOUS CONCENTRATIONS OF SEVIN

Test concentration, mg/liter	Test number					
	Experiment 1		Experiment 2		Experiment 3	
	(1)	(2)	(1)	(2)	(1)	(2)
0	93.5%	90.0%	90.0%	96.0%	88.0%	83.5%
0.19	87.5	82.5	81.7	84.5	68.5	84.0
0.26	89.7	88.5	--	76.3	--	79.5
0.65	72.1	71.0	72.5	74.3	67.0	78.3
1.3	65.5	35.5	--	62.0	36.5	40.5
3.3	6.5	2.0	0	2.5	0	2.0
48-hour EC_{50}, mg/liter	1.48		1.80		1.21	

Although the effect of the pesticides on larval growth was studied by measuring 20 larvae from each treatment group every other day for a minimum of 30 days (Appendix B), we elected to analyze statistically the data collected on the 10th and 20th days. This period covers growth of the veliger and pediveliger larvae. During the study, larval measurements were discontinued in several test jars because of insufficient numbers of survivors. Since only a few jars were terminated during this period, size comparisons were possible among nearly all the treatment levels. The difference between the means of the control larvae and the pesticide-exposed larvae were tested for significance by subjecting the data to a Student's t-test, using a 95% level of significance. The concentrations of Sevin used were 0, 0.33, 0.65, 1.30, and 2.61 mg/liter. Only the highest concentration was measured; the lower concentrations were estimated using the mean of the highest concentration as a starting point. The mean of 2.61 mg/liter for the highest concentration was calculated using values from 45 determinations. The standard deviation was 0.39 mg/liter.

Table 5 presents the mean shell lengths of larvae exposed to Sevin for 10 and 20 days. Growth was a more sensitive measure of toxic

Table 5. MEAN SHELL LENGTH OF MUSSEL LARVAE EXPOSED TO SEVIN FOR 10 AND 20 DAYS

Test concentration, mg/liter	No. of larvae measured/ test	No. of tests/ experiment	Mean shell length, μ			
			Day 10		Day 20	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	20	2	170	174	309	244
0.33	20	2	162	138	218	194
0.65	20	2	128	126	204	165
1.30	20	2	122	109	164	130
2.61	20	2	124	99	--	--

effect than embryo shell development. After 10 days of exposure, 0.33 mg/liter of Sevin reduced larval size by 20.7% in one of the tests (Experiment 2). In the same experiment, a 29.5% reduction in growth was observed at the same concentration after 20 days of exposure. The lowest concentration that inhibited growth in both experiments was 0.65 mg/liter. As much as a 27.5% reduction in size was observed after 10 days of exposure, and as much as a 32.6% reduction in size was observed after 20 days of exposure. At the highest concentration of 2.61 mg/liter, the duplicate jars used in both tests did not contain enough larvae to warrant their continued use after 14 days. By the tenth day, the larvae exposed to this concentration of Sevin were about 46% smaller than controls.

The effect of Sevin on metamorphosis to the juvenile stage was studied in two ways. First, the effect of continuous exposure to Sevin from the 48-hour stage to larval metamorphosis was investigated by continuing the growth experiments beyond 30 days. Second, the effects were studied by delaying exposure until the larvae were 29 days old.

In the first set of experiments, larvae exposed to a measured Sevin concentration of 1.3 mg/liter did not survive longer than 39 days. Larvae exposed to the highest test level of 2.61 mg/liter survived

no longer than 14 days. Juvenile mussels did not develop at either concentration. Conditions in Experiment 1 (Table 6) were apparently unsuitable for larval development, since only two of the control larvae metamorphosed. Conditions in Experiment 2 were somewhat better; in the control groups, a total of 159 larvae developed dissoconch shells. Of larvae exposed to the lowest Sevin concentration of 0.33 mg/liter--the only pesticide-exposed group remaining--only 11 developed into juvenile mussels. Larvae in this group did not begin to undergo metamorphosis until six days after the control larvae had started. The juvenile larvae were also smaller in this group than larvae in the control group.

Table 6. NUMBER, AGE, AND SIZE OF JUVENILE MUSSELS DEVELOPING IN LARVAL CULTURES EXPOSED AT 48 HOURS TO VARIOUS CONCENTRATIONS OF SEVIN

Test concentration, mg/liter	Experiment 1				Experiment 2			
	No. of juveniles	Age,[a] days	Length, μ		No. of juveniles	Age,[a] days	Length, μ	
			Mean	SD			Mean	SD
0	2	37	457	93.0	159	32	434	66.3
0.33	9	30	304	44.0	11	38	414	108.2
0.65	4	32	279	26.3	0	--	--	--
1.30	0	--	--	--	0	--	--	--
2.61	0	--	--	--	0	--	--	--

[a]Number of days required by treatment group to begin metamorphosing.

Table 7 presents the data obtained from the second set of experiments in which exposure was initiated after the larvae were 29 days old. Although each test chamber contained an initial number of 100 swimming larvae, many were lost or crushed during renewal of the test solutions. Because the larvae in this category were lost

Table 7. EFFECT OF SEVIN ON MUSSEL LARVAE METAMORPHOSIS AFTER A 40-DAY EXPOSURE INITIATED 29 DAYS AFTER FERTILIZATION OF THE EGGS
(Presence of Dissoconch Shell Used as Indication of Metamorphosis)

	Test concentration, mg/liter									
	Control		0.36		0.72		1.45		2.9	
	1	2	1	2	1	2	1	2	1	2
Number of larvae										
Initial	100	100	100	100	100	100	100	100	100	100
Lost or crushed	16	19	22	35	27	24	35	16	40	31
Experimental	84	81	78	65	73	76	65	84	60	69
Metamorphosing, percent	94.0%	92.6%	91.0%	84.6%	82.2%	88.2%	81.6%	83.3%	80.0%	75.4%
Nonmetamorphosing, percent	1.2%	0	6.4%	9.2%	2.7%	3.9%	1.5%	2.4%	0	0
Dead, percent	4.8%	7.4%	2.6%	6.2%	15.1%	7.9%	16.9%	14.3%	20.0%	24.6%
Days to metamorphosis of 50% of test larvae	23	24	21	24	19	23	17	24	16	17
Shell length of juvenile mussels, μ	455.4 μ	458.6 μ	499.2 μ	483.5 μ	450.8 μ	406.5 μ	391.6 μ	393.3 μ	350.2 μ	339.3 μ

through procedural error, they were not included in the analysis of the data. Percentages of metamorphosing, nonmetamorphosing, and dead larvae presented in Table 7 are based on the number of experimental larvae, not on the initial number of larvae. Only the highest concentration was monitored throughout the test. The mean and standard deviation of the highest test concentration, based on 31 determinations, were 2.9 ± 0.76 mg/liter.

Sevin-exposed larvae as a group did not undergo metamorphosis as extensively as the controls. An average of 93.3% of the control larvae developed into juveniles, whereas the average for all groups exposed to Sevin was 83.3%. The percentages of metamorphosing larvae decreased with increasing levels of Sevin. The mean percentages of metamorphosed larvae found in the test chambers containing 0.36, 0.72, 1.45, and 2.9 mg/liter of Sevin were 93.3, 87.8, 85.2, 82.4, and 77.7, respectively.

Mortality also increased with increasing levels of Sevin. An average of 6.1% of the controls died during the course of the 40-day exposure period. A smaller percentage died in the chambers with 0.36 mg/liter of Sevin; however, at greater concentrations, a higher percentage of the larvae died. Mortality among larvae exposed to the highest test concentration of 2.9 mg/liter amounted to an average of 22.3% of the experimental population.

In general, the pesticide-exposed larvae metamorphosed at a faster rate than the control larvae. This enhanced rate was particularly evident in larvae exposed to 2.9 mg/liter of Sevin (Figure 4). Of those that metamorphosed, 50% had metamorphosed at this concentration in about 16.5 days, whereas the controls required an average of 23.5 days.

The average size of the larvae at metamorphosis also was affected by exposure to Sevin. The mean size of the control larvae was 457 μ. Larvae exposed to 0.36 mg/liter of Sevin were about 10 μ larger; however, at concentrations above 0.36 mg/liter, the larvae decreased progressively in size as the pesticide concentration increased. Larvae exposed to 0.72, 1.45, and 2.9 mg/liter were 6.2, 14.1, and 24.6% smaller than controls.

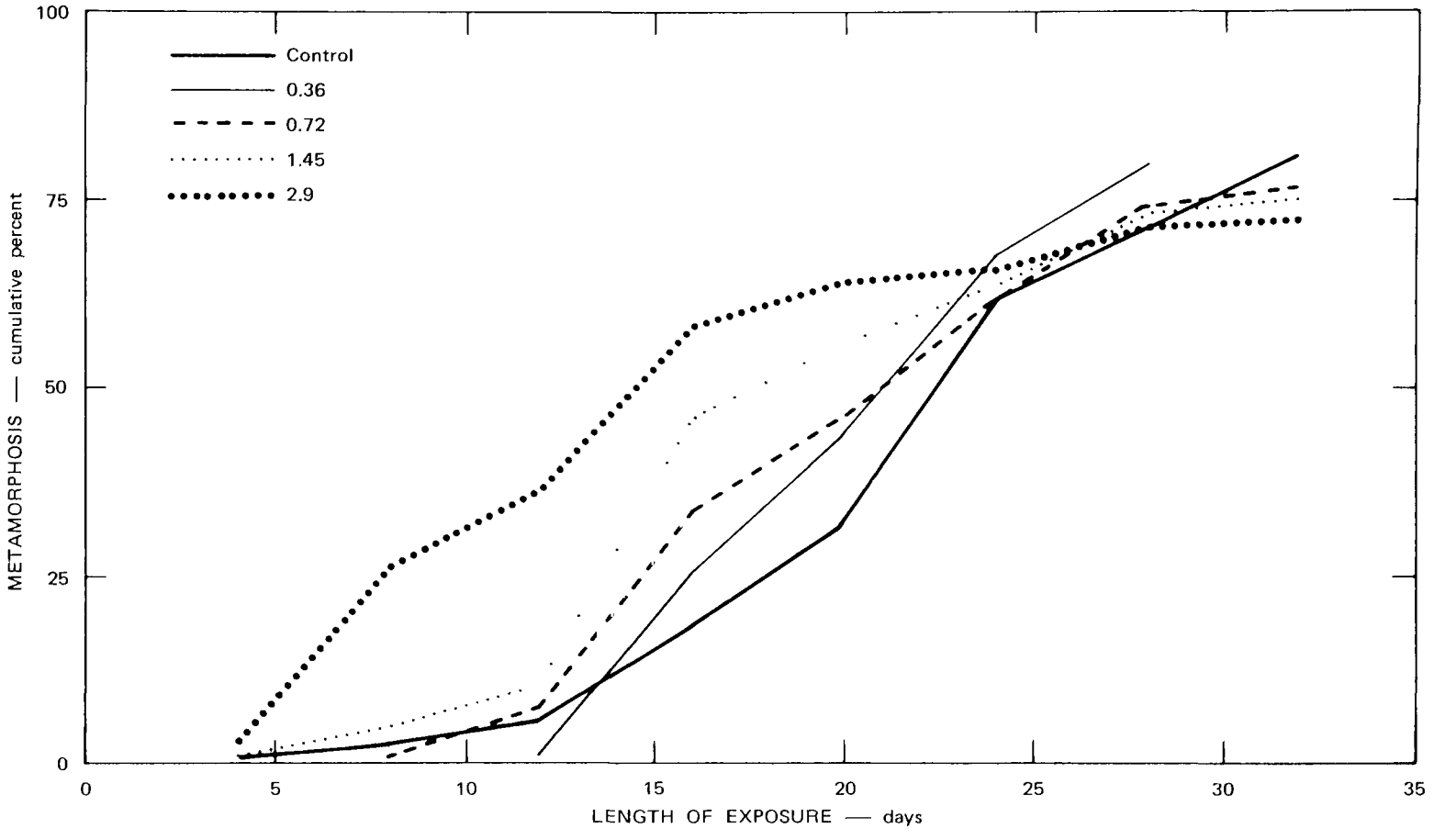

FIGURE 4 EFFECT OF EXPOSURE TO SEVIN ON PERCENTAGE OF MUSSEL POPULATION COMPLETING METAMORPHOSIS

Larvae were 29 to 30 days old when exposure was initiated. Data from the two experiments were pooled.

The 96-hour TL_{50} estimates we obtained for adult mussels are higher than those reported for adult forms of a number of other marine invertebrate organisms. The test organisms for most of the studies on adult forms have been marine arthropods, which, perhaps because of their close phylogenetic relationship to terrestrial insects, show a relatively high sensitivity to Sevin. In one study using the mollusc Clinocardium nuttalli (cockle clam), the 96-hour TL_{50} was reported as 3.85 mg/liter (Butler et al., 1968). In a more recent field study, Armstrong and Millemann (1974) observed reductions of up to 69% of controls in populations of the gaper clam (Tresus capax) and reductions of up to 28% in populations of the bent-nosed clam (Macoma nasuta) in field plots to which Sevin was applied. The pesticide concentration was not measured but was assumed to be less than 60 mg/liter.

Stewart and coworkers (1967) investigated the acute toxicity of Sevin and its byproduct 1-naphthol to a number of marine organisms. They found that the sensitivity of some of the organisms varied with water temperature and with the sex of the organism. The invertebrate species generally were more sensitive to Sevin than to 1/naphthol. The 24-hour TL_{50} for the mud shrimp (Callianassa californiensis) was 0.13 mg/liter at 16°C, but at 20°C it was 0.04 mg/liter. Female shore crabs (Hemigrapsis oregonensis) were more sensitive to Sevin than the male crabs: The 24-hour TL_{50} for females was 0.27 mg/liter, whereas the estimate for males was 0.71 mg/liter. The sensitivity of male and female Dungeness crabs (Cancer magister) was just the opposite of that of the shore crab. The 24-hour TL_{50} reported for male crabs was 0.60 mg/liter, whereas the estimate for female crabs was 0.63 mg/liter. This difference in response was not statistically significant, however. These investigators also reported an estimated 24-hour TL_{50} of 7.3 mg/liter for the cockle clam. That this estimate is almost twice the one reported by Butler and coworkers may be due to the differences in the length of exposure.

Stewart and associates (1967) also conducted 48-hour mussel embryo bioassays with Sevin and reported a 48-hour EC_{50} of 2.3 mg/liter. Several tests were performed with EC_{50} values ranging from 1.4 to 2.9 mg/liter. Our 48-hour EC_{50} estimate of 1.5 mg/liter is slightly lower. Using the same bioassay technique, Davis and Hidu (1969) investigated the effects of a large nuuber of pesticides to the

American oyster (Crassostrea virginica) and the hard-shell clam (Mercenaria mercenaria). Their estimates of the 48-hour EC_{50} for oyster and clam larvae were 3.0 and 3.82 mg/liter, respectively.

We were unable to locate any published reports on the effects of Sevin on mussel larvae growth or metamorphosis. Other bivalve molluscs have been studied, however. Butler and coworkers (1968) reported that three-day exposure to 0.8 mg/liter of Sevin is lethal to the larvae of the cockle clam. At concentrations as low as 0.1 mg/liter, Sevin inhibited the growth of clam larvae. In C. virginica, Davis and Hidu (1969) found that exposure to 2.0 mg/liter of Sevin for 12 days produced a marked reduction in larval growth.

We encountered many difficulties in our efforts to determine the effects of the five pesticides on metamorphosis, using survivors of the growth study. Thus, the data obtained are questionable. The study in which initiation of exposure was delayed until the larvae were 29 days old was better controlled and produced more meaningful information.

Our study indicates that adult forms of M. edulis may be more resistant to Sevin than other species of bivalve molluscs and are decidedly more resistant to the insecticide than marine anthropods. The early trochophore larvae of the mussel are as sensitive to Sevin as the larvae of several other molluscan bivalves; however, they are about 15 times less tolerant of the pesticide than the adult mussel.

Inhibition of shell growth was observed at a concentration 45% less than that which caused 50% of the larvae used in the 48-hour embryo bioassay experiments to develop abnormally. This concentration (0.65 mg/liter) also reduced the number of larvae that developed dissoconch shells and inhibited their growth rate.

Treflan

Treflan, also known as trifluralin, is a selective preemergence herbicide. Exposure of adult mussels to a measured concentration

of 0.24 mg/liter for 96 hours killed 50% of the test population (Table 8); however, all the mussels exposed to a measured level of 0.1 mg/liter of Treflan survived. Test concentrations above 0.24 mg/liter or between 0.24 and 0.1 mg/liter were not evaluated. The estimated solubility of this herbicide in seawater is 0.20 mg/liter.

Table 8. SURVIVAL AND ATTACHMENT DATA FOR ADULT MUSSELS EXPOSED TO TREFLAN FOR 96 HOURS

Test no.	Test concentration		No. of animals	Survival, percent	Attached, percent
	Nominal	Measured			
15	Control	--	9	100	100
	0.2	--	6	100	33.3
21	Control	--	10	100	100
	Control (acetone)	--	10	100	89
	0.2	--	10	100	0
43	Control	0	10	100	100
	Control (acetone)	0	10	90	100
	0.2	0.1	10	100	30
	0.4	0.24	10	50	10

Although 0.1 mg/liter of Treflan was not lethal to the adult mussels, this concentration and the higher concentration of 0.24 mg/liter reduced the number of mussels that attached to the glass test chamber. All mussels exposed to seawater alone or to seawater containing 200 µl/liter of acetone attached to the chamber during the experiment. Of the mussels exposed to 0.1 and 0.24 mg/liter of Treflan, only 30% and 10%, respectively, attached. The 96-hour EC_{50} for attachment, based on these data, is 0.35 mg/liter.

