# DIELDRIN: EFFECTS ON SEVERAL ESTUARINE ORGANISMS[1]

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## ABSTRACT

Tests were conducted to determine (1) the acute toxicity of dieldrin in flowing sea water to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus* duorarum), grass shrimp (*Palaemonetes pugio*) and sheepshead minnows (*Cyprinodon variegatus*) and (2) the rate of dieldrin uptake and depuration by spot (*Leiostomus xanthurus*). Acute (96-hour) EC50's were: oysters, 12.5 ug/l; pink shrimp, 0.9 ug/l; grass shrimp, 11.4 ug/l; and sheepshead minnows 23.6 ug/l. Spot exposed to 0.0135, 0.075, 0.135, 0.75 or 1.35 ug/l for 35 days accumulated the chemical with maximum concentrations attained in 11 to 18 days. Maximum whole-body residue (wet-weight) was 6,000X the concentration in test water. Spot contained no detectable dieldrin residues at the end of a 13-day depuration period in dieldrin-free water. Tissue alterations, such as subepithelial edema in gill lamellae and severe lysis and sloughing of the small intestine epithelium, occurred in spot exposed to 1.35 ug/l for four days.

## INTRODUCTION

The effects of dieldrin on estuarine organisms were investigated because this toxicant is present in most of this nation's estuaries. Dieldrin was the second most commonly detected organochlorine compound in molluscs from 15 coastal states during the period 1965-1972 (Butler, 1973).

Dieldrin, a chlorinated hydrocarbon insecticide, is acutely toxic to certain non-target estuarine animals under field conditions (Harrington and Bidlingmayer, 1958). Dieldrin is also acutely toxic to several estuarine animals exposed for 48 hours under laboratory conditions (Lowe, personal communication[1]).

This study was conducted to determine (1) the acute (96-hour) toxicity of dieldrin to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum),* grass shrimp (*Palaemonetes pugio*) and sheeshead minnows (*Cyprinodon variegatus*) and (2) the rate of uptake and depuration in spot (*Leiostomus xanthurus*).

## MATERIALS AND METHODS

*Test animals*

All test animals except pink shrimp were collected near the Gulf Breeze Laboratory and acclimated to laboratory conditions for at least ten days before exposure. Pink shrimp were purchased from a local bait dealer and acclimated similarly. 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, those animals were not used. Oysters tested were from 24 to 43 mm in

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height; pink shrimp, 52 to 81 mm rostrum-telson length; grass shrimp, 18 to 24 mm rostrum-telson length; sheepshead minnows, 11 to 14 mm standard length; and spot, 22 to 38 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. In the uptake and depuration study, spot were fed commercial fish food that contained no pesticide or polychlorinated biphenyl contaminants detectable by gas chromatographic analysis.

*Test Conditions*

Acute toxicity of dieldrin was determined by exposing ten animals per aquarium to different concentrations for 96 hours. Two 20 l aquaria were used for each concentration. Technical grade dieldrin (92% active ingredient) was dissolved in reagent grade acetone and metered at 0.14 ml/hr into unfiltered sea water that entered each aquarium at 75 l/hr. Two control aquaria received the same quantities of water and solvent but no dieldrin.

The rate of uptake and depuration of dieldrin by spot was determined by exposing 35 animals per aquarium in duplicate 20 laquaria to 0.0135, 0.075, 0.135, 0.75, or 1.35 ug/l for 35 days and then placing them in dieldrin-free water for 14 days. Sea water flow rate was 75l/hr per aquarium. Technical grade dieldrin (92% active ingredient) was dissolved in reagent grade acetone and metered at 15 ml/hr into the unfiltered sea water.

Effect of dieldrin was assessed by measuring reduction of shell growth of oysters (Butler, 1962), by determining mortality in shrimps and fish, and by examining for pathological changes fish from the uptake and depuration exposure.

*Histopathological examination*

Gills and viscera from live fish from the uptake and retention exposure were examined. Tissues were fixed in Davidson's fixative, stored in 70% ethyl alcohol and then processed for paraffin sections (7 u). Sections were stained with Harris hematoxylin and eosin. Six fish from each concentration were removed for tissue preparation after 4 days of exposure. Six fish from concentrations of 0.135, 0.075, and 0.0135 ug/l were removed at the end of the 35-day exposure, and six fish from concentrations of 0.135 and 0.075 ug/l and control were removed after the 13-day depuration in dieldrin-free water.

