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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

BEFORE THE ADMINISTRATOR

In re: The Dow Chemical Company, et al.

FIFRA DOCKET NOS.
415, et seq.

DIRECT TESTIMONY OF DR. ALVIN L. YOUNG ^{*/}

JUNE 1980

Prepared by:

ALVIN L. YOUNG, Ph.D.
Major, United States Air Force
Environmental Sciences Consultant
Epidemiology Division
USAF School of Aerospace Medicine
Aerospace Medical Division
Air Force Systems Command
Brooks Air Force Base
San Antonio, Texas 78235

SUBJECT OF TESTIMONY

Environmental Fate of 2,3,7,8-Tetrachlorodibenzo-p-dioxin
and 2,4,5-Trichlorophenoxyacetic acid

*/ USDA Exhibit No.

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DIRECT TESTIMONY OF DR. ALVIN L. YOUNG^{*/}

INTRODUCTION

My name is Alvin L. Young. I am a Major in the United States Air Force with a current assignment as an Environmental Sciences Consultant with the United States Air Force School of Aerospace Medicine, Aerospace Medical Division, Air Force Systems Command, Brooks Air Force Base, San Antonio, Texas. I am appearing at this United States Environmental Protecting Agency Hearing as a witness for the United States Department of Agriculture. The data I will present were obtained directly from my research while assigned with the United States Air Force. The Air Force provided me the funds and opportunities during the past ten years necessary to collect the data I will present. The data are presented in the interest of the public; however, the opinions and conclusions that I state in this testimony are mine and not necessarily those of the United States Air Force.

*/ USDA Exhibit No.

Qualifications as Expert Witness

My detailed educational and professional resume is attached. I hold the Bachelor of Science Degree in Agricultural Science and the Master of Science Degree in Crop Physiology. Both of these degrees were obtained from the University of Wyoming. My Doctor of Philosophy Degree was obtained in the speciality of Herbicide Physiology from Kansas State University (1968). My first assignment with the United States Air Force in 1968 was as a Project Scientist assigned to investigate the ecological impact of repetitive applications of phenoxy herbicides. For the past ten years I have continued to extensively research the fate of 2,4,5-T and TCDD in the environment under natural field conditions. I have also gained extensive teaching experience during these years having taught courses in three universities. The courses that I have taught include Ecology, Botany, Human Physiology, Medical Genetics, Plant and Animal Taxonomy, and Environmental Public Health. In my capacity as an Air Force expert on the environmental fate of TCDD, I have served as an advisor or consultant to the United States Environmental Protection Agency, the United States Department of Agriculture, the National Institute of Environmental Health Sciences, the Veterans Administration, the National Academy of Sciences, and the Federal Aviation Administration. In 1977, at the request of the Government of Italy, I spent five days in Seveso, Italy, participating in scientific dialogue with the Seveso Authority, a group of scientists appointed by the Italian Government to research the consequences of an accidental industrial release of TCDD in July 1976. I have most recently co-authored a book on "The Science of 2,4,5-T and Associated Phenoxy Herbicides."

Subject of Testimony

For my testimony I wish primarily to discuss the environmental fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). However, since most of my data on TCDD came from studies involving either the application or the spillage of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) herbicide formulations, I am also prepared to address some aspects of the environmental fate of 2,4,5-T.

Let me begin by proposing five questions that I believe are germane to the issues of this hearing:

1. What historical data are available on the concentration of the TCDD contaminant in formulations of 2,4,5-T herbicide?
2. Does TCDD persist in the field environment when sprayed as a contaminant of 2,4,5-T herbicide?
3. Does TCDD move in the abiotic (non-living) portion of the environment?
4. Does TCDD in the field environment bioaccumulate in biological organisms and/or biomagnify within food chains?
5. Do the levels of TCDD found in the field environment have adverse effects upon the organisms within that environment?

I wish to address each of the questions in sequence. Data to answer each question will be obtained from Air Force research projects for which I was either the principal investigator, a co-investigator, or a Project Officer.

Statements on the fate of TCDD in the environment are predicated on its detection in environmental substrates. Prior to 1973, the detection limit for

TCDD was between 0.5 and 1.0 parts per billion (ppb) for soils and 0.05 parts per million (ppm) for biological tissues. Theoretically, a one-pound-per-acre (1 lb/A or 1.1 kg/ha) application of 2,4,5-T containing 0.1 ppm TCDD applied directly to the soil could result in a maximum of 0.1 parts per trillion (ppt) in the top 6 inches (15 cm) of soil. The environmental monitoring of soils and food chains for buildup of TCDD would require a level of detection at least in the very low parts per trillion range. Obviously, because TCDD would be present in the environment only in minute levels following the normal field applications of 2,4,5-T or silvex, data on environmental fate would be very difficult to obtain. Highly sophisticated instrumentation and elaborate sample clean-up techniques were required in order to obtain the detection of such picogram (⁻¹²10 grams) quantities of TCDD. Dr. Michael Gross has previously testified to problems associated with the detection and confirmation of TCDD in the analytical laboratory. Consequently, only a few laboratories have been available to support the analytical requirements of both an extensive ecological program and a complex research program on methods to dispose of military surpluses of phenoxy herbicides. The TCDD analyses reported in my exhibits and in this testimony were obtained through Air Force contracts for analytical services from the following laboratories: The Interpretive Analytical Services, Dow Chemical Company, Midland, Michigan (1972-75); The Aerospace Research Laboratories, Wright-Patterson AFB, Ohio (1972 - 74); The Brehm Laboratory, Wright State University, Dayton, Ohio (1975-77); The Midwest Center for Mass Spectrometry, Department of Chemistry, University of Nebraska, Lincoln, Nebraska (1977-80); and The Flammability Research Center, University of Utah, Salt Lake City, Utah (1977-79).

Nature of the Research and Historical Prospective

I feel it is important to place into perspective the nature of the research that I have been involved in. My studies have centered on three major projects involving the phenoxy herbicides and TCDD. These projects are:

- a. Studies of the ecology of a unique (one of a kind) military test site that aerially received massive quantities of phenoxy herbicides.
- b. Development of ecologically safe and economically feasible methods of disposing of surplus phenoxy herbicides contaminated with TCDD.
- c. Residue monitoring of sites contaminated with phenoxy herbicides and TCDD as a consequence of the long-term storage of large quantities of herbicides.

All three projects have involved situations not typical of the normal use of 2,4,5-T. Indeed, each situation has involved residue levels hundreds and thousands of times greater than those encountered in the normal use of 2,4,5-T (and 2,4-D) herbicide. Yet, because of the nature of the situation (e.g., herbicide spill sites), these studies have provided an opportunity to evaluate the consequence of long-term exposure to a variety of organisms to readily detectable levels of residue, especially 2,3,7,8-TCDD.

Before commenting on the results from each of the above projects, I would like to discuss the herbicides used and the historical background involved in these projects. In 1961, the Office of the Secretary of Defense, Washington DC, requested the Departments of Army and Air Force to determine the technical feasibility of defoliating jungle vegetation in the Republic of Vietnam (see Technical Report OEHL-TR-78-92). Eighteen different aerial spray tests were conducted with various formulations of commercially available herbicides. The primary herbicides selected for use as defoliants in the heavy jungles of Vietnam were the butyl ester formulations of 2,4-D and 2,4,5-T. The choice was

based upon the facts that these chemicals had had considerable research, proven performance, were available in large quantities at reasonable cost, and had known or proven safety in regard to their toxicity to humans and animals. Remember, that in 1961, the chemical and toxicological evaluations conducted on these materials were not of the standards we accept today, but they were at the standards accepted at that time. The project responsible for aerially disseminating the herbicide in Vietnam was called Operation RANCH HAND. The principle equipment used for this project was the Air Force C-123 cargo aircraft with a 1,000 gallon spray tank installed and configured with spray booms under each wing and behind the cargo door. The major military herbicide from 1962-1965 was called Herbicide Purple. The formulation was a brown liquid soluble in diesel fuel but insoluble in water. One gallon of Purple contained 8.6 pounds of the active ingredient 2,4-D and 2,4,5-T. The percentages of the formulation were:

n-butyl ester of 2,4-D 50%

n-butyl ester of 2,4,5-T 30%

iso-butyl ester of 2,4,5-T 20%

Beginning in 1965 and through April 1970, the major military herbicide used in Vietnam was called Herbicide Orange. It was similar to Purple in color, solubility and pounds active ingredient. However, the percentages of the formulation were:

n-butyl ester of 2,4-D 50%

n-butyl ester of 2,4,5-T 50%

All of the spray equipment for RANCH HAND was developed, tested and evaluated at Eglin Air Force Base (AFB), Florida. The early test programs (1962-1964) involved spraying Herbicide Purple; the later programs (1964-1970) involved

spraying Herbicide Orange. I initiated research on the ecology of this unique spray-equipment test area (Test Area C-52A) at Eglin AFB in January 1969.

Following the termination of spraying Herbicide Orange in South Vietnam in April 1970, the Department of Air Force was tasked with the disposition of the surplus stocks. Those stocks (approximately 1.37 million gallons) remaining in South Vietnam were re-drummed as required and transferred to Johnston Island in April 1972. The remaining surplus of 0.85 million gallons, which had not been shipped to Vietnam, remained in storage at the Naval Construction Battalion Center (NCBC), Gulfport, Mississippi. When the herbicide was removed from these two storage sites in 1977, I was tasked the responsibility of conducting residue monitoring studies.

During the years from 1970 to 1977, the Air Force evaluated numerous options and methods for the final disposition of Herbicide Orange. One method that I evaluated was called soil biodegradation and involved incorporating massive concentrations of the herbicide into soil. These studies took place in Utah, Kansas and Florida, and began in the spring of 1972. I will provide additional details on these and the other two projects in subsequent sections of this testimony.

It should be apparent that it became necessary to identify and characterize the various stocks (lots) of herbicides used in all of the above projects. Unfortunately, the surplus returned to Johnston Island was not identifiable by manufacturer, Transportation Control Number (TCN) or even date of shipment to Vietnam. Thus, a scheme to sample the more than 25,000 55-gallon drums was based upon a randomized design. The surplus herbicide at NCBC could be characterized by Manufacturer's Lot and TCN. Thus it was possible to evaluate the

composition of product from many of the manufacturers who provided Herbicide Orange for the Department of Defense. Table 1 provides a more detailed analysis of the mean composition of Herbicide Orange. Table 2 provides data on the levels of TCDD found in the various lots produced by different manufacturers, and stored at NCBC. Table 3 provides a summation of the TCDD concentrations in all the available samples prior to the at-sea incineration of the stocks in the summer of 1977 (Project PACER HO). As noted in Table 3, only one sample of Herbicide Purple has been analyzed. The age of the sample was not known except that it was from a drum of herbicide that was sprayed on Test Area C-52A during the 1962-1964 period. When the Orange Herbicide was shipped to Johnston Island from South Vietnam redrumming of the herbicide in South Vietnam was accomplished as necessary. The project (PACER IVY) involved U.S. military personnel. One of the individuals participating in the redrumming operation at Da Nang (redrumming also occurred at Phu Cat and Bien Hoa) has stated that drums of Purple were found (although fewer than 20) and redrummed into Orange-banded drums (personal communication, Dr. Michael D. Neptune, now with the U.S. Environmental Protection Agency, Washington, DC). In addition, an analytical chemist involved in the analyses of Orange samples for 2,4-D and 2,4,5-T, reported finding significant quantities (15 percent) of the iso-butyl ester 2,4,5-T in a few of the samples collected from Johnston Island (unpublished data, personal communication, Dr. Eugene L. Arnold, then with the Clinical Sciences Division, USAF School of Aerospace Medicine, Brooks AFB, Texas). Thus, the 4 samples of Orange Herbicide containing TCDD concentrations greater than 15 ppm, may have been Purple. If these were, in fact, from drums of Purple, then the mean concentration of TCDD in 5 samples of Purple would have been 32.8 ppm.

