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CHRONIC TOXICITY OF METHOXYCHLOR, MALATHION, AND CARBOFURAN TO SHEEPSHEAD MINNOWS (Cyprinodon variegatus)

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U.S. Environmental Protection Agency
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CHRONIC TOXICITY OF METHOXYCHLOR, MALATHION, AND CARBOFURAN TO SHEEPSHEAD MINNOWS (*Cyprinodon variegatus*)

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FOREWARD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

the effects of toxic organic pollutants on individual species and communities or organisms;

the effects of toxic organics on ecosystem processes and components;

the significance of chemical carcinogens in the estuarine and marine environments.

This report describes effects of three insecticides in partial life-cycle tests with an estuarine fish, the sheepshead minnow. The data will be useful in establishing estuarine water quality criteria, and limiting effluents containing carbofuran, malathion, or methoxychlor.

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ABSTRACT

Sheepshead minnows (Cyprinodon variegatus) were exposed to each of three pesticides--methoxychlor, malathion, and carbofuran--in flowing seawater to determine the acute and chronic (partial life-cycle) effects. The calculated 96-hour LC50's and 95% confidence limits, based on measured concentrations, were: methoxychlor, 49 micrograms per liter (µg/ℓ), 37-65 µg/ℓ; malathion, 51 µg/ℓ, 41-63 µg/ℓ; and carbofuran, 386 µg/ℓ, 311-480 µg/ℓ.

Mortality of adult sheepshead minnows exposed to mean measured concentrations of methoxychlor ≥23 µg/ℓ was significantly ($P<0.05$) greater than mortality of control fish during the 140-day study. Further, hatching success of fry from eggs spawned by fish exposed to 23 µg/ℓ was significantly less than hatching success of control fry. The maximum acceptable toxicant concentration (MATC) was estimated to be >12<23 µg/ℓ and the application factor limits were 0.24-0.47.

Mortality of adult sheepshead minnows exposed to mean measured concentrations of malathion ≥18 µg/ℓ was significantly greater than mortality of control fish during the 140-day study. Mortality of fry hatched from eggs spawned by fish exposed to 9 and 18 µg/ℓ was significantly greater than mortality of control fry. The MATC was estimated to be >4<9 µg/ℓ and the application factor limits were 0.08-0.18.

Mortality of adult sheepshead minnows exposed to mean measured concentrations of carbofuran ≥49 µg/ℓ was significantly greater than mortality of control fish during the 131-day study. Hatching success of fry from eggs spawned by fish exposed to 49 µg/ℓ was significantly less than hatching success of control fry. Also, mortality of fry hatched from eggs spawned by fish exposed to 23 and 49 µg/ℓ was significantly greater than control fry mortality. The MATC was estimated to >15<23 µg/ℓ and the application factor limits were 0.04-0.06.

This report was submitted in fulfillment of Contract Number 68-03-0264 by EG&G, Bionomics Marine Research Laboratory, under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period 23 May 1973 to 23 May 1975; work was completed on 1 November 1976.

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ACKNOWLEDGMENTS

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SECTION I

INTRODUCTION

These studies were undertaken to gain information about the effects of three pesticides--methoxychlor, malathion, and carbofuran--on sheepshead minnows (Cyprinodon variegatus), a saltwater fish. Data from these studies can be used to derive application factors, first described by Mount and Stephan (1967). An application factor is obtained by dividing the concentration considered to be "safe" over a long period by a short-term toxicity value. The application factor may then be used to establish water quality criteria for each pesticide. The use of sheepshead minnows for long-term, life-cycle tests was proposed by Schimmel and Hansen (1975) and a tentative method was described by Hansen and Schimmel (1975). In our short term studies, data on 96-hour LC50's (the concentration of each pesticide estimated to be lethal to 50% of the test animals after 96 hours of exposure) were obtained. In our long-term studies, parental sheepshead minnows were exposed for 28 days or more and then effects on spawning and progeny were determined. "Safe" concentrations were estimated after evaluation of data on number of eggs spawned by parental fish, hatching success of embryos, mortality of fry, and growth of fry.

Methoxychlor is a stable, chlorinated hydrocarbon compound used to control a wide variety of insects which attack fruits, vegetables, field and forage crops, and livestock. It is also used to control certain household and industrial insects. Methoxychlor is a replacement for DDT in many applications (Anonymous, 1972). Methoxychlor is ostensibly insoluble in water but soluble in lipids (Gardner and Bailey, 1975) and thus might be expected to accumulate in fish and to be cumulatively toxic during long-term exposure.

A recent publication (Gardner and Bailey, op. cit.) provides an excellent overview of the effects of methoxychlor on environmental quality and states that although there are variances in estimates of the acute toxicity of methoxychlor to fishes, all reported LC50's ranged from 5-80 micrograms per liter (µg/ℓ), excepting three.

We know of only one chronic (partial life-cycle) toxicity study that has been conducted with methoxychlor and fish (Merna and Eisele, 1973). Data from that study were insufficient for

deriving an application factor.

Malathion is an organophosphate pesticide used throughout the U. S. to control a variety of pests. A major use of malathion is for mosquito control in both freshwater and estuarine areas. Several studies (Parkhurst and Johnson, 1955; Westman and Compton, 1960; Weiss, 1961; Lewallen and Wilder, 1962; Holland and Lowe, 1966; Wellborn, 1971; and Post and Schroeder, 1971) have shown that malathion is acutely lethal to a variety of freshwater and saltwater fishes under both field and laboratory test conditions. Sublethal effects (avoidance and inhibition of brain acetylcholinesterase) have also been observed in fishes exposed to malathion (Hansen et al., 1972; Coppage, 1972; and Coppage and Matthews, 1972).

Chronic studies have been conducted with malathion and two freshwater fishes, fathead minnows (Pimephales promelas) (Mount and Stephan, 1967), and bluegill (Lepomis macrochirus) (Eaton, 1970). The studies showed the application factor limits for the two fishes to be very similar, ranging from 0.02-0.06.

Carbofuran is a carbamate pesticide utilized both as a contact poison or as a soil-applied systemic poison. It is registered for soil-applied use on a variety of crops, including rice, and for direct use on several insects, including mosquitoes (Anonymous, 1971). Little research has been performed on the effects of carbofuran on aquatic organisms.

Carbofuran was the first compound tested. Tests were conducted from March-July 1974 in the EG&G, Bionomics Aquatic Toxicology Laboratory, Wareham, Massachusetts. Our new laboratory was opened in January 1975, and tests with methoxychlor and malathion were conducted from June-October 1975, at EG&G, Bionomics Marine Research Laboratory, Pensacola, Florida.

SECTION 2

CONCLUSIONS

Sheepshead minnows (Cyprinodon variegatus) are suitable test animals for toxicity tests which include the reproductive portion of the life cycle and the critical life stages (embryos and fry) of the successive generation.

Tests with these saltwater fish are practical means of determining maximum acceptable toxicant concentrations and application factors because of (a) the amenability of sheepshead minnows to laboratory culture and (b) the relatively short period of time required to reach sexual maturity and complete the reproductive phase of the life cycle.

The application factor limits derived for sheepshead minnows exposed to malathion are very similar to the application factor limits derived for two freshwater fishes, indicating that this saltwater fish may be used effectively to obtain data on which to base water quality criteria.

SECTION 3

RECOMMENDATIONS

Spawning groups comprising five sheepshead minnows in the ratio of 3 female fish:2 male fish are satisfactory to determine spawning success.

