# DDT RESIDUES IN THE FOOD CHAINS OF BIRDS

Thesis for the Degree of Ph. D.

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Ernest A. Boykins

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This is to certify that the

thesis entitled

DDT RESIDUES IN THE FOOD CHAINS OF BIRDS

presented by

Ernest A. Boykins

has been accepted towards fulfillment
of the requirements for

Ph. D degree in Zoology

George J. Wallace
Major professor

Date May 25, 1964

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ABSTRACT

DDT RESIDUES IN THE FOOD CHAINS OF BIRDS

by Ernest A. Boykins

The use of a 12 per cent DDT (1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl)-ethane) solution, primarily for the prevention of Dutch elm disease, has led to the death of large numbers of birds on the Michigan State University campus. Therefore, this study was undertaken to try to determine the food chains involved in the transference of DDT to birds.

Samples of soil, earthworms, fruit, and the bark, buds and leaves of the elms Ulmus americana were collected during a 2-year period. The samples were quantitatively analyzed for DDT using the Schechter-Haller method of analysis.

Earthworms showed concentrations ranging from an average of 63.6 μg of DDT/g in the fall of 1963 to an average of 70.7 in the spring of 1962. High DDT levels persisted in earthworms up to 18 months after spraying. There was no significant difference at the 5 per cent level in the amount of DDT in the two species of earthworms (Lumbricus terrestris and Helodrilus caliginosus) collected in 1964. Sprayed earthworms (Helodrilus foetidus) kept in the laboratory for 22 weeks showed initial levels of 298 μg of DDT/g, then declined to 86 μg of DDT/g. For 12 weeks the earthworms averaged 86 to 90 μg of DDT/g in unsprayed soil. This indicated that DDT can be stored, up to 22 weeks at least, in the tissues of these worms.

Residues in 78 soil samples ranged from an average of 31.0 (18 months after spraying) to 298.1 μg of DDT/g, 4 to 16 days after spraying. During two collecting periods DDT levels in the soil were lower than the concentrations found in earthworms.

The amount of DDT found on fruit averaged 2.4 μg of DDT/g prior to spraying and 44.4 μg of DDT/g following a dormant spray. Six months later the fruit averaged 1.0 μg of DDT/g. Elm bark averaged 23.6 μg of DDT/g prior to the dormant spraying, 242.1 after spraying and 92.0 μg of DDT/g 6 months after the fall spraying. Fallen elm leaves averaged 12.5 μg of DDT/g prior to spraying and 167.5 μg of DDT/g after spraying. Young elm buds in the spring following fall spraying averaged 1.5 μg of DDT/g and the new elm leaves averaged 3.1 μg of DDT/g.

Experiments in feeding House Sparrows earthworms (Helodrilus foetidus) containing 298 μg of DDT/g showed a survival time for the birds of 1 to 5 days. The brain averaged 31.4 μg of DDT/g while the liver averaged 48.4 μg of DDT/g. Japanese Quail on the same diet had a survival time of 3 to 10 days. The brain of the quail averaged 43.6 μg of DDT/g and 44.4 μg of DDT/g in the liver. However, Japanese Quail showed a preference for chick starter grain when given a choice of grain or earthworms and did not eat lethal quantities of the earthworms.

House Sparrows fed on earthworms containing 86 to 90 μg of DDT/g, had a survival time of 2 to 6 days. The brain and liver averaged 33.6 and 59.0 μg of DDT/g respectively. House Sparrows given a 12 per cent DDT solution (concentration used on elms) had a survival time of 5 to 13 days while those maintained on a 12 per cent DDT solution and tap water had a survival time of 6 to 17 days.

DDT levels in the brain and liver increased with survival time in the above groups.

The analysis of campus Robins during the spring of 1962 and 1963 showed averages of 91.7 and 130.0 μg of DDT/g respectively in the brain. The liver averaged 144.2 and 185.7 μg of DDT/g for 1962 and 1963 respectively. A Student "t" Test showed no significant difference at the 5 per cent level in DDT between the 1962 and 1963 birds. The absence or low levels of DDT in grackles found dead or dying, and the presence of wheat and/or barley seeds in their digestive tract, led to the conclusion that these birds may have consumed poisoned grain used in a sparrow control experiment.

Analysis of other species of birds showed highly variable levels of DDT in their tissues. The varied food habits of these other species show that many avenues exist for possible DDT contamination of birds.

# DDT RESIDUES IN THE FOOD CHAINS OF BIRDS

By

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# INTRODUCTION

An experimental spray program using DDT (1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl)ethane) for the control of Dutch elm disease was introduced on the Michigan State University campus in 1954. In nearly all cases a rotomist sprayer using a 12 per cent solution of DDT was employed. During the early years of the program both foliar and dormant sprays were used, but since 1959 only the dormant treatment has been employed. Usually the elms were sprayed in the spring, or in a combination of spring and fall treatments, but in 1962 all of the elm trees were sprayed during the fall and were not sprayed again in 1963.

The Michigan State University spray program was accompanied by a conspicuous decrease in campus Robins (Turdus migratorius) between 1954 and the summer of 1957 (Mehner and Wallace, 1959). Chemical analysis of Robins and other birds found dead or dying on the Michigan State University campus showed large quantities of DDT in their tissues (Bernard, 1963). Bernard (op. cit.) also showed that feeding House Sparrows DDT in chick starter mash can cause death. However, little has been done to disclose the food chains involved in the transferring of DDT to wild birds.

Several sources are believed to be responsible for chemical poisoning in birds. Barker (1958) found that living earthworms from areas treated for the control of Dutch elm disease and phloem necrosis contained DDT in amounts that could be fatal to robins. Soil samples in the spray area contained up to 18 PPM of DDT and/or DDE. Whole earthworms (Lumbricus terrestris, L. rubellus, Helodrilus spp., and Octolasium lacteum) contained from 53 to 204 PPM of DDT.

Numerous reports have shown that earthworms are resistant to most chemicals (Fleming and Hadley, 1945; Goffart, 1949; Fleming and Hawley, 1950, and Edwards and Dennis, 1960). Other reports have stated that DDT caused death or reduced earthworm activity (Baker, 1946; Martin and Wiggans, 1959).

DDT residue in soils and its consequent contamination of terrestrial organisms is another possible link in the food chains of non-target organisms. Herne and Chisholm (1958) reported that runoff from the foliage at the time of spraying was an important factor in the rate of DDT accumulation in soils. Concentrations in the soil near the trunks of the trees were significantly greater than those near the peripheries or midway between adjacent trees.

Similar results were obtained by Osborne (unpublished thesis, 1963) at the Kellogg Gull Lake Biological Station in Kalamazoo County, Michigan. Results of soil analyses following a DDT spray program showed that DDT levels in soils at the base of elm trees and from under shrubs were higher than those in soils from open areas.

In Ohio turf plots treated with an application of DDT in 1945 contained 10.9 per cent to 17.9 per cent of the original application when analyzed in 1955 (Lichtenstein, 1957). Persistence of DDT led to speculation that it is not really stored in the tissues of earthworms, but is continually reaccumulated because the earthworms are located in contaminated soils.

Birds of the bark-foraging type (e.g., Black-capped Chickadee, Parus atricapillus and White-breasted Nuthatch, Sitta carolinensis) have been found to have DDT in their tissues (Bernard, 1963). Presumably these birds feed on insects (including eggs and larvae) contaminated by DDT residues on the trunks, branches and twigs of sprayed elms. Probably some of these insects are strongly resistant

to the toxicant and thus make excellent carriers by storing significant amounts of DDT in their bodies. However, birds feeding on insects killed by small amounts of DDT are less likely to get a lethal dose than they are when feeding on the more resistant insects.

Deposits of DDT on leaves exposed to ordinary climatic conditions caused death to 75 per cent of the tsetse flies exposed after 8 weeks (Hadaway and Barlow, 1949). Analysis showed 87 mgms./sq. ft. of DDT on the surface and 17 mgm/sq. ft. on the inside of the leaves after an 8-week period.

Fruit-eating birds such as Cedar Waxwings (Bombycilla cedrorum) have shown various levels of DDT in their tissues (Wallace et al., 1961). Concentrations of DDT were high in two spring specimens (when waxwings are budding in elm trees) while two fall specimens (when waxwings are feeding on fruit) were negative. Taschenberg and Avens (1960) reported only very small amounts of DDT (6.1 to 12 PPM) on grapes 6 to 8 weeks after spraying. However, as Taschenberg and Avens pointed out, studies of this type need to include factors such as growth and weathering.

Many other studies have shown that pesticides of persistent types can accumulate in various animal and plant parts to such an extent that they will cause death to the birds that feed upon them. Concentration of pesticides by organisms in terrestrial situations have not been demonstrated so thoroughly.

Therefore, the purpose of this report was to collect and analyze for DDT the various materials believed to lead directly or indirectly to fatal poisoning in birds on the Michigan State University campus. These materials included earthworms, soils, leaves, bark, fruit and other suspected carriers of DDT. Some feeding experiments were carried out using DDT in the diet to determine its effects on birds.

## METHODS

### Procedure for Collecting

Samples of bark, earthworms, fruit and leaves were collected in the study area and analyzed for DDT. Most samples were collected between April 1962 and April 1964. The Baker and Sandford Wood Lots, which had not been sprayed since 1959 and then only aerially for mosquitoes, served as control areas. All samples were placed in plastic containers, frozen and stored until analyzed. Lichtenstein et al. (1958) report that DDT is not affected by freezing.

Earthworms were collected following heavy rains or on moist warm nights. They were identified by means of the key in Ball and Curry (1956) and Eddy and Hodson (1951) Taxonomic Keys to the Common Animal of the North Central States. Whole earthworms were analyzed for DDT.

Soil samples were taken from both dry and wet areas at depths ranging from 0 to 6 inches. No attempt was made to identify soil types. Ginsburg and Reed (1954) reported no significant variation in the amount of DDT residue in different soil types.

Bark was taken directly from the surface of American elms (Ulmus americana) 5 to 10 feet from the ground. In a few cases samples were taken at higher levels. Leaves and elm buds were collected directly from the elm trees. Fallen leaves were collected directly under the elm tree or near the periphery. Fruit materials were collected from the plants and frozen until analyzed.

## Analysis for DDT

General Considerations. --The Schechter-Haller method of analysis (Schechter et al., 1945) was used for quantitative determination of DDT. This reaction is fairly specific for DDT. Exceptions are nitrated on dehalogenated decomposition products of DDT and certain close analogues that may produce yellow to red and sometimes blue colors with Sodium Methylate. The principal known interfering insecticides are TDE (dichloro-2, 2, bis(chlorophenyl)-ethane), methoxychloro(1, 1, 1-trichloro-2, 2, bis(p-methoxyphenyl)-ethane), DNB and DNP (the nitro 1, 1, bis(p-chlorophenyl)butane and propane analogues of DDT). Other insecticidal compounds, as well as limited quantities of benzene soluble plant extractives, are oxidized or degraded in the nitration process and then removed from ether or petroleum solution by washing with alkali, or they do not react under conditions of the method.

