

PHYSIOLOGICAL SUSCEPTIBILITY OF ROBINS TO DDT POISONING

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LARGE numbers of Robins (*Turdus migratorius*) occupy major portions of their breeding range in the northern United States from March until at least October (Bent, 1949), but published records of Robin deaths attributed to DDT used for Dutch elm disease control are confined mostly to the months of April and May (Barker, 1958; Hickey and Hunt, 1960; Bernard, 1963; Boykins, 1964; Wallace et al., 1964; Wurster et al., 1965). I have previously described how peak Robin mortality on the Madison campus of the University of Wisconsin during late April coincided with maximum availability of DDT-laden earthworms influenced by moderating soil conditions (Hunt, 1968). Such observations suggested that the chronology of DDT poisoning in these birds was extrinsically influenced if not controlled by phenological responses of their toxic food supply, but counter-evidence was also presented indicating that few or no avian deaths were associated with other periods of mild climate and high worm activity. In one of the first reports of bird kills associated with elm (usually *Ulmus americana*) spraying, Blagbrough (1952) expressed the view that birds in spring were especially susceptible to poisoning if their food was highly contaminated and if their body fat, which could store high DDT concentrations, was at a minimum. It would appear that appreciable mortality will occur only when these two conditions are concurrent.

Using measurements taken on both poisoned and normal specimens from Wisconsin as well as comparisons involving data collected by others, I have as my objective in this study the presentation of quantitative support for the following argument: adult male and female Robins show a differential tolerance to DDT, and in at least the males, changes in body weight which occur naturally during the spring season are inversely correlated with changes in susceptibility to this insecticide.

METHODS AND MATERIALS

Most, but by no means all, birds dying on the Madison campus after the elm spraying began were collected; all reasonably fresh specimens were weighed, labeled, and immediately frozen in plastic bags. Most birds were later measured and examined internally, and a representative sample was chosen for chemical analysis on the basis of species, age, sex, condition, collection date, and exposure to insecticide. Of 27 adult Robins analyzed, two represented controls, 21 had known exposure to DDT, and four had known exposure to both DDT and methoxychlor, the latter group being discussed elsewhere (Hunt and Sacho, 1969). From each bird the brain and breast muscle were removed,

weighed, oven-dried to constant weight at 49°C, reweighed, and individually frozen in glass jars until analysis. All tissue analyses were made at the Wisconsin Alumni Research Foundation Insecticide Laboratory, with extraction procedures reported by Hunt and Sacho (1969). Residue concentrations of DDT, DDE, and TDE (DDD) were detected by gas chromatography and are reported here in parts per million wet weight.

Since the sample of Wisconsin Robins to be analyzed was restricted by the expense involved, residue figures from other published sources are included for purposes of comparison. Thus trends suggested by the Wisconsin material might be either confirmed or refuted. Ideally, such borrowed data should involve specimens comparable to those analyzed from Wisconsin—adult Robins of known sex presumed to have died from DDT poisoning during April or May in a Dutch elm disease-control environment. Birds matching these criteria were collected in Michigan in 1959–60 (Bernard, 1963), in Michigan in 1962–63 (Boykins, 1964; and Wallace, et al., 1964), and in New Hampshire in 1963 (Wurster, et al., 1965). The New Hampshire specimens, although not oven-dried, were analyzed by the same Wisconsin laboratory using identical procedures, while residues in all Michigan birds were determined by the Schechter-Haller colorimetric method which is apparently quantitative for DDT and TDE but not DDE (Stickel, et al., 1966). Because differences in drying and analytical procedures may have affected the retention or detection of the original insecticide content, I will use these additional data to emphasize trends and differences within individual studies rather than to stress quantitative comparisons between studies.

Body weights of apparently normal, adult Robins were obtained at my banding station in Kenosha, Wisconsin, during 1964–66. Each bird was color-banded so that its presence and nesting activities could be followed through the breeding season. Additional weights for Wisconsin Robins banded in 1963–66 were provided by E. W. Peartree of Oconomowoc, Wisconsin. Based upon my measurements and internal sexing of 170 male and 85 female Robin specimens, I chose to omit Mr. Peartree's unsexed birds with wing lengths of 124–127 mm; larger birds I called males and smaller ones females.

