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Item ID Number	03896 ☐ **Not Scanned**
Author	Thalken, C. E.
Corporate Author	Department of Life and Behavioral Science, United Stat
Report/Article Title	Absence of TCDD Toxicity to a Rodent Population Following Massive Field Applications of 2,4,5 -T Herbicide
Journal/Book Title	
Year	1975
Month/Day	July
Color	☐
Number of Images	41
Description Notes	Submitted July 1975 for publication in the Journal of the American Veterinary Medical Association

ABSENCE OF TCDD TOXICITY TO A RODENT POPULATION FOLLOWING MASSIVE FIELD APPLICATIONS OF 2,4,5-T HERBICIDE

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SUBMITTED JULY 1975 FOR PUBLICATION IN THE JOURNAL OF THE AMERICAN VETERINARY MEDICAL ASSOCIATION.

Absence of TCDD Toxicity to a Rodent Population Following Massive Field Applications of 2,4,5-T Herbicide

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SUMMARY

Field studies were conducted on populations of beach mice, Peromyscus polionotus, from a unique 92 acre military test site that received 87,186 pounds of active ingredient 2,4,5-trichlorophenoxyacetic acid herbicide (2,4,5-T). Significant levels (10-710 parts per trillion - ppt) of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were found within the top six inches of test site soils. Liver tissue from rodents inhabiting the test site contained 540-1,300 ppt TCDD. However, no gross or histological evidence of teratogenesis or toxicity was found in 106 adults and 67 fetuses. An analysis of variance of liver and spleen weights indicated significant differences between control and TCDD-exposed animals. Analysis of plant seeds revealed no detectable levels of TCDD (minimum detection limit of 1 ppt TCDD). TCDD accumulation in liver tissue was thought to be associated with pelt contamination from burrowing and subsequent ingestion of soil particles via grooming.

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The authors thank Lieutenant Colonel Harold W. Casey, Chief, Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, D. C. 20306, and his staff for histopathological contributions to this study.

The animals in this study were handled in accordance with the "Guide for the Care and Use of Laboratory Animals", 4th Ed. 1972, Institute of Laboratory Animal Resources (NAS-NRC), 2101 Constitution Avenue, N.W., Washington, D. C. 20418.

Presented before the Section on Public Health, 112th Annual AVMA Meeting July 14-17, 1975, Anaheim CA.

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)[3,4,6,10,11,13] has prompted discussion on the safety of using 2,4,5-T in forest and rangeland environments.[12] 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.[5,7,8,9,14,15,16]

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 µg 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.[8] Slight thymic atrophy, related to TCDD dose levels, was a common finding in young mice receiving a single oral dose (50 µg TCDD/kg),[7] while severe thymic atrophy in young mice receiving a single oral dose of TCDD (150 µg TCDD/kg) or four separate oral doses (25 µg TCDD/kg x 4) was reported.[5] A single oral dose of TCDD (50 µg TCDD/kg) in young adult rats and (3 µg TCDD/kg) in young guinea pigs caused severe thymic atrophy.[7] At these same dose levels slight to severe centrilobular liver necrosis and degeneration of parenchymal cells in mice,[5,7,8] rats[8] and guinea pigs,[8] together with ceroid pigment deposits and hepatic porphyria in mice given four oral doses of TCDD (25 µg TCDD/kg) at weekly intervals was seen.[5] Acute death in guinea pigs has occurred

following a single (3 μg TCDD/kg) oral dose of TCDD.[8] A recent report indicated that four doses of TCDD (25 μg TCDD/kg) given at weekly intervals to young mice induced the production of δ-aminolevinic acid (ALA) synthetase and hepatic porphyria.[5]

TCDD was not converted or metabolized by the three liver microsome NADPH systems in mice,[14] but rather, a major portion remained in the unmetabolized form in the liver, partially concentrated in the microsomal fraction of the cells.[14] It appeared that the unmetabolized compound, rather than a metabolite, was responsible for the toxic effect of TCDD and the endoplasmic reticulum of the liver was the possible site of toxic effect.[14] The fact that TCDD is a very potent porphyrogenic chemical is evidenced by the 2,000 fold increase in uroporphyrins following four oral doses of 25 μg TCDD/kg given at weekly intervals to young mice.[5] It may be through the combined direct toxic effect of TCDD on the liver endoplasmic reticulum plus the indirect toxic effect of TCDD on the ALA synthetase system and consequent production of toxic levels of porphyrins that significant liver damage is produced in mice.

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 quan-

tities of TCDD is improbable because of (1) low levels of TCDD (<0.1 ppm) found in cur ently produced phenoxy herbicide, and (2) low rates of 2,4,5-T applied for brush control on rangelands or for reforestation (1 to 2 pounds per acre). This report, however, documents the effects of residual TCDD on a rodent population inhabitating 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 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 one mile square 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 three square miles 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 one square mile and is a cleared area occupied mainly by broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), wooly 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 93 feet 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 61 inches. The mean annual temperature for the test area is ~~67.6~~ 19.8C F while the mean annual relative humidity is 70.8%. 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% slope. A typical three-foot soil core contained approximately 92% sand, 3.8% silt, and 4.2% clay with an organic matter content of 0.17%, an average pH of 5.6, and a cation exchange capacity of 0.8.