In order to correct for deviations in technique it was necessary to carry an uncontaminated sample (control) along with each test series. These samples consisted of earthworms and soil samples from control areas (unsprayed), chemically pure sand and corn meal.

## Procedure

The procedure followed for determining DDT was that of Schechter et al., as described by the Association of Official Agricultural Chemists (1956): Remove samples from storage, weigh and then macerate in a mortar containing anhydrous sodium sulfate and quartz sand. Add a mixture of diethyl ether and petroleum ether (1:4 ratio by volume of ether-petroleum ether) to the sample. Thoroughly mix with the ether to extract DDT and then filter the ether layer through a funnel into an Erlenmeyer flask.

Evaporate the solvent on a steam bath with the aid of a gentle current of air. When the flask is dry, remove it from the steam bath and chill it thoroughly in an ice bath. While the flask is immersed in ice water, add 5.0 ml of the chilled nitrating mixture (mixture of fuming $HNO_3$ with equal volume of concentrated $H_2SO_4$) from a buret or pipet, taking care to wet all portions of the residue. Then nitrate by placing the flask in a water bath and heat so that solutions reach 85°C. in 20-30 minutes (lead rings may be used to weight flask). Next, place the flasks on a steam bath for one hour. Remove the flasks and cool under the tap or leave overnight. Cool the flask and pour the cooled acid mixture slowly from each flask into a 500 ml separator funnel containing 25 ml of ice-cold water; rinse the flasks with several portions of ice water, then pour the rinsings into the separator. Rinse again with 25 ml of the ether solution ( 1:4 ether-petroleum ether) and finally with a second 15 ml portion of ether, pouring last ether rinse into a second separator. Extract by shaking the first separator vigorously for one minute; then drain the aqueous (lower) layer into the second separator and repeat the extraction. Discard the aqueous layer and drain the second separator into the first, rinsing with small portions of ether. Draw off any residual aqueous layer as completely as possible, add 10 ml of 10 per cent KOH solution (a second or even third wash may be necessary to remove all yellow color from the ether layer), and shake vigorously for 30 seconds. Allow time for solutions to separate, then drain off the KOH layer and wash with two successive 15 ml portions of saturated NaCl solution. Drain well and filter extract through a plug of Pyrex glass wool held in a funnel into a 125 ml Erlenmeyer flask containing a glass bead (the glass wool should be saturated with ether before filtration). Rinse the separator and filter with a few small portions of ether and evaporate off the ether on a

steam bath. Cool the flask, making sure that the interior is thoroughly dry, and take up the residue with exactly 10 or 25 ml of benzene, the amount depending on the quantity of DDT expected (for small quantities use 10 ml). Stopper the flask and swirl for one minute to ensure complete solution of the residue (procedure may be interrupted overnight at this stage). Transfer a 5.0 ml aliquot of the benzene solution to a small flask and develop characteristic DDT color by adding exactly 10.0 ml of the sodium methylate solution (10 per cent sodium methylate in dry C.P. methanol). Mix well, allow solution to stand for 15 minutes, and then determine the transmittancy (absorbance may also be used) at 600 mμ in a cell of appropriate length by means of a Beckman B spectrophotometer (other photometric instruments may be used). Calculate DDT content from a standard curve prepared with the 3 to 1 $p,p^1$-$o,p^1$-DDT mixture.

Preparation of Standard Curve

In order to prepare a standard curve, dilute 40.0 mg of a DDT mixture of 3 parts $p,p^1$ and 1 part $o,p^1$ in benzene to 200 ml (1 ml = 0.20 μg). Measure 1.00, 2.00, 3.00, 4.00 and 5.00 ml of the dilution into 50 ml Erlenmeyer flasks and evaporate benzene on a steam bath. Follow the same procedure as described for the unknown samples and prepare standard curve by plotting transmittancy reading against corresponding concentrations of DDT at 600 mμ. The technique should be repeated several times in order to establish a curve that can be reproducible within small error.

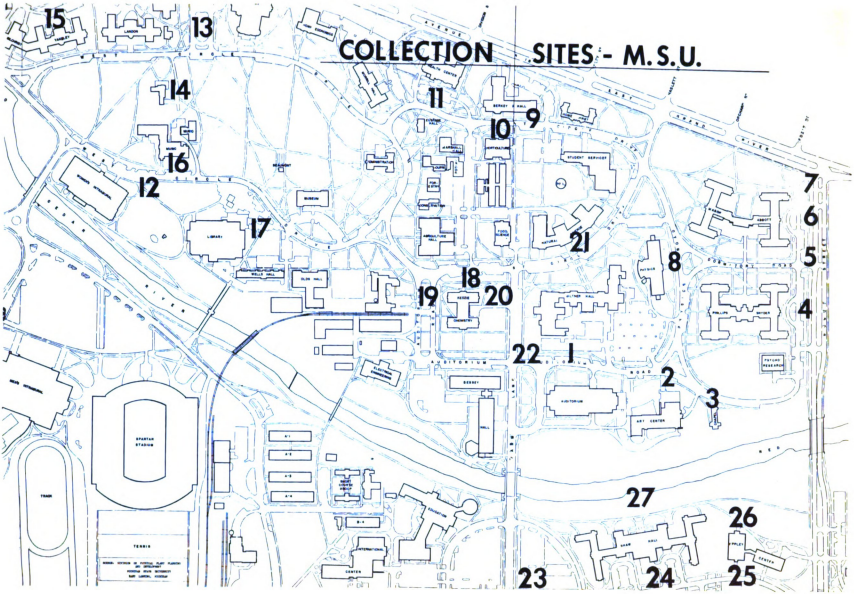
Presentation of Data

The following tables summarize the results obtained from the chemical analysis of whole earthworms, soil samples and various plant materials. The amount of DDT residue is expressed in micrograms

of DDT/gram of sample (μg of DDT/g). The spraying date given is the last known spray date for the area. These dates were obtained from the Michigan State University Department of Grounds Maintenance. Table 1 gives code numbers designating the various collecting sites on campus. Figure 1 contains a map showing more exact collecting sites referred to by the code numbers.

Table 1. Code Numbers Designating the Sampling Sites on the Michigan State University Campus

| Code Number | Location |
|---|---|
| 1 | Rear of Giltner Hall |
| 2 | Front of Kresge Art Center |
| 3 | Front of University Chapel |
| 4 | Bogue Street in Front of Snyder Hall |
| 5 | Bogue Street and Dormitory Road |
| 6 | Bogue Street in Front of Abbott Hall |
| 7 | Bogue Street and Grand River |
| 8 | Physics Road in Front of Physic Bldg. |
| 9 | South Side of Berkey Hall |
| 10 | North Side of Horticulture Bldg. |
| 11 | South Side of Health Center |
| 12 | Edge of Beal Garden |
| 13 | West Side of Union Bldg. |
| 14 | East Side of President's Home |
| 15 | Rear of Yakeley Hall |
| 16 | Front of Music Bldg. |
| 17 | Terrace on East Side of Library |
| 18 | North Side of Kedzie Chemical Laboratories |
| 19 | West Side of Kedzie Chemical Laboratories |
| 20 | East Side of Kedzie Chemical Laboratories |
| 21 | Front of Natural Science Bldg. |
| 22 | Corner of Farm Lane and Auditorium Road |
| 23 | Corner of Farm Lane and Shaw Lane |
| 24 | South of Shaw Hall |
| 25 | South of Eppley Center |
| 26 | North of Eppley Center |
| 27 | South of Red Cedar River (Rear of Shaw Hall) |
| 28 | Sanford Wood Lot |
| 29 | Baker Wood Lot |


COLLECTION SITES - M.S.U.
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# RESULTS

Earthworm samples were collected over a two and a half year period and analyzed for DDT. Only the earthworms collected during the spring of 1964 were separated and analyzed by species. The two species found were Lumbricus terrestris and Helodrilus caliginosus.

Table 2 lists residues of DDT in 22 samples of earthworms collected in the spring of 1962. Exact spray dates could not be obtained for this period but apparently most of the elms were sprayed in the fall of 1961 and only a few were sprayed in the spring of 1962. Concentrations in the earthworms ranged from 8 (S-17) to 128 μg of DDT/g (S-7). The average concentrations for the 20 experimental samples (excluding the 2 control areas) was 70.7 μg. of DDT/g. S-17 was taken from the terrace east of the Michigan State University library about 50 feet from the hearest elm tree. The flowering plants in this area were sprayed with DDT using a small hand sprayer in the fall of 1961. There was some uncertainty about the purpose of this spray. Samples S-25 and S-26 (North of Eppley Center) were from an area free of elm trees. However, this was a new construction site and apparently contaminated soil was brought in from other areas. Also, the incline on and near the collecting site may have received some runoff from the nearby sprayed areas. Areas of high elm density had high DDT concentrations in the earthworm samples (S-5, S-6, S-7, S-9, S-13, S-18 and S-19). In order to have a representative collection of earthworms, three samples were taken from each of the two control areas. All of these samples were negative for DDT.

Table 2. DDT Found in Earthworms Collected in the Spring of 1962

| Site | Date Collected | Date Last Sprayed | μg of DDT/g Earthworm |
|---|---|---|---|
| S-1 | 4-29-62 | Fall, 1961 | 78 |
| S-2 | 4-29-62 | Fall, 1961 | 65 |
| S-3 | 4-29-62 | Fall, 1961 | 55 |
| S-4 | 4-29-62 | Fall, 1961 | 54 |
| S-5 | 4-29-62 | 4-25-62* | 97 |
| S-6 | 4-29-62 | 4-25-62* | 91 |
| S-7 | 4-29-62 | Fall, 1961 | 128 |
| S-8 | 4-29-62 | Fall, 1961 | 77 |
| S-9 | 4-29-62 | Fall, 1961 | 90 |
| S-11 | 4-29-62 | Fall, 1961 | 62 |
| S-13 | 4-29-62 | Fall, 1961 | 88 |
| S-16 | 4-29-62 | Fall, 1961 | 60 |
| S-17 | 4-29-62 | Fall, 1961 | 8 |
| S-18 | 4-29-62 | 4-25-62* | 95 |
| S-19 | 4-29-62 | Fall, 1961 | 108 |
| S-20 | 4-29-62 | 4-25-62* | 85 |
| S-21 | 4-29-62 | Fall, 1961 | 50 |
| S-22 | 4-29-62 | Fall, 1961 | 88 |
| S-25 | 4-29-62 | Not Sprayed† | 23 |
| S-26 | 4-29-62 | Not Sprayed† | 12 |
| S-8 | 4-29-62 | Control Site | 0 |
| S-29 | 4-30-62 | Control Site | 0 |
| Average | | | 70.7 |

*Questionable spray date.

†Soil transported from other areas.

Table 3 lists 17 samples of earthworms and 24 soil samples collected and analyzed in the fall of 1962. The earthworm samples ranged from 12 (S-17) to 138 μg of DDT/g (S-18). The average for the 17 samples was 86.7 μg of DDT/g. Again, S-17, from the elmless library terrace, had the lowest DDT levels in earthworms and S-18, which was an area of high elm density, had the highest level for this period (fall of 1962). The average residue of 86.7 μg of DDT/g during the fall of 1962 was higher than the average recorded in the spring of 1962 (70.7 ug of DDT/g). However, the period between spraying and collecting was shorter (4 to 19 days) in the fall of 1962.

