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ENVIRONMENTAL MONITORING AFTER AERIAL APPLICATION OF 2,4,5-T HERBICIDE: THE BLODGETT FOREST ENVIRONMENTAL MONITORING PROJECT - ~~A SUMMARY~~ FINAL REPORT

REPORT NO. 81-

Compiled and Edited by
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SUMMARY

CONTENT

II. ACKNOWLEDGEMENT

The project was a cooperative effort, which was initiated by the California Forest Protective Association, in cooperation with the California Departments of Food and Agriculture and Fish and Game and the University of California, Davis. Subsequently, the Fisheries and Wildlife Management Staff, Pacific Southwest Region and the Forest Pest Management Staffs, Pacific Southwest Region and Washington, D.C., Forest Service, helped coordinate and support the project. Dr. Anthony S. Wong, California Analytical Laboratories, Inc., Sacramento, CA, under contract to the California Department of Food and Agriculture, did the chemical analyses of 2,4,5-T in soil, air, water and plant and animal (deer) tissue samples. He also did all low resolution analyses for TCDD residues from mylar panels, soil, air, water, and vegetation samples. Aerial application of 2,4,5-T was accomplished by Evergreen Helicopters, McMinnville, Oregon, under contract to the University of California, Berkeley. The Forest Insect and Disease Methods Application Group, Forest Service, Davis, CA, calibrated the spray equipment and assessed herbicide distribution. Dr. Logan Norris, Principal Chemist, Pacific Northwest Forest and Range Experiment Station, Corvallis, Oregon, provided technical assistance on deer tissue sampling procedures. Dr. Ralph Bosss, Assistant to the Administrator for Agricultural Research, Science and Education Administration (SEA), USDA, Washington, D.C. provided technical assistance on chemical analysis procedures for 2,4,5-T and TCDD and helped coordinate participation by the US Environmental Protection Agency, Michael Dellarco, Coordinator, Dioxin Monitoring Program, Special Pesticide Review Division, U.S. Engironmental Protection Agency, Washington, D.C. arranged for validation of results of TCDD analyses at the University of Nebraska Laboratory.

Principal cooperators and their roles were as follows:

1. California Department of Food and Agriculture, Environmental Monitoring and Pest Management, Sacramento, CA: Monitoring of 2,4,5-T in soil, air, water, plant and deer tissues.

   H. V. Cheyney, Area Supervisor

C. M. Walby, Agricultural Inspector
R. E. Shields, Agricultural Inspector
T. M. Mischke, Special Consultant
R. E. Gallavan, Statistician

2. California Department of Fish and Game, Pesticides Investigations Unit, Sacramento, CA: Captive deer herd, deer behavior and deer tissue sampling.

David Zeiner, Wildlife Biologisst
Arthur Bischolt, Wildlife Pathologist
William Griffith, Supervisory Biologist
Timothy Curtis, Associate Marine Biologist

3. University of California, Department of Forestry, and Conservation, Berkeley: Management of study area, aerial application of herbicide, and pre- and post-spray assessment of chanoes in plant and small animal communities.

Reginald H. Barrett, Assistant Professor, Forestry and Resource Management
Robert Heald, Asssistant Professor, Forest Management and Manger, Blodgett Experimental Forest

4. University of California, Department of Environmental Toxicology, Davis: Fate of TCDD in the Environment.

Donald G. Crosby, Professor, Environmental Toxicology

5. University of Nebraska, Department of Chemistry, Lincoln: Ultratrace analysis of TCDD in deer and plant tissues.

Michael L. Gross, Professor, Chemistry

6. U.S. Environmental Protection Agency, Pesticides Monitoring Laboratory, NSTL/NASA St. Louis, Mississippi: Preparation of deer

tissue extracts for TCDD analysis.

Aubry F. Dupuy, Jr., Chemist, Pesticides Monitoring Laboratory

7. U.S. Environmental Protection Agency, Research Triangle Park labboratory, North Carolina: Ultratrace analysis of TCDD in deer tissues.

