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Item ID Number: 00104

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Report/Article Title Persistence, Bioaccumulation and Toxicology of TCDD in an Ecosystem Treated
With Massive Quantities of 2,4,5-T Herbicide

Journal/Book Title

Year 1979

Month/Day September

Color

Number of Images 24

Description Notes SAF/OIS Document 791336; AFSC Document 79-890

PERSISTENCE, BIOACCUMULATION AND TOXICOLOGY OF TCDD IN
AN ECOSYSTEM TREATED WITH MASSIVE QUANTITIES OF 2,4,5-T HERBICIDE

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Abstract and Presentation

Symposium on the Chemistry of Chlorinated
Dibenzodioxins and Dibenzofurans

DIVISION OF PESTICIDE CHEMISTRY
AMERICAN CHEMICAL SOCIETY
178th ACS National Meeting
13 - 14 September 1979
Washington DC

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SAF/OIS DOCUMENT 791336
AFSC DOCUMENT 79-890



ABSTRACT

PERSISTENCE, BIOACCUMULATION AND TOXICOLOGY OF TCDD IN AN ECOSYSTEM TREATED WITH MASSIVE QUANTITIES OF 2,4,5-T HERBICIDE*

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Field investigations were conducted during 1973-1978 on a 3.0 Km² military test area (Test Area C-52A, Eglin Air Force Base, Florida) that received 73,000 kg 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) herbicide during the period 1962-1970. No residues of 2,4,5-T were detected (detection limit of 10 ppb) in any soil samples collected during 1971-1972. However, residues of the contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were still present in 1978.

During the period 1974-1978, 54 soil samples were collected to a depth of 15 cm from throughout the test area. TCDD levels ranged from <10 to 1,500 parts per trillion (ppt). The median concentration was 30 ppt while the mean was 165 ppt. The ecological survey extending over a five-year period documented the presence of at least 123 different plant species, 77 bird species, 71 insect families, 20 species of fish, 18 species of reptiles, 18 species of mammals, 12 species of amphibians and 2 species of mollusks. At least 250 biological samples were analyzed for TCDD, including 30 species of animals. No TCDD was found in any of the plant species examined. However, TCDD was found in nine species of animals including two rodent species: beachmouse (300-2,400 ppt, liver) and hispid cotton rat (<10-210 ppt, liver); three species of birds: meadowlark (100-1,020 ppt, liver), mourning dove (50 ppt, liver), and Savannah

*Abstract, American Chemical Society, Division of Pesticide Chemistry, Washington, D.C., 10-15 September 1979.

sparrows (69 ppt, liver); three species of fish: spotted sunfish (85 ppt, liver) mosquitofish (12 ppt, whole body) and sailfin shinner (12 ppt, whole body), and one reptile, the six-lined racerunner (360-430 ppt, muscle).

Gross pathology was done on all species collected for TCDD residue analyses. Histopathological examinations were performed on over 300 beach-mice or hispid cotton rats from the test area and a control field site. 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. Initially, the tissues were examined on a random basis without the knowledge of whether the animal was from a control or test area. All microscopic changes were recorded, including those interpreted as minor or insignificant. The tissues were then reexamined on a control and test basis, which demonstrated that the test and control mice could not be distinguished histopathologically. Similar histopathological studies were conducted on the fish and racerunner, and again no significant abnormalities were found.

PERSISTENCE, BIOACCUMULATION AND TOXICOLOGY OF TCDD IN
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Concern over the level of contamination of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) herbicide by the teratogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has prompted discussion on the safety of using 2,4,5-T in forest and rangeland environments. Although numerous reports have recently appeared in the scientific literature, most of these deal with effects of 2,4,5-T and TCDD in laboratory systems.

In general the effects and mode of action of TCDD on laboratory animals can be characterized by a relatively small number of clinical signs. It is reported that a single oral dose (25 ug TCDD/kg) caused an actual weight loss for one week in young female rats and young male rats receiving the same dose had significantly decreased weight gain over a two week period. Slight thymic atrophy, related to TCDD dose levels, was a common finding in young mice receiving a single oral dose (50 ug TCDD/kg), while severe thymic atrophy in young mice receiving a single oral dose of TCDD (150 ug TCDD/kg) or four separate oral doses (25 ug TCDD/kg x 4) was reported. A single oral dose of TCDD (50 ug TCDD/kg) in young adult rats and (3 ug TCDD/kg) in young guinea pigs caused severe thymic atrophy. At these same dose levels slight to severe centrilobular liver necrosis and degeneration of parenchymal cells in mice, rats and guinea pigs, together with ceroid pigment deposits and hepatic

porphyria in mice given four oral doses of TCDD (25 ug TCDD/kg) at weekly intervals were seen. Acute death in guinea pigs has occurred following a single (3 ug TCDD/kg) oral dose of TCDD. A recent report indicated that four doses of TCDD (25 ug TCDD/kg) given at weekly intervals to young mice induced the production of δ -aminolevulinic acid (ALA) synthetase and hepatic porphyria.