RESULTS AND DISCUSSION

The problem of providing proof that Robins were poisoned by DDT residues has been considered by Stickel et al. (1966) and by Hunt (1968) with only a resumé of evidence presented here. Elms on the Madison campus were first sprayed with 22.5 lb DDT/acre in 1959 and at half that dose in 1960 and 1961. The die-off of songbirds following the initial application was considerable (Hickey and Hunt, 1960), although no dying birds had attracted attention the previous year nor were specimens being reported from adjacent unsprayed sites. A single campus tally of 15 dead and dying Robins on 23 April 1959 indicated a highly lethal environment. Insecticide concentrations in the brains of 21 campus Robins were similar to those from other DDT-contaminated areas and from experimentally poisoned birds, but were 100 times the average concentration in two Wisconsin Robins from unsprayed habitat. Tissue residues in birds found dead did not differ significantly from those in specimens found with tremors thought to be caused by DDT. Absolute assurance that certain concentrations of specific chemicals were lethal to

TABLE 1
ADULT ROBIN MORTALITY ON THE DDT-SPRAYED MADISON CAMPUS.
Shown as a percentage of the season total

Specimens Sampled	Sample Size	April 1-15	April 16-30	May 1-15	May 16-31	June 1-30
All Robins*	232	5	52	30	12	2
Males	119	9	60	20	10	1
Females	52	6	46	35	14	0

* Includes unsexed birds not examined internally.

individual animals is beyond the realm of expectation, but an objective evaluation of the above findings will likely lead to the conclusion that most adult Robins picked up on the Madison campus during spring of 1959-61 were victims of DDT.

Seasonal pattern of mortality and residues.—From 1959 when DDT was first used on Madison campus elms until 1962 when methoxychlor was substituted in an effort to reduce songbird mortality, more than 340 apparent-DDT victims were picked up. Robins, which formed the bulk of this collection, began returning to nesting areas during late March with males preceding females by one or two weeks. The earliest trembling Robin was seen on 6 April and the latest on 20 June, but the majority were found dead or dying during late April (Table 1). The pattern was repeated each spring with 51, 59, and 47 per cent of the season's total Robin kill appearing during the same two-week period. This expression of Robin mortality should not be confused with *mortality rate* as derived from life-table computations. Treatment of these data by the latter method appeared unjustified since considerable recruitment to the initial population occurred during the spring season. As evidence of such ingress, one closely watched territory yielded a succession of seven dead, adult Robins before becoming totally vacated. While the rate of mortality may have remained consistently high through May, the magnitude noticeably declined with the approach of summer.

Other trends became apparent when the results of tissue analyses were also separated on a seasonal basis (Table 2). In the sequence from early April through May, there were considerable increases among individual and total insecticide residues in both brain and breast muscle and in breast muscle fat content as the season progressed. Only the per cent of fat in the brain failed to follow the trend. The early-April and late-April measurements did not differ significantly ($P > 0.05$), but total brain residues were significantly higher ($P < 0.05$) in May than in late April, and breast fat in May was significantly higher than in early April. When the pooled April

TABLE 2
SEASONAL TISSUE DIFFERENCES IN ROBINS FOUND DEAD AND DYING ON
THE MADISON CAMPUS

Tissue Component	April 1-15 $\bar{x} \pm SE$ (N = 6)	April 16-30 $\bar{x} \pm SE$ (N = 8)	May 1-31 $\bar{x} \pm SE$ (N = 7)
Brain			
Fat (per cent)	6.7 \pm 0.4	6.1 \pm 0.1	6.9 \pm 0.3
Residues (ppm)			
DDT	5.0 \pm 1.3	6.3 \pm 1.0	9.1 \pm 2.4
DDE	27.9 \pm 6.7	33.1 \pm 3.9	63.3 \pm 9.4
TDE	38.2 \pm 2.6	45.1 \pm 6.5	50.7 \pm 9.5
Total	71.1 \pm 7.4	84.5 \pm 8.0	123.1 \pm 11.7
Breast Muscle			
Fat (per cent)	1.2 \pm 0.2	1.8 \pm 0.3	2.1 \pm 0.3
Residues (ppm)			
DDT	0.9 \pm 0.2	3.0 \pm 1.5	4.0 \pm 1.4
DDE	35.8 \pm 9.1	57.8 \pm 8.2	133.1 \pm 17.0
TDE	63.3 \pm 9.5	125.8 \pm 45.7	184.6 \pm 45.8
Total	100.0 \pm 15.7	186.5 \pm 53.9	321.7 \pm 54.0

