

Distribution of Heptachlor Residues in Pond Ecosystems in Southwestern Virginia

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Thirty-five farm pond ecosystems in southwestern Virginia were examined for heptachlor residues. The data showed that every watershed held heptachlor residues, although in amounts less than 300 p.p.b. The only chlorinated hydrocarbon detected besides heptachlor and its

epoxide was DDT. Pond water contained residues only in the spring sampling period, while soil residues decreased during the summer months. Results showed that heptachlor is a persistent insecticide with residues detected up to 25 months after application.

Heptachlor had been widely used for a number of years to control the alfalfa weevil, *Hypera postica* (Gyllenhal), in southwestern Virginia. In 1964, heptachlor treatment of alfalfa was discontinued, and thus, an opportunity became available to study the residual properties of heptachlor under agricultural conditions. The specific purpose of the present study was to determine the existence and quantification of heptachlor in farm pond water, flora, fauna, pond bottom mud, and watershed soil.

Experimental Procedures

Thirty-five ponds and their respective watersheds were selected in three southwestern counties (Montgomery, Wythe, and Pulaski) with the aid of the county personnel of the Soil Conservation Service, United States Department of Agriculture.

Although most of the alfalfa fields had heptachlor applied either as granules in the fertilizer, or by tractor-mounted sprayers, some of the fields received both types of treatment. Most of the alfalfa was treated with heptachlor from 1959 through 1963. The watersheds averaged 144 acres with a mean alfalfa acreage of 14.5. The distance from the pond to the site of heptachlor application varied from 10 to 1500 feet, the average being 500 feet.

Sampling Technique. Fourteen ponds were sampled in the winter of 1964, and in the spring, summer, and fall of 1965. Twenty-one other ponds were sampled only in the early summer and late summer of 1965.

Five water samples and five bottom mud samples were selected at random from each pond and composite soil samples were taken at the pond edge, 100 and 300 feet from the pond and in a direction leading toward the site of heptachlor application.

Bottom mud samples were taken with an Eckmann dredge and included the bottom organisms present in the mud.

Analysis of Residues. A 300-gram sample of mud (or soil) was extracted with a *n*-hexane-isopropyl alcohol mixture (3 to 1) by tumbling the solvents and sample for 4 hours at 30 r.p.m. The solvents were decanted,

and the alcohol was removed by extraction with distilled water. The hexane was then evaporated just to dryness and the residue taken up in methylene chloride. The sample was then cleaned up with the use of the "No. 5" column and acetonitrile-hexane partitioning procedure described by Samuels (1966).

An 800-ml. portion of pond water was extracted with 150 ml. of petroleum ether and isopropyl alcohol (2 to 1) by shaking for 1 minute in a 1000-ml. separatory funnel. Three more extractions of the sample were made using 75 ml. of the solvent mixture. The petroleum ether from these extractions was set aside. Another 800-ml. aliquot from the same sample was extracted in the same manner and combined with the first portions. The ether extract was then cleaned up by using the above mentioned procedure of Samuels (1966).

The entire plant sample was macerated in a Hobart food chopper, and a 10-gram portion of the sample was blended with 100 ml. of 95% ethyl alcohol and water (1 to 1) for 2 minutes with an Omni-Mixer. One hundred milliliters of *n*-hexane was added, the sample was blended for 2 minutes, transferred to a 500-ml. centrifuge bottle, and centrifuged at 1500 r.p.m. for 5 minutes. The *n*-hexane was siphoned off, and an additional 50 ml. of *n*-hexane was added to the sample. The sample was shaken for 1 minute and recentrifuged. Final cleanup was accomplished by the previously mentioned procedure of Samuels (1966).

The method of Stemp *et al.* (1964) was used for preparing animal tissues for analysis.

A 3- to 5-gram subsample of each mud and soil sample was dried at 105° C. for 24 hours in a forced-air oven for moisture determinations. All residues are reported on a dry weight basis.

Pesticide residues were determined using the Micro-Tek Model 220 gas chromatograph equipped with a Dohrmann C-200 microcoulometric chloride ion detector. Spot checks for identification of pesticide residues were made with thin-layer chromatography using the method of Kovacs (1963).

Recovery Data. Recovery tests showed that the pesticides were being extracted satisfactorily. Results indicated a recovery of at least 80% in all samples with the exception of mud where recovery was approximately 70%.

The thin-layer chromatograms confirmed the presence of pesticides detected by the gas chromatographic system.

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Results and Discussion

Despite the variations observed in residue levels from different ponds, certain general distribution patterns or trends did emerge from the study.

Residues in Soil. The epoxidation of heptachlor has been noted in both soil and living organisms (Lichtenstein and Schultz, 1965; Westlake and San Antonio, 1960). Heptachlor epoxide was detected in every watershed. These residues ranged from less than 1.0 to 291 p.p.b. Only eight of the 35 watersheds contained heptachlor, with 24% of the residues below 20 p.p.b. The highest levels were in soil samples taken directly from the alfalfa fields. Two of the watersheds contained DDT residues of 18.3 and 67.2 p.p.b. despite the absence of any record of previous DDT application.