Robert Harless, Chemist

8. USDA Forest Service, Pacific Southwest Region: Overall coordinator.

Hugh C Black, Wildlife Biologist

(1)

INTRODUCTION

In California, in the mid-1970's, the public and the legislature were concerned about the use of phenoxy herbicides in forests and rangelands and their potential to harm human health and the environment. One of the two principal periods of release spraying occurs coincident with the fall hunting season. Attention was focused on the possibility of bioaccumulation of 2,4,5-T or its contaminant 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), ~~which occurs at minute concentrations less than 0.1 ppm in 2,4,5-T~~ in deer tissue. Because of concern about spraying of phenoxy herbicides during the deer season and because of label restrictions prohibiting "slaughter of meat animals grazing on treated areas within 2 weeks after application, the California Department of Health, in October 1976, recommended against consumption of deer harvested from sprayed areas within this time period. Thus, in 1976 and 1977, Departments of Health and Fish and Game both opposed 2,4,5-T spraying on forest lands during the deer hunting season. In July 1978, the Department of Health reversed its position, and both Departments withdrew their opposition to ~~restricting~~ phenoxy herbicide spraying ~~before or during the deer season.~~

In 1977-78, the California Departments of Food and Agriculture and Fish and Game, in cooperation with the California Forest Protection Association and the University of California, Davis, attempted to monitor release-spray projects on private forest lands in northern California. Because of public opposition to aerial application of phenoxy herbicides, restrictive regulations and inclement weather, only 117 acres were sprayed in spring 1978, and monitoring results were inconclusive.

The U.S. Forest Service, Pacific Southwest Region, joined in this cooperative monitoring program in March 1978, primarily to help resolve residue questions related to spraying during the deer hunting season.

* Obtaining conclusive data about 2,4,5-T or TCDD residues in deer is extremely difficult

1 Because deer are both resident and migratory ~~and because~~ it is difficult to determine whether deer have been directly exposed to spraying during operational conditions, when or how long they have used the sprayed area, or what portion of time has been spent feeding and resting ~~occurs on~~ sprayed vs. adjacent unsprayed areas. 2 The cooperators undertook to study a "worst case" situation, by

(2)

~~confining~~ captive deer within a fenced plantation, spraying ~~the plantation~~ with 2,4,5-T (~~one third was unsprayed~~), and harvesting deer at intervals after spraying. The objective was to study the behavior or fate of 2,4,5-T and its contaminant TCDD in a forest environment and the possible accumulation of these compounds in tissue of deer exposed ~~under ordinary use conditions~~. Thus, the Blodgett Forest Monitoring project was undertaken in August 1978, with the California Departments of Food and Agriculture, the California Department of Fish and Game, the University of California, Berkeley, the University of California, Davis, the University of Nebraska, the EPA and the U.S. Forest Service cooperating.

(3)

- location?
Test site located on the Blodgett Experimental Forest near [illegible], Georgetown. Township & Range?

DESCRIPTION OF THE STUDY AREA

Physical characteristics of the site were similar on the untreated and treated portions of the area. The site consisted of 11 acres of ground which was flat with a gentle slope (6-9 percent) to the southeast. Mean elevation was about 1340 meters. Insolation averaged about 50 percent on both treated and untreated portions of the area.

We need to describe site enough so reader knows it is typical of that region + surroundings

The area was a 10 year old clear cut which had been replanted with white fir (Abies concolor) and giant sequoia (Sequoiadendron giganteum) which were being outgrown by an overstory of brush consisting primarily of deer brush (Ceanothus integerrimus), snowbrush, greenleaf manzanita, and whiteleaf manzanita. The brush ranged from 4 to 8 feet in height and the crop trees averaged 3 feet.

A water tower was located in the center of the rectangular plot for use by the crew.

PROCEDURES

SITE MANAGEMENT

Aerial Herbicide Application

A Hiller 12E helicopter was used to apply herbicide to approximately 7 acres of an 11-acre plantation on the University of California Blodgett Experimental Forest near Georgetown, California, on October 2, 1978. ~~Esteron 245 (2,4,5-T), provided by Dow Chemical, was opened and~~ samples were taken by the California Department of Food and Agriculture Environmental Assessment Team.

