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EFFECTS OF DDT ON *Fundulus Heteroclitus:* SURVIVAL, UPTAKE, AND DISTRIBUTION

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The teleost *Fundulus heteroclitus* was selected as an appropriate model system for the study of effects of pesticides on reproduction and embryogenesis. Therefore studies were conducted on the effects of a pesticide, DDT (1,1,1 trichloro-2,2 bis (p-chlorophenyl) ethane), on the adult organism and the distribution of DDT in the surviving animals. It is of particular interest to determine an effective route for preloading gametes with the pesticide.

Fish were kept in 1000 ml Erlenmeyer flasks containing 750 ml sea water maintained at 13° C. Under standard conditions each flask held four fish. All sea water was filtered through membrane filters (0.45 μ pore size) to eliminate the influence of other marine organisms and particles on DDT uptake by *Fundulus*. Each flask was aerated from a central pump *via* an aquarium stone. The water, whether it contained DDT or not, was changed daily. DDT solutions were prepared by adding 2.0 ml of ethanolic solutions to 1000 ml filtered sea water immediately prior to use. Control fish were kept in 0.2 percent ethanol-sea water.

Lethal dosage of DDT for *Fundulus* was determined under a variety of exposure conditions. Keeping the fish in DDT continuously gave results as seen in Table 1. The survivorship after 24 hours of treatment was over 90 percent for fish in 0.1 ppm DDT. Further reduction in concentration was not lethal within 24 hours.

TABLE 1

Percentage Deaths in Fundulus Maintained in DDT-Containing Media

DDT	Hours in DDT solution					
ppm	6	10	19	22	24	
1.0	0	0	33	67	100	(3)[a]
0.5	0	0	0	55	82	(11)
0.1	0	0	0	0	9	(56)
0.07	0	0	0	0	0	(8)
0.05	0	0	0	0	0	(24)
0.01	0	0	0	0	0	(8)
control	0	0	0	0	0	(16)

[a]Figure in parentheses refers to number of fish studied.

TABLE 2

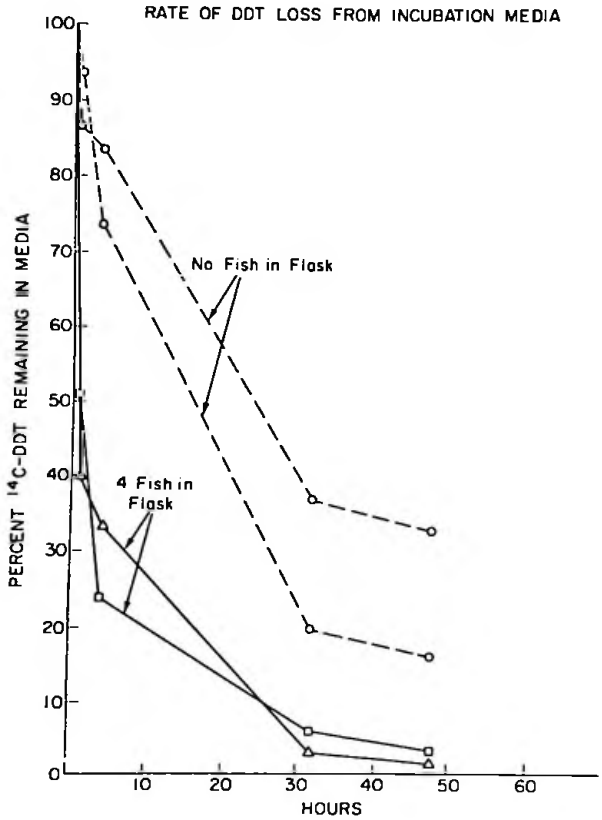
Percentage Deaths in Fundulus Given Long Term Dosages of DDT

DDT ppm	Days from first DDT dose[a] 1	3	5	7	9	11	13	15	
0.1	25	44	75	81	88	88	94	94	(16)[b]
0.05	0	6	13	38	38	38	38	56	(16)

[a]Each 24 hour dose was followed by 24 hours free of DDT. Each Dose was initiated on the even numbered days.

[b]Figure in parentheses refers to number of fish studied.

Figure 1



At time zero, each flask contained 750 ml of 0.1 ppm ^{14}C-DDT. 4 fish were added to each flask except in the no-fish controls. 1.0 ml aliquots were removed and analyzed at the indicated time intervals.

In another type of study, fish were subjected to DDT for 24-hour periods interspersed with 24 hours in sea water containing no DDT. The dosages were 0.10 and 0.05 ppm and the results are seen in Table 2. It is apparent that the fish cannot long tolerate additional doses at the 0.1 ppm level. However several fish survive four treatments at 0.05 ppm interspersed with 24 hours free of the pesticide. Thus gradual preloading of the fish with the pesticide appears possible by dividing smaller doses over a longer period of time.

Uptake of DDT was measured both by direct measurements on the fish and indirectly by determination of the loss of DDT from the media. The presence of DDT was detected by using ^{14}C-labelled material (phenyl-ring-^{14}C(U), Amersham/ Searle, sp. act = 63.9 μCi/mg). Aliquots from the ^{14}C-containing media were extracted by 18 ml of toluene scintillation solution (4 g PPO and 50 mg POPOP/liter). Fifteen ml of the extract were placed in a vial and counted in the Nuclear Chicago Mark I liquid scintillation spectrometer. The results of such analyses may be seen in Figure 1. The flasks which contained no fish showed a large decrease in ^{14}C-DDT within 24-hours due to volatilization and absorption onto the glass. However, the flasks containing fish showed approximately 80 percent diminution of DDT within four hours. Examination of these curves suggests that at least 50 percent of the dose was taken up by the fish in four hours, thus offering further confirmation of the ability of the fundulus to concentrate this pesticide.

