DDT RESIDUES IN A MOSQUITO CONTROL PROGRAM AT THE KELLOGG BIOLOGICAL STATION

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ABSTRACT

DDT RESIDUES IN A MOSQUITO CONTROL PROGRAM
AT THE KELLOGG BIOLOGICAL STATION

by David R. Osborne

Following the use of DDT sprays for the purpose of controlling Dutch elm disease, many people have reported large numbers of birds either dead or dying of symptoms commonly associated with chlorinated hydrocarbon poisoning. However, a mosquito control program using a mixture of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) and Chlordane (1,2,4,5,6,7,8, 8-octochloro-2,3,3a,4,7,7a-hexahydro-4,6-methanoidene), in effect since 1954 at the W. K. Kellogg Gull Lake Biological Station, Kalamazoo County, Michigan, has been associated with high bird populations and apparent lack of any unusual bird mortality. This study was undertaken during the summer of 1962 in an attempt to discover, under actual field conditions, the reasons for the apparent lack of bird mortality in the program at the Biological Station.

For this study samples of soils, earthworms, leaves, fruits, and elm bark were collected and quantitatively analyzed by the Schechter-Haller method for DDT (Schechter et al., 1945). The levels of DDT in eggs and tissues of

young and adult birds from the study area and similar areas were determined, and the persistence and the availability of DDT to birds was studied.

The results of the soil analyses indicated that most of the DDT was lost from soil in open areas, but persisted in low concentrations under shrubs, and increased as much as 28.5 per cent in soils at the base of elms during a 35 week interim between samplings, presumably due to run-off from the trunks of the trees.

Analyses of spring and summer specimens of earthworms showed that DDT was present in their tissues in levels below 15 μg. of DDT/g. The results also showed that earthworms often contained higher levels of DDT than did the soils from which they were taken.

The analyses of plant and animal materials disclosed DDT in variable amounts in the study area, thus making it widely available to birds with different feeding habits. DDT levels ranged from 0-1388 μg. of DDT/g. on leaves, 5.28-146.4 μg. of DDT/g. on elm bark, and 0-10 μg. of DDT/g. on fruit. A sample of camel crickets (Ceuthophilus maculatus) contained 2.16 μg. of DDT/g.

Studies of persistence in plant material showed that 62.2 per cent of the DDT was lost from the plant surface

after 19 days while a 77.2 per cent loss occurred after eight months. Loss of DDT from the trunks of elms ranged from 75.9-84.3 per cent over eight months.

DDT ranging from 2.75 to 15.2 μg. of DDT/g. was found in unhatched eggs and young birds.

Results from the analyses of the tissues of 23 birds including 13 from the study area showed that levels of DDT ranged higher in the brain than in liver or breast muscle. Analyses of brain tissues from 13 dead birds found in the study area in 1962 showed that in most cases DDT was not present in sufficient quantities to justify the conclusion that DDT was the cause of death. Only three of the birds analyzed had 50 μg. or more of DDT in the brain.

The brain tissue of eight cedar waxwings obtained from outside the study area averaged 20.6 μg. of DDT/g.

The evidence obtained in this study, plus the fact that no birds were found in tremors in the summer of 1962, supports the conclusion that lack of mortality that summer was due to the low levels of DDT present in the study area, which resulted in sublethal accumulations of DDT in the tissues of birds.

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By

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INTRODUCTION

A spray program for mosquito control, using a mixture of DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) and Chlordane (1,2,4,5,6,7,8,8-octochloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoidene), has been in progress each summer since 1954 at the W. K. Kellogg Gull Lake Biological Station, Kalamazoo County, Michigan. Several unpublished reports in 1959, 1960, and 1962 indicate this area had relatively high bird populations and good though variable nesting success (Jensen, 1959; Moldenhauer, 1960; Johnson, 1962). In contrast, spray programs on the Michigan State University Campus with DDT, for Dutch elm disease, also in progress since 1954, resulted in a sharp decline of robins by 1957 (Mehner and Wallace, 1959), and high bird mortality in 1958, 1959, and 1960 (Wallace, 1960; Wallace et al., 1961). Chemical analyses of birds and feeding experiments indicated that the high bird mortality was a direct result of ingestion of lethal amounts of DDT in areas sprayed for the control of Dutch elm disease (Bernard, 1963).

This study was undertaken to try to discover reasons for the apparent lack of mortality from DDT, under actual field conditions, at the Biological Station in contrast with the

relatively low population and obvious mortality on the Michigan State University Campus. Hence, studies were made to determine the levels of DDT present in the environment, its persistence and availability to birds, and the amounts accumulated by birds.

The study area had been sprayed for the past 9 summers with several applications of DDT. In each case the spray mixture consisted of 4 pounds of 50 per cent wetable DDT powder and 2 pounds of 40 per cent wetable Chlordane combined in 100 gallons of water. Two applications of this mixture, each covering a separate portion of the grounds, were used to spray the 32-acre site on 14 June 1962 and theoretically represented an application rate of 0.125 pounds of DDT/acre.

Spraying was by pressure apparatus (the type commonly used in orchard spray programs) connected to a 100 gallon tank-trailer which was pulled by jeep. All lawns, shrubs, and low vegetation were sprayed thoroughly. Precautions were taken not to spray known active nest sites. Although the program was primarily for mosquito control, trunks of elms were sprayed to a height of 40 feet.

Chemical analyses for residues of DDT in field programs for mosquito control are scarce. Most studies are based on

field observations only. In most instances there has been no observed bird mortality in mosquito control areas receiving one pound or less of DDT/acre (Adams et al., 1949; Couch, 1946; Erickson, 1947). Two to three pounds of DDT/acre, in repeated treatments, have been found to affect insectivorous birds directly, or indirectly through their food supply, eliminating flying insects on which they feed (Rogers, 1948; Rudd et al., 1956). Bird mortality is severe at an application rate of five pounds of DDT/acre (Hotchkiss et al., 1946).

George (1957) has shown that where formerly multiple applications of 0.1 to 0.5 pounds of DDT/acre were used, now dosages may range up to 2 pounds of DDT/acre for mosquito control; the increase in dosage was necessary because of resistance to DDT developed by insects.

In the type of spray program used for mosquito control, the application may not be uniform; that is, one spot may receive more DDT than an adjacent one and some shrubs more than others. Even at a low average application rate of 0.125 pounds of DDT/acre, lethal amounts of DDT may be available to birds due to the variability of application and also to accumulations in plant and animal tissues. Kendeigh (1947) has suggested that bird mortality might occur even

when application rates are below the acute toxicity level for a given species. He found that due to uneven coverage, DDT dosages obtained after spraying were 15 pounds/acre in some areas supposedly sprayed at a rate of 1 pound of DDT/ acre.

Availablility of DDT to birds is possible in many ways, depending upon their feeding habits. Animals or plants may act as carriers of the poison. The fact is now well-known that earthworms build up DDT residues and serve as a source of contamination to robins (Barker, 1958; Bernard, 1963).

Therefore, the purpose of this study was to determine the levels of DDT in various bird tissues, soils, plants, fruits, and other carriers which might serve as a source of DDT to birds.

This investigation does not include an analysis or a determination of the effects of Chlordane used in the site. Nor was the effect of DDT storage in fat of summer birds studied.

GENERAL DESCRIPTION AND LOCATION OF THE STUDY AREA

The W. K. Kellogg Biological Station of Michigan State University is situated on the north shore of Gull Lake in Kalamazoo County, Michigan. The main grounds cover an area of approximately 32 acres, bounded by county roads on the north and east sides, Gull Lake on the south, and a township park on the west. A small additional acreage used as a rifle range is located adjacent to and just north of the main entrance. The main area is enclosed by a fence except for the Gull Lake shoreline. Roads, dormitories, laboratories and other buildings have been constructed on the grounds.

