Localization of DDT in the Body Organs of Pink and White Shrimp[1]

by D. R. NIMMO, A. J. WILSON, JR., and R. R. BLACKMAN
Bureau of Commercial Fisheries Center for Estuarine and Menhaden Research
Pesticide Field Station
Gulf Breeze, Florida

Introduction

Bioassays conducted at this laboratory have shown commercial penaeid shrimp to be one of the most sensitive crustaceans to organochloride pesticides (1). Nevertheless, little is known about residues in the organs of shrimp or the sites of pesticide localization. We therefore exposed shrimp to low concentrations of DDT (0.05 to 0.2 p.p.b., parts per billion) in the laboratory for extended periods to compare residues accumulated under controlled conditions with residues in natural populations.

Methods and Materials

Adult pink shrimp, Penaeus duorarum, and white shrimp, Penaeus setiferus, were collected from Escambia and West Bays (Escambia and Bay Counties, Florida) for the experiments. The animals were selected for uniformity of length and weight. Shrimp were acclimated to laboratory conditions and maintained through the experiments in plastic aquaria supplied with flowing sea water and a sand substrate. They were fed cubes of frozen mullet muscle each day. Periodic analysis of the mullet for DDT and its metabolites showed the average residue to be 1.58 p.p.m. (parts per million). We do not believe this contributed significantly to the residue in shrimp since the amount of DDT in most control shrimp did not exceed 0.1 p.p.m.

DDT (p,p'-DDT) was dissolved in polyethylene glycol (200 molecular weight) and infused into the flowing-water aquaria by means of syringe pumps to obtain the desired concentration. A water sample was removed each week and analyzed by gas chromatography to determine the concentration of toxicant.

Shrimp exposed to the desired concentration of DDT were removed from the aquaria at predetermined intervals and frozen immediately to prevent autolysis of organs.

[1] Contribution No. 104

Bulletin of Environmental Contamination & Toxicology,
Vol. 5, No. 4, 1970, published by Springer-Verlag New York, Inc.

Gas chromatographs were used to analyze residues in shrimp used in this study. Each sample of tissue was ground with anhydrous sodium sulfate in a blender and extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and partitioned with acetonitrile. The acetonitrile was evaporated to dryness and the residues transferred to a Florisil column (2) with petroleum ether. DDT and its metabolites were eluted from the column with 6% ethyl ether in petroleum ether. DDT was identified and measured on three columns (DC-200, QF-1, mixed DC-200-QF-1) equipped with electron capture detectors. When polychlorinated biphenyls interfered with the quantification of DDT, thin layer chromatography and gas liquid chromatography were employed.

Five experiments were conducted to determine accumulation of DDT in shrimp after they were subjected to selected concentrations. A summary of test conditions, including the purpose of each experiment, is given in Table 1.

The purpose of the first experiment was to determine the rate of accumulation of DDT in whole shrimp. Ninety shrimp were exposed to 0.14 p.p.b. and 90 were "controls". Every third day 10 shrimp were removed from each group, combined, and analyzed for total-body residues.

In earlier experiments, we established that 0.1 p.p.b. kills shrimp within 4 weeks at summer temperatures (20-30°C.). Therefore, in the second experiment, we exposed 36 shrimp to 0.05 p.p.b. to determine if this also was a lethal concentration. We terminated the experiment on the 56th day and survivors from both the treated and untreated groups were analyzed for total-body and individual-organ residues.

The objective of the third and fourth experiments was to determine the amount of DDT that would accumulate in individual organs. Equal numbers of untreated and treated shrimp were maintained in each experiment. Fourteen pink shrimp were exposed to 0.12 p.p.b. and 9 white shrimp to 0.2 p.p.b. DDT. Treated shrimp were removed and frozen when they exhibited symptoms of acute poisoning. The last pink shrimp was removed after 28 days and the last white after 18 days. Untreated shrimp were removed and frozen on the last day of each experiment. A composite sample of each organ was analyzed for residues in both experiments.