The distribution of DDT in various tissues of fundulus was determined. Flask media were made 0.1 ppm with ^{14}C-DDT. Four fish were placed in each flask containing 750 ml of medium and removed at the appropriate time for assay. Tissues were removed, weighed, and aliquots placed in 2 ml of NCS Tissue Solubilizer at 50°. When the tissue dissolved, 18 ml of toluene scintillation solution were added and the samples were counted. The remaining carcass was homogenized and aliquots representing approximately 100 mg were prepared with NCS and scintillation solution in the usual manner.

TABLE 3

DDT Incorporation and Distribution in Fundulus[a]
(single 24-hour DDT dose)

	1 Day Post DDT Dose		8 Days Post DDT Dose	
Tissue	ppm DDT	% absorbed dose in tissue	ppm DDT	% absorbed dose in tissue
Intestine	5.11 ± 0.88[b]	7.83 ± 1.80	3.08 ± 0.44	11.80 ± 1.71
Liver	4.91 ± 1.01	8.70 ± 1.33	2.92 ± 0.33	8.06 ± 0.95
Eggs	0.25 ± 0.09	3.02 ± 1.12	0.57 ± 0.12	10.92 ± 2.60
Ovaries	--------	--------	9.33 ± 1.86	42.85 ± 2.28
Brain	2.63 ± 0.66	0.88 ± 0.10	1.74 ± 0.21	1.00 ± 0.13
Heart	2.07 ± 0.55	0.23 ± 0.04	0.97 ± 0.12	0.24 ± 0.04
Spleen	2.37 ± 0.50	0.15 ± 0.04	0.56 ± 0.08	0.15 ± 0.03
Gills	2.17 ± 0.64	0.97 ± 0.24	1.59 ± 0.17	1.69 ± 0.38
Muscle	1.50 ± 0.36	--------	0.83 ± 0.06	--------
Carcass	1.54 ± 0.20	72.83 ± 3.65	0.79 ± 0.08	52.50 ± 5.38

[a]Dosage and assay performed as described in the text.

[b]Standard deviation of the mean. 7 fish used in each study.

Tables 3 and 4 show the distribution results in two types of experiments, differing in the time of exposure of the fish to the DDT. These data indicate the concentration of DDT in tissues and the percent of absorbed dose in each tissue both one and eight-day post dose. The fish of Table 3 were exposed to 0.1 ppm DDT for 24 hours while the fish of Table 4 were in the DDT 24 hours, in sea water 24 hours, and then returned to the DDT solution for another 24 hour exposure. It is clear that over half of the dose is found in the carcass (presumably within muscle). However it is of special interest to note that the major quantity of DDT accumulates in the ovaries and that significant quantities appear in the eggs, indicating a degree of maternal transport of substances across

TABLE 4

DDT Incorporation and Distribution in Fundulus[a]
(two 24 hour DDT doses)

Tissue	1 Day Post DDT Dose		8 Days Post DDT Dose	
	ppm DDT	% absorbed dose in tissue	ppm DDT	% absorbed dose in tissue
Intestine	14.24 ± 4.42[b]	18.07 ± 3.80	2.57 ± 0.30	5.69 ± 1.51
Liver	8.18 ± 1.03	7.43 ± 1.17	5.19 ± 0.31	6.67 ± 0.33
Ovaries	6.80 ± 0.82	15.20 ± 1.12	10.40 ± 3.00	25.16 ± 6.29
Brain	3.59 ± 0.60	0.77 ± 0.10	3.07 ± 0.53	0.78 ± 0.14
Heart	2.39 ± 0.18	0.17 ± 0.03	1.82 ± 0.42	0.20 ± 0.04
Spleen	1.84 ± 0.38	0.15 ± 0.02	0.94 ± 0.20	0.17 ± 0.07
Gills	2.50 ± 0.29	0.98 ± 0.16	2.32 ± 0.40	1.41 ± 0.33
Muscle	1.76 ± 0.33	--------	1.23 ± 0.16	--------
Carcass	1.73 ± 0.09	55.58 ± 2.90	1.35 ± 0.15	56.17 ± 7.58

[a]Dosage and assay performed as described in the text.
[b]Standard deviation of the mean. 5 fish used in each study.

egg membranes. Experiments with male fish show incorporation of DDT into testes at levels similar to those shown for eggs. Furthermore it should be noted that a major portion of the labelled material is still present in the fish eight days following removal from the DDT-containing medium. It therefore would appear that suitable preloading of fundulus eggs with DDT can be accomplished by administration of the pesticide to females *via* uptake from the water. Furthermore, our results suggest that more DDT is absorbed with less maternal toxicity if each 24-hour exposure is followed by 24 hours in DDT-free water.

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THE DISSOCIATION BETWEEN RENAL HCO_3^- REABSORPTION AND H^+ SECRETION

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Using micropuncture technique and the appropriate kinetic data, it has been possible to study the rates of H^+ secretion in the kidney of the skate *Raja erinacea* and to compare these with rates of HCO_3^- reabsorption. *Raja* and other sea water fish offer particular advantages for such a study since they have a fixed urinary pH (5.8) and no renal carbonic anhydrase. In this situation, *Squalus* acanthias excretes (per kg fish) about 15 μeq H^+ per hour and reabsorbs essentially all of the filtered HCO_3^-, 10 μeq per hour (J. Hodler, H.O. Heinemann, A.P. Fishmann and H.W. Smith, Am. J. Physiol. 138:155, 1955). Both of these basal rates are susceptible to increase: H^+ output by administration of phosphate; and HCO_3^- reabsorption when large amounts of $NaHCO_3$ are injected, none of which appears in the urine. The problem is to find the mechanism underlying each of these processes.