The landscape consists mainly of rolling hills of grass, dotted with trees and clumps of shrubs. Native trees are mainly elm (*Ulmus americana*), oak (*Quercus sp.*), and maple (*Acer sp.*). Shrubs are primarily of honeysuckle (*Lonicera sp.*), lilac (*Syringa sp.*) and hazel (*Corylus sp.*), found in isolated clumps, in open expanses next to buildings, and along fence rows. A mature apple orchard has been established at the west end of the study area. Two wooded areas (see figure 1), primarily of oak, are also present. A stand of tall elms shades the west side of the tennis court.

Many exotic plants, introduced by W. K. Kellogg, are also found on the grounds.

Figure 1 indicates the major vegetation of the study area. Isolated trees in open grassy areas are not shown. The symbols used are as follows:

A. shrubs

B. woods

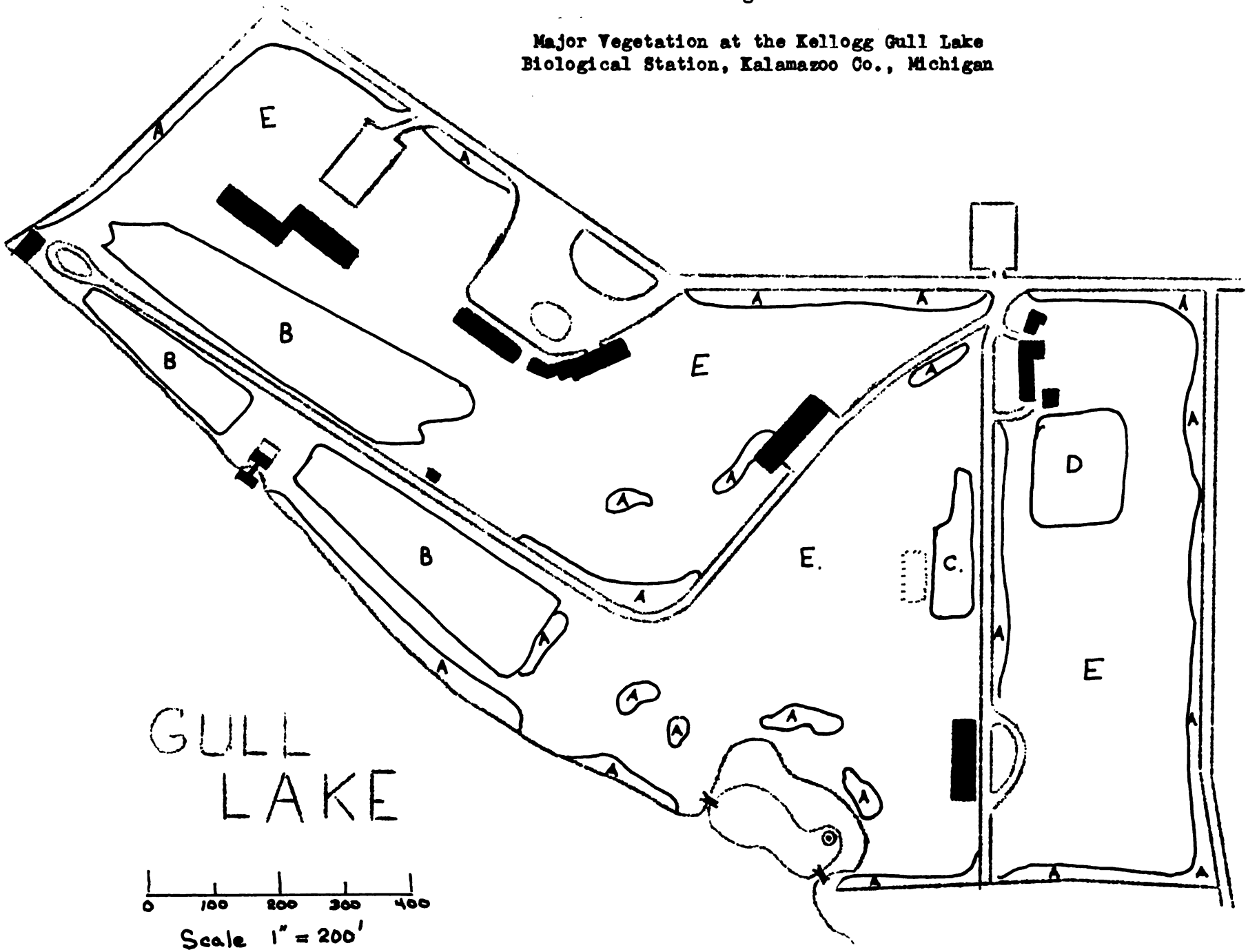
C. stands of elms

D. orchard

E. grass

Figure 1

Major Vegetation at the Kellogg Gull Lake
Biological Station, Kalamazoo Co., Michigan



METHODS

Samples of soil, bark, plants, fruits, earthworms, eggs, nestlings, and adult birds in the study area were collected and analyzed for DDT. Most specimens were collected from 28 June to 8 August 1962. However, a collection of plant and animal material was made on 7 April 1963, before any spraying was done that year. At no time did any of the birds analyzed show symptoms of tremors commonly associated with chlorinated hydrocarbon poisoning.

Chemical determination of DDT residues was made by the Schechter - Haller method of analysis (Schechter et al., 1945). Since facilities for analysis were not available at the station all samples were placed in plastic bags, frozen, and stored. The samples remained frozen until determinations for DDT could be made in an on-campus laboratory of the Department of Biochemistry. Freezing does not alter the stability of DDT (Lichtenstein et al., 1958).

The earthworms collected were identified by means of the key in Ball and Curry (1956). Harlow's Trees of the Eastern United States and Canada was used for identification of trees and shrubs. The scientific names for the birds mentioned are from Zimmerman and Van Tyne (1959).

ANALYSIS FOR DDT

Procedure. - Briefly, the method of analysis used consisted of macerating the material with anhydrous sodium sulfate and quartz sand, removing the DDT with an organic solvent (diethyl ether- petroleum ether mixture), nitrating the residue, and separating the nitrated DDT from the acidified aqueous solution with ether. DDT in the ether extract was evaluated by the procedure of Schechter et al. as prescribed by the Association of Official Agricultural Chemists (1955). The ether-petroleum ether portion containing DDT was filtered, evaporated, and the residue taken up in Benzene. Finally, alcoholic sodium metholate was added to an alequot of the benzene solution to develop the characteristic blue-colored complex. The per cent transmittancy was determined with a Beckman B spectrophotometer at a wave length of 600 millimicrons. DDT was then calculated from a standard curve prepared with a 3 to 1 p,p' o,p' - DDT mixture and the results expressed in micrograms of DDT/gram of sample (μg. of DDT/g.).

Interference. - The Schechter-Haller method is generally specific and accurate. However, other insecticides may interfere in the nitration process or with the colormetric

evaluation of DDT. The principal insecticides known to interfere are DNB and DNP (the nitro 1, 1 bis (p-chlorophenyl) butane and propane analogues of DDT), TDE (dichloro-2,2-bis (Chlorophenyl) ethane), DDE (1, 1-dichloro-2,2 bis (p-chlorophenyl) ethylene), and Methoxychlor (1,1,1-trichloro-2,2-bis (p-methoxyphenyl) ethane). However, none of these were used in the control program in this area.