TABLE 1. Composition, Percent, of Selected Samples of Herbicide Orange in Relation to Military Specifications*

Component	NCBC Inventory Number ^a			Mean Composition	Approximate Military Specification ^b
	ASN 8	ASN 10	ASN 14		
Number of Gallons	123,695	383,955	145,860		
Level of TCDD	<0.02 ppm	0.30 \pm 0.06 ppm	<0.02 ppm		
n-Butyl ester 2,4-D	42.6%	46.2%	43.7%	44.2%	49.5%
n-Butyl ester 2,4,5-T	39.3	44.9	42.2	42.1	48.8
Other Butyl esters of chlorophenoxyacetic acids	7.96	4.01	9.05	7.0	-
Octyl esters of chlorophenoxyacetic acids	5.76	0.25	-	2.0	-
Acid, 2,4-D	0.78	0.19	0.65	0.5	0.1
Acid, 2,4,5-T	0.84	0.13	0.78	0.6	1.0
Inert Ingredients ^c	2.76	4.32	3.62	3.6	0.6

^aSelected samples of Herbicide Orange were collected from the surplus inventory maintained at the Naval Construction Battalion Center (NCBC), Gulfport, Mississippi. Samples represented lots produced by different manufacturers. Analyses for TCDD and sample composition were performed by the Aerospace Research Laboratories, Wright-Patterson AFB, Ohio. [See Technical Report ARL-TR-75-0010]

^bMilitary specifications for manufacture of Herbicide Orange were based on Specifications MIL-H-51147A (MU) and MIL-H-51148A (MU) dated 7 Nov 1966.

^cInert ingredients included butanol, toluene, butylchloride, dichlorophenol, trichlorophenol, butoxydichlorobenzene, and butoxytrichlorobenzene.

*Source: Technical Report OEHL-TR-78-92.

TABLE 2. Identification Data on Herbicide Orange Stocks Stored at the Naval Construction Battalion Center, Gulfport MS^a

Manufacturer	Transportation Control No. (TCN)	b Analysis Sequence No.	Total Number of Drums with Same TCN	*TCDD ^c (ppm)
Hercules Co	9464 8156 0001	8	500	<0.05
Hercules Co	9464 8192 001	14	2,152	NA ^d
Diamond Co	FY9461 7165 0001AA	18	60	14.2 ^e
Diamond Co	FY9461 8156 001AA	11	421	8.62 ^f
Thompson Hayward Co	9463 8155 X032	1	1,546	0.32
Dow Chemical Co	9463 8155 X052	10	6,976	0.12
Thompson Co	9463 7184 X011	3	46	NA
Thompson Co	9463 8155 X012	5	808	0.17
Monsanto Co	FY9463 7163 X0001XX	4	563	NA
Monsanto Co	FY9463 8183 X002XX	6	<u>2,185</u> 15,257	7.62

^aSource: Fee, et al. Technical Report ARL-TR-75-0010 Reprinted with modifications in OEHL-TR-79-169.

^bEach separate purchase of herbicide was designated by a separate TCN

^cTetrachlorodibenzo-p-dioxin (TCDD) content. Results reported in this column are the average of six samples collected from six different barrels of Herbicide Orange having the same TCN.

^dNot Analyzed.

^eAverage value of five samples: 12, 17, 12, 15, 15. Other sample value was 0.07 with rechecks.

^fAverage value of four samples: 8.0, 8.1, 8.7, and 9.7. Other two samples each averaged <0.05 with rechecks.

*On the basis of 280 samples of Herbicide Orange taken from the Gulfport inventory, the weighted mean concentration of TCDD was 2.06 ppm.

TABLE 3. Concentration, ppm, of TCDD in samples of Herbicides Orange and Purple ^{a*}

Source of Samples	Number of Samples		Range of TCDD (ppm)	Mean TCDD, Concentration (ppm)
	<u>Orange</u>	<u>Purple</u>		
Johnston Island Inventory, 1972 ^b	200	(4) ^c	0.05-47	1.91
Johnston Island Inventory, 1974	10		0.07-5.3	1.68
NCBC, Gulfport Inventory, 1972 ^d	42		0.05-13.3	1.77
NCBC, Gulfport Inventory, 1975	238		0.02-15	2.11
Eglin AFB Archived Sample		1 ^e	-	45
Eglin AFB Inventory, 1972	2		-	0.04
The Weighted Mean Concentration of TCDD in Orange = 1.98 ppm				

^aAnalyses for TCDD performed by Interpretive Analytical Services, Dow Chemical U.S.A., Midland Michigan; Aerospace Research Laboratories, Wright-Patterson AFB, Ohio; and The Brehm Laboratory, Wright State University, Dayton Ohio.

^bSurplus Herbicide Orange was shipped from South Vietnam to Johnston Island for storage in April 1972.

^cFour of 200 samples may have been Herbicide Purple.

^dThe Naval Construction Battalion Center (NCBC) Gulfport, Mississippi served as a storage site for Surplus Herbicide Orange from 1969 to 1977.

^eHerbicide Purple was extensively used in the evaluation of aerial spray equipment on Test Area C-52, Eglin Air Force Base Reservation, Florida, 1962-1964.

*Source: Technical Report OEHL-TR-78-92

It can be concluded with reasonable certainty that the weighted mean concentration for all of the Herbicide Orange remaining after Vietnam (including the inventory on Johnston Island) was 1.98 ppm. Individual lots may have contained higher (≥ 15 ppm) or lower (≤ 0.02 ppm) concentrations of TCDD, but the weighted mean was 1.98 ppm.

The first question I posed on page 1 of this testimony was:

"What historical data are available on the concentration of the TCDD contaminant in formulations of 2,4,5-T?"

From the previous tables and discussion we can conclude:

a. Some samples of 2,4,5-T produced in either the late 1950's or very early 1960's were significantly contaminated with TCDD. One drum of Purple contained 45 ppm TCDD. The 2,4,5-T used in that formulation must have contained 90 ppm TCDD.

b. Samples of 2,4,5-T produced after 1964 contained much lower levels of TCDD. Drums of Herbicide Orange stored at NCBC, Gulfport, Mississippi, were found to contain levels of TCDD from <0.02 to 15 ppm. The 2,4,5-T used in these formulations must have contained <0.04 to 30 ppm TCDD.

c. The concentration of TCDD varied between and within the Lots of Herbicide Orange procured from the various chemical companies contracted to produce it.

d. The mean weighted concentration of TCDD in 2.3 million gallons of Herbicide Orange was approximately 2 ppm TCDD. These data were based upon the analyses of 488 drums, selected for analyses so as to permit inferences about the entire inventory.

Eglin AFB, Florida Ecological Studies

Laboratory data for rodents strongly suggest a correlation between histological lesions in the liver and lymphatic system and the amount of TCDD ingested. Unfortunately, data relating to any actual effects on wild populations in their natural habitat are lacking. The problem of finding a field site where a wild population of rodents has been exposed to significant quantities of TCDD is improbable because of (1) low levels of TCDD (<0.1 ppm) found in currently produced phenoxy herbicide, and (2) low rates of 2,4,5-T applied for brush control on rangelands or for reforestation (1.1 to 2.2 kg/ha).

The Eglin Reservation has served various military uses, one of them having been development and testing of aerial dissemination equipment in support of military defoliation operations in Southeast Asia. It was necessary for this equipment to be tested under controlled situations that would simulate actual use conditions as near as possible. For this purpose an elaborate testing installation, designed to measure deposition parameters, was established on the Eglin Reservation with the place of direct aerial application restricted to an area of approximately 3.0 km² within Test Area C-52A in the southeastern part of the reservation. Massive quantities of herbicide, used in the testing of aerial defoliation spray equipment from 1962 through 1970, were released and fell within the instrumented test area. The uniqueness of the area has promoted continued ecological surveys since 1967. As a result, few ecosystems have been so well studied and documented.

Description of Field - Test Area C-52A (TA C-52A) covers an area approximately eight square kilometers and is a grassy plain surrounded by a forest stand that is dominated by longleaf pine (Pinus palustris), sand pine (Pinus clausa), and turkey oak (Quercus laevis). The actual area for test

operations occupies an area of approximately three square kilometers and is a cleared area occupied mainly by broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), woolly panicum (Panicum lanuginosum) and low growing grasses and herbs. Much of the center of the range was established prior to 1960, but the open range as it presently exists was developed in 1961 and 1962. The test grid is approximately 28 m above sea level with a water table of six to ten feet. The major portion of this test area is drained by five small creeks whose flow rates are influenced by an average rainfall of 150 cm. The mean annual temperature for the test area is 19.7°C while the mean annual relative humidity is 70.8 percent. For the most part, the soil of the test grid is a fine white sand on the surface, changing to yellow beneath. The soils of the range are predominantly well drained, acid sands of the Lakeland Association with 0 to 3 percent slope. A typical three-foot soil core contained approximately 92 percent sand, 3.8 percent silt, and 4.2 percent clay with an organic matter content of 0.17 percent, an average pH of 5.6, and a cation exchange capacity of 0.8.

Although the total area for testing aerial dissemination equipment was approximately 3.0 km², the area actually consisted of four separate testing grids. The primary area was located in the southern portion of the testing area and consisted of 37 ha instrumented grid. This was the first sampling grid and was in operation in June 1962. It consisted of four intersecting straight lines in a circular pattern, each being at a 40° angle from those adjacent to it. Although this grid was discontinued after two years it received the most intense testing program. From 1962 to 1964, this grid (called Grid I) received 39,550 kg 2,4-D and 39,550 kg of 2,4,5-T as the Herbicide Purple formulation. Two other testing grids were sprayed with Herbicide Orange. Grid II was an area of 37 ha and located immediately north of Grid I. Grid II received 15,890 kg 2,4,5-T

from 1964 through 1966. Grid IV was the latest and final Grid established on Test Area C-52A. It was approximately 97 ha and received 17,570 kg 2,4,5-T from 1968 through 1970. These data are presented in Table 4. Figure 1 is an aerial photograph of the Test Area in 1971, one year after the last application of herbicide.

Vegetation Studies - The first extensive ecological studies of Test Area C-52A were on the vegetation. Numerous technical reports have been written on the early studies and are summarized in exhibits 5 and 11 (Technical Reports AFATL-TR-74-12 and AFATL-TR-75-142). Photographic records of numerous sites on the Test Area have been maintained through the years of the study. Figure 2 shows the vegetative succession that has occurred between May 1964 and June 1974 on Grid 2, the grid receiving massive amounts of 2,4,5-T during 1962-1964.

Detailed studies of the vegetative composition of the Test Area were initiated in June 1971, nine months after the last defoliant-equipment test mission. The Test Area was divided into a grid of 169 sections (each 122 by 122m), and within each section the percentage vegetative coverage was visually ranked as Class 0, 0-5%; I, 5-20%; II, 20-40%; III, 40-60%; IV, 60-80%; and V, 80-100%. Three sections within each class were selected at random and surveyed for dicotyledonous plants. An unsprayed area 0.2 miles northwest of the test areas was again surveyed, but in addition, a square-foot (0.093m^2) analysis technique was performed in 15 additional sections. These sections were randomly selected and within each section, nine areas, each 0.093m^2 , were analyzed for species composition and ground cover density. Both methods of vegetative survey were repeated in June 1976. The number of dicotyledonous species increased from 74 in 1971 to 107 in 1973, and to 123 in 1976. In 1971,

Table 4. Approximate amount of 2,4,5-T and estimated amount of TCDD applied to Test Area C-52A, Eglin AFB Reservation, Florida, 1962-1970

Test Grid	Grid Area (ha)	2,4,5-T ^a (kg)	TCDD ^b (kg)
1	37.25	39,550 (1962-1964) ^c	2.613
2	37.25	15,890 (1964-1966)	0.078
4	97.0	17,570 (1968-1970)	0.087
Total	171.5	73,010	2.778

a
Amount of 2,4,5-T calculated on weight of active ingredient 2,4,5-T in the military herbicides Orange or Purple.

b
Amount of TCDD calculated from data on mean concentration of TCDD in the formulation of Herbicides Purple or Orange, i.e., 32.8 ppm TCDD in Purple and 1.98 ppm TCDD in Orange.

c
Years when the specific grid received the herbicide/TCDD.