In the 48-hour embryo bioassay experiments, Treflan had no effect on shell development in the trochophore larvae at the highest measured test concentration of 0.12 mg/liter (Table 9). The mean of the percentages of normal control larvae for three duplicated experiments (six tests) was 88.6%. For larvae exposed to 0.12 mg/liter of Treflan, the mean was about 82%. The difference between the means was not statistically significant ($p = 0.05$).

Table 9. PERCENTAGE OF NORMAL LARVAE DEVELOPING IN 48 HOURS FROM MUSSEL EGGS FERTILIZED IN VARIOUS CONCENTRATIONS OF TREFLAN

Test concentration, mg/liter	Test number					
	Experiment 1		Experiment 2		Experiment 3	
	(1)	(2)	(1)	(2)	(1)	(2)
0	82.0	75.0	91.2	94.5	95.5	93.5
0 (acetone)	84.5	85.0	93.3	91.5	--	93.5
0.018	85.5	86.5	--	77.8	90.0	93.8
0.036	75.5	88.5	79.2	90.5	90.5	90.0
0.070	88.0	83.5	83.5	90.5	89.0	91.5
0.120	81.5	79.5	87.5	75.3	83.5	84.5
48-Hour EC_{50}, mg/liter	0.12		0.12		0.12	

In the larval growth study, the concentrations tested were 0.024, 0.048, 0.096, and 0.192 mg/liter. Seawater control and seawater-acetone control groups also were included. The acetone concentration was 50 µl/liter in all test chambers except those containing the seawater control groups. Thirty-five determinations were performed on the highest test concentration. The mean and standard deviation were 0.192 ± 0.037 mg/liter.

Reduced shell length was not observed in any of the Treflan-exposed treatment groups during the first 10 days of exposure (Table 10). To the contrary, the average shell length of larvae

Table 10. MEAN SHELL LENGTH OF MUSSEL LARVAE EXPOSED TO TREFLAN FOR 10 AND 20 DAYS

Test concentration, mg/liter	No. of larvae measured/ test	No. of tests/ experiment	Mean shell length, μ			
			Day 10		Day 10	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	20	2	146	182	270	288
0 (acetone)	20	2	154	179	252	302
0.024	20	2	178	200	222	320
0.048	20	2	176	218	255	262
0.096	20	2	168	188	200	254
0.192	20	2	160	196	190	207

exposed to all levels of Treflan was greater than that of the larvae reared in both control media. The degree of growth enhancement was different in the two tests. In Experiment 1, all the pesticide-exposed larvae were larger than the seawater control larve ($p = 0.05$) Except for those exposed to the highest Treflan concentration of 0.192 mg/liter, the pesticide-exposed larvae also were larger than the seawater-acetone control larvae. The average size of the seawater control larvae was less than that of the seawater-acetone larvae, although the difference was not statistically significant. In Experiment 2, there was no significant difference between mean sizes of larvae exposed to 0.096 mg/liter of Treflan and of those reared in the two control solutions; however, all other Treflan-exposed groups were larger than the control larvae.

After 20 days of exposure, differences in response were still apparent between tests. For example, in Experiment 2 the seawater-acetone control larvae were larger than the seawater control larvae ($p = 0.05$), but in Experiment 1 the opposite effect was observed. The only response consistent to both tests was the reduction in mean shell length observed in groups exposed to the two highest Treflan concentrations. Larvae exposed to 0.096 mg/liter of Treflan were

up to 25.9% smaller than seawater control larvae and up to 15.9% smaller than the seawater-acetone control larvae. Larvae exposed to 0.192 mg/liter of Treflan were up to 29.6% smaller than seawater control larvae and up to 28.1% smaller than seawater-acetone control larvae. These differences were statistically significant.

As with the data collected on metamorphosis of the larvae used in the Sevin growth studies, the data on metamorphosis of larvae exposed continuously to Treflan from the time they were 48 hours old were inconsistent and difficult to interpret. The data from duplicate experiments were pooled for analysis, but results from the two tests were analyzed separately. These data are shown in Table 11. Mean age values in the table refer to the age of the larvae at the time the first larva or group of larvae completed metamorphosis.

Table 11. NUMBER, AGE, AND SIZE OF JUVENILE MUSSELS DEVELOPING IN LARVAL CULTURES EXPOSED AT 48 HOURS TO VARIOUS CONCENTRATIONS OF TREFLAN

Test concentration, mg/liter	Experiment 1				Experiment 2			
	No. of juveniles	Age,[a] days	Length, μ		No. of juveniles	Age,[a] days	Length, μ	
			Mean	SD			Mean	SD
0	54	24	425	77.0	178	26	456	78.7
0 (acetone)	87	27	402	54.3	81	26	410	61.0
0.024	84	28	439	84.0	146	26	444	72.6
0.048	167	26	379	50.7	150	26	431	80.1
0.096	0	--	--	--	43	32	360	34.4
0.192	0	--	--	--	0	--	--	--

[a]Number of days required by treatment group to begin metamorphosing.

Larvae exposed to the highest test concentration of 0.192 mg/liter did not undergo metamorphosis. None of the larvae exposed to this concentration lived beyond 26 days of exposure. Nor did the larvae exposed to 0.096 mg/liter of Treflan in Experiment 1 survive longer than 26 days. Inspection of the number of larvae that did undergo metamorphosis in Experiment 1 does not reveal any deleterious effects. In Experiment 1, metamorphosis appeared to have been delayed by two to four days in groups exposed to 0.024 and 0.048 mg/liter when the age data for these groups were compared with those for the seawater controls; however, comparison with the age data for the seawater-acetone controls revealed no delay.

In Experiment 2, twice as many seawater control larvae developed into juveniles than seawater-acetone control larvae; however, because this effect was not observed consistently in the two studies (Treflan and methoxychlor) in which seawater-acetone controls were employed, we believe the effect may be an artifact. In this experiment, the number of juvenile mussels developing from larvae exposed to 0.096 mg/liter of Treflan was only 24% of the number of juvenile seawater control larvae and about half the number of juvenile seawater-acetone control larvae. The Treflan-exposed larvae also began to undergo metamorphosis six days later than the controls and the larvae exposed to the next lower concentration, 0.048 mg/liter. In this experiment, the larvae exposed to 0.096 mg/liter were also about 21% smaller than the seawater control larvae at the time of metamorphosis.

These effects were not observed in the metamorphosis study in which exposure to Treflan was not initiated until the larvae were 30 days old (Table 12). The concentrations tested in this study were slightly lower than those used in the growth study, although the same concentrations were intended. The mean and standard deviation of 34 determinations made on the highest test concentration, nominally set at 0.4 mg/liter, were 0.16 ± 0.072 mg/liter. The lower concentrations were estimated at 0.08, 0.04, and 0.02 mg/liter.

Juvenile Traflan-exposed mussels were somewhat smaller than the seawater control larvae. As a group, they were 9.6% smaller than the seawater controls, although their size did not appear to be related to the pesticide concentration.

Table 12. EFFECT OF TREFLAN ON MUSSEL LARVAE METAMORPHOSIS AFTER A 40-DAY EXPOSURE INITIATED 30 DAYS AFTER FERTILIZATION OF THE EGGS
(Presence of Dissoconch Shell Used as Indication of Metamorphosis)

	Test concentration, mg/liter											
	Seawater control		Solvent control		0.02		0.040		0.080		0.160	
	1	2	1	2	1	2	1	2	1	2	1	2
Number of larvae												
Initial	100	100	100	100	100	100	100	100	100	100	100	100
Lost or crushed	24	16	22	31	27	29	6	16	23	12	20	2
Experimental	76	84	78	69	73	71	94	84	77	88	80	98
Metamorphosing, percent	89.5%	92.8%	89.7%	87.0%	84.9%	78.9%	85.1%	86.9%	79.2%	81.8%	87.5%	75.5%
Nonmetamorphosing, percent	7.9%	4.8%	0	0	0	0	1.1%	0	1.3%	2.3%	0	1.0%
Dead, percent	2.6%	2.4%	10.3%	13.0%	15.1%	21.1%	13.8%	13.1%	19.5%	15.9%	12.5%	23.5%
Days to metamorphosis of 50% test larvae	21	21	16	12	12	14	12	11	16	16	16	17
Shell length of juvenile mussels, μ	449 μ	427 μ	424 μ	387 μ	383 μ	403 μ	412 μ	424 μ	397 μ	383 μ	402 μ	406 μ

An average of only 2.5% of the seawater control larvae died during the 40-day experiment; however, an average of 18.1% died at the lowest concentration of 0.02 mg/liter, and the same percentage of larvae died at the highest concentration of 0.16 mg/liter. Although this indicates that larval mortality is not proportional to pesticide concentration, it also implies that mortality may not have been caused by Treflan. Exposure to 50 µl/liter of acetone alone killed an average of 11.6% of the larvae. The high mortality observed among the seawater-acetone controls implicates acetone; however, mortality among the seawater-acetone controls employed in the tests on methoxychlor was less than that of the seawater controls; hence, some other factor must be involved.

The rate of metamorphosis of Treflan-exposed larvae was greater than that of the seawater control larvae (Figure 5). The metamorphosis rate for Treflan-exposed larvae was not related to the test concentration. As a group, 50% of the pesticide-exposed larvae reached the juvenile stage in an average of 14.2 days, whereas the average for controls was 21 days. Here acetone appears definitely to be involved. Of the seawater-acetone controls that did undergo metamorphosis, 50% reached the juvenile stage in in an average of 14 days. Hence, all larvae exposed to acetone metamorphosed at a more rapid rate than those that were not exposed to acetone. The same effect was observed in the experiments on methoxychlor.

Acetone also appeared to inhibit growth. Metamorphosed larvae that had been exposed to 50 µl/liter of acetone alone averaged 406 µ--7.3% smaller than the controls that had been exposed to seawater alone. Metamorphosed larvae from the methoxychlor-laden test chambers averaged 401 µ, or 8.4% less than the seawater controls. These larvae also were exposed to 50 µl/liter of acetone, which was used as a pesticide-dispersing agent. The mean size of the larvae for each pesticide-exposed group did not vary with pesticide concentration; hence, Treflan did not appear to be a factor in inhibiting growth in this test.

Although the effects of exposure to Treflan have been studied in aquatic organisms, we know of no studies using marine invertebrates. Sanders (1970) determined the toxicity of a number of herbicides to

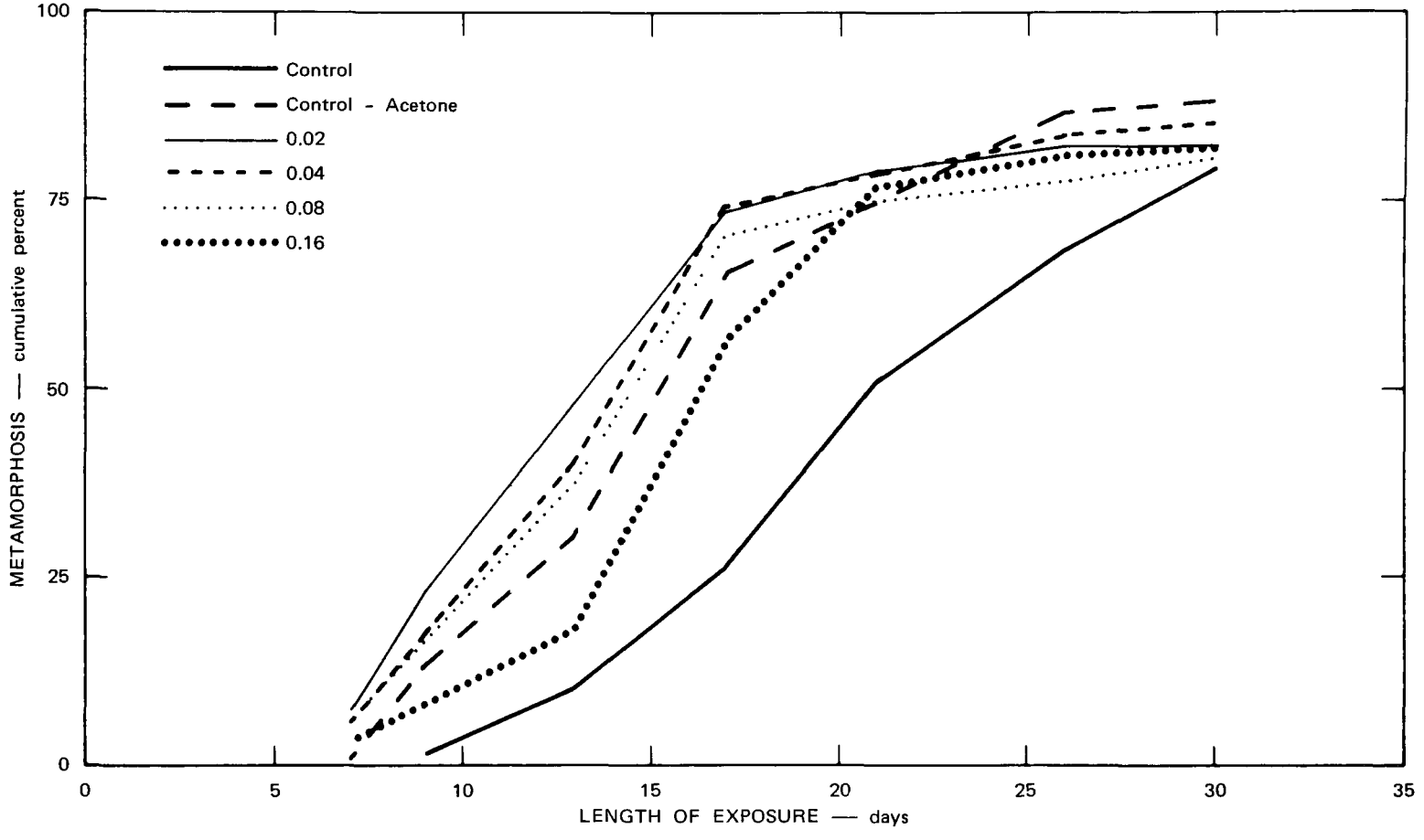

FIGURE 5 EFFECT OF EXPOSURE TO TREFLAN ON PERCENTAGE OF MUSSEL POPULATION COMPLETING METAMORPHOSIS

Larvae were 29 to 30 days old when exposure was initiated. Data from the two experiments were pooled.

six freshwater arthropods and reported 48-hour TL_{50} values ranging from 0.25 mg/liter for the seed shrimp (Cyprinodopsis vidua) to 3.2 mg/liter for the grass shrimp (Paleomonetes kadiakensis) and 50 mg/liter for the crayfish (Orconectes nais). The toxicity of Treflan to various species of freshwater fish also has been reported (Macek et al., 1969; Parka and Worth, 1965; Bohmont, 1967; Worth and Anderson, 1965).

Although our study showed that Treflan may be lethal to adult mussels exposed to 0.24 mg/liter for four days and can inhibit shell growth in larval mussels at a concentration as low as 0.096 mg/liter if exposure exceeds 10 days, under nonlaboratory conditions it us unlikely that Treflan exerts serious deleterious effects on the bay mussel or perhaps on other bivalve molluscs. Its solubility in seawater with a salinity of 25‰, pH of 8, and temperature of 20°C is about 0.2 mg/liter. Its half-life in seawater is only 82 hours. A selective preemergence herbicide designed for use with a variety of farm crops, particularly vegetables, Treflan is applied by mixing it into the soil. When applied at recommended levels, it usually disappears in four to six months but not by leaching (Herbicide Handbook, 1970). These characteristics suggest that, unless Treflan is applied directly into the aquatic habitat, the possibility of toxic levels entering the aquatic environment is low.

Methoxychlor

In terms of survival, methoxychlor was not toxic to adult mussels exposed to concentrations as high as 0.092 mg/liter for 96 hours (Table 13). This concentration is almost twice that estimated for methoxychlor in methoxychlor-saturated, 20°C seawater. This level of insecticide also had no effect on attachment.

