Chlorinated Hydrocarbon Insecticide Residues in Selected Insects and Birds Found in Association with Cotton Fields

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Insecticide residues were detected in 10 of the 15 insect species examined. The most common insecticide residues were *p,p'*-DDT and its metabolite *p,p'*-DDE. The concentrations ranged from 0.10 to 9.16 p.p.m. and from a trace to 12.89 p.p.m., respectively. Aldrin, dieldrin, endrin, heptachlor epoxide, chlordane, and toxaphene also were present in some samples. Laboratory studies on the persistence of DDT in *Heliothis* spp. showed the fast disappearance of DDT, followed by the disappearance of DDE. There was no change in the level of *p,p'*-DDE in diapausing bollworm pupae over a period of 5 months. All of the eight bird species examined showed residues of *p,p'*-DDT and/or *p,p'*-DDE with concentrations ranging from 0 to 132.50 p.p.m. and from a trace to 37.50 p.p.m., respectively. In most cases, the fat tissue contained higher levels of residues than the other tissues.

Chlorinated hydrocarbon insecticides have been used extensively during the past 20 years in controlling various insect pests. As a result, contamination of the environment by these residual insecticides is almost universal (George, 1963).

Cotton fields represent an ecological area where large amounts of chlorinated hydrocarbon pesticides are used annually. Exactly how much of these chemicals are present in beneficial (predaceous) and pest (phytophagous) insects of cotton, nearby aquatic insects, and birds feeding in cotton fields on insects, nectar, and seeds is unknown. In order to establish the magnitude of contamination and to obtain basic information on the residue problem, a survey of the chlorinated hydrocarbon insecticide residues in selected insects and birds found in association with cotton fields in Louisiana was undertaken.

EXPERIMENTAL

Collection and Preparation of Samples. Insect samples were collected from six locations in Louisiana during the period from July 19 to Nov. 12, 1964. Samples collected at Alexandria and Curtis were from cotton fields. Collections at Baton Rouge were from an area used by Louisiana State Agricultural Experiment Station on which pesticides have been applied annually for many years. Samples from Krotz Springs and Washington were from within the town limits. Washington is a diversified farming area where chlorinated hydrocarbons are used each year. Krotz Springs is on the Atchafalaya River several miles from any agricultural operation. The collections from Bayou Sorrel were removed by several miles from any row-cropping areas.

Insects were collected primarily through the use of light traps and/or sweep nets. Many insects were collected by hand around light traps, street lights, and store windows. A total of 15 species with various feeding habits from both aquatic and terrestrial habitats was collected. Samples were held in polyethylene freezer bags and frozen until they could be extracted. The only exception was a sample of diapausing bollworm pupae which was held for 5 months under a 10-hour light and a 14-hour dark photoperiod regimen at 20° C.

Bird samples were collected from three locations in Louisiana by shooting the animals as they fed or rested in or adjacent to cotton fields. All three locations (Alexandria, Curtis, and St. Joseph) are in cotton-producing areas where annual insecticide use is heavy. A total of eight species were collected during a period from Aug. 8 to Oct. 23, 1964. Most bird species were dissected and the following tissues collected: fat, brain, kidney, heart, liver, gizzard, and muscle. A representative sample of each tissue was weighed and frozen until it could be extracted. Some samples consisted of the whole bird except for the head, feathers, and legs below the femur.

In the case of both the insects and the birds, a sample consisted of all of one species collected on a given date at a given locality. The only exception was the combining of the hummingbird samples from Alexandria and St. Joseph. The samples varied in size from 0.5 gram for some of the small insects to about 1 kg. for some of the birds.

Persistence of DDT in Two Species of *Heliothis*. In an effort to ascertain how long *p,p'*-DDT and its metabolite *p,p'*-DDE persist in *Heliothis*, 40 μg. of *p,p'*-DDT was applied topically to fifth and sixth instar bollworms, *H. zea*, and tobacco budworms, *H. virescens*, using the technique described by Graves *et al.* (1963). The insects were reared using the procedures and diet described by Berger (1963).

After treatment the larvae were held until pupation, and a sample of these pupae was removed for analysis. The remaining pupae were held until the moths emerged. A sample of the emerging moths was removed and held for analysis. The remaining moths were placed in cages where mating and oviposition occurred. The eggs were oviposited on cheesecloth. A sample of the eggs was separated from the cheesecloth by dipping first in a 5.25% sodium hypochlorite for about 3 seconds and then in distilled water. The eggs were removed from the water by filtration and air dried. The main portion of the eggs was allowed to hatch, and larvae were allowed to develop until the fifth and sixth instars when another sample was obtained. Control sam-

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ples were taken at these same intervals from a corresponding untreated culture.