Since Chlordane was used in the spray formulation and was undoubtedly present in the samples analyzed, its effect on the chemical analysis for DDT was determined. Known amounts of DDT and Chlordane, in various concentrations, were mixed and analyzed by the Schechter-Haller method. Chlordane, even in high concentrations, did not affect the DDT values beyond the normal limits of reproducability with this procedure.

General Considerations. - In order to correct for deviations in technique and effects of the individual samples it was necessary to carry a blank (uncontaminated sample) along with each test series. Due to a lack of soil, plants, and birds known to be free of DDT, substitute blanks were used. These consisted of benzene solutions, chemically pure sand, "Poll feed", and birds. The birds used were "road-kills" found outside the spray area at Gull Lake, and found to be free of DDT.

PRESENTATION OF DATA

DDT RESIDUES IN SOIL

Samples. - The soil samples analyzed were taken from the areas shown in figure 2 at depths ranging from 0-6". The depth of the samples, which were obtained by shovel, was measured by tape. The soils analyzed consisted of 5-10 composite samples which were thoroughly mixed, placed in plastic bags, and frozen. Prior to analysis the soils were thawed, classified as to type, and then air-dried for 24 hours. Ten soil samples were collected on 9 July 1962, and ten were collected on 7 April 1963. Of the first ten, five were from open areas, two from under elms, two from under thick shrubbery, and one from a wooded area. In the second series two were collected from open areas, three from under elms, and four from beneath shrubs. Muck from the lagoon was also collected.

Results and Discussion. - Table 1 shows the amounts of DDT found in 20 soil samples obtained from the study area. DDT levels in the soil were low (below 7μg. of DDT/g.). Soil samples collected in open areas on 9 July 1962 contained from 0-1.67μg. of DDT/g., those beneath elms had 1.62-1.65 μg. of DDT/g., and those under shrubs 2.78-2.79 μg. of DDT/g.

Location of Soils Collected at the Kellogg Biological Station, Kalamazoo County, Michigan

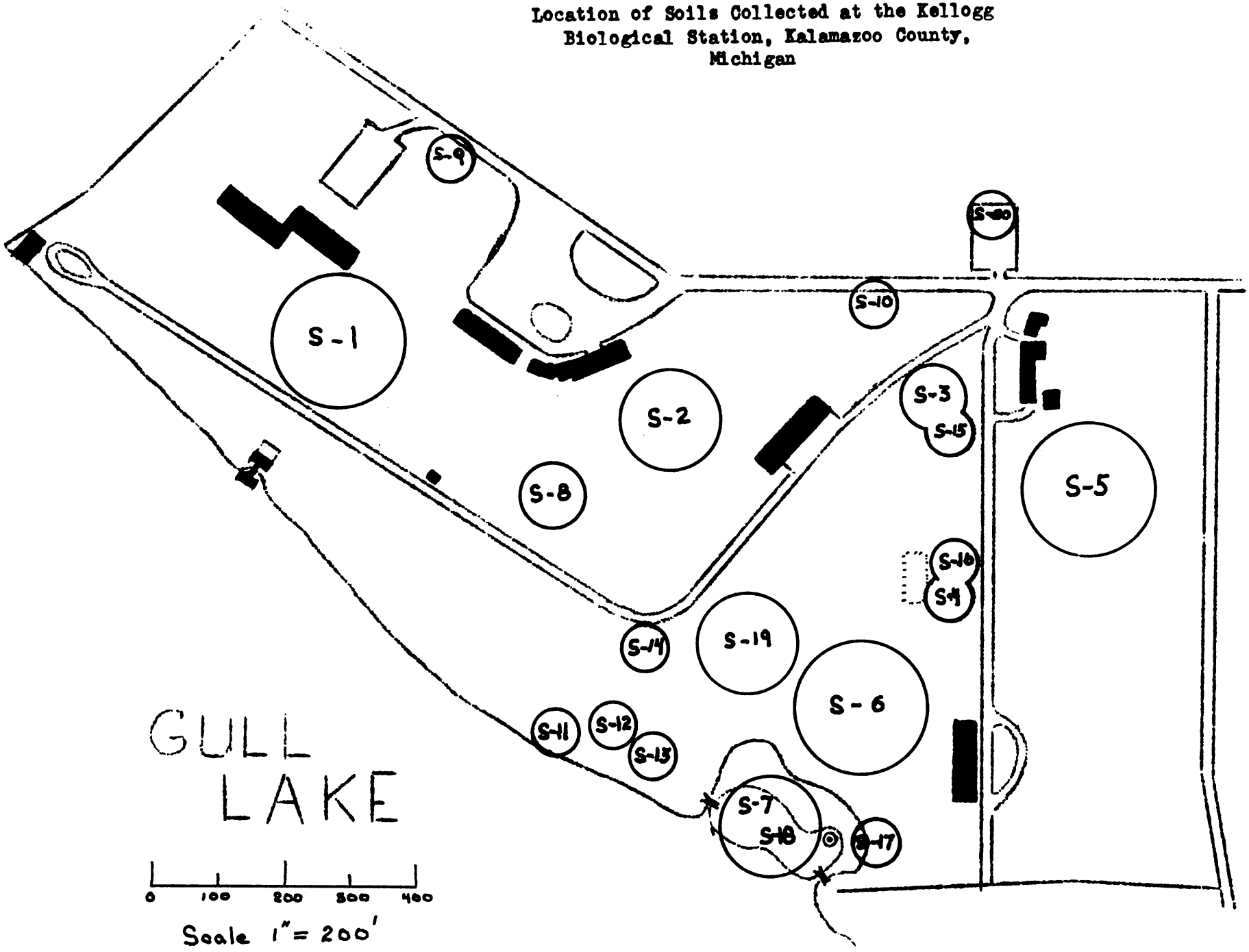


TABLE 1

DDT FOUND IN SOILS

No.	Date obtained	Soil type	Source	Depth in inches	µg. of DDT/g.
S-1	7/9/62	Loam	Wooded area	0-2	2.81
S-2	7/9/62	Sandy loam	Open area	1-3	0.00
S-3	7/9/62	Sandy loam	Under elms	0-1	1.62
S-4	7/9/62	Loam	Base of elms	0-1	1.65
S-5	7/9/62	Sandy loam	Open area	0-1	0.00
S-6	7/9/62	Loamy sand	Open area	0-1	1.15
S-7	7/9/62	Loamy sand	Open area	0-1	1.67
S-8	7/9/62	Loamy sand	Open area	0-4	0.00
S-9	7/9/62	Loam	Under shrubs	0-2	2.78
S-10	7/9/62	Sandy loam	Under shrubs	0-2	2.79
S-11	4/7/63	Loamy sand	Under shrubs	0-1	6.25
S-12	4/7/63	Loam	Under shrubs	1-2	1.16
S-13	4/7/63	Loam	Under shrubs	4-6	0.00
S-14	4/7/63	Loamy sand	Base of elms	0-1	5.57
S-15	4/7/63	Loam	Base of elms	4-6	1.09
S-16	4/7/63	Sandy loam	Base of elms	0-1	2.12
S-17	4/7/63	Muck	Lagoon	0-3	0.00
S-18	4/7/63	Loamy sand	Open area	0-1	0.00
S-19	4/7/63	Sandy loam	Open area	0-1	0.00
S-20	4/7/63	Gravelly, loamy sand	Under shrubs	1-2	1.15

Soils collected on 7 April 1963, nearly a year after spraying,were negative in open areas, contained 1.09-5.57 μg. of DDT/g. under elms, and 0-6.25 μg. of DDT/g. under shrubs.

