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BIRD MORTALITY FOLLOWING DDT SPRAY FOR DUTCH ELM DISEASE

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Abstract. Avian populations in Hanover, N. H., a town that has sprayed its elms with DDT for many years in an attempt to control Dutch elm disease, were compared with those in Norwich, Vt., a town 1 mile (1.6 km) west of Hanover that has never sprayed. Hanover applied 1.9 lb DDT/acre (2.1 kg/hectare) in April 1963, then used Methoxychlor in April 1964. Population surveys were taken regularly during spring and early summer of these years, dead birds were collected in both towns, and 106 birds were analyzed for DDT, DDE, and DDD.

Severe mortality of both resident and migrant birds occurred in Hanover during spring 1963, and the evidence implicates DDT as its cause. Robin loss was estimated at 70% of the resident population, or 350 to 400 individuals, but mortality among other species of widely varied feeding habits was also substantial. Feeding habits suggest that some birds acquired the toxicant by eating living insects carrying DDT, presenting the paradox of survival of the intended DDT victims, and death, instead, of insectivorous birds.

Organ and whole bird analyses are presented and criteria for establishing cause of death are discussed. Most of the DDT had been converted to DDE and DDD, and residues were found in all organs analyzed.

Robin mortality was reduced, but not eliminated following Methoxychlor application in 1964; these losses were believed caused by residual DDT in the soil. There was no evidence DDT poisoning among other species in 1964, though the dead birds collected were not analyzed.

Introduction

In an attempt to control Dutch elm disease, the use of DDT[2] has become increasingly widespread in the United States during the past two decades. Dutch elm disease, caused by the fungus, *Ceratocystis ulmi,* is characterized by obstruction of the xylem vessels, and is usually fatal to elms (*Ulmus* sp.). The vectors are bark beetles (*Scolytus multistriatus* and *Hylurgopinus rufipes*) that breed in dead or dying elm wood and feed mainly on healthy elm twigs, thus inadvertently transmitting the spores to healthy trees. The New York State College of Agriculture showed that DDT gave limited control of the disease when sprayed under ideal circumstances (Matthysse 1959). At the same time, Matthysse described the superior control achieved by many cities in New York State employing sanitation, i.e., removal and destruction of dead and dying elm wood without use of insecticide. In contrast, Whitten and Swingle (1964) compared an untreated check plot with a study plot on which sanitation alone was used, and concluded that sanitation was not effective. They then sprayed the "sanitated" area and obtained good results. These conclusions are questionable, however, since it is impossible to separate the residual effects of sanitation from the effects of spraying. Furthermore, Matthysse (1959) showed that the beneficial effects of sanitation take several years to become fully evident. Whereas Doane (1958) demonstrated in the laboratory the ineffectiveness of DDT in preventing the emergence of bark beetles, or in killing them after emergence, Hafstad and Reynolds (1961) produced evidence that both DDT and Methoxychlor[2] as dormant sprays in the field were effective in killing the beetles as they fed on elm twigs brought into the laboratory. Welch and Matthysse (1960) concluded that sanitation "is always the first step in the practical control of the disease" and that "spraying is not recommended as a community-wide project."

Although the beneficial effects of DDT in con-

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[2] DDT = 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane
DDE = 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene
DDD = TDE = 1,1-dichloro-2,2-bis(*p*-chlorophenyl ethane
Methoxychlor = 1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl) ethane.

trolling Dutch elm disease are both uncertain and controversial, evidence of bird mortality as a result of DDT poisoning has become extensive. Shortly after initial use of DDT for Dutch elm disease control (Whitten and Parker 1945), reports of bird mortality following use of the chemical began appearing in the literature (Hotchkiss and Pough 1946; Coburn and Treichler 1946; George and Stickel 1949; Robbins and Stewart 1949; Benton 1951; Robbins, Springer, and Webster 1951; Blagbrough 1952; Mitchell, Blagbrough, and VanEtten 1953). In recent years the evidence has been greatly expanded by studies related to Dutch elm disease in the Midwest (Barker 1958; Mehner and Wallace 1959; Hickey and Hunt 1960a, b; Hickey 1961; Wallace, Nickell, and Bernard 1961; Wallace 1960, 1962; Bernard 1963; Wallace, Etter, and Osborne 1964). Wallace (1959) reported, for example, that by 1958, following several years of DDT application, elimination of the Robin (*Turdus migratorius*) from the campus of Michigan State University was virtually complete. Similarly, Hunt (1960) showed that breeding bird populations of many species were 31 to 90% lower in sprayed communities than in unsprayed areas. In several reports, DDT was directly implicated as a causative factor.

Both Wallace et al. (1961) and Hickey et al. (1960a) noted that no study comparable in scope to those of the Midwest had been done in the eastern USA. In fact, Wallace stated that DDT had been sprayed for many years in the East "apparently without the heavy mortality that has been observed in the Midwest." During recent years, however, residents of Hanover, N. H., a town that has sprayed its elms annually for about 15 years, have reported unusual numbers of dead birds, suggesting that abnormal mortality might have been occurring, that it may have been caused by DDT, and that Hanover was an ideal site in which to study the problem. This investigation was therefore undertaken to evaluate the effects of DDT on bird populations in Hanover, N. H., using Norwich, Vt., as an unsprayed reference area. Norwich is across the Connecticut River 1 mile (1.6 km) west of Hanover and has never had a community spray program. The study was conducted in three phases in Hanover and Norwich: population studies of birds; collection of dead or dying birds; and analyses of dead birds for DDT and its metabolites. A preliminary report of this investigation has been published (Wurster, Wurster, and Strickland 1965).

Materials and Methods

Spray procedures.—During the nights of 15 to 18 April 1963, approximately 1,285 lb (583 kg) of DDT were sprayed with a Bean Rotomist on 2,000 to 2,500 elms in Hanover. These trees occurred in an area of about 670 acres (271 hectares), or just over 1 sq mile, which included most of the town. Average application was therefore 1.92 lb/acre (2.15 kg/hectare), but, because of very uneven distribution of elms, DDT dosage varied widely from this average. As in past years, this was a dormant (prefoliar) spray. Composition of the spray, upon emergence as an emulsion from the Rotomist, was as follows: DDT, 11.5%; xylenes, 10%; methylated naphthalenes, 10.7%; inert materials, 1.2%; and water, 66.6%. Methoxychlor was sprayed during the nights of 13 to 18 April 1964, the composition and procedure being similar to the 1963 DDT application.

