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**FIELD STUDIES OF WILDLIFE
EXPOSED TO TCDD CONTAMINATED SOILS**

ENVIRONICS & HUMAN FACTORS OFFICE

Bartleson, F.D.

MARCH 1975

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<p>A study was made of the birds, other wildlife, and soils in two areas of the Eglin Air Force Base Reservation, Florida, which previously had been exposed to massive quantities of military herbicides containing the highly toxic chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The studies were conducted on a three-square-mile test site (Test Area C-52A) where aerial dissemination equipment was tested and around a hardstand where the spray-aircraft were loaded. TCDD was found to be persistent and widespread in these areas. No positive deleterious effects from the TCDD were found. Evidence was found, however,</p>		

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to suggest that selected species of wildlife, in restricted habitats, might have been affected. TCDD had entered certain food chains, and bioaccumulation was demonstrated. Transient wildlife and certain herbivorous animals appeared to suffer no ill effects. Recommendations were made for a method to accelerate degradation of TCDD in terrestrial environments and for specific follow-up studies on TCDD wildlife effects.

PREFACE

This report is the result of research conducted by the Air Force Armament Laboratory from 29 March 1974 to 1 February 1975 under Air Force Exploratory Development Project 50660101.

Dr. W. J. Potts and Mr. R. A. Hummel, Interpretative Analytical Services, Dow Chemical USA, were responsible for the chemical analysis. Mr. Louis Jeeter, Florida Game and Fresh Water Fish Commission, provided invaluable assistance in the collection of specimens and revelation of wildlife habits. Captain Alvin L. Young and Lt Colonel William E. Ward, USAF Academy, provided general background information and data from six years of study in the test area and assisted immeasurably in this project. Lt Colonel E. L. Massie, Veterinary Officer, and his staff from the USAF Regional Hospital, Eglin Air Force Base, assisted in post-mortem examinations and preparation of samples. Captain Wayne Pask, Eglin Air Force Base Entomologist, identified insect remains. Acknowledgements are also made to Vitro Services and Jackson Guard Ranger Station for their assistance and services. Mr. Richard C. Crews, Ms Sandra Lefstad, Dr. Jimmy Cornette, and MSgt Gary Wyman, Environics and Human Factors Office, Air Force Armament Laboratory, Armament Development and Test Center, assisted in the collection of samples and data in the preparation of this report.

Because of the subjective nature of this study dealing with dynamic ecosystems, the use of controls was not always possible and the results may not be duplicable. The conclusions are those of the authors and do not necessarily reflect the position of the United States Air Force.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER:



JOE A. FARMER
Chief, Environics and Human Factors Office

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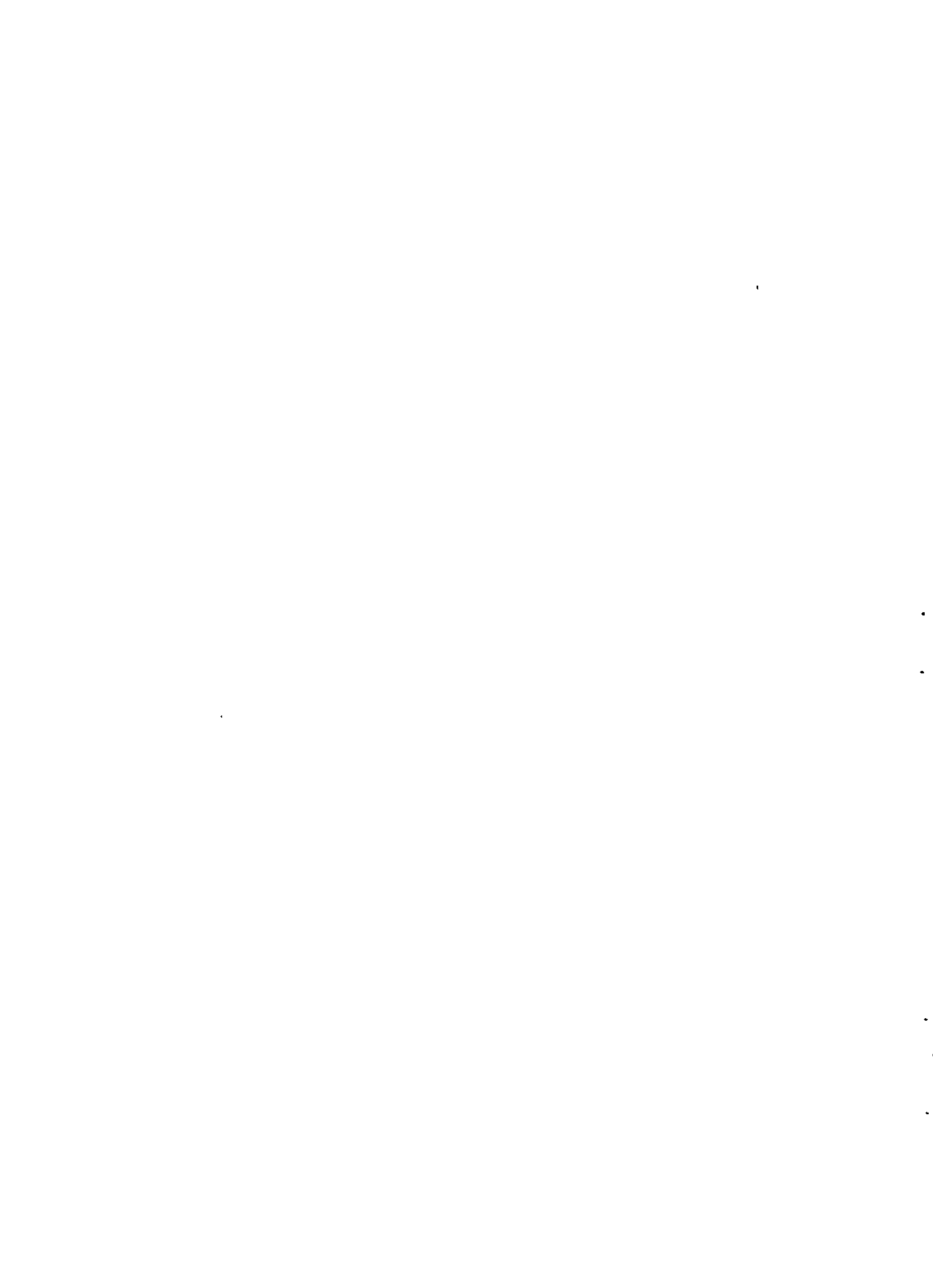


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SECTION I

INTRODUCTION

A study was made of the wildlife in areas of the Eglin Air Force Base Reservation, Florida, which previously had been exposed to high concentrations of military herbicides. This study was prompted by Young's discovery (Reference 1) in 1973, that a highly toxic chemical, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), was present in mouse liver tissue and soil samples from Test Area C-52A (TA C-52A). TCDD is an impurity formed during the manufacture of certain herbicides. It is much more toxic than strychnine, and experiments have demonstrated that it is teratogenic (causes birth defects) in laboratory animals.

The objectives of this study were to assess, if possible, the extent of contamination and the ecological effects of TCDD on the terrestrial environment of TA C-52A and the aquatic environment near a hardstand used for loading herbicides into spray aircraft. This study was made by observing wildlife and assaying selected biological specimens and soil samples for TCDD.

The analysis for traces of TCDD, in the parts per trillion (ppt) range, required undesirably large sample quantities to insure a significant detection limit. Therefore, tissues from several biological specimens had to be combined in some cases to form sufficient quantities for analysis. Furthermore, an ecological assessment of this type is quite subjective due to the dynamic nature of environmental factors which render the use of experimental controls, in most cases, of questionable value. The results of this study probably could not be duplicated, but the findings are important for focusing controlled experimentation on the ecological effects of TCDD.

Reference:

1. AFATL-TR-74-12, Ecological Studies on a Herbicide-Equipment Test Area (TA C-52), Eglin AFB Reservation, Florida, Air Force Armament Laboratory, January 1974.

SECTION II

BACKGROUND

This section provides background information on the physical characteristics of the study areas and their role in previous test operations using TCDD-containing herbicides. The chemical properties and currently known biological effects of TCDD are summarized.

1. DESCRIPTION OF STUDY AREAS

The two areas selected for this study were on the Eglin Air Force Base Reservation located in Northwest Florida (Figure 1). Test Area C-52A, located approximately ten miles east of the main base, was used to test military herbicide equipment between 1962 and 1970. Hardstand 7, located on the main base airfield, was used as one of the principal sites for loading of the spray aircraft.

Test Area C-52A, a man-made grassy plain covering approximately three square miles (Figure 2), was bounded on three sides by a dense forest dominated by sand pine (*Pinus clausa* (Engelm Vasey), but had large numbers of longleaf pine (*Pinus palustris* Mill.), turkey oak (*Quercus laevis* Walt.), and live oak (*Quercus virginiana* Mill.). The northern side of TA C-52A bordered another large cleared test area. The cleared area of TA C-52A was occupied mainly by broomsedge (*Andropogon virginicus* L.) and low growing grasses and herbs (Figures 3 and 4). In the center of the test area was a one-square-mile instrumented grid containing permanent markers spaced 400 feet apart (Figure 5). Immediately south of this large grid was a smaller, 1000-foot radius, circular test site, called Grid 1.

Both of these grids were used to test aerial spray equipment for military herbicides, but Grid 1 received a greater quantity of herbicides. Two of the herbicides used, Orange and Purple, contained 50 percent 2,4,5-Trichlorophenoxyacetic Acid (2,4,5-T) which was contaminated by TCDD. The quantities of herbicides delivered on these areas which contained 2,4,5-T are given in Table 1. The precise amount of TCDD in the herbicides used was unknown, but probably was in the range of 1 to 47 parts per million (ppm) (Reference 2).

Large areas of these test grids were quite arid and sandy (Figure 6 and 7). However, young trees, principally oaks (*Quercus spp*) averaging less than two feet in height, were beginning to reappear on the large grid and a few widely scattered pines (*Pinus spp*) were on Grid 1. Two small ponds were present on the one-square-mile grid: a permanent pond (Gator Pond) near the center of the grid and an intermittent pond (East Pond) near the eastern border (Figures 8 and 9). The area surrounding the two grids was drained by bayheads or ravines that form the headwaters of Mullet, Basin, and Trout Creeks (Figure 10).

Hardstand 7 (HS-7) was an asphalt and concrete aircraft parking area located west of the north-south runway on the main Eglin airdrome (Figure 11). The hardstand was connected to the airdrome by an asphalt taxiway. Directly behind the hardstand was a ravine that dropped off approximately 50 feet to a small pond. Erosion of the hardstand area had been a constant

Reference:

2. Department of the Air Force, Final Environmental Statement on Disposition of Orange Herbicide by Incineration. November 1974.