The results showed that soils in areas receiving the heaviest spraying (shrubs and elms) had greater quantities of DDT than soils receiving chiefly drift (open areas).

S-11 contained 6.25 μg. of DDT/g., the highest concentration found in soils, while S-14, from the base of an elm was next highest with 5.57 μg. of DDT/g. Both were surface soils collected at a depth of 0-1". S-12 contained 1.16 μg. of DDT/g. and S-13 was negative. S-12 and S-13 were obtained in the same area as S-11 but at different depths, 1-2" and 4-6", respectively. This indicates that most of the DDT residue was present in the first inch of soil, even 38 weeks after spraying. Normally soil samples used for analysis consist of core samples to a depth of 6". Ginsburg has shown that DDT does not penetrate vertically downward beyond a depth of 6" (Ginsburg et al., 1954). Since samples in this study from 4-6" depths contained very small amounts of DDT, it is evident that DDT was retained primarily in the first inch.

Numerous studies have been made to determine the persistence of DDT in the soil. Foster (1951) has shown that DDT

residues in the soil were not affected by soil types or rain over a period of four years. However, under certain conditions of high temperature (Lichtenstein et al., 1958), and humidity (Barlow et al., 1956), some DDT decomposition does occur. Microbial organisms also cause a breakdown of DDT; 50 per cent wetable DDT powder in low concentrations is affected the most (Jones, 1952, 1956). Sunlight may decompose DDT, with the rate of decomposition greatest for the lowest dosage (Ginsburg, 1952, 1953).

S-19 and S-18, taken in the same areas as S-6 and S-7 respectively, showed a complete loss of DDT 38 weeks after spraying. Since the entire 32-acre site was sprayed only once the year this investigation was being conducted, the effect of repeated applications could not be studied. Repeated applications of DDT have been shown to increase the concentration of DDT in soil (Chisholm, 1955). In this study, soil at the base of elms was found to increase in DDT over a period of 35 weeks. Comparison of amounts of DDT found in S-16 and S-4, from the base of the same elm, showed this increase to be 28.5 per cent. Presumably, the increase was due to run-off from the trunk or decomposition of elm leaves containing DDT after the leaves fell to the ground. Barker (1958) has shown that the quantity of DDT in soil does not

decrease over winter, but actually increases in some cases with the fall of sprayed leaves. This may also be the explanation for the higher amounts of DDT found in soil under shrubs and bushes, where fallen leaves were not removed as they were in open areas.

DDT RESIDUES IN EARTHWORMS

Samples. - Ten earthworm samples, representing three different species, were collected and analyzed for DDT. Each sample consisted of 5-10 individuals of Helodrilus with the exception of W-9, which was a single specimen of Lumbricus terrestris. During the period of the investigation earthworms were difficult to find, although robins had little trouble finding them early in the morning. Most attempts to find worms during and after rains resulted in failure.

Of the 10 samples analyzed, seven were collected on 20 June 1962 and three on 7 April 1963. Most of the samples, specimens of the small garden worm (Helodrilus sp.), were found in a wooded area in moist loamy soil beneath fallen leaves. Red worms (Helodrilus foetidus) were abundant in a decaying pile of leaves left from the sanitation and clean-up program conducted yearly on the grounds. Sites from which earthworm samples were taken are plotted in figure 3.

TABLE 2

DDT FOUND IN EARTHWORMS

No.	Date obtained	Species	Source	µg. of DDT/g.
W-1	6/20/62	*Helodrilus sp.*	Island	8.33
W-2	6/20/62	*Helodrilus sp.*	Wooded area	0.00
W-3	6/20/62	*Helodrilus sp.*	Wooded area	2.75
W-4	6/20/62	*Helodrilus sp.*	Wooded area	12.50
W-5	6/20/62	*Helodrilus sp.*	Wooded area	2.98
W-6	6/20/62	*Helodrilus sp.*	Wooded area	14.00
W-7	6/24/62	*Helodrilus sp.*	Base of elms	6.25
W-8	4/ 7/63	*Helodrilus sp.*	Under shrubs	3.18
W-9	4/ 7/63	*Lumbricus terrestris*	Under shrubs	0.00
W-10	4/ 7/63	*Helodrilus foetidus*	Leaf litter	3.27

Results. - Table 2 presents the results of the analyses of 10 earthworm samples collected at the Kellogg Biological Station. No attempt was made to determine the levels of DDT in the intestinal organs. The greatest amount was 14μg. of DDT/g. in tissue found in W-6 in a wooded area. Samples W-2 and W-9 from the same area, collected at the same time, were negative. Samples W-1, W-4, W-5, and W-6, also from the same wooded area and collected on the same day, had higher levels of DDT than the soil in which they were found (compare values to S-1, table 1).

This supports the findings of Barker (1958). Barker found that earthworms can accumulate DDT and concentrate it in their body tissues by obtaining the residues from sprayed soil or leaf litter from sprayed trees. Similarly, sample W-7, obtained near the base of an elm, contained higher levels of DDT than the soil from which it was obtained (see S-4, table 1).

The results also show that DDT was present in earthworm tissues not only in summer but also in spring specimens 38 weeks after spraying. Hence, DDT was either retained by the worms in some measure over winter, or it was reaccumulated in the spring from contaminated leaf litter or soil.

Discussion. - No correlation was made between the three different species of earthworms collected and the levels of DDT they accumulated. It seems unlikely that the quantities of DDT found in the earthworms analyzed approach the lethal levels necessary to cause immediate mortality in robins. The lethal level of DDT in the brain for adult robins is greater than 50 μg. of DDT/g. (Bernard, 1963). Earthworms analyzed from the Michigan State University campus, an area of high robin mortality, ranged up to 88 μg. of DDT/g. (Bernard, Ph. D. thesis, 1962). Boykins (pers. comm.) also found greater than 50 μg. of DDT/g. in campus earthworms analyzed in 1963. The average concentration found in robins at the station was 8.6 μg. of DDT/g. in brain tissue (see p. 30). However, robins consuming numerous earthworms containing sublethal levels of DDT may actually be concentrating it in their tissues over long periods of time. This has not been investigated, but analyses of brains of eight robins fed DDT-injected earthworms (by J. J. Hickey at the University of Wisconsin) at a dosage of 110 mg. of DDT/Kg. of body weight, disclosed the tremoring birds died with greater than 50 μg. of DDT/g. in the brain (Bernard, 1963). Thus, earthworms analyzed in this investigation appear to have had DDT levels sublethal to robins feeding on them. The lethal

amount for robins, in earthworms, must be somewhere between 14 and 110 μg. of DDT/g.