In the 48-hour embryo bioassay experiments, the test solutions contained measured levels of 0.011, 0.018, 0.041, and 0.075 mg/liter of methoxychlor. Exposure to methoxychlor did not affect the development of normal larvae significantly at any of these concentrations (Table 14). The mean percentages of normal larvae found in the seawater control and seawater-acetone control groups were 89.4 and 88.7, respectively. The mean percentage of normal larvae found

Table 13. SURVIVAL AND ATTACHMENT DATA FOR ADULT MUSSELS EXPOSED TO METHOXYCHLOR FOR 96 HOURS

Test no.	Test concentration		No. of animals	Survival, percent	Attached, percent
	Nominal	Measured			
14	Seawater control	--	6	100	100
	Acetone control	--	6	100	100
	0.050	--	6	100	100
42	Seawater control	0	10	100	90
	Acetone control	0	10	90	90
	0.050	0.007	10	100	100
	0.100	0.020	10	100	100
45	Seawater control	0	10	100	100
	0.050	0.055	10	100	100
	0.100	0.0925	10	100	100

Table 14. PERCENTAGE OF NORMAL LARVAE DEVELOPING IN 48 HOURS FROM MUSSEL EGGS FERTILIZED IN VARIOUS CONCENTRATIONS OF METHOXYCHLOR

Test concentration, mg/liter	Test number							
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
0	88.5	84.5	91.5	91.0	89.3	86.0	94.5	89.5
0 (acetone)	86.0	89.5	89.0	90.0	89.0	89.5	90.5	85.8
0.011	89.5	88.0	90.5	87.0	86.0	86.5	93.4	93.0
0.018	87.5	90.0	89.0	88.5	91.5	86.0	94.0	93.5
0.041	80.5	83.0	90.5	90.0	89.3	95.3	84.0	--
0.075	94.0	98.5	84.0	90.0	91.3	75.1	88.0	91.3
48-Hour EC_{50}, mg/liter	>0.075		>0.075		>0.075		>0.075	

in the containers with the highest methoxychlor concentration of 0.075 mg/liter was 89.0.

The mean and standard deviation for the highest test concentration used in the growth study was 0.062 ± 0.0078 mg/liter, based on 43 analyses. Estimates of the lower test concentrations are 0.031, 0.015, and 0.008 mg/liter. Seawater control and seawater-acetone control groups were employed in each series of tests. The acetone concentration in all test chambers except the seawater control chambers was 50 µl/liter.

The results obtained from the two experiments were slightly different (Table 15). In Experiment 1, the mean size of the larvae exposed to all levels of methoxychlor for 10 days was similar to that of the seawater control larvae, except for those reared in 0.032 mg/liter of methoxychlor. These larvae were 7.7% larger than the seawater controls. The difference was statistically significant ($p = 0.05$). All larvae exposed to methoxychlor were larger than the seawater-acetone controls. Analysis of the data by applying the t-test showed that this difference was significant. The same

Table 15. MEAN SHELL LENGTH OF MUSSEL LARVAE EXPOSED TO METHOXYCHLOR FOR 10 AND 20 DAYS

Test concentration, mg/liter	No. of larvae measured/ test	No. of tests/ experiment	Mean shell length, μ			
			Day 10		Day 20	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	20	2	182	195	246	270
0 (acetone)	20	2	173	191	257	283
0.008	20	2	186	196	252	264
0.015	20	2	184	196	262	293
0.031	20	2	196	196	261	273
0.062	20	2	188	188	228	249

test indicated, however, that the seawater-acetone controls were not significantly smaller than the seawater controls. In Experiment 2, the mean sizes for all groups were similar.

After 20 days of exposure, the larvae exposed to the highest concentration of 0.062 mg/liter were smaller than the seawater controls by about 7.5% and smaller than the seawater-acetone controls by about 11.5%--statistically significant differences. Inhibition of growth was not observed in any other methoxychlor-exposed group.

Marked reductions in the number of larvae that developed into juvenile mussels were observed when 48-hour-old larvae were exposed to methoxychlor for 38 days (Table 16). In Experiment 1, the numbers of metamorphosed larvae recovered from all chambers containing methoxychlor were about the same. On the average, these chambers contained 82.5% fewer juvenile mussels than those containing the seawater-acetone controls. In Experiment 2, the effect appeared to be related to the pesticide concentration. Of larvae exposed to 0.008, 0.015, 0.031, and 0.062 mg/liter of methoxychlor, the numbers that completed metamorphosis were 37, 50, 56, and 88% less, respectively, than the number of seawater-acetone controls that completed metamorphosis.

Table 16. NUMBER, AGE, AND SIZE OF JUVENILE MUSSELS DEVELOPING IN LARVAL CULTURES EXPOSED AT 48 HOURS TO VARIOUS CONCENTRATIONS OF METHOXYCHLOR

Test concentration, mg/liter	Experiment 1				Experiment 2			
	No. of juveniles	Age,[a] days	Length, μ		No. of juveniles	Age,[a] days	Length, μ	
			Mean	SD			Mean	SD
0	24	26	395	64.9	126	28	448	74.3
0 (acetone)	99	26	427	71.4	68	26	419	68.4
0.008	14	26	401	71.4	43	26	373	55.0
0.015	27	26	412	61.9	34	26	389	62.6
0.031	12	26	345	48.0	30	26	395	50.0
0.062	15	26	385	59.5	8	28	358	27.2

Exposure to methoxychlor also appeared to inhibit growth at all pesticide levels. The degree of inhibition did not appear to be related to the pesticide concentration. In both experiments, the pesticide-exposed larvae were about 9.5% smaller than the seawater-acetone controls.

In the metamorphosis study in which exposure to methoxychlor was delayed until the larvae were 29 days old (Table 17), the concentrations tested were insignificantly different from those used in the growth and metamorphosis experiments. The mean and standard deviation of the highest test concentration were 0.0595 ± 0.0081 mg/liter based on 31 analyses. The larvae were exposed to the pesticide for 41 days.

A slight decrease in the number of larvae that completed metamorphosis was observed at concentrations of 0.015 mg/liter or higher. For larvae exposed to 0.015, 0.03, and 0.06 mg/liter, the percentages of metamorphosed larvae, averaged for the two tests, were 84, 88.4, and 82.8, respectively. For the seawater controls and the seawater-acetone controls, the percentages were 93.4 and 96.2, respectively.

Table 17. EFFECT OF METHOXYCHLOR ON MUSSEL LARVAE METAMORPHOSIS AFTER A 41-DAY EXPOSURE INITIATED 29 DAYS AFTER FERTILIZATION OF THE EGGS (Presence of Dissoconch Shell Used as Indication of Metamorphosis)

	Test concentration, mg/liter											
	Seawater control		Solvent control		0.008		0.015		0.03		0.060	
	1	2	1	2	1	2	1	2	1	2	1	2
Number of larvae												
Initial	100	100	100	100	100	100	100	100	100	100	100	100
Lost or crushed	21	39	17	20	18	20	0	27	20	11	11	24
Experimental	79	70	86	73	82	80	100	73	80	89	89	76
Metamorphosing, percent	92.4%	94.3%	96.5%	95.9%	95.1%	97.5%	79.0%	89.0%	93.8%	83.1%	80.0%	85.5%
Nonmetamorphosing, percent	1.3%	0	0	0	0	0	0	0	0	0	0	0
Dead, percent	6.3%	5.7%	3.5%	4.1%	4.9%	2.5%	21.0%	11.0%	6.2%	16.8%	19.1%	14.5%
Days to metamorphosis of 50% of test larvae	20	22	11	14	11	14	16	11	16	17	16	14
Shell length of juvenile mussels, μ	480 μ	457 μ	408 μ	406 μ	386 μ	411 μ	377 μ	376 μ	377 μ	390 μ	364 μ	400 μ

Exposure to methoxychlor also increased larval mortality. This parameter was not affected at 0.008 mg/liter; however, mortality percentage means for larvae exposed to 0.015, 0.03, and 0.06 mg/liter of methoxychlor were 16, 11.5, and 17, respectively. For the seawater controls and the seawater-acetone controls, the mean percentages for mortality were 6.0 and 3.8, respectively.

As observed in the study of Treflan, the larvae in test chambers containing acetone completed metamorphosis more rapidly than the larvae reared in seawater alone (Figure 6). For larvae exposed to acetone, the number of days required for 50% of the animals to undergo metamorphosis ranged from 11 to 17; the mean was 14 days. The mean for seawater control larvae was 21 days.

In addition to influencing the rate of larval metamorphosis, acetone appeared to inhibit growth. This effect also was observed in the study of Treflan, in which acetone was used as a dispersing agent. In the study of methoxychlor, the growth-inhibiting effect of acetone was more pronounced. Metamorphosed larvae exposed to seawater containing 50 µl/liter of acetone alone were 13% smaller than the seawater controls. As a group, the methoxychlor-exposed larvae, which were also exposed to 50 µl/liter of acetone, averaged 17.7% smaller than the seawater controls. The size of the larvae did not appear to be related to the pesticide concentration; hence, methoxychlor, like Treflan, did not appear to be involved in inhibiting the growth of the larvae in this test.

The toxicity of methoxychlor to marine bivalve molluscs has not been studied extensively. Most of the toxicological information on this pesticide concerns freshwater organisms, particularly fish. Eisler and Weinstein (1967) reported that exposure of the hard-shelled clam (M. mercenaria) to 1.1 mg/liter of methoxychlor was not lethal, although the insecticide was highly toxic to marine crustaceans. The 48-hour TL_{50} estimates for the grass shrimp, sand shrimp, and hermit crab ranged from 0.004 to 0.012 mg/liter. According to Butler (1963), the brown shrimp (Panaeus aztecus) is also highly sensitive to low concentrations of methoxychlor, having a 48-hour TL_{50} value of 0.006 mg/liter. Butler also reported that exposure of C. virginica to 0.097 mg/liter for four days resulted in a 50% growth reduction.

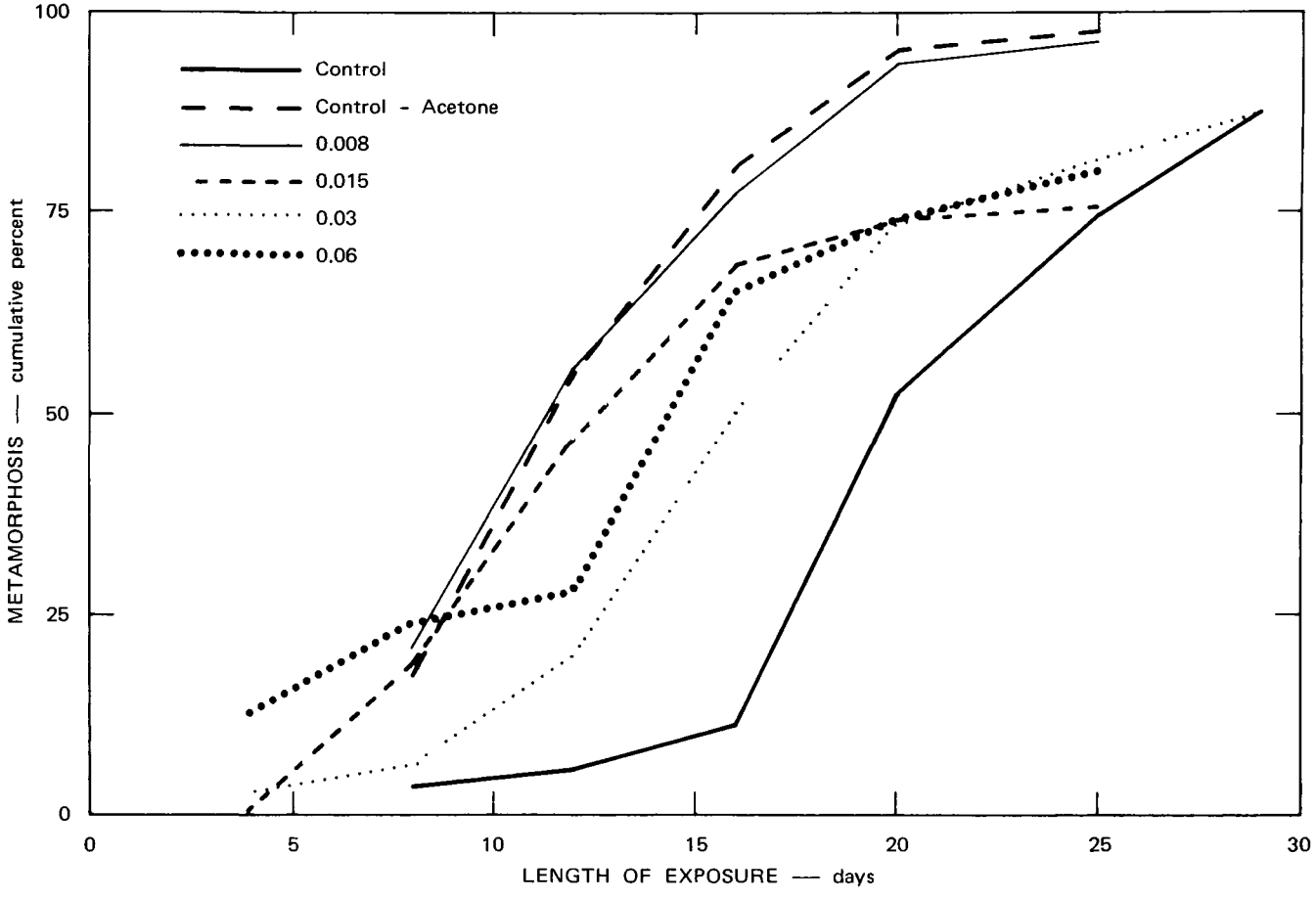

FIGURE 6 EFFECT OF EXPOSURE TO METHOXYCHLOR ON PERCENTAGE OF MUSSEL POPULATION COMPLETING METAMORPHOSIS

Larvae were 29 to 30 days old when exposure was initiated. Data from the two experiments were pooled.

Our study indicates that the bay mussel has considerable tolerance to methoxychlor. Survival and attachment by byssus threads in small adult mussels (3 to 4 cm) were not affected by exposure for 96 hours to methoxychlor concentrations as high as 0.092 mg/liter, which is almost twice the estimated solubility of the pesticide in 20°C seawater. Nor did the pesticide have an adverse effect on the percentage of normal larvae developing from eggs fertilized in water containing as much as 0.075 mg/liter. Exposure of 48-hour-old larvae to concentrations as high as 0.062 mg/liter for 10 days did not reduce growth. Only after relatively prolonged exposure to methoxychlor were adverse effects observed. Exposure to 0.062 mg/liter of the pesticide reduced growth by 11.6% after 20 days. The same concentration also reduced by 88% the number of larvae developing into juvenile mussels during exposure for 38 days.

The chemical stability of methoxychlor and, thus, its relatively high environmental persistence suggest that, in spite of its relatively low acute toxicity to the bay mussel, the pesticide cannot be considered nonhazardous to the mussel or other bivalve molluscs.

2,4-D

The acid form of 2,4-D was used in this study. Several other forms are commercially available, including the amine salt, the butoxyethanol ester, and the acetamide salt. The solubility of the acid form in distilled water is 600 to 700 mg/liter (Herbicide Handbook, 1970). Our estimate of solubility in 20°C seawater was about 1100 mg/liter.

Exposure of small adult mussels (3 to 4 cm) to measured concentrations of 331 and 334 mg/liter of 2,4-D for 96 hours resulted in 100% mortality (Table 18). Mortality may have resulted from low pH, which averaged 6.5 at 331 mg/liter and 6.4 at 334 mg/liter (Table 19). The 96-hour TL_{50}, estimated from data obtained from Test 12, was 259 mg/liter, with 95% confidence limits of 232 to 289 mg/liter. Pesticide determinations were not performed on the test solutions used in Test 7. The 96-hour TL_{50}, based on the nominal concentrations, was 290 mg/liter. [The 95% confidence limits could not be calculated using the method of Litchfield and Wilcoxon (1949).] The number of mussels that attached themselves

Table 18. SURVIVAL AND ATTACHMENT DATA FOR ADULT MUSSELS EXPOSED TO 2,4-D (ACID) FOR 96 HOURS

Test no.	Test concentration		No. of animals	Survival, percent	Attached, percent
	Nominal	Measured			
7	0	--	6	100	--
	50	--	6	83	--
	100	--	6	100	--
	200	--	6	83	--
	400	--	6	33	--
	600	--	6	0	--
12	0	0	12	100	92
	100	--	6	100	100
	200	142	6	100	100
	300	--	12	92	83
	400	274	6	50	33
	450	--	12	42	25
	500	331	6	0	0
	600	334	12	0	0
41	0	0	10	100	80
	250	240	10	100	50
	300	275	10	100	80
	350	340	10	30	10
	400	395	10	0	0
	450	445	10	0	0
40	0-450	--	10	All animals died within 24 hours.	

Table 19. WATER QUALITY DATA FOR 96-HOUR ADULT MUSSEL SURVIVAL TEST NO. 12 ON 2,4-D

Test concentration, mg/liter	Temperature, °C		Dissolved oxygen, mg/liter		Salinity, ‰		pH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	18.1	0.72	6.4	0.74	23.3	0.74	7.8	0.11
--	18.0	0.67	7.6	1.54	23.4	0.25	7.9	0.11
142	18.0	0.66	7.8	1.55	22.9	0.75	7.8	0.11
--	17.9	0.68	8.0	1.54	23.5	0.41	7.7	0.01
274	18.0	0.56	8.2	0.91	24.0	0.00	7.4	0.54
--	17.2	0.00	9.1	0.08	24.5	0.00	7.6	0.01
331	18.4	0.35	8.7	0.43	24.5	0.00	6.5	0.22
334	18.6	0.35	8.7	0.14	24.5	0.00	6.4	0.07

to the test chamber was less than that of the controls at concentrations above 142 mg/liter. The 96-hour EC_{50} for attachment, based on data from Test 12, was 262 mg/liter.

The trochophore larvae were slightly more sensitive to 2,4-D than the adults. The 48-hour EC_{50} estimates ranged from 210 to 213 mg/liter for three embryo bioassay experiments in which each treatment group was tested in duplicate (Table 20). The mean was 211.7 mg/liter.