*Chemical analyses*

Concentrations of dieldrin in water and animals were determined by electron capture gas chromatography. Unfiltered water samples from each concentration were analyzed once during the 96-hour exposures and weekly during the uptake and depuration exposure. Concentrations in animals that survived the 96-hour exposures were determined as whole-body residures. In the uptake depuration exposure, six fish were removed from each connentration after 4, 11 18, 25 and 35 days and after 13 days in dieldrin-free water. Concentrations were determined for pooled samples of liver, muscle (all muscle above lateral line on left side of fish with scalless skin), and remaining tissues. Results from the two pooled samples of each tissue from each concentration were averaged. Residues in all tissues were summed to compute concentrations of dieldrin in the whole fish.

Tissue samples that weighed more than 5 g were prepared for analysis by mixing with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred in 3- to 4-ml portions to a 400 x 20 mm chromatographic column that contained 76 ml of unactivated Florisil. After

each portion settled in the column, vacuum was applied until all solvent was evaporated. This was repeated with three 5-ml rinses. The residue was eluted from the column with 70 ml of a 9:1 mixture (v/v) of acetonitrile and distilled water. The eluate was evaporated to dryness and the residue transferred to a Florisil column (Mills, et al., 1963) with petroleum ether. Dieldrin was eluted in the 15% ethyl ether-in-petroleum ether fraction.

Tissue samples that weighed 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).

Water samples were extracted with petroleum ether, the extracts dried with anhydrous sodium sulfate and evaporated to approximately 1 ml. The concentrates were transferred to a size 7 Chromaflex[1] column containing 1.6 g Florisil topped with 1.6 g anhydrous sodium sulfate. Dieldrin was eluted with 20 ml of 10% ethyl ether in hexane and the eluates were adjusted to an appropriate volumn for analysis.

All samples were analyzed by electron capture gas chromatography using a 182 cm x 2 mm 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 temperature was 210° C. Recovery exceeded 85%; data were not adjusted for recovery. All tissue residues were determined on a wet-weight basis.

*Statistical analyses*

Data from the acute (96-hour) exposures were analyzed statistically. Oyster shell growth data were analyzed by unweighted least squares and shrimp and fish mortality data were analyzed by maximum liklihood profit analysis (Finney, 1971).

## RESULTS AND DISCUSSION

*Acute (96-hr) exposures*

Dieldrin was acutely toxic to the estuarine organisms tested (Tables 1 and 2). Shell growth in oysters was appreciably inhibited by exposure to 32 ug/l for 96 hours. Pink shrimp were more sensitive to dieldrin than were grass shrimp, but significant numbers of both these crustaceans died when exposed to concentrations in the low parts-per-billion (ug/l) range.

All animals accumulated dieldrin (Table 1). The quantities accumulated depended on the species and the exposure concentration. In live oysters, whole-body (meats only) concentrations ranged from 2,000 to 5,000X nominal concentrations in test water and 2,400 to 21,500X measured concentrations. In an earlier experiment at this laboratory, oysters chronically exposed to 0.01mg/l of dieldrin accumulated 8,000X the concentration in test water after 8 weeks exposure (Parrish, 1973). In live pink shrimp, whole-body concentrations ranged from only 240 to 250X nominal concentrations in test water and 280 to 420X measured concentrations. In live grass shrimp, whole-body concentrations ranged from 330 to 660X nominal concentrations in test water and from 470 to 750X measured concentrations. In live sheepshead minnows, whole-body concentrations ranged from 2,000 to 4,000X nominal concentrations in test water and from 3,500 to 7,300X measured concentrations.

[1]Mention of commercial products or trade names does not constitute endorsement by the U. S. Environmental Protection Agency.

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*Uptake and depuration*

Spot exposed to 0.0135, 0.075, 0.135, 0.75 or 1.35 ug/l of dieldrin for 35 days accumulated the chemical, maximum concentrations being attained in 11 to 18 days (Table 3). Fish in some concentrations began to lose dieldrin after body concentrations had peaked, even though the exposure continued and dieldrin concentrations in test water remained constant (Table 4). Unlike our findings, DDT concentrations in pinfish (*Lagodon rhomboides*) and Atlantic croaker (*Micropogon undulatus*) exposed to 0.1 and 1.0 ug/l increased for 14 days, then remained relatively constant for 21 days (Hansen and Wilson, 1970).