Although the actual area for testing aerial dissemination equipment was

approximately one square mile, the area of interest in this study was located in the southern portion of the testing area and consisted of a 92-acre 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 45^0 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 87,186 pounds of 2,4-dichlorophenoxyacetic acid (2,4-D) and 87,186 pounds 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). 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 on Grid I was initiated in 1969 (five years after the last mission).[17] At that time traces (parts per billion) of 2,4,5-T were detected.[17] Analyses for TCDD were initiated in 1972. By midsummer 1973 analysis of soil samples indicated that TCDD was detected only in the top ~~six inches~~ 15 cm of soil (e.g., analysis of soil cores at ~~6 inch~~ 15 cm increments to a depth of ~~three feet~~ 90 cm indicated no detectable TCDD in increments below ~~six inches~~ 15 cm).[17] Therefore, six sites on Grid I were sampled for TCDD in the top ~~six inch~~ 15 cm increment. One of the sites was also subsampled at increments of 0-1, 1-2, 2-4, and 4-6 inches. Analysis

of soil samples for TCDD was accomplished by a commercial laboratory.[a]

Animals.---The beach mouse, Peromyscus polionotus, is also referred to as the old field mouse. It is a small mouse weighing about 13 g, approximately 120 mm in length, with brown (adult) or dark gray (juvenile) fur on the back, and pale gray to white fur on the ventral region and legs.[1] It may be found in old field habitats and in areas of 5% to 60% vegetative cover, but prefers sandy areas.[2]

Burrow and Diet.---The structure of fifteen mouse burrows was determined by either preparing plaster casts of the tunnels and nests or by careful excavation of the burrow complex. When plaster of Paris was used it was poured into the hole, allowed to dry for 24 hours, and then the cast was freed by removing the soil around it. Data on the diet of the beach mouse were obtained by examination of litter both outside and within the tunnel and nest. In addition, an analysis of stomach content was performed on ten mice. Four 20 g samples of seed, composites from mature plants adjacent to burrows, were sent to a commercial laboratory for TCDD analysis.[a] The samples were taken from four areas on the test site.

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[a]Interpretive Analytical Services, Dow Chemical, U.S.A., Midland, Michigan, 48640. An LKB 9000S computerized gas chromatographic-mass spectrometer combination was employed.

Traps---Live traps,[b] sizes 0 and 1, for small mammals were used to obtain the rodents. These traps were baited with peanut butter and oatmeal. Some traps were randomly placed on the test grid where 20 to 80% vegetative coverage was present, while others were placed near openings to mouse burrows. Still other traps were placed in rectangular patterns of five rows of traps, each row located 20 paces apart, and containing five traps per row, at 15 pace intervals. Four areas approximately 200 to 1,000 yards off the grid were designated as control areas, and were trapped in the same manner as on the test grid. Traps were checked daily and were moved to other locations after four days failure to catch an animal. Captured mice were taken to the laboratory for histopathologic examination and chemical analysis of the tissues.

Tissue Preparation---All animals were prepared for examination using a cervical dislocation procedure to accomplish humane euthanasia. Euthanatized animals were photographed, weighed, measured, skinned, and systematically examined for developmental defects such as cleft palate, cleft lip, polydactyly and microophthalmia. All internal organs were examined for gross lesions, and individually weighed. Representative sections of each tissue were placed in neutral 10% buffered formalin and processed for microscopic study.[c]

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[b]Havahart Traps, Department 1, P.O. Box 551, Ossining, N.Y. 10562.

[c]Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, D. C. 20305.

All remaining liver tissues and the pelts from mice captured in the test and control areas were pooled according to sex ans maturity, placed in glass jars, frozen, and submitted for TCDD analysis.

RESULTS

Soil Analysis---Analysis of 6-inch soil cores for TCDD taken at six locations on the 92-acre area (Grid I) indicated wide fluctuations in TCDD concentrations. The results for the uniformly mixed top 6-inch increments were 10, 25, 70, 70, 110, and 710 parts per trillion (ppt) TCDD. Further analysis of a duplicate core, obtained from the site having 110 ppt TCDD concentration, indicated that TCDD was stratified within the top six inches of soil. The analysis for depths of 0-1, 1-2, 2-4, and 4-6 inches resulted in detectable levels of 150, 160, 700, and 44 ppts TCDD, respectively. These data are shown in Table I and are placed in perspective with hypothetical concentrations of TCDD which might occur with currently produced 2,4,5-T herbicide formulations containing 0.1 parts per million (ppm) TCDD and applied at normal rangeland or reforestation rates for brush control.

Trapping Data---In the eight weeks of trapping beach mice during the summer (June-July) of 1973 and six weeks during the summer (June-July) of 1974, 106 specimens were collected from either Grid I or a portion of a grid immediately north and slightly overlapping Grid I, and a control site. Since many of the females were pregnant at the time of collection, 67 fetuses were

recovered. This brought the total number of beach mice collected and examined over a period of two years to 173 (Table 2).

