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ENDRIN: EFFECTS ON SEVERAL ESTUARINE ORGANISMS[1]

by

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ABSTRACT

Acute (96-hour) bioassays were performed with endrin and the following estuarine organisms: American oyster (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sailfin molly (*Poecilia latipinna*) and sheepshead minnow (*Cyprinodon variegatus*). Endrin was acutely toxic to all organisms tested, except oysters, whose shell growth was appreciably inhibited by 56 ug/l (parts per billion) of the chemical. Pink shrimp were the most sensitive animal tested, but significant numbers of both species of shrimps and fishes died when exposed to concentrations of one ug/l or less. In a separate test, embryos and fry of the sheepshead minnow were exposed to concentrations of endrin ranging from 0.046 to 1.0 ug/l (nominal) for 33 days in an intermittant-flow bioassay. Embryos were not affected by the concentrations to which they were exposed, but the estimated LC50 (probit analysis, a=.05) of fry was 0.27 ug/l.

INTRODUCTION

Widespread use of the organochlorine insecticide, endrin, has prompted numerous investigations to determine the effects of this compound on aquatic organisms. Several studies involving marine organisms have shown that endrin is acutely toxic at low levels. Eisler (1969) found endrin acutely toxic to sand shrimp (*Crangon septemspinosa*), grass shrimp (*Palaemonetes vulgaris*), and hermit crabs (*Pagurus longicarpus*). The 96-hour LC50's were 1.7 ug/l, 1.8 ug/l, and 12 ug/l, respectively. Davis and Hidu (1969) assessed the effects of endrin on oysters by (1) determining the number of fertilized eggs that developed into normal larvae after 48-hours exposure to a given concentration of endrin, and (2) observing survival and growth of larvae over a period of 12 days. At concentrations greater than 0.025 mg/l, endrin reduced the number of eggs developing, survival, and growth of larvae. Katz (1961) and Katz and Chadwick (1961) found the 96-hour LC50 of endrin to threespine stickleback (*Gasterosteus aculeatus*) ranges from 0.5 to 1.5 ug/l. Salinity had little effect on toxicity, but temperature markedly affected toxicity - the higher the temperature the greater the toxicity. Eisler and Edmunds (1966) found that acute exposure to sublethal concentrations (1 ug/l or less) of endrin impaired liver function in northern puffers (*Sphoeroides maculatus*).

Few data have been published concerning the effects of endrin on larval marine fishes. One such study by Johnson (1967) on the threespine stickleback (*Gasterosteus aculeatus*) demonstrated that endrin immobilizes hatching fry at 15.0 ug/l and

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produced behavioral changes in fry reared in water containing 2 and 5 ug/l.

Our study was conducted to determine (1) the 96-hour LC50 of endrin to pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*), and sailfin mollies (*Poecilia latipinna*), (2) the effect of endrin on shell growth of American oysters (*Crassostrea virginica*), and (3) the effect of endrin on egg fertility, hatching success of embryos, and survival of fry of the sheepshead minnow.

We thank Johnny Knight for his chemical analyses of water samples and Steven S. Foss for his work on the illustration.

MATERIALS AND METHODS

Test Animals

All test animals were collected near the Gulf Breeze Environmental Research Laboratory in Florida and acclimated to laboratory conditions for at least ten days before exposure. If mortality in a specific lot of animals exceeded 1% in the 48 hours immediately preceding the test or if abnormal behavior was observed during acclimation, the entire lot was discarded. Oysters tested were from 27 to 54 mm in height; pink shrimp, 39 to 70 mm rostrum-telson length; grass shrimp, 19 to 36 mm rostrum-telson length; sheepshead minnows, 15 to 25 mm standard length; and sailfin mollies, 36 to 49 mm standard length. Animals were not fed during acute toxicity tests, but they could obtain food (plankton and other particulate matter) from the unfiltered sea water in which they were maintained. Adult sheepshead minnows, 35 to 50 mm standard length were used to produce eggs used in studies of fertility, hatching success, and fry survival. Fry were fed daily, using live brine shrimp (*Artemia salina*) nauplii which contained no pesticide or polychlorinated biphenyl detectable by our gas chromatographic analysis.