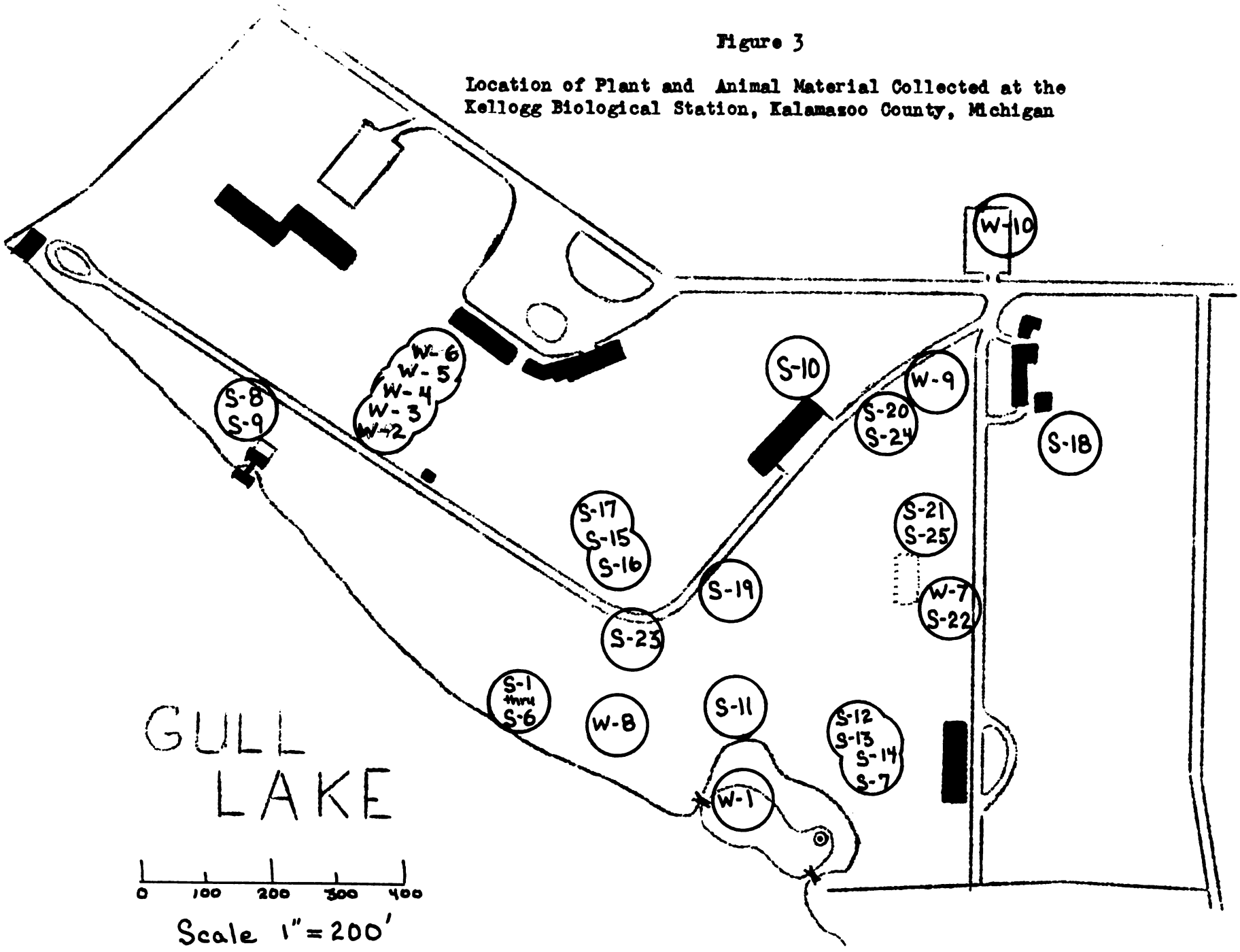
Applications of 100 pounds of DDT/acre are ineffective for the control of the exotic earthworm, (Pherentima sp.) (Fleming et al., 1945), and some earthworms survive at applications of 214 pounds of DDT/acre (Richards et al., 1946). The lack of abundance of earthworms and the difficulty in finding them was probably a result of a combination of environmental factors: low temperature, lack of rain, and dry soil, rather than of earthworm mortality caused by the toxicity of DDT.

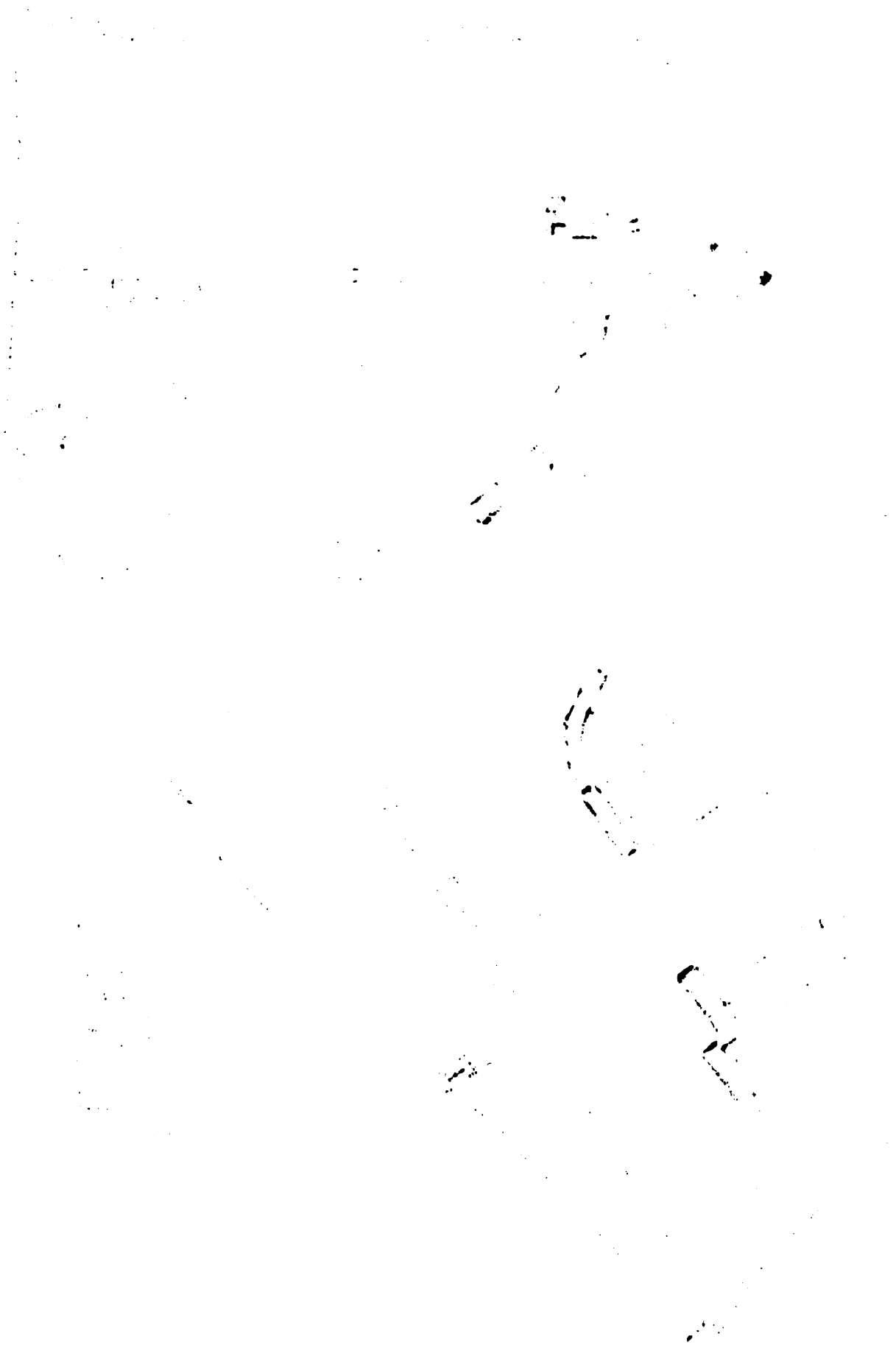
DDT RESIDUES IN LEAVES, FRUIT, AND ELM BARK

Samples. - A total of 26 samples, primarily leaves, fruit, and elm bark, were collected and analyzed for DDT residues. The samples, for the most part, were randomly selected at three foot heights in areas which had received the heaviest spraying. In many instances the dried, milky-white residue of DDT could be seen on the plant surface. The DDT levels in leaves and fruit of crab apple (Malus coronaria), honeysuckle (Lonicera sp.), and choke cherry (Prunus virginiana) were determined. Leaf samples of juneberry (Amelanchier canadensis), lilac (Syringa vulgaris), and honey locust

Figure 3

Location of Plant and Animal Material Collected at the Kellogg Biological Station, Kalamazoo County, Michigan





(Geditsia triacanthos), were collected as were fruits from mulberry (Morus rubra), sweet cherry (Prunus avium), and ornamental bittersweet (Celastrus obiculata).