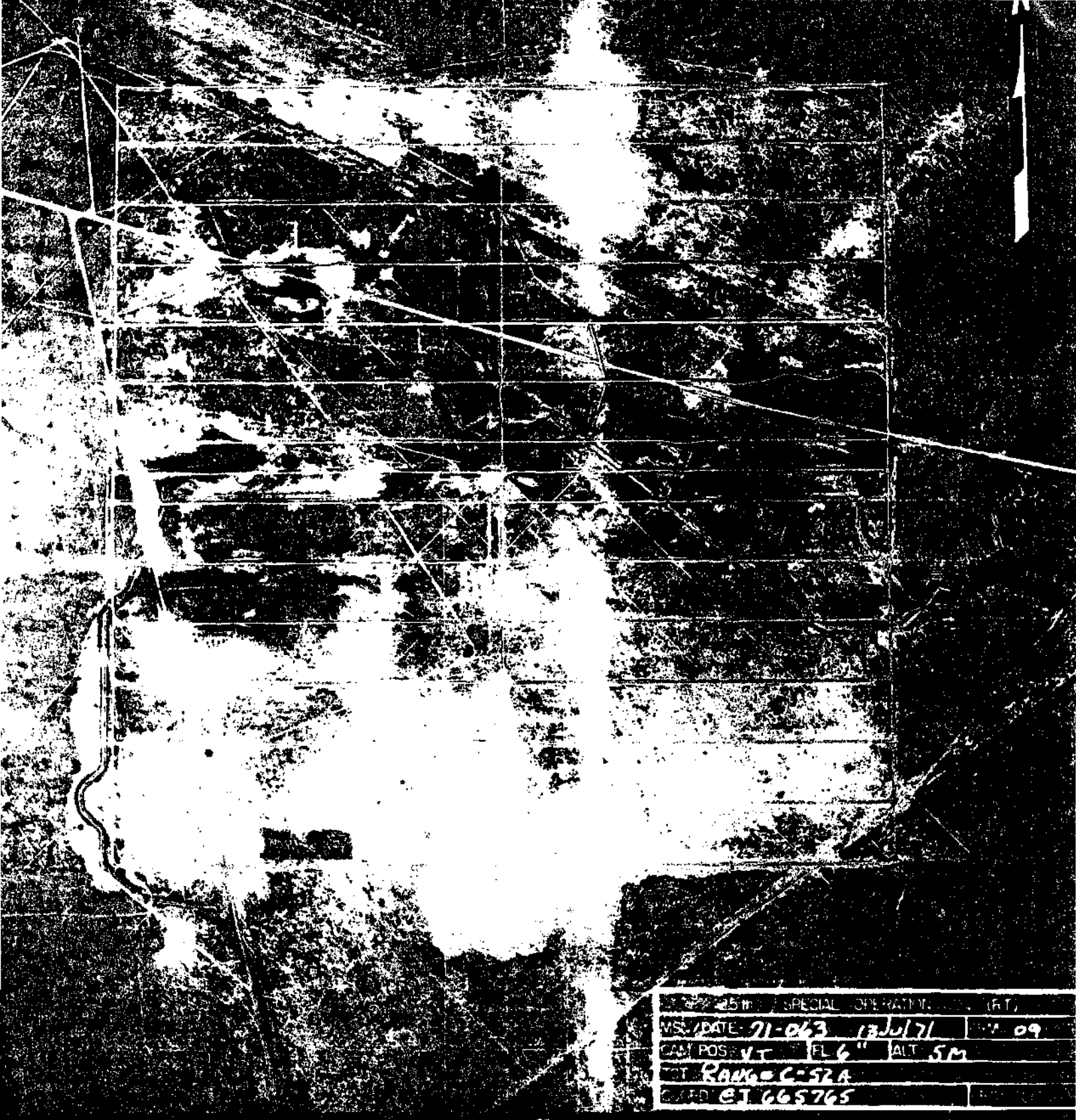
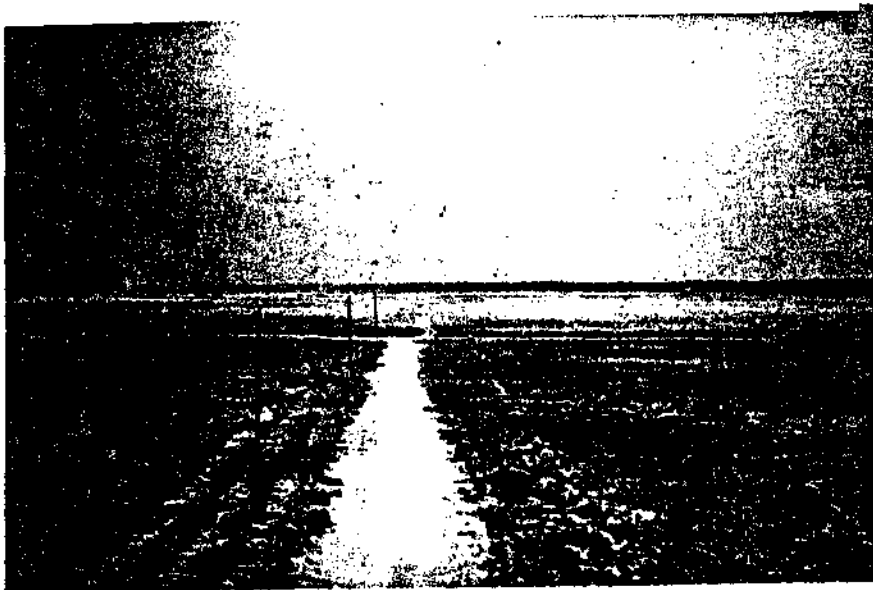


Figure 1. Photograph of the 3 km² spray-equipment testing complex on Test Area C-52A, Eglin AFB, Florida. Photograph taken at 1.7 km above ground level on 13 July 1971.



Figure 2. Vegetative succession on Grid I, Test Area C-52A, Eglin AFB, Florida. All photographs taken from the same location.

Grid 1, April 1969



Grid 1, June 1974



20% of the test area had less than 20% vegetative cover, while 26% of the test area had more than 60% vegetative cover (see Figure 3). In 1976, no sections had less than 20% vegetative cover, but over 73% of the test area had a cover of more than 60% (see Figure 4). The major grass species were Panicum virgatum and Panicum lanuginosum. The major dicotyledon was Diodia teres in 1971, but was replaced by Chrysopsis graminifolia in 1976. The data demonstrate the rapid invasion of dicotyledonous species despite the unusually heavy applications of phenoxy herbicides.

Soil Residue Studies - The first residue studies of Test Area C-52A involved analyses of soils for phenoxy herbicides by both chemical and bioassay techniques. Exhibit 5 (Technical Report AFATL-TR-74-12) provides the details of these complex studies. The problem we encountered in the residue studies centered on the heterogeneity of the test girds. Not only were there small geologic differences (soil types, contours, organic matter and pH), and differences in vegetation density and locations of water, but most important the herbicides had been sprayed on specific test arrays (i.e., along dictated flight paths) over a span of eight years. By considering the flight paths, the water sources and the terracing effects, it was possible to divide the test area into fifteen vegetation areas. These areas formed the basis for the random selection of 48 soil cores, plus three additional cores collected from a control site. The distribution of the core sample is shown in Figure 5. Sampling at each site consisted of removing 15 cm increments (6 inches) of soil to a total depth of 90 cm (3 feet). Each increment from each of the 51 cores was bioassayed used soybeans and cucumbers. Many were analyzed by gas chromatography. The initial experiment was conducted in April 1970. Selected sites were re-sampled in December 1970. Figure 6 illustrates the results we obtained for selected sites.

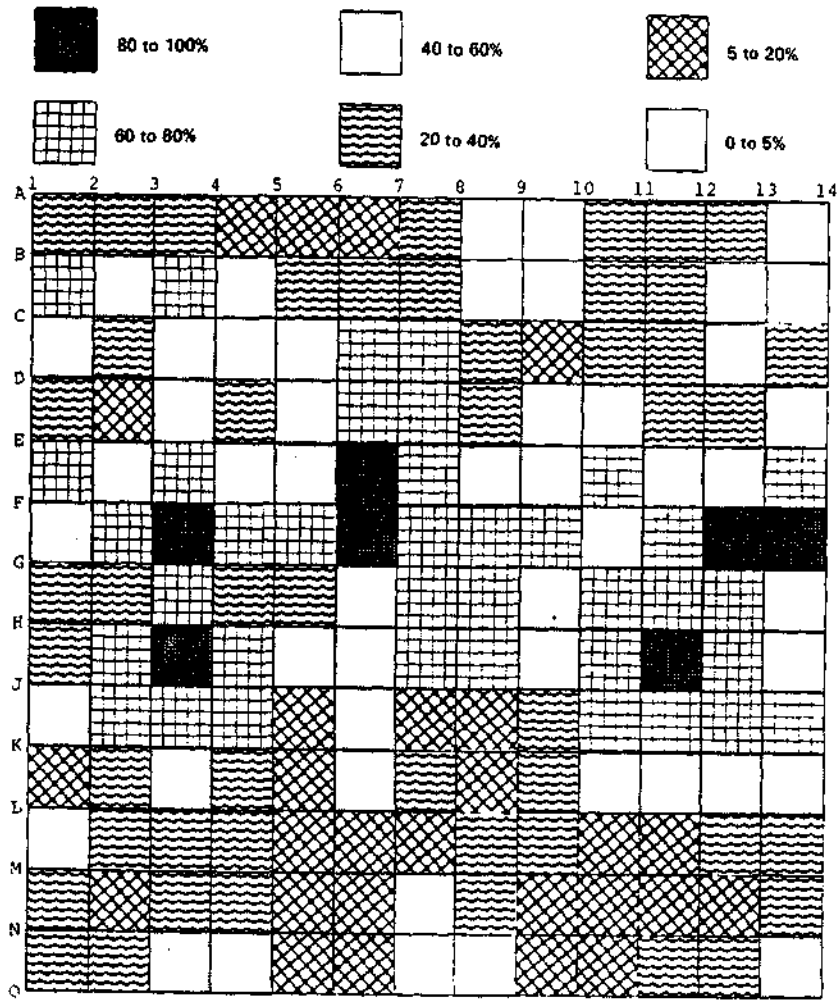


Figure 3. The 1971 density map of vegetative cover for the 3.0 km² Spray-equipment test site, Test Area C-52A, Eglin AFB, Florida.

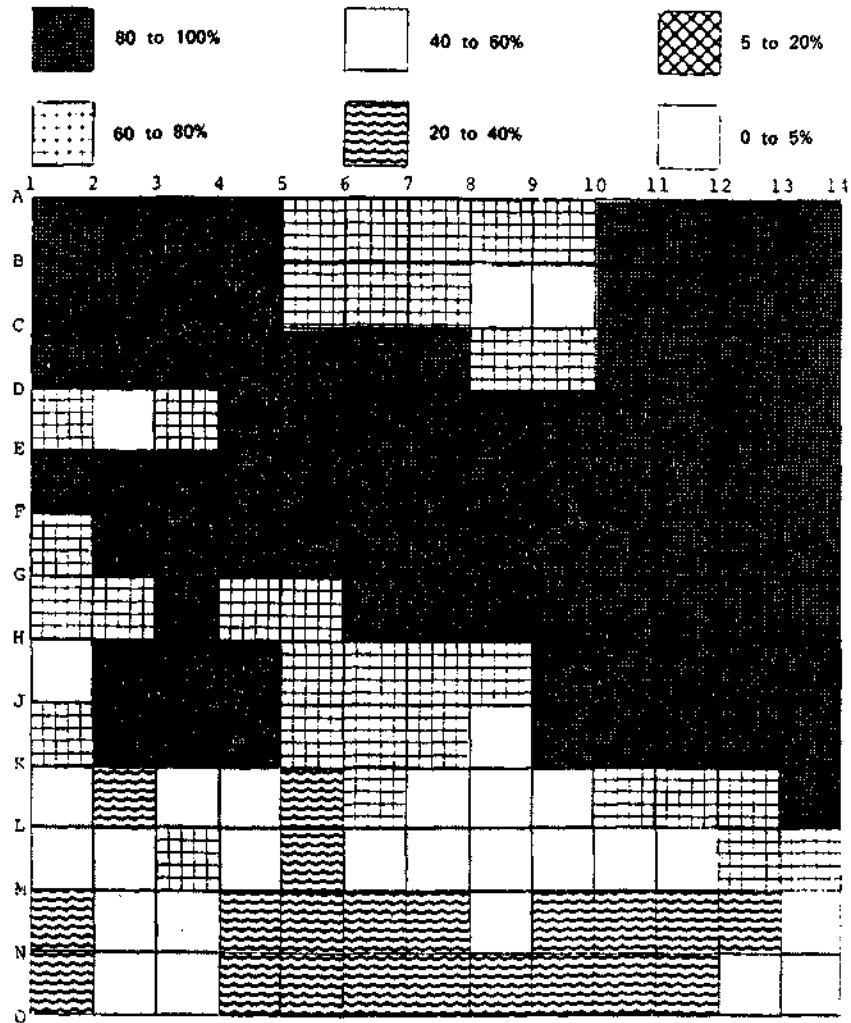


Figure 4. The 1976 density map of vegetative cover for the 3.0 km² Spray-equipment test site, Test Area C-52A, Eglin AFB, Florida.