96-Hour Test Conditions

Acute toxicity of endrin was determined by exposing 20 animals per aquarium (except 15 sailfin mollies per aquarium) to logarithmic concentrations for 96 hours. A 30-liter aquarium was used for each concentration. Technical grade endrin (96% active ingredient) was dissolved in reagent grade acetone and metered by Milroyal® pumps (Lowe, et al., 1972) at 60 ml/hr into unfiltered seawater that entered each aquarium at 150 l/hr. A control aquarium received the same quantity of water and solvent, but no endrin.

Effect of endrin was assessed by measuring reduction of shell growth of oysters (Butler, 1962) and by determining mortality in shrimps and fishes.

Sheepshead Minnow Embryo and Fry Test Conditions

The exposure apparatus used in the sheepshead minnow embryo and fry test was a modification of the dilutor designed by Mount and Brungs (1967). Each discharge of our dilutor delivered 500 ml of salt water and a constant concentration of carrier (polyethylene glycol 200) to control aquaria and to aquaria receiving each of five concentrations of toxicant. Another control aquarium received 500 ml of salt water without carrier. The Mount and Brungs apparatus consists of two tiers of dilution cells and a mixing chamber; in our apparatus, (Figure 1), we added a tier of three cells (F) situated above the W-cell series of Mount and Brungs. One of these cells (F-1) provided 500 ml of seawater without carrier directly to the exposure tank. One of the larger cells (F-2) delivered 2250 ml of seawater to the toxicant-mixing box (M-2); the other 2250 ml cell (F-3) to the carrier-mixing box (M-1) which discharged into the W-cell series. Two injectors with 50 ml syringes were used: one provided endrin in 0.018 ml of carrier per cycle to the toxicant-mixing box; the second provided an identical quantity of

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carrier without endrin to the carrier-mixing box. Both injectors were activated mechanically by the filling and draining of a bucket (not illustrated) situated below the excess water drain in the reservoir (R). A float switch in the bucket shut off the water pump (P) at the beginning of each cycle and opened it at the end of the cycle. Since volumes of carrier were equal in each injection, the same amount of polyethylene glycol 200 was administered to each exposure tank, regardless of the dilution. Excess water containing endrin was discarded prior to cycling of the dilutor.