Population survey techniques.—Population studies during 1963 were performed on ecologically comparable areas in Hanover and Norwich. Hanover study areas (15 acres; 6 hectares) represented 2¼% of the sprayed portion of the town. Simultaneous surveys of these study areas were made by four observers, two in each town. All birds identified by sight or sound were counted. To minimize possible differences in efficiency of detecting birds, observers surveyed the areas in rotation. Thus each observer counted birds for an equal period with each of the other three observers, and spent the same amount of time in both Hanover and Norwich. Counts were made from 7 to 8 AM daily from April 8 until mid-May, every 2 to 3 days until mid-June, and at irregular intervals thereafter until July 13.

During 1964 surveys were done in two different ways. One method involved an observer in each town simultaneously walking a line transect along approximately 0.5 mile (0.8 km) of elm-lined street, noting all birds seen or heard. Observers again were rotated. Counts were made daily from April 13 until mid-June, then every 3 to 5 days until July 13. The other method consisted of an "automobile survey" specifically for Robins, and was performed entirely by one of us (D. H. W.). All Robins observed from a slowly moving automobile were counted along an ecologically similar 3-mile (5-km) route in each town, i.e., comparable lengths of elm-lined street, lawns, open fields, etc. This type of survey was conducted daily from April 13 to June 1, every other day until June 19, and every 3 to 5 days thereafter until July 13.

Collection, handling, and analysis of birds.—Dead or dying birds from both towns were collected from mid-April through July of both years, with the aid of residents responding to an appeal through two local radio stations and three newspapers. All birds were labeled with species, date, time and place found, condition of bird, and sex, and were then stored frozen until analysis. Only birds from 1963 were analyzed, except three reference Robins collected in April 1964. Because of the high cost of chemical analysis, not every bird could be analyzed, and the number of reference birds was necessarily limited. All birds observed with tremors before death, birds representing a variety of species and feeding habits, some birds showing injuries, and five reference Robins were analyzed. The latter were collected from unsprayed areas outside of Hanover, and included one dead 1963 specimen from Norwich.

A selection of 48 Robins was prepared for analysis as follows. Skin, beak, entire digestive tract, feet and wings (disjointed at ankle and elbow, respectively) were removed from the partially thawed bird and discarded. The brain, liver, breast muscle, heart, and gonads were dissected free, weighed and wrapped individually in foil, as was the remainder of the bird. The six small packets were then packaged together in foil and refrozen. Fifty-eight other birds, including 2 Robins and individuals of 24 other species, were skinned, stripped of legs, wings, and digestive tracts as described above, and analyzed as whole birds. The sum of the six Robin tissues is equivalent to a whole bird. Specimens were shipped frozen to the Wisconsin Alumni Research Foundation, Madison, Wis., for analysis of DDT, DDE, DDD[2], fat, and water content. Samples were analyzed by electron capture gas chromatography (Klein, Watts, and Damico 1963, with modification), with 17 random duplicate analyses; 6 cross checks were made with paper chromatography.

TABLE I. Dead and tremoring birds recovered from Hanover and Norwich, 1963 and 1964. Numbers in parentheses = no. of species represented. Hanover spray dates: DDT, 15-18 April 1963; Methoxychlor, 13-18 April 1964

Birds and localities	1963 – DDT Birds recovered, April 16 to July 30		1964 – Methoxychlor Birds recovered, April 14 to July 30	
	Total[a]	Tremors	Total[a]	Tremors
HANOVER (sprayed)				
Robin	61	19[b]	26	6
Chipping Sparrow	15	5	1	0
Myrtle Warbler	8	7[c]	0	0
Other species	67 (31)	3 (3)[d]	45 (25)	0
Totals	151 (34)	34 (6)	72 (27)	6 (1)
NORWICH (unsprayed)				
Robin	4	0	2	0
Chipping Sparrow	0	0	1	0
Myrtle Warbler	0	0	0	0
Other species	6 (6)	0	5 (5)	0
Totals	10 (7)	0	8 (7)	0

[a]Includes birds with tremors.
[b]Includes three Robins seen with tremors but not recovered.
[c]Includes two Myrtle Warblers seen with tremors but not recovered.
[d]One Song Sparrow, one Catbird, and one White-breasted Nuthatch.

The data were processed by a General Electric 235 computer at the Dartmouth College computation center.

RESULTS AND DISCUSSION

Recovery of dead and dying birds

In Table I the numbers of dead and dying birds collected in Hanover and Norwich during 1963 and 1964 are summarized. Reference to "tremors" means the loss of coordination, and excessive activity of voluntary muscles resulting in continuous fluttering of the wings and trembling of all parts of the body. This condition is typical of DDT poisoning (Bernard 1963), and usually progresses from a limited ability to fly or flutter along the ground to total disability and a short period of violent clonic convulsions before death. The corpse is thereafter immediately stiff with legs rigidly extended. Tremoring birds never survived, and usually died within 1 hour. All birds with tremors came from Hanover; no such birds were found in unsprayed areas. When sizable numbers of tremoring birds are observed in an area sprayed with substantial quantities of DDT, tremors may be considered strong evidence for DDT as the cause of mortality (Bernard 1963).

It is important to realize that only a small portion of the dead birds in a large area will be found. The difficulty of finding dead birds in the field was noted by Wallace (1962). Rosene and Lay (1963) experimented with planted dead Bobwhite Quail (*Colinus virginianus*); they concluded that finding even a small or moderate number of dead quail is reason to suspect a heavy mortality. The data in Table I therefore presumably represent only a minor portion of the total dead birds. Furthermore, recovery of small, obscure birds such as sparrows, warblers, or chickadees will be much lower than the recovery of large, conspicuous birds such as Robins (estimated below at a 16% maximum in Hanover in 1963).

Population studies

Determination of bird populations is difficult, especially when migration, nesting, and other seasonal factors are involved. Nevertheless, with daily surveys, the number of resident species and individuals in an area can be estimated with reasonable accuracy.