A 10-day spawning period for a spawning group is sufficient to monitor spawning success.

Studies should be conducted with sheepshead minnows and other toxicants in chronic (full life-cycle) tests because tests with this saltwater fish appear to provide accurate estimates of MATC's in a shorter time and with less effort than do tests with most freshwater fishes.

SECTION 4

MATERIALS AND METHODS

TEST MATERIALS

Methoxychlor used in this study was obtained from E. I. du Pont de Nemours & Company, Biochemicals Department, Wilmington, Delaware. It was contained in a plastic jar labeled "Methoxychlor Technical, 1 Kg." Although active ingredient was not listed on the label, a technical data sheet dated April 1972 which accompanied the chemical stated that "...methoxychlor technical...contains 88% (minimum) 2,2-bis-(p-methoxypheny1)-1, 1, 1-trichloroethane and 12% (maximum) other isomers and reaction products.

Malathion was obtained from the American Cyanamid Company, Agricultural Division, Princeton, New Jersey. It was contained in a metal bottle, apparently aluminum, with a transluscent cap. The bottle was refrigerated at all times. The material was labeled "MALATHION Technical, Active Ingredient: Malathion* 95%; Inert Ingredients 5%. *0,0-dimethyl phosphorodithioate of diethyl mercaptosuccinate; (1 Gallon contains 9.7 lb of malathion)."

Carbofuran was obtained from FMC Corporation, Agricultural Chemical Division, Middleport, New York. It was contained in a plastic bag labeled "Carbofuran Technical (99%), FURADAN® Insecticide, Mr L514, 2Kg. C4717-54-A, 9/14/73."

Concentrations of each pesticide are reported here as micrograms (µg) of the technical material described above per liter (ℓ) of seawater.

Stock solutions of all three pesticides were prepared on a weight:volume basis by dissolving them in reagent grade acetone. These 1-ℓ stock solutions were placed in amber glass bottles and stored in the laboratory. New stock solutions were prepared as required.

TEST WATER

Methoxychlor and Malathion

All water used for holding, acclimation, and testing was natural seawater which was pumped from Big Lagoon into the

laboratory. The pump intake was 85 meters (m) offshore at a depth of approximately 3 m. Water was pumped by a #316 stainless steel pump through hard polyvinylchloride (PVC) pipes into an elevated fiberglass reservoir. En route, the water passed through a fiberglass, sand filter and a 10-micrometer (μm) polypropylene bag filter. From the reservoir, in which the water was continuously and vigorously aerated, water flowed by gravity through PVC pipes to the diluters.

No attempt was made to alter the salinity of the water, but temperature was maintained at 30±1 degrees Celsius (°C) by heating the incoming seawater in small fiberglass-coated plywood boxes above the diluters with electric quartz heaters and by placing test aquaria in constant-temperature water baths.

Carbofuran

All water used for holding, acclimation, and testing was synthetic seawater, formulated according to the methods of LaRoche et al. (1970). Freshwater was pumped from a 120-m deep bed-rock well at the Wareham, Massachusetts, laboratory into two 1,500-ℓ fiberglass tanks. Ingredients were added, mixed with freshwater, and aerated. Water was drained from one tank at the rate of approximately 1,200 ℓ per day until it was empty. Then, water was drained from the second tank while fresh synthetic seawater was being prepared in the first tank.

All water flowed by gravity through PVC pipes to the diluter. Temperature was maintained at 30±1°C by placing the test aquaria in constant-temperature water baths.

TEST ANIMALS

Methoxychlor and Malathion

All sheepshead minnows used in these studies were collected from Big Lagoon, near Bionomics Marine Research Laboratory. They were held in sand-banked ponds on the laboratory grounds and in fiberglass tanks in the laboratory. All fish were acclimated to test conditions for 14 days before testing according to the conditions of U. S. Environmental Protection Agency (1975). Mortality was <3% during acclimation. During holding and acclimation, fish were fed frozen or live Artemia salina (San Francisco Bay Brand) which contained <0.1 μg/g of chlorinated hydrocarbon pesticides or polychlorinated biphenyls as determined by our electron-capture gas chromatograph analyses.

Size of fish was: methoxychlor--acute test, 1.7-3.8 centimeters (cm) standard length (SL) and chronic test, 1.0-1.9 cm SL and 0.15 g mean weight (determined in water); malathion--acute test, 0.8-1.8 cm SL and chronic test, 1.0-1.8 cm SL and 0.11 g mean weight (determined in water).

Carbofuran

Fish were obtained from the U. S. Environmental Protection Agency's Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Florida, where they had spawned naturally in outside earthern ponds. Fish for the acute and chronic tests were 1.8-4.0 cm SL.

TEST METHODS

Acute Tests

All procedures followed methods of APHA et al. (1976) and U. S. Environmental Protection Agency (1975), except as stated. The 96-hour tests were conducted in an intermittent-flow system by using a proportional diluter (Mount and Brungs, 1967) constructed to deliver 1 ℓ/cycle at a dilution ratio of 75%. The average number of cycles was approximately 5/hour, providing 99% replacement in 24 hours (Sprague, 1969). A mechanical injector (manufactured by George Frasier, Duluth, MN), equipped with a 50-milliliter (mℓ) glass syringe and stainless steel needle, pumped methoxychlor, malathion, or carbofuran stock solution through polyethylene tubing to the mixing cell. Test containers were 30 X 30 X 61-cm glass aquaria. Each contained 20 fish and approximately 28 ℓ of water.

Chronic Tests

The tests were conducted as described above except that the proportional diluter was constructed for 50% dilution. It delivered 1 ℓ/cycle at a rate of approximately 5 cycles/hour. For the methoxychlor and malathion tests, the diluter was modified to include a solvent control wherein the same volume of solvent/carrier (acetone) was added to methoxychlor- or malathion-free seawater as was added to the highest pesticide concentration. One injector, equipped with a 30-mℓ glass syringe and a stainless steel needle, metered the respective stock solutions through polyethylene tubing into the mixing chamber. A second injector, equipped with a 50-mℓ glass syringe with stainless steel needle, metered acetone to each solvent control. Maximum solvent concentration was 29 μℓ/ℓ (parts per million, ppm).

To begin each test, 20 acclimated fish were impartially selected and placed in the test aquaria (a total of 40 fish per treatment) after the toxicant delivery system had been operational for several days. Fish were daily fed flaked commercial fish food (BiOrell® and Tetramin®) ad libitum. Salinity and dissolved oxygen were measured daily throughout the tests. Light for all tests was provided by two 3.7-m fluorscent bulbs suspended 46 cm above the test containers, providing approximately 1,100 lux incident to the water surface. Photoperiod was 16 hours light, 8 hours dark. Survival was monitored daily by visually inspecting each test container. Growth was monitored biweekly according to the photographic method of McKim and Benoit

(1971) and average weight was determined monthly by weighing each group in water.

Effects of each pesticide on spawning were determined after fish began to exhibit signs of sexual maturity.