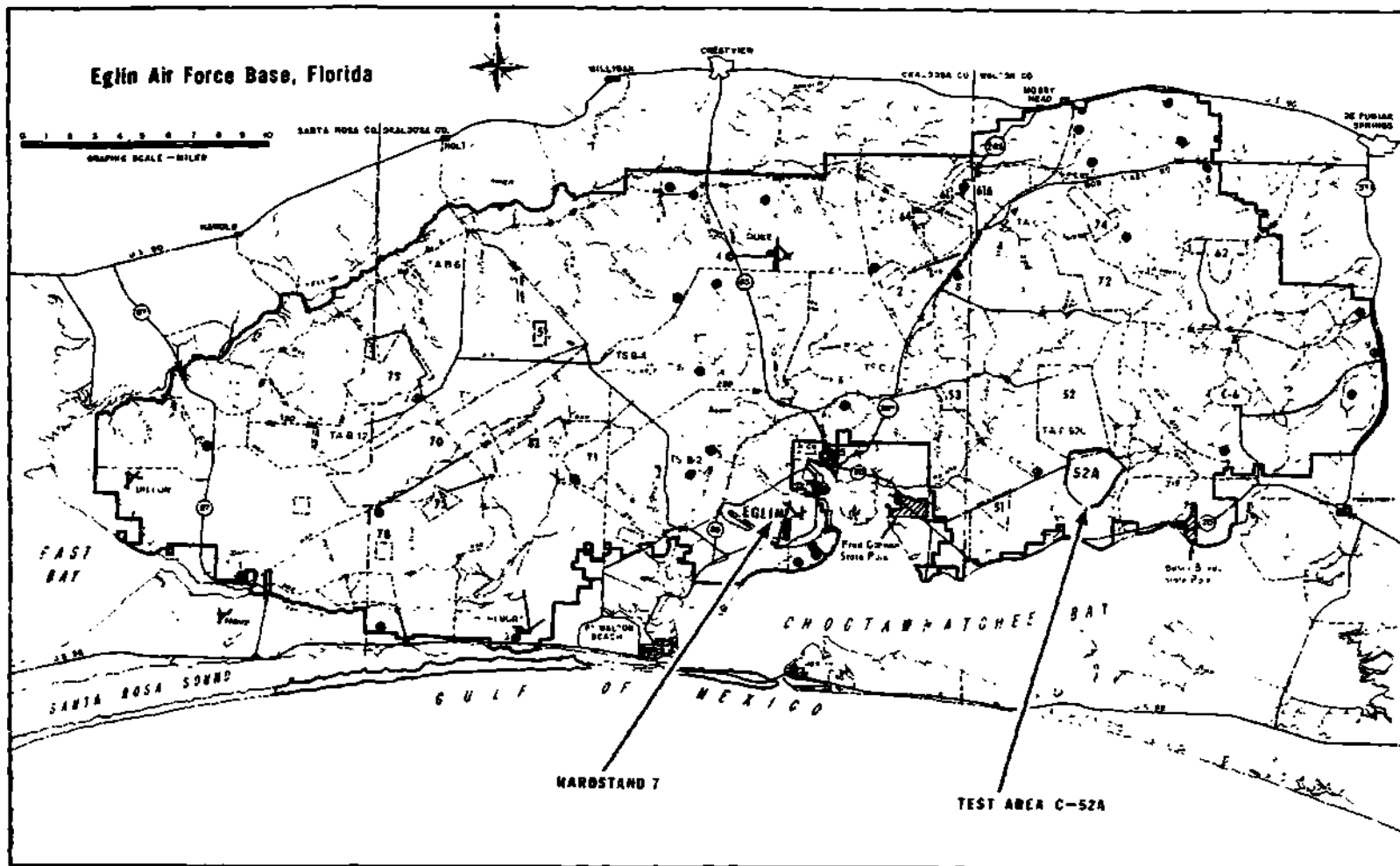


Figure 1. Areas of Study on Eglin Air Force Base, Florida

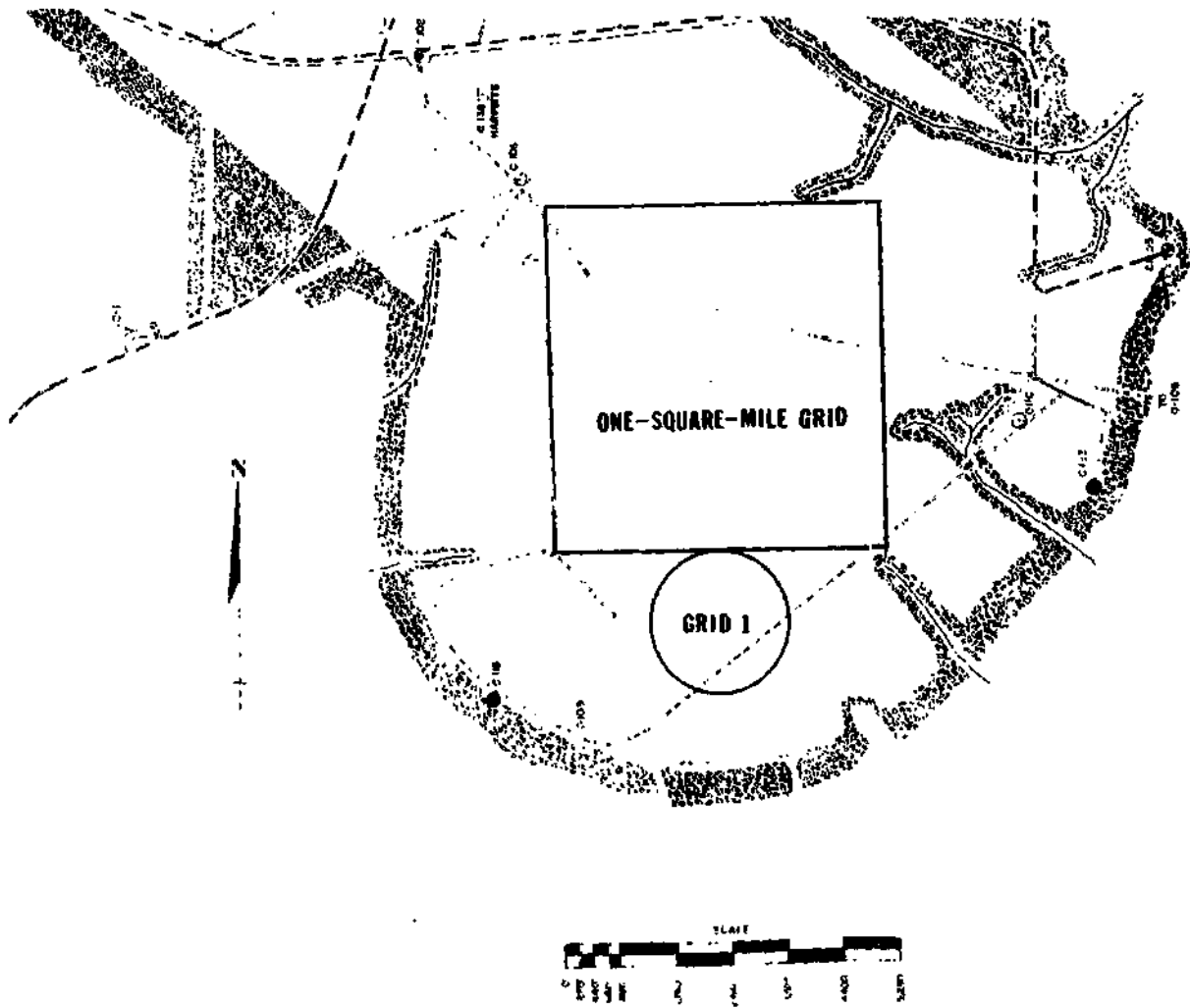


Figure 2. Test Area C-52A



Figure 3. Typical Vegetation Looking North from One-Square-Mile Grid

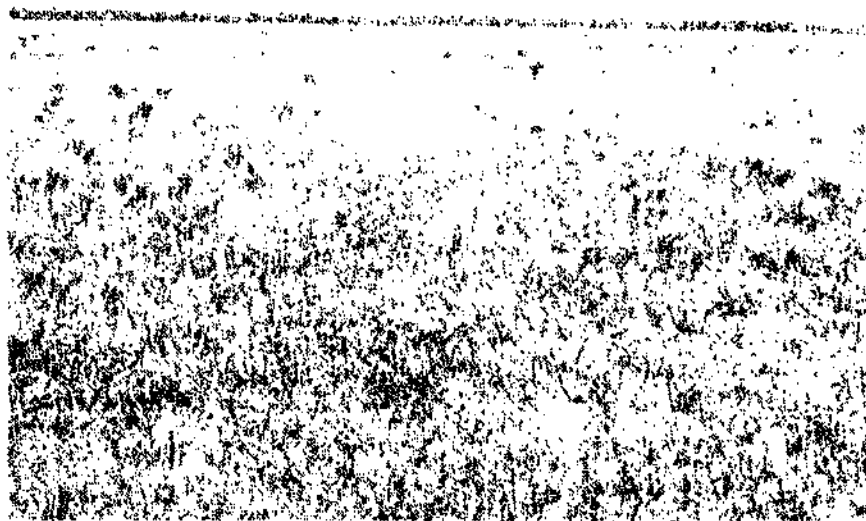


Figure 4. Typical Vegetation Looking East from One-Square-Mile Grid

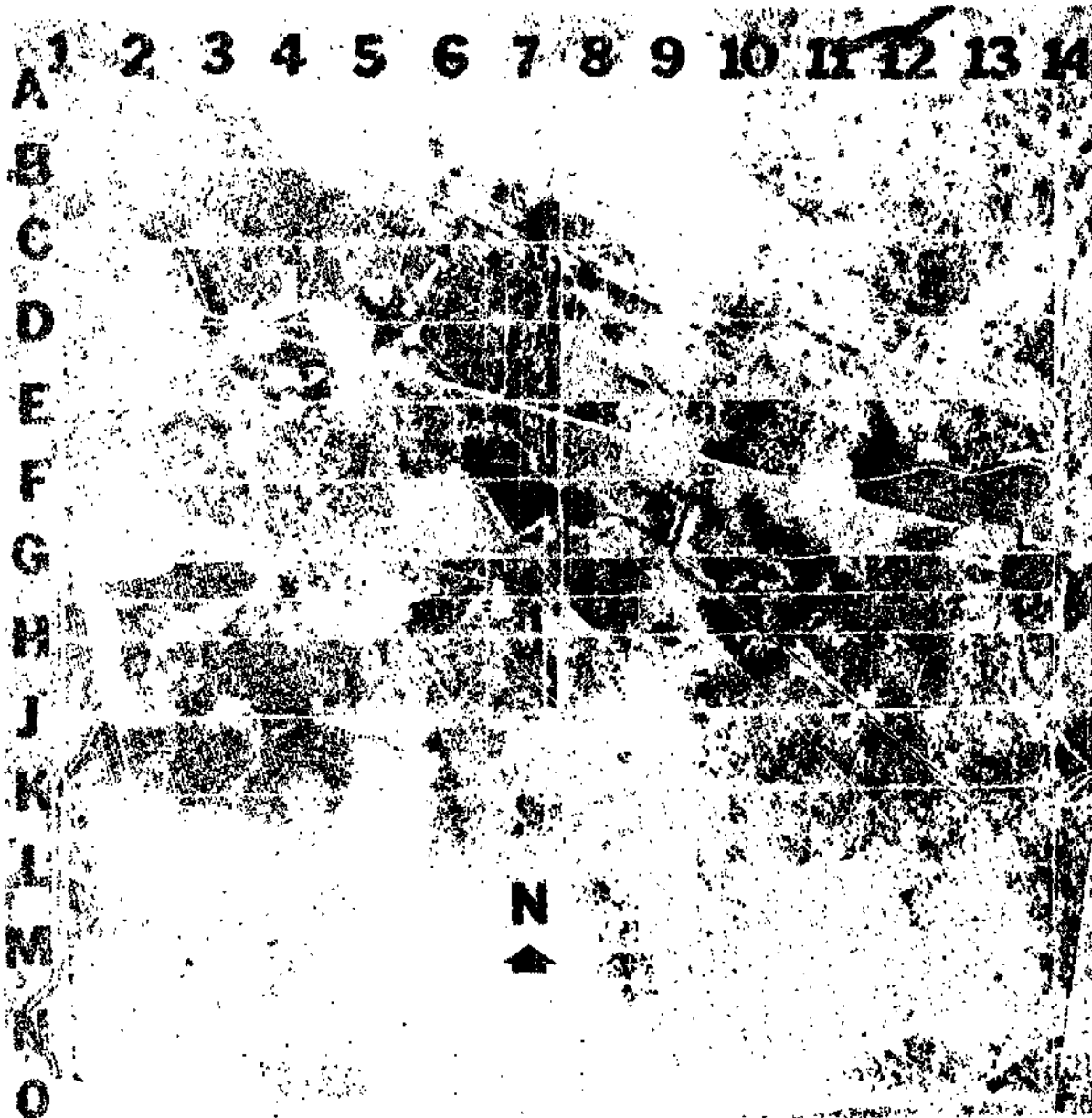


Figure 5. 1971 Photograph of the One-Square-Mile Grid of Test Area C-52A (Permanent Markers are Placed 400 Feet Apart. Markers are Labeled with Coordinates as Shown along Borders.)