data were compared with May data, differences in the total insecticide residues in both brain and breast muscle were highly significant ($P < 0.01$). It is thus apparent that birds collected later in spring contained higher fat reserves and tolerated higher tissue levels of poison before succumbing, but it is not obvious whether these increases were due to actual seasonal changes or rather to changes relating to the sex ratio of specimens as the weeks passed.

Analyses of male and female Robins.—The disproportionately high number of males occurring in my collection (Table 1), as well as in other DDT studies, suggested the tendency for excess males to repopulate rapidly vacated territories where a breeding population had been depleted (see discussions in Stewart and Aldrich, 1951; Hensley and Cope, 1951), but it might also indicate a greater susceptibility among males to DDT poisoning. Similarly the lag in female mortality could be the result of later arrival in the contaminated environment, or it could point to physiological differences in the reproductive roles of the two sexes resulting in unique stresses conducive to insecticide poisoning.

The tissue analyses for Wisconsin specimens, along with residue data from sources cited earlier were grouped according to sex (Table 3). In all seven comparisons, the concentrations in the females exceeded those in the males

TABLE 3

TOTAL DDT-COMPLEX RESIDUES IN ROBINS SUSPECTED OF DYING FROM DDT.
Shown in parts per million wet weight.

Tissue Where Studied	DDT Complex	Males Mean \pm SE (N)	Females Mean \pm SE (N)
Brain			
Michigan-1963	DDT-TDE	79.5 \pm 6.1 (25)	110.9 \pm 17.7 (11) *
Michigan-1964	DDT-TDE	96.5 \pm 9.6 (23)	120.5 \pm 8.4 (19)
New Hampshire	DDT-TDE-DDE	119.4 \pm 16.7 (25)	169.7 \pm 27.3 (9)
Wisconsin	DDT-TDE-DDE	81.0 \pm 5.1 (14)	118.6 \pm 15.1 (7) **
Breast Muscle			
Michigan-1963	DDT-TDE	108.3 \pm 12.6 (21)	120.8 \pm 17.0 (10)
New Hampshire	DDT-TDE-DDE	96.5 \pm 11.2 (25)	194.5 \pm 36.0 (9) **
Wisconsin	DDT-TDE-DDE	149.5 \pm 26.0 (14)	321.6 \pm 68.2 (7) **

* Difference is significant ($P < 0.05$).

** Difference is highly significant ($P < 0.01$).

although only four comparisons were statistically significant. Wurster et al. (1965) did find significant brain differences in their New Hampshire birds when two June specimens were included in the tests. On the other hand, when Bernard (1963) limited his comparisons in Michigan to trembling birds only, he found higher breast residues among males, but the differences were not significant. From insecticide tests on penned Ring-necked Pheasants (*Phasianus colchicus*), both Genelly and Rudd (1956) and Azevedo, Hunt, and Woods (1965) found males to be more vulnerable than females to DDT in the diet.

Comparisons of fat content in tissues submitted for insecticide analysis help to shed some light on possible reasons for higher DDT tolerance among the females (Table 4). While brain lipids from New Hampshire birds did not differ significantly, fat content in the three remaining comparisons was significantly greater among females. Since fats serve as reservoirs where insecticides may temporarily accumulate, even seemingly insignificant changes in the lipid balance of various tissues might have a direct bearing on the immediate fate of ingested toxins. Furthermore, the following studies revealed that stored poisons could later be released when fats are metabolized to meet energy demands. A rise in the DDT level of body tissues during periods of stress was shown to be lethal in starvation experiments with rats by Dale, Gaines, and Hayes (1962), with House Sparrows (*Passer domesticus*) by Bernard (1963), and with American Woodcock (*Philohela minor*) by Stickel et al. (1965). Although no DDT deaths were noted, Mussehl and Finley (1967) reported that residue-laden, fatty tissues in male Blue Grouse (*Den-*

TABLE 4
FAT CONTENT IN MALE AND FEMALE ROBINS EXPOSED TO DDT.
Shown as a per cent of tissue wet weight.