The 2,4-D concentrations evaluated in the growth study were 182.8, 91.4, 45.7, and 22.8 mg/liter. The standard deviation of the highest test concentration--the only test level monitored throughout the experiment--was 10.2 mg/liter. Only seawater controls were employed. Table 21 presents data from the study.

After 10 days of exposure, a statistically significant reduction in growth was observed in larvae exposed to a concentration of 91.4 mg/liter of 2,4-D (Experiment 2). These larvae were 11.6% smaller than the controls. Reduction in growth was not observed at this concentration in Experiment 1. At 182.8 mg/liter, inhibition

Table 20. PERCENTAGE OF NORMAL LARVAE DEVELOPING IN 48 HOURS FROM MUSSEL EGGS FERTILIZED IN VARIOUS CONCENTRATIONS OF 2,4-D

Test concentration, mg/liter	Test number					
	Experiment 1		Experiment 2		Experiment 3	
	(1)	(2)	(1)	(2)	(1)	(2)
0	88.5	86.0	95.5	98.5	90.5	92.0
47	90.0	90.5	91.0	91.0	93.5	93.5
98	88.0	93.5	88.8	96.0	92.3	88.0
120	92.5	93.5	91.0	92.5	92.5	95.5
140	87.5	91.5	86.5	87.0	93.0	--
160	88.0	89.5	82.5	88.0	82.5	88.5
190	74.5	71.0	86.0	80.5	81.5	86.5
240	0	0.5	0	0	0	0
48-Hour EC_{50}, mg/liter	210		212		213	

Table 21. MEAN SHELL LENGTH OF MUSSEL LARVAE EXPOSED TO 2,4-D FOR 10 AND 20 DAYS

Test concentration, mg/liter	No. of larvae measured/ test	No. of tests/ experiment	Mean shell length, μ			
			Day 10		Day 20	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	20	2	182	172	297	259
22.8	20	2	198	188	287	278
45.7	20	2	182	170	293	268
91.4	20	2	178	152	231	234
182.8	20	2	124	112	--	206

of growth was observed in both tests. In Experiments 1 and 2, respectively, larvae were 31.9 and 34.9% smaller than the controls. Continued exposure of the larvae for 20 days resulted in statistically significant decreases in larval size at 91.4 and 182.8 mg/liter. In Experiments 1 and 2, the larvae exposed to 91.4 mg/liter of 2,4-D were smaller than the controls by 22.5 and 9.6%, respectively. All the larvae exposed to 182.8 mg/liter of 2,4-D in Experiment 1 died within 12 days of exposure. Larvae exposed to this concentration in Experiment 2 were 20.5% smaller than the controls. The differences were statistically significant ($p = 0.05$).

The growth study was extended to 32 days to determine the effect of 2,4-D on larval metamorphosis. The data, presented in Table 22, varied considerably between the two tests and did not follow any readily identifiable trend. Fewer controls than pesticide-exposed larvae developed into juvenile mussels. The only observation consistent to both experiments was that the larvae exposed to 182.8 mg/liter failed to undergo metamorphosis. At this concentration, the four larval groups tested did not survive longer than 22 days.

Table 22. NUMBER, AGE, AND SIZE OF JUVENILE MUSSELS DEVELOPING IN LARVAL CULTURES EXPOSED AT 48 HOURS TO VARIOUS CONCENTRATIONS OF 2,4-D

Test concentration, mg/liter	Experiment 1				Experiment 2			
	Total number	Age,[a] days	Length, μ		Total number	Age,[a] days	Lemgth, μ	
			Mean	SD			Mean	SD
0	14	26	372	57.6	25	22	386	56.7
22.8	99	22	453	81.7	72	22	444	77.6
45.7	124	24	361	52.3	67	24	356	48.1
91.4	3	24	339	81.3	43	26	343	41.6
182.8	0	--	--	--	0	--	--	--

[a]Number of days required by treatment group to begin metamorphosing.

Metamorphosis of larvae exposed from age 30 days to age 70 days was not markedly affected by exposure to 2,4-D concentrations as high as 176 mg/liter (Table 23). Variability in the number of larvae that completed metamorphosis increased at concentrations above 22 mg/liter. The mean percentages of larvae that completed metamorphosis while exposed to 2,4-D varied from 68.6 for larvae exposed to 44 mg/liter to 70.6 for those exposed to 22 mg/liter. The mean for all 2,4-D-exposed groups was 70%. Of the control larvae, 79% in both tests developed into juvenile mussels.

Generally, larvae exposed to 2,4-D exhibited higher mortality than controls. Between-test mortality percentages varied considerably more in the pesticide-exposed groups than in the control groups; however, mortality did not vary with concentration. As a group, the 2,4-D-exposed larvae had a mortality average of 12.7%, compared with 7% for the controls. During the first 22 days of exposure, the larvae exposed to the two higher levels of 2,4-D metamorposed at a faster rate than any other group (Figure 7). However, no significant differences in rate were observed by the end of the experiments.

Few toxicity studies on the acid form of 2,4-D have been performed on marine invertebrate organisms. Butler (1963) reported that 2.0 mg/liter of 2,4-D acid and the same concentration of the diethylamine salt did not affect shell growth in adult oysters (C. virginica); however, 3.75 mg/liter of the butoxyethanol ester reduced shell growth by 50%, and 5 mg/liter of the 2-ethylhexyl ester of 2,4-D reduced it by 38%. Davis and Hidu (1969) tested two forms of 2,4-D using C. virginica and reported 48-hour EC_{50} values, based on embryo bioassays, of 8 mg/liter for the butoxyethanol ester and 20.44 mg/liter for the diethylamine salt. They also reported 14-day TL_{50} estimates, based on larval survival, of 0.74 mg/liter for the ester and 64.29 mg/liter for the salt. Marine crustacea appear to have much more tolerance to 2,4-D acid than marine bivalve molluscs. The 96-hour TL_{50} for the fiddler crab (Uca pugnax) is reported to be 5000 mg/liter (Sudak and Claff, 1960).

Table 23. EFFECT OF 2,4-D ON LARVAL METAMORPHOSIS AFTER A 40-DAY EXPOSURE INITIATED 30 DAYS AFTER FERTILIZATION OF THE EGGS
(Presence of Dissoconch Shell Used as Indication of Metamorphosis)

	Test concentration, mg/liter									
	Control		22		44		88		176	
	1	2	1	2	1	2	1	2	1	2
Number of larvae										
Initial	100	100	100	100	100	100	100	100	100	100
Lost or crushed	18	23	20	16	20	3	33	3	49	26
Experimental	82	77	80	84	80	97	67	120	51	74
Metamorphosing, percent	79.3%	79.2%	71.2%	70.3%	77.5%	59.8%	73.2%	66.7%	80.4%	60.8%
Nonmetamorphosing, percent	14.6%	13.0%	11.3%	21.4%	13.7%	22.7%	14.9%	24.2%	9.8%	20.3%
Dead, percent	6.1%	7.8%	17.5%	8.3%	8.8%	17 5%	11.9%	9.1%	9.8%	18.9%
Days to metamorphosis of 50% of test larvae	27	28	23	25	22	27	21	24	22	31
Shell length of juvenile mussels, μ	428 μ	468 μ	446 μ	484 μ	456 μ	463 μ	486 μ	463 μ	390 μ	412 μ

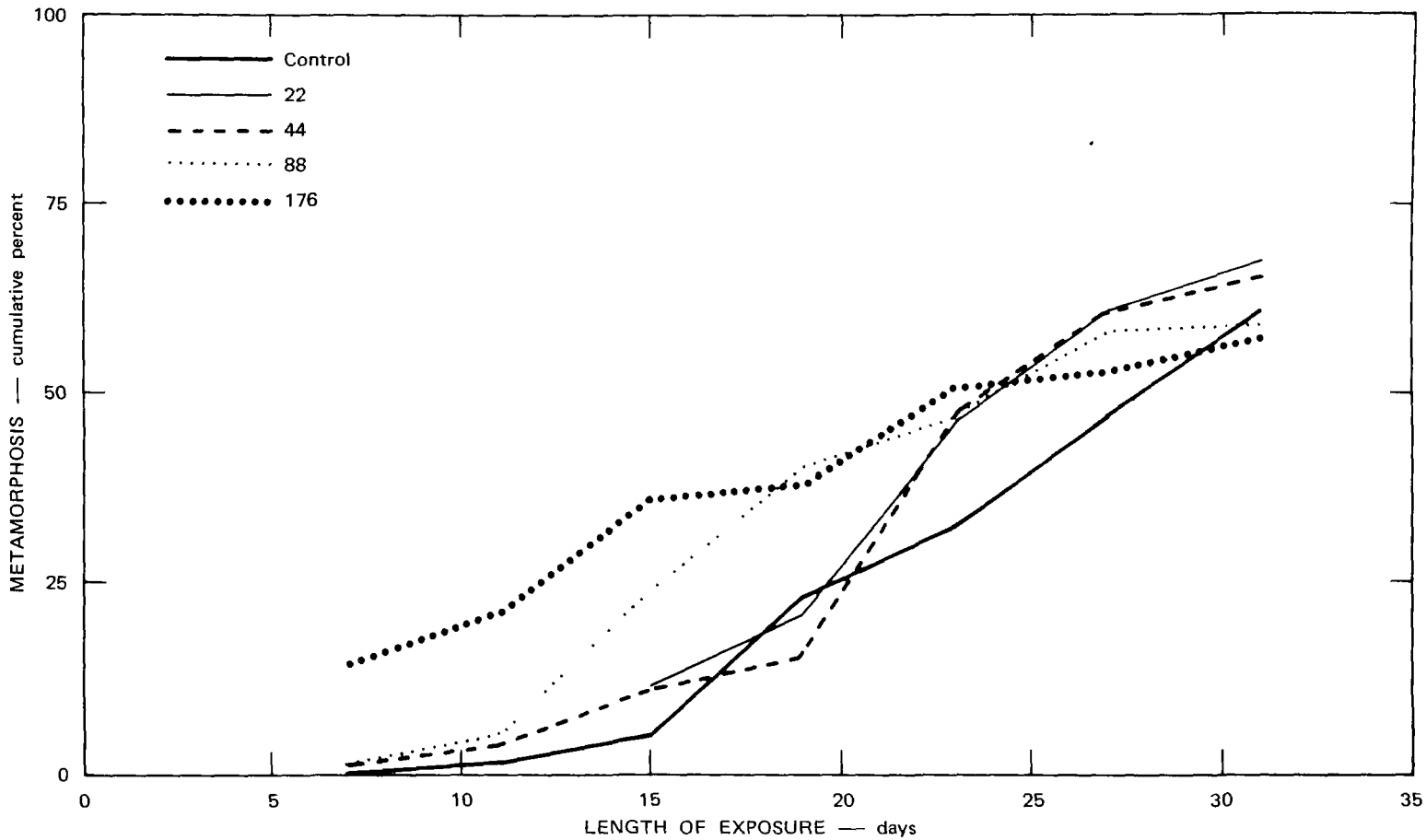

FIGURE 7 EFFECT OF EXPOSURE TO 2,4-D ON PERCENTAGE OF MUSSEL POPULATION COMPLETING METAMORPHOSIS

Larvae were 29 to 30 days old when exposure was initiated. Data from the two experiments were pooled.

Malathion

Malathion is an organophospate insecticide that degrades relatively rapidly upon application to soil or water. In a silt-loam soil, 3.2 ppm persisted for eight days, with about 0.1 ppm remaining (Lichtenstein and Schultz, 1964). In water, 10% of 10 μg/liter remained after 14 days (Rumker et al., 1972).

The insecticide appears to be much more toxic to aquatic crustacea than to aquatic molluscs. The 24-hour TL_{50} for the grass shrimp, sand shrimp, and hermit crab ranges from 0.118 to 0.246 mg/liter (Eisler, 1969), whereas the clam, M. mercenaria, tolerated four days of exposure to a concentration of 37 mg/liter (Eisler and Weinstein, 1967). Using shell growth as a measure of effect, Butler (1963) found that exposure of C. virginica to 0.097 mg/liter of malathion reduced growth by 50%. The number of normal larvae developing from eggs of the same species of oyster, fertilized in water containing 9.07 mg/liter of malathion, also was reduced by 50% in 48 hours (Davis and Hidu, 1969). The same investigators reported a 14-day TL_{50} of 2.66 mg/liter for oyster larvae survival.

Although several tests were performed, we were unable to determine the TL_{50} for adult mussels using survival or attachment as the measured parameter. Response was inconsistent within and between tests (Table 24). For example, in one test only 20% of the mussels survived at a measured concentration of 9.0 mg/liter, and 100% mortality was observed at 17 mg/liter. In another test, all the mussels survived at a measured concentration of 39.4 mg/liter. The response pattern for attachment was also inconsistent.

The response of the embryos to malathion was much more consistent than that of the adults. The 48-hour EC_{50} values, estimated from the data from four duplicated embryo bioassays, were 13.5, 17.2, 12.5, and 10.5 mg/liter. The mean was 13.4 mg/liter. Table 25 presents data from these tests.

In the growth tests, the highest test concentration for malathion averaged 12.3 ± 2.3 mg/liter. The mean and standard deviation were calculated from 35 determinations. Estimates of the lower test concentrations are 6.2, 3.1, 1.5, and 0 mg/liter.

Table 24. SURVIVAL AND ATTACHMENT DATA FOR ADULT MUSSELS EXPOSED TO MALATHION FOR 96 HOURS

Test no.	Test concentration		No. of animals	Survival, percent	Attached, percent
	Nominal	Measured			
49	0	0	10	90	80
	5	9.0	10	20	20
	10	17.0	10	0	0
	20	23.5	10	0	0
	40	48.0	10	0	0
	80	69.0	10	0	0
52	0	0	10	100	100
	0.75	0.74	10	50	50
	1.25	1.4	10	60	50
	2.50	2.8	10	80	80
	5.0	6.9	10	100	90
	10.0	13.0	10	80	80
56	0	0	10	100	100
	1.25	1.2	10	100	90
	2.5	2.5	10	100	90
	5.0	5.0	10	100	100
	10.0	8.6	10	100	90
	20.0	18.5	10	100	30
59	0	0	10	100	90
	6.25	0.34	10	90	90
	12.5	6.3	10	100	100
	25.0	12.2	10	90	30
	50.0	19.4	10	70	0
	100.0	39.4	10	100	0

Table 25. PERCENTAGE OF NORMAL LARVAE DEVELOPING IN 48 HOURS FROM MUSSEL EGGS FERTILIZED IN VARIOUS CONCENTRATIONS OF MALATHION

Test concentration, mg/liter	Test number							
	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
0	95.5	98.5	88.0	91.0	85.5	87.0	85.0	86.5
1.95	96.5	93.0	--	--	--	--	--	--
2.75	--	--	80.0	83.5	73.5	73.0	82.0	78.8
3.95	93.5	96.0	--	--	--	--	--	--
5.50	--	--	85.8	87.3	70.3	70.8	64.0	58.5
8.15	82.5	94.0	--	--	--	--	--	--
12.0	--	--	77.5	79.5	42.3	48.0	37.0	37.5
15.5	36.0	32.5	--	--	--	--	--	--
24.0	--	--	0	0	0	0	0	0
48-Hour EC_{50}, mg/liter	13.5		17.2		12.5		10.5	

Larvae exposed to concentrations of up to 3.1 mg/liter for 10 and 20 days exhibited a growth rate similar to that of the controls (Table 26). At the higher concentrations, the larvae exposed to 6.2 mg/liter for 10 days were about 5% smaller than the controls in both experiments; at 12.3 mg/liter, the larvae were 18 to 19% smaller than controls. After 20 days of exposure, the differences in size increased. The greatest difference was observed in Experiment 2, in which the larvae were 24.4% smaller than the controls at 6.2 mg/liter and 39.7% smaller at 12.3 mg/liter. The percentage difference noted for these two concentrations at 10 and 20 days was statistically significant ($p = 0.05$).

Continued exposure for a total of 30 days after shell development of the larvae used in the growth study resulted in a marked decrease in the number of larvae developing into juvenile mussels. This effect on metamorphosis was particularly evident in larval populations exposed to 6.2 and 12.3 mg/liter of malathion (Table 27). In

Table 26. MEAN SHELL LENGTH OF MUSSEL LARVAE EXPOSED TO MALATHION FOR 10 AND 20 DAYS

Test concentration, mg/liter	No. of larvae measured/test	No. of tests/experiment	Mean shell length, μ			
			Day 10		Day 20	
			Experiment 1	Experiment 2	Experiment 1	Experiment 2
0	20	2	156	159	214	262
1.5	20	2	155	162	211	248
3.1	20	2	164	154	220	264
6.2	20	2	148	151	174	198
12.3	20	2	126	130	160	158

Table 27. NUMBER, AGE AND SIZE OF JUVENILE MUSSELS DEVELOPING IN LARVAL CULTURES EXPOSED AT 48 HOURS TO VARIOUS CONCENTRATIONS OF MALATHION

Test concentration, mg/liter	Experiment 1				Experiment 2			
	Total number	Age,[a] days	Length, μ		Total number	Age,[a] days	Length, μ	
			Mean	SD			Mean	SD
0	276	20	393	76.1	87	20	415	72.0
1.5	168	20	396	71.6	140	22	451	71.2
3.1	166	20	397	82.5	228	20	461	98.4
6.2	14	22	369	87.9	43	24	392	100.0
12.3	0	--	--	--	2	22	410	--

[a]Number of days required by treatment group to begin metamorphosing.