Six samples of elm bark from the trunks of elms (Ulmus americana) were analyzed as well as a sample of leaf mulch, and a composite sample containing 12 camel crickets (Ceuthophilus maculatus). Sites of the samples are shown in figure 3 and listed in table 3.

Results. - The levels of DDT found in leaves, fruit, and bark were variable, as can be seen in table 3. The highest concentration of DDT was from a sample of juneberry leaves collected soon after spraying. The leaves contained the astonishing level of 1388 µg. of DDT/g. High concentrations were also found on lilac leaves. A sample collected 20 June 1962, yield 254 µg. of DDT/g., while another sample collected 9 July 1962, from the same bush, had 94.5 µg. of DDT/g., indicating a 62.2 per cent decrease of DDT over a period of 19 days.

Low levels of DDT (4.11 µg. of DDT/g.) were found on crab apple leaves collected on 8 August 1962. Dried crab apples found on the ground in another area on 7 April 1963 were negative. Leaves from a honeysuckle obtained 8 August

TABLE 3

DDT FOUND IN LEAVES, FRUIT, AND BARK

No.	Date obtained	Kind of plant material	Source	µg. of DDT/g.
S-1	6/20/62	Mulberry, fruit	On ground	4.41
S-2	6/20/62	Mulberry, fruit	1' off ground	3.97
S-3	6/20/62	Mulberry, fruit	2' off ground	1.62
S-4	6/20/62	Mulberry, fruit	3' off ground	1.12
S-5	6/20/62	Mulberry, fruit	5' off ground	0.00
S-6	6/20/62	Mulberry, fruit	8' off ground	0.00
S-7	6/24/62	Juneberry, leaves	2' off ground	1388.00
S-8	6/20/62	Lilac, leaves	3' off ground	254.00
S-9	7/ 9/62	Lilac, leaves	3' off ground	94.50
S-10	8/ 8/62	Crab apple, leaves	3' off ground	4.11
S-11	4/ 7/63	Crab apple, fruit	Dried, on ground	0.00
S-12	8/ 8/62	Honeysuckle, leaves	3' off ground	21.10
S-13	4/ 7/63	Honeysuckle, leaves	Dried, on ground	4.80
S-14	8/ 8/62	Honeysuckle, fruit	3' off ground	10.20
S-15	8/ 8/62	Choke cherry, leaves	3' off ground	9.46
S-16	8/ 8/62	Choke cherry, fruit	3' off ground	5.50
S-17	8/ 8/62	Honeylocust, leaves	3' off ground	52.50
S-18	6/20/62	Sweet cherry, fruit	3' off ground	1.00
S-19	4/ 7/63	Ornamental bitter-sweet, fruit	Dried, on vine	0.00
S-20	8/ 8/62	Elm bark	3' off ground	21.80
S-21	8/ 8/62	Elm bark	3' off ground	111.40
S-22	8/ 8/62	Elm bark	3' off ground	146.40
S-23	4/ 7/63	Elm bark	3' off ground	16.90
S-24	4/ 7/63	Elm bark	3' off ground	5.25
S-25	4/ 7/63	Elm bark	3' off ground	17.50

1962 had 21.1 μg. of DDT/g. A sample of leaf mulch collected eight months later beneath the same bush had 4.08 μg. of DDT/g., a 77.2 per cent loss from the original source. Loss of DDT from the plant surface was not 100 per cent as shown by the level obtained in the leaf mulch. Prior to its decomposition, the DDT present in the dried leaves was very probably incorporated in the soil or became incorporated in soil organisms such as earthworms.

Generally, the fruits contained lower levels of DDT than leaves from the same species. Leaves of a choke cherry had 9.46 μg. of DDT/g. while the berries contained 5.5 μg. of DDT/g. In samples from a honeysuckle the leaves contained 21.1 and the berries 10.2 μg. of DDT/g.

Robins had been observed eating cherries near the orchard. A sample of partially eaten cherries disclosed only 1 μg. of DDT/g. Similarly, a sample of fruit of bittersweet, which cedar waxwings were feeding on, was analyzed and found negative.

The extreme differences in the amounts of DDT found on leaves and fruits obtained at the same height probably was due to the lack of uniformity of the spray application as well as continued growth of the fruit. Lower levels in the fruit and a decrease in the amount of DDT found on leaves

over a period of eight months could be attributed to weathering, and plant and fruit growth. Taschenberg (1960) has shown that weathering and plant growth are factors which affect DDT deposits on grapes. Weather causes a loss in the initial deposit, while growth affects the final level of DDT found when expressed on a weight-weight basis. The level of DDT obtained by analysis of a mature fruit would be less than the level obtained from an immature fruit due to the increase in size and weight resulting from plant growth, even though the initial deposits of DDT on the fruit may have been the same.

Six samples of ripe mulberries, obtained on 20 June 1962 from the same tree at various heights, showed that the concentration of DDT was inversely proportional to the height of the berries from the ground. Those berries collected on the ground contained the highest levels of DDT - 4.41 μg. of DDT/g. - while those at five and eight feet were negative.

DDT levels in elm bark were relatively high. Of three samples collected on 8 August 1962, at three foot heights, one had 21.8, another 111.4, and another 146.4 μg. of DDT/g. DDT levels were lower in three samples collected on 7 April 1963; these were 5.25, 16.9, and 17.5 μg. of DDT/g., indicating a general decrease of DDT residue on elm bark over a

period of eight months. Comparison between samples S-24 and S-20 (table 3), obtained from the same tree, shows a 75.9 per cent decrease, while comparison between S-25 and S-21, also from the same tree, indicates a 84.3 per cent decrease.

A sample of camel crickets, not listed in table 3, contained 2.16μg. of DDT/g. in their tissues, indicating the possibility of insecticidal resistance in this species since they were collected alive.

Discussion. - DDT was available to many birds as shown by the residues present in the samples tested. Bark foragers (woodpeckers, chickadees, nuthatches, and creepers) may have obtained high and possibly lethal levels of DDT from insects in bark crevices. Foliage gleaners (orioles, vireos, and warblers) could receive low to very high concentrations of DDT by gleaning leaves for insect prey. Birds feeding on fruits and berries (cardinals, grosbeaks, waxwings) could obtain low and probably sublethal levels of DDT. Dried leaf mulch contained DDT available to wrens and other ground feeders. Also, insect-feeding birds may have obtained the poison by feeding on DDT-resistant insects which contained the toxin in their tissues.

The effect of translocation of DDT from the soil to various parts of the plants was not determined. Most investigations of this sort have been with field crops where the effects on plants are dependent upon soil type and concentration of DDT (Lindgreen et al., 1954; Lichtenstein, 1958). There is no evidence that DDT is translocated in woody plants. Hence, it was assumed that the results obtained above were due to the amount of DDT residue present on the plant surface after spray application.

DDT RESIDUES IN EGGS

Samples. - Five unhatched eggs - three robin, one barn swallow, and one brown thrasher - were analyzed for DDT. Both the robin and the barn swallow eggs were laid after the spraying of 14 June 1962. Prior to analysis the egg shells were removed to eliminate the possibility that DDT was present only on the egg surface.

Results and Discussion. - The DDT residue in these eggs is shown in table 4.