Burrows and Diet---From an examination of the burrows it was apparent that no two burrows were identical. However, most were characterized by a small mound of soil on the surface with a 2-inch diameter tunnel near the center, leading down and away from the surface at a 45 angle. A plug of soil was usually found within the first 10-12 inches of the tunnel entrance. Eight to ten inches beyond the plug, the tunnel leveled and continued horizontally for another 18 inches. There it expanded into a spherical chamber with a diameter of about 6 inches in which a nest was usually found: this placed the nest approximately 12 inches below the soil surface. Frequently, beyond the nest, the burrow turned upward and to one side while at the same time narrowing to its original 2-inch diameter. This "escape tunnel" normally extended to within 2-6 inches of the soil surface.

The nests were contructed of dried grasses (stems, blades, and inflorescences). The dominant grasses used for bedding were broomsedge and low panicum. The litter within the nests consisted of caryopsis hulls, leaf fragments, twigs, insect exoskeletons, insect wings, and snake scales. From a detailed examination of the caryopsis hulls, six species comprised the bulk of the vegetative portion of the diet: rough button weed, Diodia teres; spotted spurge, Euphorbia maculata; bitter polygala, Polygala polygama; common

polypremum, Polypremum procumbens, and low panicum and switchgrass. Examination of insect parts indicated insects of the Orders Coleoptera (Beetles), Hemiptera (True Bugs), and Homoptera (Cicadas and Leaf-Hoppers, etc.). Based upon number of insect parts and seed hulls and upon each of their estimated weights, it was assumed that about 90% of the beach mouse diet was seeds, and the remaining 10% was made up of insects.

The results from the TCDD analysis of the four composite seed samples indicated that TCDD was not detectable in any sample (minimum detection limit of 1 ppt TCDD). The insects were not analyzed for TCDD.

Liver and Pelt Analysis---Results of liver and pelt analysis for TCDD are shown in Table 3. Samples of liver, as well as pelts, of mice taken from Grid I in which significant soil levels of TCDD were found, exhibited positive evidence of accumulation of TCDD. TCDD was also found in the pooled liver samples of both male and female control animals, although no TCDD was detected on their pelts.

Histopathology---A series of histological 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 mouse was from a control or test area. All microscopic changes, including those interpreted as minor or

insignificant, were recorded. For example the following types of lesions were interpreted as not significant or of a common finding when large populations of wild animals are surveyed histologically: variation of nuclear size (poikilotosis) and of cytoplasmic staining in liver tissue from both control and TCDD-exposed animals; subacute, focal myocarditis and sialitis in two separate mice captured from a control area; poikilotosis of acinar cell nuclei in a mucous salivary gland and poikilotosis of nuclei from the adrenal cortex of two separate TCDD-exposed mice; and sarcosporidiosis of skeletal muscle in one TCDD-exposed mouse. Following the recording of all microscopic findings, the tissues were re-examined on a control and test basis. Results of both studies determined that the test and control mice could not be distinguished on a histopathologic basis. Significant lesions were only found in two mice, one from a control area (Fig 1) and one from a test area (Fig 2). Both had a moderately severe, multifocal, necrotizing hepatitis. Sections from the liver of these animals were stained with a variety of stains in attempts to identify an etiologic agent. Neither bacterial nor fungal organisms nor ceroid pigments were demonstrated and the lesions were considered to be virus induced, as they resembled the lesions seen in viral hepatitis of laboratory mice. The gross lesions observed in the kidney of one other beach mouse proved to be severe ectasia of the renal veins (Figs 3, 4, 5, 6, 7). Microscopically, the vascular dilation was interpreted as being of little functional

significance. All other lesions observed in both control and test mice were minor and insignificant and of the type normally observed when a large group of animals are examined at the microscopic level.

Due to the small size of the beach mouse and since both mature and immature specimens were collected, it was most difficult to grossly dissect the thymus gland from salivary glands, fat, and regional lympth tissue. Upon histological examination it was found that the tissue samples contained one or more of the four tissues. Nevertheless, in those specimens that contained thymus tissue, no histological evidence of thymus atrophy was noted in either control or TCDD-exposed animals. Data on the gross weight of thymus tissue were not statistically analyzed because of the uncertainty as to what other tissues might be in the specimen.

Statistical Analysis---Tables 4 through 10 present statistical data for physical parameters on beach mice collected from a control site and the TCDD-exposed field site on Test Area C-52A. Pregnant females and sexually immature animals were excluded from statistical comparisons because of the widely varying differences in both body and organ weights. Although microscopic examination for ovarian follicles or sperm production confirmed sexual maturity, animals with total body weights of 10 grams or greater were considered mature and were included in statistical evaluations. Fourteen of 46 females were pregnant. Since their body weights ranged from 11.48

to 18.68 grams, inclusion of these animals into the population used for statistical comparisons would have distorted the data for body and organ weights. Table 4 presents the actual number (57) of beach mice used in the statistical analyses. Unfortunately, all females from the 1973 control site were either pregnant or immature.