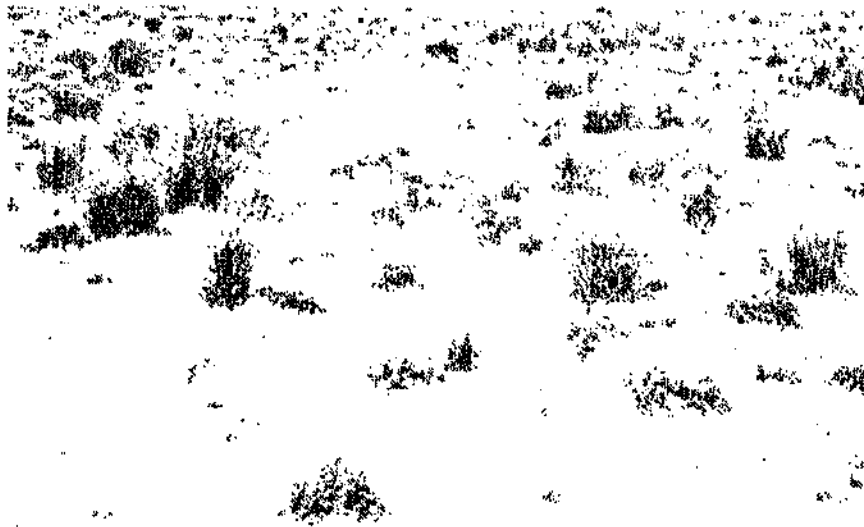


Figure 6. Arid Section of Large Grid

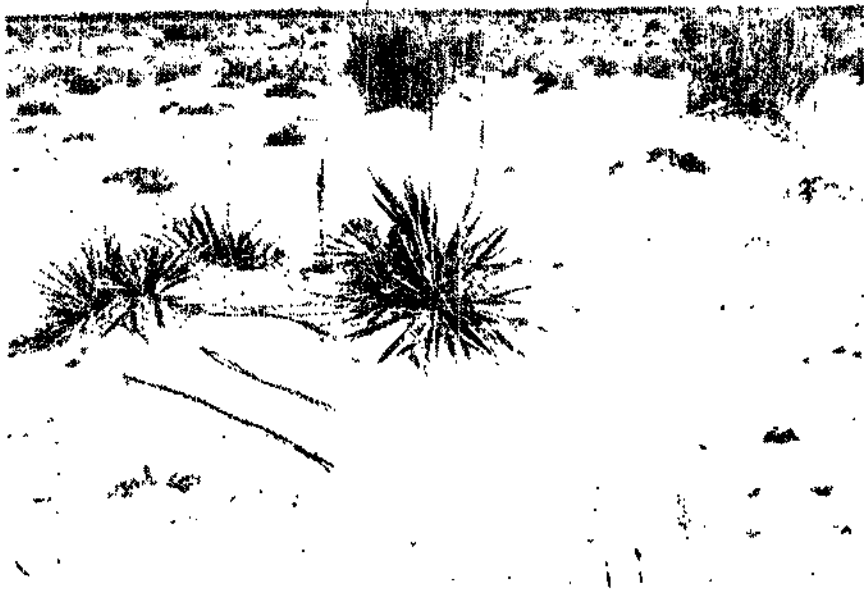


Figure 7. Arid Section of Grid 1

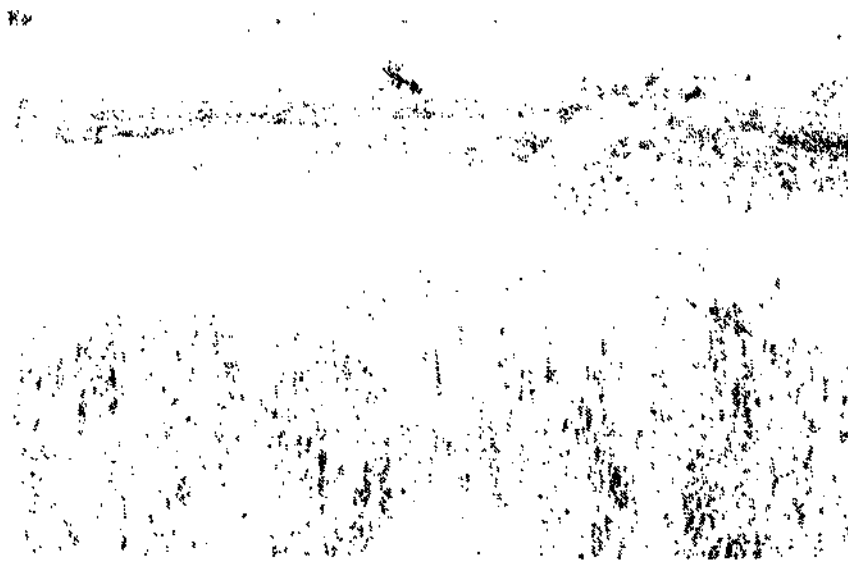


Figure 8. Gator Pond



Figure 9. East Pond



Figure 10. Trout Creek Bayhead in Southeast Corner of TA C-52A

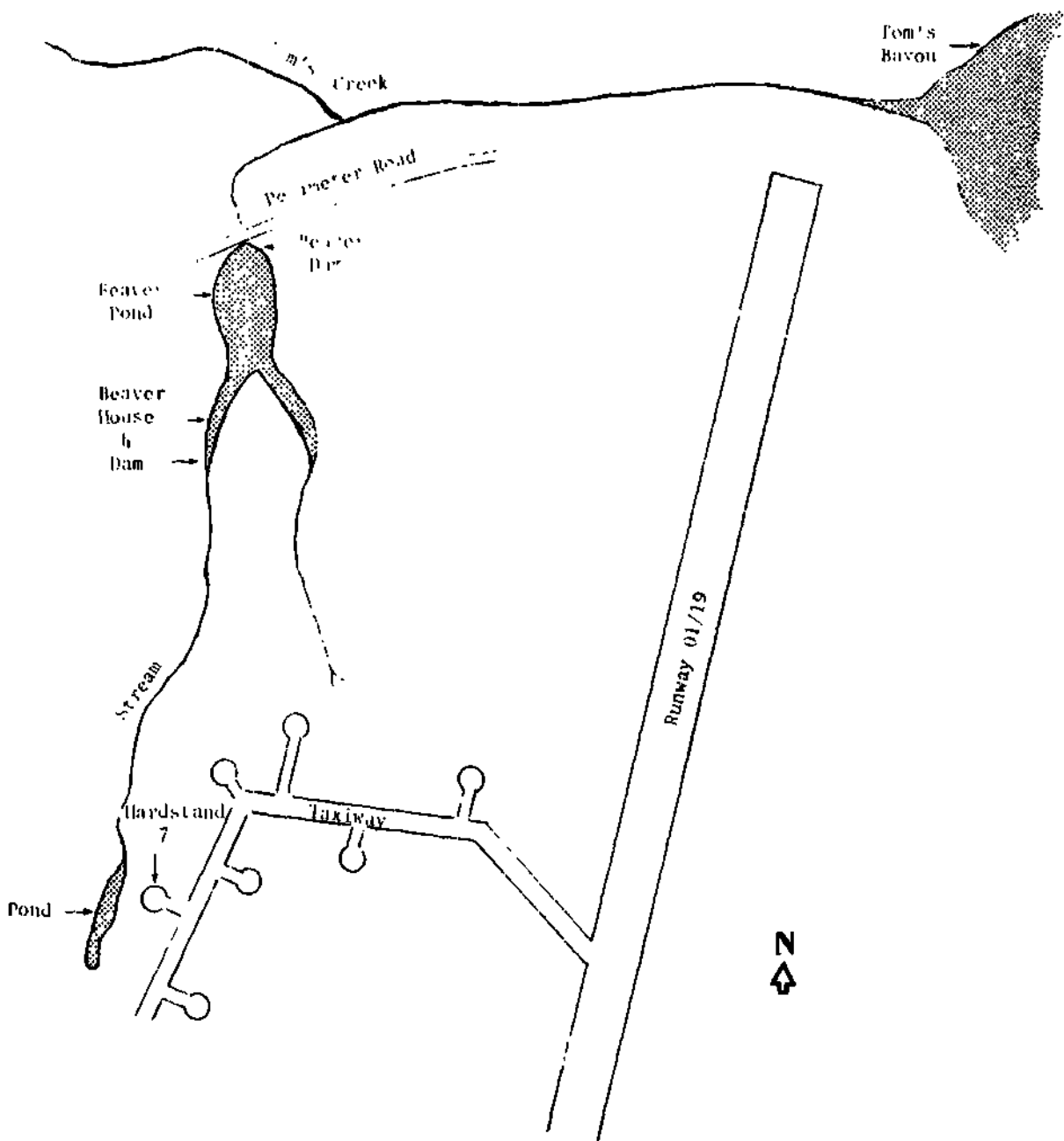


Figure 11. Map of Area Around Hardstand 7

TABLE 1. QUANTITIES OF HERBICIDE 2,4,5-T SPRAYED ON TA C-52A

Years	Area Sprayed	Approximate Number of Acres Sprayed	Lb/Acre
1962-1964	Grid 1	92	947
1964-1966	Southwest Section of One-Square-Mile Grid	92	584
1968-1969	One-Square-Mile Grid	240*	160

*Only about 240 of the 640 acres was sprayed

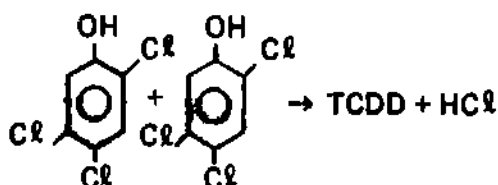
problem and required the use of fill dirt. Asphalt had been poured over the fill dirt on the rim of the ravine for soil stabilization (Figure 12). During the time herbicide operations were being conducted, spray aircraft were loaded and herbicide drums were stored on this hardstand. The pond behind HS-7 (Figure 13) was drained by a small shallow stream which flowed north for 1,000 meters before entering Beaver Pond (Figure 14).

2. CHEMICAL PROPERTIES AND EFFECTS OF TCDD

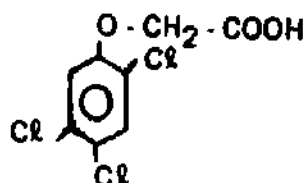
The chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)



is a synthetic chlorinated hydrocarbon derived from the condensation of two molecules of 2,4,5-trichlorophenol (TCP),



Since TCP is the precursor for some phenoxy herbicides, notably 2,4,5-T



formulations of 2,4,5-T herbicides may contain TCDD as a contaminant. This has been found to be the case (Reference 3). Since the condensation reaction in which TCDD is formed takes

Reference:

3. Woolson, E. E., et al, J. Agr. Food Chem 20 (2), 351 (1972).



Figure 12. Aerial Photograph of Hardstand 7



Figure 13. Pond by Hardstand 7

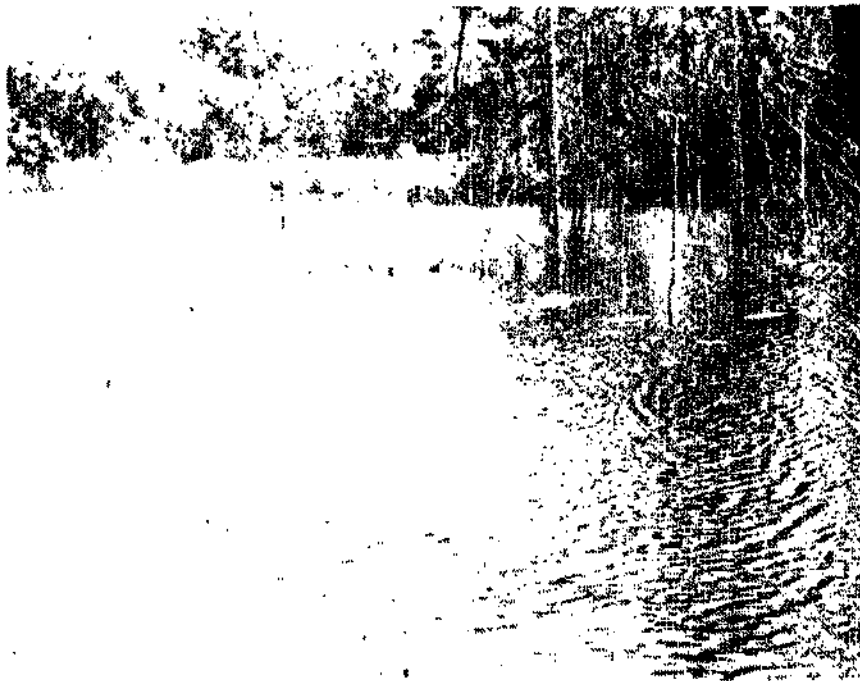


Figure 14. Beaver Pond North of Hardstand 7

place only under strong reaction conditions (strong base and high temperature), TCDD is a remarkably stable compound. It is resistant to many of the processes by which simple organic molecules are broken down.

TCDD has been shown to be an extraordinarily toxic substance. Its biological effects include being a teratogen (Reference 4), an enzyme inducer (Reference 5), and an organic poison (Reference 6). TCDD was first implicated as a teratogen in 1969 when the results of the Bionetics study (Reference 7) were published. Since very little was known about the biological effects of phenoxy herbicides, the National Institute of Health contracted with the Bionetics Laboratory of Litton Industries to study the effects of these herbicides on developing fetuses. The results showed that 2,4,5-T contaminated with 30 ppm TCDD produced significant abnormalities in both mouse and rat fetuses and mothers. Subsequent studies with contaminated 2,4,5-T and purified TCDD have largely corroborated and extended these findings.

Thus, since TCDD possesses the dual properties of being extremely toxic and extremely stable, it may pose an environmental hazard by accumulating or possibly magnifying in food chains to harmful concentrations.

The analytical determination of the presence of TCDD is usually made by the combination of gas chromatography and mass spectrometry. Generally, detection in the ppt range is required since TCDD is active in these concentrations. Two laboratories (Reference 8) have reported capabilities for ppt determination of TCDD. The method involves treatment of contaminated samples with strong acid to remove interfering pesticides, further separation by silica gel, hexane-chloroform, and gas liquid chromatography and mass spectrometric analysis using the 322 (321.894) mass-to-charge (m/e) peak. Other peaks of TCDD's mass spectrum can be compared to increase sensitivity and ³⁷C1 TCDD (m/e 328) can be used for calibration and to improve ease of analysis.

References:

4. Sparschu, G. L., et al, Food Cosmet. Toxicol. 9 (3), 405 (1971).
5. Buu-Hoi, N. P., et al, Naturwissenschafter 59 (4) 173 (1972).
6. IBID, p. 174.
7. Courtney, K. K., et al, Science 168, 864 (1970).
8. Baughman, R., and Meselson, M., Environmental Health Perspectives, Experimental Issue No. 5, September (1973).

SECTION III

WILDLIFE OBSERVATIONS ON TA C-52A

A survey of the wildlife on TA C-52A was conducted between 29 March 1974 and 1 February 1975. Visits to the study area were made on an average of twice each week at various times of the day and night. A small collection of specimens was made for species identification and for TCDD analysis.

The study was a follow-up to Young's summary (Reference 1) of five years' ecological investigation of TA C-52A. The observations reported in this section were restricted to those made within the boundary of the cleared area (including the bayheads) of TA C-52A, and unlike Young's report, does not include observations made on the large test area north of TA C-52A.

The emphasis of this study was upon the birds because, due to their primarily diurnal activities, they were more apparent and could be observed with less disturbance to the ecosystems. Observations of other classes of wildlife were also made during the course of this study.

1. BIRDS

Seventy-seven species of birds were observed on TA C-52A. Of this number, 44 species were observed on Grid 1. The remaining birds were seen in the surrounding clearing and bayheads projecting into the clearing.

Only three species can be classified as residents which nest on the large grid. These are the southern meadowlark (*Sturnella magna*), the mourning dove (*Zenaidura macroura*), and the bobwhite quail (*Colinus virginianus*) (Figure 15). There were no nesting resident birds on Grid 1.

At the beginning of the study, in the spring of 1974, the meadowlark was the dominant bird of the large grid and the surrounding cleared area. They could be found in nearly all areas containing low shrubs and were most abundant in the areas around the northeastern and northwestern corners of the large grid. These birds were rarely seen south of the middle of the large grid except near the bayheads. Since meadowlarks were the most numerous birds of the study area, they were selected for analysis of TCDD in liver tissue. Nine specimens were collected during the second week of May along the F and G rows of the one-square-mile grid.

During the late spring and summer there was a gradual but marked decrease in the number of these birds. Part of the decline was due to the collection of specimens and to the normal spring migration of winter visitors, but the number continued to decline after these events. A nest containing five eggs was discovered on 11 July in the northeastern section of the large grid (near marker B-12), but the nest was molested and eggs disappeared on the night of 17 July. The decline in the meadowlark population continued during and after the breeding season. Young meadowlarks were first seen in the area in late May and appeared to outnumber adults by the end of June. By mid-summer, meadowlarks had become quite scarce and no more than a dozen birds were observed in one day; more frequently, none was seen or heard.



Figure 15. Resident Birds of the One-Square-Mile Grid (Left to Right: Meadowlark, Mourning Dove, and Bobwhite Quail)

Three more specimens (one adult and two immature birds) were collected in the first week of August. The adult specimen, a male, was in an apparent bad state of health (Figure 16). The feathers were badly frayed and sparse. The skin was very reddened, and the scales of the feet and legs were cracked, swollen, and rough. The tip of the normally pointed, horny tongue was truncated and frayed. The testes and liver were both enlarged, but other internal structures appeared normal. The stomach contents were largely insect remains, with a small amount of vegetative matter. Darkling beetles (Family Tenebrionidae) were the principal insect remains. The two immature meadowlarks appeared normal; their livers and stomachs, including contents were analyzed for TCDD.

Until mid-September meadowlarks were very scarce, but then they appeared in rapidly increasing numbers. On both 24 and 26 September, over 150 meadowlarks were seen on and around the northern edge of the large grid. Most of these birds were obviously transient migrants passing through the area because by mid-November the numbers had dropped to about 50 or 60. In early December only 15 to 20 remained in the immediate area and occasionally were found on the large grid, but in January they were rarely seen.

Mourning doves were seen regularly during most of the study, either as singles or in groups up to four, but were rare in late December and January. These seed-eaters ranged over a larger area than the meadowlarks and could even be found occasionally on Grid 1. In the spring, at the beginning of the study, mourning doves were much less common than the meadowlarks, but they gradually became more abundant than the meadowlark. Local breeding of the doves was obviously quite successful. Surprisingly, four dove nests, each containing two eggs, were discovered on the ground of the large grid (Figure 17). This is quite unusual, but ground nests have been reported by Howell (Reference 9) and Weston (Reference 10). By mid-August well over 100 doves were present on TA C-52A. With the arrival of migrants, this number was doubled by mid-September. By December the population of doves had returned to approximately 25, and by January they were seldom seen. Six mourning doves were collected from the large grid in May, and three additional specimens were collected from Grid 1 in November for TCDD analysis.