Dieldrin was accumulated in greatest quantity in the liver of spot, where maximum concentration was 113,000X that in test water. Maximum concentration in muscle was 11,000X that in test water and maximum concentration in whole-body was 6,000X that in test water.

Spot lost all detectable dieldrin residues after a 13-day depuration period in dieldrin-free sea water (Table 3). Pinfish lost 87% of DDT residues and Atlantic croaker lost 78% of accumulated DDT when held in pesticide-free water for 56 days (Hanson and Wilson, 1970). Similarly, goldfish (*Carassius auratus*) have been reported to eliminate $^{14}$C-dieldrin from various tissues more rapidly than DDT (Grzenda et al., 1972). Thus, the flushing rate of dieldrin in fish appears to be faster than that of DDT.

Fish exposed to 1.35 ug/l showed degenerative changes in gill and visceral tissue after 4 days of exposure. Gill lamellae from three of six fish exhibited subepithelial edema (Fig. 1). A similar condition was observed in gills of cutthroat trout (*Salmo clarki*) exposed chronically to endrin (Eller, 1971) and in gills of goldfish exposed chronically to mirex (Van Valin et al., 1968). Alteration of visceral tissue included severe lysis and sloughing of the mucosal epithelium of the anterior small intestine (Fig. 1) and apparent inflamation of the underlying lamina propria in three of six fish.

Fish examined at the end of the exposure (from concentrations of 0.135, 0.075, and 0.0135 ug/l) and at the end of the depuration (from concentrations of 0.135 and 0.075 ug/l) showed no significant differences from control fish.

Dieldrin is a persistent chlorinated hydrocarbon insecticide (Wurster, 1971) and, as shown by our study, is acutely toxic to an estuarine mollusc, two crustaceans and a fish. Concentrations of dieldrin shown by our study to be acutely toxic to estuarine animals, as well as concentrations which are chronically toxic, should be kept out of the estuarine environment.

Table 1. Acute toxicity of dieldrin to and uptake by American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), and sheepshead minnows (*Cyprinodon variegatus*) during 96-hour exposures. Effect is expressed as percentage reduction in shell growth for oysters and death for shrimps and fish. Whole-body residues are from animals alive at end of exposure.

| SPECIES | WATER CONCENTRATION (ug/l) | | EFFECT (%) | WHOLE-BODY RESIDUE |
|---|---|---|---|---|
| | Nominal | Measured | | (ug/g, wet weight) |
| *C. virginica* | Control | <0.01 | 0 | 0.022 |
| | 1.0 | 0.23 | 18 | 4.95 |
| | 3.2 | 5.8 | 0 | 13.85 |
| | 10.0 | 6.7 | 24 | 20.0 |
| | 32.0 | 13.0 | 61 | 80.5 |
| *P. duorarum* | Control | <0.01 | 0 | 0.016 |
| | 0.01 | 0.014 | 0 | <0.01 |
| | 0.32 | 0.19 | 25 | 0.08 |
| | 1.0 | 0.9 | 55 | 0.25 |
| | 3.2 | 2.5 | 70 | 0.76 |
| | 10.0 | 11.4 | 100 | - |
| *P. pugio* | Control | ND[1] | 0 | 0.09 |
| | 3.2 | 2.8 | 20 | 2.1 |
| | 10.0 | 7.1 | 30 | 3.3 |
| | 32.0 | 27.1 | 85 | - |
| | 100.0 | 57.4 | 100 | - |
| | 320.0 | 65.7 | 100 | - |
| *C. variegatus* | Control | <0.01 | 0 | 1.1 |
| | 1.0 | 0.52 | 0 | 3.8 |
| | 3.2 | 2.2 | 0 | 12.8 |
| | 10.0 | 6.0 | 10 | 34.0 |
| | 32.0 | 17.6 | 65 | 62.4 |
| | 100.0 | 13.1 | 100 | - |

[1]Not detectable; <0.005 ug/l.