The helicopter was equipped with Spraying Systems Co. D8 nozzles mounted on a 40-foot boom. ~~Calibration of~~ the aircraft was ~~accomplished by charging the spray system with water, spraying until a pressure drop was noted, then adding a known amount of water to the system and measuring the time necessary to spray the known volume (detected by the second pressure drop). Boom pressure was maintained at 30 psig. After several such calibration trails, it was determined that 13 nozzles would be needed. Nozzle configuration was 45° to the rear. Calibration was completed at 9:30 a.m.~~ The pilot made a reconnaissance flight over the area, and spraying began at approximately 9:45 a.m. The herbicide was applied ~~at a rate of 5 gallons per acre, and the~~ area ~~was flown~~ twice to meet the desired application rate of 3 lbs. active ingredient in 10 gallons of water per acre. The first application was made with a north-south flight pattern, and the second and east-west pattern.

Meteorological conditions at 9:45 a.m. were almost perfect for spraying. The day was cloudless and sunny. The temperature at the time of spray application was 68° F, and winds were less than 3 mph. Conditions seemed very stable at ground level.

MONITORING OF 2,4,5-T IN PLANT TISSUES, WATER, SOIL AND AIR.

The study area, which was rectangular in shape and oriented along a

this should be in the site description page 3

north-south axis, was subdivided into a sprayed (8 acres) section and an unsprayed (3 acres) section (Figure ___), the south end of the area was not sprayed because of the presence of a small intermittent spring. A water tank was placed in the northeastern corner of the plantation (within the sprayed area) for use by the deer. A 9-foot high woven wire fence was installed to confine the deer to the area.

Sampling stations for all media were systematically located in a grid pattern consisting of three rows of seven stations each, which was distributed across the entire plantation (Figure ___). Stations were selected at random as required for sampling each of the media sampled.

what is breakdown between treated & untreated areas.

Intensive monitoring was conducted during the 6-week period from September 25, 1978 to November 2, 1978. Pre-application control samples were taken 1 week before herbicide spraying. The control sample consisted of one high-volume air sample from the site using XAD-4 resin as the sample media, one sample from each foliage sample station; one soil sample from each soil sample station, and one water sample from the spring in the southeast corner of the site.

Soil Sampling

Soil samples were taken from four randomly selected sample stations (Figure ___). Collected samples include the prespray control sample and soil samples taken 0, 1, 3, 8, 15, 21, 31, and 227 days after spraying. These samples were analyzed for 2,4,5-T residues by _____ ?

Foliage Sample

At each sampling period,
Two samples were taken from each of four foliage sampling stations (Figure ___). The first sample was taken on the south side of the sample station at about chest height. The second sample was taken on the north side of the sample station at about knee height. Only the foliage of deer brush (Ceanothus integerrimus) was collected, with each sample consisting of a combination of leaves and tender tips of twigs weighing about 20 grams. Foliage samples were collected day 0, 1, 3, 8, 15, 21, 31, and 227 days after spraying. These samples were sent to _____ for 2,4,5-T residue analysis.

An additional series of foliage samples were taken using a 1-inch diameter leaf punch. These also were taken from the four established foliage sample stations. Each sample consisted of 200 1-inch leaf punches, and each site was sampled on post-application day 1, 2, 4, and 8. Only greenleaf manzanita (Arcotostaphylos patula) was sampled with this technique because of the need for a leaf large enough to obtain a 1-inch punch. These samples were analyzed for TCDD residue by — ———.

Air Sampling

Air samples were obtained using both high and low volume air samplers. The high volume samplers (Staplex Model TF1A) were powered by portable gasoline generators located downwind of the instruments. High volume samplers were originally calibrated at 70 cubic feet per minute (cfm) at the factory but were not recalibrated before each sampling period because of a lack of calibration equipment and facilities. Air was drawn through 30 gram beds of Ambberlite XAD-4 (polystyrene, divinylbenzene copolymer) macroreticular polymer resin beeads (20/50 mesh Rohm and Haas, Philadelphia, PA) for two hours. After the samples were drawn, the resin was transferred to clean glass jars and placed on ice ~~in chests~~ for transport to the laboratory. The XAD-4 resin used as the capture media was cleaned before use by washing the resin beads with hydrochloric acid and water, then extracting with acetone in a soxhlet for 8 hours and finally drying the beads overnight in an oven.