Experiment 1, the number of juvenile mussels recovered from larval populations exposed to 6.2 mg/liter was only 5.1% of that recovered from the control population. In the same experiment, none of the larvae exposed to 12.3 mg/liter survived longer than 28 days, and during this period none of the larvae developed a dissoconch shell.

In Experiment 2, the number of control larvae metamorphosing was low; however, the number among those exposed to 6.2 mg/liter of malathion was even lower, amounting to about 50% of the number of metamorphosed controls. The test chambers with 12.3 mg/liter of malathion contained only two juvenile mussels, or 2.3% of the number found in the control chambers. Metamorphosis of the two larvae occurred when the larvae were 22 days old. None of the other larvae exposed to 12.3 mg/liter survived for longer than 28 days.

Exposure of 30-day-old larvae to malathion did not affect the number developing into juvenile mussels (Table 28). However, the larvae exposed to the highest test malathion concentration of 12.1 mg/liter exhibited a higher rate of metamorphosis than any other treatment group (Figure 8). On the average, about 27 days was required for 50% of the controls to undergo metamorphosis; however, the larvae exposed to 12.1 mg/liter required only 13 days. In all other malathion-exposed groups, the rate of metamorphosis did not differ significantly from that of the controls. In general, mortality among the malathion-exposed larvae was higher than among controls. Mortality percentages for the two tests differed considerably, with some pesticide-exposed groups showing about the same mortality as controls in one test and higher percentages in the other test. Mortality did not appear to be related to the malathion concentration. As a group, the malathion-exposed larvae exhibited 12.6% mortality, whereas average mortality for the controls amounted to 5.4% of the total number of experimental animals.

EVALUATION OF THE STUDY

The bay mussel shows considerable promise as a test organism for the laboratory evaluation of potential marine and estuarine water pollutants. This opinion is shared by Dimick and Breese (1965).

Table 28. EFFECT OF MALATHION ON MUSSEL LARVAE METAMORPHOSIS AFTER A 40-DAY EXPOSURE INITIATED 30 DAYS AFTER FERTILIZATION OF THE EGGS (Presence of Dissoconch Shell Used as Indication of Metamorphosis)

	Pesticide concentration, mg/liter									
	Control		1.51		3.02		6.05		12.1	
	1	2	1	2	1	2	1	2	1	2
Number of larvae										
Initial	100	100	100	100	100	100	100	100	100	100
Lost or crushed	36	20	28	16	18	15	10	25	14	16
Experimental	64	80	72	84	82	85	90	75	86	84
Metamorphosing, percent	78.1%	87.5%	75.0%	79.8%	75.6%	82.4%	67.8%	89.3%	90.7%	82.1%
Nonmetamorphosing, percent	17.2%	6.3%	13.9%	15.4%	9.8%	9.4%	7.8%	0	0	0
Dead, percent	4.7%	6.2%	11.1%	4.8%	14.6%	8.2%	24.4%	10.7%	9.3%	17.8%
Days to metamorphosis of 50% of test larvae	28	26	28	28	25	22	33	22	13	13
Shell length of juvenile mussels, μ	456 μ	429 μ	448 μ	443 μ	434 μ	439 μ	417 μ	413 μ	371 μ	366 μ

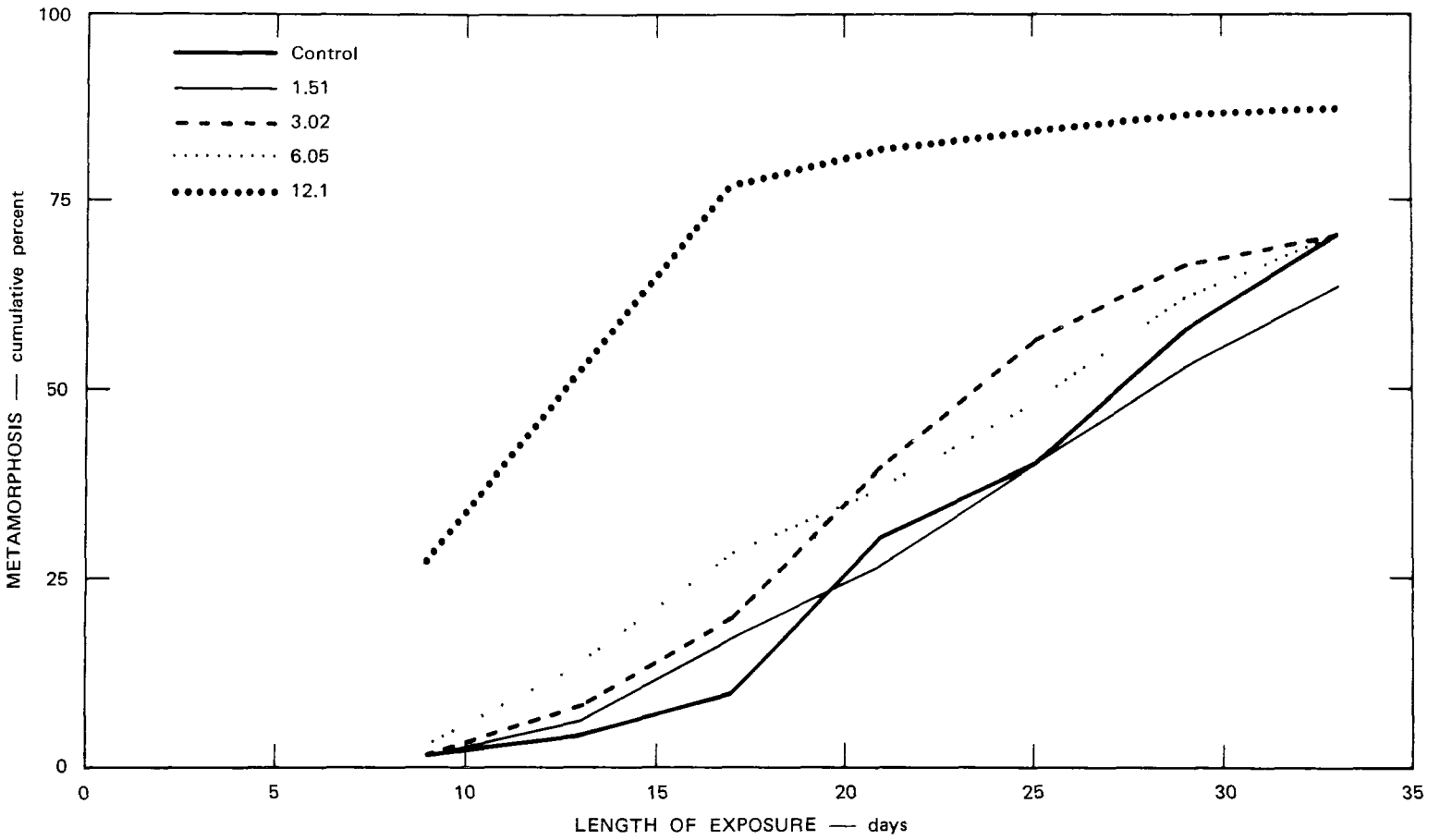

FIGURE 8 EFFECT OF EXPOSURE TO MALATHION ON PERCENTAGE OF MUSSEL POPULATION COMPLETING METAMORPHOSIS

Larvae were 29 to 30 days old when exposure was initiated. Data from the two experiments were pooled.

We believe, however, that the full potential of the bay mussel will not be realized until toxicological testing procedures are refined to such a degree that most laboratories having a source of mussels and of natural seawater can perform the studies described herein with greater efficiency and reliability of results.

Except in the adult survival tests and the 48-hour embryo bioassay experiments, much of the project effort was directed toward developing methods, often resulting in considerable delays in proceeding from one phase of the project to the next. Because of requisite project deadlines, the procedures actually employed--particularly in the studies on larval growth and metamorphosis--were far from efficient.

In spite of the difficulties encountered and the limitations of the study, the investigation revealed important information on the relative sensitivity of various stages in the life history of the bay mussel to the pesticides evaluated. Regardless of the type of pesticide, larval growth was the most sensitive indicator of toxicity--which is readily apparent on inspection of Table 29, a summary of the toxicity data. Table 29 presents the 96-hour TL_{50} and EC_{50} estimates for adult survival and attachment, the 48-hour EC_{50} estimates for embryo shell development, and the minimum effective concentrations that produced statistically significant reductions in larval growth and marked reductions in the number of larvae developing into juvenile mussels. For some of the pesticides, statistically significant reductions in larvae size did not appear until after 20 days of exposure; for others, size reductions were observable by the tenth day. These two periods were the only ones for which effects on growth were examined in detail; it is likely that statistically significant effects would have been detected earlier if all the growth data had been evaluated. The greater sensitivity of growth as a measure of effect is probably the outcome of longer periods of exposure, which usually permit effects on biochemical and physiological processes time to express themselves in grosser terms.

The adult mussel was the least sensitive of the life history stages investigated, and attachment by byssus-thread formation was usually a more sensitive indicator of effect. Byssus-thread formation and attachment to a solid substrate have a certain behavioral significance,

Table 29. SUMMARY OF TOXICITY DATA FROM 96-HOUR ADULT MUSSEL SURVIVAL, 48-HOUR EMBRYO SHELL DEVELOPMENT, LARVAL GROWTH, AND LARVAL METAMORPHOSIS STUDIES
(Pesticide Levels in mg/liter)

Pesticide	96-hr TL_{50} (adults)	96-hr EC_{50} (attachment)	48-hr EC_{50} (embryos)	Growth			Metamorphosis		
				Minimal effective concentration	Percentage of reduction	Days	Minimal effective concentration	Percentage of reduction	Days
Sevin	22.7	10.3	1.5	0.33	20.7	10	0.33	93.1	50
Treflan	>0.24	0.035	>0.12	0.096	15.9	20	0.096	Death	26
Methoxychlor	>0.092	>0.092	>0.075	0.062	11.6	20	0.008	37.0	36
2,4-D (acid)	259.0	262.0	211.7	91.4	11.6	10	182.8	Death	22
Malathion	--	--	13.4	6.2	5.0	10	6.2	94.9	32

particularly in the pediveligers and perhaps in the juveniles and adults. According to Bayne (1965) and others (Green, 1968; De Blok and Geelen, 1959), swimming and crawling movements of the pediveliger represent efforts to locate a suitable area in which to attach by byssus threads. Metamorphosis can be delayed by the lack of a suitable substrate or of the proper environmental conditions (Bayne, 1965). Larvae evidently can detect subtle changes or differences in their environment. Wisely (1963) showed that crawling mussel larvae, Mytilus planulatus Lamarck, could detect antifouling paint before they touched it. After detection, the mussel ceased crawling and remained closed. The shelled mussel must open its shell to secrete byssus threads, and, in doing so, exposes itself to the environment. Refusal to attach may indicate that the environment is unsuitable and may reflect a defense mechanism used by the mussel during periods of adversity. Reish and Ayers (1968) determined the number of byssus threads laid down by the bay mussel under various conditions of chlorinity and dissolved oxygen. They discovered that, although the mussel can tolerate low oxygen and chlorine levels, the number of byssus threads formed varies with these two factors.

The embryo was sometimes more sensitive than, and sometimes only as sensitive as, the adult to the pesticides. The concentration of the insecticide Sevin that caused 50% of the embryos to develop abnormally was 15 times less than the concentration that produced 50% mortality among the adults. However, with 2,4-D, the statistics were about the same. Concentrations of methoxychlor and Treflan that had no effect on adult survival also had no effect on embryonic shell development.

Larval metamorphosis or, more specifically, the formation of the dissoconch shell appeared to be about as sensitive an indicator of toxic effect as shell growth; however, because of inconsistencies in the data on metamorphosis obtained from the survivors of the growth study, and because of the lack of information on the size of the test population and mortality rate, the evidence is not conclusive. Data from some of the experiments support this idea, whereas data from others do not.

The study could have been aided greatly if knowledge concerning differences in the biology of the various M. edulis populations in the San Francisco Bay region had been available before initiation of the project. This information would have been of particular value in all the phases involving use of the larvae. To assure that a sufficient number of eggs from different females were avail able to conduct any series of experiments requiring eggs or larvae, several hundred mussels were usually obtained during each collection trip. Sources close to the testing laboratory, such as Treasure Island and St. Francis Yacht Harbor, naturally were favored. As shown in Table 30, mussels from Treasure Island, Moss Landing, and St. Francis Yacht Harbor did not respond well to spawn-induction procedures, and egg viability was poor. Thirty-nine groups of eggs, each from a different St. Francis Harbor female, were used in the 48-hour embryo bioassays; only 7.7% of these groups contained the minimum of 85% normal larvae in the control test. Sixty-one egg groups from Treasure Island were tested; 11.7% produced the acceptable number of normal larvae.

Mussels from Tomales Bay, located relatively far from the laboratory, proved to be much more responsive to our spawn-induction procedure, and their gametes were in better condition than those of mussels collected at the other sites. On the average, about 35% of the mussels subjected to spawn-induction procedures spawned. In general, the gametes were produced in abundance. Of 42 different egg groups used in the 48-hour embryo bioassays, about 40% produced acceptable percentages of normal larvae. Performance in the growth and metamorphosis studies was even better.

These data illustrate the importance of knowing which available mussel population is best suited for the contemplated study. Early frustrations encountered during this project could have been avoided if the Tomales Bay population had been identified as the most suitable for the project.

With the exception of the 48-hour embryo bioassay tests, all the experiments performed in this investigation would have been improved if continuous-flow toxicant dilution and delivery systems as well as special larval test chambers could have been developed and used. Although only two small adult mussels, measuring between

Table 30. SPAWNING SUCCESS AND VIABILITY OF EGGS OF MUSSELS COLLECTED FROM VARIOUS SOURCES

Mussel source	Percentage of spawn		Performance in 48-hour embryo bioassays	
	Mean	Range	No. of different egg groups tested	Percentage of groups with minimum of 85% normal larvae
Tomales Bay	35.3	6-66	42	40.5
St. Francis Yacht Harbor	8.7	2-15	39	7.7
Treasure Island	18.2	2-39	61	11.5
Moss Landing	10.8	1-28	Number of eggs insufficient to run tests	

3 and 4 cm, were used per 15 liters of test solution in the adult survival tests, dissolved oxygen concentrations reached dangerously low levels during some of the tests. Severe reductions in oxygen levels were particularly evident whenever acetone was used as an initial pesticide solvent. Although the situation was rectified by aerating the test solutions, use of a continuous-flow system would have eliminated the need for direct aeration of the test solutions, which could have caused pesticide loss by volatilization. Use of a continuous-flow system also would have eliminated the need to employ five 5-gal. jars per treatment group in each series of tests.

In our extended growth and metamorphosis studies, conducted for 30 to 40 days, variations in toxicant levels due to adsorption, metabolism, volatilization, and chemical degradation were minimized by renewing the test solutions at intervals of approximately 40 hours. Because the larvae were extremely small, the old solution and the larvae were siphoned from the test chamber into a tube containing nylon screen (53-μ mesh) to catch them. Although the test chamber was rinsed several times with fresh seawater, and the rinse water was poured through the screen, we know that larvae were accidentally lost. On occasion, we discovered larvae caught in the surface film

of water remaining in the test chamber; we also found larvae caught on the screen after it was rinsed several times. When several toxicants are tested simultaneously, each concentration in duplicate, such frequent handling of the larvae results in their accidental loss. Even when enough manpower is available to inspect carefully each test chamber, screen, or siphon, the task of accounting for the 3000 larvae in each test chamber is enormous.

If further studies on growth of mussel larvae are anticipated, we recommend that a method for applying the continuous-flow technique be developed. In laboratories having a large supply of natural seawater--especially those that pump seawater directly from the ocean--continuous-flow systems should not be difficult to install. Maintaining a sufficient food level in the larval chambers may present a problem, although large-scale culturing of algae for constant metering into the system is not infeasible. The major problem probably would be the design of the test chamber. Upon developing a shell, a larva measures about 100 μ in length and somewhat less in width. Fairly finely meshed screens are needed to retain the larvae in the chamber in a continuous-flow system. Although screened test chambers are used commonly in toxicity tests employing various invertebrate organisms such as Daphnia magna, the use of screens with mussel larvae could present cleaning difficulties, especially when the larvae reach the pediveliger stage.

We found that, despite frequent renewal of the toxicant solutions, a considerable amount of debris accumulated in the test chambers. This debris usually was composed of a whitish, amorphous material, lint, and other unidentifiable matter, none of which would pass through 53-μ mesh screens. This material, especially the fibrous type, seemed to attract the pediveligers, and this attraction made it difficult for us to clean the screens effectively without injuring or killing some of the larvae. Accumulation of this debris, in addition to feeding the larvae a continuous supply of algae, most likely would necessitate frequent screen cleaning.

The ability of the pediveligers to attach themselves rather firmly to the sides of the test chamber with a ciliated foot presents problems in obtaining a representative sample of the larval population for measurements. Vigorous mixing, swirling, and agitation of the test solution usually are not sufficient to dislodge all the larvae.