Table 2. Acute toxicity of dieldrin to American oysters (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*), grass shrimp (*Palaemonetes pugio*), and sheepshead minnows (*Cyprinodon variegatus*). Effect is expressed as percentage reduction in shell growth for oysters and death for shrimp and fish. Confidence limits (95%) are in parentheses.

| SPECIES | 96-HOUR EC50 (ug/l) | | TEMPERATURE (°C) | | SALINITY (o/oo) | |
|---|---|---|---|---|---|---|
| | Nominal | Measured | Mean | Range | Mean | Range |
| *C. virginica* | 12.50 (4.80-20.2) | 31.20 0.60-61.80) | 16.6 | 14.5-19.0 | 30.8 | 30.0-32.5 |
| *P. duorarum* | 0.93 (0.52-1.48) | 0.70 (0.39-1.15) | 19.6 | 18.2-21.0 | 26.0 | 22.0-30.0 |
| *P. pugio* | 11.39 (7.47-16.71) | 8.64 5.92-12.05) | 22.5 | 21.4-23.5 | 30.8 | 28.5-33.0 |
| *C. variegatus* | 23.57 (17.47-32.03) | 10.00 - | 13.8 | 12.0-15.5 | 31.5-33.0 | |

Table 3. Uptake and depuration of dieldrin by spot (*Leiostromus xanthurus*) exposed to 0.135, 0.075, 0.135, 0.74 or 1.35 ug/l in flowing sea water. Residue concentrations (wet-weight) are the average of two samples of pooled tissue from three fish.

| LIVER | DAYS | CONCENTRATION, ug/g | | | | | |
|---|---|---|---|---|---|---|---|
| | Exposure | Control | .0135 | .075 | .135 | .75 | 1.35 |
| | 4 | ND | 0.08 | 0.52 | 0.98 | 1.8 | 10.2 |
| | 11 | ND | -[b] | 3.90 | 15.3 | 12.9 | - |
| | 18 | ND | 0.42 | 1.10 | 2.0 | 17.5 | - |
| | 25 | ND | 0.15 | 1.20 | 1.4 | 5,8[c] | - |
| | 35 | ND | 0.31 | 0.55 | 0.47 | - | - |
| | Depuration | | | | | | |
| | 13 | ND | - | ND | ND | - | - |
| MUSCLE | Exposure | | | | | | |
| | 4 | ND | 0.029 | 0.07 | 0.16 | 0.81 | 2.6 |
| | 11 | ND | -[b] | 0.44 | 1.45 | 1.40 | - |
| | 18 | ND | 0.029 | 0.12 | 0.15 | 1.20 | - |
| | 25 | ND | 0.029 | 0.11 | 0.24 | 0.81[c] | - |
| | 35 | ND | 0.030 | 0.15 | 0.20 | - | - |
| | Depuration | | | | | | |
| | 13 | ND | - | ND | ND | - | - |
| | Exposure | Control | .0135 | .075 | .135 | .75 | 1.35 |
| | 4 | ND | 0.029 | 0.43 | 0.63 | 1.2 | 2.9 |
| WHOLE-BODY | 11 | ND | -[b] | 0.15 | 0.52 | 1.9 | - |
| | 18 | ND | 0.031 | 0.07 | 0.23 | 2.0 | - |
| | 25 | ND | 0.033 | 0.15 | 0.29 | 0.6[c] | - |
| | 35 | ND | 0.045 | 0.12 | 0.27 | - | - |
| | Depuration | | | | | | |
| | 13 | ND | | ND | ND | - | - |

[a] Not detectable; <0.005 ug/g.

[b] Sample lost.

[c] Analysis of one sample only.

Table 4. Concentration (ug/l) of dieldrin in test water on days 4, 11, 18, 25 and 35 of uptake and depuration exposure of spot (*Leiostomus xanthurus*).

| NOMINAL | MEASURED | | | | | AVERAGE |
|---|---|---|---|---|---|---|
| Control | ND | ND | ND | ND | ND | ND |
| 0.0135 | 0.015 | 0.019 | 0.010 | 0.016 | 0.016 | 0.015 |
| 0.075 | 0.075 | 0.052 | 0.070 | 0.057 | 0.067 | 0.064 |
| 0.135 | 0.11 | 0.12 | 0.13 | 0.15 | 0.11 | 0.12 |
| 0.75 | 0.55 | 0.45 | 0.50 | 0.68 | - | 0.55 |
| 1.35 | 0.70 | - | - | - | - | 0.70 |

