The Residue Up Take and Histology of American Oysters (*Crassostrea virginica* Gmelin) Exposed to Dieldrin

Mads Emanuelsen[1]**, Jeffrey L. Lincer[1]*, and Erik Rifkin[2]

[1] *Mote Marine Laboratory*
9501 Blind Pass Road
Sarasota, Fla. 33581

[2] *Environmental Planning and Research Institute*
100 North Pineapple Avenue
Sarasota, Fla. 33577

Long-term sublethal effects resulting from chronic exposure of organisms to low levels of toxicants probably have the single most important impact on estuarine life (BUTLER 1966). It has been shown that numerous commercially available pesticides markedly affect bivalve shell growth in exposures of less than 1.0 ppm (BUTLER 1961). PARRISH et al. (1973) found shell growth inhibited in oysters (C. virginica) exposed to 12.5 ppm dieldrin for 96 hours. These animals concentrated the pesticide from 2,000X to 5,000X the nominal concentration in test water. Numerous investigators have concerned themselves with the ability of estuarine organisms to take up, store and depurate themselves of pesticides (LINCER et al., in press), but relatively few attempts have been made to determine what histopathological effects these environmental insults have on estuarine organisms. However, where histological studies have been undertaken, they have often shown pesticide-induced tissue damage. For instance, LOWE et al. (1971) found that C. virginica exposed to 1 ppb DDT, toxaphene, and parathion (in combination) exhibited tissue damage. Histological change has also been shown in estuarine fishes. PARRISH and co-workers (1973) observed tissue alterations in spot, Leiostomus xanthurus after a 4-day exposure to 1 ppb dieldrin in water. ELLER (1971) also found tissue damage in cutthroat trout (Salmo clarkii) after intermittent exposure to endrin. In view of the widespread use and occurrence of dieldrin, it was deemed desirable to look at its histopathological effect on the American oyster (C. virginica).

MATERIALS AND METHODS

Oysters were collected from the seawall of a residential community on the eastern shore of Sarasota Bay, Sarasota, Florida. The shells were carefully cleaned and the animals measured and weighed. The test animals were from 21.3 to 57.9 mm in height (mean = 36.6 mm). The oyster weights (out of water) were calculated as the average wet weight of the oysters in each batch of 12 individuals, and were found to be from 7.82 to 11.01 gm (mean = 8.95 gm). Test animals were acclimated for 2 weeks prior to experimentation in the test tanks using running sea water and aeration. Animals were not fed during the experimental period, since the seawater was only partially filtered, providing plankton and other particulate matter.

** Present address: Texas A & M University, Marine Laboratory, Bldg. 311 Fort Crockett, Galveston, Texas 77550.

* Present address. National Wildlife Federation, 1412 16th Street N.W. Washington, D.C. 20036. Reprints available from this author.

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Exposure to dieldrin was carried out in a static system, where each of the eight 78-liter tanks was drained, filled and recontaminated every 48 hours. Aeration was continuous. Two aquaria were used for each of three concentrations of dieldrin plus two control tanks. Technical dieldrin* (87% active ingredient) was serially diluted in pesticide grade acetone such that 1 ml acetone resulted in 0.1, 0.01 or 0.001 ppm for each of the test aquaria. The solutions were made up in and stored in acid-washed vials, which after being emptied were washed twice with 2 ml aliquots of pesticide grade acetone, thus delivering a total of 5 ml acetone to each tank (i.e., 64 ppm acetone in each tank). Control tanks received the same amount of solvent only and were subjected to the same schedule of water changes. The eight groups (each initially containing twelve oysters) were exposed to the pesticide for 43 days. Each was sampled 4 times (after 8, 14, 30 and 43 days) for histological studies and residue analysis. Although some initial mortalities were observed, none could be directly attributable to dieldrin.

Four water quality parameters were monitored during the experimental period: dissolved oxygen, temperature, pH, and salinity (Table 1).

