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FATE OF 2,3,7,8-TCDD IN AN ECOSYSTEM TREATED WITH MASSIVE  
QUANTITIES OF 2,4-D AND 2,4,5-T HERBICIDES

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PRESENTATION TO THE  
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FATE OF 2,3,7,8-TCDD IN AN ECOSYSTEM TREATED WITH MASSIVE  
QUANTITIES OF 2,4-D AND 2,4,5-T HERBICIDES

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Since 1970 hundreds of laboratory studies have been conducted on the toxic contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) found in trichlorophenol. Although numerous commercial products are made from trichlorophenol, including the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5,-T), controversy remains as to what extent the TCDD found in these products has impacted humans or their environment.

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 or their natural habitat are lacking. The problem of finding a field site where a wide population of animals 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 rangeland or for reforestation (1.1 to 2.2 kilogram (kg)/hectare (ha)). This presentation summarizes the effects of residual TCDD on the ecology of a unique test site: a site previously treated with massive quantities of 2,4,5-T and 2,4-D herbicides and located on the Eglin Air Force Base Reservation, Florida.

The Eglin Reservation has served various military uses, one of them having been the development and testing of aerial dissemination equipment in support of

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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 square kilometers ( $\text{km}^2$ ) 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 prompted 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 of approximately  $8 \text{ km}^2$  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  $3 \text{ km}^2$  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 at 1.5 to 3 m. 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 19.7C 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 a 0 to 3 percent slope. A typical one-meter 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 km<sup>2</sup>, 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 a 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 45° angle from those adjacent to it. Although this grid was discontinued after 2 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 (50 percent n-butyl 2,4-D, 30 percent n-butyl 2,4,5-T and 20 percent iso-butyl 2,4,5-T). Two other testing grids were sprayed with Herbicide Orange (50 percent n-butyl 2,4-D and 50 percent n-butyl 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-D and 15,890 kg 2,4,5-T from 1964 through 1966. Grid IV was the largest and final Grid established on Test Area C-52A. It was approximately 97 ha and received 20,000 kg 2,4-D and 17,570 kg 2,4,5-T from 1968 through 1970. Grid III was an experimental circular grid that received 1,300 kg 2,4-D from 1966 through 1970. Thus, for the four spray equipment calibration grids, a total of 73,000 kg 2,4,5-T and 77,000 kg 2,4-D were aeri ally disseminated during the period 1962-1970. No residues of 2,4,5-T or 2,4-D were detected (detection limit of 10 ppb) in any soil samples collected during 1971-72.

Results - Analysis of archived samples of the formulations sprayed on the calibration grids indicated that approximately 2.8 kg 2,3,7,8-TCDD were applied

as a contaminant of the herbicide. However, 2.6 kg of this TCDD were applied to a 37 ha test grid (Grid I) from June 1962 through July 1964. Levels of < 10 to 1,500 parts per trillion (ppt) could be found in the top 15 cm of soil 14 years after the last application of herbicide on this site. Nevertheless, analysis of 61 soil samples suggested that less than 1 percent of the TCDD remained on the test area. Photodegradation at the time of and immediately after aerial application probably accounted for much of the disappearance of TCDD, although wind and water erosion and biological removal may have also contributed to its disappearance. 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, a "list of species" was initiated for the test grids. Whenever a species was observed on or associated with the grids, that species was recorded. Over the years of observation, approximately 341 species or organisms have been observed and identified as 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. 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 (21) of the different species examined for TCDD residue have been positive. In general, the levels of TCDD in the organisms appeared to be close to the mean levels of TCDD found in the soils.

The ecological survey extending over a 5-year period (1973-1978) 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. No TCDD was found in any of the seeds of the plant species examined. However, TCDD was found in nine

species of animals including two rodent species: beachmouse (300-2,900 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 sparrows (69 ppt, liver); three species of fish: spotted sunfish (85 ppt, liver), mosquito fish (12 ppt, whole body) and sailfin shinner (12 ppt, whole body); two reptiles: six-lined racerunner (360-430 ppt, muscle), and the Eastern coachwhip (150 ppt, fat); and one amphibian (Southern toad, 1,360 ppt, whole body). In addition ground-borne spiders ere positive for TCDD (115 ppt) as were crickets (18-25 ppt) and insect grubs (240 ppt). These data verify that both bioaccumulation and bioconcentration of TCDD occurs. Indeed, in a special examination of liver tissue from 36 individual beachmice, a close relationship between soil and liver levels of TCDD was observed, i.e., high liver levels of TCDD were consistent with high soil levels of TCDD. Moreover, for beachmice bioconcentration factors (mean liver concentrations divided by mean soil concentrations) ranged from 6 for females to 18 for males. Whole body analysis of fetuses from test area females indicated apparent placental transport of TCDD. Histopathological examinations were performed on 255 adult or fetal beachmice from the test area and a control area. 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 blind study basis. All microscopic changes were recorded including those interpreted as minor or insignificant. The issues were then re-examined on a control versus test basis, which demonstrated that the test and control mice could not be distinguished histopathologically. 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 a litter every 26 days. At



this frequency, the animals collected in 1978 could have been 50 generations removed from the population studies in 1973. A two-factor (treatment and year) disproportional analysis of covariance of organ weights revealed that liver weights for pregnant beachmice from the test area were significantly heavier ( $P < .01$ ) than liver weights of pregnant females from the control area, and these differences were consistent over the 5 years of observation. 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.