The most intensive air sampling occurred on the day of the herbicide application. Three low volume air samplers were placed within the site along with two high volume air samplers. Three additional high volume air samplers were located at 1, 100 and 200 feet downwind of the site (Figure ___). An air sample was obtained by operating a sampler for 2 hours. Sampling began with commencement of herbicide application, and a second 2 hour sampling period began within 15 minutes of the completion of the first sampling period. ~~This was followed by second period of sampling which began within 15 minutes of the first period.~~ ~~Transpo Plots~~ Post-spray air samples were collected ~~about~~ from ~~four~~ samplers located at the edge of the spray plot at 2, 4, 8, 15 — 21 days after treatment. The samplers were operated for 2 hours from 6-8 AM and 12-1 PM providing two samples per sample day.

DEER BEHAVIOR AND DEER TISSUE SAMPLING

~~Experimental Animals.~~ Free-roaming, semi-tame or captive black-tailed deer

Water Sampling

Nylon Panels

(Odocoileus hemionus columbianus), were obtained for placement within the enclosure; two wild deer were collected by shooting for controls. A Palmer cap-chur gun with transmitter-equipped darts (wildlife materials, Carbondale, Illinois) was used to capture three of the free-roaming deer; all others were live-trapped or restrained by hand. Darts contained a mixture of 3.8 mg M-99 and 30 mg Rompun with 150 IU hyaluronidase. These darts, when used with a 3cc syringe, weighed about 30 grams and measured 16.4 cm in length plus a 19 cm trailing wire antenna. Because of the size of the darts, their effective range was restricted to a maximum of 40 feet (Zeiner 1979).

Two free-roaming deer were captured with darts and one animal was trapped at Growlersburg, less than 15 miles from the deer enclosure at Blodgett Forest. The remainder of the deer used in the pen study were captive animals obtained from the Placer Ecology Center, and Bidwell Park, Chico. One of the Chico deer was darted and the second was restrained by hand, the deer at the Ecology Center were held in 6-foot by 15-foot cyclone fenced pens with concrete floors. The Chico deer were in a 2-3 acre pen containing grass and shrubs.

All except one of the deer put in the enclosure were weighed and all had a fat biopsy taken for control. A fat biopsy was obtained from a small incision made over the cranial aspect of the thigh in the area of the prefemoral lymph node. This area contains a large amount of readily accessible fat, easily located and removed surgically in animals in good condition.

The surgical site was clipped and shaved and prepared with two scrubs of iodinated soap (Betadine Surgical Scrub), a rinse with 75 percent ethyl alcohol and a paint of tamed iodine (Betadine Solution). A disposable drape was placed over the site and a 2-3 ince incision was made with a scalpel. The prefemoral lymph node, surrounding fat and connective tissue were isolated and removed. Heat cautery on the vessels feeding the node was used and the incision was closed with a continuous pattern of #1 Dexon suture. A small gap for drainage was left at the distal aspect of the incision and Foracin antibacterial dressing was packed in the site at this point. Benzathine Penicillin, Procaine Pennicillin and Streptomcycin were injected intra-muscularly.

How long were the deer in the enclosure before treatment.

A radio collar was attached to each deer to assist in locating the animal in the enclosure. After being released in the enclosure, the deer were located at least once each week to establish if they were alive and well and to determine what plant species were being eaten and what areas of the enclosure were utilized. Observations were intensified before and after herbicide application, with the deer being located the day before, during, immediately after, and the day after application.

Deer Tissue Collection

Three deer were collected by shooting 48 hours after spraying. One of these deer had been in the enclosure 21 days, while the other two had only 13 days to become acclimated to the enclosure. Two of the deer taken at this time were wild deer and the other was from the Placer Ecology Center. To minimize the possibility of contamination from the enclosure environment, the deer were transported from the enclosure to the Blodgett headquarters where they were weighed, hung and skinned by a person who had not been on the spray site. A second person who wore rubber surgical gloves and who had not been in contact with the outside of the deer took samples of lung, liver, muscle, kidney, feces, rumen content, thyroid, blood and urine (when available) and placed them in prelabeled bottles for 2,4,5-T and TCDD analysis. The samples were then frozen. The middle incisor of each deer was removed for age determination.

Same
The deer collection, tissue sampling and handling process was repeated 2 weeks after spraying. The wild deer taken in this collection had been in the enclosure for 35 days; the other two deer obtained from Bidwell Park had been in the enclosure for 20 days before this collection. The final collection was made 4 weeks after spraying. These animals were all from the Placer Ecology Center and had been in the enclosure for 39 days before removal.