TABLE 1

Characteristics of water used in experiment

Temp. (°C)		D.O. (% Sat.)		pH		Salinity (°/oo)	
Mean	Range	Mean	Range	Mean	Range	Mean	Range
24.16	22.26-26.48	94.94	87.69-99.91	7.9	7.8-7.95	35.6	35.53-35.65

On the sampling day, 2 or 3 oysters were collected from each tank. An estimated 15 percent of the tissue from the animal (cross-section through the digestive diverticulum) was removed and fixed in either 70% ethanol or 10% formalin in seawater for at least 72 hours before going through an ethanol dehydration series. Sections were embedded in paraffin and sectioned at 10 µm. Five general histological and histochemical stains were used. Hematoxylin and Eosin, and a slightly modified Masson Trichrome were used for general histological studies. Three histochemical stains were utilized to permit study of potential changes in intra- and intercellular elements: (1) the periodic acid-Schiff (PAS) reaction (MCMANUS 1946); (2) the Toluidine Blue method for metachromasia (KRAMER and WINDRUM 1954), and; (3) Tainzer-Unna's Orcein stain for elastic fibers as given by EDWARDS (1950). As controls for the PAS reaction, additional sections

*Manufactured by Shell Development Company (Div. of Shell Oil Co., Modesto, Cal.)

subjected to a 1% malt diastase solution in PO_4 buffer for one hour at 37°C were subsequently exposed to the reaction (HUMASON 1972).

Slides were observed in bright field Köhler illumination on a Zeiss Photomicroscope I with achromatic objectives. Each slide was initially scanned at 25-40X to ascertain presence, location and gross appearance of the various tissues. Selected portions of the tissues were then examined at 100X magnification to determine incidence and distribution of leucocytes and to scan for abnormal or damaged tissue. Individual organs were then checked at 400-500X with emphasis on changes in the tissues and cells. On occasion, higher magnifications (1000 - 1600X) were used to observe evidence of phagocytic activity of the leucocytes.

Tylocephalum sp. metacestodes were found to be infecting most of the oysters examined (RIFKIN and CHENG 1968). Since the distribution of these parasites was uniform between controls and experimentals, and they were already in various stages of encystment and resorption, it was felt that the presence of these parasites would not affect the outcome of the experiment.

The remaining tissue was frozen for subsequent pesticide residue analysis. The analytical procedure used for pesticide residue analysis was a modification by W.A.R.F. (Wisconsin Alumni Research Foundation - personal communication, D.L. HUGHES) of an earlier one and has been described in detail elsewhere (LINCER 1975). Samples were oven dried for at least 48 hours at 40-45°C. Dried samples were ground with coarse anhydrous Na_2SO_4, placed into Soxhlet thimbles and extracted for 8 hours with a 1:3 mixture of ethyl ether and petroleum ether. The extracts were concentrated to 50 ml and a 5 ml (10 percent) portion placed in tared beakers, evaporated to dryness at 40-45°C for 2 hours, and total fat calculated. The clean-up procedure followed is that outlined in the "Pesticide Analytical Manual" which employs florisil columns to provide pesticide separation and remove interfering substances and some fats (U.S. D.H.E.W. 1971).

A Varian Aerograph Model 2100 chromatograph equipped with two Ni^{63} electron capture detectors was used for pesticide quantification. The gas chromatographic columns and nitrogen flow rates used for quantifying the dieldrin are listed in Table 2. Injector, column and detector oven temperatures were 225, 200 and 290°C, respectively. Two columns were used simultaneously and quantification was based on peak height, since the dieldrin eluted early with a symmetrical peak. The residue micrograms in each sample were calculated for both columns and the lower of the two figures routinely chosen as the basis for expressing parts per million (ppm). This procedure resulted in a disparity between columns ranging from 0 to 16 percent, with a calculated mean of 5.8 percent. Although a recovery study was not carried out for this experiment, recovery of similar organochlorines, using the above procedure, exceeded 88 percent (LINCER 1975). No corrections were made for recovery.

TABLE 2

Column	N_2 Flow Rate (ml/min)
1.5% OV-17 + 2.95% QF 1 Chrom W 80/100	60
4% SE 30 + 6% OV 210 Chrom W 80/100	60
3% SE 30 Varaport 30 100/120	65
1% XE 60 Varom 30 100/120	65

RESULTS AND DISCUSSION

Dieldrin Uptake

Figure 1 shows the uptake of dieldrin by the various dosage groups of oysters. The initial rapid uptake of dieldrin by the oysters (in the 0.1 and 0.01 ppm dieldrin groups) is comparable to that described by MASON and ROWE (1976). It is of interest to note that the rate of uptake for the 0.1 ppm group is approximately one order of magnitude greater than that of the .01 ppm group. The phenomenon did not repeat itself in the lower concentrations, and the only explanation that can be offered is that because of the small sample size, the residues were too near the limits of detectability. The control groups were analyzed for pesticide residues twice during the course of the experiment. The first was after 8 days and the second was at the conclusion of the experiment. The initial control sample was found to contain 3.8 ppm dieldrin (based on oven dry or OD weight of sample). In the second sample (at the end of the experiment) dieldrin was not detectable. It is also interesting to note that the 0.1 ppm group appears to have reached a plateau at approximately 536 ppm whereas the residues in the 0.01 ppm group appear to still be ascending at day 43.

