DDT Residues Absorbed from Organic Detritus by Fiddler Crabs

Abstract. *DDT and its metabolites accumulate in organic plant detritus within estuaries and may persist there for many years. The residues appear to be most abundantly associated with particulates having diameters from 250 to 1000 microns. Detritus particles of this size are ingested by many organisms, and associated DDT residues may enter diverse food chains. Fiddler crabs* Uca pugnax *were fed natural detritus containing DDT residues (10 parts per million) during an 11-day experiment and showed grossly modified behavior associated with a threefold increase in concentration of DDT residues in the muscle of the large claw.*

Residues of the chlorinated hydrocarbon pesticides now occur in biological systems in widely separated parts of the earth (*1*). The residues are concentrated in lipids, especially among scavengers and carnivores (*1–3*). They also accumulate in soils and persist there for long periods (*2, 4*). In California, Keith and Hunt (*5*) reported that the mean concentrations of DDT and its metabolites (*6*) were 0.62 part per billion (ppb) in water, 4.44 parts per million (ppm) in bottom sediments, and 14.7 ppm in particulate matter. Residues in particulates ranged up to 78 ppm and were 10,000 to 100,000 times higher than amounts in the filtrate.

The association of pesticides with organic particulate matter in estuaries is significant because many organisms rely on such particles for part or all of their energy requirements (*7*). This is especially true in areas where the bulk of primary production is composed of slowly decaying plant material such as marsh grasses, rushes, and mangroves that are not consumed directly by herbivores (*8*) but enter the plant detritus food chain. This material decomposes into smaller particles, becomes covered with microorganisms, and presents a uniquely enriched food source.

We examined the association of DDT residues with organic detritus particles of various sizes and the availability of these residues to consumers of plant detritus such as the fiddler crab (*Uca pugnax*). Samples of detritus were collected from the Carmans River marsh in Brookhaven, Long Island, New York. The marsh was sprayed regularly for more than 15 years with DDT for mosquito control, and the soils of certain areas contain residues totaling several kilograms per hectare (*2*). Spraying with DDT ceased in 1966 (*9*).

A series of soil cores (4.8 cm in diameter and 5 cm long) taken with a tube sampler were used to determine the pattern of association of DDT and its metabolites with detritus particles of various sizes. The particles were sorted by size by washing the detritus through standard geological screens (*10*). Samples for DDT analyses were dried in air and ground with mortar and pestle to assure uniform extraction of pesticide residues. Residues were extracted from a mixture of the particles with Florisil; petroleum ether–diethyl ether was used as the solvent (*11*). Analyses for DDT, DDE, and DDD were by electron-capture gas chromatography; certain identifications were confirmed by thin-layer chromatography (*11*).

Detritus particles from 500 to 1000 μ contained the highest concentration of DDT residues, the average being about 50 ppm (Fig. 1). The 250- to 500-μ fraction contained almost as much with about 45 ppm; the three smaller size classes had lower concentrations, ranging between 30 and 35 ppm. Thus, small particles of detritus (< 4000 μ in diameter), contained 20 to 50 times the concentrations that occurred in the living shoots of the plants that ultimately decay to form the detritus, and three to five times the concentrations in larger undecayed pieces.

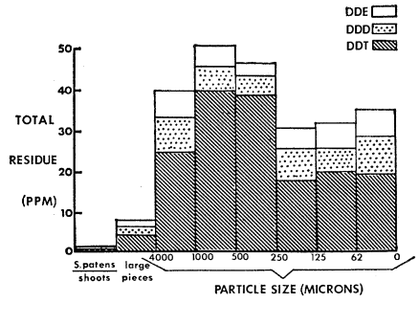

Fig. 1. Distribution of DDT residues in organic detritus of the Carmans River marsh, Brookhaven, Long Island, New York. The sample was pooled from four cores, each 5 cm long.

We selected the 500- to 1000-μ fraction for a survey of the distribution of DDT and its metabolites (DDE and DDD). Samples were collected at the edges of mosquito control ditches and in the sediments of shallow waters, in and adjacent to the marsh. Total residues in this size class in ten samples from the ditches ranged from 1.44 to 51.93 ppm, with a mean and standard deviation of 13.85 ± 19.24 ppm. The ten samples taken from submerged sediments ranged from 0.69 to 18.81 ppm, with a mean of 8.77 ± 8.0 ppm. Wilcoxson's nonparametric two-sample test showed that the probability is 95 percent that there is no significant difference between the two groups. This means that the detritus particles had not lost their associated pesticide residues when they entered the water. The DDT residues were present in material that was readily available to both deposit and filter feeders.

To determine whether DDT residues attached to organic particles could be transferred to consumers of detritus, we conducted an experiment on 45 fiddler crabs collected in a marsh on the north shore of Long Island. These crabs no longer occur on the Carmans River marsh on the south shore. Muscle of the large claw, the only tissue analyzed, was chosen to eliminate the possibility that surface contamination contributed to the DDT residues.

Fifteen crabs were killed immediately; DDT residues in their claw muscle averaged 0.235 ppm (51 percent DDT, 19 percent DDE, 30 percent DDD). The remaining 30 crabs were divided into control and experimental groups of 15 crabs each. Both groups were placed in 25-gallon (94.6-liter) aquaria containing equal volumes of sifted detritus with particles no larger than 500 μ. The control group received detritus (containing 0.01 ppm DDT residues) from the north shore where the crabs had been collected. The experimental group received detritus (containing 10.0 ppm DDT residues) from the Carmans River marsh.