Table 5 gives the mean total body weight by sex for mature beach mice captured in 1973 and 1974 from control and test sites. A matrix of F-values from the analysis of variance for total body weight for all possible combinations of treatment (location), sex, and year are given in Table 6. Statistically significant differences (95% probability level) existed only for sex, or a sex-year interaction. No treatment differences were noted. In general, female beach mice are heavier than male beach mice.

Table 7 presents the mean values for liver weight by sex for mature beach mice captured in 1973 and 1974 from control and test sites. The matrix of F-values from the analysis of variance for all possible combinations of treatment, sex and year are given in Table 8. Statistically significant differences were found for liver weights between sex (e.g., comparing control females collected in 1974, comparison II and III) and with the sex year interaction (e.g., comparing control 1973 males with control 1974 females). However, the comparison of 1973 or 1974 control males with 1973 or 1974 Grid I males (I with IV and II with V, respectively) yielded no significant

differences in liver weights. The comparison of liver weights for females collected from a control site in 1974 (III) with females collected in 1974 from the TCDD-exposed site (VII) indicated statistical significance at the 99% confidence level. Note from Table 7 that the mean values for liver weights would have suggested the results of the above comparisons.

Table 9 presents mean values for heart, lung, kidney and spleen weights collected in 1974 from beach mice inhabiting control and TCDD-exposed field sites. Statistical analyses of the weights for heart, lung, or kidney indicated no significant differences in the weights of these organs between sex or treatment. However, significant differences were noted for spleen weights between control and test site animals. A matrix of the F-values from the analysis of variance for spleen weights is given in Table 10. Note that the comparison of sex within the same treatment is not significant; e.g., I with II or III with IV. Differences are noted for all treatment comparisons; e.g., I with [redacted], I with IV, or II with [redacted], II with IV.

DISCUSSION

Soil Analysis---The application of massive quantities of 2,4,5-T herbicide to Test Area C-52A has created a unique field site in which to assess not only the ecological impact of the herbicide (2,4,5-T) but also the toxic contaminant TCDD. The method of application; i.e., by aerial dissemination, resulted in unequal distribution of the herbicide. Three major flight paths

intersected the 92-acre instrumented grid. If a soil sample was obtained from an area not under one of the flight paths or if it was obtained near the intersection of all three flight paths then the residue levels would be expected to vary significantly.

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 in Table 1, 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 4600 pounds of butyl esters of 2,4-D and 2,4,5-T applied per acre (equivalent to 947 pounds of 2,4,5-T acid) in the years from 1962-1964, the amount of TCDD applied was 0.0368 pounds per acre. This is 12,267 ppt TCDD in the top six inches of soil.[d] At the 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.

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[d]The value of 0.0368 pounds per acre = $4600 \times 8 \times 10^{-6}$. 0.0368 pounds of TCDD in 3×10^6 pounds per acre-foot of soil = $0.0368 \times 10^{12}/3 \times 10^6 = 0.0368 \times 10^6/3 = 36{,}800/3 = 12{,}267$ ppt.

Liver and Pelt Analysis---The presence of TCDD in the liver samples of both male and female mice collected from the control site in 1974 may have been due to high levels in one or more specimens in the pool of samples. Mice from the test area could have migrated to the periphery of the grid and wandered into the area designated as control. The closest point from the control site to the test area was 200 yards. A previous trapping study on this test site[17] reported that the mean random travel distance (or average habitat radius) for the beach mouse was 65 yards. The distance traveled on the longest radius observed was over 1000 yards, but this unusual observation was regarded as a freak occurence. However, it emphasizes that a mouse (or mice) could have been contaminated in this way, and thus have contaminated pooled samples analyzed for TCDD. Nevertheless, the use of these data as truly control data must be viewed with caution.

The levels of TCDD in the livers of beach mice collected from Grid I 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.

Although the concentration of TCDD on the pelts of beach mice from the test area was only 10-15% of that in their livers, it was apparent that the mice continually contaminated themselves by repeated movement in an out of their burrows. The soil data substantiated the presence of a zone of TCDD within the top six inches of the surface (Table 1). Thus, whenever an animal burrowed into the soil it was exposed to TCDD. Moreover, the sand plug located within a short distance of the tunnel entrance suggested that recurrent burrowing activity occurred even in established burrows. Likewise, the location of the escape tunnel suggested that even the nest itself may contain detectable levels of TCDD.

Histopathology---The only significant lesions seen on histopathologic examination of 173 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 <20 ppt to 1,300 ppt (Table 3) 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 previous study[17] 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.[2] 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.[1] It is further reported that beach mice on Santa Rosa Island, Florida (within 20 miles of Test Area C-52A), may have produced 10 generations per year.[1] 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 1974. The absence of pathological signs in mice collected in 1974 indicated that TCDD was neither mutagenic (somatic or germinal) nor carcinogenic in the field at the concentrations noted in Table 1. Since none of the 34 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.