The 24 soil samples (Table 3) ranged from a low of 42 μg of DDT/g to a high of 1190 μg of DDT/g, with an average of 298.1 μg of DDT/g. S-9 (1190 μg of DDT/g) was taken 4 days after spraying and remains of the excess solution used could still be observed on the soil surface. Residues on this site decreased to 755 μg of DDT/g (wet weight) 11 days after spraying.

The average ratio of soil sample residue to the earthworm levels was 3.4 to 1.0. There seems to be no standard ratio between each soil sample and each earthworm sample. However, it is interesting to note that earthworms and soil from S-17 had the lowest levels of DDT. Though the soil residue for S-9 in the fall of 1962 was very high immediately after spraying, the earthworm concentrations for this same period did not change appreciably from the spring of 1962.

Table 4 lists the DDT levels for 15 earthworm samples and 18 soil samples taken in the spring of 1963. The earthworm concentration ranged from 13 to 100 μg of DDT/g with an average of 67.7 μg of DDT/g. Earthworms taken at S-12 in a spray area were negative for DDT. The average earthworm concentration for this period

Table 3. DDT Found in Earthworms and Soil Collected in the Fall of 1962

| Site | Date Collected | Date Last Sprayed | µg of DDT/g Earthworm | µg of DDT/g Soil |
|---|---|---|---|---|
| S-1 | 11-18-62 | 11-2-62 | 87 | 242 |
| S-2 | 11-18-62 | 11-2-62 | 89 | 201 |
| S-3 | 11-18-62 | 11-2-62 | 65 | 215 |
| S-4 | 11-18-62 | 11-2-62 | 60 | 173 |
| S-5 | 11-18-62 | 11-2-62 | 93 | 118 |
| S-6 | 11-18-62 | 11-2-62 | 88 | 135 |
| S-7 | 11-18-62 | 11-2-62 | 130 | 258 |
| S-8 | 11-28-62 | 11-2-62 | | 344* |
| S-9 | 11-28-62 | 11-24-62 | | 1190* |
| S-10 | 11-28-62 | 11-24-62 | | 727* |
| S-11 | 11-28-62 | 11-24-62 | | 336* |
| S-7 | 12-5-62 | 11-24-62 | 75 | 192 |
| S-8 | 12-5-62 | 11-24-62 | 88 | 280 |
| S-9 | 12-5-62 | 11-24-62 | 96 | 755 |
| S-10 | 12-5-62 | 11-24-62 | 108 | 387 |
| S-11 | 12-5-62 | 11-24-62 | 90 | 304 |
| S-16 | 12-5-62 | 11-24-62 | 67 | 112 |
| S-17 | 12-5-62 | Fall, 1961 | 12 | 42 |
| S-18 | 12-5-62 | 11-24-62 | 138 | 330 |
| S-19 | 12-5-62 | 11-16-62 | 105 | 222 |
| S-20 | 12-5-62 | 11-16-62 | -- | 118 |
| S-21 | 12-5-62 | 11-16-62 | -- | 97 |
| S-22 | 12-5-62 | 11-16-62 | 83 | 193 |
| S-23 | 12-5-62 | 11-16-62 | -- | 182 |
| S-29 | 12-5-62 | Control | 0 | 0 |
| Average | | | 86.7 | 298.1 |

*Dry weight.

Table 4. DDT Found in Earthworms and Soil Collected in the Spring of 1963

| Site | Date Collected | Date Last Sprayed | µg of DDT/g Earthworm | µg of DDT/g Soil |
|---|---|---|---|---|
| S-1 | 5-4-63 | 11-2-62 | 82 | 107 |
| S-2 | 5-4-63 | 11-2-62 | -- | 88 |
| S-3 | 5-4-63 | 11-2-62 | 54 | 95 |
| S-4 | 5-4-63 | 11-2-62 | -- | 38 |
| S-5 | 5-4-63 | 11-2-62 | 69 | 77 |
| S-6 | 5-4-63 | 11-2-62 | 58 | 67 |
| S-7 | 5-4-63 | 11-2-62 | 62 | 70 |
| S-9 | 5-4-63 | 11-24-62 | 85 | 214 |
| S-11 | 5-4-63 | 11-24-62 | 85 | 158 |
| S-12 | 5-4-63 | 11-24-62 | 0 | 12 |
| S-16 | 5-4-63 | 11-24-62 | 72 | 165 |
| S-17 | 5-4-63 | Fall, 1961 | 13 | 26 |
| S-18 | 5-4-63 | 11-16-62 | 98 | 118 |
| S-19 | 5-4-63 | 11-16-62 | 100 | 94 |
| S-20 | 5-4-63 | 11-16-62 | -- | 48 |
| S-21 | 5-4-63 | 11-16-62 | 44 | 85 |
| S-22 | 5-4-63 | 11-16-62 | 85 | 102 |
| S-23 | 5-4-63 | 11-16-62 | 42 | 58 |
| S-29 | 5-5-63 | Control | 0 | 0 |
| Average | | | 67.7 | 90.1 |

represents a 21 per cent decrease over the average recorded during the last period (fall of 1962). The site of low DDT residue is the same as in the other sampling periods, namely, S-17. The highest residue site (S-19) was the second highest in the fall of 1962 (Table 3) and had the highest levels in the spring of 1962 (Table 2).

Eighteen soil samples had DDT residues ranging from 12 to 214 μg of DDT/g with an average of 90.1 μg of DDT/g. The low site (S-12) was an area not directly under the sprayed elms but where some runoff or drift could occur. The average soil residue for this period (spring of 1963) represents a 70 per cent decrease over the fall of 1962 sampling period.

All of the samples recorded in Table 5 (collected in the fall of 1963) were sprayed at least 12 months earlier. Eighteen earthworm samples ranged from 10 to 95 μg of DDT/g during the fall of 1963. The average DDT level was 63.6 μg of DDT/g. S-17 was again the site of the lowest earthworm level and S-19 the site with the highest earthworm concentrations.

Soil residues ranged from 9 (S-17) to 90 μg of DDT/g (S-3 and S-9) in the fall of 1963. The average residue in the soil was 50.1 μg of DDT/g for 19 samples. This represents a 44.5 per cent decrease over the spring 1963 period. It is interesting to note that the average soil residue was 21 per cent lower than the average amount of DDT in the earthworms collected during the same period.

Table 6 shows the DDT levels found in two species of earthworms collected in the study area during the spring of 1964, namely, Lumbricus terrestris and Helodrilus caliginosus. Although no actual count was made, L. terrestris far outnumbered H. caliginosus. The DDT level for L. terrestris ranged from 8 to 78 μg of DDT/g with an average of 62.9 μg of DDT/g while H. caliginosus ranged from 11 to 83 μg of DDT/g and averaged 64.8 μg of DDT/g. The Student "t" test

Table 5. DDT Found in Earthworms and Soil Collected in the Fall of 1963

| Site | Date Collected | Date Last Sprayed | μg of DDT/g Earthworm | μg of DDT/g Soil |
|---|---|---|---|---|
| S-1 | 11-19-63 | 11-2-62 | 80 | 51 |
| S-2 | 11-19-63 | 11-2-62 | 77 | 68 |
| S-3 | 11-19-63 | 11-2-62 | 48 | 90 |
| S-4 | 11-19-63 | 11-2-62 | 55 | 45 |
| S-5 | 11-19-63 | 11-2-62 | 70 | 35 |
| S-6 | 11-19-63 | 11-2-62 | 65 | 13 |
| S-7 | 11-19-63 | 11-2-62 | 70 | 28 |
| S-8 | 11-19-63 | 11-2-62 | 32 | 82 |
| S-9 | 11-19-63 | 11-24-62 | 80 | 90 |
| S-10 | 11-19-63 | 11-24-62 | 54 | 17 |
| S-11 | 11-19-63 | 11-24-62 | 78 | 48 |
| S-16 | 11-19-63 | 11-24-62 | 75 | 78 |
| S-17 | 11-19-63 | Fall, 1961 | 10 | 9 |
| S-18 | 11-19-63 | 11-16-62 | 88 | 65 |
| S-19 | 11-19-63 | 11-16-62 | 95 | 62 |
| S-20 | 11-19-63 | 11-16-62 | -- | 36 |
| S-21 | 11-19-63 | 11-16-62 | 40 | 48 |
| S-22 | 11-19-63 | 11-16-62 | 84 | 70 |
| S-23 | 11-19-63 | 11-16-62 | 45 | 16 |
| S-29 | 11-19-63 | Control | 0 | 0 |
| Average | | | 63.6 | 50.1 |

Table 6. DDT Found in Earthworms According to Species and Soil Collected in the Spring of 1964

| Site | Date Collected | Date Last Sprayed | µg of DDT/g | | |
|---|---|---|---|---|---|
| | | | Lumbricus terrestris | Helodrilus caliginosus | Soil |
| S-1 | 3-20-64 | 11-2-62 | 72 | 78 | 25 |
| S-1 | 3-25-64 | 11-2-62 | 75 | 76 | 28 |
| S-2 | 3-20-64 | 11-2-62 | 70 | 62 | 30 |
| S-2 | 3-25-64 | 11-2-62 | 73 | 80 | 33 |
| S-4 | 3-25-64 | 11-2-62 | 60 | -- | 35 |
| S-5 | 3-25-64 | 11-2-62 | -- | -- | 30 |
| S-6 | 3-25-64 | 11-2-62 | 62 | 55 | 23 |
| S-7 | 3-25-64 | 11-2-62 | 75 | 72 | -- |
| S-9 | 3-25-64 | 11-24-62 | 74 | 70 | 38 |
| S-11 | 3-25-64 | 11-24-62 | 65 | -- | 18 |
| S-13 | 3-25-64 | 11-24-62 | 60 | 62 | 25 |
| S-14 | 3-25-64 | 11-24-62 | 52 | -- | -- |
| S-16 | 3-25-64 | 11-24-62 | 65 | 71 | 48 |
| S-17 | 3-25-64 | Fall, 1961 | 8 | 11 | 10 |
| S-18 | 3-25-64 | 11-16-62 | 78 | 83 | 40 |
| S-19 | 3-25-64 | 11-16-62 | 75 | 72 | 44 |
| S-20 | 3-25-64 | 11-16-62 | 55 | -- | 32 |
| S-21 | 3-20-64 | 11-16-62 | 38 | 47 | 25 |
| S-22 | 3-20-64 | 11-16-62 | 76 | 69 | 43 |
| S-29 | 3-20-64 | Control | 0 | -- | 0 |
| Average | | | 62.9 | 64.8 | 31.0 |

showed no significant difference at the 5 per cent level in the amounts of DDT found in the two species of earthworms.

The 17 soil samples collected during the spring of 1964 had residues ranging from 10 to 48 μg of DDT/g with an average of 31.0 μg of DDT/g (Table 6). Again, the earthworm concentrations for both species were at least twice the levels found in the soil.