Bobwhite quail were frequently heard calling and occasionally seen in the northern half of the large grid and around the bayheads in spring and early summer. The numbers of these birds residing on or visiting the grid were apparently quite small. One quail nest containing 10 chicks was discovered near marker D-5 in the northwest section (Figure 18). The only other group of quail seen on the grid was a covey of five. No quail were heard or seen on the grid after July.

In mid-October, hundreds of Savannah sparrows (*Passerculus sandwichensis*) migrated into TA C-52A and could be found in numbers throughout the area, including Grid 1. Twelve specimens (three from the large grid and nine from Grid 1) were collected in mid-November and analyzed for TCDD.

References:

9. Howell, A. H., Florida Bird Life, Coward-McCann, Inc., N.Y. (1932).
10. Weston, F. M., A Survey of the Birdlife in Northwestern Florida, Tall Timbers Research Station, Tallahassee, Florida (1965).



Figure 16. Meadowlark Speciman Showing Conditions of: a. Body, b. Throat, c. Feet, and d. Leg



Figure 17. Mourning Dove Nest Found on the One-Square-Mile Grid

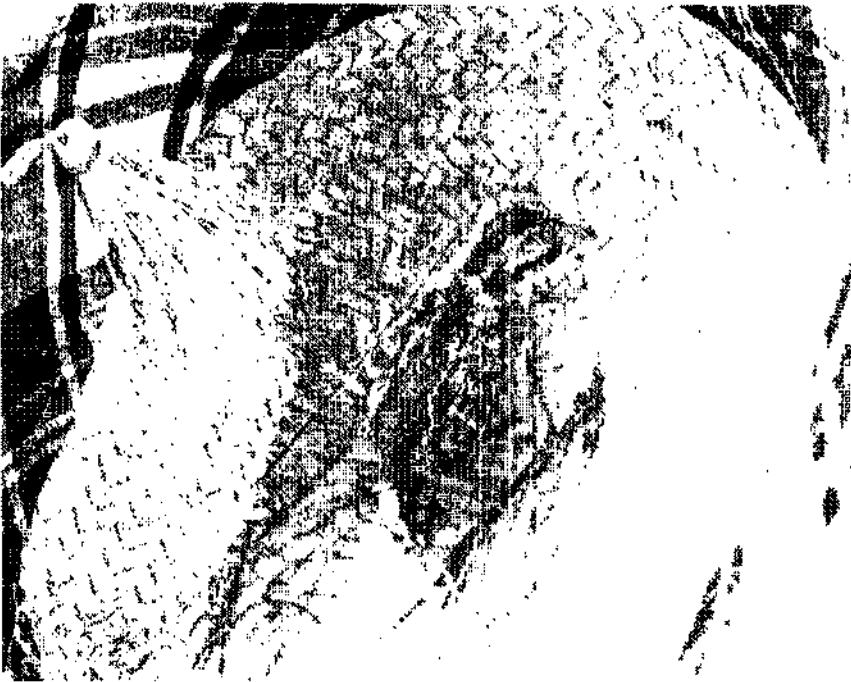


Figure 18. Bobwhite Quail Chick Found on the One-Square-Mile Grid

Other birds observed on the two grids and surrounding areas of TA C-52A are recorded in the following annotated list of birds of TA C-52A.

2. ANNOTATED LIST OF BIRDS OF TA C-52A

The following is a list of all species of birds observed on TA C-52A. The order of listing and nomenclature follow the American Ornithologists' Union Checklist on North American Birds (Reference 11). Species not previously recorded for the area by Young (Reference 1) are marked by an asterisk.

Green Heron: *Butorides virescens* (Linnaeus)

This species was not observed during this study but was reported on the grid by Young.

Little Blue Heron: *Florida caerulea* (Linnaeus)

Two birds were observed on East Pond on 6 June. Single birds were seen on several subsequent occasions. This species was not recorded on the grid by Young until 1973.

Catt'e Egret: *Bubulcus ibis* (Linnaeus)

These birds were occasional visitors to Gator Pond. Four birds were observed on 3 and 5 July.

American Egret: *Casmerodius albus egretta* (Gmelin)

This species was not observed during this study but was reported on and off the grid by Young.

American Bittern: *Botaurus lentiginosus* (Rackett)

This species was not observed during this study but was reported on and off the grid by Young.

*Wood Ibis: *Mycteria americana* (Linnaeus)

A single sighting of the wood ibis was made on Gator Pond, 25 June. A wood ibis was seen on an Eglin golf course pond on 23 June. Both of these sightings were probably of the same bird because it was outside its normal range, and Weston (Reference 10) cited only four occurrences in Northwest Florida.

*Pintail (*Anas acuta* (Linnaeus))

A single female pintail was observed on East Pond 24 September and on Gator Pond 26 September and 2 October. This migrant duck was accompanied by a male blue-winged teal on each occasion.

Reference:

11. American Ornithologists' Union, Check-list of North American Birds, Lord Baltimore Press, Inc., Baltimore, Maryland (1957).

In addition to the male teal cited above, a female blue-winged teal was seen on Gator Pond 24 September.

Turkey Vulture: Cathartes aura (Linnaeus)

These birds were frequent visitors, both on and off the grid. They were seen in numbers up to five, but usually singly, searching for carrion. None was observed feeding or on the ground, but they occasionally perched on the spotting tower located northwest of the grid.

Black Vulture: Coragyps atratus (Bechstein)

A single black vulture was seen soaring with two turkey vultures on 2 May.

Swallow-tailed Kite: Elanoides forficatus (Linnaeus)

This species was not observed during the study but was reported on and off the grid by Young.

Mississippi Kite: Ictinia mississippiensis (Wilson)

This summer resident was first seen on 8 May and was last seen on 6 August. Between these dates, one to three birds were normally observed perched in the surrounding trees or flying over the clearing. This protected hawk-like bird is entirely insectivorous. Despite Sprunt's allegation (Reference 12) that "deforestation has made inroads on the population", this kite appears to prefer the large cleared test ranges in the area. Seven kites were seen feeding over Test Area D-51 (four miles southwest) on 5 June, and nine were seen over the large grid of TA C-52A on 9 July. During the latter part of July and early August, the birds were seen less frequently, and some may have departed earlier than the end of their normal sojourn reported by Weston (Reference 10) as mid-August.

Sharp-shinned Hawk: Accipiter striatus (Vieillot)

This species was not observed during this study but was reported on and off the grid by Young.

Red-tailed Hawk: Buteo jamaicensis (Gmelin)

Young reported this species off the grid but not on the grid. During this study, several red-tails were regularly observed in the vicinity, but none was seen in the study area until 8 May. After that date, a single male was regularly seen on and around the grid. For about two weeks in late May it was joined by a female. Mating was apparently unsuccessful, and the female left. The male red-tail had leather leg bands and was identified by Dr. J. C. Foster, a nearby resident, as being his trained bird which had escaped in that area on 28 April. This red-tailed hawk usually hunted the southern and eastern sections of TA C-52A, including the southern half of the large grid and Grid 1. Unfortunately his hunting range covered areas which were used for trapping mice for TCDD studies in June and July. This probably accounted for less

Reference:

12. Sprunt, A., Florida Bird Life, Coward-McCann, Inc., N.Y. (1954).

successful trapping than had been expected. This hawk was last seen on 25 June. However, on 1 October it was learned from Vitro Services personnel that a dead hawk had been found on the grid "a month or so ago". After receiving the location, the remains of the bird were found next to a utility pole near the center of the large grid. The skeleton and feathers were recovered and examination revealed no fractures. The cause of death remains unknown.

Red-shouldered Hawk: Buteo lineatus (Gmelin)

This species was not observed during this study but was reported by Young off the grid.

*Marsh Hawk: Circus cyaneus (Linnaeus)

This species was first observed during the annual hawk migration when two female marsh hawks were observed on the grid on 24 September. One of these large hawks remained in the area and was seen regularly up until mid-November, hunting the entire area of TA C-52A. This species is principally a rodent eater but does devour some birds, including the bobwhite quail. The occurrence of this hawk for about two months may partially account for the apparent disappearance of quail.

*Peregrine Falcon: Falco peregrinus (Tunstall)

This falcon was seen on one occasion 18 October off the grid.

Sparrow Hawk: Falco sparverius (Linnaeus)

A single bird was observed on a utility wire near the center of the grid on 29 March. The species was not recorded again until 14 August when three were seen on one occasion. Weston (Reference 10) states the species is rare in Northwest Florida in the summer. In late September, four sparrow hawks established themselves northeast of the grid. They usually hunted north of the grid but occasionally were seen over the entire grid. According to Sprunt (Reference 12), this bird is generally a grasshopper hawk, but may occasionally take small mammals or even birds. Of the 20 to 30 captures witnessed in the study, all the prey were grasshoppers which were abundant in the area. Three of these hawks remained in the area until the study was concluded in February 1975.

Bobwhite Quail: Colinus virginianus (Linnaeus)

Quail resided in the study area, but were distributed principally near the northwest section of the large grid and near the bayheads. This species was not seen on Grid 1. A nest containing ten chicks was discovered on the large grid on 21 June. The number of quail seen and heard in the area decreased during the summer and fall. The last sighting of a quail on the grid was in July, and the last one heard calling was off the grid near the northwest corner on 24 September.

*Turkey: Meleagris gallopavo (Linnaeus)

This was a rare visitor to the study area, but single birds were seen several times in areas surrounding the clearing. On 26 July, a turkey was observed flying from a pine tree onto the clearing near the Trout Creek bayhead.

*Killdeer: Charadrius vociferus (Linnaeus)

Three birds of this species were seen on 7 November on the East Pond, and two more were seen on Gator Pond on 19 December.

*American Woodcock: Philohela minor (Gmelin)

One woodcock was flushed from a sand dune on Grid 1 6 November.

*Wilson's Snipe: Capella gallinago (Linnaeus)

Single birds were seen on East Pond on 18 October and 1 November, and two were observed on Gator Pond 2 January 1975.

*Pectoral Sandpiper: Erolia melanotos (Vieillot)

On 8 May, a flock of twelve sandpipers was seen on East Pond. Four specimens, including two pectoral and two white-rumped sandpipers, were collected. The remaining birds stayed on the pond until 16 May but were not seen after that date. Howell (Reference 9) lists the pectoral sandpipers as an uncommon spring migrant in Florida, and Weston (Reference 10) lists the bird as rare and irregular since 1948.

White-rumped Sandpiper: Erolia fuscicollis (Vieillot)

These sandpipers were observed in the company of the pectoral sandpipers cited above. According to both Howell (Reference 9) and Weston (Reference 10), this bird is a rare migrant in the area.

*Black-necked Stilt: Himantopus mexicanus (Muller)

One female specimen was collected 8 May on East Pond. This is the first known specimen collected in Northwest Florida. The bird was obviously an accidental stray outside of its recorded range (Reference 5). However, Weston (Reference 10) reported three sightings near Pensacola, Florida.

Mourning Dove: Zenaidura macroura (Linnaeus)

A resident population of approximately 25 mourning doves was found distributed throughout the study area. The number of these birds increased after the breeding season and during migration but then decreased during the winter.

*Ground Dove: Columbigallina passerina (Linnaeus)

This dove was an occasional visitor. A single bird was seen on the grid 17 June. Pairs of birds were observed on 25 June, 24 September, and 2 October.

*Yellow-billed Cuckoo: Coccyzus americanus (Linnaeus)

This resident of the wooded area surrounding TA C-52A was occasionally seen flying over the clearing near bayheads.

*Barred Owl: Strix varia (Barton)

This owl was heard occasionally in the woods near Trout Creek bayhead. Single birds were seen leaving the clearing south of Grid 1 on 19 July and on the ground east of Grid 1 on 2 November.

Whippoorwill: Caprimulgus vociferus (Wilson)

This species was heard within the wooded area but was not seen or heard on the clearing. It was reported off the grid by Young (Reference 1).

Common Nighthawk: Chordeiles minor (Foster)

This species was frequently observed in courtship flights during the spring. Up to 20 birds could be seen flying over the entire clearing at all times of day. During the remainder of the study these birds were seen infrequently, generally in the early morning or evening, in small groups near the tree line. Although no nests were found, one nighthawk appeared to be nesting in the clearing near the tree line, east of the grid.

*Chimney Swift: Chaetura pelagica (Linnaeus)

Swifts were occasional visitors to the study area. This species was first seen off the grid on 6 May and on the grid on 7 May.

*Ruby-throated Hummingbird: Archilochus colubris (Linnaeus)

Numerous hummingbirds were seen in the Trout Creek bayhead on 20 August. From mid-September to mid-October, they were abundant in all the bayheads and along the southern tree line. None was observed on the grid.

*Belted Kingfisher: Megasceryle alcyon (Linnaeus)

A single bird was observed briefly, hovering over East Pond on 24 September.

*Yellow-shafted Flicker: Colaptes auratus (Linnaeus)

This ground dwelling member of the woodpecker family was seen on a number of occasions northwest of the grid, but never on the grid.

*Hairy Woodpecker: Dendrocopos villosus (Linnaeus)

A single bird was seen crossing the clearing near Trout Creek on 6 May.

*Eastern Kingbird: Tyrannus tyrannus (Linnaeus)

This species was seen only once on the northwest corner of the grid. A pair of these kingbirds successfully nested west of the grid and were frequently seen on utility wires in that area.

*Great Crested Flycatcher: Myiarchus crinitus (Linnaeus)

This bird was a common summer resident in the surrounding forest. It was frequently seen along the edge of the clearing but not on the grid.

*Eastern Phoebe: Sayornis phoebe (Latham)

A single bird was observed in the Basin Creek bayhead just north of the grid on 18 October.

*Yellow-bellied Flycatcher: Empidonax flaviventris (Baird and Baird)

These flycatchers are casual migrants in this area of Florida. One bird of this species was seen in the Basin Creek bayhead on 18 October.

*Eastern Wood Peewee: Contopus virens (Laird)

A peewee was seen only once on 18 October near the middle of the grid.

*Tree Swallow: Iridoprocne bicolor (Vieillot)

Three to five tree swallows were seen regularly during the month of October mainly over the two ponds on the grid.

*Rough-winged Swallow: Stelgidopteryx ruficollis (Vieillot)

A single bird was observed traversing the grid on 9 July.