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). This report, however, documents the effects of residual TCDD on selected animal populations inhabiting a unique test site: a site previously treated with massive quantities of 2,4,5-T herbicide and located on the Eglin Air Force Base Reservation, Florida.

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.

MATERIALS AND METHODS

Description of Field - Test Area C-52A (TA C-52A) covers an area of 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 of 2,4-dichlorophenoxyacetic acid (2,4-D) and 39,550 kg of 2,4,5-T. The herbicide was disseminated as the water insoluble n-butyl and iso-butyl esters (their military code names were Orange and Purple). Two other testing grids were sprayed with 2,4,5-T. 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 17,440 kg 2,4,5-T from 1968 through 1970. Both Grids II and IV received Herbicide Orange (a 50:50 mixture of the n-butyl esters of 2,4,-D and 2,4,5-T).

Despite excellent records as to the number of missions and quantity of herbicide per mission, there was no way to determine the exact quantity of herbicide deposited at any point on the instrumented grid. The first extensive soil sampling for residues of herbicides was initiated in 1969 for Grid I (five years after the last mission) and in 1970 for Grids II and IV. At that time traces (parts per billion) of 2,4,5-T were detected in soils of Grid I and in parts per million for Grids II and IV. Analyses for TCDD were initiated in 1972. By midsummer 1973 analysis of soil samples indicated that TCDD was detected only in the top 15 cm of soil (e.g., analysis of soil cores at 15 cm increments to a depth of one meter indicated no detectable TCDD in increments

below 15 cm. Therefore, fifty four sites on three Grids were sampled for TCDD in the top 15 cm increment. One of the sites was also subsampled at increments of 0-2.5, 2.5-5, 5-10 and 10-15 cm. Analysis of soil samples for TCDD was accomplished by three different commercial laboratories.

Vegetation - Detailed studies of the vegetative composition of Test Area C-52A were conducted in 1971, 1973 and 1976. Transect analysès were conducted on all test grids. Frequencies and densities of monocotyledonous and dicotyledonous species were determined. Representatives of all plant species were collected and mounted. Photographic records of numerous sites were maintained through the years of study.

Animals - Studies of the animals began in 1970. However, detailed investigations of the beachmouse, hispid cotton rat, and six-lined racerunners were conducted in 1973 and 1974. The beachmouse was further studied in 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 diets and residue analysis were conducted throughout all years of study.

RESULTS

Slides:

1. Test Area C-52A
2. Application Rates of 2,4,5-T
3. Types of Ecological Investigations
4. Survey of Species
5. Concentration of TCDD in Test Grid Soils
6. Disappearance of TCDD from Soils of Grid I
7. Investigations of Bird Species
8. Investigations of Insect Families

9. Investigations of Mammals
10. Studies of the Beachmouse - Control Area
11. Studies of the Beachmouse - Test Grid I
12. Mean Liver Weights of Pregnant Females
13. Histopathological Examination of Beachmice

DISCUSSION

The data suggest that TCDD may persist for long periods of time in the environment. However, caution must be exercised in making such a statement. As noted from the slides, it was probable that Grid I received highly contaminated herbicide. The herbicide was most likely produced in the 1950s or early 1960s and thus was subjected to preparation treatment different from those controlled procedures subsequently used. A conservative estimate for TCDD contamination may be 8 ppm in the formulation. Using the 8 ppm figure for all of the herbicide applied to Grid I, then the amount of TCDD applied would have been at a concentration equal to 12,267 ppt TCDD in the top 15 cm of soil. At least, this has declined to 710 ppt in about 8 years. This is a loss of about 95 percent. Thus, the apparent high residue is probably due to the massive quantities applied rather than to the resistance of TCDD to biological and/or physical degradation.

The levels of TCDD in the livers of beach mice and birds collected from the test grids substantiated bioaccumulation of TCDD; i.e., an accumulation of TCDD in an organism from its environment. In general, levels of TCDD in the livers were no greater than the most concentrated zones of TCDD in the soil; there are no data from this study to support biomagnification of TCDD; i.e., an increase in concentration of TCDD in successive organisms ascending the trophic food chain.