Methoxychlor and Malathion--

Monitoring of spawning activity was begun on day 54 of the methoxychlor test and on day 87 of the malathion test. Spawning chambers were constructed by lacing pieces of 6.5-millimeter (mm) square mesh #316 stainless steel screen together with #316 stainless steel wire. The chambers were 30.5-cm square X 25.5-cm high, and were supported by 5-cm high extensions of the screen ends. Beneath each spawning chamber, a 29.5-cm square X 4.7-cm high egg collection tray was placed to retain the demersal eggs that sank through the bottom of the spawning chamber. The tray was constructed of plate glass and silicone sealant, with a 4-cm wide strip of 480-µm square mesh nylon screen along one side of the bottom to facilitate consolidation of eggs. Spawning groups, which consisted of two male and three female fish, were placed in the spawning chambers for a 10-day period. All possible 2:3 ratios in each aquarium were spawned once and extra, unspawned fish from each replicate aquarium were combined whenever possible to form a 2:3 spawning group. Each day, one end of each spawning chamber was lifted slightly and the egg collection tray was removed from the aquarium. The eggs spawned during the previous 24 hours were washed with seawater, transferred by large-bore glass pipette into glass Petri dishes, counted, and separated into groups of 50 eggs. Each 50-egg group was placed in an egg incubator cup (a 100-mℓ glass jar with the bottom cut off and 480-µm square mesh nylon screen attached with silicone sealant). Each egg cup was then placed in the same aquarium as the spawning group which produced it. The egg incubator cups were suspended from a rocker-arm apparatus (Mount, 1968) which gently oscillated them in the test aquaria. Eggs were removed from each egg incubator by pipette daily, counted, and the cups washed with bursts of freshwater to clean the screens. This procedure was repeated until all living embryos hatched. Then, 40 fry were placed in glass chambers (14-cm wide X 20.5-cm high X 26-cm long with 381-µm square mesh #316 stainless steel screen over one end). Survival was monitored daily and growth (standard length and average weight) was measured after 28 days. At least two groups of fry per duplicate from each test concentration and controls were monitored, except in the higher concentrations where toxicant-induced mortality made it impossible to obtain spawning groups and subsequent eggs and fry.

Carbofuran--

Effects on spawning were determined by monitoring spawning activity of individual pairs of fish, beginning on day 28. Each pair was placed in a 14 X 25 X 25-cm glass and #316 stainless steel mesh spawning chamber similar to the fry chambers described

above. Spawning was monitored for a 62-day period, during which all possible pairs of unspawned fish in all duplicate aquaria were spawned. Eggs that sank through a false bottom of 7-mm square mesh #316 stainless steel screen were collected daily and treated as described above.

CHEMICAL ANALYSES

Seawater

For each of the three acute tests, water was collected from each aquarium at the beginning and end of the 96-hour exposure. Water was collected from alternate duplicate aquaria weekly during the chronic tests. Water samples were prepared and analyzed as follows:

Methoxychlor--

Unfiltered seawater was extracted twice with two 50-mℓ portions of Nanograde® (Mallinckrodt) dichloromethane. Volumes extracted were:

Nominal concentration (µg/ℓ)	Volumes (mℓ)
6	500
11	300
22	300
45	100
90	100

The combined extracts were dried by elution through anhydrous sodium sulfate (heated at 100°C for 24 hours), concentrated to approximately 1 mℓ in a Kuderna-Danish evaporator, and solvent-exchanged with Nanograde petroleum ether. The extract volumes were adjusted to obtain a sensitivity of 0.05 ppm (nanograms [ng] per µℓ) by using a Perkin-Elmer Model 2100 gas chromatograph equipped with a Ni63 electron-capture detector.

Operating conditions were:

Column (glass)--2 m X 4 mm ID 3% OV-101 on 80/100 mesh Gas Chrom Q

Oven temp.--210°C Detector temp.--275°C

Injector temp.--250°C Carrier gas--Nitrogen

Malathion--

Unfiltered seawater samples of 500 mℓ were extracted twice with two 50-mℓ portions of Nanograde dichloromethane. The extracts were dried by eluting through anhydrous sodium sulfate

(heated at 100°C for 24 hours) and concentrated in a Kuderna-Danish evaporator. The extract volumes were adjusted to obtain a sensitivity of 1.0 ppm by using a Perkin Elmer Model 2100 gas chromatograph equipped with a flame photometric detector operating in the phosphorus mode.

Operating conditions were:

Column (glass)--2 m X 4 mm ID 3% OV-101 on 80/100 mesh Gas Chrom Q

Oven temp.--210°C Bead setting--500

Injector temp.--250°C Carrier gas--10% argon/methane

Carbofuran--

Unfiltered water samples (approximately 500 mℓ) were measured volumetrically in a graduated cylinder and placed in a 1-ℓ separatory funnel equipped with a Teflon® stopcock. The water was extracted three times with separate 30-mℓ portions of Nanograde dichloromethane and the combined extract was passed through an anhydrous sodium sulfate column to remove moisture from the solvent. The sodium sulfate was rinsed with a portion of dichloromethane and the extract and rinse were placed in a Kuderna-Danish evaporator equipped with a three-ball Synder column. The solvent was evaporated to approximately 3 mℓ over an 80°C water bath, the extract was transferred to a 15-mℓ centrifuge tube with a Teflon-lined cap, and evaporated to dryness at room temperature by using a gentle stream of clean dry air. The extract was then dissolved in an accurately known volume of Nanograde benzene and stored in a freezer at 15°C prior to analysis by gas/liquid chromatography under the following conditions:

Instrument--Perkin-Elmer Model 3920 gas chromatograph

Detector--Nitrogen/phosphorus thermionic detection

Column--0.6 m X 2 mm ID glass packed with 20% SE-30 coated on 60/80 Chromasorb W. The column was conditioned at 235°C for two weeks prior to use. Several injections (3 X 50 µℓ) of Silyl-8, a column-silanizing agent, were made over the two-week conditioning period.

Gas flows--38 cubic centimeters (cc) N_2/minute (min.) carrier, 7 cc H_2/min. and 100 cc air/min. to the N/P detector.

Temperatures--Injection port: 225°C
Column: 155°C
Transfer line: 245°C

Recorder--Leeds & Northrup dual pen, 0-1 mV range, 5 mm/min. chart speed

Response--30 ng of carbofuran and 80 ng of 3-hydroxycarbofuran gave half-scale response with retention times of 3.4 and 6.4 min., respectively

Extraction efficiency and mean recovery for the analytical methods were 89.9±9.8% for methoxychlor, 84.5±11.7% for malathion, and 79.0±7.3% for carbofuran. Data in this report are corrected for recovery.

Fish Tissue

Fish were collected for residue analyses as follows:

a. adults alive at the end of the respective exposure;

b. fry alive at the end of the 28-day growth period; and

c. eggs randomly collected during the spawning periods.

Methoxychlor--

Tissues were analyzed by an adaptation of the methods of U. S. Environmental Protection Agency (1971 and 1974). Fish tissue was weighed to the nearest 0.01 g in a beaker. The tissue was transferred to a 100-mℓ graduated cylinder, which had been cut at the 80-mℓ mark to reduce the height of the cylinder; the beaker was rinsed with dichloromethane, which was then added to the cylinder. The tissue was homogenized with approximately 30 mℓ of dichloromethane for 20 seconds by using a Brinkman Polytron Homogenizer, Model PT 10/20.

The homogenate was filtered through No. 3 Whatman paper into a clean beaker, approximately 30 mℓ of dichloromethane was added to the graduated cylinder, and the Polytron probe was rinsed for approximately 10 seconds in the solvent while the homogenizer was operating at low speed. The probe rinse was added to the filter and finally the filter was washed with dichloromethane.