After 43 days of dieldrin exposure, oysters concentrated the pesticide approximately 5×10^3 times regardless of the actual ambient concentration (Table 3). An interesting trend regarding the relationship between accumulation of dieldrin and the percent lipid was also noted (Table 3): the higher the dieldrin residue, the higher the mean percent lipid. The differences in percent lipid between the sample groups is, however, not statistically significant. No explanation can be offered for this phenomenon at this time.

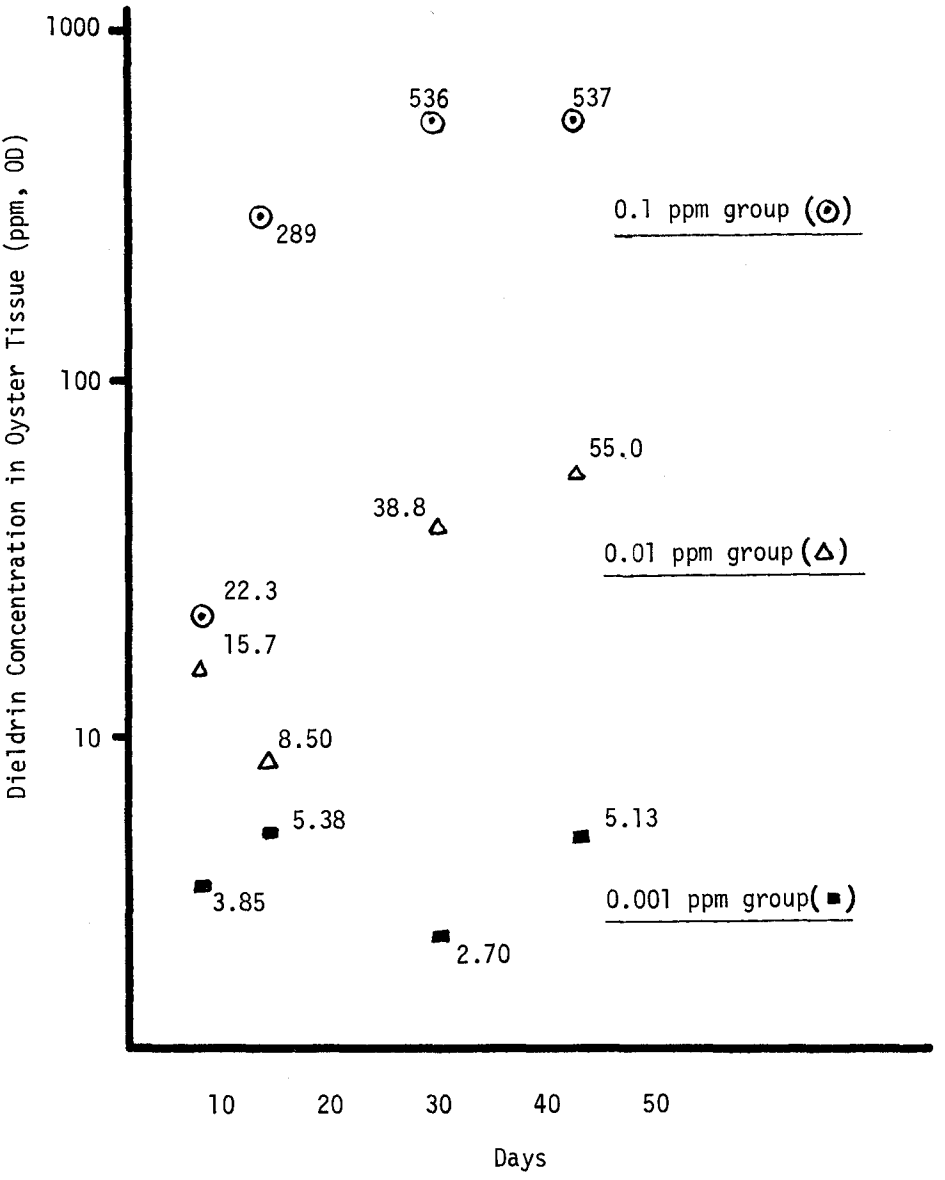


Figure 1. Dieldrin uptake over time by oysters exposed to three different dieldrin levels.