The two groups of crabs were kept for 10 days under identical conditions. Filtered water without measurable amounts of pesticide was added each day to compensate for evaporation. Crabs were often observed feeding on the detritus; no other food was present. All crabs lived to the end of the experiment. Those in the control tank appeared normal in all respects, but

some crabs in the experimental tank developed poor coordination on day 4. By day 5 all experimental crabs were uncoordinated. Instead of scurrying away when threatened by a hand, as usual with the control crabs, they moved a few centimeters, lost coordination, and rolled over once or twice before regaining equilibrium. This was repeated several times as the crab moved 10 or 15 cm. Such awkward and sluggish behavior is unusual and would almost certainly affect survival under natural conditions.

On day 11, the crabs were killed and the muscles of the large claw were analyzed. Controls showed no change in residue concentration (0.240 ppm) or metabolite distribution. The mean of the DDT residues found in the crabs that had fed on the contaminated detritus was 0.885 ppm (34 percent DDT, 58 percent DDE, 8 percent DDD), representing a threefold increase during the 10-day experiment. Wilcoxson's two-sample test showed that the probability was 95 percent that this difference was not due to chance. The poor coordination coincided with the increase in residues within the experimental group, a factor that may explain the disappearance of fiddler crabs from the Carmans River marsh more than a decade ago.

Organic detritus particles with their associated bacteria and other microorganisms in marsh sediments appear to be a reservoir of DDT residues in the environment, small particles sometimes containing residues thousands of times greater than the concentration occurring in water. Fiddler crabs assimilate and concentrate these DDT residues in their muscle tissues from the organic detritus, a process that probably also occurs among other marsh inhabitants.

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6. The DDT residues include DDT and its decay products (metabolites) DDE and DDD: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; DDD, also known as TDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane.
7. Plant detritus consumers include: amphipods, isopods, harpacticoid copepods, various filter- and deposit-feeding bivalves, annelid worms, caridean and penaeid shrimp, fiddler crabs, and fish such as the mullets (*Mugil* spp.).
8. Generally less than 5 percent of these plant materials is consumed on the stalk by herbivores, leaving 95 percent to the detritus food chain [A. E. Smalley, *Ecology* **41**, 785 (1960); E. J. Heald, personal communication].
9. Use of DDT by the Suffolk County Mosquito Control Commission was halted in 1966 by court injunction and later by agreement of the commission when local scientists demonstrated that the accumulation of DDT residues was deleterious to diverse natural resources of the area (*2*).
10. Inorganic particles were separated by washing each size class in a beaker; the organic debris, being lighter, was decanted. The technique could not be used with particles smaller than about 50 μ, but examination showed that more than 95 percent of these fine particles were organic matter.
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Encephalitogenic Protein: Structure

Abstract. *Amino acid sequences of encephalitogenic proteins from bovine cord and rabbit brain are reported. The bovine protein contains 45 residues. The rabbit protein is identical except for two isopolar substitutions, a dipeptide and amino acid deletion. Analysis of this protein and a 140-residue myelin basic protein indicates that the smaller protein is a portion of the larger encephalitogen. The larger myelin protein contains at least two encephalitogenic sites.*

Experimental allergic encephalomyelitis (EAE) was initially produced in animals by injection of whole central nervous system tissue (*1*). It has now been shown that the encephalitogenic activity of whole tissue resides in a basic protein (or proteins) of myelin (*2*).

We have reported the isolation and partial amino acid sequence of proteins of small molecular weight (4700) from bovine and rabbit central nervous system tissues that are encephalitogenic in rabibts (*3, 4*). The full sequences of these proteins have now been ascertained, and their relation to the basic protein of myelin has been established.

Isolation of the proteins used in these structural studies has been described (*3*). Bovine spinal cord or rabbit brain was defatted in a mixture of acetone and ether and extracted in sodium citrate buffer, *p*H 4.3; the extract was then chromatographed on carboxymethyl-cellulose and Sephadex G-50.

Twenty-four-hour tryptic and chymotryptic digests of the protein were separated by two-dimensional chromatography and electrophoresis. The papers were stained with 0.01 percent trinitrobenzenesulfonic acid (TNBS) in butanol, developed in pyridine vapor, and viewed against a black light (*5*). As soon as the spots became visible they were eluted from the paper in 0.01*N* HCl. Excess TNBS was removed by chromatography on Sephadex G-10. The following procedures were carried out on each peptide: hydrolysis in 6*N* HCl at 110°C for 24 and 96 hours; amino-terminal analysis by the fluorodinitrobenzene method; analysis of amino acid sequence by the subtractive Edman degradation; and timed digestion with leucine aminopeptidase and carboxypeptidases A and B. All amino acid analyses were carried out on the Beckman-Spinco model 120C analyzer used in an accelerated system (*6*).

The results are shown in Fig. 1. All seven of the primary tryptic peptides except T_2 and T_6 were sequenced in a straightforward manner. The sequence of peptide T_2, Gly-Ala-Pro-Lys, was assumed to be correct since leucine aminopeptidase should not have removed glycine if the order were Gly-Pro-Ala-Lys. Peptide T_6 proved resistant to attack by both carboxypeptidase and the Edman degradation, and digestion with leucine aminopeptidase was limited. This problem was resolved by treatment of the peptide with 0.03*N*