Robin populations.—It was evident that by early May, resident Robin populations had been established; to compare population changes between Hanover and Norwich, early May populations were equated to 100% in each town, and population trends are shown for 1963 (Fig. 1) and 1964 (Fig. 2). In both years the arrival of Robins during April was apparent, as was a subsequent

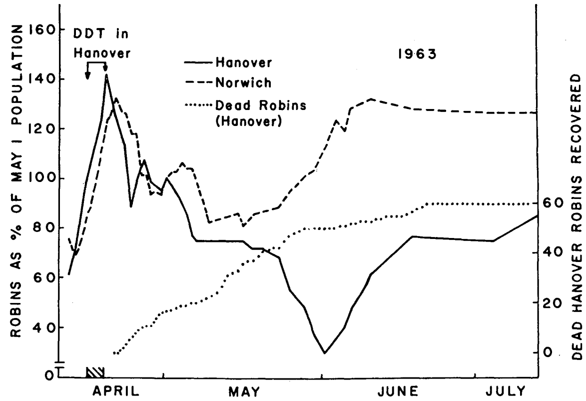

FIG. 1. Robin populations, 1963, in Hanover and Norwich study areas, plotted as moving average of five surveys, with total numbers of dead Robins recovered in Hanover. Actual numbers on May 1: Hanover 12, Norwich 16.

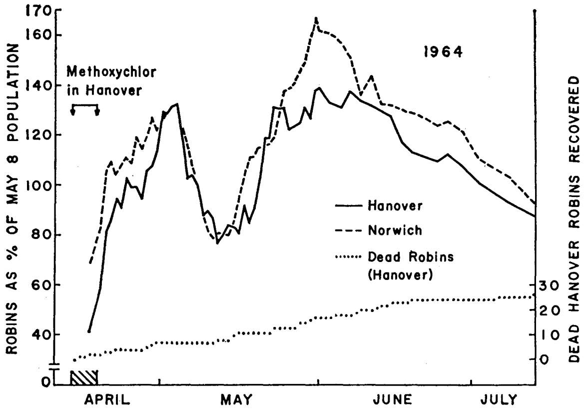

FIG. 2. Robin populations, 1964, in Hanover and Norwich by automobile survey, plotted as Figure 1, with total numbers of dead Robins recovered in Hanover. Actual numbers on May 8: Hanover 23, Norwich 32.

decline representing migrant departure and resident nesting. Failure to detect birds on nests in both towns contributed to the apparent early May decline.

In the latter half of May 1963, when adult and young Robins appeared in the open, the observed population in Norwich recovered to prenesting levels. In contrast, the population in Hanover dropped sharply, and by June 1 had reached 30% of the original May 1 resident population; this decline was inversely related to the number of dead Robins found. Such a population decline following DDT application is consistent with the findings of Wallace (1959), Hickey and Hunt (1960a, b), and Hunt (1960).

During June 1963, the population in Hanover rose through influx of new Robins, but, partly due to continued mortality, it did not recover to the prenesting level. Such population replacement from outside an evacuated area was demonstrated by Stewart and Aldrich (1951), and Hensley and Cope (1951). Furthermore, Wallace (1960) stated that total Robin loss may be greater than the entire population present at any one time, i.e., the complete elimination of the original breeding population plus one or more replacement populations.

Figure 2 shows the similarity of the Hanover and Norwich Robin population trends in 1964. Both towns showed a post-nesting population increase, but that of Hanover lagged slightly. This is consistent with the reduction of mortality in 1964 (Table I) from the 1963 level.

Bark feeder populations.—Like Figure 1 for Robins, Figure 3 illustrates a divergence in 1963 for bark feeders, i.e., chickadees, nuthatches, creepers, and woodpeckers, which were grouped because of low absolute numbers and the similarity of their population curves. Bark feeder popula-

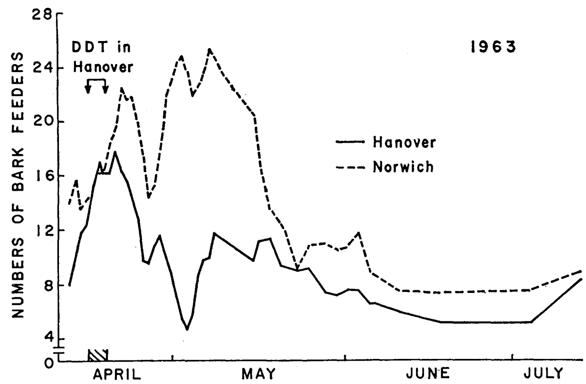

FIG. 3. Bark feeder populations, 1963, in Hanover and Norwich study areas, plotted as moving average of five surveys. Species included: Yellow-shafted Flicker (*Colaptes auratus*), Hairy Woodpecker, Downy Woodpecker, Yellow-bellied Sapsucker (*Sphyrapicus varius*), Black-capped Chickadee, White-breasted Nuthatch, Red-breasted Nuthatch (*Sitta canadensis*), and Brown Creeper (*Certhia familiarus*).

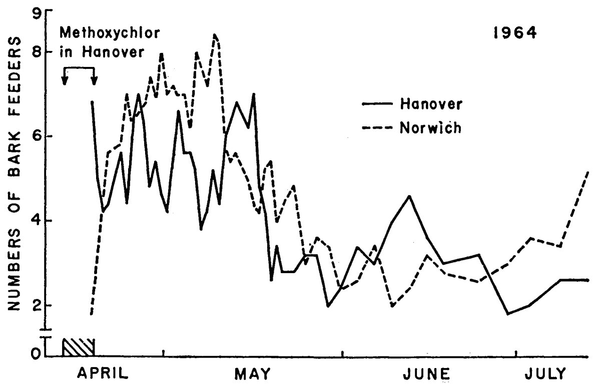

FIG. 4. Bark feeder populations, 1964, in Hanover and Norwich by line transect. Plotting and species as Figure 3.

tions were approximately equal in Hanover and Norwich study areas at the time of DDT application. Within 3 to 4 weeks, Hanover areas showed a net decline in these species, while Norwich areas showed a net increase. Hanover areas then contained only one-third as many birds of these species as did Norwich areas. Dead and tremoring birds of some of these species were found in Hanover. Later in the season populations declined in both towns, presumably because of quiet behavior and dispersion of bark feeders during summer. Figure 4 shows the lack of divergence of the two populations during 1964, but recurrence of the summer decline. The curves gyrate considerably because the sample size was smaller in 1964 than 1963.