The solvent was evaporated to approximately 5 mℓ over a steam bath, cooled, and evaporated to dryness at room temperature by using a gentle stream of clean air. At this point the extract was cleaned by florisil column chromatography according to U. S. Environmental Protection Agency (1974). Methoxychlor eluted quantitatively from the 6% ether-in-petroleum ether fraction and was sufficiently free of interfering substances to permit analysis by electron capture detection. The 6% ethyl-petroleum ether fraction containing methoxychlor was evaporated to approximately 3 mℓ in a Kuderna-Danish evaporator equipped with a three-ball Snyder column, and the unit was cooled to room temperature. The receiver was disconnected, the remainder of the solvent was evaporated to dryness at room temperature by using a gentle air flow, and a known volume of hexane was added to the

receiver to dissolve the residue.

An aliquot of the extract was analyzed by gas chromatography under the following operating conditions:

Instrument--Tracor Model MT-550 gas chromatograph

Detector--Electron capture with 15 millicuries of Ni63

Column--2 m X 2 mm ID glass packed with 3% OV-101 on 100/120 mesh HMDS-treated Supelcoport

Gas flows--30 cc N_2/min. carrier, 60 cc N_2/min. scavenger

Temperatures--Column: 200°C Inlet: 230°C
Transfer: 270°C Detector: 302°C

Recorder--Corning Model 841, 0-1 mV, 0.5 cm/min. chart speed

Response--2.5 ng of methoxychlor gave half-scale pen deflection at an attenuation of 1.6 X 10^{-9} amperes

Three fish, weighing approximately 1 g each, were spiked with 100 ng of methoxychlor and were analyzed by the above method. The average precentage recovery of methoxychlor was 100±4.6%. The analytical results were not corrected for recovery which was considered quantitative.

Malathion--

Tissues were analyzed in the manner described for methoxychlor except that malathion eluted from the florisil column quantitatively in the 1:1 ethyl ether-in-petroleum ether fraction. An aliquot of the extract was analyzed by gas chromatography under the following operating conditions:

Instrument--Perkin-Elmer Model 3920 gas chromatograph

Detectors--Electron capture with 15 millicuries of Ni63 and nitrogen/phosphorus thermionic detection

Effluent Splitter--10 parts to N/P and 3 parts to ECD

Column--2 m X 2 mm ID glass packed with 3% Dexsil 300 GC on 80/100 mesh DMHS-treated Supelcoport

Gas flows--36 cc N_2/min. carrier, 7 cc N_2/min. and 100 cc air/min. to the N/P detector

Temperatures--Injection port: 250°C
Column: 220°C
Transfer line and splitter: 265°C

Recorder--Leeds & Northrup dual pen, 0-1 mV range, 1.0 cm/min. chart speed

Response--0.30 ng of malathion gave half-scale recorder pen deflection using the N/P detector at an attenuation of 16 X 1. Retention time was 2.0 min.

Three whole fish, weighing approximately 1 g each, were spiked with 100 ng of malathion and were analyzed by the above method. The average percentage recovery of malathion was 103± 6.8%. The analytical results were not corrected for recovery which was considered quantitative.

Carbofuran--

Results of previous research in which fish were continuously exposed to radiolabeled C14 carbofuran for 28 days indicated that the maximum tissue concentrations were reached within 3-10 days, after which an equilibrium concentration was observed. A concentration factor of 5-20X was calculated (FMC, 1976). In view of the rapid equilibrium, the low concentration factor, and the absence of a routine gas-chromatographic analytical method for fish tissues, fish from the carbofuran chronic test were not analyzed for residues.

STATISTICAL ANALYSES

In the acute tests, the LC50's and 95% confidence limits were calculated by linear regression analysis after probit transformation (Finney, 1971).

In the chronic tests, differences between treatments were determined by chi-square (X^2) and analysis of variance (Sokol and Rohlf, 1973). Differences were considered significant at the 95% ($P<0.05$) confidence level. Post-hoc tests were conducted on treatment means by using the Student-Newman-Keuls range test (Keuls, 1952).

SECTION 5

RESULTS AND DISCUSSION

CHEMICAL ANALYSES

Mean measured concentrations of methoxychlor in seawater were from 57-109% of nominal during the 96-hour test and from 45-55% of nominal during the chronic test (TABLE 1). Because this chlorinated hydrocarbon pesticide is "essentially insoluble in water (0.10 mg/ℓ @ 25°C)" (Anonymous, 1972) and because exposure to light and the addition of particulate matter and microorganisms hastens its degradation (Gardner and Bailey, 1975), these mean measured concentrations were within an expected and acceptable range.

TABLE 1. NOMINAL AND MEASURED CONCENTRATIONS OF METHOXYCHLOR DURING ACUTE AND CHRONIC EXPOSURES OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN FLOWING SEAWATER.

Test	Concentration (µg/ℓ)					
	Nominal	Measured				
		0 hour	96 hour	Mean	% of nom.	
Acute	Control	<0.01	<0.01	-	-	
	22	13	21	17	77	
	30	11	29	20	67	
	40	25	26	26	65	
	53	33	35	34	64	
	70	26	54	40	57	
	93	67	86	78	84	
	125	62	209	136	109	
		Mean	S.D.	Range	% of nom.	# samples
Chronic	Control	0.2	±0.5	0-2	-	18
	Sol. control	-[a]	-	-	-	-
	6	3	±2	1-7	50	15
	11	5	±4	2-18	45	15
	22	12	±7	4-30	55	15
	45	23	±19	9-85	51	15
	90	48	±10	34-53	53	4

[a]Not analyzed.

Malathion is readily soluble in water and mean measured concentrations during both the acute and chronic tests reflected this characteristic; concentrations were 72-143% of nominal (TABLE 2).

TABLE 2. NOMINAL AND MEASURED CONCENTRATIONS OF MALATHION DURING ACUTE AND CHRONIC EXPOSURES OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN FLOWING SEAWATER.

Test	Concentration (µg/ℓ)					
	Nominal	Measured				
		0 hour	96 hour	Mean	% of nom.	
Acute	Control	<0.1	<0.1	-	-	
	22	16	16	16	72	
	30	26	27	26	87	
	40	25	37	31	78	
	53	43	50	46	89	
	70	62	67	64	91	
	94	78	99	88	95	
	125	108	111	109	87	
		Mean	S.D.	Range	% of nom.	# samples
Chronic	Control	<0.1	-	-	-	19
	Sol. control	-[a]	-	-	-	-
	4	4	±2	1-6	100	20
	8	9	±4	4-17	112	20
	15	18	±6	8-28	120	20
	30	37	±12	20-57	123	10
	60	86	±15	70-101	143	3

[a]Not analyzed.

Mean measured concentrations of parent carbofuran were from 44-62% of nominal during the acute test and from 18-24% of nominal during the chronic test (TABLE 3). Evaluation of the analyses of water samples collected during days 1-30 of the chronic test shows that concentrations of parent carbofuran were approximately 40% of nominal. Thereafter, despite the use of a flowing-water exposure system, measured concentrations of parent material decreased to approximately 10% of nominal. Concurrently, however, we observed a pattern of increasing concentrations of hydrolyzed carbofuran derivitives (TABLE 4). The rapid decline of measured concentrations of parent material in the 4-day acute test and the stability of parent carbofuran in a stock solution over a 12-day period (confirmed by chemical analyses) is further evidence that carbofuran was degraded in seawater.

TABLE 3. NOMINAL AND MEASURED CONCENTRATIONS OF CARBOFURAN DURING ACUTE AND CHRONIC EXPOSURES OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN FLOWING SEAWATER.