Tissue Where Studied	Males Mean \pm SE (N)	Females Mean \pm SE (N)
Brain		
New Hampshire	5.4 \pm 0.3 (25)	4.8 \pm 0.5 (9)
Wisconsin	6.3 \pm 0.2 (14)	7.0 \pm 0.3 (7)*
Breast Muscle		
New Hampshire	0.6 \pm 0.0 (25)	1.1 \pm 0.2 (9)**
Wisconsin	1.4 \pm 0.1 (14)	2.3 \pm 0.4 (7)**

* Difference is significant ($P < 0.05$).

** Difference is highly significant ($P < 0.01$).

dragapus obscurus) were heaviest early in the breeding season but declined at the breeding peak, while lipid content in females was highest at peak laying with rapid declines during incubation and hatching. Breitenbach and Meyer (1959) presented evidence that fat reserves in hen pheasants became so depleted during incubation and brooding that these females would be highly vulnerable to stress factors; previously sublethal concentrations of stored DDT could well impose such stress late in the breeding cycle.

Seasonal changes in Robin tissues.—When male Robins from the several studies were separated according to collection date (Table 5), there was a tendency for May victims to contain higher insecticide residues, although the

TABLE 5
TOTAL DDT-COMPLEX RESIDUES (PPM WET WEIGHT) IN MALE ROBIN VICTIMS.

Tissue Where Studied	April Mean \pm SE (N)	May Mean \pm SE (N)
Brain		
Michigan-1963	75.5 \pm 9.1 (13)	83.8 \pm 8.3 (12)
Michigan-1964	98.8 \pm 17.5 (12)	94.0 \pm 7.3 (11)
New Hampshire	90.5 \pm 7.0 (12)	146.1 \pm 30.2 (13)
Wisconsin	77.4 \pm 5.1 (12)	102.6 \pm 8.6 (2)
Breast Muscle		
Michigan-1963	105.1 \pm 21.2 (9)	110.8 \pm 15.9 (12)
New Hampshire	68.0 \pm 9.2 (12)	122.9 \pm 17.1 (13)*
Wisconsin	117.0 \pm 11.7 (12)	344.1 \pm 91.5 (2)**

* Significant difference ($P < 0.05$).

** Highly significant difference ($P < 0.01$).

TABLE 6
TOTAL DDT-COMPLEX RESIDUES (PPM WET WEIGHT) IN FEMALE ROBIN VICTIMS.

Tissue Where Studied	April Mean \pm SE	(N)	May Mean \pm SE	(N)
Brain				
Michigan-1963	140.0 \pm 35.7	(5)	86.7 \pm 6.6	(6)
Michigan-1964	117.0 \pm 16.2	(9)	123.7 \pm 7.2	(10)
New Hampshire	109	(1)	177.3 \pm 29.7	(8)
Wisconsin	86.9 \pm 33.4	(2)	131.4 \pm 14.9	(5)
Breast Muscle				
Michigan-1963	130.0 \pm 21.6	(5)	111.6 \pm 28.1	(5)
New Hampshire	38.1	(1)	214.1 \pm 34.3	(8)
Wisconsin	343.8 \pm 211.6	(2)	312.7 \pm 72.2	(5)

results were far from conclusive. The probability of significant brain differences between most monthly pairs was reduced by the considerable variability in both periods, and in the second Michigan group even the general trend was reversed. Changes in male breast residues were more convincing, especially with the New Hampshire results approaching a highly significant difference. Among the females represented in Table 6, there was a high degree of variability within most subgroups; neither common trends nor significant differences were present. It appeared that residue levels from different females may have varied with the particular stage and metabolic demands of the individual nesting cycles rather than exhibiting seasonal tendencies as such.