Extraction and Cleanup Procedures. Acetonitrile extraction procedure and Florisil column cleanup were used in this study (Mills *et al.*, 1963). The extraction method was modified as follows: Up to 10 grams of the sample was added to a 250-ml. homogenization cup containing 30 ml. of acetonitrile, then mixed at high speed for 1 to 2 minutes using a Virtis 45 homogenizer and filtered with suction. The sample was re-extracted twice by blending with 15 ml. of acetonitrile and filtering. The combined contents of the suction flask were transferred to a 1-liter separatory funnel containing 450 ml. of distilled water and 4 to 5 ml. of concentrated hydrochloric acid. The acetonitrile was mixed with the acidified water and extracted three times with 24 ml. of redistilled petroleum ether, shaking for 1 minute each time. The petroleum ether extract was combined in a 250-ml. beaker and evaporated, then passed through a funnel (5.5-cm. O.D.) packed with a 1-inch layer of anhydrous sodium sulfate. The extract was then cleaned up using Florisil chromatography if needed, adjusted to volume, and injected into the gas chromatograph (Bonner, 1965).

Gas Chromatography. A Micro-Tek Model GC 2000-R gas chromatograph employing an electron-capture detector was used for residue analysis. The gas chromatograph was connected to a Westronics Model LS 11 A/MAZ-1/DV 7.5 H strip chart recorder equipped with a disk chart integrator Model 222. Glass U-shaped 3-foot × 1/4-inch O.D. columns were employed in the analysis. The columns were packed with Chromosorb coated with 5% Dow 11 silicone. All tests were run with a carrier gas mixture of 95% argon and 5% methane. The operating conditions of the apparatus were as follows:

Detector temperature	156° C.
Oven temperature	200° C.
Inlet temperature	210° C.
Attenuation	16
Chart speed	30 inches per hour

Injection of microliter quantities were made with a Hamilton 10-μl. syringe. The sensitivity level was in the range of 0.01 p.p.m. for most insecticides. Two analyses were made for each sample and the results averaged. The range in the results was generally less than 10%.

Thin-Layer Chromatography. Thin-layer chromatography was used to verify the qualitative phase of the results. The TLC technique described by Kovacs (1963) was used. The method was modified to include the prewashing of the absorbent material before coating the plates. Spraying with the chromatographic reagent was eliminated (Damaska, 1964).

RESULTS AND DISCUSSION

Residues of one or more chlorinated hydrocarbon insecticides occurred in 10 of the 15 insect species examined (Tables I and II). Of the five species where no residues were detected (Table III), three were collected at Bayou Sorrel. The three species involved were: the cone-headed grasshopper, the dog-day cicada, and the giant water bug. The absence of detectable residues in these samples was probably due to the fact that Bayou Sorrel is somewhat removed from row-cropping areas. The other two species in which no residues were detected were *Anax junius*, a dragonfly collected in small numbers at Alexandria and Baton Rouge and a species of damselfly of the genus *Enallagms* collected at Alexandria.

Table I. Insecticide Residues in Some Insects from Various Locations in Louisiana during 1964

Sample	Locality	Residues, P.P.M. *p,p'*-DDE	*p,p'*-DDT
	LEPIDOPTERA		
Noctuidae:			
Bollworm moths, *Heliothis zea*	Alexandria	1.02	0.00
Bollworm pupae	Curtis	1.17	0.00
Tobacco budworm moths, *H. virescens*	Alexandria	0.38	0.00
Cabbage looper moths, *Trichoplusia ni*	Baton Rouge and Krotz Springs	Trace[a] 2.85	0.00 0.00
	COLEOPTERA		
Carabidae:			
Calosoma alternous	Alexandria	2.93	4.34
	Krotz Springs and Washington	Trace[b] 0.15[a]	0.00 0.00
Harpalus pennsylvanicus	Alexandria and Krotz Springs	5.25 12.89	0.10 0.00
Agonoderus lecontei	Baton Rouge and Curtis	0.00 0.70	9.16[a] 0.00
Hydrophilidae:			
Tropisternus lateralis	Alexandria and Washington	2.48 0.12	0.00 0.69
Hydrophilus triangularis	Baton Rouge and Washington	Trace 0.22	4.32[a] 1.32[a]
Dytiscidae:			
Thermonectes basilaris	Alexandria and Washington	0.16 2.58	0.00 0.00

[a] Toxaphene present in unmeasurable amounts.
[b] Aldrin residues present at the level of 0.52 p.p.m.