As animals mature, the thymus gland undergoes gradual regression until in the adult it is often found only as a rudimentary structure. Because of the

age differences in animals captured from the field, it was impossible to obtain mean thymus weights. In the literature where thymic atrophy is reported following single oral doses of TCDD, young animals of a similar age were used for the laboratory study.[7] Microscopic examination of all thymus gland tissue from both control and TCDD exposed animals further substantiated the lack of thymic lesions in the field situation where animals were exposed, via their burrowing and grooming habits, to soil levels of TCDD as seen in Table 1.

Statistical Analysis---Although 32 control and 74 test animals were collected from the field, the population selected for statistical analyses was necessarily smaller (19 and 48, respectively). Removal of all immature mice and pregnant females from the population reduced the range of variability between individuals. However, it also eliminated the data on control females collected in 1973. This is important to note since the only remaining comparison of liver weights between females were those collected in 1974 from the control site and Grid I and which were significant at the 99% confidence level. Since the population numbers were low for this comparison (4 versus 4), caution must be used in the interpretation of the results (histological examination did not support differences in liver tissue between control and test animals).

Data on spleen weights between the control and Grid I mice were statistically significant (Table 10). It has been reported[6] that rats given 10.0 μg/kg became moribund or died between 17 and 31 daily treatments. Remarkable changes were consistently observed in the spleen and lymph nodes. These changes consisted of a relative depletion of lymphoid cells and pyknosis of the nuclei and degenerative change in the multinucleated megakaryotic type giant cells of the spleen. In the present study, an increase in spleen weight was found in those animals (male and female) collected from the TCDD-exposed field site. However, as with the liver data, histological examination (gross and microscopic) of the spleens did not support differences between the control and test animals. It is interesting to note the magnitude of the standard deviations between spleen weights from the two locations. The magnitude of the differences in the standard deviation may reflect the fluctuations in soil levels of TCDD throughout Grid I. Thus, not all animals from the test site received the same exposure levels. Because the population numbers were relatively large for this comparison (19 versus 48), and hence a measure of their reliability, the data suggest that the spleen may be the most sensitive organ by which to assess field exposure to TCDD.

This report has reaffirmed that results from laboratory experiments and field studies present widely varying differences in biological organisms' responses to chemicals found in the environment. It should further serve as a ceveat regarding conclusions reached from laboratory experiments alone.

List of Figures

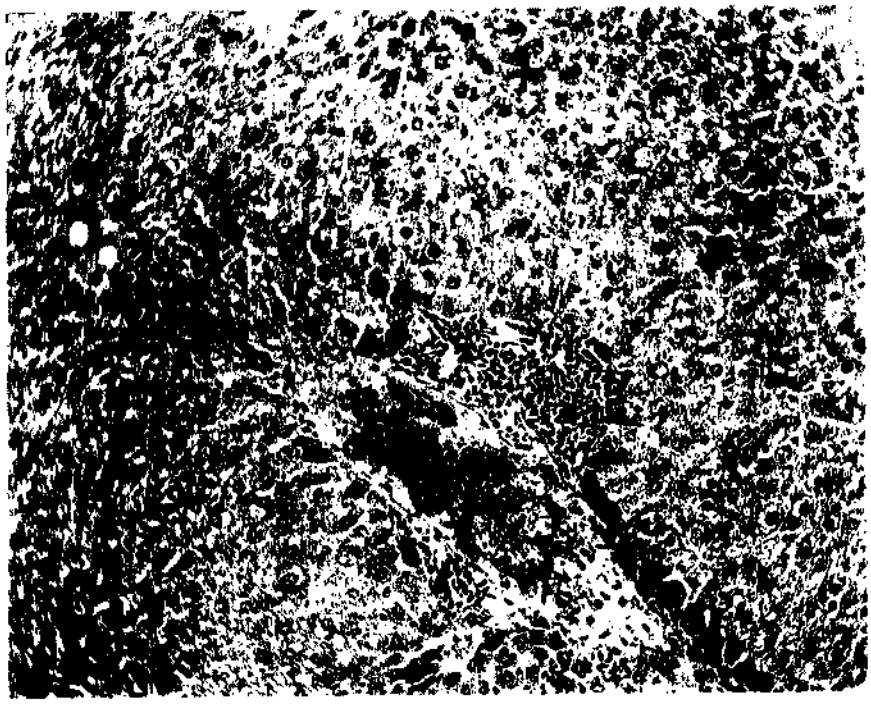

Fig 1---Hepatitis in a beach mouse captured from a control area. Inflammatory cells can be seen in the sinusoids and periportal areas. H&E stain; x 130. AFIP Negative No. 74-14883.