Residues in Mud. Residues in the pond bottom mud were lower than those in the watershed soil. Heptachlor epoxide residues were found in mud samples from 14 of the 35 ponds, and these residues ranged from less than 1.0 to 59.9 p.p.b. Heptachlor residues (less than 5.0 p.p.b.) were detected in a few ponds.

Residues in Water. Only 10 of the 35 ponds contained residues in the water. The highest heptachlor epoxide residue was 10.8 p.p.b., while heptachlor residues never exceeded 5.0 p.p.b. A few ponds contained DDT, but only in trace amounts (less than 1.0 p.p.b.).

Residues in Plants. Various aquatic plants were sampled, but none showed the presence of any chlorinated hydrocarbons. The plants analyzed included the following genera: *Lemna*, *Typha*, *Carya*, *Potamogeton*, and *Spirogyra*.

Residues in Fish and Animal Tissues. Three bass (*Micropterus salmoides salmoides*, Lacopede) and three blue gills (*Lepomis macrochirus*, Rafinesque) taken from ponds containing residues were devoid of any chlorinated hydrocarbons. A snapping turtle (*Chelydra serpentina*) taken from a pond that had no residues in the water or bottom mud contained a total of 5100 p.p.b. of heptachlor epoxide in the body fat, egg yolk, and liver tissues (Table I).

The small sample size probably does not reflect accurately the residues present in the fish populations of these ponds. Cottam (1965) reported that fish are capable of concentrating pesticides from the surround-

ing water. A larger sample size could have shown this expected concentrating ability by the fish. On the other hand, the turtle was not restricted to the pond and could have come in closer contact with the pesticide elsewhere.

Seasonal Variation of Residues. Considering all samples from the ponds sampled seasonally (14 ponds), there was a seasonal difference among the samples containing residues. The highest percentage of samples showing residues was found in the winter. This percentage decreased through spring and summer and increased slightly in the fall. Bailey and White (1964) showed that low rainfall increased the adsorption of pesticides onto soil particles. This adsorption could partly account for the low percentage of samples containing residues during the summer months (Figure 1), though the length of time since the heptachlor application was probably the largest factor governing the number of samples containing residues. That the seasonal effect might be cyclic is suggested by the data, though the seasonal fluctuations would probably be less apparent in subsequent sampling periods because of the increasing time since heptachlor application.

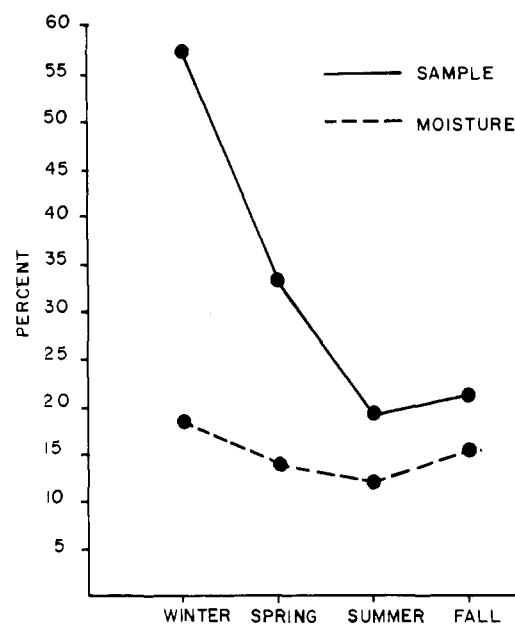


Figure 1. Effect of season on moisture content of soil samples and per cent of soil samples containing residues

Table I. Residues of Heptachlor Epoxide in Faunal Samples in P.P.B.

Pond	Species	Homogenate	Fat	Egg Yolk	Liver	Striated Muscle
6	Blue gill	None
17	Blue gill	None
19	Blue gill	None
24	Bass	None
24	Bass	None
24	Bass	None
35	Snapping turtle	...	3471	1134	504	None

Residues in mud samples decreased throughout the study (Figure 2) perhaps reflecting the more stable environmental conditions in the pond bottom. The water samples contained residues only in the spring. This fact was attributed to spring runoff.

Apparently, pesticide residues were not evenly distributed throughout the pond water and pond mud. In fact, 10 of the ponds showed residues in only one of the water or mud samples taken, and only five ponds had residues in all mud and water samples analyzed in a given season. If the pesticide was concentrated by the plankton in these waters, its uneven distribution, due to wind action and the settling of debris to the pond bottom during the late fall dieoff, may have helped to account for both the high percentage of samples containing residues in the winter mud samples and the "pocketing" effect. Spring rains and accompanying silt may have buried and diluted these residues to account for the fewer samples containing residues in subsequent samples.