*Barn Swallow: Hirundo rustica (Linnaeus)

These swallows were occasional summer visitors to the entire study area. They were usually seen in small numbers, but sometimes 20 to 30 birds would pass through the area. They were first seen on 2 May and were last seen on 25 October.

*Purple Martin: Progne subis (Linnaeus)

Approximately 24 martins nested in the martin houses erected near the Range Control Complex on the northern border of TA C-52A. These birds were first seen on 18 April and normally remained within one-quarter of a mile of the nests. However, these birds sometimes were seen in the north-west section of the grid. After the young could fly, the martins left their houses but were seen in the area until 9 July. On 11 May an immature female was found on the ground with an apparently broken wing. When approached the right wing was flapped, but the left wing was held tightly against the body. This specimen was collected and examined. The bird was fully grown, but the proximal ends of some primary flight feathers were still partially ensheathed. The martin had a large amount of visceral fat and moderate cermatitis, but otherwise appeared normal. No lesions or fractures were found when dissecting the pectoral girdle and left wing.

***Blue Jay: Cyanocitta cristata (Linnaeus)**

This jay resided in abundance in the surrounding forest and was occasionally seen on the edge of the clearing. It was never seen on the grid.

Common Crow: Corvus brachyrhynchos (Brehm)

This species was seen occasionally flying over the grid and surrounding area during the entire period of this study.

***Fish Crow: Coryus ossifragus (Wilson)**

A single fish crow was seen crossing the grid on 2 May. No other observations of this species were made.

***Winter Wren: Troglodytes troglodytes (Linnaeus)**

One winter wren was seen on a single occasion in the Mullet Creek bayhead 16 October.

***Carolina Wren: Thryothorus ludovicianus (Latham)**

This resident of the region occasionally was seen and heard in all the bayheads and along the tree line. None was observed on the grid.

***Mockingbird: Mimus polyglottos (Linnaeus)**

Occasionally mockingbirds were sighted along the edge of the clearing, and one pair nested in the Trout Creek bayhead.

***Catbird: Dumetella carolinensis (Linnaeus)**

This winter resident was seen first on 24 September in the Trout Creek bayhead. Two days later the species was abundant in all the bayheads.

***Brown Thrasher: Toxostoma rufum (Linnaeus)**

Thrashers are common residents of the region, but they were not observed until 26 September in Mullet Creek bayhead.

Robin: Turdus migratorius (Linnaeus)

Robins were not observed during this study but were reported both on and off the grid by Young.

***Loggerhead Shrike: Lanius ludovicianus (Linnaeus)**

Shrikes were frequently seen on utility wires in the northwest section of the clearing. A single bird was observed on the grid near marker G-2 on 22 September.

*Yellow-throated Vireo: Vireo flavifrons (Vieillot)

This species was seen throughout the summer in Trout Creek bayhead. On 24 September several of these birds were found in all the bayheads. They were not seen on the grid.

*Yellow Warbler: Dendroica petechia (Linnaeus)

A specimen of this early migrant was collected on 20 August in the Mullet Creek bayhead.

*Pine Warbler: Dendroica pinus (Wilson)

Only one pine warbler was seen in the study area, east of the grid. The species was frequently seen, sometimes in large numbers, in the adjacent wooded area.

*Palm Warbler: Dendroica palmarum (Gmelin)

A number of these warblers were seen in the Basin Creek bayhead on the northern border of the grid between 18 October and 24 October.

*Kentucky Warbler: Oporornis formosus (Wilson)

Two of these birds were seen in the Trout Creek bayhead on 9 July.

*Yellowthroat: Geothlypis trichas (Linnaeus)

Several of these birds were observed in the Trout Creek bayhead throughout the period of study.

*Yellow-breasted Chat: Icteria virens (Linnaeus)

Resident birds were found in both Trout and Mullet Creek bayheads. One adult and two young chats, just barely able to fly, were observed on 9 June.

Eastern Meadowlark: Sturnella magna (Linnaeus)

The meadowlark is a resident of the study area, but was uncommon on Grid 1 and the southern half of the large grid.

Red-winged Blackbird: Agelaius phoeniceus (Linnaeus)

This species inhabited the bayheads in large numbers during the spring and early summer. After the young were raised, these birds banded together in a large flock and left the area about 20 August. Young (Reference 1) reported that this species was first seen on the grid in 1973. From the beginning of this study, one male and two females were regularly seen around Gator Pond, and a single male was seen on East Pond on 3 and 5 June. By 16 May there was only a single pair of red-wings on Gator Pond, and it appeared that they might nest. These birds were then closely watched. On 18 June, the female exhibited the kind of behavior that is typical of a bird when trying to protect a nest, but on the following day, both birds had disappeared. An exhaustive, fruitless search was then made for the nest. The nesting attempt was obviously unsuccessful, but the cause could not be determined.

*Brown-headed Cowbird: Molothrus ater (Boddaert)

Small numbers of cowbirds were seen at irregular intervals throughout the study. A single specimen was observed on Grid 1 on 3 November.

*Summer Tanager: Piranga rubra (Linnaeus)

Tanagers were frequently observed near the tree line and crossing the study area in the spring and were seen on a few occasions during the summer. One tanager was seen on the eastern part of the grid on 19 April.

*Cardinal: Richmondia cardinalis (Linnaeus)

This bird was a common resident of the surrounding forest. It was not seen on the grid but occasionally was observed flying across the study area into the bayheads.

*Blue Grosbeak: Guiraca caerulea (Linnaeus)

During the spring, this was the most abundant bird along the tree line and in the bayheads, but it was not observed on the grid. These grosbeaks were less common in the summer and were last seen on 18 October.

*Rufous-sided Towhee: Pipilo erythrophthalmus (Linnaeus)

This species was seen occasionally throughout the study in Mullet Creek bayhead and in Basin Creek on 2 October.

*Savannah Sparrow: Passerculus sandwichensis (Gmelin)

These winter visitors arrived in enormous numbers in mid-October and were scattered over the entire study area. These were the only birds which were seen regularly or in numbers on Grid 1 or on the southern half of the large grid. The birds remained on the grids throughout the remainder of the study, but the numbers decreased significantly.

Grasshopper Sparrow: Ammodramus saviannarum (Gmelin)

This sparrow was not observed during this study, but Young reported the capture of one in a mouse trap.

*Vesper Sparrow: Pooecetes gramineus (Gmelin)

At least 12 birds were seen near the center of the large grid on 1 February 1975.

*Bachman's Sparrow: Aimophila aestivalis (Leichtenstein)

This species was seen only on 18 October in Basin Creek bayhead with a large mixed flock of sparrows.

*Field Sparrow: Spizejja pusilla (Wilson)

Field sparrows were seen on 18 October in Basin Creek bayhead and along the northern edge of the grid.

*Swamp Sparrow: Melospiza georgiana (Latham)

This sparrow was seen only on 18 October in Basin Creek bayhead.

*Song Sparrow: Melospiza melodia (Wilson)

Five birds were seen near East Pond on 1 February 1975.

3. OTHER WILDLIFE

Observations of wildlife other than birds were also recorded, and selected specimens were collected from TA C-52A. The principal concern was to determine which species were most prevalent and if it were likely that any animals contaminated with TCDD might enter the human food chain.

Reference 1 listed the following numbers of species identified on TA C-52A: mammals - 18; birds - 22; reptiles - 18; amphibians - 18. Follow-up studies by Young in the summer of 1974 concentrated heavily on rodents and fish. For this reason, these two groups of animal life were not considered in this study.

a. Mammals

The most conspicuous mammal in the area was the whitetail deer (Odocoileus virginianus). Sightings were made on nearly every visit, and fresh tracks could always be found on the two grids and surrounding area. These deer usually resided in the surrounding forest during the middle of the day and grazed in the clearing from late afternoon until early morning. Hunting is permitted during season in the surrounding forest but not in the clearing. Mr. Louis Jeter, Florida Game and Fresh Water Fish Commission, has studied these deer for several years. His estimate of the average population of this area is about 30 deer. Radio tagging has shown that the range of any one animal is generally not over one mile. Mr. Jeter collected two specimens from the grid for TCDD analysis. Another deer struck by a car four miles southwest of the grid was used as a control. In addition to deer and small rodents, only two other mammals were sighted on the large test grid. A red fox (Vulpes fulva) was seen twice near the center of the grid. Eastern cottontail rabbits (Sylvilagus floridanus) were seen on several occasions, and two were captured in traps on the grid. One of the rabbits was analyzed for TCDD.

In addition to the above, identification of tracks on the grid confirmed the presence of armadillo (Dasypus novemcinctus), bobcat (Lynx rufus), striped skunk (Mephitis mephitis), and raccoon (Procyon lotor). Over twenty-five large burrows were found on the large grid. All of these were deserted except two that were inhabited by rabbits (Figure 19).

In the clearing around the grid, deer, opossum, armadillo, and raccoon were sighted. Just off the northwest corner of the grid, a colony of pocket gophers (Geomys pinetus) was found. One opossum was trapped southeast of the grid near the edge of Trout Creek bayhead and analyzed for TCDD. A complete and unmolested raccoon skeleton was found 200 yards southeast of the grid.



Figure 19. Deserted Animal Burrow Found on Large Grid

b. Reptiles

The prevalent reptile on the grid and surrounding area was the six-lined racerunner (Cnemidophorus sexlineatus). Specimens were collected and analyzed for TCDD. A four-foot alligator (Alligator mississippiensis) inhabited Gator Pond on the grid. Only three snakes were seen during the study. A pigmy rattlesnake (Sistrurus miliarius) and a diamondback rattlesnake (Crotalus adamanteus) were found northwest of the grid, and an eastern coachwhip (Masticophis flagellus) was seen in the southeastern section of the grid. A Gulf Coast box turtle (Terrapene carolina bauri) was found southeast of the grid.

c. Amphibians

Only two species of amphibians were observed during this study. The cricket frog (Acris gryllus) was found in both Gator and East Ponds, and the leopard frog (Rana pipiens) was found in Gator Pond.

4. HARDSTAND 7 OBSERVATIONS

At the beginning of this study, it was considered highly probable that the area around Hardstand 7 was contaminated with TCDD. It was known that herbicides were stored, loaded, and spilled in the area, and there was still a strong odor resembling that of herbicide Orange (a 50-50 mixture of 2,4-D and 2,4,5-T). A wide variety of wildlife was found in the area, but it was felt that it would be unlikely that many of the animals would regularly frequent the small contaminated area. Consequently, the study in this area was restricted to the fish in the ponds and streams that drained the area. The following is a list of fish collected: mosquito fish, Gambusia affinis (Baird and Girard); bluegill sunfish, Lepomis macrochirus (Rafinesque); longear sunfish, Lepomis megalotis (Rafinesque); and starhead topminnow Fundulus notti (Agassiz). TCDD analyses were run on Gambusia and Lepomis specimens.

SECTION IV

TCDD ANALYSIS

1. METHODS

The following is a description of the methods used by Interpretive Analytical Services, Dow Chemical USA, for TCDD analysis.

a. Soil Samples

Ten grams of soil plus 7 ml of 1 percent aqueous ammonium chloride were extracted with a 100-ml portion, then a 30-ml portion of 1:1 hexane:acetone. The extracts were combined, and the acetone was removed by extraction with water. The hexane extract was cleaned up by washing with 10 ml of concentrated sulfuric acid, followed by two chromatographic operations. The first chromatographic separation was done on a 0.4 x 5 cm silica gel column, eluting with 1:4 benzene:hexane. The appropriate eluate fraction was then transferred to a 0.4 x 5 cm alumina column. Partial removal of DDE and PCB's was accomplished by washing the alumina column with 1:4 carbon tetrachloride:hexane. Then the TCDD was eluted from alumina column with 1:4 methylene chloride:hexane. The effluent from the alumina column was evaporated to dryness, and the residue dissolved in 20 μ l of xylene for subsequent determination of TCDD.

The determinations were made with an LKB-9000S gas chromatograph-mass spectrometer. The GC column was a 6-foot by 2 mm glass column packed with 3 percent OV-3 silicone on Gas Chrom Z. Column temperature (isothermal) was 230°C. Five μ l injections of the above xylene solutions were made. The mass spectrometer was set to monitor m/e = 320 and m/e = 322 (molecular ions $C_{12}H_4O_2Cl_4^{35}$ and $C_{12}H_4O_2Cl_4^{35}Cl^{37}$).

b. Biological Samples

Ten grams of sample were digested in a potassium hydroxide solution, which was then extracted with several portions of hexane; the combined hexane extracts were washed with four 10 ml portions of concentrated sulfuric acid, cleaned up, and analyzed by the same procedures as described above.

2. SOIL SAMPLE RESULTS

a. Large Grid

Soil samples were taken from 25 locations on the one-square-mile grid, and one sample was taken from Basin Creek just north of the grid (Table 2 and Figure 20). The analysis of the samples revealed that TCDD was spread over the entire grid, even in areas that were not near the spray aircraft flight paths. The highest concentration of TCDD found in soil samples from the large grid was 470 ppt. This sample was taken in the southwest section, which probably had received heavier deposition of 2,4,5-T. Six of the samples had no detectable amount of TCDD. These included the sample from Basin Creek and two samples from the highly organic muck of Gator Pond. The other three samples that contained no TCDD were from the northwest, southwest, and east central sections of the grid. One of these sample sites was in a low, sandy area. The other two were in high, barren areas. One drained toward the location where 190 ppt TCDD was recorded, and the other drained to the location where 31 ppt was found.