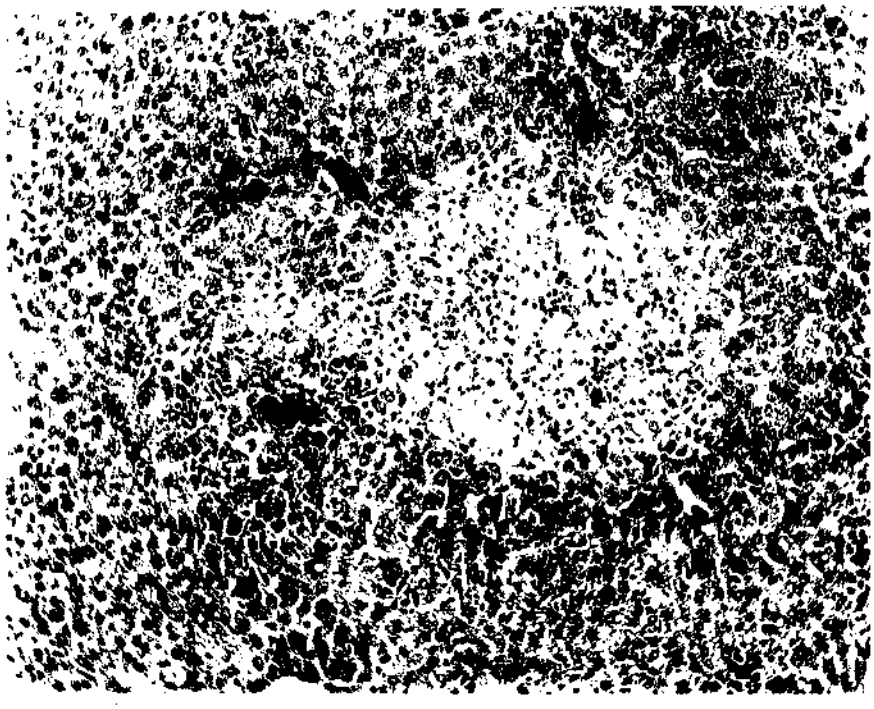

Fig 2---Acute necrosis and inflammation in the liver of a beach mouse captured from the test area. H&E stain; x 130. AFIP Negative No. 74-14874.

Fig 3---Gross illustration showing ectasia of renal veins on the left with the normal contralateral kidney on the right. Beach mouse was collected from test area. AFIP Negative No. 74-86541.

Fig 4---Close-up view of veins illustrated in Fig 3. AFIP Negative No. 74-86542.

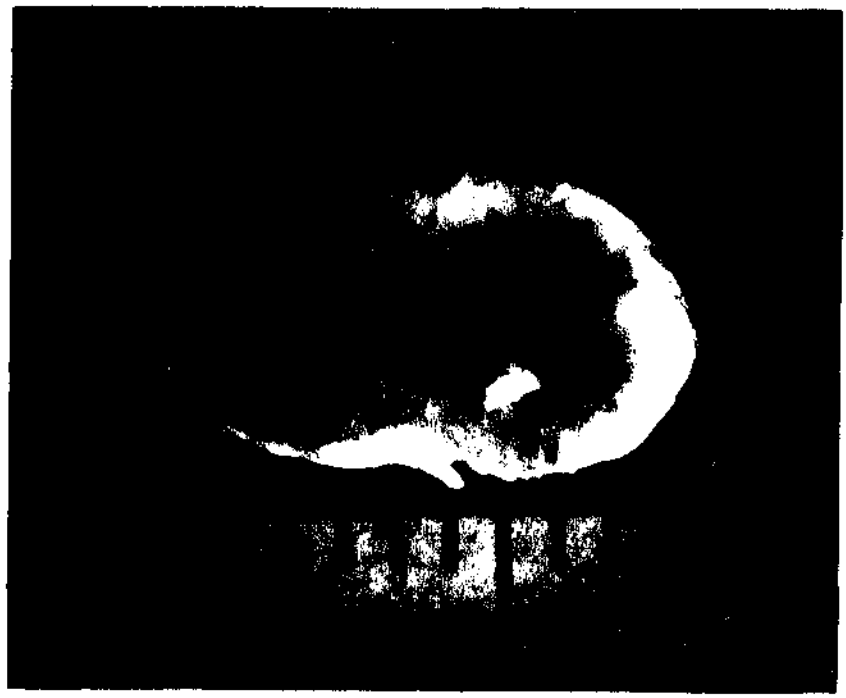

Fig 5---Close-up view of normal contralateral kidney illustrated in Fig 3. AFIP Negative No. 74-86543.

Fig 6---Microscopic appearance of venous ectasia in the kidney of the beach mouse shown in gross illustration Fig 3. H&E stain; x 90. AFIP Negative No. 74-14876.