Efforts to document the movement and occurrence of residues in the ponds with environmental factors yielded no apparent correlations. Dean (1960) found that under laboratory conditions different soils had different degrees of attraction for pesticides. Three general soil types were present on the watersheds studied. These soils were derived from shale, sandstone, or crystalline rock. In this study, no correlation between soil type and movement of residues through the soil was detected. Other factors such as slope, vegetative cover, and sample size may have masked any correlation. Despite these variations, the residues in the pond ecosystem decreased when the pond's distance from the treated alfalfa increased (Figure 3).

Limnological studies on these ponds were conducted for possible correlations with the presence and movement of pesticide residues. Determination of major anions and cations in the water was made, but the fluctuations of these species in no way reflected the behavior of pesticide residues. Bottom organisms were also examined; however, they seemed to exist independently of pesticides in the ecosystem and revealed, in general, the eutrophic nature of the water. These efforts to equate pesticide residues in pond water and mud with other factors in the ecosystem show that generalizations are not evident.

Epoxidation of Heptachlor. As previously stated, the epoxidation of heptachlor is known to occur in the soil (Lichtenstein and Schultz, 1965). Nearly 10% of the pond edge soil, bottom mud, and water samples contained heptachlor residues, while less than 3% of the watershed soil samples held residues. Westlake and San Antonio (1960) state that the mechanism for epoxidation is not known. Bottom organisms dredged in this study were mostly chironomid larva and tubificid worms. The presence of these organisms suggests polluted waters and low oxygen levels (Hynes, 1963), which may influence the slower epoxidation rate in the water and mud samples.

The conversion of heptachlor to heptachlor epoxide in the soil occurred at a faster rate in this study than in other studies (Barthel *et al.*, 1960; Lichtenstein and

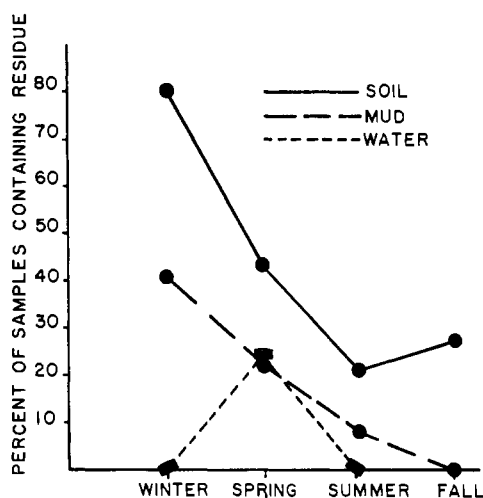


Figure 2. Per cent of water, mud, and soil samples containing detectable (>1.0 p.p.b.) heptachlor epoxide residues by season in ponds 1 through 14

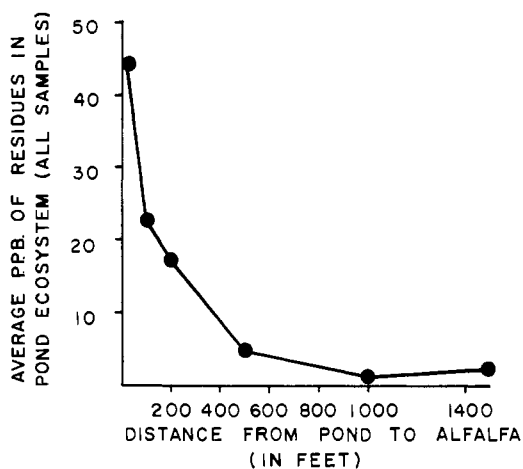


Figure 3. Correlation between average p.p.b. of heptachlor epoxide in the pond ecosystem and the distance of the treated alfalfa from the pond (data from all ponds)

Schultz, 1965). Since the vegetative cover on most of these watersheds, other than the alfalfa, was overgrazed pasture, this lack of cover would enhance both volatilization and epoxidation of heptachlor (King *et al.*, 1966).

Conclusions

These data support the concept that heptachlor is a persistent insecticide when used under normal agricultural conditions since residues were found in every ecosystem studied at periods up to 25 months after application. However, these residues were low, with only a snapping turtle having residues in excess of 300 p.p.b. With the exception of small amounts of DDT, no other chlorinated hydrocarbon pesticides were found.

Levels of heptachlor and its epoxide were somewhat variable owing to unknown factors in the ecosystem causing a pocketing behavior.

Residues were considerable distances from the site of application, demonstrating that substantial movement of heptachlor had occurred.

The tendency for heptachlor residues to move and persist for long periods of time was observed under usual agricultural conditions.

These data, based on 1200 residue analyses and one year of field investigations, point to the large numbers of samples required to locate accurately, quantify, and describe the behavior of residues in a pond ecosystem.

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