We also determined the depuration of DDT from the hepatopancreas and other organs. Six shrimp were removed for determination of background residues whereas the remainder were exposed to 0.17

TABLE 1

Summary of test conditions

Purpose of Test	Species of Shrimp	Average Test Concentration p.p.b. DDT	Length of Exposure	Average Length of Shrimp	Average Weight of Shrimp	Number Used		Temp. Range °C	Salinity Range ‰
						Treated	Untreated		
Accumulation in Total Body	pink	0.14	22 days	6.4 cm	3.6 g	90	90	8-15	28-33
Accumulation in Organs	pink	0.05	56 days	7.6 cm	3.8 g	36	36	15-28	23-34
ditto	pink	0.12	28 days	13.3 cm	21.1 g	14	14	21-29	24-33
ditto	white	0.20	18 days	16.2 cm	28.0 g	9	9	6-15	29-33
Depuration	pink	0.17	5 days*	9.5 cm	7.6 g	42	6	18-25	24-32

* Shrimp were exposed to 0.17 p.p.b. DDT in the water for 5 days, then held in pesticide-free water.

p.p.b. DDT for 5 days. After exposure, the shrimp were held in uncontaminated aquaria. Four were removed each week for residue analysis. No "control" shrimp were maintained in this experiment.

Data were obtained on total-body residues of shrimp from the National Pesticide Monitoring Program (Table 6). These shrimp were shipped as homogenized samples with a sodium sulfate desiccant plus 10% by weight of Quso, a micro-fine, precipitated silica. Also, shrimp representing wild populations were captured in Texas, Florida, and South Carolina in 1969 for analyses of total-body residues (Table 6) and two bays were sampled extensively for residues in hepatopancreatic tissues of shrimp (Table 7). These shrimp were collected by personnel at the Bureau of Commercial Fisheries Laboratories, Galveston, Texas and Gulf Breeze (near Pensacola), Florida. Samples were analyzed immediately after capture or frozen until the analyses could be performed.

Residues of DDT in Shrimp After Exposure to Test Concentrations

Residues of DDT in the hepatopancreas were consistently greater than those in the remaining organs (Tables 3, 4, and 5). Maximum total-body residues was 0.21 p.p.m.; the maximum amount in the hepatopancreas was 40.4 p.p.m. Concentrations in the hepatopancreas ranged from 1.4×10^4 to 3.0×10^5 times greater than that in the test water (Tables 3 and 4). A loss of DDT (98% from hepatopancreas and 100% from other organs) occurred after the shrimp were placed in clean flowing water for 6 weeks (Table 5). DDE was usually present in the organs after the shrimp were exposed to DDT. DDD was usually absent, but when present, it was at concentrations of 0.05 p.p.m. or less (Tables 2 and 5).

Stress related to laboratory confinement was probably responsible for a portion of the mortalities in the group of shrimp exposed to 0.05 p.p.b. After 56 days, 30% of the treated and 17% of the untreated had died. Residues in the hepatopancreas of treated shrimp did not exceed 0.7 p.p.m. (Table 3). All shrimp exposed to 0.2 p.p.b. DDT died within 18 days and all shrimp exposed to 0.12 p.p.b. DDT died within 28 days. Analysis of organs confirmed that DDT was concentrated in the hepatopancreas (Table 4)

Implications of DDT Residues from Natural Populations of Shrimp

To date, about half of the shrimp samples sent to our laboratory or collected by our staff contained 0.01 p.p.m. or more of DDT

TABLE 2

Accumulation of DDT (in p.p.m.) in living pink shrimp exposed to 0.14 p.p.b. DDT. Untreated shrimp contained less than 0.01 p.p.m.