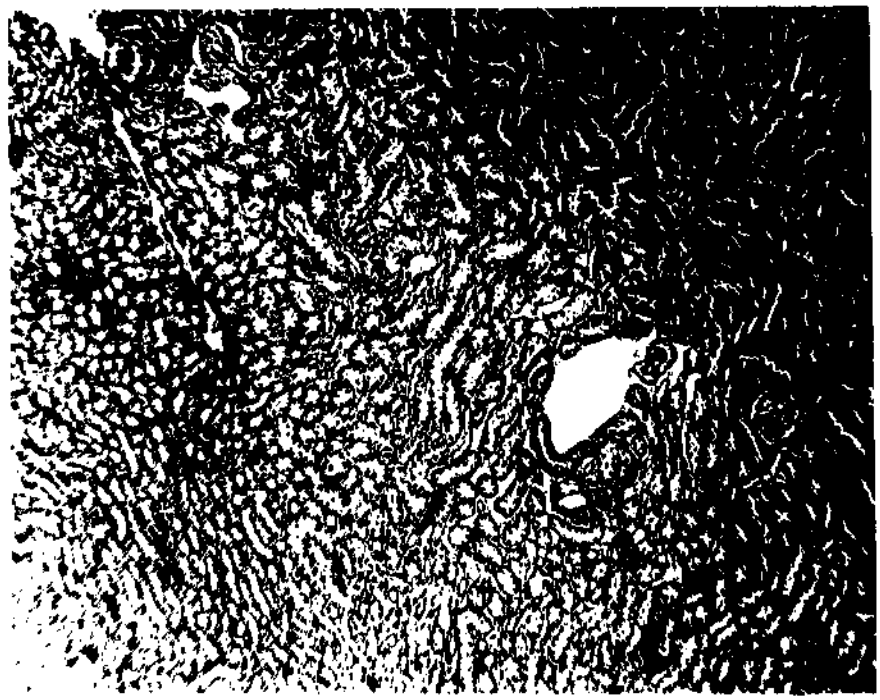

Fig 7---Microscopic appearance of a normal kidney from a beach mouse captured on the test area. Compare this figure with Fig 6. H&E stain; x 90. AFIP Negative No. 74-14881.

TABLE 1. Comparison of 2,4,5-T/TCDD Application Rates to Rangelands (Normal Use) Versus Rates Applied to Grid I, Test Area C-52A, Eglin AFB, Florida (Military Aerial Spray Equipment Test Program)

SUBJECT	NORMAL RANGELAND USE	GRID I APPLICATIONS (1962-1964)
Pounds 2,4,5-T Active Ingredient Per Acre	2	947
Total For 92 Acre Area	184	87,186
TCDD Concentration of 2,4,5-T Formulation	< 0.1 ppm (Current Production Standards)	< 0.1 - 47 ppm*
Concentration of TCDD in Soil Profile (parts per trillion):		
0-1 inch	0.8 ppt**	150 ppt***
1-2 inches	Not Detectable	160 ppt
2-4 inches	Not Detectable	700 ppt
4-6 inches	Not Detectable	44 ppt
Below	Not Detectable	Not Detectable

* Range of TCDD Contamination in Herbicide Stocks Returned from Southeast Asia in 1971 and stored on Johnston Island, Pacific Ocean. (In Disposition of Orange Herbicide by Incineration, November 1974, Department of the Air Force Final Environmental Statement.)

** Assuming no TCDD degradation and the application of 2,4,5-T to bare soil. If two pounds 2,4,5-T, containing 0.1 ppm TCDD, are applied and uniformly mixed into top one inch of an acre of soil, then $2 \times 0.1 \times 10^{-6}$ pound TCDD per acre in one inch of soil weighing 3×10^{6} pound per acre-foot or about 0.25×10^{6} pound per inch acre equals $0.2 \times 10^{-6} / 0.25 \times 10^{6}$ or 0.8×10^{-12}.

*** Soil profile samples collected in 1974.

TABLE 2---Numbers of Beach Mice Collected During the Summer 1973 and 1974 Studies of Control and TCDD-Exposed Field Sites

	1973	1974	
CONTROL			
MALE	5	12	17
FEMALE	5	10	15
FETUSES	12	11	33
		SUBTOTAL =	65
TEST			
MALE	26	17	43
FEMALE	18	13	31
FETUSES	25	9	34
		SUBTOTAL =	108
		TOTAL =	173

TABLE 3---Concentration (parts per trillion) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in Liver and Pelt Samples From Beach Mice, Peromyscus polionotus Collected From Control and TCDD-Exposed Field Sites, 1973 and 1974.

TREATMENT	YEAR	SEX	LIVER	PELT
CONTROL	1973	MALE AND FEMALE	< 20*	N.D.**
CONTROL	1974	MALE	51	< 40*
		FEMALE	83	< 40*
GRID I	1973	MALE AND FEMALE	540	N.D.**
GRID I	1974	MALE	1,300	130
		FEMALE	960	140

* Minimum level of detection.

** Not determined.

TABLE 4---Number of Peromyscus polionotus Used in Statistical Comparisons of Populations Collected From Control Site and TCDD-Exposed Field Site. Pregnant Females and Immatures Excluded

LOCATION	SEX	1973	1974
CONTROL	MALE	4	11
	FEMALE	0*	4
TREATMENT	MALE	12	14
	FEMALE	8	4

* All females were either pregnant or immature (See text).

TABLE 5---Mean Values For Total Body Weight (Grams) of Peromyscus polionotus Collected From Control Site and TCDD-Exposed Field Site. Pregnant Females and Immatures Excluded.