Table 7 shows DDT levels in earthworms collected in the spring of 1962 adjacent to the Forest Akers Golf Course. Although the golf course is a part of the Michigan State University campus the collection sites along the roadway were not. Approximately 6 months after spraying the DDT levels for 5 samples of earthworms ranged from 54 to 72 μg of DDT/g and averaged 62.6 μg of DDT/g.

Table 8 shows the DDT levels in earthworms and soil samples taken from the same area listed in Table 7. These samples were collected in the summer of 1963 approximately 18 months after spraying. The residue ranged from 50 to 70 μg of DDT/g in the earthworms and from 7 to 45 μg of DDT/g in the soil. The averages for the earthworms and soil were 58.5 and 24.3 μg of DDT/g respectively.

## DDT Residue in the Tissues of Cultured Earthworms

It has often been suspected that earthworms containing DDT may be positive for the toxicant as a result of constant exposure to DDT contaminated soils. On the other hand the possibility of DDT storage in the tissues of these terrestrial animals exists.

In order to clarify this situation earthworms (Helodrilus foetidus) were obtained from Nelon Homer Cutstone & Hardware in Lansing, Michigan. These worms were kept in confinement in containers with peat moss and fed Quaker's Corn Meal. The earthworms only were sprayed directly with a 12 per cent solution of DDT. Immediately

Table 7. DDT Found in Earthworms Adjacent to MSU Golf Course in Spring of 1962

| Sample | Date Collected | Date Last Sprayed | µg of DDT/g Earthworm |
|---|---|---|---|
| G-1 | 5-3-62 | Fall, 1961 | 72 |
| G-2 | 5-3-62 | Fall, 1961 | 54 |
| G-3 | 5-3-62 | Fall, 1961 | 68 |
| G-4 | 5-3-62 | Fall, 1961 | 62 |
| G-5 | 5-3-62 | Fall, 1961 | 57 |
| Average | | | 62.6 |

Table 8. DDT Found in Earthworms and Soil Adjacent to MSU Golf Course in Summer of 1963

| Sample | Date Collected | Date Last Sprayed | µg of DDT/g | |
|---|---|---|---|---|
| | | | Earthworm | Soil |
| G-6 | 6-27-63 | Fall, 1961 | 51 | 38 |
| G-7 | 6-27-63 | Fall, 1961 | 70 | 11 |
| G-8 | 6-27-63 | Fall, 1961 | 55 | 17 |
| G-9 | 6-27-63 | Fall, 1961 | 64 | 45 |
| G-10 | 6-27-63 | Fall, 1961 | 50 | 7 |
| G-11 | 6-27-63 | Fall, 1961 | 61 | 28 |
| Average | | | 58.5 | 24.3 |

following spraying a sample of earthworms was taken and analyzed for DDT. At various intervals the earthworms were removed from the now contaminated peat moss and placed in uncontaminated peat moss. Subsequently, samples were taken at weekly intervals and the DDT levels determined. The earthworms were kept for 22 weeks. The results are recorded in Table 9 and Figure 2.

Results. -- The initial sample taken immediately after spraying showed 290 μg of DDT/g. After one week and one soil change a sample of earthworms showed a decrease to 201 μg of DDT/g. After 10 weeks and three additional soil changes 94 μg of DDT/g was present in the earthworms. For the next 12 weeks the DDT residue ranged from 86 to 92 μg of DDT/g (Table 9). This narrow range seems to indicate a leveling off of the residue at 10 to 12 weeks (Figure 2)..

## DDT Residue in Plant Material

Leaves, fruit, elm bark and buds were collected and analyzed for DDT residue during the fall of 1962 and the spring of 1963. The samples taken in the fall were collected both before and after the dormant spraying. DDT levels were determined in the bark, buds and leaves of Ulmus americana and the fruit of High Bush Cranberry (Viburnum opulus) and Rosebud Crab Apple (Malus spp.).

Results. --The 5 fruit samples taken before the fall spraying are listed in Table 10. In three collection sites (S-1, S-9, and S-22) the fruit was negative for DDT; the average for the 5 samples was 2.4 μg of DDT/g.

The elm bark residue prior to the fall spraying ranged from 7 to 48 μg of DDT/g with an average of 23.6 μg of DDT/g. in 5 samples.

Table 9. DDT Determined at Weekly Intervals in Helodrilus foetidus Sprayed with 12 Per Cent DDT

| Number of Weeks | µg of DDT / g of Earthworm |
|---|---|
| 0 | 290 |
| 1* | 201 |
| 2 | 185 |
| 3* | 147 |
| 4 | 122 |
| 5 | 126 |
| 6 | 120 |
| 7 | 112 |
| 8* | 107 |
| 9 | 106 |
| 10 | 94 |
| 11 | 92 |
| 12 | 90 |
| 13 | 87 |
| 14* | 88 |
| 15 | 87 |
| 16 | 86 |
| 17 | 88 |
| 18* | 90 |
| 19 | 88 |
| 20* | 87 |
| 21 | 88 |
| 22 | 86 |

*Soil changes.

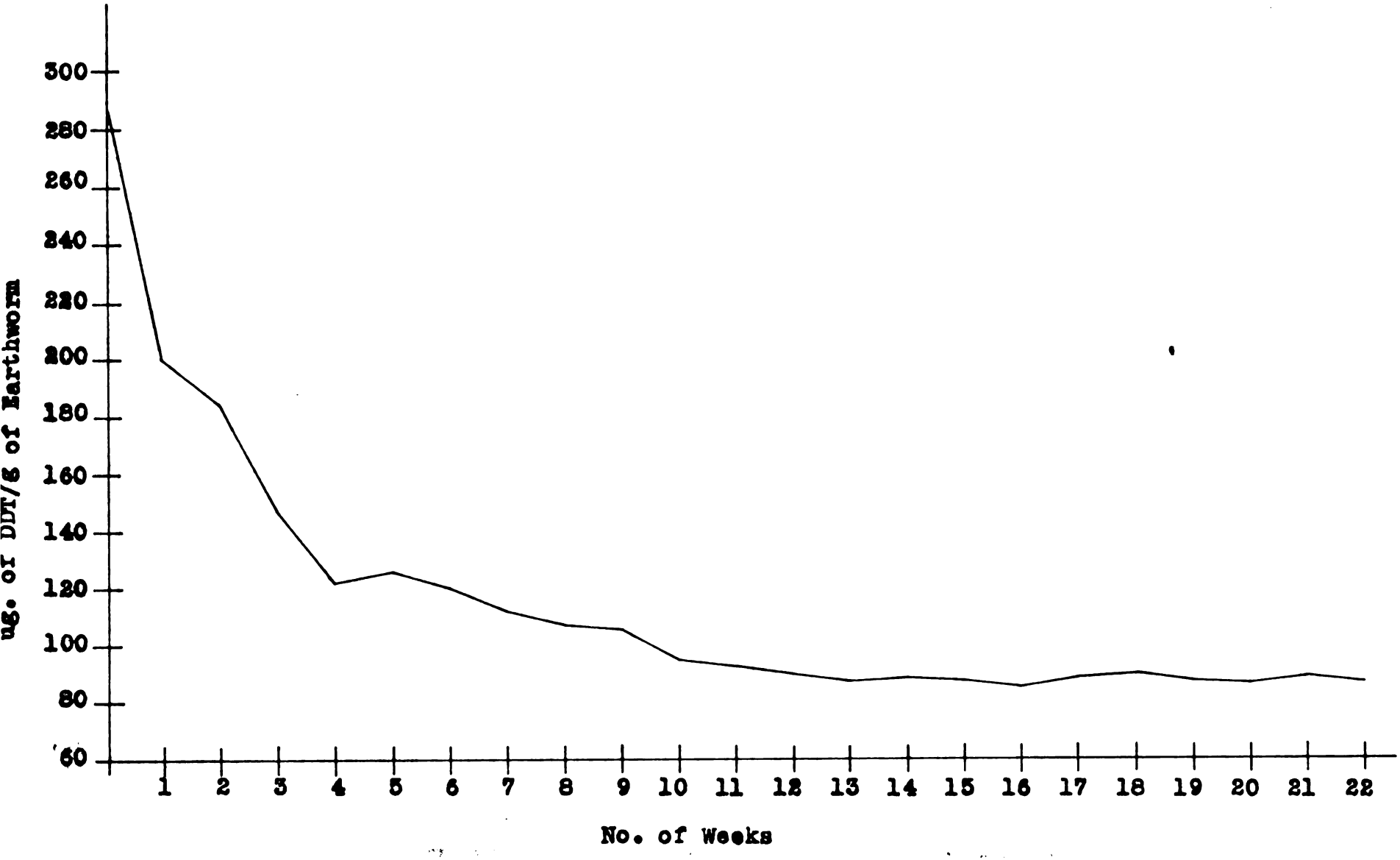


Figure 1. Concentrations of DDT In Earthworms Sprayed With 12 Per Cent DDT.

Table 10. DDT Residue on Plant Material Prior to Fall Spraying 1962

| Site | Plant Material | Date Collected | Date Last Sprayed | μg of DDT/g |
|---|---|---|---|---|
| 1 | Crab Apple Fruit | 10-29-62 | Fall, 1961 | 4 |
| 1 | Cranberry Fruit | 10-29-62 | Fall, 1961 | 0 |
| 1 | Fallen Elm Leaves | 10-29-62 | Fall, 1961 | 33 |
| 1 | Elm Bark | 10-29-62 | Fall, 1961 | 16 |
| 3 | Fallen Elm Leaves | 10-29-62 | Fall, 1961 | 18 |
| 3 | Elm Bark | 10-29-62 | Fall, 1961 | 48 |
| 5 | Fallen Elm Leaves | 10-29-62 | 4-25-62 | 15 |
| 8 | Cranberry Fruit | 10-29-62 | Fall, 1961 | 8 |
| 9 | Cranberry Fruit | 10-29-62 | Fall, 1961 | 0 |
| 9 | Fallen Elm Leaves | 10-29-62 | Fall, 1961 | 12 |
| 9 | Elm Bark | 10-29-62 | Fall, 1961 | 7 |
| 16 | Fallen Elm Leaves | 10-29-62 | Fall, 1961 | 10 |
| 19 | Fallen Elm Leaves | 10-29-62 | Fall, 1961 | 0 |
| 19 | Elm Bark | 10-29-62 | Fall, 1961 | 19 |
| 22 | Fallen Elm Leaves | 10-29-62 | Fall, 1961 | 0 |
| 22 | Elm Bark | 10-29-62 | Fall, 1961 | 28 |
| 22 | Cranberry Fruit | 10-29-62 | Fall, 1961 | 0 |

Fallen elm leaves ranged from no DDT residue on 2 samples (S-19 and S-22) to a high of 33 μg of DDT/g; the average was 12.5 μg of DDT/g. All of the samples listed in Table 10 were sprayed approximately one year earlier except S-5 which was sprayed April 25, 1962.