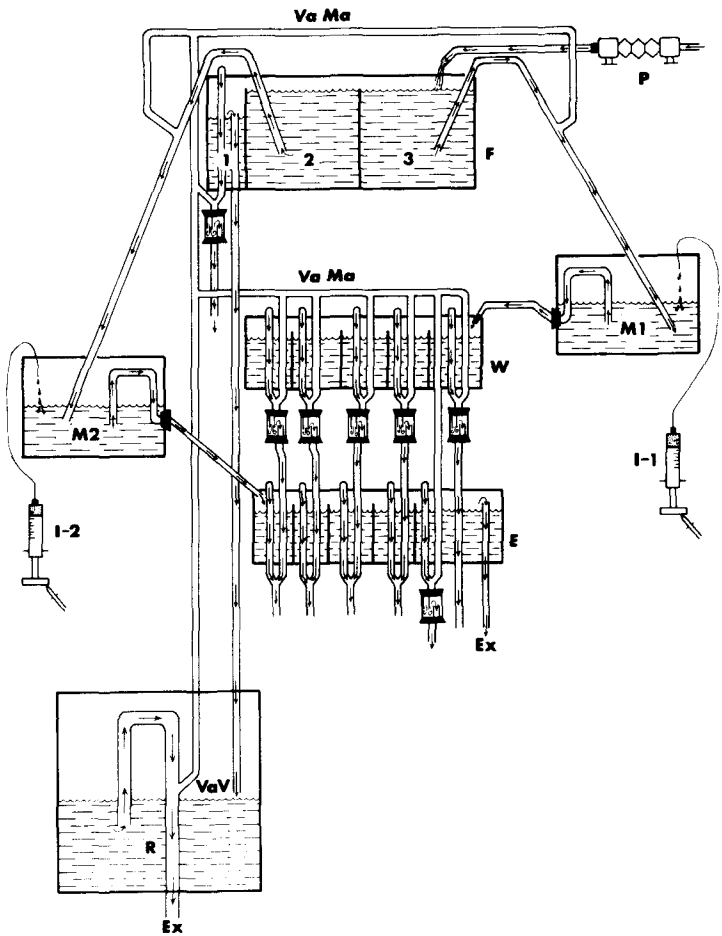

Figure 1. Exposure apparatus:
E - Endrin and seawater cells; Ex-Excess water; F - Filtered seawater cells; I-1 - carrier injector; I-2 - carrier and endrin injector; M-1 - carrier mixing box; M-2 - carrier and endrin mixing box; P - Seawater pump; R - Reservoir; Va Ma - vacuum manifolds; Va V - Vacuum venturi; W - Seawater with carrier.

Seawater used in this bioassay was pumped from Santa Rosa Sound, Florida through a swimming-pool sand filter and a 1 u-pore polypropylene filter into a constant head box in the laboratory. In the box, the water was heated to 30 C + 1 C while the salinity varied with that of the Sound water (15 to 28 o/oo, average 23.4 o/oo). The water was then pumped to the toxicant-delivery apparatus. Our dilutor cycled approximately 80 times each day, delivering 125 ml water to each of four 1200 ml exposure chambers per concentration per cycle.

Eggs of the sheepshead minnow were obtained and fertilized by procedures described by Schimmel *et al.* (1974). Twenty eggs were placed in 10-cm Petri dishes to which a 9 cm high nylon screen collar (0.5 mm mesh) was attached. This collar permitted water exchange while preventing escape of fry. Exposure of the embryos began one hour after fertilization and lasted for 33 days.

Concentrations of endrin were calculated to give 1.0, 0.46, 0.21, 0.1 and 0.046 ug of endrin/liter of water. Concentrations, measured weekly were typically within 35-60% of the intended concentration. Dissolved oxygen concentrations, determined weekly by the modified Winkler method of Strickland and Parsons (1968), were above 50% saturation and appeared adequate.

Chemical Analyses

Concentrations of endrin in water and animals were determined by electron-capture gas chromatography, using a 182 cm x 2mm ID glass column packed with 2% OV-101 on 100-120 mesh Gas Chrom Q. Nitrogen flow rate was 25 ml/min, the oven temperature was 190°C, and the injector and detector temperatures were 210°C. Recovery exceeded 85%; data were not adjusted for recovery. Sensitivity of this method was 0.004 ppb when using a 1-liter sample. Unfiltered water samples from each concentration and control were analyzed once during the acute 96-hour bioassays. In the study of sheepshead minnow fertility, hatching success, and survival, water samples from each concentration and controls were analyzed weekly. Whole-body concentrations in surviving animals were determined on wet weight basis.

Tissue samples that weighed more than 5 g. were prepared using the methods described by Lowe et al. (1972) except that endrin was eluted in the 15% (v/v) ethyl ether-in-petroleum ether fraction. Samples from 1-5 g. were analyzed using the semi-micro method described by Hansen et al. (1974) except that endrin was eluted in 20% (v/v) ethyl ether/hexane fraction, while those less than 1 g were analyzed by the micro method described in the Pesticide Analytical Manual, Volume III (U. S. Food and Drug Administration, 1970). Sensitivity of each method was 0.010 ppm when using a 1 gram sample.

Statistical Analyses

Data from the 96-hour oyster shell growth study were analyzed by linear regression; shrimp and fish mortality data were analyzed by the probit method of Litchfield and Wilcoxon (1949). None of the bioassay results were rejected by the Chi-square test for variation.