TABLE II. Analyses of 48 dissected Robins. Organ analyses in ppm wet weight are expressed as geometric means ($\bar{x}$); standard errors of the mean (SE) are in logarithmic form.[a] Categories: A, reference Robins; B, dead, 0-5 ppm RES in brain; C, dead, 5-50 ppm RES in brain; D, dead, > 50 ppm RES in brain; E, tremors. No. = number of Robins in each category. RES = DDT + DDE + DDD

Organ	No.	%H_2O[b]	%Fat[b]	DDT $\bar{x}$(ppm)	DDT SE	DDE $\bar{x}$(ppm)	DDE SE	DDD $\bar{x}$(ppm)	DDD SE	RES $\bar{x}$(ppm)	RES SE
Brain											
A	5	67.8	6.7	0.11[a]	*1254*[a]	0.64	*1925*	0.12	*1448*	0.87	*1782*
B	4	68.5	4.8	0.13	*1186*	0.61	*1267*	0.28	*2334*	1.07	*1434*
C	3	69.8	6.1	1.93	*2264*	12.5	*1614*	3.49	*2394*	18.0	*1812*
D	20	71.0	5.1	13.0	*0589*	69.1	*0530*	33.1	*0281*	120	*0373*
E	16	68.9	5.5	14.6	*0692*	56.5	*0777*	38.8	*0471*	114	*0614*
Liver											
A	5	66.2	2.5	0.08	*0966*	1.31	*1850*	0.22	*1902*	1.64	*1790*
B	4	63.9	2.3	0.12	*0818*	1.08	*1416*	0.55	*3004*	1.92	*1724*
C	3	68.0	2.7	0.24	*2157*	28.7	*2832*	13.3	*3017*	42.4	*2880*
D	20	64.0	2.6	0.70	*0717*	174	*0635*	115	*0745*	300	*0629*
E	16	60.6	2.7	1.04	*1011*	165	*0619*	139	*0929*	316	*0740*
Breast Muscle											
A	5	67.9	1.1	0.08	*0982*	0.52	*2478*	0.15	*2207*	0.78	*2246*
B	4	66.5	1.7	0.21	*2009*	0.83	*1190*	0.55	*1833*	1.69	*1322*
C	3	68.3	1.5	0.66	*1593*	8.56	*0402*	3.68	*0541*	13.0	*0340*
D	20	69.5	0.7	0.38	*0922*	58.4	*0760*	44.2	*0781*	107	*0705*
E	16	69.2	0.7	0.65	*1202*	40.5	*0812*	42.3	*0902*	87.0	*0806*
Heart											
A	5	71.2	5.0	0.28	*2640*	1.68	*2918*	0.25	*2011*	2.27	*2756*
B	4	70.3	4.0	0.34	*1674*	1.44	*0930*	0.48	*2045*	2.45	*0983*
C	3	71.7	1.8	2.68	*1948*	19.7	*0740*	7.58	*1958*	31.5	*0801*
D	20	70.7	1.1	3.33	*1747*	74.2	*0660*	49.0	*0470*	135	*0571*
E	16	72.3	1.1	4.15	*1681*	52.4	*0777*	48.1	*0613*	110	*0697*
Testes											
A	3	80.8	5.6	0.10	*1714*	0.40	*2193*	0.13	*1939*	0.63	*2056*
C	2	75.9	2.1	0.52	*1095*	19.0	*1985*	9.35	*0678*	29.1	*1544*
D	11	80.1	2.4	0.70	*0865*	48.0	*0764*	33.7	*0392*	85.3	*0529*
E	13	80.1	1.8	0.92	*0899*	38.8	*0534*	38.0	*0239*	79.9	*0316*
Ovaries											
B	1	70.3	11.6	1.29		4.79		0.53		6.60	
D	7	68.3	8.5	23.0	*3879*	301	*1035*	105	*0433*	505	*1332*
E	3	72.6	8.0	110	*3966*	168	*2535*	65.3	*1350*	359	*2864*
Remainder											
A	5	65.5	3.6	0.34	*2906*	2.88	*2930*	0.41	*3084*	3.79	*2910*
B	4	60.2	4.8	0.73	*2765*	3.32	*0641*	0.89	*1982*	5.46	*1046*
C	3	65.2	2.5	11.9	*0550*	52.1	*0313*	9.77	*0493*	74.3	*0141*
D	20	66.3	1.1	15.3	*0970*	55.5	*0706*	24.8	*0667*	101	*0673*
E	16	66.3	1.1	19.5	*1117*	42.4	*0751*	23.2	*0738*	90.8	*0761*
Whole Bird											
A	5	66.5	3.0	0.27	*2569*	2.09	*2875*	0.34	*2812*	2.79	*2817*
B	4	62.5	3.9	0.56	*2430*	2.47	*0649*	0.77	*1914*	4.15	*1003*
C	3	66.5	2.4	7.84	*0386*	38.5	*0261*	8.56	*0628*	55.2	*0100*
D	20	67.4	1.2	10.8	*0984*	69.3	*0613*	38.7	*0649*	125	*0583*
E	16	67.2	1.2	13.8	*1107*	51.8	*0677*	37.7	*0764*	110	*0697*

[a]Calculated by converting ppm values to logarithms and computing arithmetic mean and standard error of the mean for these logs; the mean has been reconverted to ppm. All SE's are 0.——, so the characteristic and decimal have been omitted and only the mantissa is given. For further mathematical treatment, therefore, use log $\bar{x}$ ± SE (e.g., log 0.11 ± 0.1254 = −0.9586 ± 0.1254).
[b]Arithmetic means.