Table II. Insecticide Residues in Adult Mayflies (Ephemeroptera) in Louisiana during 1964

Residues, P.P.M.	Krotz Springs	Alexandria
p,p'-DDE	0.38	0.0
Dieldrin	0.21	0.0
Endrin	0.46	0.0
Heptachlor epoxide	0.02	0.0
Chlordane	Trace	0.0
Toxaphene	0.00	Trace

Except for a trace of toxaphene in a sample of cabbage looper moths, the only residue detected in lepidopterous insects was *p,p'*-DDE (Table I). These residues were associated with the place of collection since most of the samples that contained *p,p'*-DDE came from cotton fields. Diapausing bollworm pupae dug from the soil of a cotton field and sampled on Nov. 12, 1964, and April 12, 1965, for insecticide residues contained the same amounts of *p,p'*-DDE (1.17 p.p.m.). Thus, apparently residues can persist in diapausing pupae for a long period of time with little or no diminution under these special conditions.

To understand better the persistence of DDT and its metabolite DDE in *Heliothis* spp., a laboratory experiment was undertaken in which fifth and sixth instar larvae were treated with 40 μg. of *p,p'*-DDT. Analysis of the resulting

Table III. Insects From Various Locations in Louisiana during 1964[a]

Sample	Locality	Sample	Locality
Bollworm moths, *Heliothis zea*	Alexandria and Krotz Springs	Cone headed grasshopper, *Neoconocephalus ensiger*	Bayou Sorrel
Dragonfly, *Anax junius*	Alexandria and Baton Rouge	Dog day cicada, *Tibicen chloromera*	Bayou Sorrel
Damsel fly, *Enallagms* sp.	Alexandria	Giant water bug, *Lethocerus uhleri*	Bayou Sorrel
Ground beetle, *Harpalus pennsylvanicus*	Bayou Sorrel		

[a] Insects were free of insecticide residues at the level of sensitivity and sample size used (0.5 to 3 grams).

Table IV. Insecticide Residues in Ruby-Throated Hummingbirds, *Archilochus colubris*, and Eastern Meadowlarks, *Sturnella magna*

Sample	Locality	Residues, P.P.M.[a] *p,p'*-DDE	*p,p'*-DDT
Ruby-throated hummingbird	Alexandria and St. Joseph	0.01	0.08
	Curtis[b]	0.26	0.08
Eastern meadowlarks	St. Joseph	0.08	0.02

[a] Whole carcass.
[b] Aldrin present at the level of 0.43 p.p.m.

pupae (about 5 days later) revealed *p,p'*-DDE at a level of 5.05 p.p.m. but no *p,p'*-DDT. Apparently, DDT is changed quite rapidly to DDE and other metabolites. Also the amount of *p,p'*-DDE in the treated pupae was about four times greater than in the diapausing pupae dug from the soil of a cotton field. Analysis of moths resulting from the treated larvae revealed that *p,p'*-DDE was still present although the amount was reduced (3.46 p.p.m.). Again this level exceeds that in moth samples collected in the field by about threefold. Analysis of eggs laid by the moths resulting from treated larvae showed no detectable residue of *p,p'*-DDE. However, Atallah and Nettles (1966) found *p,p'*-DDE and *p,p'*-DDT in eggs of a lady beetle, *Coleomegilla maculata*, 5.5 days after topical treatment with *p,p'*-DDT.

Residues were generally higher in the carabids (two predaceous species and one seed feeder, *Harpalus pennsylvanicus*) than in the lepidopterous moths. Similar data were presented by Giles and Peterle (1964). One reason might be traced to the feeding habits of the two groups. Residues in aquatic insects were somewhat higher than those in the lepidopterous insects and lower than those in carabids (Table I). There was no apparent difference in the residue picture of two species of water scavenger beetles (*Tropisternus lateralis* and *Hydrophilus triangularis*) that supposedly feed principally on organic matter and one species of predaceous diving beetle (*Thermonectes basilaris*). Five different insecticide residues were in a sample of Mayflies collected at Krotz Springs. Only toxaphene was found in a small sample from Alexandria (Table II). The residue complex in the sample from Krotz Springs can be attributed to the very large sample size (approximately 400 times greater than the other Mayfly sample and most of the other insect samples).

