Insecticide Residues in Two Turtle Species Following Treatment with DDT

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INTRODUCTION

Widespread environmental contamination by DDT has led to numerous investigations of the metabolism of this compound in tissues of non-target organisms. The principal metabolites DDD and DDE have been found widespread in the environment (BARKER 1964; DALE 1963; HAYES 1965; BITMAN 1971). Since DDD and DDE are rarely used as a pesticide, the residues found in the environment are from applications of DDT.

The two species of turtles used in this study were collected from the Reelfoot Lake area of northwest Tennessee. This lake was formed by the New Madrid Earthquake of 1811-12. A major portion of the 16,000 acre lake forms part of a National Wildlife Refuge. Reelfoot Lake is used by commercial and sports fishermen as well as duck hunters. A major portion of the land surrounding Reelfoot Lake is used for agricultural purposes where in the past, DDT has been used extensively.

HENSON (1973) found p, p' -DDT, p, p' -DDD and p, p' -DDE present in several species of sport fishes and p, p' -DDE in tissues of the mallard duck collected from Reelfoot Lake. The levels of pesticide found in the fishes were below FDA guidelines. However, the pesticide levels from fishes collected from tributaries of Reelfoot Lake had levels exceeding FDA guidelines.

Biological magnification of organochlorine insecticides has been shown to be a factor in controlling diversity and size of fish populations (JOHNSON 1968). Turtles feed on fishes, but very few predators feed on turtles. Therefore the turtle is of value in determining pesticide concentrations at a higher trophic level for the purpose of determining potential levels that might be a menace to health. The purpose of this study was to determine the residual levels of DDT and the metabolites when turtles were fed high concentrations of DDT.

METHODS AND MATERIALS

Two species of turtles, the red-eared, Chrysemys scripta, and the midland painted, Chrysemys picta, were collected in the Reelfoot Lake area of northwest Tennessee. All turtles were maintained in a plastic swimming

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pool at a water depth of 5 cm at a temperature of 23° - 27°. All turtles treated with DDT had a plastron length of 6-8 cm. Eighteen red-eared (14 males and four females) and 17 painted (11 males and six females) turtles were assayed.

Three groups each of four turtles from each of the two species were treated with 100 mg DDT/kg of body weight by forced feeding DDT in corn oil. Each turtle was weighed and then anesthetized with ethyl ether until limp. A 0.5 ml syringe equipped with a delivery tip from an Eppendorf pipet was inserted into the esophagus of the turtle on a depth of approximately 8 cm and the DDT (95% p, p' -DDT, 5% o, p' -DDT) injected. Each turtle was placed in an 8 l glass aquarium containing 80 ml of water. Three turtles from each of the two species were sacrificed at 3, 6, 12, and 24 hr after forced feeding. A second group of three turtles from each of the two species was forced fed 100 mg DDT/kg of body weight at weekly intervals and sacrificed at the end of 3 weeks. A group of three red-eared and two painted turtles was assayed for background DDT residues.

Following timed treatments with DDT, each turtle was anesthetized again and dissected for brain, liver, and fat. Wet weights of each tissue were used to express pesticide residue as ppm (ng pesticide/mg wet weight). All excrement was also collected and the volume recorded and used to express pesticide residue as ppm (ng pesticide/ul of water containing excrement).

Acetonitrile extraction procedure and Florisil column cleanup were used (MILLS 1972) with the following modifications. Each tissue was extracted three times with acetonitrile followed by extraction three times with hexane. Elution of the pesticide residues from a Florisil column was with hexane followed by 1% methanol/hexane.

A Beckman GC-45 Gas Chromatograph employing an electron capture detector was used for residue analysis. Two glass columns for GLC were used: a 6' x 2 mm (ID) packed with 10% DC-200 (100-200 mesh) and a 6' x 2 mm (ID) packed with 11% OV-17 + QF-1 (80-100 mesh). The operating temperatures were as follows: column temperature, 230°C and detector line temperature, 250°C. The carrier gas was prepurified helium maintained at a flow rate of 80 cc/min. Carbon dioxide flow rate was 3 cc/min. The electron capture detector was operated under the following conditions: polarizing voltage, 800, bias voltage zero with 7 ma current. Recovery of residues using this procedure was approximately 90%.

Residues were quantitated by comparison with standards (Perrine Primate Laboratory) of known concentration. The study was limited to the insecticide DDT and its metabolites.