It was unfortunate that the substrate preference studies were not initiated until after completion of the definitive studies on the effect of the pesticides on growth and metamorphosis. Information gained from the substrate preference studies could have helped measurably in the definitive tests. This change in the original study schedule occurred early in August 1973 when we had to decide whether to stay on the original schedule and, because of the uncertainty of a supply of readily spawnable mussels during the winter months, jeopardize the initiation of the growth and metamorphosis studies or to conduct the growth and metamorphosis studies first, knowing that the substrate preference studies might be abandoned. Because the project was toxicological in nature, we decided to proceed first with growth and metamorphosis.

Information on the kinds of substrates that attract and repel larvae that are about to undergo metamorphosis can decrease considerably the tedium and experimental error associated with the experimental procedures we employed during the study. Ideally, larvae that are about to undergo metamorphosis should be presented with an attractive substrate that can be removed periodically for assessment of the percentage of the population that have become juvenile mussels. In addition, use of a test chamber composed of a nontoxic, repellent material would ensure maximum use of the presented substrate.

Our substrate preference study indicated that the larvae were attracted to rigid PVC plastic, particularly at larva-to-volume (ml) ratios of 1:3 to 1:1, the highest ratio tested. Attraction to PVC was evident in the later stages of the growth study, when larvae left submerged in the screened PVC pipes often were difficult to remove because of their attachment to the pipe.

Of the rigid substrates evaluated, frosted glass was the least attractive at the higher larval densities; however, clean Plexiglas was about as poor and probably was more suitable as a material for test chamber construction because of its transparency. Polyethylene, although not evaluated and generally available only in the opaque form, may be even less attractive than Plexiglas or frosted glass.

The debris problem is one that must be considered in studies on larval metamorphosis. The pediveliger larvae were attracted to

the debris, and many were found attached by byssus threads to fibrous substances in the debris. Many of these larvae had undergone metamorphosis. The source of the debris is still in question. During the studies, all test jars were well covered with sheets of clear polyethylene film. In view of the virtual impossibility of avoiding formation of debris and the attractiveness of the debris to the larvae, the usefulness of an ideal setting substrate is somewhat decreased.

During our studies of larval metamorphosis, we discovered that settling--the act of attaching to a substrate by means of byssus threads--was not a meaningful measure of the rate or extent of metamorphosis by larval populations. It is well known and we frequently observed that, in selecting a spot to settle and complete metamorphosis, mussel larvae often secrete and break their byssus threads several times and move to another location. Thus, the number of larvae found attached at one time does not reflect the number that can attach or have attached at another time.

A more accurate method for determining the number of larvae that have undergone metamorphosis is to inspect each larva for the presence of the dissoconch, or "adult," shell. This shell is a lighter color than the larval shell and is readily discernible from the latter. It appears along the entire edge of the original shell and grows rapidly.

This evaluation of the study is included for use as a guide by others who may be planning to perform similar studies using the bay mussel.

SECTION VII

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SECTION VIII

INSTRUMENTS USED IN THE STUDY

1. Gas chromatograph (Microtek, Model 220) with electrolytic conductivity detector; used in the quantitative analysis of the pesticides.

2. Particle counter (Coulter, Model B); used in counting mussel eggs and algal cells.

3. Refractometer (Goldberg); used in measuring salinity.

4. pH meter (Radiometer, Type PHM26).

5. Oxygen meter (Yellow Springs Instrument Co., Model 54); used to determine dissolved oxygen levels and temperature of the test solutions.

6. Ultraviolet liquid purifier (Ultradynamics, Model 500); used to reduce bacterial population in natural seawater used in the tests.

7. Sartorius analytical balance.

8. Sonifier (Branson Instruments, Inc., Model LS75).

SECTION IX

APPENDICES

Appendix A

Table 1. WATER QUALITY DATA FROM REPRESENTATIVE 96-HOUR ADULT SURVIVAL EXPERIMENTS

Pesticide*	Temperature, °C			Dissolved oxygen, mg/liter			Salinity, °/oo			pH		
	Mean	SD	Analyses	Mean	SD	Analyses	Mean	SD	Analyses	Mean	SD	Analyses
Sevin (53)	19.5	1.62	125	7.2	0.45	125	25.4	0.24	20	7.95	0.14	20
Treflan (43)	20.2	0.30	100	4.9	2.11	100	26.0	0.10	24	7.83	0.10	36
Methoxychlor (45)	19.1	0.60	75	7.1	0.50	75	26.1	0.49	6	8.09	0.10	6
2,4-D (12)	18.0	0.63	42	8.0	1.30	42	23.6	0.67	25	7.50	0.52	26
Malathion (56)	19.5	1.18	150	6.7	0.38	150	24.2	0.36	24	7.87	0.09	12

*Experiment numbers in parentheses.

Table 2. WATER QUALITY DATA FROM REPRESENTATIVE 48-HOUR EMBRYO BIOASSAY EXPERIMENTS
Means Based on 5 to 24 Determinations

Pesticide	Temperature, °C		Dissolved Oxygen, mg/liter		Salinity, °/oo		pH	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Sevin	22.0	0.55	6.6	0.22	25.9	0.20	7.80	0.10
Treflan	21.6	0.84	6.7	0.31	25.1	0.32	7.97	0.11
Methoxychlor	21.9	0.43	6.6	0.27	25.3	0.56	8.02	0.11
2,4-D	21.8	0.52	6.9	0.19	25.4	0.40	7.97	0.09
Malathion	21.6	1.09	6.7	0.31	25.7	0.36	8.02	0.08

Table 3. WATER QUALITY DATA FROM GROWTH AND METAMORPHOSIS EXPERIMENTS

Pesticide	Temperature, °C			Dissolved Oxygen mg/liter			Salinity, °/oo			pH		
	Mean	SD	Analyses	Mean	SD	Analyses	Mean	SD	Analyses	Mean	SD	Analyses
Sevin	20.8	1.09	774	6,80	0.41	533	Not recorded			8.04	0.11	502
Treflan	19.0	0.81	797	6.57	0.80	599	25.4	0.87	239	8.03	0.08	569
Methoxychlor	19.8	1.04	873	6.30	0.98	645	25.3	1.00	321	7.98	0.97	644
2,4-D	20.4	0.93	670	6.87	0.40	445	Not recorded			7.89	0.29	419
Malathion	20.0	0.99	627	6.80	0.13	484	25.3	1.11	132	8.02	0.10	477

Table 4. WATER QUALITY DATA FROM METAMORPHOSIS EXPERIMENTS

Pesticide	Temperature, °C			Dissolved Oxygen mg/liter			Salinity, °/oo			pH		
	Mean	SD	Analyses	Mean	SD	Analyses	Mean	SD	Analyses	Mean	SD	Analyses
Sevin	19.7	1.76	260	7.00	0.51	180	25.3	0.34	175	8.04	0.12	175
Treflan	19.6	1.29	282	6.40	0.91	195	25.5	0.59	180	8.01	0.11	189
Methoxychlor	19.6	1.12	240	6.60	0.94	181	25.2	0.33	181	8.00	0.12	181
2,4-D	19.6	1.29	240	6.70	0.55	160	25.4	0.50	153	8.06	0.15	160
Malathion	19.7	1.27	240	6.70	0.47	160	25.4	0.51	153	8.06	0.10	155

Appendix B

Table 1. MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF MUSSEL LARVAE REARED IN SEAWATER WITH VARIOUS CONCENTRATIONS OF SEVIN
Means Based on Measurement of 20 Larvae
(microns)

Day	Seawater control				Concentration, mg/liter															
					0.33				0.65				1.3				2.6			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
										Experiment 1										
0	104	10.6	104	10.6	104	10.6	104	10.6	104	10.6	104	10.4	104	10.6	104	10.6	104	10.6	104	10.6
2	111	20.1	102	22.5	97	16.9	89	15.0	107	22.4	117	18.6	113	11.2	107	13.7	105	13.8	103	14.0
4	121	34.1	134	15.7	125	17.9	111	22.1	116	16.6	118	23.0	100	15.8	109	18.1	105	15.5	107	9.1
6	147	36.4	141	28.7	131	18.3	139	30.5	127	24.7	113	29.8	116	19.5	111	22.4	103	19.4	105	23.6
8	152	47.9	159	51.9	125	26.9	127	35.8	147	22.8	129	28.3	131	16.4	116	20.5	120	17.0	114	14.4
10	179	70.5	162	67.4	157	41.6	167	51.5	116	49.1	141	35.2	135	29.6	109	22.4	117	21.8	131	21.6
12	213	50.5	233	43.5	147	46.6	182	30.0	157	33.0	177	17.0	99	27.2	127	19.5	96	17.5	99	20.0
14	199	78.6	174	68.4	182	49.6	213	46.2	188	37.8	180	37.5	139	21.0	141	22.9	---		---	
16	281	19.7	272	30.4	213	34.9	201	33.5	216	26.3	197	23.5	158	31.4	152	23.4	---		---	
18	236	56.3	300	65.0	215	34.4	234	26.2	185	34.4	215	33.9	160	31.1	150	16.2	---		---	
20	297	32.7	322	26.9	211	27.1	226	30.9	181	25.0	227	19.6	171	23.0	157	17.9	---		---	
22	317	42.0	342	22.4	234	32.3	251	29.2	196	16.0	207	25.8	176	47.7	166	19.6	---		---	
24	306	81.3	251	59.7	220	25.4	251	27.7	200	20.1	227	38.2	171	19.0	---		---		---	
26	326	61.0	315	45.7	196	37.1	247	24.0	179	29.4	219	21.5	---		101	8.1	---		---	
28	324	86.4	373	45.8	241	66.0	259	30.6	229	38.3	250	26.1	---		180	28.4	---		---	
30	343	50.2	386	21.8	224	22.7	261	27.5	231	29.2	259	31.5	---		253	34.3	---		---	
32	---		---		---		---		---		---		---		---		---		---	
34	397	45.7	420	14.1	235	23.6	287	25.1	267	24.4	277	33.2	---		---		---		---	
36	395	30.5	425	33.0	317	89.3	307	19.4	213	34.3	298	1.4	---		---		---		---	
38	372	74.2	431	35.7	263	36.7	319	21.8	286	34.2	317	22.6	---		---		---		---	
										Experiment 2										
0	98	8.0	98	8.0	98	8.0	98	8.0	98	8.0	98	8.0	98	8.0	98	8.0	98	8.0	98	8.0
2	116	13.0	113	16.4	112	14.4	111	21.5	117	13.4	117	8.7	96	17.3	99	20.0	98	9.1	95	13.5
4	128	15.6	109	19.2	113	18.2	122	9.4	119	12.9	116	14.8	110	11.3	99	15.1	102	12.7	99	13.3
6	123	33.1	175	24.9	115	22.0	114	18.7	114	17.8	118	22.0	115	13.2	117	13.4	106	10.5	110	14.7
8	160	22.0	140	17.6	115	20.0	107	21.9	107	11.9	112	17.5	106	15.1	99	17.1	103	10.0	102	11.5
10	173	30.0	175	25.2	132	26.5	143	16.3	132	21.9	120	18.1	111	12.3	107	13.0	95	16.1	103	8.7
12	206	54.6	176	51.3	162	25.1	153	33.8	142	35.1	143	31.9	93	19.2	110	7.2	96	13.7	---	
14	176	47.5	187	49.3	168	46.1	171	44.3	151	38.3	151	23.4	131	20.1	123	22.9	---		---	
16	218	55.1	213	46.5	181	38.1	163	25.7	169	35.1	160	20.0	122	22.1	122	15.7	135	22.4	---	
18	231	48.1	182	42.0	182	34.1	195	26.4	160	25.2	170	25.3	128	15.3	127	10.6	101	11.9	---	
20	264	34.2	225	36.4	179	35.4	208	33.3	175	18.0	155	23.0	122	13.7	139	16.4	---		---	
22	297	33.0	273	31.8	207	38.1	219	36.5	195	38.0	173	21.2	---		---		---		---	
24	301	33.4	272	27.2	223	27.5	224	30.2	217	31.7	201	36.5	---		281	26.6	---		---	
26	310	33.5	263	33.8	179	49.2	179	33.3	160	42.5	162	31.8	---		229	33.5	---		---	
28	346	29.3	305	39.3	219	31.0	256	37.1	246	39.5	179	26.5	---		287	44.1	---		---	
30	347	36.9	315	46.3	235	35.3	224	54.9	232	60.0	212	49.6	---		311	37.3	---		---	
32	---		---		---		---		---		---		---		---		---		---	
34	387	33.8	349	49.9	291	62.8	256	57.8	273	42.5	277	64.5	---		269	46.0	---		---	
36	370	24.1	367	30.7	233	24.4	273	40.4	225	24.1	234	43.7	---		280	61.8	---		---	
38	423	41.9	377	36.9	305	59.0	306	39.5	324	28.7	261	81.4	---		---		---		---	
40	404	40.2	361	56.2	299	80.9	299	64.9	---		239	45.5	---		---		---		---	
42	435	36.1	367	52.0	315	43.8	334	38.6	---		304	46.6	---		---		---		---	
44	---		---		---		---		---		---		---		---		---		---	
46	355	28.9	399	37.5	407	41.2	---		---		289	42.5	---		---		---		---	
48	359	56.0	401	29.4	368	32.1	---		---		---		---		---		---		---	
50	350	53.2	388	54.5	418	44.8	---		---		---		---		---		---		---	

Table 2. MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF MUSSEL LARVAE REARED IN SEAWATER WITH VARIOUS CONCENTRATIONS OF TREFLAN
Means Based on Measurement of 20 Larvae
(microns)

	Seawater controls				Solvent controls				Concentration, mg/liter															
									0.024				0.048				0.096				0.192			
Days	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
											Experiment 1 1													
0	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9	105	2.9
2	103	7.0	107	7.0	109	3.7	106	6.3	109	5.4	109	7.4	110	3.5	109	7.2	107	4.1	110	3.5	104	10.5	108	6.5
4	110	5.3	108	7.5	115	8.2	117	8.4	122	9.7	127	6.5	124	7.6	123	5.7	118	8.8	118	11.7	119	10.5	112	7.1
6	121	10.4	126	10.7	133	13.4	142	13.0	134	15.7	140	13.0	137	20.7	133	13.3	137	10.0	134	18.0	129	13.7	130	14.6
8	141	25.5	141	12.6	148	16.1	152	14.4	147	21.9	172	17.2	167	14.6	162	11.6	162	16.2	166	13.3	155	13.1	145	12.8
10	142	22.5	150	21.1	152	18.2	156	30.2	168	29.9	189	15.8	173	19.1	179	21.0	160	15.7	176	20.4	166	18.8	154	22.8
12	179	15.2	177	20.8	179	17.3	189	19.0	184	31.7	216	25.9	202	29.9	191	31.7	178	16.4	198	20.4	176	28.1	179	20.2
14	186	27.9	187	32.8	177	25.1	206	26.7	203	37.0	236	25.8	219	24.7	205	14.8	177	30.5	203	23.2	186	22.4	185	20.0
16	206	37.0	200	37.9	193	22.8	221	27.4	232	20.0	239	26.7	246	21.7	220	27.7	194	32.4	206	25.1	185	25.5	177	26.1
18	230	35.7	210	44.0	230	30.2	233	32.5	191	29.5	243	23.0	222	38.1	231	26.9	187	33.9	188	25.8	193	31.8	177	25.8
20	274	40.4	266	41.6	249	21.2	256	34.3	216	40.0	227	45.3	271	29.2	239	29.0	188	24.4	213	18.3	196	26.4	183	28.5
22	260	29.9	277	36.2	240	26.6	251	27.7	220	23.9	249	35.0	271	41.0	226	47.8	230	47.2	217	17.7	196	24.4	195	21.5
24	275	28.9	268	43.6	241	41.1	231	33.4	233	29.6	272	38.6	297	44.5	266	44.9	---		209	23.4	---		---	
26	286	42.5	280	51.3	280	35.0	281	42.6	236	32.2	294	24.6	317	46.2	269	50.8	---		239	41.2	---		---	
28	---		271	56.5	302	40.6	288	52.9	275	40.3	331	32.8	345	33.5	303	47.5	---		---		---		---	
30	---		309	45.3	353	43.4	301	42.5	269	39.7	325	25.2	361	22.0	324	38.9	---		---		---		---	
32	---		332	42.2	345	30.9	331	37.5	---		340	37.2	358	41.5	311	46.8	---		---		---		---	
34	---		330	35.0	354	38.9	342	34.7	---		333	47.6	351	43.6	291	59.3	---		---		---		---	
36	---		340	30.3	351	33.6	362	28.9	---		349	37.5	353	33.9	---		---		---		---		---	
38	---		338	41.3	---		334	42.6	---		329	49.6	324	41.6	---		---		---		---		---	
											Experiment 2													
0	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9	103	4.9
2	113	6.9	114	4.6	114	4.8	113	5.4	112	8.7	113	5.4	112	7.7	111	5.4	115	7.4	116	6.4	111	8.4	111	5.8
4	123	14.3	122	9.3	126	20.5	122	9.6	130	13.9	120	14.3	133	11.1	134	8.0	133	10.0	129	19.7	127	7.3	128	12.1
6	141	16.9	129	13.0	148	19.8	139	15.2	151	13.9	147	13.3	145	12.4	160	18.5	154	14.9	149	16.5	136	17.1	140	12.0
8	162	23.8	165	18.2	183	35.8	197	16.0	192	15.8	169	28.6	173	28.9	187	22.3	177	19.7	179	17.6	170	20.0	175	23.5
10	193	19.6	172	24.2	170	33.8	188	41.9	207	33.8	192	32.7	211	18.4	226	14.9	202	36.9	174	40.5	196	10.6	195	17.3
12	198	22.1	192	23.9	209	25.4	191	41.9	217	32.4	230	21.7	198	26.6	219	32.3	221	28.0	162	30.4	186	21.4	202	21.1
14	234	22.5	211	30.8	202	38.3	223	31.9	247	34.9	224	52.1	205	37.5	243	32.3	242	24.3	159	33.5	180	29.0	212	21.1
16	222	46.6	225	49.3	214	53.1	231	31.8	237	47.2	248	47.5	222	47.8	247	50.3	260	43.6	197	29.2	206	19.6	227	22.2
18	292	19.5	260	28.2	251	24.8	273	24.8	298	28.8	254	41.5	262	25.1	274	33.1	271	27.8	216	34.9	205	19.3	210	23.0
20	302	46.8	273	36.9	305	35.2	299	24.1	327	37.2	313	27.1	257	45.5	267	42.5	277	27.5	232	40.5	195	22.5	219	19.0
22	335	36.0	301	45.4	296	51.8	261	50.5	332	47.8	294	43.3	250	44.8	298	41.6	298	21.5	204	26.9	205	18.5	204	35.8
24	363	33.5	286	58.7	348	30.2	308	26.4	342	35.6	326	34.4	259	40.4	294	64.6	269	23.6	229	36.2	222	24.9	224	16.8
26	354	56.5	349	49.5	328	51.6	319	64.3	348	42.8	311	56.0	271	42.9	311	40.4	266	37.9	245	27.9	222	19.6	222	31.5
28	356	33.3	346	35.1	373	48.2	278	51.6	373	32.6	---		276	34.5	331	33.4	275	44.0	255	30.9	---		230	23.3
30	---		362	40.6	274	44.3	301	44.2	374	40.8	---		271	30.4	353	33.9	293	39.3	286	51.7	---		233	22.8
32	---		376	43.1	367	35.6	315	43.9	364	33.8	---		278	17.9	343	43.2	297	21.4	283	33.6	---		---	
34	---		379	29.0	353	39.9	282	41.6	353	55.1	---		---		320	45.3	303	31.7	---		---		---	