Table 11 lists DDT residues for plant samples taken 4 to 26 days after the dormant spraying in the fall of 1962. Residues on 7 fruit samples ranged from 3 to 83 μg of DDT/g with an average of 44.4 μg of DDT/g. Supposedly, these plants were not sprayed directly; they are merely located in the immediate area of the sprayed elms. S-8 and S-19, collected 4 days after spraying, had 80 and 83 μg of DDT/g respectively, whereas S-1 and S-2 collected 26 days after spraying had 37 and 58 μg of DDT/g. However, 4 days after spraying, S-9 had only 15 μg of DDT/g. Weathering, as well as location of the fruit with respect to the sprayed elms, are factors associated with the disappearance and concentration of surface deposits.

Elm bark residues after the fall spraying ranged from 130 to 392 μg of DDT/g with an average level of 242.1 μg of DDT/g (Table 11). There is no apparent correlation between levels of DDT and the time that elapsed between spraying and collecting in the fall of 1962.

Nine samples of fallen elm leaves ranged from 75 to 308 μg of DDT/g (Table 11). Presumably, these elm leaves had fallen since the last fall clean up period or were missed by the ground crews at that time. The average DDT level was 167.5 μg of DDT/g. Variation in both elm bark and elm leaf residue is probably due to the lack of uniform spraying as well as weathering and growth.

Table 12 summarizes the levels of DDT in plant materials collected during the spring of 1963, 6 to 7 months after spraying. Four samples of elm leaves taken directly from the trees (S-8, S-9

Table 11. DDT Residue on Plant Material After Fall Spraying 1962

| Site | Plant Material | Date Collected | Date Last Sprayed | μg of DDT/g |
|---|---|---|---|---|
| 1 | Cranberry Fruit | 11-28-62 | 11-2-62 | 37 |
| 1 | Elm Bark | 11-28-62 | 11-2-62 | 205 |
| 1 | Fallen Elm Leaves | 11-28-62 | 11-2-62 | 92 |
| 2 | Cranberry Fruit | 11-28-62 | 11-2-62 | 58 |
| 2 | Fallen Elm Leaves | 11-28-62 | 11-2-62 | 118 |
| 3 | Fallen Elm Leaves | 11-28-62 | 11-2-62 | 75 |
| 3 | Elm Bark | 11-28-62 | 11-2-62 | 255 |
| 5 | Fallen Elm Leaves | 11-28-62 | 11-2-62 | 193 |
| 8 | Fallen Elm Leaves | 11-28-62 | 11-24-62 | 308 |
| 8 | Elm Bark | 11-28-62 | 11-24-62 | 392 |
| 8 | Cranberry Fruit | 11-28-62 | 11-24-62 | 80 |
| 9 | Fallen Elm Leaves | 11-28-62 | 11-24-62 | 276 |
| 9 | Elm Bark | 11-28-62 | 11-24-62 | 318 |
| 9 | Cranberry Fruit | 11-28-62 | 11-24-62 | 15 |
| 15 | Cranberry Fruit | 11-28-62 | ? | 3 |
| 16 | Fallen Elm Leaves | 11-28-62 | 11-24-62 | 185 |
| 16 | Elm Bark | 11-28-62 | 11-24-62 | 208 |
| 18 | Fallen Elm Leaves | 11-28-62 | 11-24-62 | 175 |
| 19 | Elm Bark | 11-28-62 | 11-24-62 | 187 |
| 19 | Cranberry Fruit | 11-28-62 | 11-24-62 | 83 |
| 22 | Fallen Elm Leaves | 11-28-62 | 11-24-62 | 86 |
| 22 | Elm Bark | 11-28-62 | 11-24-62 | 130 |
| 22 | Cranberry Fruit | 11-28-62 | 11-24-62 | 35 |

Table 12. DDT Residue on Plant Material in the Spring of 1963

| Site | Plant Material | Date Collected | Date Last Sprayed | μg of DDT/g |
|---|---|---|---|---|
| 1 | Elm Leaves | 6-10-63 | 11-2-62 | 7 |
| 1 | Elm Buds | 6-10-63 | 11-2-62 | 0 |
| 1 | Elm Bark | 6-10-63 | 11-2-62 | 84 |
| 1 | Cranberry Fruit | 6-10-63 | 11-2-62 | 4 |
| 3 | Elm Leaves | 6-10-63 | 11-2-62 | 0 |
| 3 | Elm Buds | 6-10-63 | 11-2-62 | 0 |
| 3 | Elm Bark | 6-10-63 | 11-2-62 | 78 |
| 5 | Elm Leaves | 6-10-63 | 11-2-62 | 8 |
| 5 | Elm Bark | 6-10-63 | 11-2-62 | 112 |
| 5 | Elm Buds | 6-10-63 | 11-2-62 | 4 |
| 8 | Elm Leaves | 6-10-63 | 11-24-62 | 0 |
| 9 | Elm Leaves | 6-10-63 | 11-24-62 | 0 |
| 9 | Elm Buds | 6-10-63 | 11-24-62 | 5 |
| 15 | Cranberry Fruit | 6-10-63 | 11-24-62 | 0 |
| 16 | Cranberry Fruit | 6-10-63 | 11-24-62 | 0 |
| 18 | Elm Leaves | 6-10-63 | 11-24-62 | 2 |
| 18 | Elm Bark | 6-10-63 | 11-24-62 | 65 |
| 19 | Elm Leaves | 6-10-63 | 11-24-62 | 0 |
| 19 | Elm Buds | 6-10-63 | 11-24-62 | 0 |
| 22 | Elm Leaves | 6-10-63 | 11-24-62 | 8 |
| 22 | Elm Buds | 6-10-63 | 11-24-62 | 0 |
| 22 | Elm Bark | 6-10-63 | 11-24-62 | 121 |
| 22 | Cranberry Fruit | 6-10-63 | 11-24-62 | 0 |

Table 13. Average DDT Residue (μg of DDT/g) On Plant Material

| Plant Material | Fall 1962 Prior to Spraying | Fall 1962 After Spraying | Spring 1963 |
|---|---|---|---|
| Fruit | 2.4 | 44.4 | 1.0 |
| Elm Bark | 23.6 | 242.1 | 92.0 |
| Fallen Elm Leaves | 12.5 | 167.5 | |
| Elm Buds | | | 1.5 |
| Elm Leaves | | | 3.1 |

and S-19) were negative for DDT. Residues in the other samples ranged from 2 to 8 μg of DDT/g with an average of 3.1 μg of DDT/g.

Of the 6 elm bud samples taken 4 were negative. S-5 and S-9 had 4 and 5 μg of DDT/g respectively. Similarly leaves and buds from S-3 and S-19 were negative for DDT.

Five elm bark samples collected 6 to 7 months after spraying ranged from 78 to 121 μg of DDT/g with an average of 92.0 μg of DDT/g. This represents a 61 per cent decrease in bark residue from the previous sampling period.

## DISCUSSION

DDT contamination of the diet is probably received from several sources. Some 50 species of Michigan birds were analyzed by Bernard (1963; Bernard and Wallace, in press) of which 38 species with widely varying food habits had at least some DDT in their tissues. This would not only suggest several sources of contamination but possible links between these sources.

The data in this investigation show that earthworms are highly resistant to DDT. The fact that they can be sprayed directly with a 12 per cent DDT solution and show DDT levels up to 290 μg of DDT/g in their tissues, with no apparent deleterious effects, supports results from other investigations. Edwards and Dennis (1960) demonstrated no apparent effect on earthworms in soils treated with 200 lb/acre of 5 per cent DDT dust. My experiments demonstrate that earthworms can survive up to 18 months in contaminated soil without any observable effects.

The high residue recorded in the soil following spraying and appreciable quantities found during subsequent periods offer an excellent pathway for DDT contamination by means of terrestrial earthworms. Although there was a rapid decrease of residues in the soil, from an average of 298.1 μg of DDT/g (4 to 19 days after spraying), to 31.0 μg. of DDT/g a year and a half after spraying a source of contamination still exists. Foster (1951) has shown that quantities of DDT deposits were not severely affected by soil types or rain over a period of 4 years. Lichtenstein et al. (1958) report that high temperatures can cause some DDT decomposition. Differences recorded in various sample sites can probably be attributed to lack of

uniform spraying, difference in runoffs and drippings and degree of adsorption by the soil. Also, a direct correlation between high elm tree density and high soil residue exists. This conceivably could increase soil and earthworm deposits because of the increased sprayed foliage in the area. Regardless of the quantitative changes in DDT soil residue, there is still enough available to earthworms to show high amounts of DDT in their tissues.

Earthworms do reside at depths where soil deposits exist. During warm periods earthworms can be found at depths of 3 to 8 inches. Lichtenstein (1958) reports that 17 months after spraying 84 to 96 per cent of the DDT was found in the upper 3-inch level, 4 to 12 per cent in the 3 to 6 inch layer and 0 to 5 per cent in the 6 to 9 inch layer of soil. Even if the animals are not present in the apparently high DDT residue layers, earthworms habitually crawl out of the soil after a heavy rain or on moist warm nights thereby picking up the toxicant. Also, they could feed on contaminated organic matter near the surface.

The large amount of DDT residue on elm bark immediately following spraying (242.1 μg of DDT/g; Table 13) indicates excellent possibilities for runoff of DDT into the soil. The decrease in DDT residue in the bark the following spring to an average of 92.0 μg of DDT/g tends to indicate some weathering effect, including runoff. Also, disintegrating leaves, which averaged 167.5 μg of DDT/g, are another possible avenue for soil contamination as well as a food source for earthworms. Barker (1958) demonstrated DDT accumulation in the soil after spraying. Samples of fresh leaves contained up to 206 μg of DDT/g.

The presence of high DDT residue on the bark of elm trees offers an excellent source of contamination for bark foraging birds (woodpeckers, nuthatches and chickadees). Birds of these species

found dying or dead had DDT in their tissues (Wallace, Nickell and Bernard, 1962; Bernard, 1963). These birds may get the DDT directly from the sap of the sprayed trees, or from insects such as bark beetles.

Translocation of DDT from the soil to the leaves of elm trees is probably a very minor or non-existent factor. Lichtenstein (1959) has shown small amounts of DDT and other hydrocarbons in carrots grown in soil having the insecticide. However, no satisfactory evidence exists for supporting translocation of DDT from the soil to the leaves of elms. This investigation showed little or no DDT in young elm leaves and elm buds in the spring following fall spraying. The small amounts recorded probably came from overhead runoff or drippings from the above branches. All of these elm buds or leaves were collected near the lower levels of foliar growth and in no case higher than one-third distance up the tree. Also, it is possible that some DDT could be carried out into the buds and leaves with the new spring growth.

The fact that DDT is practically insoluble in water probably keeps translocation at a minimum. It does appear from these data, however, that the high DDT residue on fallen leaves was due to run-off or drippings (Table 13) and not through translocation of the insecticide. One must realize that any compound with even some water soluble properties and stability may possess some degree of systemic action. In fact, almost all organic insecticides are capable of penetrating into plant tissues and systemic action is, therefore, a matter of degree rather than a specific property.

As was pointed out earlier, differences in the amount of DDT found on fruit was probably due to lack of uniformity of the spray application as well as growth and weathering. The low levels and the early disappearance of the DDT after spraying is probably the

reason for low mortality in fruit-eating birds such as Cedar Waxwings. These and other birds have been observed feeding on High Bush Cranberries and other fruit.