Populations of other species.—Total numbers of other species were insufficient to show any population trends, but many individuals of other species were found dead or with tremors in Hanover during 1963. Substantial numbers of tremoring and dead Chipping Sparrows (*Spizella passerina*) were recovered in Hanover, but population curves failed to show divergence between Hanover and Norwich, presumably owing to continuous influx of new individuals. Why this should be true of Chipping Sparrows, but not Robins or bark feeders, is not entirely clear. Dead and tremoring Myrtle Warblers (*Dendroica coronata*) also were noted, but the species is migratory in Hanover and population trends could not be established.

DDT residue analyses

Interpretation.—Experimental attempts have been made to define lethal concentrations of DDT residues in avian tissues. DeWitt, Derby, and Mangan (1955) used residue concentration in breast muscle as a criterion for determining cause of death in pheasants and quail; Bernard (1963), however, concluded from his experiments that residue in brain is a better criterion. The action of DDT in animals is manifested almost entirely through the nervous system; the concentration of DDT in rat brain can be directly correlated with the appearance of symptoms, i.e., tremors, convulsions, and death (Dale, Gaines, Hayes, and Pearce 1963). These findings tend to support Bernard's conclusion.

The feeding of DDT to captive Robins and House Sparrows (*Passer domesticus*) produced symptoms of DDT poisoning (tremors) identical to those observed in field birds exposed to DDT through spraying for Dutch elm disease (Bernard 1963). Brains of both captive and field specimens contained not less than 50 ppm for Robins and 60 ppm for House Sparrows. Bernard concluded that these figures represented approximately the minimum lethal concentration in the brain for each of these species, and that dead birds containing these amounts probably died as the result of DDT poisoning.

Since the susceptibility varies among individual birds, about 50 ppm DDT in Robin brain might be considered representative of a zone in which the likelihood of DDT as the cause of death is very high, rather than a definite level applicable to every individual. Observation of tremors prior to death in a bird with such a DDT concentration leaves little doubt that DDT was the cause of death.

Robin analyses.—Table II presents the results of the analyses for DDT, DDE, DDD, fat, and water content for 48 Robins. These birds were divided into five categories. Reference Robins collected outside of Hanover formed one group (A). Robins found dead in Hanover were placed in three categories based on the brain content of DDT residues (RES)[3]: 0 to 5 ppm (B), chosen because the reference Robins all contained residues within this range; 5 to 50 ppm (C), intermediate concentrations; and > 50 ppm (D), chosen because Bernard (1963) indicated 50 ppm DDT (including metabolites) to be a lethal concentration in Robins. Tremoring Hanover Robins formed the fifth category (E).

Further justification for grouping into these five categories can be seen from the frequency distribution of all 48 Robins based on brain RES, shown in Figure 5. This distribution clearly

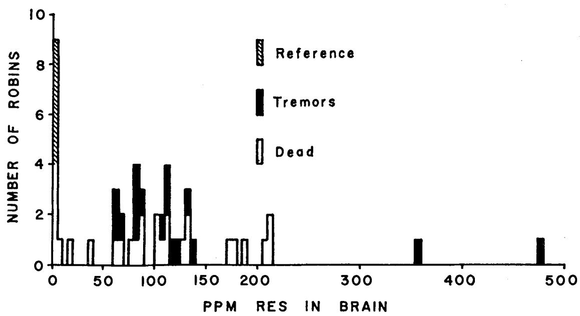

FIG. 5. Frequency distribution of 48 Robins based on brain RES within 5-ppm intervals. RES = DDT + DDE + DDD.

is biphasic. One phase (0 to 5 ppm) contains all five reference birds (A) plus four other Robins recovered dead in Hanover (B). The other phase (> 50 ppm) contains 20 Robins found dead in Hanover (D), plus 16 tremoring Robins (E). The three remaining dead Robins (C) are intermediate between the two phases, and could be considered part of the tail of either phase. The skewed nature of the distribution is evident, but when ppm values are converted to logarithms, the distribution is normal. For this reason the data have been given as geometric means, i.e., arithmetic mean and standard error of the mean have been calculated using logarithms of the ppm values.

Since the 16 Robins recovered with tremors all contained > 50 ppm RES in the brain (minimum

[3] RES = combined value of DDT and the two metabolites, DDE and DDD. DDT and these metabolites have been combined to make results comparable to those of Bernard (1963); his analyses were done by the Schechter-Haller (1945) method, which is believed to give a combined value (Mattson, Spillane, Baker and Pearce 1953) that Bernard termed "DDT." Values for various analyses in this study are generally higher than those of Bernard; this is attributed to a difference between gas chromatographic analysis and the Schechter-Haller method, which may not be quantitative for DDE and/or DDD.

TABLE III. Whole bird analyses of 106 birds representing 25 species, including 48 Robins from Table II. Results are averages expressed as in Table II, but without standard errors. Categories: A, reference Robins; B, dead, 0-10 ppm RES in whole bird; C, dead, 10-30 ppm RES in whole bird; D, dead, > 30 ppm RES in whole bird; E, tremors. RES = DDT + DDE + DDD