Test	Concentration (µg/ℓ)					
	Nominal	Measured				
		0 hour	96 hour	Mean	% of nom.	
Acute	Control	<0.1	<0.1	-	-	
	420	230	120	175	44	
	560	380	270	325	58	
	750	480	220	350	47	
	1,000	640	280	460	46	
	1,300	860	760	810	62	
		Mean	S.D.	Range	% of nom.	# samples
Chronic	Control	<0.1	-	2-12	-	7
	Sol. Control	-[a]	-	-	-	-
	31	6	±4	2-12	19	7
	62	15	±11	1-29	24	10
	125	23	±21	1-65	18	12
	250	49	±44	2-150	20	12
	500	100	±93	20-270	20	10

[a]Not analyzed.

TABLE 4. ANALYSIS OF PARENT AND HYDROLYZED CARBOFURAN IN SEAWATER SAMPLES COLLECTED FROM THE NOMINAL CONCENTRATION OF 500 µg/ℓ DURING A 131-DAY EXPOSURE OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS).

Test Day	Parent Carbofuran (µg/ℓ;ppb)	Hydrolyzed Carbofuran[a]
12	200	<1.0
27	120	<1.0
34	210	6.1
41	79	<1.0
55	29	<1.0
62	37	2.1
70	28	9.5
76	38	10.0
84	85	17.0
105	20	18.1

[a]The percentage recovery of the hydrolyzed carbofuran from water is unknown. Therefore, these values are relative to each other.

Our justification for and methodology of determining the hydrolyzed products of carbofuran are as follow:

Carbofuran and 3-hydroxycarbofuran were completly hydrolyzed within 24 hours when saturated solutions were made pH 9.2 and stored at 25°C (FMC, 1969). Similar to the base-catalyzed degradation of Sevin® to 1-hydroxynaphthol, carbofuran was expected to eliminate the methylcarbamate group to form an hydroxy-sub-stituent at the benzyl-oxygen. Therefore, an analytical procedure featuring derivatization of 1-naphthol was utilized to verify any degradation products of carbofuran which possess active hydroxy-substituents. Parent carbofuran is not detected by this analyses since it does not contain the reactive hydroxy substituent.

Approximately 2 g of carbofuran were added to 300 mℓ of normal sodium hydroxide and heated to 60°C for six hours. The solution was cooled, hydrochloric acid was added to adjust the solution to pH 4.0, and the hydrolyzed carbofuran was extracted into methylene chloride. The solvent was evaporated and a portion of the hydrolyzed carbofuran was weighed and dissolved in benzene to produce the hydrolyzed carbofuran working standard solution.

An analytical procedure (U. S. Environmental Protection Agency, 1974) designed for the gas chromatographic determination of 1-naphthol in urine, following derivatization with chloroacetic anhydride, was utilized to verify the hydrolysis of carbofuran. Working standards of hydrolyzed carbofuran were derivatized and chromatographed under operating conditions previously described (except by using electron capture detection) with the following results:

Weight of hydrolyzed Carbofuran/7 mℓ benzene (µg)	Peak height response (mm)	
	Retention time = 3.0 min.	Retention time = 4.2 min.
0	4	3
1	5	9.5
2	6	18
5	15	41
10	31	116

An aliquot of the seawater sample extracts (see TABLE 4) was derivatized and the gas chromatograms were examined for the presence of quantity of hydrolyzed carbofuran. A graph of peak height versus weight of hydrolyzed carbofuran was constructed,

by using the peak eluting in 4.2 minutes, and any hydrolyzed carbofuran found was determined with the graph. The data are presented as relative concentrations found in the seawater since the extraction efficiency of the hydrolyzed carbofuran moiety from the seawater was unknown.

The concentrations of hydrolyzed carbofuran in seawater continued to increase during the test period. It is important to note that sample extracts were not treated with an aqueous base, but were derivatized directly. Therefore, any hydrolysis product of carbofuran detected was extracted from the seawater sample. Additionally, parent carbofuran working standards survived the derivatization procedure virtually unchanged and the peaks at 3.0 and 4.2 minutes due to hydrolyzed carbofuran were not observed.

ACUTE TOXICITY

The acute toxicity of methoxychlor and malathion to sheepshead minnows was similar; carbofuran was one order of magnitude less toxic (TABLE 5).

TABLE 5. ACUTE TOXICITY OF THREE PESTICIDES TO SHEEPSHEAD MINNOWS (*CYPRINODON VARIEGATUS*) EXPOSED IN FLOWING SEAWATER. SEAWATER FOR THE METHOXYCHLOR AND MALATHION TESTS WAS NATURAL; THAT FOR THE CARBOFURAN TEST WAS SYNTHETIC. CALCULATIONS WERE BASED ON MEASURED CONCENTRATIONS OF EACH PESTICIDE.

Compound	96-hour LC50, µg/ℓ	95% confidence limits, µg/ℓ	Salinity (°/oo)	Temperature (°C)
Methoxychlor	49	37-65	23	30
Malathion	51	41-63	20	29
Carbofuran	386	311-480	21	22

The acute toxicity of methoxychlor to sheepshead minnows tested under dynamic conditions was within the range reported for other estuarine fishes under static conditions, where estimated 96-hour LC50's ranged from 12-150 µg/ℓ. In static tests with two cyprinodontid fishes, Eisler (1970) estimated 96-hour LC50 values of 30 and 36 µg/ℓ for the striped killifish (*Fundulus majalis*) and mummichog (*F. heteroclitus*), respectively.

In flowing water tests, malathion was more acutely toxic to sheepshead minnows than to the freshwater fathead minnow (96-hour LC50 9,000 µg/ℓ) (Mount and Stephan, 1967) or bluegill

(96-hour LC50 108 µg/ℓ) (Eaton, 1970). Similarly, sheepshead minnows were more sensitive than were all but one of the seven estuarine fishes tested under static conditions by Eisler (1970), including the striped killifish and mummichog, for which the 96-hour LC50's were 250 and 240 µg/ℓ, respectively.

Carbofuran was of the same order of toxicity to sheepshead minnows as to three freshwater fishes tested under static conditions. Reported 96-hour TLM's (median tolerance limits; same as LC50) for rainbow trout (Salmo gardneri), channel catfish (Ictalurus punctatus), and bluegill were 280, 210, and 240 µg/ℓ, respectively (Anonymous, 1971).

CHRONIC TOXICITY

Methoxychlor

Methoxychlor affected parental fish in the 140-day study. Exposure to 48 µg/ℓ was lethal to 100% of the fish in one duplicate after 10 days and after 15 days in the other. Mortality of fish exposed to 23 µg/ℓ was significantly greater than mortality of control fish (TABLE 6).

TABLE 6. PERCENTAGE MORTALITY OF PARENTAL SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO METHOXYCHLOR IN FLOWING SEAWATER. MORTALITY IS THE AVERAGE FROM DUPLICATE AQUARIA AND DOES NOT INCLUDE DEATHS WHICH OCCURRED IN THE SPAWNING CHAMBERS.

	Concentration (µg/ℓ)						
Day	Control	Solvent Control	3	5	12	23	48
1-30	0	0	0	2	0	10	100
31-60	0	0	0	0	0	8	-
61-90	0	0	0	0	0	2	-
91-120	0	0	0	0	0	0	-
121-140	0	0	0	0	0	0	-
Total	0	0	0	2	0	20[a]	100[a]

[a]Significantly different from the control.

Growth of parental fish exposed to methoxychlor was not significantly different from growth of control fish. Although growth was monitored biweekly, only measurements at the beginning, middle, and end of the exposure are presented (TABLE 7).