RESULTS

Residual levels of p,p' -DDE were detected in all tissues of Chrysemys scripta (Table 1). There was no p,p' -DDT or p,p' -DDD

TABLE 1. Insecticide residues in liver, brain, fat, and excrement from Chrysemys scripta treated with 100 mg DDT/kg body weight. Turtles assayed at 3, 6, 12, and 24 hr were treated with DDT at time 0. Turtles assayed after 3 weeks were treated with DDT at weekly intervals. Mean values expressed as ppm ± SE for three turtles.

	Control	3 hr	6 hr	12 hr	24 hr	3 weeks
LIVER						
p,p'-DDT	ND	0.179 ± 0.091	1.077 ± 0.852	0.112 ± 0.058	0.202 ± 0.146	1.063 ± 0.437
p,p'-DDD	ND	0.014[a]	0.204 ± 0.157	0.244 ± 0.203	0.141[a]	0.109 ± 0.034
p,p'-DDE	0.394 ± 0.174	0.256 ± 0.007	0.292 ± 0.107	0.361 ± 0.136	0.237 ± 0.130	0.203 ± 0.025
BRAIN						
p,p'-DDT	ND	2.727[a]	1.723 ± 0.793	ND	ND	1.656 ± 0.437
p,p'-DDD	ND	3.166[a]	2.041	2.333[a]	ND	17.194 ± 3.081
p,p'-DDE	0.333[a]	0.284 ± 0.150	0.423 ± 0.236	0.411[a]	0.007[a]	0.725 ± 0.174
FAT						
p,p'-DDT	ND	ND	3.625[a]	ND	ND	34.805 ±16.064
p,p'-DDD	ND	ND	ND	ND	ND	55.550 ±22.694
p,p'-DDE	4.558 ± 3.886	7.682[a]	14.104 ± 5.884	9.003[a]	8.343 ± 0.704	7.580 ± 2.689
EXCREMENT						
p,p'-DDT	ND	0.116 ± 0.112	0.190 ± 0.116	0.094 ± 0.034	0.096 ± 0.073	0.616 ± 0.002
p,p'-DDD	ND	0.049 ± 0.048	0.041 ± 0.035	0.049 ± 0.045	0.048 ± 0.046	0.221 ± 0.002
p,p'-DDE	ND	0.001[a]	0.004[a]	ND	0.001[a]	0.038 ± 0.007

[a]Residue detected in one sample
ND None detected

detected in any tissues assayed prior to treatment with DDT. All metabolites of DDT were detected in the liver of Chrysemys scripta at all hours assayed. Relatively high concentrations of p,p' -DDD were detected in the brain of Chrysemys scripta 3, 6, and 12 hr after treatment with p,p' -DDT. The p,p' -DDT injected was detected 3 hr and 6 hr after treatment; p,p' -DDD was detected at all assay times. The highest concentration of p,p' -DDE was detected in fat from Chrysemys scripta. Some p,p' -DDE was present at all hours tested. The excrement contained some p,p' -DDT and p,p' -DDD at all hours of assay in both species. The p,p' -DDT and all the metabolites were detected in excrement of Chrysemys scripta 3 hr and 6 hr after treatment. Residues of p,p' -DDT, p,p' -DDD, and p,p' -DDE were detected in all tissues assayed in Chrysemys scripta following treatment for 3 weeks.

Residual p, p' -DDT was detected in the liver of one specimen of Chrysemys picta, (Table 2). Residual p, p' -DDT was not detected in the

TABLE 2. Insecticide residues in liver, brain, fat, and excrement from Chrysemys picta treated with 100 mg DDT/kg body weight. Turtles assayed at 3, 6, 12, and 24 hr were treated with DDT at time 0. Turtles assayed after 3 weeks were treated with DDT at weekly intervals. Mean values expressed as ppm ± SE for three turtles.