LOCATION	SEX	1973	1974
CONTROL	MALE	11.88 ± 1.03	12.02 ± 1.21
	FEMALE	---*	11.77 ± 1.13
TREATMENT	MALE	12.17 ± 1.13	11.49 ± 0.93
	FEMALE	13.28 ± 2.27	14.05 ± 2.21

* All females were either pregnant or immature.

TABLE 6---Matrix of F-Values From Analysis of Variance of Total Body Weight for Peromyscus polionotus Collected From Control Site and TCDD-Exposed Field Site.

MATRIX NUMBER	LOCATION	SEX	YEAR	MATRIX NUMBER I	II	III	IV	V	VI	VII
I	CONTROL	MALE	1973	---	F = 1.37 ns*	F = 1.19 ns*	F = 1.20 ns	F = 1.24 ns	F = 4.80 P .10	F = 4.58 ns
II	CONTROL	MALE	1974		---	F = 1.15 ns	F = 1.14 ns	F = 1.70 ns	F = 3.50 P .05	F = 3.34 P .10
III	CONTROL	FEMALE	1974			---	F = 1.01 ns	F = 1.47 ns	F = 4.04 ns	F = 3.85 ns
IV	GRID I	MALE	1973				---	F = 1.48 ns	F = 4.01 P .025	F = 3.82 P .05
V	GRID I	MALE	1974					---	F = 5.94 P .005	F = 5.67 P .025
VI	GRID I	FEMALE	1973						---	F = 1.05 ns
VII	GRID I	FEMALE	1974							---

*Not significant at a probability of less than .10 (i.e., 90% confidence level)

TABLE 7---Mean Values For Liver Weight (Milligrams) of Peromyscus polionotus Collected From Control Site and TCDD-Exposed Field Site. Pregnant Females and Immatures Excluded.

LOCATION	SEX	1973	1974
CONTROL	MALE	707.50 ± 143.61	611.00 ± 111.34
	FEMALE	--- *	678.25 ± 26.29
TREATMENT	MALE	861.11 ± 263.36	664.79 ± 150.54
	FEMALE	1115.00 ± 355.65	860.25 ± 151.90

* All females were either pregnant or immature.

TABLE 8---Matrix of F-Values From Analysis of Variance of Liver Weight For *Peromyscus polionotus* Collected From Control Site and TCDD-Exposed Site.

MATRIX NUMBER	LOCATION	SEX	YEAR	MATRIX NUMBER I	II	III	IV	V	VI	VII
I	CONTROL	MALE	1973	---	F = 1.66 ns*	F = 29.85 P .01	F = 3.36 ns	F = 1.10 ns	F = 6.13 P .10	F = 1.12 ns
II	CONTROL	MALE	1974		---	F = 17.94 P .025	F = 5.59 P .01	F = 1.83 ns	F = 10.20 P .005	F = 1.86 ns
III	CONTROL	FEMALE	1974			---	F = 100.39 P .005	F = 32.80 P .01	F = 183.07 P .005	F = 33.39 P .01
IV	GRID I	MALE	1973				---	F = 3.06 P .05	F = 1.82 ns	F = 3.01 ns
V	GRID I	MALE	1974					---	F = 5.58 .005	F = 1.02 ns
VI	GRID I	FEMALE	1973						---	F = 5.48 P .10
VII	GRID I	FEMALE	1974							---

*Not significant at a probability less than .10 (i.e., 90% confidence level).

TABLE 9--- Mean Values for Heart, Lung, Kidney and Spleen Weights (Milligrams) for Peromyscus polionotus Collected in 1974 from Control Site and TCDD-Exposed Field Site. Pregnant Females and Immatures Excluded.

		ORGAN			
LOCATION	SEX	HEART	LUNG	KIDNEY	SPLEEN
CONTROL	MALE	100.55 ± 12.98	98.64 ± 13.82	191.27 ± 20.12	16.82 ± 6.42
CONTROL	FEMALE	91.75 ± 19.82	114.25 ± 33.98	197.25 ± 30.90	21.75 ± 4.65
GRID I	MALE	93.93 ± 20.75	99.93 ± 21.04	193.50 ± 20.33	23.07 ± 14.66
GRID I	FEMALE	104.50 ± 13.23	96.00 ± 17.15	226.50 ± 24.77	24.75 ± 20.34

TABLE 10---Matrix of F-Values from Analyses of Variance of Spleen Weight for Peromyscus polionotus Collected in 1974 from Control Site and TCDD-Exposed Field Site.

MATRIX NUMBER	LOCATION	SEX	MATRIX NUMBER I	II	III	IV
I	CONTROL	MALE	---	F = 1.91 ns*	F = 5.22 P .01	F = 10.05 P .005
II	CONTROL	FEMALE		---	F = 9.96 P .05	F = 19.16 P .025
III	GRID I	MALE			---	F = 1.92 ns
IV	GRID I	FEMALE				---

* Not significant at a probability of less than .10 (i.e., 90% confidence level).

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