TABLE 7. GROWTH OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 140 DAYS TO METHOXYCHLOR IN FLOWING, NATURAL SEAWATER. MEAN STANDARD LENGTH AND STANDARD DEVIATION ARE GIVEN IN CENTIMETERS AND WERE DETERMINED PHOTOGRAPHICALLY. AVERAGE WEIGHT IS GIVEN IN GRAMS AND WAS DETERMINED IN WATER.

Concentration (µg/ℓ)	Day 0		Day 58		Day 140	
	Length (cm)	Wt. (g)	Length (cm)	Wt. (g)	Length (cm)	Wt. (g)
Control	1.4±0.2	0.1	2.9±0.2	0.6	3.7±0.5	1.3
Sol. control	1.4±0.2	0.1	2.8±0.3	0.6	3.6±0.4	1.2
3	1.4±0.2	0.1	2.8±0.3	0.3	3.5±0.5	1.2
5	1.4±0.2	0.1	2.7±0.3	0.5	3.5±0.4	1.0
12	1.5±0.2	0.1	2.8±0.3	0.6	3.5±0.4	1.2
23	1.5±0.3	0.1	2.8±0.4	0.7	3.7±0.4	1.5
48	1.5±0.2	0.1	-[a]	-	-	-

[a]All fish had died.

Fecundity (total eggs spawned) of exposed fish was not significantly different from that of control fish (TABLE 8). Because female fish were killed by male fish in spawning chambers in all treatments except 23 µg/ℓ, we calculated eggs per female spawning day. These values were obtained by dividing the number of eggs obtained from a spawning chamber during a 24-hour period by the number of live female fish in the spawning chamber during the same period. There was no significant difference between eggs per female spawning day in any treatment because of variability within the treatments, but fewer eggs were spawned per female spawning day by fish exposed to 5, 12, and 23 µg/ℓ than were spawned by control fish (TABLE 9).

No female fish were killed by male fish in spawning chambers in 23 µg/ℓ, although 1 to 6 females were killed in spawning chambers in lower concentrations and controls. Based on observations of fish in the spawning chambers, a probable reason is that exposure to 23 µg/ℓ of methoxychlor decreased aggressive spawning activity of male fish.

Hatching success of fry from eggs spawned by fish exposed to 23 µg/ℓ of methoxychlor was significantly less than hatching success of control fry (TABLE 10).

Neither fry mortality to 28 days posthatch nor growth of the fry was significantly affected by exposure to methoxychlor (TABLE 11).

TABLE 8. NUMBER OF EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO METHOXYCHLOR IN FLOWING, NATURAL SEAWATER DURING THREE 10-DAY SPAWNING PERIODS. FIVE UNSPAWNED FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN EACH DUPLICATE AQUARIUM, A AND B.

	Concentration (µg/ℓ)											
Day	Control		Solvent Control		3		5		12		23	
Day	A	B	A	B	A	B	A	B	A	B	A	B
54 to 63	443	253[a]	244[a]	809	746	921	49	575	162	399	91	5
101 to 110	778	514	86[a]	1,183	322[a]	991	476	277[a]	94	384	189	570
116 to 125	342[a]	413[a]	367[a]	934	528	793	563[a]	829	517	163[a]	38	1,085
SUB-TOTAL	1,563	1,180	697	2,926	1,596	2,705	1,088	1,681	773	946	318	1,660
TOTAL	2,743		3,623		4,301		2,769		1,719		1,978	

[a]Deaths occurred in spawning chamber.

TABLE 9. NUMBER OF EGGS SPAWNED PER DAY PER FEMALE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) EXPOSED TO METHOXYCHLOR IN FLOWING, NATURAL SEAWATER DURING THREE 10-DAY SPAWNING PERIODS. FIVE UNSPAWNED FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN EACH DUPLICATE AQUARIUM, A AND B.

Day	Concentration (µg/ℓ)											
	Control		Solvent Control		3		5		12		23	
	A	B	A	B	A	B	A	B	A	B	A	B
54-63	15	11	12	27	25	31	2	19	5	13	3	0
101-110	26	17	5	39	11	38	16	11	3	13	6	19
116-125	16	15	12	31	18	26	20	28	17	8	1	26
Mean of duplicate	19	14	10	32	18	32	13	19	8	11	3	18
Mean of treatment	17±5		21±13		25±9		16±9		10±5		11±14	

TABLE 10. HATCHING SUCCESS OF FRY FROM EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO METHOXYCHLOR IN FLOWING, NATURAL SEAWATER. MEAN PERCENTAGE HATCH AND STANDARD DEVIATION REPRESENTS POOLED DATA FROM DUPLICATE AQUARIA DURING THREE 10-DAY SPAWNING PERIODS.

Concentration (µg/ℓ)	Mean percentage hatch and S.D.	Numbers of eggs examined
Control	98±3	1,200
Sol. control	98±4	2,040
3	98±3	2,400
5	95±6	1,450
12	97±2	700
23	73±18[a]	1,055

[a]Significantly different from the control.

TABLE 11. PERCENTAGE MORTALITY, AVERAGE STANDARD LENGTH, AND WEIGHT (DETERMINED IN WATER) OF 28-DAY OLD SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) FRY HATCHED FROM EGGS SPAWNED BY FISH EXPOSED TO METHOXYCHLOR FOR 54-63 DAYS.

Concentration (µg/ℓ)	Number of fry	Mortality (%)	Length (cm)	Weight (g)
Control	160	0	1.4±0.1	0.07
Sol. control	160	0	1.4±0.1	0.05
3	160	1	1.4±0.1	0.07
5	80	0	1.3±0.1	0.06
12	120	4	1.4±0.1	0.06
23	59	4	1.5±0.2	0.06

Methoxychlor was accumulated by adult fish exposed continuously for 140 days. The pesticide was also accumulated in eggs spawned by these fish. Accumulation was dependent upon water concentration during exposure (TABLE 12). Concentration factors (based on measured water concentrations) ranged from 113-264. These values are much lower than concentration factors for other chlorinated hydrocarbon pesticides and marine fishes (TABLE 13). A maximum concentration of 1.1 µg/g was detected in eggs spawned by fish exposed to 12 µg/ℓ.

TABLE 12. CONCENTRATIONS OF METHOXYCHLOR IN SURVIVING ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 140 DAYS. MEAN TISSUE RESIDUES ARE WHOLE-BODY, WET-WEIGHT. DUPLICATE ANALYSES OF EACH POOLED SAMPLE (AT LEAST 2 FISH PER SAMPLE) WERE PERFORMED.

Concentration		Concentration factor	Number of samples
Water (µg/ℓ)	Tissue (µg/g)		
Control	<0.1	-	4
Sol. control	<0.1	-	4
3	0.34±0.24	113	6
5	1.32±0.24	264	6
12	1.38±0.25	115	6
23	3.18±0.53	138	6

TABLE 13. ACCUMULATION OF ORGANOCHLORINE PESTICIDES BY MARINE FISHES. CONCENTRATION FACTORS WERE DERIVED BY DIVIDING CONCENTRATIONS IN FISH (WHOLE-BODY, WET-WEIGHT) BY CONCENTRATIONS IN TEST WATER.

Pesticide	Fish	Concentration factor (maximum)	Exposure (days)	Source
DDT	Atlantic croaker	16,300[a]	21-35	Hansen and Wilson, 1970
	Pinfish	40,000[a]	14	
Dieldrin	Spot	6,700[b]	35	Parrish et al., 1973
Endrin	Sheepshead minnows	4,800[b]	33	Schimmel et al., 1975

[a]Based on nominal water concentration.
[b]Based on measured water concentration.