Earthworms kept in confinement indicate that they can store DDT in their tissues without being constantly exposed to contaminated soil. This fact is supported by previous results which showed lower levels of DDT in the soil than in earthworms (Tables 5, 6 and 8). Similarly, Barker (1958) sampled soil and earthworms from areas sprayed for Dutch elm disease and phloem necrosis and found that the soil contained up to 18 PPM of DDT and/or DDE while earthworms (Lumbricus terrestris, L. rubellus and Helodrilus spp.) contained 53 to 204 PPM.

## FEEDING EXPERIMENTS

In order to support as well as to clarify the data on earthworms earlier in this report, captive House Sparrows (Passer domesticus) and Japanese Quail (Coturnix coturnix) were fed earthworms (Helodrilus foetidus) containing different levels of DDT. The study involved determining if earthworms containing various levels could cause death, the survival time if death occurred, and the quantities of the toxicant in the tissues of the birds. Other birds were given DDT contaminated water to see if such solutions might be toxic to them. It has often been assumed that drinking from "puddles" of excess spray might be lethal to birds.

### Method

Adult House Sparrows were captured on the Michigan State University campus by means of a mist net. The birds were kept approximately two weeks before subjecting them to tests. A representative sample of the captive birds was taken in each case and the brain and liver analyzed for DDT. In no case did the DDT residue exceed 5 μg of DDT/g. Also, the brain and liver of control birds were used as control samples during the analysis for DDT.

The House Sparrows were divided into two groups, an experimental and control. Each group was fed approximately 25 grams of redworms (Helodrilus foetidus) twice daily. These redworms were purchased from a local bait shop, raised in moist peat moss and fed Quaker's Corn Meal. The earthworms fed to the experimental groups were sprayed with a 12 per cent DDT solution. This is the same

formulation used by the Michigan State University in the control of Dutch elm disease. The control groups were fed uncontaminated worms in similar fashion.

Coturnix quail ranging from 5 to 10 days of age were obtained from the Michigan State University Poultry Department. They were kept 4 to 5 weeks before being subjected to feeding tests. Two experimental and two control groups were maintained. One experimental group was fed DDT contaminated redworms while the other group was fed contaminated redworms and uncontaminated chick starter crumbles. One control group was fed non-toxic redworms and the other group fed non-toxic redworms and chick starter crumbles.

Other House Sparrows were divided into three groups. One group was given 12 per cent DDT solution and regular feed. A second group was given 12 per cent DDT solution, tap water and regular feed. The control group had tap water and regular feed.

In all cases the birds were observed for symptoms of DDT poisoning (tremors) and the survival time recorded. The brain and liver of the birds were analyzed for DDT.

## Analytical Procedure

The brain and liver of the birds that died or were sacrificed (Group 4, Table 17) during the experiment were analyzed for DDT. Control birds were used as blanks in the analysis. The procedure used was the method of Schechter et al. as described by the Association of Official Agriculture Chemists.

## Results

Group 1.--Table 14 summarizes the results obtained from 10 birds maintained on a diet of earthworms containing approximately 298 μg of DDT/g. The survival time ranged from 1 to 5 days with

Table 14. DDT Determined in House Sparrows Fed a Diet of Earthworms Containing 298 μg of DDT/g. Group 1

| No. | Specimen Data | | | μg of DDT/g of Tissue | |
|---|---|---|---|---|---|
| | Date * | Sex | Condition | Brain | Liver |
| H-1 | 5-8-62 | M | Dead | 19 | 22 |
| H-2 | 5-9-62 | F | Tremors | 26 | 29 |
| H-3 | 5-10-62 | M | Tremors | 22 | 48 |
| H-4 | 5-10-62 | M | Dead | 21 | 47 |
| H-5 | 5-10-62 | F | Tremors | 28 | 58 |
| H-6 | 5-10-62 | M | Tremors | 42 | 40 |
| H-7 | 5-10-62 | F | Tremors | 43 | 57 |
| H-8 | 5-11-62 | F | Dead | 38 | 62 |
| H-9 | 5-11-62 | M | Tremors | 35 | 79 |
| H-10 | 5-12-62 | M | Tremors | 40 | 42 |
| | Average | | | 31.4 | 48.4 |

*Starting date, May 7, 1962.

half (5) of the birds dying on the third day. Seven of the 10 experimental birds were observed in tremors at least 12 hours prior to death. The level of DDT in the brain ranged from 19 to 43 μg of DDT/g with an average concentration of 31.4 μg of DDT/g. DDT levels in the liver ranged from 22 to 79 μg of DDT/g and averaged 48.4 μg of DDT/g. The lowest level of DDT found in the brain (19 μg of DDT/g) and liver (22 μg of DDT/g) was in the bird with the shortest survival time (H-1). The residue level tends to increase with survival time. However, H-10 deviates from this pattern. Some variation may be anticipated since the liver has high storage abilities.

The fact that half of the birds died in three days rules against the idea that one bird may have eaten most of the worms at one feeding.

Group 2.--Table 15 shows the results of feeding Coturnix coturnix on redworms with approximately 298 μg of DDT/g. The survival time ranged from 3 to 10 days, Three of the birds died within 3 days while the maximum survival time for 5 other birds was 10 days. Three birds did not exhibit tremors prior to death. The period of tremors lasted about 48 hours in Q-5 and about 72 hours in Q-7 and Q-8. However, the tremors were not as severe as those observed in House Sparrows.

The level of DDT in the brain ranged from 32 to 51 μg of DDT/g with an average residue of 43.6 μg of DDT/g. The liver showed a range of 37 to 57 μg of DDT/g with an average level of 44.8 μg of DDT/g. It is interesting to note that the average concentrations for the brain and liver, 43.6 and 44.8 respectively, is very similar. Even so, in both the brain and liver there is an increase in DDT with an increase in survival time.

Table 15. DDT Determined in Japanese Quail Fed a Diet of Earthworms Containing 298 μg of DDT/g. Group 2

| No. | Specimen Data | | | μg of DDT/g of Tissue | |
|---|---|---|---|---|---|
| | Date * | Sex | Condition | Brain | Liver |
| Q-1 | 5-13-62 | Unknown | Dead | 38 | 37 |
| Q-2 | 5-13-62 | | Tremors | 32 | 44 |
| Q-3 | 5-13-62 | | Tremors | 41 | 47 |
| Q-4 | 5-14-62 | | Dead | 44 | 51 |
| Q-5 | 5-16-62 | | Tremors | 48 | 43 |
| Q-6 | 5-18-62 | | Dead | 51 | 42 |
| Q-7 | 5-20-62 | | Tremors | 44 | 38 |
| Q-8 | 5-20-62 | | Tremors | 51 | 57 |
| | Average | | | 43.6 | 44.8 |

*Starting date, May 10, 1962.

Group 3.--In order to determine the effects of starvation on survival time of quail fed DDT contaminated earthworms, a group of Coturnix coturnix was fed redworms containing 298 μg of DDT/g and regular chick starter feed. Table 16 summarizes these results.

The birds were allowed ad libitum to the chick starter while earthworms were furnished twice daily. In no case did the group eat more than 25 per cent of the worms. In most cases only one or two worms were consumed. This indicates that earthworms are not a preferred item in the diet of C. coturnix.

None of the birds died or exhibited tremors, so they were sacrificed after 4 weeks and the brain and liver analyzed for DDT. Four birds showed no DDT in the brain. The range for the other 6 birds was 4 to 18 μg of DDT/g with an average of 5.6 μg of DDT/g. Three of the birds showed no DDT in the liver. The other birds showed levels ranging from 3 to 16 μg of DDT/g. The average for the liver was 6.3 μg of DDT/g.

Group 4.--Table 17 summarizes the results of feeding 5 House Sparrows earthworms kept in the laboratory to determine the amount of DDT in their tissues (Table 9 and Figure 2). The DDT in the earthworms ranged from 86 to 90 μg of DDT/g. The survival time of the sparrows ranged from 2 to 6 days with 2 birds dying after 3 days. All of the birds were observed in tremors prior to death.

DDT in the brain ranged from 18 to 43 μg of DDT/g. The average level was 33.6 μg of DDT/g. There was a definite increase in DDT levels with increase in survival time. The liver had levels ranging from 47 to 66 μg of DDT/g with an average of 59.0 μg of DDT/g.

Group 5.--The results of feeding 12 House Sparrows a 12 per cent DDT solution are summarized in Table 18. Only one bird (H-3) was not observed in tremors prior to death. The survival time ranged

Table 16. DDT Determined in Japanese Quail Fed DDT Earthworms (298 μg of DDT/g) and Regular Chick Starter Grain *
Group 3

| Number | μg of DDT/g | |
|---|---|---|
| | Brain | Liver |
| Q-1 | 0 | 5 |
| Q-2 | 0 | 0 |
| Q-3 | 6 | 8 |
| Q-4 | 0 | 0 |
| Q-5 | 4 | 7 |
| Q-6 | 10 | 16 |
| Q-7 | 11 | 14 |
| Q-8 | 7 | 0 |
| Q-9 | 18 | 10 |
| Q-10 | 0 | 3 |
| Average | 5.6 | 6.3 |

*Sacrificed after 4 weeks of feeding.

Table 17. DDT Determined in House Sparrows Fed a Diet of Earthworms Containing 86-90 g of DDT/g * Group 4

| No. | Specimen Data | | | g of DDT/g of Tissue | |
|---|---|---|---|---|---|
| | Date † | Sex | Condition | Brain | Liver |
| H-1 | 10-5-62 | F | Tremors | 18 | 47 |
| H-2 | 10-6-62 | M | Tremors | 32 | 63 |
| H-3 | 10-6-62 | M | Tremors | 35 | 58 |
| H-4 | 10-8-62 | M | Tremors | 40 | 61 |
| H-5 | 10-9-62 | F | Tremors | 43 | 66 |
| | Average | | | 33.6 | 59.0 |

*Earthworms sprayed with 12% DDT solution and fed to House Sparrows after 22 weeks.

†Starting date October 3, 1962.

Table 18. DDT Determined in House Sparrows Given 12% DDT Solution Group 5

| No. | Specimen Data | | | Ml. of Solution Consumed DDT | µg of DDT/g | |
|---|---|---|---|---|---|---|
| | Date* | Sex | Condition | | Brain | Liver |
| H-1 | 2-8-64 | F | Tremors | 9.55 | 9 | 17 |
| H-2 | 2-8-64 | M | Tremors | 9.55 | 14 | 12 |
| H-3 | 2-9-64 | F | Dead | 11.46 | 16 | 20 |
| H-4 | 2-9-64 | M | Tremors | 11.46 | 15 | 18 |
| H-5 | 2-14-64 | F | Tremors | 21.01 | 26 | 33 |
| H-6 | 2-14-64 | M | Tremors | 21.01 | 32 | 48 |
| H-7 | 2-14-64 | M | Tremors | 21.01 | 25 | 38 |
| H-8 | 2-14-64 | F | Tremors | 21.01 | 30 | 44 |
| H-9 | 2-15-64 | M | Tremors | 22.92 | 28 | 52 |
| H-10 | 2-15-64 | M | Tremors | 22.92 | 33 | 48 |
| H-11 | 2-16-64 | F | Tremors | 24.83 | 35 | 56 |
| H-12 | 2-16-64 | M | Tremors | 24.83 | 38 | 55 |
| | Average | | | | 25.1 | 36.7 |

*Starting date, February 3, 1964.

from 5 to 13 days. The average daily consumption of the DDT solution per bird was 1.91 ml. The longer the survival time the greater the amount of DDT present in the brain and liver. DDT concentrations in the brain ranged from 9 to 38 μg of DDT/g while the liver ranged from 12 to 56 μg of DDT/g. The brain and liver averaged 25.1 and 36.7 μg of DDT/g respectively.