Table 3. MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF MUSSEL LARVAE REARED IN SEAWATER WITH VARIOUS CONCENTRATIONS OF METHOXYCHLOR
Means Based on Measurement of 20 Larvae
(microns)

Days	Seawater control				Solvent control				Concentration, mg/liter															
									0.008				0.015				0.031				0.062			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
											Experiment 1													
0	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1	113	7.1
2	114	7.2	116	11.0	111	9.0	118	9.2	119	6.5	122	8.6	117	12.9	113	10.4	121	9.7	117	7.0	119	8.8	126	9.6
4	127	16.1	135	10.0	129	9.2	135	10.9	132	9.4	134	12.6	141	13.8	136	9.7	146	10.7	139	11.6	142	15.5	141	9.5
6	146	16.1	140	19.3	147	17.2	142	20.0	143	14.0	157	18.0	146	19.5	150	16.5	160	14.4	153	20.2	150	19.4	137	15.6
8	165	17.9	185	21.1	153	20.1	171	22.3	167	15.3	164	17.4	178	22.6	168	22.3	159	24.5	176	26.5	169	18.4	173	15.5
10	177	20.1	188	24.9	159	27.8	187	12.8	185	20.8	186	26.5	186	23.7	181	19.8	194	22.4	197	18.6	190	12.5	187	15.0
12	186	15.9	190	23.0	180	31.9	191	28.9	175	31.5	181	28.6	201	20.1	193	24.6	183	28.7	171	27.4	171	17.9	167	19.2
14	223	43.5	225	22.4	217	25.6	232	36.7	215	30.7	189	34.0	211	29.5	204	20.6	214	38.7	213	24.7	184	22.3	197	31.9
16	226	22.0	207	34.6	224	25.1	230	34.1	211	33.3	205	25.7	219	39.1	227	31.6	204	44.6	218	40.5	189	29.0	210	26.6
18	235	30.2	236	26.3	237	30.2	243	17.7	242	26.1	216	29.3	255	43.0	240	31.9	228	33.9	224	44.3	199	38.5	225	41.6
20	227	30.9	264	32.2	248	26.5	266	27.7	261	50.4	244	45.9	267	30.5	258	36.0	281	52.8	241	38.0	235	41.6	221	42.2
22	270	40.0	291	39.7	---		275	39.4	230	39.3	233	35.0	269	41.8	289	28.9	290	49.7	271	40.4	239	33.7	226	33.0
24	275	25.5	306	23.9	---		279	55.0	256	38.5	262	58.3	260	54.9	288	40.1	283	45.9	268	50.1	242	39.7	239	50.6
26	304	36.1	323	34.7	---		294	41.2	283	38.1	223	44.7	323	24.6	327	28.2	276	48.5	331	35.9	280	31.4	299	45.9
28	308	44.2	340	34.6	---		318	55.7	258	45.4	260	57.1	322	38.4	310	60.8	273	50.2	294	54.0	265	53.7	262	50.1
30	331	61.7	371	25.1	---		263	52.8	281	48.5	277	46.6	347	30.4	334	43.5	297	55.0	331	50.6	287	38.8	281	38.6
32	366	29.4	381	28.6	---		294	64.8	334	26.3	247	60.4	319	42.2	344	47.5	317	43.7	328	36.2	259	37.6	255	35.1
34	357	53.7	383	40.1	---		306	51.7	321	42.9	258	59.7	307	53.3	321	68.1	321	49.1	328	56.4	271	42.2	274	37.2
36	373	26.2	383	63.3	---		296	50.7	316	50.5	269	39.9	349	47.3	356	55.7	317	32.8	348	40.4	281	33.6	260	40.1
											Experiment 2													
0	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9	110	7.9
2	109	10.0	113	12.3	110	13.5	113	12.8	120	16.1	120	15.0	120	10.4	126	14.6	117	10.9	118	12.9	127	12.2	118	15.1
4	115	12.6	116	16.3	115	10.7	116	13.8	106	9.5	115	17.0	130	16.7	127	21.1	135	21.3	134	16.3	141	20.8	137	16.1
6	173	26.2	161	38.3	136	22.0	141	19.4	153	19.7	136	27.7	145	28.4	162	13.7	153	17.0	153	27.1	148	21.1	153	18.6
8	146	31.2	152	28.5	149	23.3	164	19.3	171	28.3	165	19.4	175	15.0	172	20.3	168	21.9	181	19.8	167	22.0	187	15.6
10	197	44.6	193	26.6	195	21.6	187	18.1	207	20.1	185	25.6	190	24.5	201	28.6	198	19.9	195	23.7	175	23.3	202	23.1
12	189	33.1	174	30.9	192	26.6	186	33.9	189	33.9	212	26.0	204	29.0	213	21.1	196	19.2	192	22.6	178	20.4	178	19.4
14	197	30.9	191	31.6	189	49.3	208	41.0	203	35.6	214	35.3	213	37.7	219	24.7	228	21.1	215	28.9	196	26.4	202	21.1
16	228	17.0	225	29.8	231	36.2	248	22.5	219	31.2	240	30.5	235	24.4	254	24.0	230	25.5	232	18.4	205	25.9	220	26.7
18	224	28.6	244	26.7	256	30.2	267	37.3	235	34.0	238	40.4	271	28.7	265	37.5	262	29.3	252	41.0	225	21.0	218	35.0
20	259	25.0	280	17.6	264	50.6	303	39.7	267	39.3	262	41.6	292	35.4	294	37.8	265	30.4	281	24.7	236	48.7	262	19.9
22	282	27.9	280	47.6	257	58.0	299	53.6	276	45.8	252	59.0	293	43.4	302	30.7	277	41.4	279	37.5	275	29.0	284	31.9
24	275	31.5	295	38.5	324	46.8	317	36.8	280	47.7	283	42.6	311	39.5	252	39.6	277	31.7	293	40.4	275	46.7	261	34.5
26	306	35.0	299	49.9	319	38.0	347	44.4	303	38.1	306	42.9	325	33.0	328	32.8	302	35.6	301	42.2	273	39.2	291	41.9
28	314	49.8	303	54.1	316	44.6	336	42.5	334	38.3	329	35.4	346	34.5	348	28.9	319	33.6	310	37.1	284	32.6	298	38.1
30	301	46.5	330	40.8	338	33.4	346	56.2	313	54.2	322	50.4	323	52.2	349	40.7	302	28.9	299	43.0	298	41.0	298	49.1
32	351	38.7	338	63.7	330	61.6	381	30.5	369	38.5	329	53.9	354	37.8	359	47.9	310	56.5	338	24.9	312	33.4	296	42.8
34	358	37.9	---		339	48.6	---		359	45.8	---		353	56.5	361	58.2	306	43.6	325	44.0	278	41.0	280	35.4

Table 4. MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF MUSSEL LARVAE REARED IN SEAWATER WITH VARIOUS CONCENTRATIONS OF 2,4-D
Means Based on Measurement of 20 Larvae
(microns)

Day	Seawater control				Concentration, mg/liter															
					22.8				45.7				91.4				182.8			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
									Experiment 1											
0	---		---		---		---		---		---		---		---		---		---	
2	121	7.1	121	5.3	121	4.5	116	5.5	115	5.7	112	5.4	109	4.6	106	3.3	113	5.2	113	4.7
4	125	7.6	121	10.1	123	11.7	124	7.2	128	11.4	111	7.9	115	10.9	114	8.7	109	5.7	104	6.2
6	130	8.5	135	7.1	136	11.7	139	13.8	140	18.4	142	10.3	140	17.9	127	10.7	115	8.0	113	9.9
8	155	22.8	151	20.5	152	23.8	151	26.1	150	22.3	150	23.1	152	21.8	139	16.1	116	11.9	119	5.4
10	176	21.8	188	32.2	198	31.4	197	25.3	191	17.5	174	27.5	179	24.5	176	23.4	124	33.1	---	
12	207	27.8	215	33.5	217	24.6	224	25.6	205	35.8	205	23.6	171	25.3	158	22.1	---		---	
14	214	26.6	227	30.2	257	18.5	223	29.4	225	22.7	235	30.3	201	42.7	184	30.9	---		---	
16	255	33.3	253	36.9	288	30.9	264	26.3	265	18.8	241	22.5	203	24.6	216	35.0	---		---	
18	273	31.9	287	34.5	293	29.4	259	35.5	237	38.2	261	27.8	242	40.2	262	43.2	---		---	
20	280	53.2	314	32.6	304	28.1	270	36.1	293	34.8	293	31.9	240	53.6	223	37.4	---		---	
22	---		---		---		---		---		---		---		---		---		---	
24	344	42.7	320	41.1	373	32.5	282	34.4	292	44.5	292	28.9	258	48.2	249	35.7	---		---	
26	329	38.3	340	35.9	363	36.2	311	33.0	294	35.4	286	38.7	269	66.5	---		---		---	
28	324	54.8	317	71.0	374	48.6	294	47.0	307	60.4	309	37.8	242	37.8	224	55.2	---		---	
30	353	31.4	354	48.7	384	24.0	334	28.9	306	42.6	296	54.0	281	16.4	255	37.0	---		---	
32	---		347	46.9	418	84.3	328	23.4	328	27.9	---		274	47.8	217	75.1	---		---	
									Experiment 2											
0	114	4.7	114	4.7	114	4.7	114	4.7	114	4.7	114	4.7	114	4.7	114	4.7	114	4.7	114	4.7
2	124	6.9	123	5.7	124	4.9	125	5.5	123	5.3	125	5.4	119	5.8	119	4.4	114	6.9	116	6.5
4	131	7.9	127	9.0	125	4.9	125	5.4	122	4.1	135	11.8	129	6.5	128	7.3	116	5.0	117	3.9
6	144	9.1	137	9.6	144	11.2	139	14.6	137	16.5	151	12.4	127	16.9	131	12.5	116	3.3	116	3.4
8	147	14.5	154	17.5	154	23.7	148	22.6	166	18.6	160	21.3	145	15.1	146	13.4	117	6.9	114	6.1
10	166	27.9	177	18.6	195	20.3	182	28.5	175	22.6	166	22.2	150	15.6	154	9.7	112	10.6	111	9.0
12	199	26.8	193	20.9	206	22.5	214	27.3	194	20.5	107	25.2	179	19.4	200	28.6	163	18.3	118	9.3
14	197	21.7	204	26.3	233	28.4	211	27.6	206	28.8	238	19.8	186	16.9	184	17.2	194	10.1	125	14.9
16	225	25.4	221	23.4	257	30.7	242	29.6	227	33.9	239	24.0	210	21.7	216	22.0	205	20.9	---	
18	245	23.4	252	34.5	271	36.1	240	38.5	242	33.4	250	39.4	245	36.5	258	28.6	242	24.7	---	
20	255	31.2	263	38.8	280	34.8	277	31.8	261	29.8	274	38.5	232	26.9	235	32.2	206	28.1	---	
22	---		---		---		---		---		---		---		---		---		---	
24	302	28.5	305	36.8	316	51.4	301	35.1	271	56.0	298	30.5	241	32.1	258	39.5	162	16.2	---	
26	348	20.6	323	36.0	345	37.6	317	34.5	282	45.7	310	50.1	227	52.4	265	45.4	---		---	
28	345	39.8	296	48.8	347	33.8	323	50.7	285	37.2	318	49.4	256	41.4	269	38.9	---		---	
30	324	67.6	298	50.7	350	72.3	323	54.5	310	51.6	348	42.7	275	63.0	270	48.4	---		---	
32	---		339	35.0	364	42.1	358	39.9	335	27.9	343	40.0	271	41.8	276	38.2	---		---	

Table 5. MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF MUSSEL LARVAE REARED IN SEAWATER WITH VARIOUS CONCENTRATIONS OF MALATHION
Means Based on Measurement of 20 Larvae
(microns)

Day	Seawater control				Concentration, mg/liter															
					1.5				3.1				6.2				12.3			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
									Experiment 1											
0	---		---		---		---		---		---		---		---		---		---	
2	120	6.9	120	7.3	119	7.2	117	7.5	119	9.3	116	6.9	113	8.6	113	9.1	116	6.9	115	6.9
4	134	7.6	135	12.3	136	6.3	128	10.7	133	10.2	135	8.6	132	8.0	135	9.0	124	9.5	127	7.5
6	142	13.8	144	11.4	140	19.5	140	9.9	144	11.3	154	13.2	136	11.9	133	15.4	121	9.2	121	8.2
8	151	12.0	146	18.9	150	14.9	146	15.8	166	13.7	167	11.4	141	15.6	147	17.6	127	13.1	130	11.9
10	159	17.8	152	16.4	154	15.8	155	15.9	159	14.9	168	16.0	148	16.9	147	13.9	124	13.2	127	14.1
12	177	21.3	176	20.2	165	16.1	163	18.2	168	17.5	170	21.9	164	26.8	152	13.2	166	17.7	141	19.5
14	195	19.6	174	29.1	167	18.7	168	28.6	173	21.5	172	15.1	140	22.1	141	17.2	132	12.0	132	13.0
16	178	27.7	180	23.8	175	26.8	177	27.0	176	27.7	187	17.0	167	26.4	151	13.6	142	14.7	130	11.2
18	210	21.6	188	27.9	186	32.6	171	28.1	195	35.1	208	18.1	186	20.1	163	19.9	147	23.3	134	12.2
20	225	31.6	204	32.3	210	40.1	212	26.6	225	34.8	216	22.2	190	20.6	157	12.1	185	46.7	135	15.6
22	242	31.4	227	35.7	223	31.4	230	25.8	211	23.8	242	39.9	214	25.7	207	29.7	161	17.4	145	17.4
24	263	30.3	226	20.6	234	25.8	230	49.1	243	34.4	254	32.9	194	27.2	160	11.6	163	14.6	150	17.1
26	297	30.7	294	33.9	284	41.6	259	42.7	264	33.6	268	30.4	238	35.9	---		---		163	37.3
28	296	37.5	291	32.2	262	35.1	246	33.0	265	36.2	271	36.0	230	25.4	---		---		212	39.9
30	329	52.1	326	33.4	308	45.8	286	46.1	279	33.9	323	30.3	262	35.0	---		---		---	
32	328	66.9	344	56.6	311	55.3	301	32.3	279	33.9	342	38.4	261	25.2	---		---		---	
									Experiment 2											
0	---		---		---		---		---		---		---		---		---		---	
2	116	6.0	116	6.0	114	5.9	109	5.6	113	6.4	107	8.3	115	7.6	113	8.0	112	5.9	107	5.9
4	140	9.5	138	9.4	136	7.8	138	10.4	124	8.1	125	7.4	138	7.3	127	8.5	118	6.8	117	7.7
6	146	15.6	131	14.3	184	17.6	160	13.3	141	11.6	143	16.4	143	15.1	140	15.2	121	7.7	119	10.9
8	161	26.8	147	28.6	142	22.6	157	14.8	140	21.0	158	19.4	155	15.1	131	9.3	132	15.1	132	18.7
10	166	37.6	152	25.9	160	15.9	165	20.8	145	18.4	162	21.4	147	23.9	155	15.3	133	13.8	128	8.8
12	196	43.0	179	37.2	186	25.2	145	34.1	158	26.6	158	25.6	156	26.1	146	17.3	145	23.4	134	17.9
14	216	30.3	185	28.1	206	19.6	181	22.9	157	19.7	174	29.1	160	17.3	154	21.6	132	18.5	146	14.8
16	216	40.9	173	31.1	200	33.3	218	25.6	171	23.8	229	32.1	165	13.6	166	16.5	149	23.5	157	24.7
18	237	46.2	244	41.0	226	30.9	223	28.8	193	30.8	215	28.9	178	23.8	180	18.0	153	24.4	155	27.8
20	273	38.4	252	31.1	229	32.9	266	31.4	267	31.9	261	26.3	210	37.0	185	12.2	159	41.3	156	19.3
22	278	33.6	281	24.9	278	41.9	273	36.6	239	54.9	246	60.3	224	22.8	217	31.9	176	24.5	163	22.2
24	309	37.7	290	34.4	309	31.5	318	25.4	269	47.7	297	41.0	255	26.4	223	24.7	170	36.4	208	47.7
26	311	43.3	319	33.6	306	43.8	330	31.6	227	66.5	318	38.0	244	37.3	238	31.1	---		200	54.8
28	289	59.9	333	36.1	297	56.6	333	56.8	308	56.0	311	41.2	253	41.9	259	38.9	---		268	74.9
30	330	66.0	335	52.2	362	33.6	365	33.0	305	40.4	294	50.1	305	42.2	268	36.9	---		---	
32	333	61.2	356	24.5	375	37.4	358	48.4	299	40.0	342	36.2	298	36.4	264	34.6	---		---	