Descriptions	No.	% H_2O	% Fat	DDT	DDE	DDD	RES
Ground feeders							
Robin, *Turdus migratorius*							
A[a,b]	5	66.5	3.0	0.27	2.09	0.34	2.79
B	4	62.5	3.9	0.56	2.47	0.77	4.15
D	25	67.3	1.3	9.82	61.7	30.5	107
E	16	67.2	1.2	13.8	51.8	37.7	110
Chipping Sparrow, *Spizella passerina*							
B[b]	4	58.1	9.1	0.29	1.76	0.21	2.31
D	4	64.0	4.1	22.4	10.6	23.9	61.4
E	5	66.2	3.0	28.7	15.2	40.1	85.4
Song Sparrow, *Melospiza melodia*							
B[b]	3	58.9	8.4	0.68	2.15	0.27	3.36
E	1	68.7	9.5	4.8	68	10	82.8
White-throated Sparrow, *Zonotricha albicollis*							
B[b], Norwich	1	54.6	10.3	0.70	1.3	1.0	3.00
B	1	59.5	6.8	0.10	2.6	0.09	2.79
Veery, *Hylocichla fuscescens*							
B[b]	1	59.7	6.6	0.40	1.2	0.21	1.81
C	1	64.8	2.0	5.0	18	5.6	28.6
Wood Thrush, *Hylocichla mustelina*							
B[b]	2	56.6	5.2	0.10	0.25	0.10	0.46
Catbird, *Dumetella carolinensis*							
E	1	68.2	1.0	12	19	21	52
Swainson's Thrush, *Hylocichla ustulata*							
B	1	55.5	20.2	0.18	3.8	0.11	4.09
Slate-colored Junco, *Junco hyemalis*							
C	1	65.6	5.2	5.4	12	3.1	20.5
Common Grackle, *Quiscalus quiscula*							
D	1	67.3	1.1	4.8	64	72	141
Brown-headed Cowbird, *Molothrus ater*							
B	1	67.1	1.7	0.10	2.0	0.23	2.33
Treetop feeders							
Myrtle Warbler, *Dendroica coronata*							
B	1	68.7	1.4	0.48	6.3	0.51	7.29
D	2	66.9	2.6	19.5	6.27	29.5	60.2
E	5	63.7	1.7	26.9	15.4	88.4	136
Chestnut-sided Warbler, *Dendroica pensylvanica*							
B, Norwich	1	66.4	2.9	0.17	1.7	0.41	2.28
D	1	64.8	1.8	19	6.9	41	66.9
Rose-breasted Grosbeak, *Pheucticus ludovicianus*							
B[b]	2	60.4	3.7	0.45	0.91	0.57	2.02
Canada Warbler, *Wilsonia canadensis*							
B[b]	1	59.4	5.2	0.91	3.2	0.45	4.56
Yellowthroat, *Geothlypis trichas*							
B	1	57.9	7.6	0.58	2.7	0.44	3.72
Pine Siskin, *Spinus pinus*							
B	1	60.9	5.7	0.10	0.24	0.10	0.44
Bark feeders							
Black-capped Chickadee, *Parus atricapillus*							
B	3	56.7	8.3	0.12	0.92	0.14	1.24
C	1	56.9	10.9	3.2	24	1.1	28.3
D	1	67.6	1.7	19	34	36	89

(Table III cont.)

Descriptions	No.	% H_2O	% Fat	DDT	DDE	DDD	RES
White-breasted Nuthatch, *Sitta carolinensis*							
E	1	65.1	1.6	25	115	102	242
Hairy Woodpecker, *Dendrocopos villosus*							
B	1	67.1	4.9	0.53	1.4	1.3	3.23
Downy Woodpecker, *Dendrocopos pubescens*							
D	1	63.3	3.2	2.1	27	2.9	32.0
Yellow-bellied Sapsucker, *Sphyrapicus varius*							
B	1	67.5	3.3	0.14	0.26	0.10	0.50
Aerial feeders							
Tree Swallow, *Iridoprocne bicolor*							
B	1	64.3	1.0	0.50	6.5	0.43	7.43
D	1	67.8	1.4	1.2	38	0.98	40.2
Olive-sided Flycatcher, *Nuttallornis borealis*							
B	1	61.1	15.3	0.18	0.72	0.10	1.00
Eastern Kingbird, *Tyrannus tyrannus*							
B	1	51.9	4.8	0.10	1.4	0.10	1.60

[a]Includes one dead Robin from Norwich.
[b]Includes birds showing evidence of accidental death.

62 ppm), after Bernard (1963), we may infer that these birds died of DDT poisoning. Among those Robins found dead, 20 out of 27 analyzed contained > 50 ppm in the brain (minimum 64.7 ppm), and these also, therefore, most likely died of DDT poisoning. The four Robins containing 0 to 5 ppm RES presumably did not die from DDT, while cause of death of the three Robins containing 5 to 50 ppm is uncertain.

The RES content of all organs and remainders of the Robins found with tremors (E) was significantly greater (0.1% level) than the corresponding content of the reference birds (A). Among the dead birds, RES concentrations in 20 birds in group D did not differ significantly from the birds recovered with tremors (E), and RES concentrations in four birds in group B did not differ significantly from the reference birds (A). Therefore, 20 dead Robins (D) can reasonably be assumed to have died with tremors from DDT poisoning, and 4 (B) to have died of other causes. Again, cause of death in the three remaining Robins (C) cannot be determined.

Whole bird analyses.—Whole bird analyses of 106 individuals of assorted species, including the 48 Robins from Table II calculated as whole birds, are presented in Table III, grouped where possible. Sizable numbers of three species were recovered and analyzed, i.e., Robins (50), Chipping Sparrows (13), and Myrtle Warblers (8). The range of RES content for tremoring birds of all species, when analyzed as whole birds, was 30 ppm (Robin) to 295 ppm (Myrtle Warbler). Since RES ranged between 30 and 283 ppm in tremoring Robins, and between 40 and 295 ppm in tremoring Myrtle Warblers, it would appear that differences within a species are greater than differences between species.

Residue concentration in brain is believed to be the best criterion for establishing cause of death. In the few cases where these data lead to ambiguity, analyses of additional organs or whole birds still may not permit complete clarification of the interpretation. This is illustrated by several Robin analyses presented in Table IV. Cause of death for birds #153, #30, and #43 is not immediately obvious. Brain RES concentrations in these three birds were lower than in any tremoring bird in this study or Bernard's (1963), yet whole body residues were comparable to, or higher than, those of some tremoring birds (e.g., #83, #18). Using concentration in brain as the criterion, cause of death for these three birds is uncertain, whereas the whole body analyses suggest that DDT was responsible for death. Fortunately, the data from most birds are not as confusing as these, and can be easily categorized by any criterion used (e.g., #52, #11). Note that reference bird #422 had somewhere accumulated a moderate quantity of RES; it was mainly concentrated in remainder and heart (sites of most depot fat), rather than in brain. This is consistent with Bernard (1963), who showed that DDT could be stored without apparent harm in adipose tissue, but that fat utilization caused transfer of the toxin to more vital organs, with subsequent onset of symptoms.