These seasonal comparisons were based upon the premise that some time after their arrival on northern breeding grounds, resident Robins would gradually accumulate fat reserves enabling them to store greater toxicant concentrations before the dosage became lethal. This assumption was not entirely borne out by seasonal comparisons of the fat content in analyzed tissues of either males or females, but there was reason to suspect that tissue lipids from DDT victims might be a poor index to normal body fat prior to the onset of poisoning symptoms. First, when Wisconsin Robins found in tremors and dispatched were compared with those already dead, tissue insecticides did not differ significantly, but the dead birds had significantly lower brain fat and also lower breast muscle fat which approached significance. Such findings suggested that fat reserves were being metabolized as energy demands increased during periods of violent terminal tremors. Second, for 170 Robins, body weights at death for each sex were remarkably constant with 118 males averaging 69.4 ± 0.4 g and 52 females averaging 72.8 ± 0.8 g.

TABLE 7

SPRING BODY WEIGHTS IN WILD-TRAPPED, BANDED ROBINS.
Shown in grams \pm standard error and (sample size).

Period	Males		Females Kenosha
	Kenosha	Oconomowoc	
March 16-31	89.3 \pm 2.0 (13)	—	—
April 1-15	82.5 \pm 2.0 (5)	85.3 \pm 2.6 (6)	82.5 \pm 2.1 (5)
April 16-30	78.6 \pm 2.3 (7)	75.0 \pm 1.6 (8)	88.9 \pm 3.1 (9)
May 1-15	82.8 \pm 3.7 (3)	77.6 \pm 1.5 (2)	86.1 \pm 3.6 (3)
May 16-31	78.6 \pm 1.3 (9)	79.1 \pm 1.7 (4)	85.3 \pm 2.2 (7)
June 1-30	80.1 \pm 1.3 (14)	—	78.7 \pm 2.0 (4)

These differences were highly significant, but within each sex there were no significant differences between any two intervals in April or May. All 21 analyzed Robins and 94 per cent of the 223 Robin specimens examined were assigned to fat class "none," the rest being judged as having "little" fat according to the criteria of Wolfson (1954). Together, these observations point to a situation where a series of birds, regardless of sex or collection date, underwent such a reduction in fat reserves prior to death that all may have approached the homeostatic, fat-free, body weight described by Odum, Rogers, and Hicks (1964), even though these authors excluded from their remarks severely stressed birds. If changes in DDT susceptibility were to be correlated with changes in fat deposits, then clues to this relationship should logically be taken from apparently healthy Robins showing no poisoning symptoms.

Body weights of normal Robins.—Robin weights from both Kenosha and Oconomowoc are shown in Table 7 since the distance of some 50 air miles separating the two Wisconsin stations was not considered sufficient to interfere with seasonal comparisons. Peartree's Oconomowoc data included no repeat weights from the same year, and the weights of females were too few and unequally distributed to be useful here. Kenosha totals did include repeat weights but only those taken more than 48 hours after the previous capture, since Rogers and Odum (1966) attributed rapid weight losses in several species to stress from handling. Among Kenosha males, the high mean weight for late-March birds did not differ significantly from that for the first half of April, but taken in pairs, differences between March males and males weighed during each of the entire later months were highly significant. From Oconomowoc, the heaviest males were trapped in early April, and the weight difference between these and the late-April males was also highly significant.

For females in Table 7, the early-April mean weight was identical to that

TABLE 8

INITIAL AND REPEAT WEIGHTS OF MALE ROBINS TRAPPED IN KENOSHA.

Year Bird	March 16-31	April 1-15	April 16-30	May 1-15	May 16-31	June 1-30
1964						
*	80.7	-----	72.4			
2	78.1	-----	69.9			
3			81.0	-----	81.6	----- 83.1
1965						
4		78.4	----- 88.1	-----	84.1; 79.4	
5		76.9	-----	-----	-----	82.4
6*			80.4	-----	83.1	
1966						
7	98.6	-----	88.5			
8	91.7	----- 84.4; 84.4	-----	89.2		

* Banded but not weighed in 1963.

of Kenosha males, but there followed a sizable weight increase in late April during the period of greatest egg laying on a Kenosha study area in both 1964 and 1965 (no census in 1966). This same relationship between peak weight and peak egg production was also detected in hen pheasants (Breitenbach and Meyer, 1959). From early to late April, when mean weights of two male populations declined 3.9 and 10.3 g, the mean weight for females increased 6.4 g. A portion of this female change would result from the process of ovulation, for a fully formed, 5.1-g egg found in the lower oviduct of one DDT specimen added appreciably to the total weight of that individual. Nevertheless, it is unlikely that all late-April females were ready to lay when weighed, and an important part of the additional weight must be explained as increases in adipose deposits. Due in part to small sample size, the only statistically significant weight difference in Kenosha females was between pooled, monthly samples representing May and June.