The three robin eggs found 21 June 1962 were from a nest which was abandoned by the parents. They contained 10.8, 11.3, and 15.2 μg. of DDT/g.

The barn swallow egg, from a nest containing five eggs found 20 June 1962, was the only egg which did not hatch. It contained 2.70 μg. of DDT/g.

A brown thrasher egg was found on 6 July 1962, in an abandoned nest. This egg had 7.85 μg. of DDT/g.

Thus, all of the eggs analyzed contained DDT. The robin eggs contained the highest levels. The DDT present in eggs could have been passed on by the parent, and may have contributed to the failure of the eggs to hatch.

TABLE 4

DDT FOUND IN EGGS

No.	Date obtained	Species	μg. of DDT/g.
E-1	6/20/62	Barn swallow (*Hirundo rustica*)	2.70
E-2	6/21/62	Robin (*Turdus migratorius*)	15.20
E-3	6/21/62	Robin (*Turdus migratorius*)	10.80
E-4	6/21/62	Robin (*Turdus migratorius*)	11.30
E-5	7/ 6/62	Brown thrasher (*Toxostoma rufum*)	7.85

In the summer previous to this study, Moldenhauer found 15 non-hatching catbird eggs out of 52. Mitchell (1946) has shown that direct spraying of nests and eggs with high concentrations of DDT will not affect egg hatch, but

exposure of adult pheasants and quail to DDT immediately prior to the breeding season results in lower egg fertility and high chick mortality (Dewitt, 1955; Cross et al., 1962). Unfortunately, the minimum levels of DDT required to affect the various stages of development and reproduction have not been determined.

Of special interest was the finding of DDT in the barn swallow egg. Published records of insect-eating birds which feed on the wing having accumulated DDT were not to be found. Two swifts, a martin, and a nighthawk analyzed by Bernard (1962) were negative. It seems probable that the DDT in the barn swallow egg was passed on by the parents which had fed on DDT-resistant insects.

DDT RESIDUES IN BIRDS

Samples. - Twenty-three birds, representing 11 different species, were examined for DDT. Of these, 13 were obtained from the study area, six from the Michigan State University campus at East Lansing, two from Potterville, and two from Dearborn. Of the 13 birds obtained from the study area, eight were found dead, while five were caught in a mist net. None of the birds captured alive exhibited tremors commonly associated with hydrocarbon poisoning.

In most cases, the brain, breast muscle, and liver of each bird were analyzed separately. In the nestling barn swallow, where the individual tissues were too small to analyze separately, the whole carcass was used.

Results and Discussion. - The results of these analyses are shown in table 5.

The results show that only three of the birds found dead had 50 μg. or more of DDT/g. in the brain. All of the six robins (Turdus migratorius) analyzed (four from the study area and two from Potterville) had low concentrations of DDT in their tissues. The highest level was in a robin trapped by mist net. The brain contained 22 μg. of DDT/g., higher than in brains of some robins found dead. The average concentration of DDT found in the brains of robins was 8.6 μg. of DDT/g. In 1960 three robins from the study area, trapped by mist net, and analyzed by Bernard (1963) contained low levels of DDT on the brain.

Eight cedar waxwings (Bombycilla cedrorum) averaged 20.6 μg. of DDT/g. in the brain. Wallace (1961) suggested that waxwings accumulate high levels of DDT in the spring when budding on sprayed elms, but obtain little or no DDT in fall or winter when their diet consists primarily of fruit.

TABLE 5

DDT FOUND IN BIRDS

Specimen Data						µg. of DDT/g. of Tissue			
No.	Species	Date obtained	Location	How obtained	Sex**	Brain	Muscle	Liver	Carcass
B-1	Yellow-shafted flicker	7/17/62	KBS*	Found dead	M	18.5	13.5	0	-
B-2	Eastern wood pewee	7/16/62	KBS	Found dead	M	43.7	28.5	134.0	-
B-3	Barn swallow	6/29/62	KBS	Found dead	-	-	-	-	5.5
B-4	White-breasted nuthatch	7/23/62	KBS	Found dead	M	137.0	35.25	84.3	-
B-5	Catbird	7/16/62	KBS	Found dead	M	5.9	4.56	15.6	-
B-6	Robin	6/27/62	KBS	Found dead	M	10.3	2.1	12.7	-
B-7	Robin	7/ 5/62	KBS	Found dead	-	7.8	3.3	6.2	-
B-8	Robin	7/16/62	KBS	Mist net	-	22.0	2.5	2.6	-
B-9	Robin	8/ 1/62	KBS	Found dead	M	5.27	6.97	9.33	-
B-10	Robin	6/ 5/61	Potter-ville+	Found dead	F	3.33	4.14	7.50	-
B-11	Robin	6/11/61	Potter-ville+	Found dead	M	2.94	2.92	7.32	-

TABLE 5 (continued)

Specimen Data						µg. of DDT/g. of Tissue			
No.	Species	Date obtained	Location	How obtained	Sex	Brain	Muscle	Liver	Carcass
B-12	House sparrow	7/17/62	KBS	Mist net	M	18.4	4.84	29.4	-
B-13	Common grackle	6/19/62	KBS	Mist net	-	25.7	25.6	15.4	-
B-14	Rose-breasted grosbeak	7/18/62	KBS	Mist net	F	1.4	0.0	0.0	-
B-15	Starling	6/23/62	KBS	Found dead	F	9.50	7.0	12.0	-
B-16	Cedar waxwing	10/29/62	MSU Campus	Found dead	M	6.25	0.00	8.14	-
B-17	Cedar waxwing	11/ 7/62	MSU Campus	Found dead	M	23.3	1.37	9.26	-
B-18	Cedar waxwing	12/ 1/62	MSU Campus	Found dead	F	27.8	10.4	10.6	-
B-19	Cedar waxwing	12/ 1/62	MSU Campus	Found dead	M	0.0	2.3	10.7	-
B-20	Cedar waxwing	12/ 6/62	MSU Campus	Found dead	F	0.00	0.00	0.00	-
B-21	Cedar waxwing	1/ 8/63	Dear-born+	Found dead	-	57.6	10.4	7.4	-
B-22	Cedar Waxwing	1/ 8/63	Dear-born+	Found dead	M	0.00	6.2	7.6	-
B-23	Cedar Waxwing	4/18/63	MSU Campus	Found dead	F	50.0	22.6	35.0	-

*Kellogg Biological Station **Sex undetermined (-) +Mosquito program

However, in this study the level of DDT in the brain of a winter specimen surpassed the level found in the brain of a spring bird (see table 5).

Other station birds with low levels of DDT in their tissues were single specimens of the following: yellow-shafted flicker (Colaptes auratus), catbird (Dumetella carolinensis) banded in 1960 and trapped by mist net 16 July 1962, house sparrow (Passer domesticus), rose-breasted grosbeak (Pheucticus ludovicianus), and starling (Sturnus vulgaris).

The highest levels of DDT in bird tissues were found in two insect-eating species. An eastern wood pewee (Contopus virens), banded in 1960 and found dead 16 July 1962, had 134 μg. of DDT/g. in the liver, while a white-breasted nuthatch (Sitta carolinensis) also found dead had 137 μg. of DDT/g. in the brain. The wood pewee must have obtained its DDT by feeding on DDT-resistant insects. The white-breasted nuthatch could have accumulated DDT by foraging in the bark crevices of sprayed elms.