Days Exposed	DDE	DDD	DDT	Total
1	< 0.01	< 0.01	< 0.01	< 0.01
4	"	"	0.02	0.02
7	"	"	0.06	0.06
10	0.02	"	0.17	0.19
13	< 0.01	"	0.21	0.21
16	"	"	0.16	0.16
19	"	"	0.14	0.15
22	0.02	"	0.12	0.15

TABLE 3

Localization of DDT in organs of living pink shrimp exposed to 0.05 p.p.b. DDT for 56 days. Residues are expressed as p.p.m. DDD was less than 0.01 p.p.m.

Tissues	Treated			Untreated		
	DDE	DDT	Total	DDE	DDT	Total
Hepatopancreas	0.27	0.43	0.70	0.13	< 0.01	0.13
Ventral Nerve	0.06	0.34	0.40	0.06	"	0.06
Heart	0.04	0.13	0.17	0.03	"	0.03
Digestive Tract	0.03	0.06	0.09	0.04	"	0.04
Gills	0.02	0.05	0.07	0.02	"	0.02
Exoskeleton	< 0.01	0.02	0.02	< 0.01	"	< 0.01
Muscle (Tail)	"	< 0.01	0.02	"	"	"
Total-Body*	0.02	0.04	0.06	"	"	"

* Four treated and four untreated shrimp were analyzed for total-body residue of DDT.

TABLE 4

Accumulation of DDT in organs of white shrimp killed by 0.2 p.p.b. and pink shrimp killed by 0.12 p.p.b. DDT.
Residues are expressed as p.p.m. DDT and include DDD and DDE.

Organ	White Shrimp		Pink Shrimp	
	Treated	Untreated	Treated	Untreated
Hepatopancreas	11.00	0.10	40.40	1.10*
Gills	0.36	<0.01	2.20	0.02
Digestive Tract	0.04	"	1.97	0.04
Ventral Nerve	0.55	"	1.86	0.06
Heart	0.46	"	1.69	0.03
Exoskeleton	0.86	"	0.66	<0.01
Muscle (Tail)	0.02	"	0.19	"

* This value appears to be high and may be due to contamination.

TABLE 5

Loss of DDT from hepatopancreas and other organs of living pink shrimp. Shrimp were previously exposed to 0.17 p.p.b. DDT for 5 days, then held in pesticide-free water.
Residues are expressed as p.p.m.

Time	Hepatopancreas				Remaining Tissue			
(weeks)	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total
Background	0.23	<0.01	0.10	0.33	<0.01	<0.01	<0.01	<0.01
Post Exposure (Initial)	0.68	0.49	11.00	12.17	"	"	0.10	0.12
1	1.80	*	7.60	9.40	"	"	0.13	0.13
2	0.40	0.05	0.50	0.95	0.03	"	0.06	0.09
3	0.65	0.05	0.26	0.96	<0.01	"	<0.01	<0.01
4	0.36	0.03	0.22	0.61	"	"	"	"
6	0.17	0.03	0.11	0.31	"	"	"	"

* Sample was destroyed.

and its metabolites (Table 6). Our findings show that the magnitude of contamination in some of the field samples approached that of our treated shrimp (Tables 2, 3, and 5). For example, we found that shrimp exposed to 0.14 p.p.b. DDT accumulated 0.21 p.p.m. total-body residue after 13 days and 0.15 p.p.m. after 19 days. Shrimp that died during this exposure accumulated 0.13 p.p.m. One sample from field collections in Florida contained total-body residue of 0.14 p.p.m.; others from Texas contained 0.06 and 0.09 p.p.m. Shrimp exposed to 0.05 p.p.b. in the laboratory accumulated 0.06 p.p.m. (Table 3). Three residues from shrimp reported in Table 6 equal or exceed this amount; two samples have 0.04 p.p.m.

Analysis of shrimp from field collections confirmed our laboratory observations that DDT was localized in the hepatopancreas (Table 7). Also, six of nine samples listed in Table 7 contained residues in the hepatopancreas greater than those that occurred in shrimp exposed to 0.05 p.p.b. in the laboratory (Table 3).