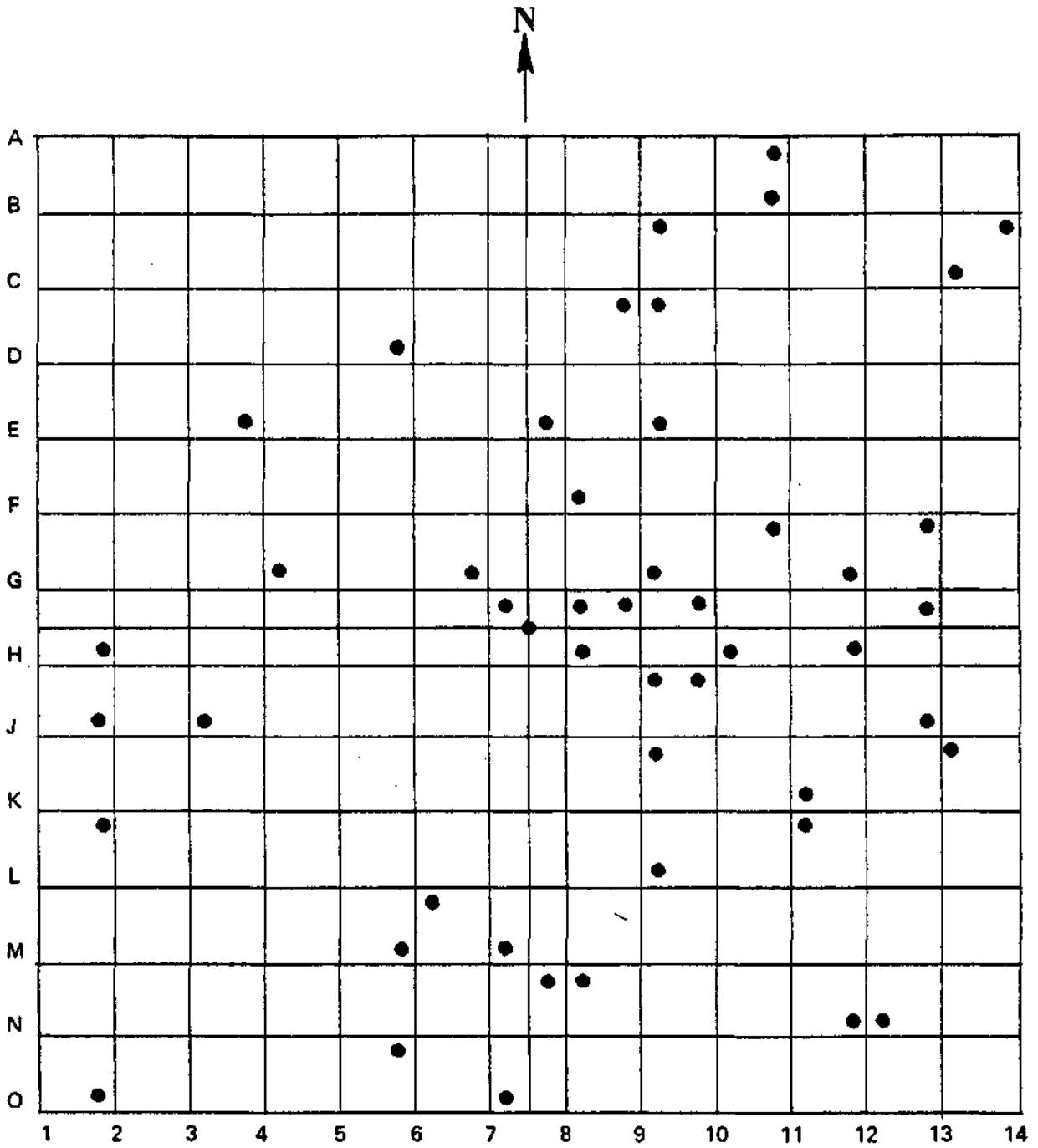
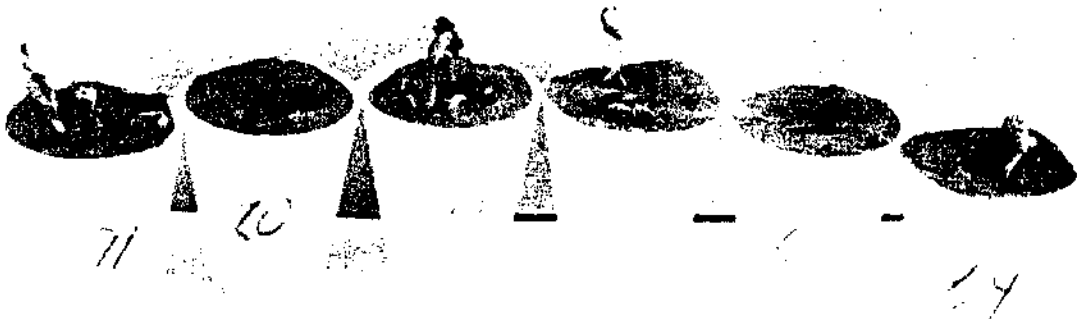


Figure 5. The 1970 sample sites for the collection of 90 cm (3 foot) cores for bioassay of Phenoxy Herbicides on the 3 km² Test Area C-52A.

DAY 3 TREATMENT
14 DAY BIOASSAY
AREA 13
APR. 70



SOIL CORE C-9
AREA 13
DEC. 70



Figure 6. Bioassay of 15 cm increments of soil cores collected in April and December 1970 from Test Area C-52A. Depth of increments within the core decreases from left to right.

Samples collected in April 1970 had levels of 2,4-D and 2,4,5-T in the 1 to 10 ppm throughout most of the soil profile. Samples from the same site in December 1970 had levels between 7 and 20 parts per billion (ppb). These data demonstrate the rapid disappearance (degradation) of the phenoxy herbicides despite the massive quantities repetitively applied to this test area. Indeed, the residue and vegetative studies are highly complementary. As residue levels of herbicides rapidly dropped, a rapid invasion of dicotyledonous plant species occurred.

What about the dioxin residues? In the spring of 1971, we selected soil cores that had had high levels of herbicide residues in them as indicated by the bioassay studies. These cores were collected from Grid 4, an area sprayed with Herbicide Orange from 1968 to early 1970. The samples were analyzed by the USDA Pesticide Degradation Laboratory, Beltsville, Maryland, and found to be negative at a detection limit of 0.0005 ppm TCDD. No additional samples were collected until 1973. Data from studies conducted by other researchers suggested that TCDD does persist in the soil. We critically reviewed all our data and the history of the test range. It became obvious that if the herbicides produced in the late 1950s and early 1960s were more contaminated with TCDD than materials produced later in 1960s, the site we should search would be Grid 1. Simultaneously the detection limit for TCDD was now approaching the level of parts per trillion (ppt). Table 5 is from Technical Report AFATL-TR-74-15 (Exhibit 5) and shows the first positive analysis of TCDD on the test area. The data in the table suggested soil penetration of TCDD. Hence, the following summer (1974), this site on Grid was re-sampled. The data (from Technical Report OEHL-TR-78-92, Exhibit 7) in Table 6 show that TCDD was confined to the top 15 cm (6 inches) and that sampling procedures accounted for the previous results showing contamination below 15 cm. Over the next few years, numerous soil samples were collected from each of the test grids that had received 2,4,5-T

Table 5. TCDD Results published in Exhibit 5.

TABLE II-10. LEVELS (PARTS PER TRILLION) OF 2,3,7,8 TETRACHLORODIBENZO- p-DIOXIN (TCDD) IN SOIL FROM TA C-52A COLLECTED IN JUNE OR OCTOBER 1973 ^a					
DEPTH, Inches	LOCATION				
	C-9	F-7	O-7	GRID 1	CONTROL
0-6	<10 ^b	11	30	710	<20 ^c
6-12	ND	ND	<10	140	<10
12-18	ND	ND	<10	72	<10
18-24	ND	ND	<10	<10	<10
24-30	ND	ND	<10	<10	<10
30-36	ND	ND	<10	<10	<10

^aMethod described in text.
^bLower limit of detection in parts per trillion TCDD.
^cProbable interference from excessive organic matter.

TABLE 6. Concentration of TCDD in soil profile of Grid 1, Test Area C-52A, Eglin AFB, Florida.^a

Depth of (inch)	Parts per Trillion (ppt) TCDD
1	150
1 - 2	160
2 - 4	700
4 - 6	44
6 - 36	ND ^b

^aGrid 1 received 1,894 pounds of Herbicide Purple per acre during 1962-1964. The soil samples were collected and analyzed in 1974.

^bNone detected, minimum detection limit - 10 ppt.

Source: Young et al. [Exhibit 7]

Herbicide. A summary of these data is shown in Table 7. Although more 2,4,5-T was applied to Grid 1 than to the other grids (see Table 4), it was applied years earlier. Note that the mean value between Grids 1 and 4 is approximately 1 magnitude in difference as is the maximum value between the two grids. Considering the time differences (periods of application) the data suggest that indeed levels of TCDD in Herbicide Orange were significantly less than the levels of TCDD in Herbicide Purple.

In August 1974 we established five 1-m² plots on Grid 1. Five subsamples were collected from each plot to a depth of 10 cm and those subsamples from each plot were mixed. The soil samples were analyzed for TCDD. In January 1978, we re-sampled in a similar manner from the same plots. The data from this sampling program are shown in Table 8. The data suggest that the half-life (the time required for one-half of the chemical to disappear/degrade) for TCDD is approximately three (3) years, under the environmental conditions encountered at this site at Eglin AFB, Florida.

The second question I posed on page 1 of this testimony was:

"Does TCDD persist in the field environment when sprayed as a contaminant of 2,4,5-T Herbicide?"

From the previous tables, figures and discussion we can conclude:

a. When massive quantities of 2,4,5-T were aerially applied to a spray-equipment testing grid at Eglin AFB, Florida, detectable levels of TCDD could be found in some soils 14 years after the last application of herbicide.

b. Field studies on the half-life of TCDD in sandy soils with low organic matter in Northwest Florida and in the absence of detectable levels of phenoxy herbicides was approximately three (3) years.