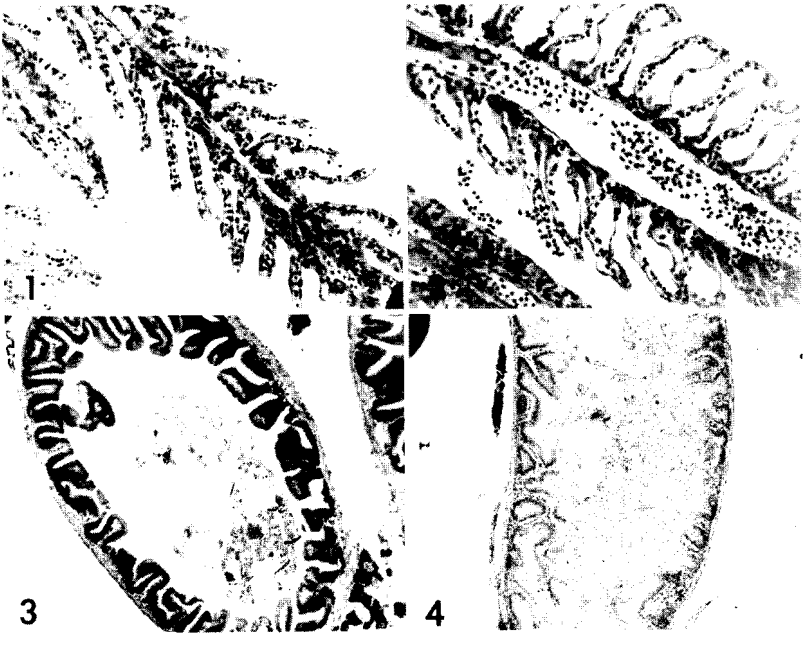


Figure 1. Photomicrographs of tissues from spot (*Leiostomus xanthurus*) exposed to dieldrin. 1: Normal gill tissue from fish exposed 35 days to 0.075 ug/l. (X450) 2: Gill tissue from fish exposed 4 days to 1.35 ug/l. Note subepithelial edema in lamellae. (X450) 3. Normal small intestine tissue from fish exposed 35 days to 0.075 ug/l. (X450) 4: Small intestine tissue from fish exposed 4 days to 1.35 ug/l. Note severe lysis and sloughing of mucosal epithelium. (X450).

## LITERATURE CITED


Butler, Philip A. 1973. Organochlorine residues in estuarine mollusks, 1965-72 -- National Pesticide Monitoring Program. Pestic. Monit. J. 6(4): 238-362.

. 1962. Reaction of some estuarine mollusks to environmental factors. *In*: Biological problems in water pollution. Third Seminar. U. S. Dept. Health, Educ., Welfare, Public Health Serv. Publ. No. 999-WP-25, 1965: 92-104.

Eller, Lafayette L. 1971. Histopathologic lesions in cutthroat trout (*Salmo clarki*) exposed chronically to the insecticide endrin. Am. J. Pathol. 64(2): 321-336.

Finney, D. J. 1971. Probit analysis. (Third ed.) Cambridge Univ. Press, Cambridge, England. 333 p.

Grzenda, A. R., W. J. Taylor, and D. F. Paris. 1972. The elimination and turnover of $^{14}C$ - dieldrin by different goldfish tissues. Trans. Amer. Fish. Soc. 101(4): 686-690.

Hansen, David J. and A. J. Wilson, Jr. 1970. Significance of DDT residues from the estuary near Pensacola, Fla. Pestic. Monit. J. 4(2): 51-56.

Harrington, R. W. and W. L. Bidlingmayer. 1958. Effects of dieldrin on fishes and invertebrates of a salt marsh. J. Wildl. Manage. 22(1): 76-82.

Mills, P. A., J. F. Onley and R. A. Gaither. 1963. Rapid method for chlorinated pesticide residues in non-fatty foods. J. Assoc. Offic. Agric. Chem. 46(2):

Parrish, Patrick R. 1973. Aroclor$^{R}$ 254, DDT and DDD, and dieldrin: accumulation and loss by American oysters (*Crassostrea virginica*) exposed continuously for 56 weeks. (Abstract) Proc. Natl. Shellfish. Assoc. (In Press).

U. S. Food and Drug Administration. 1970. Pesticide Analytical Manual. U. S. Dept. of Health, Educ., Welfare, Sect. H212.

Van Valin, Charles C., A. K. Andrews, and L. L. Eller. 1968. Some effects of mirex on two warm-water fishes. Trans. Amer. Fish. Soc. 97(2): 185-196.

Wurster, Charles F. Aldrin and dieldrin. Environment 13(8): 33-45.