TABLE IV. RES analyses of selected Robins, ppm wet weight. RES = DDT + DDE + DDD

Description of bird	Brain	Liver	Breast muscle	Heart	Gonads[a]	Remainder	Whole bird
Reference #410	0.33	0.56	0.23	0.74	0.26 (T)	0.68	0.54
Reference #422	3.86	7.10	5.16	25.2	6.2 (O)	38.7	27.3
Tremors # 83	69.9	98.3	25.4	45.2	83.5 (T)	21.8	30.3
Tremors # 18	62.0	160	21.1	52.1	80.8 (T)	61	56.8
Tremors # 20	62.4	355	91.2	82.5	72.9 (T)	62.1	82.6
Tremors # 64	480	578	247	176	124 (T)	158	215
Tremors # 82	111	383	151	520	186 (O)	325	283
Dead[b] #153	9.22	11.3	11.4	22.7	— (T)	76.4	55.5
Dead # 30	16.3	85.4	13.0	31.9	20.4 (T)	69.6	53.0
Dead # 43	38.8	79.4	14.9	43.1	41.7 (T)	77	57.3
Dead # 52	90	135	67.2	66.5	58.8 (T)	46.2	58.8
Dead # 11	0.46	0.79	1.03	1.53	— (O)	3.31	2.53

[a](T) = testes; (O) = ovaries.
[b]Immature bird.

Furthermore, Table II shows that birds believed to be DDT victims (categories D and E) contained substantially less depot fat (remainder and heart) than did those birds that died of other causes (categories A and B).

In the interpretation of whole bird analyses, DDT poisoning must be suspected when the concentration is 30 or more ppm RES, since Robin #83 died with tremors and contained 30.3 ppm in the whole bird and 69.9 ppm in the brain. Residue values of 30 to 60 ppm might thus be considered a minimum lethal zone in which the likelihood of DDT as cause of death increases greatly as residue values increase. When tremors have been observed prior to death in birds with such concentrations, the likelihood of any other cause of death is remote.

Mortality summation based on analyses.—The data in Tables II and III, interpreted as in the previous three sections, indicate that among the birds collected and analyzed, the following died in 1963 as a direct consequence of Hanover's DDT application: 41 Robins, 9 Chipping Sparrows, 7 Myrtle Warblers, 1 White-breasted Nuthatch (*Sitta carolinensis*), 1 Downy Woodpecker (*Dendrocopos pubescens*), 1 Catbird (*Dumetella carolinensis*), 1 Song Sparrow (*Melospiza melodia*), 1 Black-capped Chickadee (*Parus atricapillus*), 1 Chestnut-sided Warbler (*Dendroica pensylvanica*), 1 Tree Swallow (*Iridoprocne bicolor*), and 1 Common Grackle (*Quiscalus quiscula*). This represents 65 of 99 Hanover birds analyzed.

Table III also shows that 33 birds (excluding reference birds) had whole body RES content of 0 to 10 ppm; these birds are presumed to have died of causes unrelated to DDT. All birds showing evidence of accidental death (e.g., bloody bill, internal hemorrhage, other injuries), and two Norwich birds, were in this group. Cause of death for three birds containing 10 to 30 ppm RES is uncertain.

Sublethal effects.—It is possible that additional nonresident birds, e.g., Myrtle Warblers, may have died after their departure from Hanover. Since fat depletion occurs during breeding season (Baldwin and Kendeigh 1938; Breitenbach and Meyer 1959), sublethal quantities accumulated by migrants may then become lethal. Furthermore, it has been shown that sublethal amounts of DDT caused an impairment of reproductive success in caged Ring-necked Pheasants (*Phasianus colchicus*) and quail (Genelly and Rudd 1956; DeWitt 1955, 1956), and in American Woodcock (*Philohela minor*) under field conditions (Wright 1965). In view of the large number of birds receiving a lethal dose of DDT in Hanover, many others probably accumulated sublethal quantities. The effect of sublethal quantities on reproductive capacity of birds passing through or nesting in Hanover may have been substantial, but evaluation of this factor was beyond the scope of this investigation.

Distribution of DDT residues.—Table II indicates that in each category, RES was distributed through all organs; no individual tissue analyzed was entirely free of RES. Among probable DDT victims (D and E) RES concentrations in liver and ovaries (but not testes) were especially high. An egg taken from the oviduct of a Robin with 109 ppm RES in brain contained 355 ppm RES; ovaries of this bird contained 1,439 ppm RES, while those of another had 1,884 ppm. The data show further that in all birds analyzed, most DDT had been converted to the two metabolites, DDE and DDD, transformed by the bird itself, by organisms in its food chain, or both. In liver, RES was found almost entirely in the form of DDE and DDD, suggesting this organ as a site of degradation. Brains, ovaries (but not testes), and remainders generally contained > 10% of the RES in the form of DDT. Presumably DDT, DDE, and DDD are toxic to birds in varying degrees, and death likely results from the combined toxic action of all three compounds.

General observations

Mortality in spring.—Mortality in 1963 occurred chiefly in late April and May, and diminished during early summer; this finding is consistent with other studies (Barker 1958; Hickey and Hunt 1960b; Wallace 1962). Mortality thus coincides with breeding season, when increased fat utilization (Baldwin and Kendeigh 1938; Breitenbach and Meyer 1959) may quickly concentrate RES in vital organs. Spring Robin mortality also could be explained by territorial behavior (Young 1951); a bird may then be restricted to feeding in a contaminated area for at least a month.

Male-female ratio.—Among the 41 Robins believed to have died from DDT (Table III, D and E), males outnumbered females by 29 to 12. Other studies have shown a similar imbalance among dead birds (Bernard 1963; Stewart and Aldrich 1951; Hensley and Cope 1951). Furthermore, peak mortality of males preceded that of females; males outnumbered females 18 to 2 from April 22 through May 11, but only 11 to 10 from May 12 to June 20. These differences probably result from a combination of factors. Males arrive on the breeding grounds earlier than females (Young 1951), and are therefore exposed longer to DDT. Males move into a vacated territory more rapidly than females (Stewart and Aldrich 1951; Hensley and Cope 1951). Finally, males appear more susceptible to DDT than females. This can be seen from Table V, where RES content in males is significantly lower at the 1% level in heart, breast muscle, remainder, and the whole bird, and at the 5% level in brain. Only in liver did RES content not differ significantly between males and females. Identical circumstances may explain the 5:1 ratio of males to females among the 24 probable DDT victims of other species. Whole bird RES content of this group showed geometric means of 75.7 ppm for 20 males and 134 ppm for 4 females; this difference was significant at the 10% level.