	Control	3 hr	6 hr	12 hr	24 hr	3 weeks
LIVER						
p,p'-DDT	0.094[a]	0.336[a]	0.883[a]	0.044[a]	ND	3.936 ± 1.388
p,p'-DDD	0.066[a]	0.165[a]	0.070[a]	0.040[a]	ND	0.610 ± 0.151
p,p'-DDE	0.151 ± 0.046	0.064[a]	0.182 ± 0.096	0.163 ± 0.025	0.171 ± 0.034	0.786 ± 0.168
BRAIN						
p,p'-DDT	ND	ND	2.828[a]	ND	2.890[a]	2.890[a]
p,p'-DDD	0.308[a]	ND	0.208[a]	ND	ND	1.502 ± 0.368
p,p'-DDE	ND	ND	0.277[a]	ND	0.179[a]	0.300 ± 0.160
FAT						
p,p'-DDT	ND	0.454[a]	ND	ND	ND	33.068 ± 5.980
p,p'-DDD	0.617[a]	ND	ND	ND	ND	5.295 ± 0.293
p,p'-DDE	2.672 ± 0.589	1.084 ± 0.294	1.036 ± 0.526	2.553 ± 1.170	2.406 ± 1.650	6.576 ± 1.059
EXCREMENT						
p,p'-DDT	ND	0.045 ± 0.025	0.028 ± 0.019	0.143 ± 0.019	0.081 ± 0.035	0.237 ± 0.018
p,p'-DDD	ND	0.002 ± 0.001	0.028 ± 0.019	0.143 ± 0.019	0.081 ± 0.035	0.083 ± 0.003
p,p'-DDE	0.004[a]	ND	ND	0.001[a]	0.003 ± 0.002	0.018 ± 0.001

[a] Residue detected in one sample
ND None detected

brain, fat, or excrement. Clearance of p, p' -DDT and p, p' -DDD from the liver was accomplished 24 hr after injection. The excrement contained p, p' -DDT and p, p' -DDD throughout the assay period following the initial injection.

DISCUSSION

The turtles used in this study were collected in an area of West Tennessee which had a history of DDT usage. Officials at the Reelfoot Lake Biological Station indicated the DDT had been used in fruit orchards. They also reported a widespread use of DDT in general farming. Residual metabolites of DDT detected in both turtle species substantiate this report.

In an earlier report (HERALD 1949), one soft shell turtle (Amyda ferox) was found dead in a test pond due to ingestion of DDT contaminated

food. Hard shell turtles were relatively immune. In the present study one Chrysemys scripta died after 16 days when treated with 100 mg DDT/kg body weight at weekly intervals. The two remaining Chrysemys scripta and the three Chrysemys picta did not show any symptoms of DDT poisoning during the three weeks treated. All turtles treated during the other time period showed no ill effects. This study with a cold-blooded animal parallels the work of LAUGER et al. (1945) which indicated that the uptake in several warm-blooded organisms treated with DDT reached a maximum in 2 - 5 hours after a single large oral dose. In the present study DDT reached a maximum in 3 - 6 hours after a single large dose.

There was no pattern as to the fate of p, p' -DDT or its metabolites in the tissues assayed following treatment. As with other chordates the major organ of degradation was the liver. Both p, p' -DDT and its metabolites were detected in the liver of both species 3, 6, 12 hr after treatment. Most of the residues detected in the brain before the 24-hr treatment period appeared to be residual. However, following the 3-week treatment, higher amounts of DDT and its metabolites were detected, indicating delayed penetration of the brain by the insecticide.

These findings support other studies which have shown that fat serves as a storage depot for insecticides due to the high solubility of insecticides in lipid solvents. In the present study large quantities of residues were detected in fat removed from the turtle mucosae, thus serving to partition the insecticide in a relatively inactive tissue.

These data indicate that both species are capable of degrading DDT to less toxic metabolites. The metabolites as well as the DDT injected can be excreted immediately. Removal of metabolites by excrement may also occur long after exposure as evidenced by residues detected in turtles which were not treated with DDT in the laboratory, but which apparently had been exposed to DDT in the field or had eaten contaminated organisms. The uptake of DDT was greater in Chrysemys scripta than in Chrysemys picta. Chrysemys scripta also excreted and metabolized DDT to a greater extent.

The 1965 United States-Japan meeting on pesticide research concluded that widespread use of pesticides had caused damage to fisheries, beneficial insects and a number of non-target organisms including humans. Numerous fish kills have been reported to be caused by pesticides. The food habits of turtles are varied. Chrysemys picta eats considerable plant material but also feeds on fishes and has been trapped using carrion. Chrysemys scripta is herbiverous and also eats carrion. In the aquatic environment, the turtle occupies the highest tropic levels. The food habits of turtles should make then a perfect non-target organism for pesticides due to biological magnification throughout the aquatic food chain. However, it appears that the health of at least two species of turtles is not affected by high concentrations of DDT. PEARSON et al. (1973) injected dieldrin (intraperitoneal) into turtles and sacrificed at various times up to 70 days. Since dieldrin is usually more toxic to non-target organisms than is DDT, the effect on

the health of turtles should be greater. However, this was not the case. Most of the dieldrin accumulated in fat and no mortality was reported. In the present study, high concentrations of DDT were found to have little effect on turtle activity. Based on these data, the concentrations of DDT previously used in the environment would not pose a health problem for turtles.

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