Where sampled

Two additional deer were collected by shooting from areas known to have not been sprayed with 2,4,5-T. Tissues from these deer were used for standards, spike samples and blank samples for the analyses.

2,4,5-T ANALYSIS IN DEER AND PLANT TISSUE

Tissue samples were received frozen and stored at -10°C for analysis. The samples (2-10 g) were thawed, homogenized with a Virtis Homogenizer, and a 2.0 g (± 0.05 g) subsample was placed in a disposable pyrex tube (1 cm x 10 cm). Methanolic potassium hydroxide (5 ml of a 0.1% solution) was added, the contents vortex mixed for 5 minutes, centrifuged, and the extract removed with a pipette. The tissues were again blended with 5 ml of methanolic potassium hydrxide, the combined extracts transferred to a 125 ml separatory funnel. After the addition of 50 ml of hexane-rinsed water, the mixture was adjusted to pH 10, rinsed with two 10 ml portions of diethyl ether (discarded), adjusted to pH 2 with sulfuric acid and then extracted with two 10 ml portions of diethyl ether. The pooled extracts were concentrated in a round-bottomed flask under vacuum with a rotary evaporator at 35°C. Ethereal diazomethane (prepared from Diazald according to the manufacturer's instructions, Aldrich) was added and the flasks were left at room temperature for about 30 minutes. The contents were transferred with hexane rinses to a graduated test tube, adjusted to the appropriate volume and analyzed by electron-capture gas-liquid chromatography using a MicroTek MT-220 instrument equipped with a 1.4 m x 2 mm (id) glass cloumn containing 1.5% OV-17/1.95% QF-1 on Gas Chrom Q under the following conditions: column, injector and detector temperatures were 140°C, 220°C and 290°C, respectively; the carrier gas (nitrogen) was held at about 30 ml/min.

What was the detection limit of this procedure?

Sample peak heights in the 50-200 pg range were compared with those of an authentic 2,4,5-T standard (Dow) methylated under identical conditions. One sample set (deer 3) was also analyzed with a 3 percent OV-225 column to confirm the correct identification of 2,4,5-T residues.

The validity of the analytical method was checked by spiking the control deer subsamples at 1.0 ppm. Results of this study confirmed the lack of interference at the 0.1 ppm level (all control samples were blank) and the validity of the method. After all the samples had been analyzed, a recovery study whereby each organ was spiked at the average level found for that organ was conducted. Since the average recovery exceeded 80 percent, the levels of 2,4,5-T found in the treated samples were not corrected (Table 2).

Statistical Methods

An analysis of variance (ANOVA) was performed on the data to evaluate the effects of the two factors, time and body part on concentration. Duncan's pair-wise comparison procedure was used to test for differences among treatments. When necessary, simple averages of available data were used to estimate missing units in order to complete the analysis of variance. Only one of the control deer was used for the 2,4,5-T analysis, and for this reason, the control was not included in the statistical treatment because of the two missing values from the first collection.

ANALYSIS OF TCDD IN DEER AND PLANT TISSUES

Sample Preparation and Custody

The various deer tissue samples and controls were digested in base, extracted with hexane, washed with sulfuric acid and submitted to small scale liquid chromatography ("acid-base" work-up) by Dr. Aubry Dupuy and his coworkers at the Pesticide Monitoring Laboratory, USEPA, in Bay St. Louis, Mississippi. Split samples of the extracts along with any controls were coded without regard to origin and shipped frozen in dry ice to the University of Nebrasks. Upon reception, the samples were stored in a freezer until they were analyzed. All arrived in perfect condition.

Various samples of washings of six Mylar panels and five leaf samples, which were collected after herbicide application at Boldgett Forest, were passed through a silica gel column and concentrated by Dr. Anthony S. Wong of California Analytical Laboratories.

Duplicate samples of extracts were retained in cold storage at the EPA Pesticide Monitoring Laboratory in Mississippi for analysis by an independent laboratory. Subsequently, a subset of these extracts were forwarded to the EPA Laboratory at Research Park Triangle, North Carolina, for analysis. Excess tissue samples were retained in cold storage at the EPA Laboratory in Mississippi.