As noted from the above answers, the conditions under which the TCDD persists must be carefully defined. An explanation is in order. If the estimates for the amount of TCDD applied to Test Grid 1 is close to the actual

TABLE 7. CONCENTRATION OF TCDD (PPT) IN TEST GRID SOILS

<u>GRID</u>	<u>NO. SAMPLES*</u>	<u>RANGE</u>	<u>MEDIAN</u>	<u>MEAN</u>
I	22	< 10 - 1,500	110	326
II	6	< 10 - 470	30	117
IV	26	< 10 - 150	19	27

*0 - 15 CM INCREMENT

** SOURCE: YOUNG ET AL. 1979.

EXHIBIT 10

TABLE 8. DISAPPEARANCE OF TCDD FROM SOILS OF GRID I
(PARTS PER TRILLION)

<u>PLOT*</u> <u>NUMBER</u>	<u>AUGUST</u> <u>1974</u>	<u>JANUARY</u> <u>1978</u>
1	1,500	420
2	610	300
3	1,200	580
4	270	100
5	<u>440</u>	<u>400</u>
MEAN	804	360

*FIVE SUBSAMPLES FROM EACH 1-M² PLOT
COMPOSITED (0-10 CM DEPTH)

** SOURCE. YOUNG ET AL. 1979.

EXHIBIT 10.

amount disseminated (the amount was based upon a TCDD concentration of 32.8 ppm rather than the 45 ppm known to be present in a sample of Purple sprayed on Grid 1), then the amount (mean level) found on Grid 1 in the 1978 time period suggests that over 99% of the TCDD applied to Grid 1 has disappeared and/or degraded. Indeed half-life calculations based upon the three-year figure cannot account for the total amount of TCDD applied (i.e., it under estimates the amount). A different degradative mechanism may have resulted in significant loss of TCDD from the test area at the time of application. Most spray-equipment test missions occurred in the mornings. The soil of Grid 1 was without vegetation (see photograph in Figure 2). Perhaps when the herbicide was applied to the bare (and probably warm) soil, volatilization and/or photodegradation resulted in a significant loss of TCDD.

Another explanation that could account for the apparent persistence and high levels of TCDD in Grid 1 soils is the abiotic movement of TCDD in this environment. Technical Report AFATL-TR-75-142 (Exhibit 11) noted that during some seasons the wind frequently blew from the north across Grids 2 and 4 to Grid 1. In the absence of vegetation, the wind could readily move small sand particles contaminated with TCDD from one location to another. Figure 7 is a typical profile of the soil on Grid 1. The color and texture change at the 10 cm level strongly suggests that the upper level of soil was deposited probably by wind upon the lower level. Note in Table 6 that the high level of TCDD in such a profile was in the top 10 cm. This movement of soil particles by the wind might also reduce the amount of photodegradation of the TCDD that would occur on the surface of the particle immediately after application of the herbicide. I don't believe, however, that the wind movement from Grids 2 and 4 would have continued to contaminate Grid 1 through 1970. I do believe wind movement of the soil would account for the apparent soil penetration of TCDD.

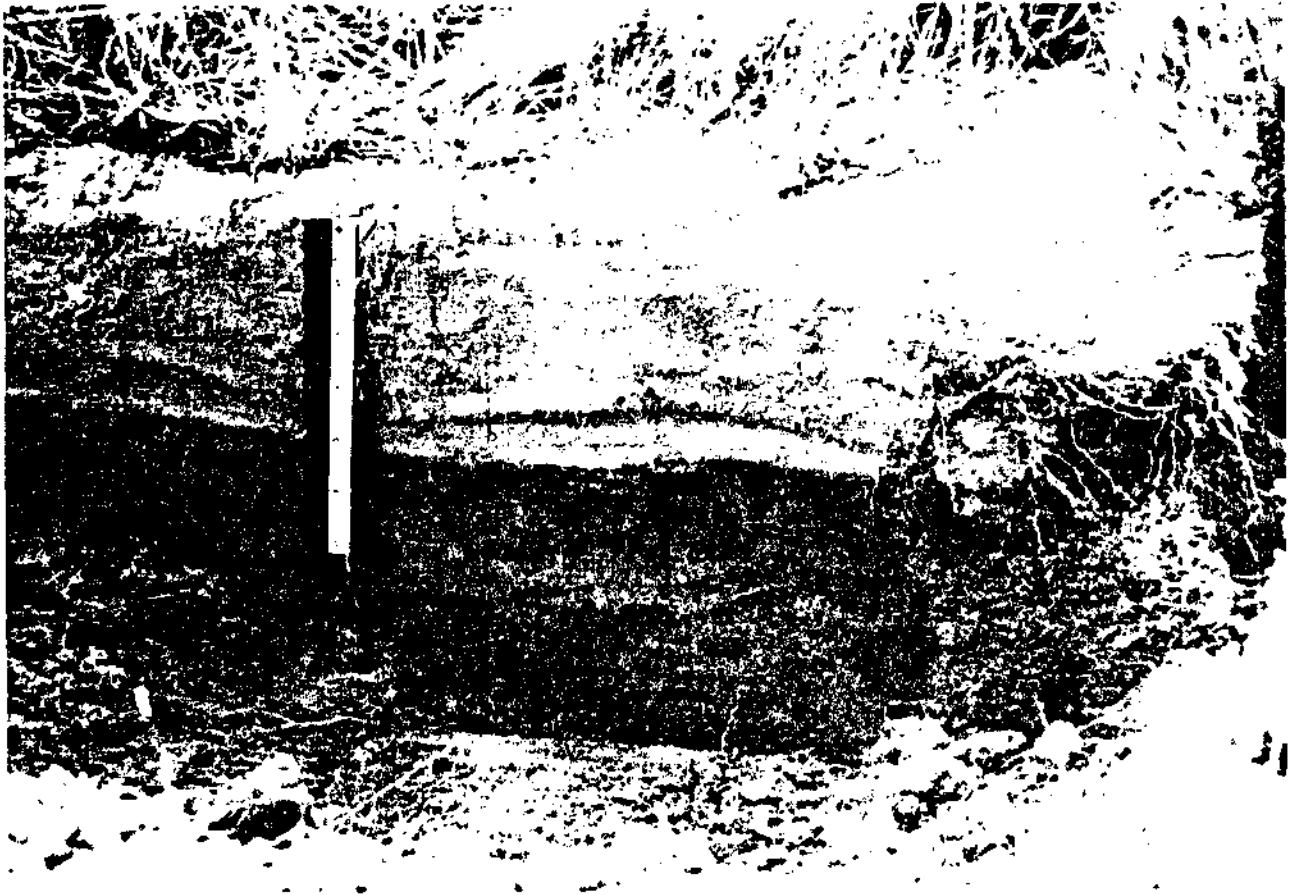


Figure 7. Photograph of soil profile from Grid 1, Test Area C-52A showing that the top 10 cm of soil is of different texture than the lower depth, suggesting that this upper level of soil was wind deposited.

A series of small springs are located near the center of the 3.0 km² Test Area. The water moves through a series of small ditches diverging east and west across Grid 3, going adjacent to Grid 1 on both sides and entering into the bayheads of 2 streams (Trout and Mullet Creeks). Residue studies of this drainage system were conducted in 1974 and published in Technical Report AFATL-TR-75-142 (Exhibit 11). The search for detectable levels of TCDD in the silt of these aquatic ecosystem was positive. Figure 8 illustrates the situation in which TCDD was found in the bayhead of Trout Creek. The maximum level of TCDD found in this situation was 35 ppt and was confined to the immediate area where the eroded soil entered the bayhead. A pond associated with one of the springs on Grid 3 had 65 ppt TCDD in silt at a site where erosion had occurred.

The third question I posed on page 1 of this testimony was:

"Does TCDD move in the abiotic (non-living) portion of the environment?"

From the previous tables, figures and discussion we can conclude:

a. In areas where the vegetative density is low and the soil is comprised of light sand, the wind may play a significant role in moving TCDD-contaminated particles into adjacent areas away from where spraying occurred. Data from the Eglin studies suggest that this mechanism may also result in deposition of soil into a depth profile that may reduce degradation.

b. The erosion of soil by water has been shown to be a mechanism of removing TCDD-contaminated soil particles to locations away from where spraying occurred. Dilution of these contaminated particles results in rapid dispersal of the residue unless the particles are washed into a silt accumulating site, where build-up of residue may occur.

Animal Studies - Studies of the animals of Test Area C-52A began in 1970. However, detailed investigations of key species (e.g., the beachmouse, Peromyscus polionotus and the six-lined racerunner, Cnemidophorus sexlineatus) did not

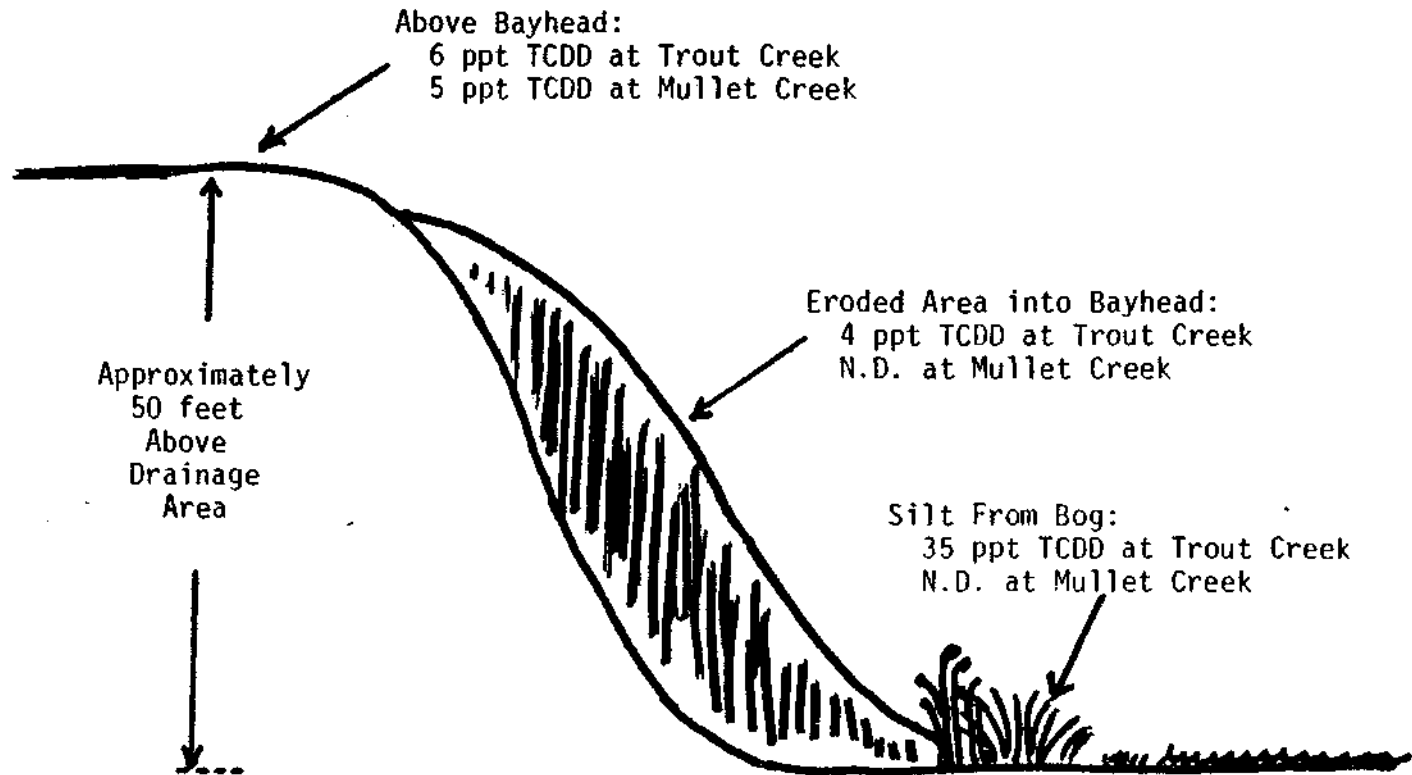


Figure 8. Concentration of TCDD (Parts Per Trillion) in an X-Section of the Bayheads of Trout and Mullet Creeks; Located Approximately One-Quarter Mile from Edge of Test Grid 1, East or West, Respectively

Source: Young, A.L. et al. 1975. Technical Report AFATL-TR-75-142.

Exhibit 11.

begin until 1973. Key species have been repeatedly studied in subsequent years (1974, 1975 and 1978). The birds were studied in 1974 and 1975. The insect studies were conducted in 1971 and 1973, while the aquatic communities were initially examined in 1970 and again in 1973 and 1974. List of species, description of habitats and TCDD residue analysis were conducted throughout all years of study.