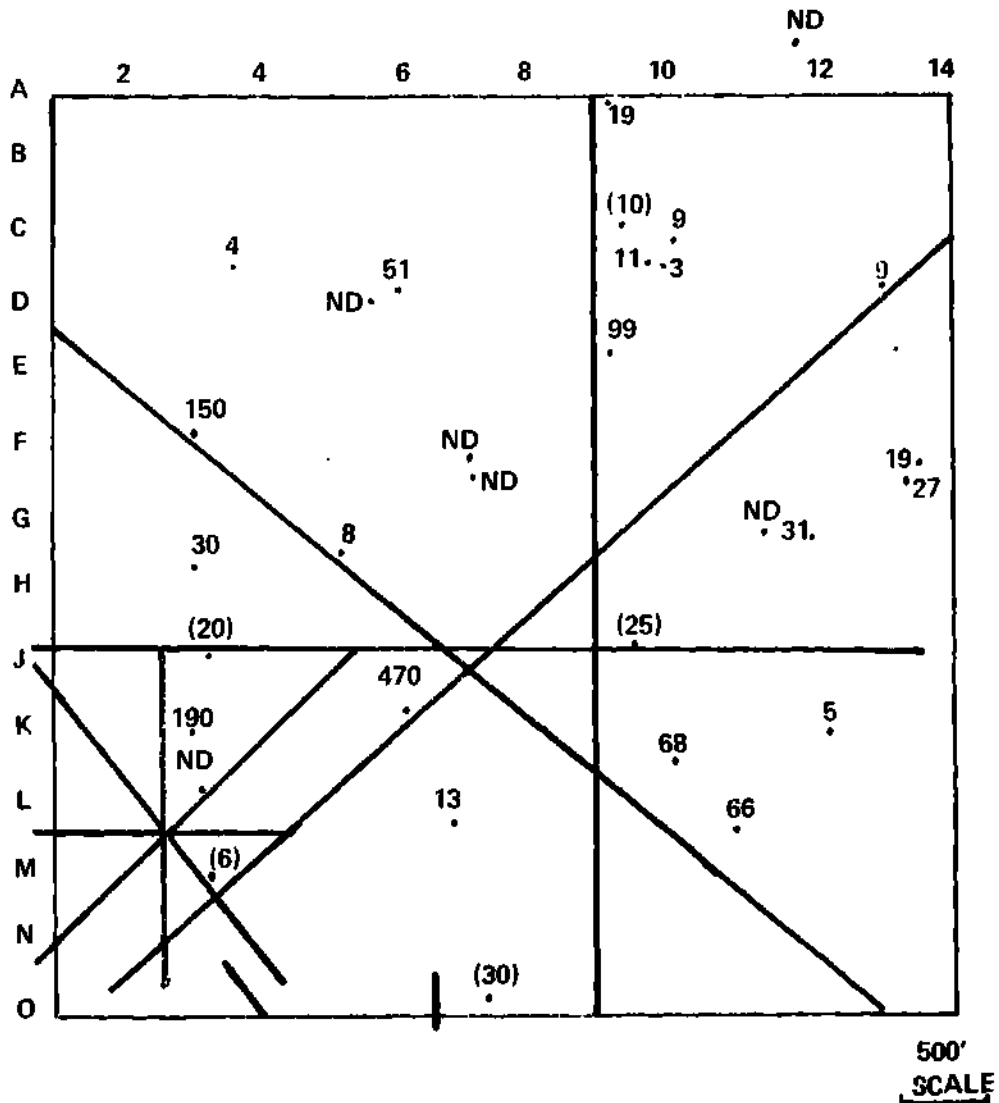


Figure 20. Distribution of TCDD in Soil Samples on the One-Square-Mile Grid (TA C-52A)

[TCDD Expressed in ppt values in parentheses are from Young's 1973 data using 6-inch core samples. Dark lines indicate flight paths of aircraft spraying 2,4,5-T between 1962 - 1969.]

TABLE 2. TCDD CONTENT OF FOUR-INCH SOIL CORE SAMPLES ON THE ONE-SQUARE MILE GRID (TA C-52A)

Sample Number	Location (Compass Direction and Feet from Permanent Markers)	TCDD (ppt)	Detection Limit (ppt)	Remarks
1	F-13, 200' SE	19		Center of East Pond
2	G-14, 200' NW	27		Drainage to East Pond
3	F-7, 50' S	ND*	8	Muck of Gator Pond
4	F-7, 100' S	ND*	10	Muck of Gator Pond
5	G-12, 150' WSW	31		Low area
6	G-11, 150' ESE	ND*	5	High spot
7	D-13, 100' N	9		Level area
8	C-10, 150' SSW	3		Low barren area
9	C-10, 150' SSW	9		High sandy area
10	C-10, 150' SW	11		Depression
11	D-6, 100' N	51		Level area
12	D-6, 250' WSW	ND*	5	Low sandy area
13	C-4, 200' SW	4		High barren area
14	L-3, 50' NE	ND*	6	High barren area
15	K-3, 50' SW	190		Drainage from Sample 14
16	L-7, 100' SW	13		Sandy knoll
17	L-12, 150' SSE	66		High knoll
18	K-12, 200' ESE	5		Low area below Sample 17
19	A-9, 100' S	19		Level area
20	A-11, 300' NE	ND*		Silt from creek (off grid)
21	F-3, 100' N	150		High area
22	G-3, 150' S	30		Low area with muck
23	J-6, 200' S	470		Sandy depression
24	H-5, 150' N	8		High eroded sandy area
25	E-9, 150' N	99		Level sandy clearing
26	L-10, 150' N	68		High sandy area

*ND - Not Detected

b. Grid 1

Soil samples from Grid 1 contained significantly higher concentrations of TCDD than did those from the large grid (Table 3 and Figure 21). One sample, containing 1,500 ppt TCDD, was taken about 400 feet northwest of the location where Young had found 710 ppt TCDD in 1973. The higher concentrations on Grid 1 were found in low areas, and the lower concentrations were generally found in areas of loose sand.

One cubic yard of soil was collected from Grid 1 for degradation and agronomic crop studies. The soil was collected from the top 4 inches of a slight depression just over 200 feet south of marker P-7. This was within a few feet of the location where 610 ppt TCDD had been found. The soil was removed and mixed by shovels. Four samples were then taken directly from the cubic yard of soil, analyzed, and found to contain 1,100 ppt (2 samples) and 1,300 ppt (2 samples) TCDD. Additional soil was placed in painted metal pots (8 inches deep and 8 inches in diameter) and divided into two groups of four pots. The soil in one group was analyzed for TCDD after 9 weeks, and the other group was analyzed after 23 weeks. The four pots in each group were treated as follows: two were left outside and exposed to natural elements, and two were placed in a greenhouse and watered with a nutrient solution. One of the two containers in each location was left undisturbed, and the other was stirred (tilled) weekly with a spatula. This stirring was not complete, and soil in the bottom of the pots was relatively undisturbed. The soil in each of the pots was emptied into a clean tray and mixed thoroughly before samples were collected and analyzed for degradation of TCDD (Table 4).

c. Hardstand 7 (HS-7)

The distribution of TCDD around HS-7 loading area was spotty and highly variable (Table 5 and Figure 22). In a low spot on HS-7 where the asphalt had decomposed, 170,000 ppt TCDD was detected. This was apparently due to spillage of herbicide on the hardstand. Two other areas containing 11,000 and 1,300 ppt were found near the hardstand and were assumed to be areas where drums of the herbicide had been stored. These highly contaminated areas were fairly localized. As expected, TCDD was not detected on the sandy slope from the hardstand to the pond because this sand had been hauled in to repair eroded areas. TCDD was found in samples taken around the pond and about 600 yards north in the stream draining the pond. The highest concentration of TCDD detected in the HS-7 pond silt was 85 ppt. Silt from the stream had 11 ppt.

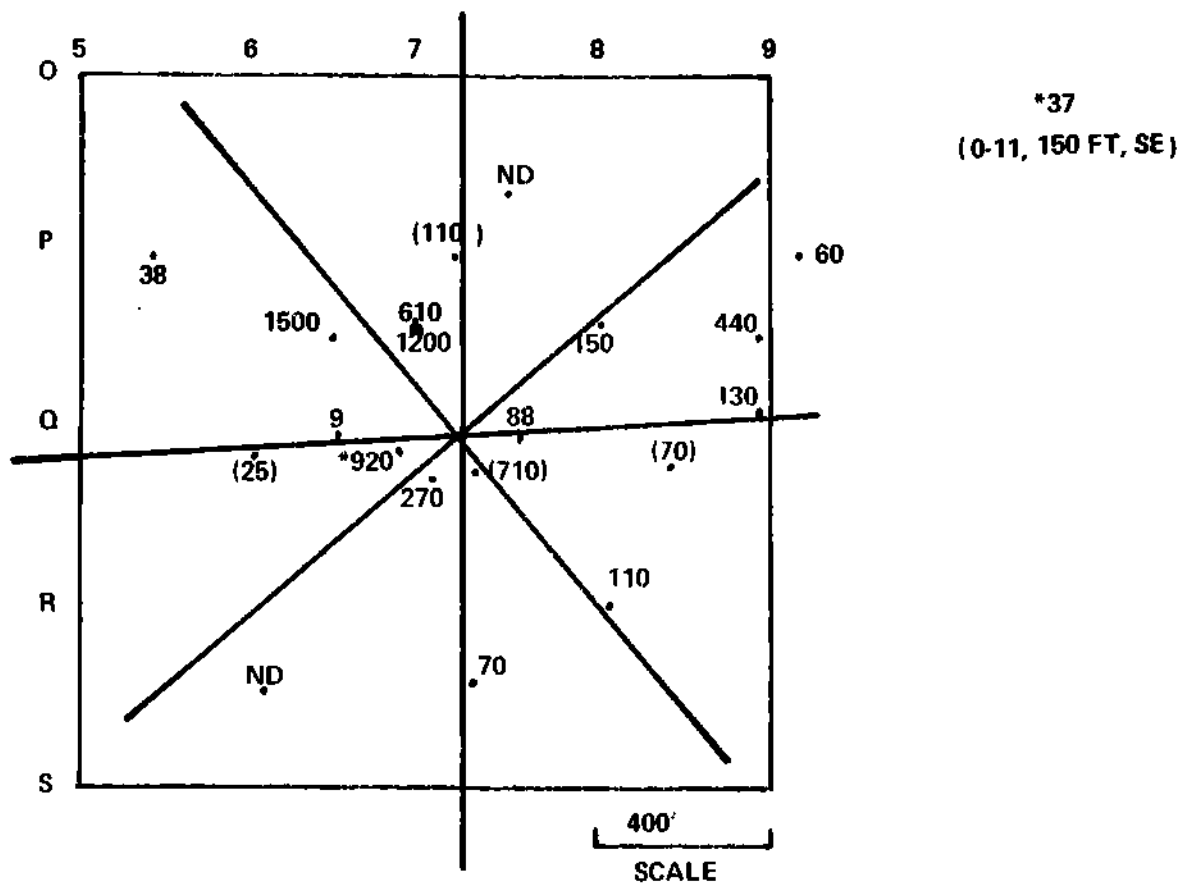
3. BIOLOGICAL SAMPLE RESULTS

Twenty-two biological samples from TA C-52A and six samples (all fish) from the area near HS-7 were analyzed for the presence of TCDD. Unfortunately, it was necessary to use composite samples in order to get large enough samples to insure a low detection limit. This procedure limited the study in many respects but permitted identification of species that accumulated TCDD.

Samples of deer and rabbit taken from the grid and opossum taken a few hundred feet away from the grid had no detectable TCDD. Meadowlarks showed the highest liver concentration of TCDD (1,020 ppt). Doves and sparrow also showed TCDD uptake. Whole body analysis showed the presence of TCDD in the sixlined racerunner and in a sample of several hundred

small insects, but it was not detected in a sample of large grasshoppers. The results of TCDD analysis of TA C-52A biological samples are shown in Table 6.

Analysis of fish from the area near Hardstand 7 (Table 7) showed that TCDD was present in whole body samples (150 ppt) and in liver tissue (740 ppt).



- - Average of 4 samples from 1 cubic yard of soil collected
- * - Samples of 1/4 inch soil.

Figure 21. Distribution of TCDD in Soil Samples of Grid 1 (TA C 52A)

[TCDD expressed in ppt. Values in parentheses are from Young's 1973 data using 6-inch core samples. All other are 4-inch core samples. Dark lines indicate flight paths of aircraft spraying 2,4,5-T between 1962 - 1969.]

TABLE 3. TCDD CONTENT OF SOIL SAMPLES ON GRID 1 (TA C-52A)

Sample Number	Location (Compass Direction and Feet from Temporary Markers)	TCDD (ppt)	Detection Limit (ppt)	Remarks*
1	R 6, 200' S	ND**	20	Interference raised detection limit
2	P-9, 200' S	440		Low spot
3	P-9, 100' E	60		Edge of sand dunes
4	Q-7, 200' S	270		Soil packed and level
5	P-7, 200' S	610		Low area
6	Q-7, 200' E	88		Level area
7	P-8, 200' S	150		Near edge of sandy area
8	P-7, 225' ENE	ND**	4	Center of high sandy area
9	P-5, 200' E	38		Rut of old road
10	P-6, 225' SE	1500		Large depression
11	Q-6, 100' E	9		Level sandy area
12	R-8	110		Level area, sand and clay
13	Q-9	130		Level area, sand and clay
14-17	P-7, 200' S	1100-1300		Four samples from 1 square yard of soil collected
18	Q-7, 50' SW	920		Top 1/4 inch soil collected with spatula
19	O-11, 150' SE	37		Top 1/4 inch soil collected with spatula

*Samples were taken with 4-inch core sampler except for numbers 14 through 19.

**ND - Not Detected

TABLE 4. DEGRADATION OF TCDD IN SOIL SAMPLES (ALL SOIL FROM GRID 1,200 FEET SOUTH OF MARKER P-7)

	Length of Exposure		
	0	9 Weeks	23 Weeks
Controls (4)	1,100-1,300 ppt		
Exposed outside to full sun (Soil mixed weekly untilled)		1,100 ppt 1,000 ppt	520 ppt 530 ppt
Exposed in Greenhouse* (Soil mixed weekly untilled)		640 ppt 810 ppt	460 ppt 530 ppt

*Soil in greenhouse was watered with a nutrient solution.