Malathion

Malathion affected survival of parental fish but did not affect their growth or fecundity (TABLES 14-17). Exposure to 86 µg/ℓ was lethal to 100% of the fish in one duplicate and 95%

in the other after 5 days; exposure to 37 µg/ℓ was lethal to 100% in one duplicate and 80% in the other after 30 days. Exposure to 18 µg/ℓ was lethal to 50% of the fish in both duplicates after 86 days. No deaths occurred in any concentration or control after day 90 of the 140-day study.

TABLE 14. PERCENTAGE MORTALITY OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO MALATHION IN FLOWING, NATURAL SEAWATER FOR 140 DAYS. MORTALITY IS THE AVERAGE FROM DUPLICATE AQUARIA AND DOES NOT INCLUDE DEATHS WHICH OCCURRED IN THE SPAWNING CHAMBERS.

	Concentration (µg/ℓ)						
Day	Control	Solvent Control	4	9	18	37	86
1-30	0	0	0	0	5	90	100
31-60	0	0	0	0	23	8	-
61-90	2	0	5	0	22	2	-
91-120	0	0	0	0	0	-	-
121-140	0	0	0	0	0	-	-
Total	2	0	5	0	50[a]	100[a]	100[a]

[a]Significantly different from the control.

TABLE 15. GROWTH OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 140 DAYS TO MALATHION IN FLOWING, NATURAL SEAWATER. MEAN STANDARD LENGTH AND STANDARD DEVIATION ARE GIVEN IN CENTIMETERS AND WERE DETERMINED PHOTOGRAPHICALLY. AVERAGE WEIGHT IS GIVEN IN GRAMS AND WAS DETERMINED IN WATER.

Concentration	Day 0		Day 61		Day 140	
(µg/ℓ)	Length	Wt.	Length	Wt.	Length	Wt.
Control	1.4±0.2	0.1	2.8±0.4	0.7	4.1±0.4	1.4
Sol. control	1.3±0.2	0.1	2.7±0.6	0.8	4.1±0.5	1.4
4	1.4±0.2	0.2	2.7±0.3	0.7	3.9±0.4	1.2
9	1.3±0.2	0.1	2.8±0.3	0.7	3.9±0.4	1.3
18	1.3±0.2	0.1	2.6±0.4	0.6	3.7±0.3	-[a]
37	1.4±0.2	0.1	2.4±0.2	0.5	-[b]	-
86	1.4±0.2	0.1	-[b]	-	-	-

[a]All fish were used for acetylcholinesterase inhibition analyses.
[b]All fish had died.

TABLE 16. NUMBER OF EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO MALATHION IN FLOWING, NATURAL SEAWATER DURING TWO 10-DAY SPAWNING PERIODS. FIVE UNSPAWNED FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN EACH DUPLICATE AQUARIUM, A AND B.

Day	Concentration (µg/ℓ)									
	Control		Solvent Control		4		9		18	
	A	B	A	B	A	B	A	B	A	B
87-96	154[a]	747[a]	777	626	422	445	341[a]	481[a]	299	353
119-128	538	258	364	325	535	729	738	390	-[b]	374[a]
SUBTOTAL	692	1,005	1,141	951	957	1,174	1,079	871	299	727
TOTAL	1,697		2,092		2,131		1,950		1,026	

[a]Death(s) occurred in spawning chamber.
[b]No spawning chamber; 50% mortality had occurred.

TABLE 17. NUMBER OF EGGS SPAWNED PER DAY PER FEMALE SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) EXPOSED TO MALATHION IN FLOWING, NATURAL SEAWATER DURING TWO 10-DAY SPAWNING PERIODS. FIVE UNSPAWNED FISH (2 MALES AND 3 FEMALES) WERE PLACED IN A SPAWNING CHAMBER IN EACH DUPLICATE AQUARIUM, A AND B.

Day	Concentration (µg/ℓ)									
	Control		Solvent Control		4		9		18	
	A	B	A	B	A	B	A	B	A	B
87-96	11	30	26	21	14	15	16	21	10	21
119-128	18	9	12	11	18	24	25	13	-[a]	19
Average of duplicate	14	20	19	16	16	20	20	17	10	16
Mean of treatment	17±9		18±7		18±4		19±5		14±5	

[a]No spawning chamber; 50% mortality had occurred.

Malathion did not affect hatching success of fry from eggs

spawned (TABLE 18) but did affect the fry. Mortality of fry hatched from eggs spawned by fish exposed to 9 and 18 µg/ℓ of malathion was significantly greater than mortality of control fry. Growth of surviving fry was not affected (TABLE 19).

TABLE 18. HATCHING SUCCESS OF FRY FROM EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO MALATHION IN FLOWING, NATURAL SEAWATER. MEAN PERCENTAGE HATCH AND STANDARD DEVIATION REPRESENTS POOLED DATA FROM DUPLICATE AQUARIA DURING TWO 10-DAY SPAWNING PERIODS.

Concentration (µg/ℓ)	Percentage hatch	Number of eggs examined
Control	97±5	850
Sol. control	99±2	700
4	97±3	1,000
9	97±2	900
18	96±6	350

TABLE 19. PERCENTAGE MORTALITY, AVERAGE STANDARD LENGTH, AND WEIGHT (DETERMINED IN WATER) OF 28-DAY OLD SHEEPSHEAD MINNOW (CYPRINODON VARIEGATUS) FRY HATCHED FROM EGGS PRODUCED BY FISH EXPOSED TO MALATHION FOR 87-96 DAYS.

Concentration (µg/ℓ)	Number of fry	Mortality (%)	Length (cm)	Weight (g)
Control	160	7	1.0±0.2	0.04
Sol. control	160	10	-[a]	0.03
4	160	9	1.1±0.2	0.04
9	160	14[b]	1.2±0.2	0.04
18	120	15[b]	1.2±0.1	0.03

[a]No data.
[b]Significantly different from the control.

Malathion was not detectable (<0.1 µg/g) in fish sampled at the end of the 140-day study. This is not surprising because fish readily convert malathion to the mono- and dicarboxylic acids of malathion (Cook and Moore, 1976).

Carbofuran

Carbofuran affected survival of parental fish exposed to the pesticide for 131 days. Exposure to 100 µg/ℓ was lethal to

100% of the fish in one duplicate and 95% in the other after 14 days. Mortality of fish exposed to 49 ppb was significantly greater than mortality of control fish after 30 days of exposure (TABLE 20).

TABLE 20. PERCENTAGE MORTALITY OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO CARBOFURAN IN FLOWING, SYNTHETIC SEAWATER. MORTALITY IS THE AVERAGE FROM DUPLICATE AQUARIA AND DOES NOT INCLUDE DEATHS FROM SPAWNING ACTIVITY.

	Concentration (µg/ℓ)					
Day	Control	6	15	23	49	100
1-30	0	5	2	5	40	100
31-60	5	5	8	10	10	-
61-90	2	0	5	0	0	-
91-131	0	0	0	0	0	-
Total	7	10	15	15	50[a]	100[a]

[a]Significantly different from the control.

Carbofuran did not significantly affect growth of parental fish or number of eggs spawned in any concentration (TABLES 21-22).

TABLE 21. GROWTH OF SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED FOR 131 DAYS TO CARBOFURAN IN FLOWING, SYNTHETIC SEAWATER. MEAN STANDARD LENGTH AND STANDARD DEVIATION ARE GIVEN IN CENTIMETERS AND WERE DETERMINED PHOTOGRAPHICALLY. AVERAGE WEIGHT IS GIVEN IN GRAMS AND WAS DETERMINED IN WATER.