Group 6.--Ten House Sparrows were given a choice of a 12 per cent DDT solution or tap water; the results are shown in Table 19. Each bird consumed an average of 1.00 ml of DDT solution per day and 1.40 ml of tap water. The survival time ranged from 6 to 17 days. Again, as the survival time increased so did the DDT residue in the brain and liver. The DDT level for the brain ranged from 20 to 51 μg of DDT/g, averaging 31.3 μg of DDT/g. The level in the liver ranged from 28 to 68 μg of DDT/g with an average of 49.5 μg of DDT/g.

## Discussion

The results of the feeding tests indicate that earthworms containing DDT as well as DDT solutions can cause bird mortality. The birds were quickly affected and died both from feeding on DDT contaminated earthworms and from drinking DDT solutions directly. Also, the presence of high levels of the toxicant in the brain and liver indicates clearly that the birds died directly as a result of DDT poisoning.

Birds such as Robins that feed on large numbers of earthworms are probably highly susceptible to DDT poisoning. Earlier results have shown high levels of DDT in earthworms up to a year and a half after spraying. Earthworms do not comprise a major portion of the diet of House Sparrows or Japanese Quail but both species were found

Table 19. DDT Determined in House Sparrows Given 12% DDT Solution and Tap Water Group 6

| No. | Specimen Data | | | Ml. of Solution Consumed | | μg of DDT/g | |
|---|---|---|---|---|---|---|---|
| | Date* | Sex | Condition | DDT Solution | $H_2O$ | Brain | Liver |
| H-13 | 2-9-64 | M | Tremors | 6.00 | 8.40 | 20 | 32 |
| H-14 | 2-9-64 | F | Dead | 6.00 | 8.40 | 26 | 38 |
| H-15 | 2-9-64 | M | Tremors | 6.00 | 8.40 | 23 | 28 |
| H-16 | 2-12-64 | M | Tremors | 9.00 | 12.60 | 28 | 42 |
| H-17 | 2-12-64 | F | Tremors | 9.00 | 12.60 | 30 | 55 |
| H-18 | 2-13-64 | F | Tremors | 10.00 | 14.00 | 25 | 44 |
| H-19 | 2-16-64 | M | Dead | 13.00 | 18.20 | 34 | 58 |
| H-20 | 2-18-64 | M | Tremors | 15.00 | 21.00 | 36 | 65 |
| H-21 | 2-20-64 | M | Tremors | 17.00 | 23.80 | 40 | 65 |
| H-22 | 2-20-64 | F | Dead | 17.00 | 23.80 | 51 | 68 |
| | Average | | | | | 25.1 | 36.7 |

*Starting date, February 3, 1964.

to be excellent laboratory animals and easy to keep. Bernard (1963) was unsuccessful in an attempt to keep adequate numbers of Robins in captivity.

Although the amount of DDT in some of the earthworms used (298 μg of DDT/ g) was much higher than the amount of the toxicant recorded in earthworms collected on campus (highest sample 138 μg of DDT/g), the data indicate that lower levels are similarly toxic. Table 17 shows quite clearly that House Sparrow mortality can result when the birds are fed earthworms containing 86 to 90 μg of DDT/g. Also, the survival time for these birds was approximately the same as the House Sparrows fed earthworms with the larger dosage. Survival time was 1 to 5 days on the higher dosage and 2 to 6 days with the lower dosage. However, when the lower dosage was used, the brain and liver averaged 33.6 and 59.0 μg of DDT/g respectively while birds on the higher dosage had concentrations of 31.4 and 48.4 μg of DDT/g in the brain and liver.

The survival time for House Sparrows on a contaminated earthworm diet was much shorter than the survival time recorded by Bernard (1963) for House Sparrows on a chick starter mash diet. Sixteen House Sparrows fed chick starter mash with 300 PPM of DDT survived from 7 to 29 days. At 200 PPM it took 22 to 49 days to kill 12 House Sparrows. Cross et al. (1962) had a mean survival time of 20.6 days for Japanese Quail fed 300 PPM of DDT in cracked corn. At 100 PPM the survival time of the quail was 30.0 days. All of the quail used in the experiment were between 50 and 80 days of age.

The short survival time demonstrated in my experiments can probably be attributed to a more concentrated distribution of the toxicant in earthworms, or in a DDT solution, than with chick starter mash or cracked corn. Also, the digestion of earthworms is obviously

much easier and more thorough than the digestion of chick mash or cracked corn. This would suggest quicker dispersal within the body of the feeding animal and consequently earlier effects. The quantity of fecal material and the accumulation of fat would be at a minimum.

Bernard (1963) found minimum DDT levels in the brain of House Sparrows fed chick starter mash at 65 μg of DDT/g. My results showed a maximum of 43 μg of DDT/g in sparrows on an earthworm diet. The short survival time in my birds probably prevented high DDT accumulations. Bernard (1963) found higher DDT levels in birds having longer survival times which compares favorably with my findings.

Although there appears to be a direct correlation between survival time and DDT levels in the brain and liver, the data are not adequate for determining exact lethal levels. The amount of DDT accumulated is quite variable not only in experimental birds but also in birds found dying or dead from DDT poisoning. The amount of the toxicant consumed at a given period of time as well as the rate of digestion are probably the most important factors causing bird mortality.

When Coturnix coturnix were given a choice between earthworm and chick starter grain, they chose the latter. The small amounts of DDT consumed by the quail produced no apparent effects and only low levels of DDT was found in their tissues. It is conceivable that birds receiving low levels of DDT in their diet can break down, destroy or pass off such small quantities. It has been observed that birds with more specialized food habits (e.g., Robins or other animal feeders) are the birds showing high mortality. Birds such as Common Grackles and Starlings, with varied food habits, show a lower incidence of DDT poisoning.

Drinking as a pathway for DDT poisoning is very possible. Birds may drink from "insecticide puddles" formed immediately after spraying. Also, contamination of water is possible by spraying the sites directly, or by runoff or drippings. Here again there is a definite correlation between survival time, the amount of liquid drunk and amount of DDT in the brain and liver. It is noteworthy that when the birds were given both DDT and tap water there was only a slight difference in intake. Both the sense of smell and of taste are apparently of little importance to birds. The appearance of the milky DDT solution may have produced some selection factors.

When the birds received both tap water and the DDT solution, their survival time and DDT in their tissues were greater than when they received the DDT solution alone (Table 18 and 19). Again, it is possible that when tap water was available the birds consumed smaller amounts of the DDT solution, thereby lengthening their survival by breakdown of the DDT or by passing off the toxicant. Also, some physiological adjustments to lower levels could occur.

## ANALYSIS OF WILD BIRDS

Numerous investigators have shown high Robin mortality in spray areas (Barker, 1958; Wallace et al., 1961; Wallace et al., 1964). The fact that Robins feed almost exclusively on earthworms in the spring make them prime targets for DDT poisoning. On the other hand only small numbers of Common Grackles exposed to DDT were found dead until the spring of 1963. Bernard (1963) examined 4 grackles of which 3 had high levels of DDT in the brain. Wallace et al. (1964) found variable levels of DDT in 4 Common Grackles found on the Michigan State University campus in the spring of 1963. The concentrations in the brain ranged from 11 to 64.7 μg of DDT/g. Because of a large increase in grackle mortality during the spring of 1963, the digestive tract of 10 of these birds was examined for food content and their tissues analyzed for DDT. Also, birds of other species obtained from various spray areas, including the Michigan State University campus, were examined for DDT.

### Results and Discussion

Twenty-two Robins found dead or dying in the spring of 1962 were examined for DDT (Table 20). The concentrations in the brain ranged from 25 to 151 μg of DDT/g with an average of 91.7 μg of DDT/g. Levels in the liver ranged from 25 to 256 μg of DDT/g with an average of 144.2 μg of DDT/g. Only one of these birds was observed in tremors.

Fifteen Robins found dead or dying in the spring of 1963 were analyzed for DDT. Eleven of these birds were observed in tremors prior to death. The DDT levels ranged from 57 to 240 μg of DDT/g

Table 20. DDT Residue in Robins on Michigan State University Campus in Spring of 1962

| No. | Specimen Data: Date Obtained | Sex | Condition When Found | µg of DDT/g: Brain | Liver |
|---|---|---|---|---|---|
| R-1 | 4-18-62 | M | Dead | 50 | 155 |
| R-2 | 4-18-62 | M | Dead | 138 | 146 |
| R-3 | 4-18-62 | M | Dead | 28 | 68 |
| R-4 | 4-18-62 | F | Tremors | 151 | 175 |
| R-5 | 4-19-62 | F | Dead | 117 | 130 |
| R-6 | 4-20-62 | M | Dead | 85 | 140 |
| R-7 | 4-25-62 | F | Dead | 123 | 145 |
| R-8 | 4-26-62 | M | Dead | 25 | 125 |
| R-9 | 4-26-62 | M | Dead | 142 | 147 |
| R-10 | 4-27-62 | M | Dead | 121 | 153 |
| R-11 | 4-27-62 | F | Dead | 98 | 154 |
| R-12 | 5-1-62 | M | Dead | 54 | 105 |
| R-13 | 5-1-62 | F | Dead | 105 | 85 |
| R-14 | 5-4-62 | F | Dead | 85 | 109 |
| R-15 | 5-4-62 | M | Dead | 72 | 181 |
| R-16 | 5-6-62 | F | Dead | 118 | 232 |
| R-17 | 5-12-62 | M | Dead | 102 | 172 |
| R-18 | 5-17-62 | ? | Dead | 54 | 112 |
| R-19 | 5-20-62 | F | Dead | 131 | 256 |
| R-20 | 5-29-62 | M | Dead | 75 | 215 |
| R-21 | 6-7-62 | M | Dead | 53 | 25 |
| | Average | | | 91.7 | 144.2 |

in the brain and from 84 to 274 μg of DDT/g in the liver, with averages of 130.3 and 185.7 μg of DDT/g for the brain and liver respectively. Using the Student "t" Test there was no significant difference at the 5 per cent level between the DDT residue in the brain and liver of these two groups of Robins. The somewhat lower averages for the 1962 birds can probably be attributed to the inclusion of 2 Robins (R-3 and R-8) with sublethal levels.

Similar analysis of Robins found in the spring of 1963 in the same spray area by Wallace et al. (1964) showed an average of 84.8 μg of DDT/g in the brain of 4 dead Robins and an average of 101.0 μg of DDT/g in the brain of 6 tremoring Robins.