The principal residues occurring in field-collected insects

Table V. Insecticide Residues (P.P.M.) in Red-Winged Blackbirds, *Agelaius phoeniceus*, in Cotton Fields at Various Locations in Louisiana during 1964

Sample	Alexandria Aug. 9 *p,p'*-DDE	Alexandria Oct. 23 *p,p'*-DDE	Alexandria Oct. 23 *p,p'*-DDT	Curtis Sept. 16 *p,p'*-DDE	Curtis Sept. 16 *p,p'*-DDT	St. Joseph[a] Oct. 15 *p,p'*-DDE
Fat	...	4.01	9.20	34.54	0.00	6.81
Brain	...	9.35	7.40	9.28	10.61	0.57
Kidney	1.50	Trace	15.62	2.03	0.00	2.40
Liver	4.56	3.58	11.82	7.24	4.83	0.43
Heart	6.67	Trace	9.35	11.29	0.00	1.53
Gizzard	1.51	1.91	4.07	2.79	0.00	5.83
Muscle	4.09	1.73	2.50	5.15	0.00	2.33
Av. of all tissues	3.66	2.94	8.56	10.33	2.20	2.84

[a] Toxaphene present in all tissues at unmeasurable levels.

Table VI. Insecticide Residues (P.P.M.) in Some Birds Near St. Joseph, La.

Sample		Tissue Sampled: Fat	Brain	Kidney	Liver	Heart	Gizzard	Muscle	Av. of All Tissues
Common grackle, *Quiscalus quiscalus*	*p,p'*-DDE	22.10	1.82	0.00	1.13	0.69	1.97	1.24	4.13
	p,p'-DDT	31.92	17.50	14.00	6.18	15.00	16.27	7.41	15.48
House sparrow, *Passer domesticus*	*p,p'*-DDE	37.50	0.00	1.94	5.94	0.46	1.25	2.43	7.07
	p,p'-DDT	132.50	0.00	0.00	0.00	48.35	35.00	31.31	35.31
Swallows[a]	*p,p'*-DDE	0.44	Trace	0.12	0.24	0.50	0.14	0.14	0.24
	p,p'-DDT	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.05

[a] A mixture of tree swallows, *Iridoprocne bicolor;* barn swallows, *Hirundo rustica;* and bank swallows, *Riparia riparia.*

were *p,p'*-DDT and its metabolite, *p,p'*-DDE. This is in agreement with practically all of the residue data (Bridges and Andrews, 1961; DeWitt *et al.*, 1963) available in the literature regardless of the species or part of the environment examined.

All bird samples collected contained insecticide residues. As in the insects, the most common residues were *p,p'*-DDT and its metabolite *p,p'*-DDE. Generally, the residues were higher in birds than in insects.

Ruby-throated hummingbirds and Eastern meadowlarks (Table IV) contained comparatively low levels of residues. Probably this was correlated to their feeding habits. The hummingbirds feed on nectar while the meadowlarks feed primarily on insects and to a lesser degree on seeds. These two species were analyzed on a whole bird basis less the head, feathers, and legs below the femur.

All of the other bird samples were dissected and seven tissues analyzed for residues (Table V and VI). Except for the fat tissue, there was no apparent pattern in the amount of residues in the various tissues. The fat tissue, in most cases, contained higher levels of residues than the other tissues. Insecticide residues in red-winged blackbirds from three locations are shown in Table V. The reasons for the variance in the residue pattern are unknown. However, chlorinated hydrocarbon insecticides (principally toxaphene and DDT) were used in the fields where the birds were collected and birds were observed to feed on insects in the treated fields. The house sparrow sample (Table VI) collected at St. Joseph contained the greatest amount of residues in any of the bird samples. Residues of *p,p'*-DDE and *p,p'*-DDT in the fat tissue were 37.5 and 132.5 p.p.m., respectively. The birds were collected in a cotton field at the Northeast Louisiana Branch Experiment Station as they fed on treated *Heliothis* larvae.

The fairly high levels of residues in grackles (Table VI) was also probably due to their activity in cotton fields treated with insecticides. These birds were observed to perch on cotton stalks and roam about under the cotton plants. Residue levels in three species of swallows (analyzed as a single sample) were comparatively low (Table VI). These were probably migratory birds that had only recently arrived in the area. With the exception of swallows and the hummingbirds, all of the bird samples were considered to be from local populations.

The level of *p,p'*-DDT in the various bird samples would appear to be far below that causing mortality. DeWitt *et al.* (1962) reported that the approximate lethal doses for young bobwhite quail, young pheasants, and young mallards were 1600, 1100, and 1600 p.p.m., respectively. Levels of DDT stored in tissues of birds have been shown to be proportional to amounts in the diet (Newsom, 1967).

The residue levels established in this study for birds feeding in and around cotton fields do not appear to differ greatly from the levels reported from other parts of the United States involving numerous species and habitats (Ames and Mersereau, 1964; DeWitt *et al.*, 1964; DeWitt and Buckley, 1962; DeWitt *et al.*, 1963; Finley *et al.*, 1963; Pillmore *et al.*, 1964; Sheldon *et al.*, 1963; Wright, 1965).

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