TABLE 5. TCDD CONTENT OF SOIL SAMPLES, VICINITY OF HARDSTAND 7 (EGLIN AIR FORCE BASE)

Sample Number	Location	TCDD (ppt)	Detection Limit (ppt)
1	150' S of pond	7	
2	100' SSE of pond	67	
3	200' NW of HS-7, sandy slope	ND*	
4	150' E of pond	20	
5	5' E of northern edge of pond	47	
6	In pond, 300' NW HS-7	85	
7	100' NW HS-7, sandy slope	ND*	3
8	100' SW HS-7, under asphalt	11	
9	100' NE HS-7, level ground	11,000	
10	SW edge of pond	22	
11	Low area E of pond	66	
12	75' S of HS-7 center	1,300	
13	100' NE of pond on slope	ND*	15
14	Under asphalt on HS-7	170,000	
15	150' NNE HS-7	100	
16	150' NE HS-7	ND*	5
17	150' ENE HS-7	ND*	10
18	300' SE of beaver dam	ND*	7
19	At base of beaver dam	ND*	10
20	125' S of HS-7	ND*	50
21	150' S of HS-7	ND*	2
22	North exit of pond	62	
23	In NE edge of pond	ND*	50
24	Stream 150' N of pond	ND*	3
25	Stream 600' N	11	
26	Silt near beaver house dam	ND*	10
27	Silt near beaver house dam	ND*	10

* ND - Not Detected

TABLE 6. TCDD ANALYSIS OF SPECIMENS FROM
TA C-52A

Specimen	Sample Number	TCDD (ppt)	Detection Limit (ppt)	Remarks
Deer				
liver	1	ND*	5	From large grid
visceral fat	2	ND*	4	Composite from two deer
liver	3	ND*	4	Control; not from TA C-52A
liver	4	ND*	4	From large grid
kidney	5	ND*	4	Same deer as Sample 4
Meadowlark				
liver	6	1,020		Composite of 9 birds from large grid
liver	7	200		One liver, Gator Pond
liver	8	100		Two livers, Gator Pond
stomach and contents	9	46		From same birds as Sample 8
Dove				
liver	10	50		Composite of 6 birds from large grid
liver and fat	11	50		Composite of 3 birds from Grid 1
stomach and contents	12	10		From same birds as Sample 11
seed in crop	13	ND*	7	From same birds as Sample 11
Opossum				
liver	14	ND*	10	Collected SE of grid
fat and mammary tissue	15	ND*	10	From same animal as Sample 14
Rabbit				
liver	16	ND*	8	Collected at Gator Pond
pelt	17	ND*	2	From same animal as Sample 16
Grasshopper (large)	18	ND*	3	Four from large grid
Insects, miscellaneous small**	19	40		From large grid
Racerunners	20	430		One from each grid
Sparrow				
livers	21	69		12 livers, Grid 1 (9), large grid (3)
stomach and contents	22	84		Same birds as Sample 21

*ND - Not Detectable

**Insects were collected with sweep nets in areas of low growing vegetation

TABLE 7. TCDD ANALYSIS OF FISH NEAR HARDSTAND 7

Specimen	Sample Number	TCDD (ppt)	Detection Limit (ppt)	Remarks
Mosquito fish (<u>Gambusia</u>) Whole body	1	150		20 fish from HS-7 pond and 600 feet downstream
Sunfish (<u>Lepomis</u>) whole body	2	14		4 large fish (greater than 8 cm) from Beaver Pond
fillets*	3	ND**	5	8 large fish from Beaver Pond
livers	4	ND**	5	25 large fish from Beaver Pond
fish and livers	5	150		6 small fish (less than 4 cm) and 18 livers from HS-7 pond
livers and fat	6	740		12 medium (4 to 8 cm) fish from HS-7 pond

*Strips of flesh, skin, and scales from sides of fish.
**ND - Not Detected

SECTION V

DISCUSSION

1. DISTRIBUTION OF TCDD

The distribution of TCDD found on TA C-52A was widespread. TCDD was detected in virtually all sections of both grids, despite the fact that herbicides had not been used anywhere in the area for four years. The highest concentration found in a soil sample from TA C-52A (1,500 ppt) was on Grid 1, which had not been sprayed directly in 10 years.

Young's studies in 1974 indicated that there was little leaching of TCDD into the soil below the top few inches. Since TCDD is virtually insoluble in water, it is conceivable that this chemical could have been gradually forced under the top layer of soil by the mechanical action of water percolating through the soil. The higher concentrations found on the grids were in depressions with little to moderate amounts of organic material in the soil. Areas where water runoff settled generally showed higher concentrations of TCDD than the areas being drained. The mechanical effects of water movement obviously resulted in horizontal translocation of the TCDD. Samples of highly organic soils had little or no detectable TCDD. The samples of muck from Gator Pond had no detectable TCDD. Samples taken from East Pond had 19 ppt TCDD in a sample taken near the center of the pond bed and 27 ppt from an area of major drainage into the pond bed. Other samples that had no TCDD were generally from very sandy soil which obviously had been blown around. This sand may have been blown in from uncontaminated areas, or if previously contaminated, the TCDD adhering to the particles may have been dislodged or degraded by exposure.

Translocation of TCDD by the wind undoubtedly took place also. The large open area of TA C-52A was frequently quite windy, and blowing sand was often readily apparent. The movement of TCDD and the uncertainty of herbicide deposition levels made it difficult to attempt to map the TCDD concentration levels. Samples taken only a few feet apart showed wide variations in TCDD concentration.

The extremely high but localized concentrations of TCDD (up to 170,000 ppt) found around Hardstand 7 were surely the result of spillage. Some of the TCDD has been translocated down the slope behind the hardstand into the pond and stream. These aquatic habitats have a fine textured sand base of natural soil covered by a layer of loose organic material varying in depth from several inches to several feet, depending on the water current. Course grained fill dirt used to retard erosion of the hardstand could also be found, particularly in the pond directly below the hardstand. TCDD was probably still being introduced into the water through runoff. The limited sampling of silt in the water showed a maximum of 85 ppt TCDD. Also that TCDD had been transported several hundred yards downstream. Further sampling might reveal areas where the concentration is higher and found further downstream. In any event, it was obvious that TCDD was quite persistent in both areas studied.

2. DEGRADATION OF TCDD

The persistence of TCDD in the study areas is probably more important than the actual concentrations detected. High concentrations would be expected as a result of the previous large scale test program at Eglin Air Force Base.

Crosby (Reference 13) has demonstrated in the laboratory that TCDD can be broken down by ultraviolet light in the presence of organic solvents and that photodecomposition was negligible in aqueous suspension and on wet or dry soil. These results appeared to be confirmed by findings in the test area. Degradation appeared to be taking place in moist, highly organic soils exposed to direct sunlight. Initially, it was felt that light alone might have caused decomposition because TCDD was not detected in some of the sandy soil core samples. However, samples of loose sand collected with a spatula contained TCDD (up to 920 ppt).

Young (Reference 14) has estimated an 88-day half-life of TCDD in alkaline soils, under desert conditions in the presence of massive quantities of 2,4-D and 2,4,5-T. However, the conditions and results of those studies in Utah differ from those of TA C-52A. In addition to differences in climate, the soils on the test grids were quite acid, and the active ingredients of 2,4-D and 2,4,5-T have virtually disappeared. It may well be that initially the herbicides provide the organic solvents necessary (with light) to decompose TCDD, but with the disappearance of the herbicide solvents photodecomposition virtually stops.

Experimental soil samples exposed to direct sunlight and occasional heavy rain showed no significant TCDD degradation during the first nine weeks, even when tilled weekly to increase light exposure. Similar soil samples exposed for 23 weeks, both tilled and untilled, had TCDD degradation of approximately 50 percent. The increased rate of TCDD breakdown observed after the 9-week period probably is not indicative of the conditions existing in the natural study areas. The experimental soil had been aerated during the initial mixing, and the average temperature of the soil in the pots was higher than that of the subsurface soil in the study areas. These factors may have resulted in a gradual increase in microbial populations which accelerated biodegradation of the TCDD. Due to the probability of experimental errors and the insufficient number of analytic samples, the results of degradation studies were inconclusive (Figure 23). A larger number of sample data points and experimental controls are required before valid conclusions can be drawn.

In addition to the experimental soils exposed to direct sunlight, pots of soil from the grid containing approximately 1200 ppt TCDD were placed in a greenhouse. A commercial fertilizer dissolved in tap water was applied to these soils on an as-needed basis to keep them

References:

13. Crosby, D. G., et al, "Photodecomposition of Chlorinated Dibenzo-p-dioxins, *Science*, Vol 173, pp 748-749 (1971).
14. Young, A. L., Arnold, E. L., and Wachinski, A. M., Field Studies on the Soil Persistence and Movement of 2,4-D, 2,4,5-T, and TCDD. Presentation to the Weed Science Society of America, 13 February 1974, Las Vegas, Nevada, Abstract No. 226.

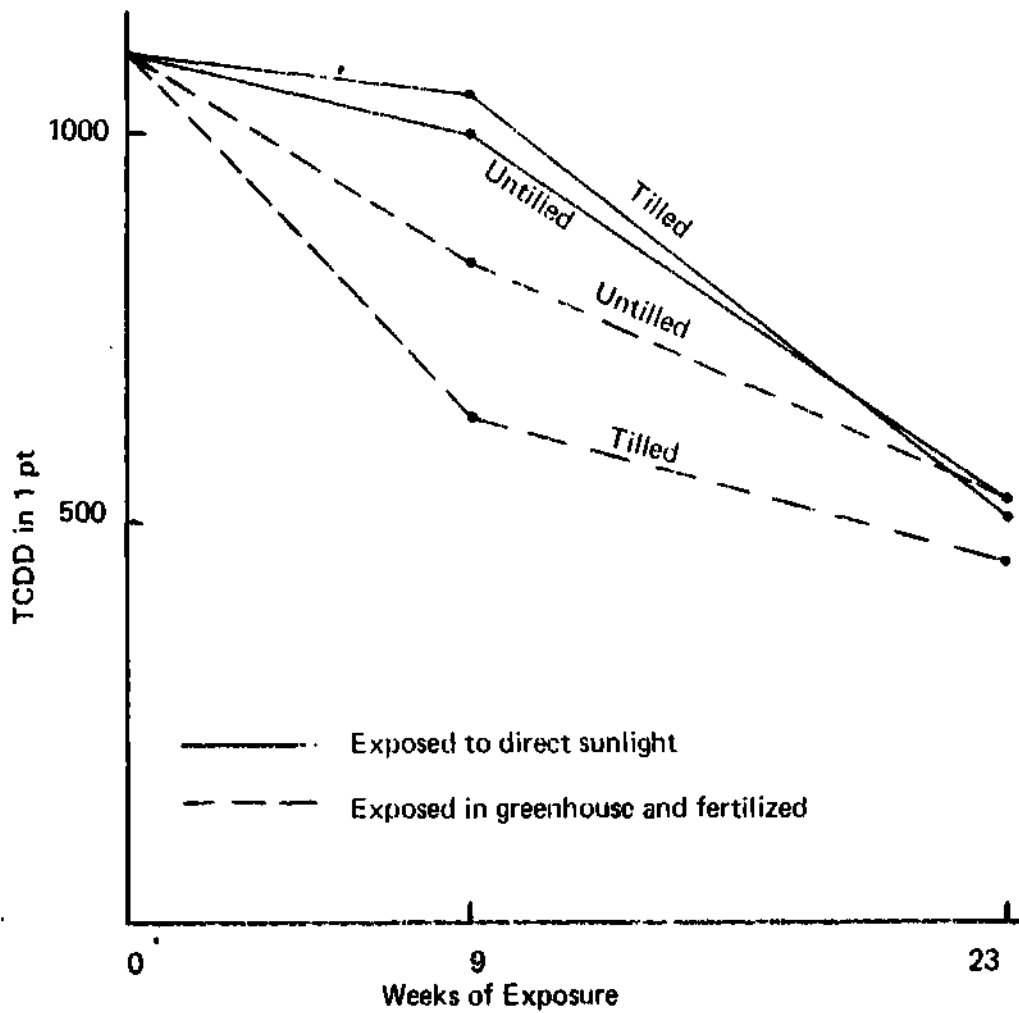


Figure 23. Degradation of TCDD in Soil Samples

Note: The average value of 1200 ppt (from four samples) was used as a starting point. Individual points plotted on chart represent samples taken from different pots.

moist but not enough to cause excessive leaching. After nine weeks, TCDD in the soil that was tilled weekly had been reduced by nearly one-half and in the undisturbed soil by approximately one-third. After 23 weeks, however, the results were not as impressive. The apparent failure of this technique to continue the rapid breakdown of TCDD may have been brought about by experimental error. TCDD may have been trapped in or leached into the soil in the bottom of the pots. The soil in the bottom of the pots was untilled and gradually compacted. TCDD in this portion of the soil was probably not affected by the treatment.

The exact mechanism of the degradation is not known, but it appears that the nutrient solution, together with light or aeration either caused a direct chemical breakdown of the TCDD or stimulated microbial proliferation which, in turn, accelerated degradation. Green algae or their byproducts, as well as bacteria, might have been involved. The soil in which TCDD apparently degraded most quickly was tilled and watered with a nutrient solution. This soil was normally covered with a green algal growth between tillings. The surface of the other tilled soils was barren, and the untilled soils had only a few weeds. The environment of the greenhouse may also have had an effect on these results. The temperature in the greenhouse averaged about 10 to 15 degrees Fahrenheit above outside air temperature, and ultraviolet light (both long and short wave) was 10 to 15 percent less than that of direct sunlight.

Further experimentation is warranted since it appears the simple use of fertilizer and/or tilling may expedite the degradation of TCDD in a strictly terrestrial environment.

3. BIOLOGICAL UPTAKE OF TCDD

Several animal species examined in this study were found to be contaminated with TCDD. These included birds, reptiles, fishes, and insects. Young (Reference 1) had previously reported contamination in mammals (rodents). Mammals analyzed in this study included deer, opossum, and rabbit. No TCDD was detected in these species. However, Reference 1 reported that a beach mouse (Peromyscus polionotus) liver sample from Grid 1 had a concentration of 540 ppt, and a sample from the large grid had 300 ppt. Cotton rats (Sigmodon hispidus) taken from the large grid in 1973 had 210 ppt in a liver sample. In an attempt to determine how these animals were being contaminated, seeds which constitute the primary diet of these rodents were analyzed and found to contain no TCDD. Young then speculated that the mice might be picking up soil containing TCDD on their fur, principally through contact with contaminated soil in the burrows. The TCDD adhering to the fur might be ingested with the animals groomed their fur.

The rabbit analyzed during this study had no detectable TCDD in the liver or on the pelt. The rabbit, like the rodents studied by Young, lived in a burrow. However, the burrow was on the edge of Gator Pond where no TCDD was detected in the soil samples. Another difference was that the rabbit food source was undoubtedly more leafy vegetation than seeds found on the ground.

The deer analyzed in this study had no detectable TCDD. Both of the deer collected from the grid were less than a year old. These animals, like rabbits, are browsers of vegetation but spend considerable time off the grid. The deer could possibly pick up TCDD on their fur when lying on the ground, but most of their resting was done in the surrounding forest. It

has been shown (Reference 15) that TCDD can be taken up by plants. These findings suggest that additional samples of grazing and browsing animals should be analyzed to make certain TCDD is not entering the food chain in this manner.