Probably, the most startling observation about Test Area C-52A, is that biological organisms are abundant. The composition of species is diverse and the distribution extensive. In February 1969, I initiated a "list of species" for the test grids. Whenever a species was observed on or associated with the grids, that species was recorded. Table 9 is a summary list of the 341 species or organisms observed and identified on or associated with the test area. The sheer number of species testifies to the extensiveness of the ecological studies that have been conducted on this unique area. All of the individual species have been variously recorded in Exhibits 1-3, 5-8, and 10-11. To date 290 biological samples (plants and animals) have been analyzed for TCDD. TCDD residues have now been found in a wide spectrum of animals collected from the test area. Approximately one-third of the different species examined for TCDD residue have been positive. Tables 10, 11, 12, 13 and 14 provide data on those species (mammals, birds, insects, reptiles and amphibians and fish respectively) found to have detectable levels of TCDD.

Why are the above species contaminated with TCDD while other species found on the same test area are not? Examination of the species by ecological niche suggest that the commonalty is a close relationship to the soil. For example the beachmouse and hispid cotton rat dig burrows. The cotton rat digs burrows near the water and in areas of high vegetation density. The beachmouse prefers

TABLE 9. ECOLOGICAL SURVEY, 1973 - 1978
TEST AREA C-52A

<u>NUMBER OF SPECIES</u>	<u>ORGANISMS</u>
123	PLANTS
77	BIRDS
71	INSECT FAMILIES
20	FISH
18	REPTILES
18	MAMMALS
12	AMPHIBIANS
2	MOLLUSCS

290 BIOLOGICAL SAMPLES ANALYZED FOR TCDD

TABLE 10. INVESTIGATIONS OF MAMMALS, TEST AREA C-52A

SPECIES	TCDD RESIDUE ANALYSIS (PPT)		
	<u>ORGAN</u>	<u>CONCENTRATION</u>	<u>DETECTION LIMIT</u>
DEER	FAT	ND	4
	LIVER	ND	5
	KIDNEY	ND	4
OPOSSUM	FAT	ND	10
	LIVER	ND	10
RABBIT	LIVER	ND	8
	PELT	ND	2
COTTON RAT	LIVER	10 - 210	
BEACHMOUSE	LIVER	300 - 2,900	
	PELT	130 - 200	

TABLE 11. INVESTIGATIONS OF BIRD SPECIES
Test Area C-52A

DOMINANT SPECIES	TCDD RESIDUE ANALYSIS (PPT)			
	No. Samples*	Organ	Range	Mean
Southern Meadowlark	3	Liver	100 - 1,020	440
	1	Stomach		10
Mourning Dove	2	Liver		50
	1	Stomach		10
Savannah Sparrow	1	Liver		69
	1	Stomach		84

*Composites from at least 6 birds

TABLE 12. INVESTIGATIONS OF INSECTS
Test Area C-52A

<u>FAMILY</u>	<u>TCDD Residue Analysis (ppt)</u>
Grasshoppers	ND (3)*
Crickets	18-26
Composite of Soil/Plant Insects	40
BURROW SPIDERS	115
INSECT GRUBS (COLEOPTERA)	238

TABLE 13. INVESTIGATIONS OF REPTILES AND AMPHIBIANS,
TEST AREA C-52A

TCDD RESIDUE ANALYSIS (PPT)

<u>SPECIES</u>	<u>ORGAN</u>	<u>CONCENTRATION</u>	<u>DETECTION LIMIT</u>
SIX-LINED RACERUNNER	VISCERA	360	50
	TRUNK	370-430	40
	WHOLE BODY	420	-
SOUTHERN TOAD	WHOLE BODY	1,360	90
EASTERN COACHWHIP	MUSCLE	ND	14
	FAT	148	64
	SKIN	20	20
PINE SNAKE (IMMATURE)	WHOLE BODY	ND	70

TABLE 14. INVESTIGATIONS OF AQUATIC SPECIES,
TEST AREA C-52A

TCDD RESIDUE ANALYSIS (PPT)

<u>SPECIES</u>	<u>ORGAN</u>	<u>CONCENTRATION</u>
MOSQUITO FISH	WHOLE BODY	12
SAILFIN SHINER	WHOLE BODY	12
SPOTTED SUNFISH	SKIN	4
	GONADS	18
	MUSCLE	4
	GUT	85

areas of low vegetation density, e.g., the centers of the old flight paths. The deer, opossum and rabbit do not burrow, but rather nest/rest upon the vegetation. The crickets, ground spiders and soil-borne insect grubs were significantly contaminated; the grasshoppers were not. The Southern Toad lives in abandoned rodent burrows. The Spotted Sunfish is a bottom feeder and its visceral mass is comprised largely of silt and detritus.

In general, the levels of TCDD in the organisms appeared to be close to the mean levels of TCDD found in the soils. Table 7 had means values of 326, 117 and 27 ppt TCDD for the top 15 cm of soil on Grids 1, 2 and 4, respectively. Most of the racerunners, snakes, toads and soil-borne insects were captured on Grid 1. The exceptions to this observation are the beachmouse, toad and the Southern Meadowlark. Observational studies of the mice and meadowlarks revealed a similar habit - they both are fastidious groomers/preeners. In the case of the beachmouse, the animal burrows in contaminated soil and during the evenings enters and leaves the burrow frequently. Each time the animal goes into or out of the burrow, it passes through the 15 cm zone of TCDD-contaminated soil. In the burrow the animal grooms the contaminated particles from the pelt. The TCDD accumulates in the lever. A laboratory study was conducted (see Exhibit 2, Technical Report FJSRL-TR-80-0008) where the fur on the central thoracic and abdominal regions, sides, back and tail on each test animal was dusted with alumina gel containing 2.5 ppb TCDD. Control animals were dusted in the same body areas but with alumina gel alone. The dusting procedure was repeated every third day for a total of 10 applications during a 28 day period. The results of the TCDD analysis are shown in Table 15. The data showed that indeed grooming may result in significant contamination.

The meadowlark kicks-up top soil and "dusts" its feathers. Preening results in the ingestion of the soil. It should, however, be noted that the meadowlark

TABLE 15. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN LIVER AND PELT SAMPLES FROM BEACH MICE, PEROMYSCUS POLIONOTUS, DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL) OR 2.5 PARTS PER BILLION TCDD (TEST)

TREATMENT	SEX	LIVER	PELT
ALUMINA GEL	MALE	8 ^a	10 ^a
	FEMALE	8 ^a	10 ^a
ALUMINA GEL + TCDD	MALE	125 ^b	45
	FEMALE	125 ^b	89
^a Minimum level of detection			
^b Male and female livers composited for analysis			

Source: Young, A.L. et al. 1975. Technical Report AFATL-TR-75-142.

Exhibit 11.

also ingests soil-borne insects. These insects probably have a significant amount of TCDD on them due to the adherence of contaminated soil particles. Thus, the role of the insect in contaminating the bird is not clear. The Southern Toad ingests primarily soil-borne insects and here too, the role of contaminated soil particles on the insect versus ingested TCDD within the insect is not clear.

It is clear from the Eglin studies that accumulation of TCDD occurs in animals. The magnitude of those levels apparently depends upon the levels of TCDD in the soil. Only two species have been adequately studied to address body-burden levels of TCDD. Samples of the six-lined racerunner have been collected and analyzed three consecutive years. The samples have been consistent for all three years, i.e., levels of 360, 370 and 430. The levels in the visceral mass and the trunk are the same, suggesting the levels are at equilibrium. If the median (not mean) soil level for TCDD is used (because of distribution - the racerunner has an extensive range within its habitat) then, a concentration factor of four (4) exists (110 ppt in soil vs. 400 ppt as body burden).

We have recently received additional TCDD analyses on components of the beachmouse and its habitat. The present analytical capability by the University of Nebraska (our contractor), permits an analysis of a single liver sample! The significance of this capability can be seen in Tables 16 and 17. The mound soil is that soil removed during the course of digging the burrow. If it represents mean exposure then the concentration factor for animals from site 0-4 is between 6 and 7 (500 divided by 75 = 6.67) for females and 18 to 19 for males (1400 divided by 75 = 18.67). The concentration factors for site 0-7 is between 6 and 7 (1900 divided by 285 = 6.67) for females and approximately 9 for males (2600 divided by 285 = 9.12). It is assumed in these studies that body burden levels of TCDD are actually liver levels of TCDD. How long does it take beachmice to accumulate these levels of TCDD, assuming no previous exposure? In 1974 we

TABLE 16. TCDD (PPT) IN SOIL AND BEACHMICE,
 SITE 0-4, GRID 1, TEST AREA C-52A
 APRIL 1978

<u>SOIL</u>	0-5 cm	=	150
	5-10 cm	=	155
	10-15 cm	=	70
	Mound Soil	=	75

BEACHMICE

BURROW 1.	FEMALE:	LIVER	=	500
		PELT	=	110
	PUPS:	LIVER	=	500
		PELT	=	150
	FETUSES:	WHOLE BODY	=	40
BURROW 2.	FEMALE:	LIVER	=	490
		PELT	=	140
	FETUSES:	WHOLE BODY	=	90
COMPOSITE	MALES:	LIVER	=	1400
		PELT	=	160

TABLE 17. TCDD (PPT) IN SOIL AND BEACHMICE,
 SITE 0-7, GRID 1, TEST AREA C-52A
 APRIL 1978

<u>SOIL</u>	0-5 cm	=	510
	5-10 cm	=	520
	10-15 cm	=	440
	Mound	=	285

BEACHMICE

FEMALE:	LIVER	=	1900
	PELT	=	160
FETUSES:	WHOLE BODY	=	150
MALE:	LIVER	=	2600
	PELT	=	150

obtained beachmice from our control site at Eglin and raised an animal colony in our laboratory. In October 1975, we transported "tagged" animals to Grid 1 and released them. Three months later, we recaptured a small number of these animals. At the time of recapture we also captured animals apparently endogenous (native) to the site. The results are shown in Table 18. The data suggest that body burden levels are obtained at this site within 3 months. Note that the body burden levels are between 5 and 8 for tagged and natives, respectively. However, the tagged consists of both sexes.

The fourth question I posed on page 1 of this testimony was:

"Does TCDD in the field environment bioaccumulate in biological organisms and/or biomagnify within food chains?"

From the previous tables and discussion we can conclude:

- a. Organisms that come into direct and intimate contact with TCDD-contaminated soil generally become contaminated themselves.
- b. From limited studies of a few animal species, animals may accumulate in selected organs from 4 to 10 times the level of TCDD that occurs in the soil. In these cases, it appears that soil is directly ingested so that accumulation occurred in the absence of food chains.
- c. From studies of organisms that ingested TCDD-contaminated organisms, the data suggest a simple concentration mechanism consisting of a single stage, e.g., birds eat insects contaminated with TCDD-contaminated soil particles. Biomagnification, i.e., orders-of-magnitude increases of residue through trophic levels, does not occur.

The Eglin test site (Test Area C-52A) is unique. Significant concentrations of TCDD have been present in the soils for at least 14 years. Animal populations are diverse and many have been shown to be heavily (by normal field standards) contaminated (up to 2900 ppt TCDD). Many generations of these animals have existed

TABLE 18. BEACHMICE TCDD EXPOSURE STUDY,
GRID 1, TEST AREA C-52A

MEAN SOIL CONCENTRATION (0-15 cm) = 326 ppt

BEACHMICE RELEASED - 16 SEPT 75

BEACHMICE CAPTURED - 22 DEC 75

RESULTS

TAGGED: LIVER = 1,700 ppt (MALE/FEMALE)
PELT = 200 ppt

NATIVE: LIVER = 2,600 ppt (MALE)
PELT = 190 ppt

on the test range. When the suspension of Herbicide Orange occurred, by the Department of Defense in April 1970, it was over the concern of toxic effects alleged to be associated with spraying 2,4,5-T contaminated with TCDD. Discussions were held with personnel who had been associated with the spray-equipment testing program on Test Area C-52A. These individuals were asked if they could remember any unusual animal deaths associated with the test grids. They could not. Never-the-less, numerous field trips were conducted on the test grids with the sole purpose of observing the wildlife and search for dead or dying animals. These studies were negative. In all subsequent trapping programs for animals, gross observations for defects, illnesses, and overall health status were made. Nothing out of the "ordinary" (e.g., parasites) have been observed. In 1973, when the data on TCDD in soils first became available, an extensive research effort was initiated on the beachmouse. Subsequent studies were conducted in 1974, 1975 and 1978. Residue levels in the liver and on the pelts of these animals have already been given. The approach we took on examining the beachmouse was to collect animals from the test grid and a control site and compare them for as many parameters as possible. In those females that were pregnant, the fetuses were also critically examined.