The carcass of a nestling barn swallow (Hirundo rustica), another insect feeder, had low amounts of DDT. It was not possible to prove that this bird died of effects of DDT poisoning, but DeWitt (1959) and Mitchell (1953) have shown

that young birds are less resistant to DDT than adults and have a lower chance of fledging when they are exposed to DDT. The nestling bird, too young to feed itself, may have had DDT passed on to it by the parent, either through the egg, or by the adults feeding it DDT-contaminated insects.

Bernard (1963) has indicated that the minimum lethal amounts of DDT for tremoring robins and house sparrows are 50 and 65 μg. of DDT/g. in brain, respectively. The liver served as an organ of storage of DDT, and often had higher levels of DDT than the brain. However, the results in this investigation indicated that the brain contained higher amounts of DDT than liver or breast muscle. This may have been due to the fact that in birds receiving small quantities of DDT in their diets, the liver was not forced to function as a storage organ until a certain level was reached on the brain. Another reason may have been that elimination of DDT was greater and faster in the liver than in the brain when low amounts were ingested. Bernard (1963) found that birds on a diet of 300 mg. of DDT/Kg. of food followed by a diet free of DDT, were able to eliminate the poison from the brain, breast muscle, and liver.

Although a few birds contained high levels of DDT in the brain, most did not. Since the levels of DDT in the brain were low, and no birds examined were known to have exhibited tremors, the conclusion is that the dead birds in this investigation had sublethal levels and did not die as a result of DDT poisoning. Return of a catbird and a wood pewee, banded on the research area in 1960, indicates that they had not accumulated enough DDT prior to 1961 to cause death during migration. However, banded returns at the station have been very low, suggesting possible migration losses from DDT.

GENERAL DISCUSSION AND CONCLUSIONS

Results of soil and earthworm analyses showed that low levels of DDT were present at the Kellogg Biological Station in 1962. In some cases earthworms contained greater concentrations of DDT than the soils from which they were taken. DDT persisted in some soil samples, increased in amounts in soils at bases of elms, and was completely lost in soils from open areas 38 weeks after spraying.

DDT was found in widely variable amounts, ranging from 0-1388 μg./g. on leaves, 5.25-146.40 μg./g. on elm bark, and 0-10.2 μg./g. on fruit. The analyses of plant and animal material indicated that DDT, though present in variable amounts, was freely accessible to leaf-gleaning, ground-feeding, bark-foraging, seed-eating, fruit-eating, and insect-feeding birds.

The results of plant analyses also showed that greatest loss of DDT from the plant surface occurred during the first month after spraying. Losses of DDT from trunks of elms ranged from 75.9 to 84.35 per cent. Low concentrations of DDT persisted in dried leaf mulch.

DDT found in unhatched eggs and young birds never having been exposed to DDT indicated the probability that DDT was

passed directly from the female to the eggs and young.

All but one of 23 birds analyzed had DDT in their tissues. All birds (13) from the study area contained DDT. Analyses of individual tissues, such as brain, breast muscle, and liver, showed that variations in concentrations of DDT existed in these tissues from different specimens. These variations may have been due to the rate of accumulation or the ability to avoid ingesting lethal amounts in their food.

Generally, the brain contained higher levels of DDT than liver or breast muscle. Of 19 birds found dead only three had 50 μg. or more of DDT/g. in the brain. Some birds live-trapped by mist net contained higher levels of DDT in their tissues than birds of the same species found dead. None of the live birds showed the symptoms commonly associated with hydrocarbon poisoning; tremoring, of course, could not be ascertained in birds found dead.

Because of the absence of birds in tremors, and the low concentrations of DDT present in the tissues of dead birds, it seems evident that most of the birds analyzed in this study did not die as a direct result of the ingested toxicant. Only the nuthatch and pewee had lethal or near lethal levels in the brain, 137 μg. and 43.7 μg. of DDT/g.

respectively. High bird populations in the study area were attributed to the fact that the birds were not exposed to lethal levels of DDT. However, this investigation has also shown that repeated applications of 0.125 pounds of DDT/acre resulted in variable amounts of DDT in plant and animal tissues, and that even at this low dosage, high concentrations were available to leaf-gleaning and bark-foraging birds.

SUMMARY

1. A DDT spray program in effect since 1954 at the W. K. Kellogg Gull Lake Biological Station, Kalamazoo County, Michigan, has been accompanied by high bird populations and apparent lack of any unusual bird mortality.
2. This investigation was undertaken during the summer of 1962 in an attempt to discover, under actual field conditions, the reasons for the apparent lack of mortality.
3. The methods used included analyses of samples of plant and animal material to determine their content of DDT, its persistence, and its availability to birds. Levels of DDT in eggs, young, and adult birds were determined. Analysis for DDT was by the Schechter-Haller method.
4. The effect of Chlordane upon analytical values for DDT was determined. Results showed that Chlordane, even in high concentrations, did not affect the determination of DDT beyond the normal limits of reproducability with the procedure.
5. Results of soil analyses showed that DDT levels were below 7 μg. of DDT/g. Soils at the base of elm trees and from under shrubs had higher levels of DDT than did soils from open areas.

6. DDT levels were greater in soils obtained at 0-1" depths than at 4-6".
7. Thirty-nine weeks after spraying, DDT was completely lost from soils in open areas, but persisted in soils under shrubs. Also, soils at the base of some elms contained more DDT (28.5 per cent more) 38 weeks after spraying than found in samples obtained soon after spraying.
8. Analyses of earthworms showed that DDT in levels below 15 μg. of DDT/g. was present in their tissues during spring and summer. Results also showed that earthworms often contained higher levels of DDT than did the soils from which they were taken.
9. The amounts of DDT found on leaves, fruit and elm bark varied greatly. Leaves contained levels of DDT ranging from 0-1388 μg.; elm bark, 5.25-146.4 μg.; and fruit, 0-10.2 μg. of DDT/g.
10. Fruits contained lower levels of DDT than did leaves from the same plant. The concentration of DDT in fruits analyzed varied inversely with the height of the fruit from the ground.
11. Live camel crickets contained 2.16 μg. of DDT/g. in their tissues indicating the possiblity of some DDT resistance in this species.

12. DDT decreased 62.2 per cent from the surface of leaves of plants tested over a period of 19 days; 77.2 per cent loss occurred after eight months. Losses of DDT from trunks of elms ranged from 75.9 per cent to 84.3 per cent over eight months. DDT persisted in dried leaf mulch in low concentrations.
13. Analyses of unhatched bird eggs showed concentrations of DDT which ranged from 2.75 to 15.2 μg. of DDT/g.
14. Of 23 birds, representing 11 different species that were analyzed, all except one had DDT. None of the birds exhibited tremors. Generally the brain contained higher levels of DDT than did liver or breast muscle.
15. Only three of the 17 dead birds analyzed had 50 or more μg. of DDT/g. in the brain. The average concentration of DDT found in brains of six robins was 8.6 μg. of DDT/g.
16. The average concentration of DDT found in brains of eight cedar waxwings obtained from outside the study area was 20.6 μg. of DDT/g. In one instance the level of DDT in the brain of a bird that died in winter surpassed the level found in a dead spring bird.
17. A catbird and eastern wood pewee, banded in 1960 and retrapped by mist net in 1962, had levels below 44 μg.

of DDT/g. in the brain.

18. The carcass of a nestling barn swallow contained low amounts of DDT.

19. The results support the conclusion that birds exposed to the varied and widely available concentrations of DDT in the study area accumulated variable levels of DDT in their tissues through their normal feeding habits.

20. Finally, this investigation showed that the dead birds analyzed in this study did not die as a direct result of ingestion of the toxicant, and that apparent lack of bird mortality due to spray treatments in the study area was attributable to the sublethal levels of DDT accumulated in their tissues.

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