It could be argued that weight differences shown in Table 7 represented different populations rather than seasonal changes within a population, even though many of the color-banded, Kenosha birds were seen repeatedly during the nesting season. To insure that only a post-migratory, resident population was involved, individual males that were weighed during more than one spring period were tabulated (Table 8); again, repeat weights after less than 48 hours were omitted. Of 21 usable weights for eight different individuals, only one bird (4) and its four weights deviated from the previously established trend of appreciable April declines from March highs followed by partial

recovery in May and June. It was possible that hourly weight changes could have influenced apparent seasonal patterns, because Nice (1937) noted daily fluctuations of less than 5 per cent with afternoon increases in the Song Sparrow (*Melospiza melodia*), but only the June weight for Robin 3 or the May weight for Robin 6 could have reflected diurnal rather than seasonal gains. All others were either reweighed during the same half-day interval or would have demonstrated improbable morning to afternoon losses. Nesting females were rarely recaptured, and the few repeat weights were trendless.

Earlier studies of bird weights and weight changes by Baldwin and Kendeigh (1938) involving over 13,500 measurements showed a pattern of annual rhythm showing a weight decline from spring to a trough in July probably caused in part by energy requirements for reproduction. These data included 28 adult Robin weights scattered over a span of eight months, and Nice (1938) reported 39 Robin weights by sex and season, pointing out the confusion caused by high and low figures during the nesting season. Wolfson (1954) warned that mean weights can obscure individual differences or changes having real meaning, and Odum (1960) stressed the significance of lipid reserves in relation to migration for, as he explained, fats are both the most variable and the most economical form of stored energy, giving useful indices to "the metabolic status of populations." Odum's research and that of King et al. (1963) was primarily concerned with pre-migratory fat accumulation, but these writers commented on the selective advantage of possessing fat reserves at the termination of migration that would permit survival during times of adverse weather or food scarcity.

Certainly, Robins nesting in the northern United States could often benefit from an adaptive store of reserve energy. For example, I observed that male Robins first appearing on my Kenosha study area on 14 March were exposed to snow showers on 25, 26, and 29 March; a Robin was not seen feeding on earthworms before 3 April. That same year two males trapped on 18 and 19 March had weight losses of 10 per cent when retrapped on 16 April (included in Table 8). The data I have presented indicate that apparently normal, male Robins were undergoing weight losses and reached their lowest body weight of the season during the same two-week period that peak male deaths associated with DDT exposure occurred in three earlier years. The frequency of dying males increased as weight representing stored fat decreased, and mortality decreased as weight began to increase. With the increasing weight in normal males went increasing concentrations of insecticide residues in Robin tissues until some higher tolerance level was apparently exceeded. Among females the relationships involving date, tissue residues, and body weight were apparently complicated by the particular stage of nesting activity, but greater fat reserves probably allowed them to accumulate and tolerate

higher insecticide concentrations and to survive later than males in the contaminated nesting environment.

SUMMARY

Following elm spraying on the Madison campus of the University of Wisconsin in 1959-61, at least 232 adult Robins apparently died of DDT poisoning between the extreme dates of 6 April and 20 June with peak mortality occurring each year during late April. Males died earlier and in greater numbers than females, and male specimens from several similar studies contained lower insecticide residues and tissue fat in both brain and breast muscle. Male specimens tended to die with lower tissue toxicants in April than in May, although the percentage of brain and muscle fat was similar in male victims of both months. Most dead and dying Robins contained almost no subcutaneous body fat, males averaging 69.4 g and females 72.8 g. Normal wild males banded soon after arrival in late March averaged 89.3 g, reached a low weight in late April, and increased slightly by June, while female weights reached a mean of 88.9 g during peak egg laying in late April before declining gradually. The seasonal loss of fat reserves in male Robins appeared to increase their susceptibility to DDT poisoning with fatter females showing greater tolerance.

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