All animals were prepared for examination using a cervical dislocation procedure to accomplish humane euthanasia. Euthanatized animals were photographed, weighed, measured, and systematically examined for developmental defects such as cleft palate, cleft lip, polydactyly, and microphthalmia. All internal organs were examined for gross lesions and individually weighed. Representative sections of each tissue were placed in neutral 10 percent buffered formalin and processed for microscopic study by the Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, D.C. 20305. All remaining tissues from mice captured in the test and control areas were pooled according to sex and maturity, placed

in glass jars, frozen, and retained for TCDD analysis.

Histopathological examinations were performed on 255 adult or fetal beachmice from the test area and a control area. The animals examined are those listed in Tables 19 and 20 for control and test, respectively. Examinations were performed on the heart, lungs, trachea, salivary glands, thymus, liver, kidneys, stomach, pancreas, adrenals, large and small intestine, spleen, genital organs, bone, bone marrow, skin and brain (Table 21). Initially, the tissues were examined on a blind study basis. All microscopic changes were recorded including those interpreted as minor or insignificant. The tissues were then re-examined on a control versus test basis, which demonstrated that the test and control mice could not be distinguished histopathologically.

Tables 22 and 23 provide body and organ weights for the pregnant female beachmice and the mature male beachmice, respectively. These were the two largest segments of the population that were captured. A two-factor (treatment and year) disproportional analysis of covariance of organ weights revealed that liver weights for pregnant females were significantly heavier ($P < .01$) between the control and test area beachmice, and these differences were consistent over the years of observation. These data are displayed in Table 24. The increase in liver weight may reflect an increase in enzymatic activity associated with low level exposure to TCDD. An ultrastructural study of liver tissue from the test and control site females found no morphologic differences (see Exhibit 2).

The mean number of fetuses per observed pregnancy was 3.1 and 3.4 for the test area and a control area, respectively. A single female beachmouse is capable of producing litters every 26 days. At this frequency, the animals collected in 1978 may have been at least 50 generations removed from the population studied in 1973.

TABLE 19. STUDIES OF THE BEACHMOUSE, PEROMYSCUS POLIONOTUS
Grid 1, Test Area C-52A, Eglin AFB FL

LOCATION	YEAR				
<u>Maturity, Sex</u>	<u>1973</u>	<u>1974</u>	<u>1975</u>	<u>1978</u>	<u>Total</u>
CONTROL AREA					
Mature					
Male	4	11	3	2	20
Female	3(3)	8(3)	3(1)	2(2)	16(9)
Immature					
Male	1	1	0	0	2
Female	0	2	0	0	2
Fetuses	12	11	3	5	31
				Total	<u>71</u>

() Number of Pregnant Females
Fetuses/Pregnancy = 3.4

TABLE 20. STUDIES OF THE BEACHMOUSE, PEROMYSCUS POLIONOTUS
Grid 1, Test Area C-52A, Eglin AFB FL

LOCATION <u>Maturity, Sex</u>	YEAR				<u>Total</u>
	<u>1973</u>	<u>1974</u>	<u>1975</u>	<u>1978</u>	
TEST GRID 1					
Mature					
Male	18	14	7	7	46
Female	15(6)	9(6)	6(4)	6(6)	36(22)
Immature					
Male	8	3	7	6	24
Female	1	4	3	3	11
Fetuses	25	9	12	21	67
				Total	<u>184</u>

() Number of Pregnant Females
Fetuses/Pregnancy = 3.1

TABLE 21. HISTOLOGICAL PARAMETERS

HEART	PANCREAS
LUNGS	ADRENALS
TRACHEA	LARGE/SMALL INTESTINE
SALIVARY GLANDS	SPLEEN
THYMUS	GENITAL ORGANS
LIVER	BONE
KIDNEYS	BONE MARROW
STOMACH	SKIN
	BRAIN

ALL MICROSCOPIC CHANGES RECORDED. TEST AND CONTROL MICE
COULD NOT BE DISTINGUISHED.

Mean body weights and organ weights of mature male beachmice,
Peromyscus polionotus, From Control and TCDD-exposed Field
 Sites, Test Area C-52A, Eglin AFB, Florida

ORGAN WEIGHTS

<u>Field Site</u>	<u>Year</u>	<u>Animals</u>	<u>Weight (g)</u>	<u>Liver (mg)</u>	<u>Heart (mg)</u>	<u>Lung (mg)</u>	<u>Spleen (mg)</u>	<u>Kidney (mg)</u>
Control	1973	4	11.88 \pm 1.03	708 \pm 114	78 \pm 15	150 \pm 14	33 \pm 10	120 \pm 27
	1974	11	12.02 \pm 1.21	611 \pm 111	101 \pm 13	99 \pm 14	17 \pm 6	191 \pm 20
	1975	3	13.25 \pm 0.24	837 \pm 103	133 \pm 6	135 \pm 1	12 \pm 3	268 \pm 6
	1978	2	11.91 \pm 0.32	667 \pm 5	135 \pm 8	163 \pm 74	21 \pm 1	225 \pm 10
Test Grids	1973	18	11.84 \pm 1.12	819 \pm 223	93 \pm 23	154 \pm 43	35 \pm 10	140 \pm 60
	1974	14	11.49 \pm 0.93	664 \pm 150	94 \pm 22	100 \pm 21	23 \pm 15	193 \pm 20
	1975	7	11.67 \pm 0.83	774 \pm 112	134 \pm 14	106 \pm 25	17 \pm 7	225 \pm 17
	1978	7	12.25 \pm 1.35	756 \pm 130	144 \pm 37	130 \pm 48	22 \pm 9	227 \pm 39

Table 23. Mean Body Weights and Organ Weights of Pregnant Female Beachmice, *Peromyscus polionotus*, from Control and TCDD-exposed Field Sites, Test Area C-52A, Eglin AFB, Florida.

Field Site	Year	Number of Animals	Body Weight (g)	ORGAN WEIGHTS				
				Liver (mg)	Heart (mg)	Lung (mg)	Spleen (mg)	Kidney (mg)
Control	1973	3	16.29±2.66	955±308	110±35	156±12	33±15	136±40
	1974	3	11.98±0.85	640±220	95± 1	103±19	21±14	186±18
	1975	1	15.84	955	149	210	56	311
	1978	2	13.94±3.48	868±140	139±13	144±48	13± 3	243±23
Test Grids	1973	6	15.45±2.00	1253±175	113±12	188±35	63±29	248±96
	1974	6	15.75±1.33	1035± 98	114±10	108±20	30±16	235±37
	1975	4	16.05±1.74	1138± 58	158± 4	138±27	26±11	308±15
	1978	6	15.67±2.20	1115±171	173±35	132±45	26±12	294±39

TABLE 24. MEAN LIVER WEIGHTS (MG) OF PREGNANT BEACHMICE
TEST AREA C-52A

<u>LOCATION</u>	<u>YEAR</u>	<u>LIVER WEIGHT (MG)</u>
CONTROL	1973	955
	1974	640
	1975	955
	1978	868
GRID 1	1973	1253
	1974	1035
	1975	1138
	1978	1115

STATISTICALLY SIGNIFICANT!

These studies suggest that long-term, low level exposure to TCDD under field conditions has had minimal effect upon the health and reproduction of the beachmouse.

We have conducted limited studies on the six-lined racerunner. Gross post mortem examinations were performed on 19 racerunners collected from either a control site or from Grid 1. No evidence of abnormality was detected in any of the specimens.

A statistical analysis was performed on the total body weights obtained at necropsy, for male and female racerunners collected in 1973 and 1974 from test and control areas. Hypotheses concerning no effect due to year of collection, no differences attributable to sex, and no effect due to treatment were tested. The analysis of variance indicated that no significant effects with TCDD were detected relative to variations in total body weights between control specimens and those collected on a site heavily contaminated.

The last question I posed on page 1 of this testimony was:

"Do the levels of TCDD found in the field environment have adverse effects upon the organisms within that environment?"

From the previous tables and discussions we can conclude:

a. The ecological studies conducted on Test Area C-52A, Eglin AFB, Florida have found no significant adverse acute toxic effects of TCDD in animal populations exposed to concentrations of TCDD in the range of 0.1 to 1.5 parts per billion.

b. The ecological studies conducted on Test Area C-52A, Eglin AFB, Florida suggest that long-term, low level exposure to TCDD under field conditions has had minimal effect upon the health and reproduction of the beachmouse.

Soil Incorporation/Biodegradation Studies

One potential method proposed for the disposal of herbicide Orange was subsurface injection or soil incorporation of the herbicide at massive concentrations. The premise for such studies was that high concentrations of the herbicides and TCDD would be degraded to innocuous products by the combined action of soil microorganisms and soil hydrolysis. In order to field test this concept, biodegradation plots were established in three climatically different areas of the United States: Northwest Florida (Eglin Air Force Base), Western Kansas (Garden City), and Northwestern Utah (Air Force Logistics Command Test Range Complex). A comparison of the soils of the three sites is given in Table 25. The Utah site had a mean annual rainfall of 15 cm, while the Kansas and Florida sites had 40 and 150 cm, respectively. Table 26 describes the experimental protocol for the three sites to include when the plots were established, the method of herbicide incorporation, the experimental design and the initial calculated herbicide concentration, ppm, at the time the plots were established.

Tables 27, 28 and 29 compare the rate of disappearance of TCDD with that of Orange herbicide for selected plots at the Utah, Kansas and Florida sites, respectively. Although the number of analyses have been extremely limited, the data have indicated that TCDD (and phenoxy herbicide) degrade more rapidly in the Kansas soils (Ulysses Silt Loam) than in the Florida soils (Lakeland Sandy Loam),, and least rapidly in the Utah desert soils (Lacustrine Clay Loam).

TABLE 25. ANALYSES OF THE TOP 15-CM LAYER FROM EACH OF THE SOIL BIODEGRADATION SITES *

LOCATION	pH	ORGANIC MATTER (%)	SAND (%)	SILT (%)	CLAY (%)	SOIL DESCRIPTION
Eglin AFB, FL ^a	5.6	0.5	91.6	4.0	4.4	Sandy loam
Garden City, KS ^b	7.0	1.7	37	42	21	Silt loam
AFLC Test Range Complex, UT ^c	7.8	1.4	27	53	20	Clay loam

^aPlots located on Test Area C-52A, Eglin AFB Reservation, Florida

^bPlots located on the Kansas Agricultural Experiment Station, Garden City, Kansas

^cPlots located 75 miles west of Salt Lake City, Utah

* Source: Exhibit 8

TABLE 26. DESCRIPTIONS OF THREE BIODEGRADATION STUDIES INVOLVING USE OF HERBICIDE ORANGE*

LOCATION	DATE ESTABLISHED	METHOD OF INCORPORATION	TREATMENT	CALCULATED INITIAL HERBICIDE CONCENTRATION (PPM) ^c
Eglin AFB, Florida	2 Apr 1972	Simulated Subsurface Injection (30 cm band width)	4,480 kg Herbicide/ha ^a	5,000
			4,480 kg Herbicide/ha, plus soil amendments ^b	5,000
			4,480 kg Herbicide/ha plus soil amendments and activated charcoal	5,000
Garden City, Kansas	10 May 1972	Preplant Incorporate (Rototiller)	2,240 kg Herbicide/ha	1,000
			4,480 kg Herbicide/ha	2,000
AFIC Test Range Complex, Utah	2 Oct 1972	Simulated Subsurface Injection (8 cm band width)	1,120 kg Herbicide/ha	10,000
			2,240 kg Herbicide/ha	20,000
			4,480 kg Herbicide/ha	40,000

^aRate of herbicide calculated as active ingredient. Herbicide injected at 10-15 cm level or preplant incorporated in the 0-15 cm level. All plots duplicated.