Table 22 shows the results of the analysis of 10 Common Grackles found dead or dying during the spring of 1963. Only one of the birds (G-10) was observed in tremors. Examination of the digestive tract revealed wheat and/or barley seeds in 7 of the birds. Generally the amount of DDT in the brain and liver was very low; 4 were negative for DDT in the brain and 3 negative for the liver. Those with DDT averaged 9.6 μg of DDT/g for the brain and 19.0 μg of DDT/g for the liver. The stomach contents of three birds were positive for DDT, two with wheat and/or barley seeds and one with unidentifiable contents. The average residue in the stomach contents was 5.0 μg of DDT/g.

Apparently poison (Senoco Thallium Corn Mix) was used on wheat and barley seeds by the Department of Farm Crops to control House Sparrows. Experimental crops were also protected from House Sparrows at various intervals by treating the seed with the same poison. Therefore, these circumstances lead me to believe that these birds died from poisons other than DDT.

Table 23 shows the levels of DDT determined in the tissues of some miscellaneous birds turned in by interested persons to

Table 21. DDT Residue in Robins on Michigan State University Campus in Spring of 1963

| No. | Specimen Data: Date Obtained | Sex | Condition When Found | g of DDT/g: Brain | Liver |
|---|---|---|---|---|---|
| R-22 | 4-9-63 | M | Tremors | 65 | 192 |
| R-23 | 4-10-63 | M | Dead | 240 | 145 |
| R-24 | 4-10-63 | F | Tremors | 165 | 170 |
| R-25 | 4-10-63 | M | Tremors | 84 | 134 |
| R-26 | 4-10-63 | M | Dead | 72 | 197 |
| R-27 | 4-11-63 | F | Dead | 57 | 188 |
| R-28 | 4-16-63 | F | Tremors | 201 | 245 |
| R-29 | 4-27-63 | M | Tremors | 135 | 177 |
| R-30 | 5-1-63 | F | Tremors | 132 | 225 |
| R-31 | 5-1-63 | F | Tremors | 160 | 136 |
| R-32 | 5-2-63 | M | Tremors | 124 | 124 |
| R-33 | 5-3-63 | F | Dead | --- | 84 |
| R-34 | 5-3-63 | M | Tremors | 124 | 227 |
| R-35 | 5-4-63 | F | Tremors | 143 | 268 |
| R-36 | 5-7-63 | M | Tremors | 123 | 274 |
| | Average | | | 130.3 | 185.7 |

Table 22. DDT Determined in Grackles Found on Michigan State University Campus in Spring 1963

| Specimen Data | | | | | | | μg of DDT/g |
|---|---|---|---|---|---|---|---|
| No. | Date Obtained | Sex | Condition When Found | Stomach Content | Brain | Liver | Stomach Content |
| G-1 | 4-11-63 | ? | Dead | Wheat and Barley | 2 | 18 | 0 |
| G-2 | 4-11-63 | ? | Dead | Insect | 0 | 0 | 0 |
| G-3 | 4-16-63 | M | Dead | Wheat and Barley | 8 | 25 | 2 |
| G-4 | 4-17-63 | ? | Dead | Wheat and Barley | 18 | 22 | 0 |
| G-5 | 4-18-63 | M | Dead | Empty | 0 | 10 | 0 |
| G-6 | 4-29-63 | F | Dead | Wheat and Barley | 7 | 15 | 0 |
| G-7 | 5-2-63 | ? | Dead | Wheat and Barley | 0 | 0 | 0 |
| G-8 | 5-2-63 | ? | Dead | ? | 11 | 15 | 8 |
| G-9 | 5-5-63 | ? | Dead | Wheat and Barley | 0 | 0 | 0 |
| G-10 | 5-22-63 | ? | Tremors | Wheat and Barley | 12 | 28 | 5 |
| | Average | | | | 9.6 | 19.0 | 5.0 |

Table 23. DDT Determined in the Tissues of Miscellaneous Birds

| Species | Specimen Data | | | | µg of DDT/g in Tissue | | |
|---|---|---|---|---|---|---|---|
| | Date Obtained | Locality | Sex | Condition When Found | Brain | Liver | Gonads |
| Mallard | 5-1-61 | Campus | M | Tremors | 28 | 56 | -- |
| Ring-billed Gull | 9-29-63 | Grand Haven | M | Paralysis | 6 | 11 | -- |
| Belted Kingfisher | 5-23-61 | Okemos | - | Tremors | 18 | 42 | -- |
| Downy Woodpecker | 12-18-60 | Bloomfield Hills | - | Tremors | 16 | 26 | -- |
| Bl-cp Chickadee | 1-15-62 | East Lansing | M | Dead | 0 | 4 | -- |
| Bl-cp Chickadee | 12-6-63 | Campus | M | Dead | 15 | 28 | -- |
| House Wren | 8-3-61 | Campus | - | Tremors | 62 | 88 | -- |
| Brown Thrasher | 5-8-61 | Campus | - | Tremors | 18 | 42 | -- |
| Robin | 4-25-61 | Campus | M | Dead | 22 | 101 | 10 |
| Robin | 4-28-61 | East Lansing | M | Tremors | 86 | 195 | 18 |
| Robin | 5-26-62 | Campus | Imm. | Dead | 0 | 0 | -- |
| Ruby-cr Kinglet | 5-4-61 | Campus | - | Tremors | 0 | 0 | -- |
| Gol-cr Kinglet | 11-3-63 | Campus | - | Dead | 38 | 60 | -- |
| Starling | 4-28-61 | Campus | - | Dead | 138 | 188 | 16 |
| Warbling Vireo | 5-10-61 | Campus | - | Tremors | 43 | 50 | -- |
| House Sparrow | 11-26-62 | Campus | F | Dead | 38 | 47 | -- |
| House Sparrow | 12-2-62 | Campus | M | Injured | 0 | 0 | -- |
| House Sparrow | 6-18-63 | Campus | M | Tremors | 48 | 65 | -- |
| Baltimore Oriole | 6-21-61 | Campus Woodlot | M | Dead | 38 | 67 | 20 |

Dr. G. J. Wallace for possible analysis. The results show a wide variability in the concentration of DDT in the different species as well as in different tissues of the same individuals. However, the highest quantities of DDT was always found in the liver.

Two Screech Owls (one with tremors) and two Sparrow Hawks (one with tremors) were checked for DDT but the tests failed because of the presence of a red interfering substance. A Barn Owl containing a bullet wound gave the same results. Bernard (1963) observed the same interference when testing most predatory birds for DDT.

## SUMMARY

1. An intensive spray program using DDT for the prevention of Dutch elm disease on the Michigan State University campus from 1954 to 1964 has resulted in high bird mortality, particularly of Robins.

2. This investigation was undertaken in 1962 in order to determine the various organisms believed to be responsible for DDT poisoning.

3. The study involved the collection and chemical analysis of soil, earthworms and plant materials to determine the levels of DDT, its persistence and its availability to birds.

4. The analytical technique used for the determination of DDT was the Schechter-Haller method of analysis.

5. Soil, plant materials and earthworms were collected over a two and a half year period.

6. Analysis of earthworms showed average levels of DDT ranging from 63.6 to 70.7 μg of DDT/g. High levels of DDT persisted in earthworms up to one and one-half years after spraying.

7. Residues in soil ranged from an average of 31.0 (18 months after spraying) to 298.1 μg of DDT/g 4 to 16 days after spraying.

8. There was no significant difference at the 5 per cent level in the amount of DDT in the two species of earthworms found on campus in the spring of 1964.

9. Earthworms along the road bordering the Forest Akers Golf Course averaged 62.6 μg of DDT/g 6 months after spraying and 58.6 μg of DDT/g 18 months after spraying. Soil residues in the same areas averaged 24.3 μg of DDT/g 18 months after spraying.

10. Earthworms kept in the laboratory and sprayed directly with 12 per cent DDT had initial deposits of 298 μg of DDT/g, then declined to 86 μg of DDT/g. For 12 weeks the earthworms averaged 86 to 90 μg of DDT/g in unsprayed soil indicating that DDT can be stored for long periods in the tissues of these animals.

11. The amount of DDT found in fruit averaged 2.4 μg of DDT/g prior to fall spraying, 44.4 μg of DDT/g after a dormant spray was applied to nearby elms, and 1.0 μg of DDT the following spring.

12. Elm bark averaged 23.6 μg of DDT/g prior to fall spraying, 242.1 after spraying and 92.0 μg of DDT/g the following spring.

13. Fallen elm leaves averaged 12.5 μg of DDT/g prior to spraying and 167.5 μg of DDT/g after spraying. This represents a possible avenue for soil and earthworm contamination.

14. Elm buds approximately 6 months after spraying averaged 1.5 μg of DDT/g; the new leaves averaged 3.1 μg of DDT/g.

15. House Sparrows fed earthworms (Helodrilus foetidus) containing 298 μg of DDT/g showed a survival time of 1 to 5 days. The brain averaged 31.4 μg of DDT/g while the liver averaged 48.4 μg of DDT/g.

16. Japanese Quail fed earthworms with 298 μg of DDT/g showed a survival time of 3 to 10 days. The brain averaged 43.6 μg of DDT/g; the liver averaged 44.8 μg of DDT/g.

17. House Sparrows fed earthworms with 86 to 90 μg of DDT/g had a survival time of 2 to 6 days. The brain averaged 33.6 μg of DDT/g and the liver 59.0 μg of DDT/g.

18. Japanese Quail given a choise of contaminated earthworms (298 μg of DDT/g) and uncontaminated chick starter grain showed no mortality, but ate very few of the worms. All birds were sacrificed and analyzed for DDT. Birds having DDT averaged 5.6 μg of DDT/g in the brain and 6.3 μg of DDT/ g in the liver. The results indicate a definite preference for grain by the quail.

19. House Sparrows given a 12 per cent DDT solution had a survival time of 5 to 13 days. Concentrations in the brain averaged 25.1 μg of DDT/g while the liver averaged 36.7 μg of DDT/g. Each bird consumed an average of 1,91 ml per day.

20. House Sparrows given 12 per cent DDT solution and tap water had a survival time of 6 to 17 days. Residues in the brain averaged 31.3 μg of DDT/g while the liver averaged 49.5 μg of DDT/g. Each bird consumed an average of 1.00 ml of the DDT solution and 1.40 of the tap water.

21. The analysis of wild robins dying in the spring of 1962 and 1963 showed an average DDT accretion in the brain of 91.7 μg of DDT/g in 1962 and 130.3 μg of DDT/g in 1963. The liver averaged 144.2 and 185.7 μg of DDT/g for 1962 and 1963 respectively. Using a Student "t" Test there was no significant difference at the 5 per cent level in DDT levels in the two groups of birds.

22. Negative or low levels of DDT in dead or dying grackles and the presence of wheat and/or barley seeds used in a poison program led to the conclusion that these birds died from causes other than DDT.

23. Obviously, many avenues exist for transfer of DDT to wild birds. Earthworm-soil relationship, the contamination of elm bark and feeding by foraging birds on insects in these contaminated areas could result in bird mortality. Also, birds with more restricted diets (e.g., Robin) have higher mortality from DDT poisoning than birds with more varied feeding habits (e.g., Common Grackle).

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