Concentration (µg/ℓ)	Day 2 Length	Day 60 Length	Day 131 Length	Day 131 Weight
Control	2.9±0.4	3.8±0.3	4.1±0.3	1.15
6	3.8±0.5	3.6±0.4	4.0±0.2	1.10
15	3.1±0.4	3.6±0.3	3.9±0.3	1.16
23	2.8±0.4	3.2±0.4	3.8±0.3	0.92
49	2.8±0.5	3.3±0.3	3.7±0.5	1.05
100	3.0±0.5	-[a]	-	-

[a]All fish had died.

TABLE 22. NUMBER OF EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO CARBOFURAN IN FLOWING, SYNTHETIC SEAWATER. ALL POSSIBLE PAIRS OF FISH IN EACH DUPLICATE AQUARIUM WERE SPAWNED.

Concentration (µg/ℓ)	Number of eggs		
	Replicate A	Replicate B	Total
Control	650	449	1,099
6	854	852	1,706
15	848	1,295	2,143
23	475	820	1,295
49	248	154	402[a]

[a]Fifty percent mortality had occurred.

Hatching success of fry from eggs spawned by fish exposed to 49 µg/ℓ was significantly less than hatching success of control fry (TABLE 23).

TABLE 23. HATCHING SUCCESS OF FRY FROM EGGS SPAWNED BY SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) EXPOSED TO CARBOFURAN IN FLOWING, SYNTHETIC SEAWATER. MEAN PERCENTAGE HATCH AND STANDARD DEVIATION REPRESENTS POOLED DATA FROM SPAWNING PAIRS IN DUPLICATE AQUARIA.

Concentration (µg/ℓ)	Percentage hatch	Number of eggs examined
Control	98±3	450
6	99±3	700
15	96±10	937
23	98±2	609
49	86±10[a]	233

[a]Significantly different from the control.

Mortality of fry hatched from eggs spawned by fish exposed to 23 and 49 µg/ℓ was significantly greater than mortality of control fry. Growth of surviving fry in all concentrations was not affected, however (TABLE 24).

TABLE 24. PERCENTAGE MORTALITY AND AVERAGE STANDARD LENGTH OF 30-DAY OLD SHEEPSHEAD MINNOW (CYRPINODON VARIEGATUS) FRY WHICH WERE HATCHED FROM EGGS SPAWNED BY FISH EXPOSED TO CARBOFURAN FOR 42-95 DAYS IN FLOWING, SYNTHETIC SEAWATER. MORTALITY AND LENGTH ARE AVERAGES OF FOUR GROUPS OF 40 FRY EXCEPT AS NOTED.

Concentration (µg/ℓ)	Number of fry	Mortality (%)	Length (cm)
Control	160	5	1.4
6	160	2	1.3
15	160	8	1.3
23	120	12[a]	1.4
49	80	41[a]	1.4

[a]Significantly different from the control.

APPLICATION FACTORS

Application factors were calculated from the results of the acute and chronic toxicity tests (TABLE 25).

TABLE 25. CONCENTRATIONS (µg/ℓ) OF THREE PESTICIDES TOXIC TO SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) IN ACUTE AND CHRONIC TESTS, AND THE RELATIONSHIP OF ACUTE TOXICITY TO CHRONIC TOXICITY.

Pesticide	96-hour LC50 (95% confidence limits)	MATC limits	Application factor limits[a]
Methoxychlor	49 (37-65)	>12<23	0.24-0.47
Malathion	51 (41-63)	>4<9	0.08-0.18
Carbofuran	386 (311-480)	>15<23	0.04-0.06

[a]Derived by dividing the Maximum Acceptable Toxicant Concentration limits by the 96-hour LC50.

We find no application factors in the literature on which to base a comparison of the sensitivity of sheepshead minnows and any freshwater fish to methoxychlor or carbofuran. For

malathion, however, studies by Mount and Stephan (1967) with fathead minnows and Eaton (1970) with bluegill show that the application factors derived for all three fishes were similar (TABLE 26).

TABLE 26. COMPARISON OF ACUTE AND CHRONIC MALATHION TOXICITY TO TWO FRESHWATER FISHES AND A SALTWATER FISH.

	Fathead minnows[a]	Bluegills[b]	Sheepshead minnows
96-hour LC50 (µg/ℓ)	9,000	108	51
MATC limits (µg/ℓ)	200-580	4-7	4-9
Application factors limits	0.02-0.06	0.04-0.06	0.08-0.18

[a]From Mount and Stephan, 1967.
[b]From Eaton, 1970.

SUMMARY

1. Carbofuran was less toxic to sheepshead minnows than were methoxychlor and malathion in acute tests. Estimated 96-hour LC50's, based on average measured concentrations in water, were 386, 49, and 51 µg/ℓ, respectively.

2. All three pesticides killed parental fish in concentrations <50 µg/ℓ during chronic tests, and the lowest concentrations of the pesticides in which toxic effects were observed were similar (TABLE 27).

3. The life stages of progeny from exposed parental fish that were sensitive to each pesticide were: methoxychlor--embryo; malathion--fry; and carbofuran--embryo and fry.

4. The relationship of acute toxicity and chronic toxicity for sheepshead minnows exposed to malathion (as expressed by application factors) was similar to that for two freshwater fishes exposed to malathion.

5. Sheepshead minnows are a suitable estuarine fish for toxicity tests which include the reproductive portion of the life cycle and the first generation.

TABLE 27. SUMMARY OF SIGNIFICANT EFFECTS OF METHOXYCHLOR, MALATHION, AND CARBOFURAN ON SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS) DURING CHRONIC EXPOSURES IN FLOWING SEAWATER.

	Generation	Life stage	Effect	Measured concentration (µg/ℓ)
Methoxychlor	Parental	Adult	Death	≥23
	F_1	Embryo	Decreased hatch	23
Malathion	Parental	Adult	Death	≥18
	F_1	Fry	Increased mortality	9 and 18
Carbofuran	Parental	Adult	Death	≥49
	F_1	Embryo	Decreased hatch	49
		Fry	Increased mortality	23

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16. ABSTRACT

Sheepshead minnows (Cyprinodon variegatus) were exposed to each of three pesticides--methoxychlor, malathion, and carbofuran--in flowing seawater to determine the acute and chronic (partial life-cycle) effects.

Mortality of adult fish exposed to concentrations of methoxychlor ≥23 µg/ℓ and hatching success of fry from eggs spawned by fish exposed to 23 µg/ℓ were significantly different from the control. The maximum acceptable toxicant concentration (MATC) was estimated to be >12<23 µg/ℓ; application factor (AF) limits were 0.24-0.47.

Mortality of adult fish exposed to concentrations of malathion ≥18 µg/ℓ and mortality of fry hatched from eggs spawned by fish exposed to 9 and 18 µg/ℓ were significantly different from the control. The MATC was estimated to >4<9 µg/ℓ; AF limits were 0.08-0.18.

Mortality of adult fish exposed to concentrations of carbofuran ≥49 µg/ℓ, hatching success of fry from eggs spawned by fish exposed to 49 µg/ℓ, and mortality of fry hatched from eggs spawned by fish exposed to 23 and 49 µg/ℓ were significantly different from the control. The MATC was estimated to >15<23 µg/ℓ; AF limits were 0.04-0.06.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
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