Physiologically, two of the principal functions of the hepatopancreas are absorption and storage of nutritive products. This capacity for absorption might explain the ability of shrimp to accumulate many times more DDT than was found in the water. We believe the amount in the hepatopancreas is a better estimate of residual DDT in shrimp than total-body residue because the concentration per unit weight is always greatest. Fortunately, DDT is localized in the hepatopancreas and this organ is discarded when the animals are processed as food for human consumption. The edible tail muscle had the least residue - well below that considered hazardous to human health.

Penaeid shrimp in some estuarine waters may be exposed to concentrations of DDT near those of our laboratory tests. Manigold and Schulze (3) reported that water in the Brazos and Colorado Rivers in Texas contained as much as 0.18 p.p.b. DDT and its metabolites. We calculated that the 3-year average was 0.03 p.p.b. Since brown shrimp are captured near the mouths of freshwater streams and white shrimp range into fresh water, shrimp in the estuaries of these rivers would be exposed to relatively high amounts of DDT. The survival of penaeid shrimp would be threatened if such concentrations reached these nursery areas in estuaries and remained relatively constant.

TABLE 6

Total body residues expressed as p.p.m. in shrimp collected from coastal waters. Each sample represents at least 5 shrimp.

Locality	Date Collected	DDE	DDD	DDT	Total
Maine	8/67[1]	<0.01	<0.01	<0.01	0.02
Maine	10/67[1]	"	"	"	<0.01
Maine	10/67[1]	"	"	"	"
Texas	10/68[1]	0.08	"	"	0.09
Texas	12/68[1]	<0.01	0.04	<0.01	0.06
Texas	7/69	"	0.02	0.02	0.04
Texas	7/69	"	0.02	<0.01	0.02
Texas	7/69	"	0.03	"	0.03
Florida	9/69	0.02	<0.01	"	0.03
Florida	9/69	0.08	<0.01	0.05	0.14
South Carolina	9/69	0.02	"	<0.01	0.02
South Carolina	9/69	0.02	"	0.02	0.04
South Carolina	9/69	0.01	"	0.02	0.03

[1] 1967-68 data from National Pesticide Monitoring Program.

TABLE 7

Residues, expressed as p.p.m., in the hepatopancreas and other organs of shrimp from Galveston Bay, Texas and Escambia Bay, Pensacola, Florida. Each sample represents at least 5 shrimp.

Organs	Galveston - July, 1969				Pensacola - Sept., 1969			
	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total
Hepatopancreas	0.25	0.81	0.30	1.36	0.89	0.11	0.50	1.50
Other Organs	<0.01	<0.01	<0.01	<0.01	0.08	<0.01	0.05	0.14
Hepatopancreas	0.06	1.20	"	1.26	0.64	0.06	0.28	0.98
Other Organs	<0.01	0.04	"	0.04	<0.01	<0.01	<0.01	<0.01
Hepatopancreas	0.17	0.47	0.16	0.80	0.23	<0.01	0.10	0.33
Other Organs	<0.01	<0.01	<0.01	<0.01	0.01	"	<0.01	0.03
Hepatopancreas	0.12	0.45	0.15	0.72	0.33	"	"	0.33
Other Organs	<0.01	<0.01	<0.01	<0.01	<0.01	"	"	<0.01
Hepatopancreas	0.06	0.08	0.04	0.18				
Other Organs	<0.01	<0.01	<0.01	<0.01				

Acknowledgments

We thank the personnel at the Bureau of Commercial Fisheries Laboratories, Galveston, Texas and Gulf Breeze, Florida who assisted in the collection of samples for this study.

Literature Cited

1. Philip A. Butler and Paul F. Springer, In Transactions of the Twenty-Eighth North American Wildlife and Natural Resources Conference, p. 378 (1963)

2. Paul A. Mills, J. H. Onley, and R. A. Gaither, J. Assoc. Off. Agr. Chem., 46, 186 (1963)

3. Douglas B. Manigold and Jean A. Schulze, Pesti. Monit. J. 3, 124 (1969)