^bThe amendments included 4.5 kg lime, 13.5 kg organic matter, and 1.4 kg fertilizer (12:4:8 for N,P,K, respectively) uniformly mixed within the top 0-30 cm of soil in the plot.

^cContained in the top 0-15 cm layer.

*Source: Exhibit 8

TABLE 27. CONCENTRATIONS OF HERBICIDE ORANGE AND TCDD IN PLOTS ORIGINALLY TREATED WITH 4,480 KG/HA, AFLC TEST RANGE COMPLEX, UTAH, AT VARIOUS SAMPLING DATES AFTER APPLICATION. (TCDD IN PARTS PER BILLION)*

DAYS AFTER APPLICATION	TOTAL HERBICIDE ^a (PPM)	TCDD (PPM $\times 10^{-3}$)
282	8,490	15.0
637	4,000	7.3
780	2,260	5.6
1,000	2,370	3.2
1,150	960	2.5

^aComposite sample from replicated plots, 0-15 cm increment

TABLE 28. CONCENTRATIONS OF HERBICIDE ORANGE AND TCDD IN PLOTS ORIGINALLY TREATED WITH 4,480 KG/HA, GARDEN CITY, KANSAS, AT VARIOUS SAMPLING DATES AFTER APPLICATION. (TCDD IN PARTS PER TRILLION)*

DAYS AFTER APPLICATION	TOTAL HERBICIDE ^a (PPM)	TCDD ^a (PPM $\times 10^{-6}$)
8	1,950	--b
77	1,070	225
189	490	--b
362	210	--b
600	40	--b
659	<1	42

^aComposite sampling from replicated plots, 0-15 cm increment

^bNot analyzed

*Source: Exhibit 8

TABLE 29. CONCENTRATIONS OF HERBICIDE ORANGE AND TCDD IN PLOTS ORIGINALLY TREATED AT 4,480 KG/HA, EGLIN AFB, FLORIDA, AT VARIOUS SAMPLING DATES AFTER APPLICATION*

DAYS AFTER APPLICATION	TOTAL HERBICIDE ^a (PPM)	TCDD ^a (PPM×10 ⁻⁶) ^c
5	4,897	375
414	1,866	250
513	824	75
707	508	46
834	438	-- ^b
1,293	<10	-- ^b

^aComposite sample from the plot containing only herbicide (i.e., no lime, organic matter, or fertilizer added). Sample from the 0-15 cm increment.

^bAnalysis not completed.

^cTCDD in parts per trillion, minimum detection limit - 10 ppt.

*Source: Exhibit 8

Note that detectable levels of TCDD were found in the field plots at all locations for the last reported analysis of TCDD. The detection limit for TCDD has generally been less than 10 ppt. Although biodegradation appears to reduce the level of herbicide and TCDD, the data did not follow simple exponential decay curves. For the mixture of 2,4-D and 2,4,5-T herbicides, disappearance was rapid initially, but slowed substantially in the later portions of the test period. With this type of decay kinetics, meaningful half lives are difficult to calculate; however, a reasonable estimate appears to be in the range of 150-210 days. The degradation of TCDD followed a similar decay pattern. Our best estimates for a half life for TCDD are between 225 and 275 days. It should be emphasized, however, that these estimates are based on limited data and on the degradation of TCDD in the presence of the chlorinated hydrocarbons 2,4-D and 2,4,5-T herbicides

The analysis of soil profiles at all three locations for biodegradation indicated that neither the herbicide nor the TCDD appreciably penetrated below the 15-30 cm (6-12 inches) level. Table 30 illustrates these observations. We believe that the disappearance of the herbicide and the TCDD can be attributed to the action of soil microorganisms, rather than leaching.

Residue Monitoring of Storage Sites

Since 1970, various Air Force and contract laboratories have been conducting environmental surveys and analyses of the soils, plants, and the aquatic system in and around the Herbicide Orange storage area at the Naval Construction Battalion Center (NCBC), Gulfport, Mississippi. As some leaking became evident and as more information became available on the TCDD contained

TABLE 30. Concentrations of TCDD, parts per trillion, in the Herbicide Orange biodegradation plots, AFLC Test Range, Utah, four years after applications.^{a*}

Depth (inch)	Original Rate of Herbicide Orange Applied		
	1,000 lb/A	2,000 lb/A	4,000 lb/A
0 - 6	650	1600	6600
6 - 12	11	90	200
12 - 18	NA ^b	NA ^b	14

^aSamples collected 6 November 1976. Plots established 5 October 1972.

^bSamples not analyzed.

*Source: Exhibit 7

in the herbicide, more extensive monitoring programs were conducted. The entire inventory was redrummed in 1972 and checked for leaks continuously thereafter. In the summer of 1977, the herbicide was transferred to a specially equipped ship and destroyed by at-sea incineration during Project PACER HO. The Air Force plan and the EPA permits for the disposal of the herbicide committed the Air Force to a follow-on storage site reclamation and environmental monitoring program. The major objectives of the subsequent program were to (1) determine the magnitude of Herbicide Orange contamination in the storage area; (2) determine the soil persistence of 2,4-D and 2,4,5-T, their phenolic degradation products and TCDD in soils of the storage area; (3) monitor for potential movement of residues from the storage area into adjacent water, sediments and biological organisms; and (4) recommend managerial techniques for minimizing any impact of the herbicides and TCDD residues on the ecology and human populations adjacent or near the storage area.

The monitoring approach used to determine storage site contamination consisted of analyzing soil samples selected from 42 different sites within the storage area. Sampling points were selected in groups depending upon whether a spill of the herbicide had occurred in that area or not. Previous studies had shown that residue did not appreciably move within the acid soil or significantly penetrate the impervious concrete-stabilized hardpan located approximately six inches below the soil surface. Soil samples were also analyzed for microorganisms.

The results (see Exhibit 9) indicated that approximately 15 percent of the 12-acre site was significantly contaminated with Herbicide Orange and TCDD. Levels of 2,4-D and 2,4,5-T in the samples, which were greater than

100,000 parts per million (ppm) in July 1977, decreased to one-third that level in 18 months. Data from spill sites monitored for this same time period also suggested that TCDD levels were decreasing but at a slower rate. The soil penetration of the herbicides was low while penetration of TCDD was negligible. Sterilization of the soil did not occur; rather, certain microflora proliferated under high levels of herbicides.

To monitor for potential movement of residue from the storage area, soil and biological samples were collected from the drainage ditch directly adjacent to the site. A November 1978 analysis of this nearby on-base drainage ditch found positive TCDD residues [0.14-3.6 parts per billion (ppb)]. The TCDD movement was presumably caused through soil erosion from the annual (Jan-Jun) heavy rain season (approximately 60 in). Drainage ditches carry heavy rain from the storage site and other parts of the base into Long Beach Canal #1, approximately 9,000 feet from the site. The canal runs from the City of Long Beach through the base carrying municipal surface drainage, and until July 1978, carried treated sewage materials. The canal eventually runs into Turkey Creek approximately 12,000 feet from the storage site. Due to the November 1978 findings, further samples were collected at varying distances from the site in January, February and June 1979. Following extensive and difficult analyses in contract laboratories, the results were received in September, November and December 1979. The results confirm the November 1978 data and indicated slightly higher levels (sediment levels of 1.7-3.6 ppb and biological levels of 0.14-7.2 ppb). Water samples collected in the same area were negative for TCDD at a detection level of 0.02 ppb. TCDD appears to move only as part of

soil sediment. Sediment and biological samples taken downstream at 3,000, 7,000, 9,000 and 12,000 feet from the site indicated that some TCDD residue was not present but at very low levels. A crayfish collected at 9,000 feet and numerous fish collected at 12,000 feet were analyzed with 0.032 ppb, the highest level detected. Additional samples collected in June 1979 confirmed contamination of the aquatic ecosystem at the 12,000-foot sampling location. Sediment samples of these various sites are currently being analyzed.

The levels of TCDD found in the soils and aquatic systems at NCBC are similar to those reported for the Herbicide Loading and Storage Site (Hardstand 7) at Eglin AFB, Florida. Hardstand 7 support the loading of the aircraft that disseminated herbicide in Test Area C-52A. Data for the Hardstand are shown in Exhibits 1 and 3.

Human Exposure to 2,4,5-T and TCDD

The Air Force studies that I have discussed have involved many researchers and support personnel. A few of these individuals were intimately associated with the handling of 2,4,5-T herbicide (as Herbicide Orange) and with TCDD (both in Herbicide Orange and in a variety of other substrates). Because of this extensive exposure, both in time and in dose, three Air Force officers volunteered to participate in the Veterans Administration (VA) Fat Biopsy Study. I was one of the participants and am listed as VA Fat Biopsy Subject Number 3 (see Exhibit 4). Two other officers participated and are listed as VA Subjects 2 and 4. A detailed exposure history was available for each of the three officers. Summaries of these exposure histories and some supporting photo documentation are provided in Appendix I of this testimony.

The levels of TCDD found in the fat of the three individuals is as follows:

Subject 2 (C.E.T.)	5 ppt TCDD
Subject 3 (A.L.Y.)	ND (detection limit 4 ppt)
Subject 4 (J.W.T.)	6 ppt TCDD

The present health of the three individuals is good (see Exhibit 4).

The VA draft manuscript did not clarify the isomer of TCDD. I do not believe that the University of Nebraska identified the TCDD as the 2,3,7,8-TCDD isomer. I believe from reviewing Exhibit 4 that our exposures to Herbicide Orange and TCDD equalled or exceeded the exposures of the other participants to Herbicide Orange. That does not imply that they may not have been exposed to TCDD from other sources nor is it intended to imply any relationship to health status.

LIST OF EXHIBITS

1. Bartleson, F. D., D. D. Harrison and J. D. Morgan. 1975. Field studies of wildlife exposed to TCDD contaminated soils. Air Force Technical Report AFATL-TR-75-49. Air Force Armament Laboratory, Eglin AFB, Florida. 53 p.
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3. Harrison, D. D., C. I. Miller and R. C. Crews. 1979. Residual levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) near herbicide storage and loading areas at Eglin AFB, Florida. Air Force Technical Report AFATL-TR-79-20. Air Force Armament Laboratory, Eglin AFB, Florida. 27 p.
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5. Young, A. L. 1974. Ecological studies on a herbicide-equipment test area (TA C-52A), Eglin AFB Reservation, Florida. Air Force Technical Report AFATL-TR-74-12, Air Force Armament Laboratory, Eglin AFB, Florida. 146 p.
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9. Young, A. L., C. E. Thalken and W. J. Cairney. 1979. Herbicide Orange site treatment and environmental monitoring: Summary report and recommendations for Naval Construction Battalion Center, Gulfport, Mississippi. Air Force Technical Report OEHL-TR-79-169, USAF Occupational and Environmental Health Laboratory, Brooks AFB, Texas. 36 p.

10. Young, A. L., C. E. Thalken and D. D. Harrison. 1979. Persistence, bioaccumulation and toxicology of TCDD in an ecosystem treated with massive quantities of 2,4,5-T herbicide. Paper presented to the Symposium on the Chemistry of Chlorinated Dibenzodioxins and Dibenzofurans. American Chemical Society, 178th National Meeting, 14 September 1979, Washington DC. 24 p.
11. Young, A. L., C. E. Thalken and W. E. Ward. 1975. Studies of the ecological impact of repetitive aerial applications of herbicides on the ecosystem of Test Area C-52A, Eglin AFB, Florida. Air Force Technical Report AFATL-TR-75-142, Air Force Armament Laboratory, Eglin AFB, Florida. 127 p.