TABLE V. RES content in ppm wet weight for 26 male and 10 female Robins containing > 50 ppm RES in the brain, expressed as geometric means ($\bar{x}$) with standard errors (SE). RES = DDT + DDE + DDD

Organ	Males	Females
	$\bar{x}$ ($\bar{x}$−SE, $\bar{x}$+SE)	$\bar{x}$ ($\bar{x}$−SE, $\bar{x}$+SE)
Brain..........	104 (95.7,114)	159 (140,180)
Liver..........	284 (246,328)	377 (337,422)
Breast muscle...	79.0 (69.7,89.5)	169 (135,210)
Heart..........	95.2 (87.5,104)	243 (209,283)
Gonads........	82.3 (77.0,88.1)[a]	456 (346,600)
Remainder.....	72.8 (65.3,81.2)	199 (174,227)
Whole bird.....	92.5 (83.6,102)	222 (200,247)

[a]Testes from 24 males.

Total Robin mortality.—The population trends shown in Figure 1 permit a rough estimation of total Robin mortality (1963) in Hanover. Since the study areas represented 2¼% of the sprayed part of town, and the May 1 resident population on these areas averaged 12 Robins, the total Hanover Robin population was approximately 500 to 550. Based on a 70% decline in the study areas by June 1, total Robin mortality in Hanover was about 350 to 400 birds. Recovery of 61 dead Robins in Hanover therefore represented 16% of the estimated total. When it is realized that mortality of this magnitude means that about one Robin per 2 acres (0.8 hectare) died during a period of several weeks, that Hanover has many wooded and brushy areas where dead birds would seldom be found, and that remains generally disappear for various reasons in a few days (Rosene and Lay 1963), some of the difficulties of recovering dead birds can be appreciated. The population decline might suggest the departure of birds owing to depletion of food chain organisms by DDT. Each avian DDT victim found, however, indicates the mortality of many, and since 16% seems a reasonable figure for recovery of dead Robins, death, rather than departure, appears to have been the primary cause of the decline. Since spraying of Hanover study areas was less than the town average, and influx of new Robins presumably swelled the survey figures, actual mortality was probably higher, and recovery lower, than the values indicated.

How birds acquire DDT.—Barker (1958) established that Robins accumulate DDT by feeding on contaminated earthworms, but many birds other than ground feeders have died following DDT applications for Dutch elm disease (Wallace et al. 1961). The enigma of the mechanisms whereby these birds of varied feeding habits acquire DDT suggests important questions. Myrtle Warblers arrived in Hanover as migrants 2 weeks after spraying. These birds feed primarily on live insects in the treetops, yet a short time in Hanover was sufficient to impart a fatal dose of DDT to substantial numbers of them. The absence of tremoring birds and the presence of low residue concentrations in birds from unsprayed areas indicates that high concentrations of DDT residues were not accumulated outside of Hanover. Other species, e.g., Chestnut-sided Warbler and Tree Swallow, present similar situations. RES in a Tree Swallow was within the minimum lethal range, but regardless of cause of death it is noteworthy that an aerial feeder somehow accumulated a considerable quantity of DDT. Nuthatches and woodpeckers forage for insects (including bark beetles) on tree trunks and limbs; ironically, a White-breasted Nuthatch was noted with tremors (242 ppm RES) and a dead Downy Woodpecker contained 32 ppm RES. Since all of these birds eat primarily live insects, it must be suspected that the toxicant was acquired by feeding on living insects carrying DDT. This presents a paradox of survival of the intended insecticide victims, and the mortality, instead, of insectivorous birds.

Effect of spray date.—Wallace et al. (1964) indicated that most treetop-feeding birds were spared when DDT was applied the previous fall, but that mortality of ground feeders was similar to that following spring spraying. Before 1963, spraying in Hanover usually was done 1 to 2 weeks later in the spring, and thus coincided with warbler migration. Under these circumstances, mortality of insectivorous treetop feeders was apparently heavier than in 1963, as judged by the accounts of numerous residents. Evidently the spray date is an important factor in mortality of treetop birds, but is of little importance to ground feeders that suffer from the persistence of DDT in soil (Woodwell and Martin 1964) and soil organisms (Barker 1958).

Methoxychlor in 1964

Application of Methoxychlor in April 1964, was followed by diminished bird mortality as compared with 1963. Six Robins were observed with tremors; total dead Robin recovery suggests that losses were one-fourth to one-third those of 1963, although Figure 2 shows only a slight population reduction as compared with Norwich. Robin mortality was both lighter and later, with the final tremoring Robin noted on 13 July 1964, against 19 May, 1963. Presumably 1964 Robin mortality was caused by residual DDT in the ground from past years (Chisholm, Koblitsky, Fahey, and Westlake 1950; Wallace et al. 1964; Woodwell and Martin 1964), rather than by Methoxychlor, which has been shown to be much less toxic to birds than DDT (Hickey 1961).

No birds of species other than Robins were known to have died in 1964 of DDT (or Methoxychlor) poisoning. While such mortality may have occurred and dead birds were collected, none was observed with tremors and no analyses were performed.

Acknowledgments

The authors express their thanks and appreciation to Hans W. Weber, who served as the fourth field observer during the spring of 1963, and to Elizabeth M. Sherrard and M. C. Abell for help with many tasks and constant encouragement. The cooperation of local news media and many residents of Hanover and Norwich, who so kindly aided in collecting dead and dying birds, is gratefully acknowledged.

For advice and criticism of the manuscript we are indebted to J. H. Copenhaver, Jr., R. W. Barratt, F. H. Bormann, G. B. Saul, T. B. Roos, and G. E. Likens of the Department of Biological Sciences, and V. H. Ferm of the Department of Pathology. Thanks are also due T. E. Kurtz and K. M. Lochner for assistance with statistical analyses, and A. S. Fox for supplying details on spraying.

The authors extend their gratitude to the Patuxent Wildlife Research Center, Laurel, Md., for defraying the expense of the chemical analyses, and thank members of its staff for suggestions throughout this investigation.

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