The only significant lesions seen on histopathologic examination of 255 adult and fetal beach mice were two instances of moderately severe multifocal, necrotizing, hepatitis and a single mouse with severe venous ectasia of the renal veins in one kidney. All other lesions were of the minor or insignificant type, normally observed in microscopic surveys of large numbers of field animals. The absence of liver lesions (necrosis and porphyria) in animals that had liver levels of TCDD from 300 ppt to 1,500 ppt is most significant in view of the massive quantities of both 2,4,5-T and TCDD that must have been applied to the test site. Moreover, a report of a previous study of this area, which terminated in the summer of 1970, indicated that a significant population of beach mice were inhabiting the test site.

The average life-span of a related species, Peromyscus maniculatus, has been recorded to be less than five months and only a few mice lived the full potential of three or more years. A single female beach mouse is capable of producing eighty or more young under laboratory conditions with litters being born at approximately 26 day intervals. It is further reported that beach mice on Santa Rosa Island, Florida (within 32 km of Test Area C-52A), may have produced 10 generations per year. At this frequency the animals collected in 1974 on Grid I may be 40 generations removed from the population first noted in 1970. However, a more conservative estimate would be 6 generations per year (giving a female 60 days to reach sexual maturity), for a total of 24 generations.

It must be stressed that the populations of beach mice noted in 1970 were probably subjected to much greater levels of residual TCDD in the soil than those animals collected in subsequent years. The absence of pathological signs in these mice indicated that TCDD was neither mutagenic (somatic or germinal) nor carcinogenic in the field at the concentrations noted and during the life

span of the animals studied. Since none of the 67 fetuses examined from animals captured on the test grid showed teratogenic defects it must also be concluded the levels of TCDD encountered failed to induce observable developmental defects.

TEST AREA C-52 A

EGLIN AIR FORCE BASE, FLORIDA

- . A TEST RANGE USED IN THE DEVELOPMENT OF
DEFOLIATION SPRAY EQUIPMENT FOR SOUTHEAST ASIA
- . HERBICIDES SPRAYED ON THE TEST AREA, 1962-1970.

TEST GRIDS AND QUANTITIES OF 2, 4, 5-T
APPLIED TO TEST AREA C-52A, EGLIN AFB FL

<u>GRID</u>	<u>AREA (HA)</u>	<u>YEARS</u>	<u>2, 4, 5-T (KG)</u>
I	37	1962 - 1964	39, 550
II	37	1964 - 1966	15, 890
IV	97	1968 - 1970	<u>17, 440</u>
		TOTAL	72, 880

ECOLOGICAL INVESTIGATIONS, TEST AREA C-52A
1973 - 1978

SOIL RESIDUES: 2,4,5-T, TCDD

TERRESTRIAL ECOSYSTEMS

VEGETATION

ANIMALS

VERTEBRATE

INVERTEBRATE

MICROORGANISMS

AQUATIC ECOSYSTEMS

ANIMALS

VERTEBRATE

INVERTEBRATE

MICROFLORA

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

170 BIOLOGICAL SAMPLES ANALYZED FOR TCDD

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

DISAPPEARANCE OF TCDD FROM SOILS OF GRID 1
(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)

INVESTIGATIONS OF BIRD SPECIES
Test Area C-52A

77 Species Observed

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

INVESTIGATIONS OF INSECTS
Test Area C-52A

71 Families Observed

<u>FAMILY</u>	<u>TCDD Residue Analysis (ppt)</u>
Grasshoppers	ND (3)*
Crickets	26
Composite of Soil/Plant Insects	40

*Detection Limit

INVESTIGATIONS OF MAMMALS , TEST AREA C-52A

<u>SPECIES</u>	<u>TCDD RESIDUE ANALYSIS (PPT)</u>		
	<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 - 1,500	
	PELT	130 - 140	

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

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	<u>67</u>
				Total	<u>184</u>

() Number of Pregnant Females
 Fetuses/Pregnancy = 3.1

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

<u>LOCATION</u>	<u>YEAR</u>	<u>LIVER WEIGHT (MG)</u>
Control	1973	929
	1974	765
	1975	934
	1978	919
Grid 1	1973	1,247
	1974	1,019
	1975	1,109
	1978	1,101

STATISTICALLY SIGNIFICANT!

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.