The opossum, an omnivore, was trapped off the grid and may not have been exposed to an area containing TCDD. Unsuccessful attempts were made to capture a carnivorous mammal on the grid. Carnivorous mammals frequently roamed across the grid, but it was not established that any actually resided on the grid.

Of the bird species analyzed for TCDD, two were breeding residents (meadowlark and mourning dove) and one was a winter resident (Savannah sparrow). The first sample of meadowlarks, taken early in the breeding season, had a liver concentration of 1,020 ppt. The nine birds, from which the sample was taken, were all collected along the east-west centerline of the large grid (rows E through H). This area had a relatively low TCDD content in soil samples. During the breeding season the range of birds probably did not exceed a few hundred yards. Birds that were flushed would simply move to another area within their nesting territory. Therefore, their feeding habitat was strictly confined, and water was available from the nearby ponds of the grid. Although it may be a minor contributing factor, the high concentration of TCDD accumulated in the liver tissue was not likely due to ingestion after preening of the feathers. Meadowlark bills are horny and smooth, and their habitat was not highly contaminated. Although some sand may have been present, none was observed in the stomachs of these birds. Meadowlarks are principally insectivorous. Therefore, in order to determine if their food was the source of TCDD, a sample of small insects was collected from the same area. This sample of several hundred small insects had 40 ppt TCDD. These insects were taken well after the meadowlark breeding season and may have been more contaminated earlier in the spring, particularly species that had just emerged from the ground.

In late summer, well after the breeding season was over and the birds were dispersed and not in specific territories, a single adult meadowlark collected had 200 ppt TCDD in the liver and a composite sample from two immature birds had 100 ppt. These birds had not been restricted to the immediate area as the birds during the breeding season had been. Since meadowlarks had only been seen irregularly in that area during the preceding few weeks, they must have been ranging over much larger areas. The young birds' stomachs and contents (insect remains) had a concentration of 46 ppt. It therefore appeared that a major source of TCDD was insects which had themselves become contaminated, probably through close contact with the soil. A sample of five large grasshoppers was also analyzed to see if they might be a potential source of TCDD for the protected Mississippi kites which were summer residents and sparrow hawks which were winter residents. The large grasshoppers collected in late summer had no detectable TCDD. Perhaps TCDD from the soil, which may have adhered to the exoskeleton of small young grasshoppers and other insects, might be removed during the numerous growth molts. On the other hand, the surface-to-volume ratio may account for the TCDD being detected in small insects and not in the large grasshoppers. Since only five grasshoppers were used in this sample, additional sampling is probably warranted. Determination as to which species of the small insects were contaminated would be useful information. However, an attempt to do this would be impractical until analytical procedures are developed that would permit the use of minute samples.

Reference:

15. Isensee, A.R. and Jones, G.E., Absorption and Translocation of Root and Foliage Applied 2,4-Dichlorophenol, 2,7-Dichlorodibenzo-p-dioxin, and 2,3,7,8-Tetrachlorodibenzo-p-dioxin, J. Agr. Food Chem., Vol 19, No. 6. (1971).

Mourning doves collected during the breeding season in the same area that the meadowlarks were collected had only 50 ppt TCDD in the composite liver sample. There are major differences in the habits of these two species which probably account for the lower TCDD content in doves. The doves ranged over a much larger area and are seed-eaters. It is possible that a small amount of the TCDD found in these birds could be attributed to preening habits, but the majority probably came from TCDD adhering to seeds or sand picked up with the seeds from the ground. A second sample of mourning doves was collected in the late fall. These birds were collected on the more intensely contaminated Grid 1, but since it was no longer the breeding season, the birds ranged over a larger territory. Seed from the crops of the doves had no detectable TCDD at a detection limit of 7 ppt, but the stomach and its seed contents had 10 ppt. Detectable traces of TCDD adhering to the seeds in the crop may have been washed into the stomach by the glandular secretions of the crop. TCDD in the livers of these doves was 58 ppt.

Twelve Savannah sparrows were collected within 30 days after they first migrated into the area. The stomach contents of these birds consisted of approximately 85 percent seed, 10 percent insect remains, and 5 percent sand by volume. Analysis revealed the stomachs and contents had a higher concentration of TCDD (84 ppt) than the livers (69 ppt) which probably reflected their recent arrival, or that these particular birds had just begun to feed on the grid.

A sample of two six-lined racerunners was analyzed and found to contain a whole body TCDD content of 430 ppt. This is a relatively high whole-body concentration as compared to other biological specimens analyzed from the test area. These two racerunners were taken from Grid 1 and the southern border of the large grid near Grid 1. An analysis of separate tissue types of this species should shed some light on the method of TCDD uptake, but it is not surprising to find a concentration like this since the reptiles were in a heavily contaminated area and probably never left it. TCDD probably adhered to the rough scales and was ingested in the insect diet.

A sample of 18 livers from small sunfish (Lepomis) was collected from the Hardstand 7 pond. The sample was not large enough to insure a low TCDD detection limit, so six whole fish from the same area were added to the sample. The composite whole body and liver concentration of TCDD was 150 ppt. The livers of medium sized (4 to 8 cm) sunfish from the same area had 740 ppt. Analysis of larger sunfish from the Beaver Pond yielded confusing results. Neither liver nor fillet (including flesh, skin, and scales) samples had detectable TCDD (detection limit 5 ppt). Whole body samples had 14 ppt. These three samples all were from different composite fish samples. Since the procedure and techniques used by Dow Chemical in the TCDD analysis had consistently been reliable and reproducible, experimental error was probably not the cause for this apparent anomaly. More likely causes were that one or more of the fish used in the whole body sample did have a trace of TCDD, or that TCDD was contained in other parts of the viscera or in the gills.

The mosquitofish (Gambusia) whole body sample contained 150 ppt TCDD. Ten of these fish came from the pond by the hardstand, and ten came from 600 yards downstream.

According to Zim (Reference 16), the diet of both of these fishes consists of insects, insect larvae, crustaceans, and small aquatic plants. The diet of the sunfish also include small fish. It is not known if the source of TCDD in these species of fishes were through the food chain or through contact with the water. Neither potential source was analyzed.

Reference:

16. Zim, H. S. and Shoemaker, H. H., Fishes, Golden Press, New York, 1955.

4. GENERAL COMMENTS

It should be remembered that this report is based largely upon field study and therefore reflects opinions of the authors.

The report should not be mis-construed as an indictment against the use of herbicides containing TCDD. Rather, it should serve as a basis for additional research; it suggests prudent use of these herbicides as well as long term monitoring of areas which receive repeated applications, particularly in areas of drainage and around aquatic habitats. The available evidence provides no positive proof that biologically deleterious effects have occurred in areas that have received even applications of these herbicides far in excess of normal practices. However, bioaccumulation of TCDD was obvious and some adverse effects were suspected.

The increase in numbers of bird species observed during the period of this study over that observed by Young in an earlier work has a simple explanation. The larger number of birds reported was due to more intense observation of this vertebrate class which resulted in the identification of more and different transients. Only the red-winged blackbird represents a potentially new breeding bird. On the other hand, the observations of the birds in the surrounding area in different habitats strongly suggest that there are no ill effects, at present, in the adjacent areas. The observed species of birds and other wildlife were generally representative of similar habitats in uncontaminated areas.

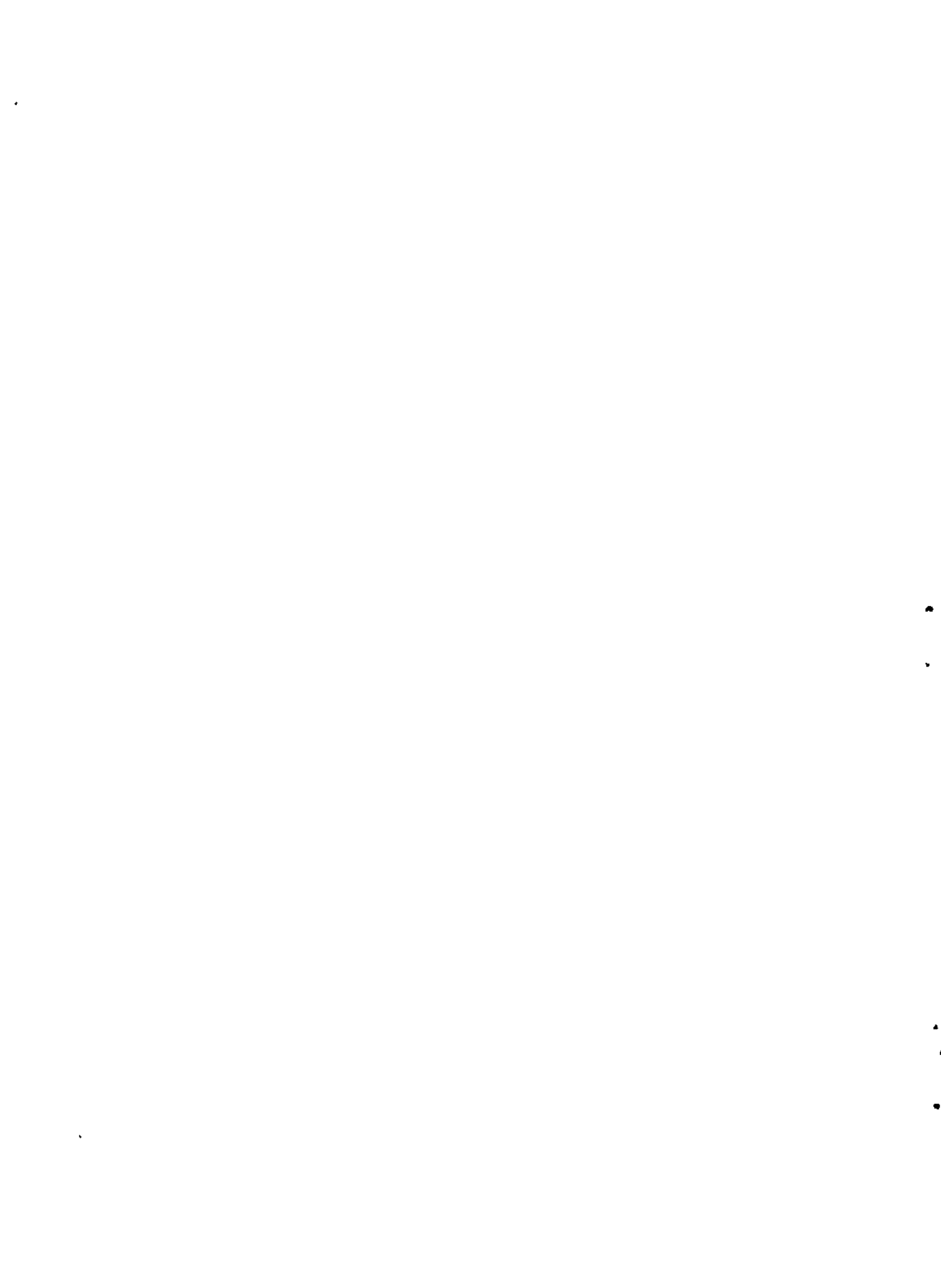
Despite the fact that the study areas had been highly contaminated with TCDD, the biological effects are apparently few and they are species dependent. On TA C-52A most of the wildlife is transitory. There appears to be no danger that any species would receive a lethal dose or concentration on a single or occasional day's exposure. The areas of highest TCDD concentration around Hardstand 7 could have adverse effects, but since the areas are so small there is little likelihood of significant wildlife poisoning. Concern should therefore be focused on species that remain in highly contaminated environments for significant periods of time. Specific examples are resident birds and rodents of TA C-52A and fish in the aquatic environment near Hardstand 7. It is unlikely that the rodents would receive the average daily dose rates of 125 - 1000 ppt necessary to be teratogenic. However, daily dose rates approaching 125 ppt could be experienced by the more voracious birds in highly contaminated areas. This could result in prenatal or postnatal lethality if adaptive mechanisms which are species related were unable to prevent liver or kidney dysfunction.

The primary areas of concern are the high concentrations in the livers of meadowlarks on the grid and fishes in the hardstand pond (as well as their predators). These birds and fishes were both in habitats of relatively low TCDD concentrations. The high concentrations in beach mouse livers is not disturbing, because they were from a heavily contaminated area and were not obviously ill-affected.

SECTION VI

CONCLUSIONS AND RECOMMENDATIONS

1. TCDD is very persistent and widespread on the grids of TA C-52A and has spread off the test grids to the immediately adjacent areas in low concentrations. TCDD was found in various kinds of wildlife.
2. TCDD contamination from the area around Hardstand 7 has entered the adjacent aquatic habitat and was found in fish. Investigation of the method of uptake by the fish and possible uptake by birds preying on the fish is recommended.
3. TCDD probably does not occur in high enough concentrations to cause teratogenesis in the wildlife, but may be present in sufficient quantities to cause a gradual liver dysfunction, in certain species. Analysis of single samples is necessary to determine the range of TCDD content, as opposed to mean values, in pooled biological specimens. The highly contaminated specimens could then be more closely evaluated for tolerance and possible harmful effects.
4. Bioaccumulation was demonstrated and it appeared that there may have been deleterious effects in selected species, in restricted and contaminated habitats. Ingestion of contaminated insects may be the major hazard to birds, such as the meadowlark, particularly during the breeding season when their range is restricted and the newly emerged insects are probably the most highly contaminated.
5. Wildlife in the areas adjacent to the locations of high contamination appear to have suffered no ill effects. Birds were obviously highly successful in their breeding in these adjacent areas. There was no reason to suspect harmful effects to wildlife transients, occasional visitors, or migrating birds.
6. The rate of degradation of TCDD in areas of high concentrations could probably be accelerated by increasing the fertility of the soil. It is not considered necessary or advisable to do this on the Eglin Reservation, however. The test range is a unique field laboratory that should be preserved for valuable research on the long term effects of TCDD. If further research reveals proven methods of decontamination of TCDD, then decontamination of Hardstand 7 might be considered. In the meantime it is advisable to restrict fishing in this habitat to prevent human consumption of the fish until all traces of the TCDD disappear.
7. The analytical procedures and techniques of Dow Chemical proved to be reliable and highly reproducible. Additional sources for TCDD analysis should be developed. Detection of TCDD, in the low ppt range, is required, and an increased capability to permit use of smaller samples sizes is desirable in order to facilitate studies on the long term environmental effects of TCDD.



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