TABLE 3

Maximum dieldrin residues, lipid levels and concentration factors

Group	Mean Lipid Percent (s.d.)	Residue* at 43 Days	Maximum Concentration Factor** ($x10^3$)
.1 ppm	3.21% (0.72)	537 ppm	5.4
.01 ppm	2.96 (1.05)	55.0	5.5
.001 ppm	1.83 (1.05)	5.13	5.1
Control	2.64 (1.42)	N.D.***	-

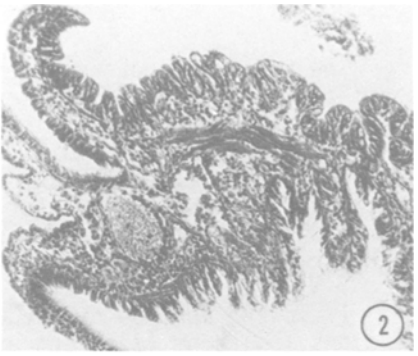
* ppm based on oven dry weight.
** Ratio of ppm OD weight in oyster to nominal ppm in water.
*** Limit of detectability is 0.41 ppb.

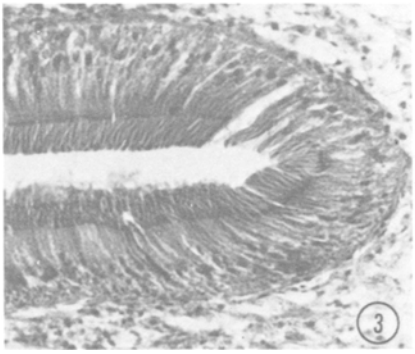
Histopathology

A comparison of fibrous and cellular components of oyster tissues exposed to 0.1 ppm, 0.01 ppm and 0.001 ppm of dieldrin with controls demonstrated no significant observable morphological differences employing general histological and histochemical stains. Organ systems of C. virginica observed included the stomach, the digestive diverticulum, gills and mantle (Plates 1-4). Although LOWE et al. (1971) found the gonads and germinal epithelium to be affected by a mixture of DDT, toxaphene and parathion, our samples did not contain a sufficient number of these tissue cross-sections to draw any conclusions as to the effect of dieldrin on them.

The characteristic inflammatory response, which would include abnormal leucocytic infiltration, was also not observed. It should be pointed out that it is difficult to compare the effects of one pesticide against another and indeed the cumulative adverse effects of a number of pesticides may produce an effect entirely different than that observed when toxic compounds are used separately.

There were no discernable differences in the affinity of intra- and intercellular components based on the histochemical stains. The PAS reaction, with and without the diastase control, showed no discernable difference between the controls and the experimental group and the staining with Orcein and Toluidine Blue also demonstrated no difference prior to and after exposure to dieldrin.





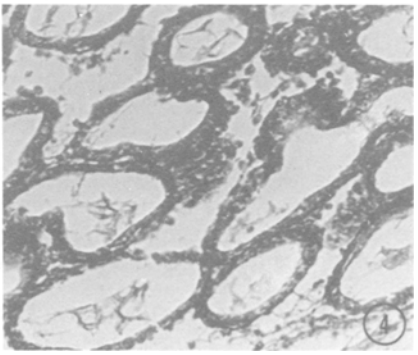


PLATE 1. Section through lower stomach/upper crystalline style sac. Exposed 0.1 ppm for 30 days. Masson Trichrome 12.5X.

PLATE 2. Section of mantle. Exposed 0.01 ppm for 43 days. Masson Trichrome 63X.

PLATE 3. Stomach ciliated columnar epithelial cells. Exposed 0.1 ppm for 43 days. H&E 200X.

PLATE 4. Digestive tubules exhibiting epithelial cells. Exposed 0.001 ppm for 30 days (a number of controls showed similar condition). Masson Trichrome 80X.

CONCLUSION AND SUMMARY

The results reported herein strongly suggest that relatively short-term exposures of low levels of dieldrin do not cause histopathological damage to C. virginica. It was found that C.virginica, after a 43-day exposure to 3 levels of dieldrin, concentrated approximately 5 x 10^3 times (based on OD weight of tissue) the level of contaminant found in the water, regardless of its nominal concentration. The percent lipid was found to vary directly with the concentration of pesticide in the water.

It is the opinion of the authors that before any definite conclusions about the long-term effects of dieldrin on C. virginica can be reached, longer exposures to sublethal concentrations should be undertaken. It is also recommended that tissues in the reproductive organs be studied to determine if these exhibit any histopathological effects.

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