Data from the study of sheepshead minnow fertility, hatching success and survival were analyzed by the Chi-square test (α =0.01) to determine differences in mortality of experimental and control animals and probit analysis to determine the LC50 (α =0.05).

RESULTS AND DISCUSSION

Acute (96-hour) Exposures

With the exception of oysters, endrin was acutely toxic to all organisms tested (Tables 1 and 2). Pink shrimp were the most sensitive, but significant numbers of both species of shrimps and fishes died when exposed to concentrations of 1 ug/l (part per billion) or less. Shell growth in oysters was appreciably inhibited by exposure to a concentration of 56 ug/l (32 ug/l measured) for 96 hours. Although the LC50 of *Palemonetes pugio* (0.73 ug/l) in our tests compares favorably with Eisler's (1969) data for *P. vulgaris* (1.8 ug/l), we found that the shrimp, *Penaeus duorarum* had an LC50 of 0.049 ug/l. This concentration is about 9 times greater than the lower limit of analytical detectibility of endrin in a 1.0 l water sample.

Table 1. Acute toxicity of endrin to and uptake by American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*), and sailfin mollies (*Poecilia latipinna*) in relation to concentration of endrin in seawater during 96-hour exposures. Whole-body residues are from animals alive at end of exposure.

SPECIES	CONCENTRATION IN WATER (ug/l)		EFFECTb	WHOLE-BODY RESIDUE
	Intended	Measured	(%)	(ug/g, wet weight)
C. virginica	Control	NDa	0	NDa
	1.8	1.6	13	1.4
	5.6	4.9	40	5.8
	18.	13.	47	20.
	56.	32.	67	52.
	180.	168.	87	124.
P. duorarum	Control	NDa	0	NDa
	0.010	0.009	5	Trace
	0.032	0.023	30	0.025
	0.10	0.077	80	0.067
	0.32	0.28	100	--
	1.0	0.88	100	--
P. pugio	Control	NDa	0	NDa
	0.032	0.024	0	0.02
	0.1	0.081	5	0.07
	0.32	0.24	25	0.19
	1.0	0.96	60	0.02
	3.2	2.4	100	--
C. variegatus	Control	NDa	0	Trace
	0.010	0.019	0	0.013
	0.032	0.026	0	0.11
	0.10	0.10	0	0.30
	0.32	0.33	20	1.5
	1.0	0.95	100	--
P. latipinna	Control	NDa	7	0.013
	0.0135	0.012	0	0.035
	0.075	0.073	0	0.17
	0.135	0.150	0	0.26
	0.75	0.60	47	1.7
	1.35	1.20	100	--

aND = 0.004 ug/l in water, 0.010 ug/g in tissue.
bEffect is expressed as percentage reduction in shell growth for oysters and death for shrimps and fishes.

Table 2. Acute toxicity (EC50, 96-hr) of endrin to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), sheepshead minnows (*Cyprinodon variegatus*) and sailfin mollies (*Poecilia latipinna*). Effect is expressed as percentage reduction in shell growth for oysters and death for shrimps and fishes. Confidence limits (95%) are in parentheses.

SPECIES	96-HOUR EC50 (ug/l)		TEMPERATURE (°C)		SALINITY (o/oo)	
	Intended	Measured	Mean	Range	Mean	Range
C. virginica	19.1 (5.3-68.78)	14.2 (3.99-50.49)	22.0	19.0-24.0	29.3	27.0-30.5
P. duorarum	0.049 (0.034-0.070)	0.037 (0.025-0.053)	14.8	12.0-16.0	28.4	26.0-31.0
P. pugio	0.73 (0.40-1.32)	0.63 (0.35-1.15)	12.6	10.0-14.5	27.2	25.5-31.0
C. variegatus	0.40 (0.30-0.53)	0.38 (0.31-0.45)	17.4	16.5-19.0	19.5	14.0-22.0
P. latipinna	0.79 (0.60-1.05)	0.63 (0.47-0.84)	19.6	18.0-21.0	28.2	23.5-30.5