Appendix C

Table 1. NUMBER, MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF METAMORPHOSED MUSSEL LARVAE EXPOSED TO SEVIN
Exposure Initiated 29 Days after Fertilization of Eggs
(microns)

	Concentration, mg/liter																											
	Seawater control			0.36			0.72			1.45			2.9															
Day	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
0	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
2	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
1	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
5	1	345	0	0	---		0	---		0	---		0	---		1	377	0	0	---		2	397	0	2	345	28	1	345	0
7	0	---		0	---		0	---		0	---		0	---		1	416	0	1	364	0	0	---		4	384	71	7	334	37
9	1	456	0	2	360	11	0	---		0	---		0	---		0	---		3	331	5	1	400	0	6	347	56	14	356	45
11	0	---		0	---		1	416	0	0	---		1	384	0	4	398	30	2	460	85	3	367	42	3	367	99	0	---	
13	3	445	18	2	484	28	0	---		0	---		1	480	0	3	374	3	1	364	0	2	456	102	7	381	28	2	348	40
15	3	407	44	1	432	0	9	458	117	4	450	14	6	391	16	2	370	8	6	363	20	3	429	99	3	381	86	4	371	83
17	12	404	54	5	442	58	19	445	68	5	460	40	14	415	43	17	433	56	20	384	35	23	384	50	14	339	59	7	320	41
19	5	444	45	3	408	119	6	428	69	5	401	65	4	419	56	10	452	80	6	392	50	6	402	59	2	296	0	3	385	86
21	6	516	76	6	547	167	3	463	49	11	443	43	3	622	250	1	360	0	2	365	2	0	---		1	302	0	2	285	17
23	13	480	86	19	479	51	6	473	61	5	423	74	7	407	67	1	358	0	3	427	127	0	---		0	---		1	260	0
25	9	482	39	9	475	117	12	444	108	11	529	82	9	435	93	5	414	38	2	514	156	7	382	41	0	---		0	---	
27	7	507	118	0	---		6	447	65	7	509	77	7	436	57	3	537	203	2	345	15	6	384	29	2	359	17	3	336	40
29	4	472	41	5	470	44	3	449	58	1	519	0	5	443	43	5	420	53	0	---		7	389	34	1	350	0	2	355	30
31	7	447	33	2	467	8	6	469	51	2	558	249	0	---		1	387	0	1	387	0	1	387	0	0	---		0	---	
33	1	451	0	6	459	29	0	---		2	---		1	535	0	1	371	0	1	339	0	1	352	0	0	---		2	---	
36	5	469	21	11	432	53	0	---		1	520	0	0	---		0	---		2	397	9	1	390	0	1	351	0	0	---	
40	2	504	51	4	507	23	0	---		1	507	0	2	442	0	12	430	40	1	442	0	7	387	66	2	---		4	377	0
Number of unmetamorphosed larvae	1			0			0			6			2			3			1			2			0			0		

Table 2. NUMBER, MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF METAMORPHOSED MUSSEL LARVAE EXPOSED TO TREFLAN
Exposure Initiated 30 Days after Fertilization of Eggs
(microns)

	Seawater control			Solvent control			Concentration, mg/liter																							
							0.202			0.040			0.080			0.160														
Day	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
0	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
2	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
4	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
5	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
7	0	---		0	---		2	330	71	0	---		3	347	32	7	361	27	3	373	55	6	393	46	5	382	38	4	300	38	2	316	40	4	375	33
9	1	488	0	1	392	0	3	403	20	14	399	52	11	389	44	12	403	25	6	367	44	16	374	36	6	363	38	13	382	28	4	378	15	4	344	34
11	1	332	0	3	360	42	0	---		16	414	54	15	403	68	3	368	37	15	400	60	22	422	50	5	370	33	16	406	45	3	377	72	7	389	51
13	7	421	44	3	397	12	2	428	74	6	467	58	11	388	41	7	441	66	3	383	14	0	---		11	391	49	3	425	24	5	403	39	3	428	63
15	6	422	108	11	479	95	5	397	47	7	403	82	7	353	83	11	344	40	2	392	68	3	384	23	5	412	73	0	---		2	394	31	4	370	63
17	6	425	38	2	469	18	35	412	42	6	353	38	7	438	54	11	362	39	34	377	38	21	415	80	23	377	55	25	385	48	32	371	48	29	360	33
19	7	459	48	8	478	43	11	402	79	1	360	0	4	383	85	2	435	8	6	441	38	1	493	0	0	---		5	382	42	15	410	60	9	400	44
21	11	477	65	14	413	62	1	452	0	1	444	0	1	318	0	1	504	0	1	624	0	0	---		2	418	8	0	---		4	456	48	8	447	98
23	4	459	38	5	464	72	2	579	133	1	358	0	0	---		0	---		0	---		1	551	0	1	424	0	0	---		1	567	0	3	467	154
24	4	449	56	5	431	49	6	398	112	4	401	60	1	480	0	2	406	124	2	366	8	2	422	78	0	---		0	---		0	---		0	---	
26	2	505	84	8	444	43	2	371	120	2	371	143	2	334	0	0	---		4	419	96	1	360	0	3	435	214	0	---		1	344	0	3	333	32
28	1	451	0	5	456	47	1	498	0	1	360	0	0	---		0	---		3	364	30	0	---		0	---		6	400	90	1	---		0	---	
30	2	469	48	10	422	50	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
32	3	449	70	4	399	45	0	---		1	313	0	0	---		0	---		2	435	0	0	---		0	---		0	---		0	---		0	---	
39	13	484	102	2	429	0	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
No. of unmetamorphosed larvae	5			1			0			1			0			0			1			0			1			2			0			1		

Table 3. NUMBER, MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF METAMORPHOSED MUSSEL LARVAE EXPOSED TO METHOXYCHLOR
Exposure Initiated 29 Days after Fertilization of Eggs
(microns)

	Seawater control			Solvent control			Concentration mg/liter																							
							0.008						0.015						0.030						0.060					
Day	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
0	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
2	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
4	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
5	2	410	14	0	---		0	---		0	---		0	---		2	406	8	0	---		0	---		3	410	61	2	348	51	12	361	39	9	416	55
8	3	405	17	0	---		3	370	44	4	322	25	4	403	39	5	363	33	0	---		6	395	34	1	432	0	2	376	0	4	356	40	3	357	17
10	1	344	0	0	---		16	408	37	6	383	75	13	401	63	9	384	30	9	397	45	14	388	34	1	328	0	1	320	0	4	416	62	6	390	40
12	1	552	0	0	---		26	439	66	17	444	58	24	407	50	11	385	31	14	399	28	14	380	40	7	391	25	0	---		2	394	13	3	366	70
14	0	---		1	424	0	10	393	61	6	424	90	12	406	59	10	427	30	4	391	48	8	433	31	16	403	58	0	---		0	---		1	424	0
16	1	448	0	1	480	0	12	399	42	3	452	121	6	395	33	7	389	56	9	394	44	6	383	27	4	404	33	7	411	52	8	380	47	25	379	33
18	3	437	156	3	459	86	9	394	54	16	387	54	9	412	42	13	408	54	10	364	62	10	357	53	18	394	38	22	396	41	20	347	34	8	378	47
20	12	429	32	9	460	22	4	448	0	13	434	74	7	404	50	13	392	42	5	359	29	2	296	11	11	382	47	21	420	60	5	371	18	5	405	83
22	23	523	78	17	460	41	2	491	124	3	495	85	1	297	0	5	464	31	4	400	42	0	---		1	403	0	6	459	88	5	373	49	0	---	
24	1	488	0	12	528	68	0	---		1	390	0	1	403	0	1	507	0	3	362	20	0	---		3	399	121	0	---		0	---		1	481	0
26	9	479	37	10	550	112	1	292	0	1	331	0	1	320	0	2	398	31	6	368	43	3	377	112	3	355	48	7	394	82	5	337	12	4	403	98
28	9	538	81	3	494	15	0	---		0	---		0	---		0	----		8	340	32	0	---		5	322	53	6	383	57	2	371	0	0	---	
30	3	634	35	4	458	16	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
32	1	488	0	1	323	0	0	---		0	---		0	---		0	---		0	---		0	---		2	273	0	0	---		3	302	0	0	---	
39	3	546	136	4	392	23	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
No. of unmetamorphosed larvae	1			0			0			0			0			0			0			0			0			0			0		

Table 4. NUMBER, MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF METAMORPHOSED MUSSEL LARVAE EXPOSED TO 2,4-D
Exposure Initiated 30 Days after Fertilization of Eggs
(microns)

	Concentration, mg/liter																											
	Seawater control						22.0						44.0						88.0						176.0					
Day	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
0	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
2	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
4	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		5	386	32	2	353	47
5	0	---		0	---		0	---		0	---		0	---		1	410	0	0	---		0	---		0	---		0	---	
7	0	---		0	---		0	---		0	---		1	344	0	0	---		0	---		1	448	0	6	371	66	3	388	59
9	1	452	0	0	---		1	436	0	1	344	0	0	---		2	464	56	3	395	17	1	536	0	1	384	0	5	369	52
11	1	312	0	0	---		3	361	51	1	572	0	2	407	105	0	---		1	507	0	3	468	18	2	381	60	1	397	0
13	0	---		1	440	0	2	420	40	2	466	76	2	372	40	1	328	0	5	459	53	4	495	107	4	410	70	4	450	50
15	3	397	15	2	452	51	3	480	56	7	458	40	4	432	22	7	489	61	10	426	81	15	438	63	4	344	55	7	403	70
17	7	436	17	6	499	86	6	463	59	1	336	0	3	456	65	3	396	75	1	567	0	27	443	87	0	---		2	468	6
19	11	450	71	7	449	44	7	499	159	0	---		1	424	0	1	424	0	2	519	150	7	328	74	0	---		1	380	0
21	5	472	62	4	498	63	10	448	56	7	490	39	14	439	51	10	445	65	3	484	17	2	462	37	2	325	9	4	361	59
23	3	384	107	3	442	118	8	425	36	18	492	66	19	460	37	12	443	40	4	491	50	3	501	85	6	380	66	3	425	22
25	4	401	40	2	456	75	5	437	74	6	494	86	11	461	99	9	486	51	7	472	56	2	640	56	1	461	0	2	456	37
27	4	424	65	13	445	42	7	421	65	6	482	19	1	334	0	2	538	146	6	573	93	1	461	0	0	---		0	---	
29	6	480	50	7	495	89	1	482	0	8	484	87	1	578	0	6	636	129	0	---		0	---		0	---		0	---	
31	4	480	64	6	490	65	1	456	0	1	871	0	0	---		2	663	135	0	---		0	---		2	432	19	3	426	43
33	8	460	28	6	419	75	1	509	0	1	321	0	2	689	0	1	488	0	5	452	6	8	366	68	2	---		5	401	33
35	4	411	52	2	449	0	0	---		0	---		1	533	0	0	---		0	---		3	449	84	0	---		0	---	
38	5	438	80	2	546	0	2	407	68	0	---		0	---		1	273	0	2	492	151	3	449	9	5	420	124	4	488	46
No. of unmetamorphosed larvae	12			10			9			18			11			22			10			29			5			15		

Table 5. NUMBER, MEAN AND STANDARD DEVIATION OF SHELL LENGTH OF METAMORPHOSED MUSSEL LARVAE EXPOSED TO MALATHION
Exposure Initiated 30 Days after Fertilization of Eggs
(microns)

	Concentration, mg/liter																											
	Seawater Control						1.51						3.02						6.05						12.1			
Day	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
0	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
2	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
4	0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---		0	---	
5	0	---		0	---		0	---		0	---		0	---		0	---		1	456	0	1	416	0	0	---		1	416	0
7	0	---		1	350	0	0	---		0	---		0	---		0	---		0	---		1	384	0	5	388	72	0	---	
9	0	---		2	396	6	1	376	0	0	---		0	---		2	412	34	2	380	23	0	---		14	350	51	26	363	32
11	0	---		2	403	147	0	---		2	371	46	0	---		7	415	62	0	---		3	394	15	8	371	40	2	371	46
13	0	---		2	438	76	2	432	11	4	412	35	2	350	110	2	488	0	3	411	61	10	408	62	18	386	47	13	349	34
15	3	464	44	0	---		2	608	34	6	468	100	5	413	44	4	410	78	6	440	56	1	392	0	12	387	54	14	369	29
17	3	433	14	1	478	0	5	421	35	5	373	76	8	447	30	2	414	190	6	398	20	11	409	63	10	345	15	7	391	48
19	6	466	61	7	422	45	7	430	51	2	500	153	9	478	49	10	527	146	5	429	75	6	360	64	3	387	32	1	307	0
21	6	433	29	11	484	90	3	395	60	3	477	18	4	432	42	11	409	46	1	409	0	2	452	17	3	317	63	1	323	0
23	5	442	91	2	394	23	7	432	41	4	436	62	4	467	50	9	436	61	2	439	59	6	396	16	2	384	28	0	---	
25	1	546	0	6	418	33	5	443	46	6	425	56	10	454	12	4	457	28	5	465	42	5	466	65	1	344	0	1	350	0
27	3	458	60	10	488	105	3	477	64	6	464	52	2	437	34	7	481	99	4	436	38	9	413	50	1	418	0	1	344	0
29	7	486	65	6	491	127	2	498	75	8	537	86	5	444	102	4	473	150	6	407	22	4	461	166	0	---		1	440	0
31	7	469	82	5	407	34	6	408	74	6	469	68	4	447	61	2	422	41	1	382	0	7	486	85	1	---		0	---	
33	2	438	4	4	435	0	0	---		5	422	64	1	---		0	---		4	401	84	1	350	0	0	---		0	---	
35	1	416	0	4	439	31	4	431	62	5	402	54	0	---		0	---		3	423	32	0	---		0	---		0	---	
38	3	438	40	4	436	78	3	459	46	3	416	85	8	411	22	2	381	50	11	376	61	0	---		0	---		0	---	
39	3	433	27	3	392	17	4	463	30	2	468	0	0	---		3	427	31	1	---		0	---		0	---		0	---	
No. of unmetamorphosed larvae	11			5			10			13			8			3			2			0			0			0		

SELECTED WATER RESOURCES ABSTRACTS
INPUT TRANSACTION FORM

1. Report No. 2

W

TOXICITY OF SELECTED PESTICIDES TO THE BAY MUSSEL (Mytilus Edulis)

5. Report Date

8. Performing Organization Report No.

Liu, David H. W.; and Lee, Jean M.

Stanford Research Institute
Menlo Park, CA 94025

68-01-0190

13. Type Report and Period Covered

12. Sponsoring Organization **Environmental Protection Agency**

Environmental Protection Agency report number EPA-660/3-75-016

The toxicity of the insecticides Sevin, methoxychlor, and malathion and of the herbicides Treflan and 2,4-D to the bay mussel (Mytilus edulis) was investigated. Toxic effects were measured in terms of survival of and byssus-thread attachment by adults, embryo shell development, and larval growth and metamorphosis.

The results indicated that growth was the most sensitive measure of toxicity. All the pesticides produced statistically significant ($p = 0.05$) reductions in larval shell length after 10 to 20 days of exposure. Relative to potency, methoxychlor was the most toxic, and 2,4-D was the least toxic.

The 96-hour TL_{50} values for each pesticide, based on adult survival and attachment data, were estimated, as were the 48-hour EC_{50} values based on data from embryo bioassays.

The effects on embryo development of delaying the time of fertilization and of using seawater larval culture media of various ages also were studied, and substrate preference by metamorphosing larvae was investigated.

A critical evaluation of the experimental approach and procedures is presented.

17a. Descriptors

Pesticide Toxicity, Mussels, Mollusks, Marine Animals

17b. Identifiers

--

VC, Group 16

19. Security Class (Report)

21. No. of Pages

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