Endrin, at the concentrations tested, appeared to have no significant effect on fertility of sheepshead minnow eggs or survival of embryos (Table 3). The LC50 for fry, however, was estimated to be 0.267 ug/l, indicating that this is the most sensitive stage. Chi-square analysis of fry-survival data showed significant mortality in experimental groups exposed to 1.0 and 0.46 ug/l. In these concentrations, most fry died within one week of the time required for a 50% hatch (approximately 5 days for all concentrations). Observed signs of endrin poisoning were: flared opercles, erratic swimming, failure to feed, lethargic behavior and loss of equilibrium. Although the group exposed to 0.21 ug/l had no significant mortality, their lethargic behavior and loss of equilibrium was evidence of effect. Survival of embryos and fry in the control aquaria was 76% and that of the control with carrier was 75%. In whole fry from experimental groups, concentration factors (concentration of endrin in the fry divided by the concentration in the water) ranged from 3.3 to 4.8 x 10^3. No detectable concentrations (0.03 ug/g) were found in the controls.

Effects from the polyethylene glycol 200 carrier at the concentrations employed were not anticipated because none was observed in preliminary bioassays of this carrier. Static tests using a control and five concentrations of the carrier (11.2, 23.6, 51.8, and 112.6 g/liter of seawater) were conducted in separate tests on embryos and on newly-hatched fry. Results of the 7-day embryo test (30° C, 25 o/oo) indicate that the LC50 of polyethylene glycol 200 was 33.0 g/liter (Probit analyses; α =0.05). The estimated 96-hour LC50 for newly-hatched fry (24 hrs.) was 18.0 g/liter. (The extended duration of the embryo test allowed for observation of fry after hatching). Because high levels of polyethylene glycol were required to produce an effect, the effect may be the result of osmotic stress rather than direct toxicity of the carrier. The maximum concentration of carrier (112.6 g/liter) when mixed with 25 o/oo seawater is osmotically equivalent to 70 o/oo seawater. Maximum solvent concentration used in the study of sheepshead minnow fertilization, hatching success and survival was only 0.009 mg/liter of water.

Table 3. Relative susceptibility of various life stages of sheepshead minnows (*Cyprinodon variegatus*) to endrin in flow-through systems. Criteria are fertility of eggs and survival of embryos, fry and juveniles. Confidence limits (95%) are in parentheses.

LIFE STAGE	EXPOSURE (days)	LC50 (ug/l) Intended	Measured
Egg fertilizationa	0.25	No observable effect at 1.0 ug/l	
Embryos	5.0	No observable effect at 1.0 ug/l	
Fry	33.0	0.267(0.08-0.62)	0.158(0.00-0.57)
Juveniles	4.0	0.40 (0.30-0.53)	0.38 (0.31-0.45)

aStatic test.

Results of this study indicate that addition of endrin to an estuarine system, even at low concentrations, could adversely affect the estuarine communities. Of particular concern in the southeastern United States is the potential effect of low concentrations of endrin on penaeid shrimp populations since our study demonstrates that the LC50 measured for *Penaeus duorarum* (0.037 ug/liter) is only 9x the present limit of analytical detectability in water. Long-term studies exposing the shrimp to ultra-low concentrations of endrin (parts per trillion) could help establish "safe" levels of this compound in estuaries.

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EFFECT OF TWO FEEDING RATES ON PRODUCTION OF ADVANCED FINGERLING STRIPED BASS

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ABSTRACT

An investigation of the effect of two feeding rates on pond production of advanced fingerling striped bass was studied at the Auburn University Fisheries Research Unit from June 27 to November 24, 1972. The mean survival for fingerlings fed a high feeding rate was 71.87% as opposed to 70.13% for fingerlings fed a low feeding rate. Mean production for fingerlings fed a high and low feeding rate was 266.44 kg/ha and 293.68 kg/ha, respectively. Food conversion for fingerlings fed a high feeding rate was 3.74 as compared to 2.51 for fingerlings fed a low feeding rate.

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