Analysis of TCDD (University of Nebraska)

At the time of analysis, each sample tube provided by Dr. Dupuy was opened, the total volume measured using a 100ul syringe, and an aliquot removed (usually between 20 and 35% of the total sample) for gas chromatography/high resolution mass spectometry (GC/HRMS). The Mylar Panel and leaf washing were analyzed by simply removing an aliquot and injecting onto the GS/HRMS.

Each of the extracts was analyzed for tetrachlorodibenzo-p-dioxin (TCDD) using (GC/HRMS). The GC was a Perkin Elmer Sigma II and the mass spectrometer was a Kratos MS-50. The interface consisted of a single glass lined capillary tube followed by a capiliary leak directly into the mass spectrometer source. The source region was pumped with a 1300 l/sec diffusion pump and, thus, no molecular separator was employed. Typical source pressures were about 1×50^{-5} torr at the ion gauge located on the 6" pump tube.

The gas chromatographic column (180 cm x 2mm ID. glass) contained 0.60% OV-17 + 0.40% Poly S-179 coated on 100% methyl silicone bonded to 80/100 mesh Chromosorb W-AW, and was operated with a helium flow rate of 15 ml/min. The temperature was held at 250° C for 1.5 min and then increased linearly to 300°C at a rate of 10°C/min; the retention time of authentic TCDD was 3.3 min. The entire effluent from the gas chromatographic column was admitted to the mass spectrometer ion source and ionized using 70eV electrons. The source temperature was 250°C, the accelerating voltage 8kV, and the mass spectral resolution was 10,000 (10% valley definition). TCDD was quantitated by dual ion monitoring using peak matching. One channel was centered at m/z 327.8848 ($^{37}Cl_4$-TCDD, the internal standard) and the other at m/z 321.8936 (the most abundant molecular ion of TCDD having natural isotopic elemental abundances). The complete peak profiles were acquired by scanning at a frequency of 2Hz, corresponding in each case to a mass range of 300 ppm (0.096 amu). The output was accumulated using a Nicolet Model 1170 signal averager and the resulting signals were submitted to a 3-point smoothing routine prior to output to an X-Y recorder.

The concentration of TCDD was calculated using the ratio of the intensities (maximum peak heights) at m/z 327.8848 and 321.8936; if no signal was observed, the detection limit was set at 2.5 times the noise amplitude (a 2.5:1 S/N criterion). The calculation is made possible by obtaining the slope

of a calibration plot based on various standard mixtures of TCDD and TCDD-$^{37}Cl_4$. These standard mixtures were interspersed between unknowns and were analyzed in the same manner. The value of the slope was 1.5 ± 0.1.

Positives were validated by analyzing a second aliquot, this time monitoring 319.8966 (TCDD-$^{35}Cl_4$) and 321.8936 (TCDD-$^{35}Cl_3{}^{37}Cl$). The theoretical ratio for the intensities of these two ions is 0.77. Uncertainty in this measurement is ± 10% relative. Those "positives" giving a ratio less than 0.60 were not considered as validated, and, therefore, they were judged to be "negatives." The validation also presented the opportunity to quantitate the amount of TCDD in each "positive" sample. This was done using absolute mass spectrometer response factors determined by injection of known amounts of TCDD (native) and measurement of peak heights at m/z 321.8936. Because most recoveries are not 100 percent, the concentration of TCDD determined in this absolute manner will be too low, and it was corrected by dividing the concentration by the recovery expressed as a fraction. The agreement between the concentration determined by this method and by the ratio method is usually good.

The concentration of TCDD in the Mylar and leaf washing was determined only by this method as no standard TCDD-$^{37}Cl_4$ had been added to the solution. Thus the internal standard method was not applicable.

In December, 1980, we reextracted and analyzed six samples and two method blanks of deer tissue as part of the Bledgett Forest Study. The coded samples were submitted by TAC scientists. The identities of the samples were not known to us until after we had completed our analyses and data interpretation (January 9, 1981). The results, obtained using our standard methods of extraction and analysis, are reported in Table 3.

Validation of UN-L Analyses. To validate their initial results, the University of Nebraska reanalyzed a subset of deer tissue sample extracts, which were selected by the Forest Service. In addition, splits of a subset of the extracts analyzed by UN-L were now submitted to Robert Harless at the EPA Research Triangle Park Laboratory for analysis using his standard method of capillary column GC/high resolution mass spectrometry (Harless, 1980). In

all, UN-L reanalyzed three deer fat samples from deer Nos. 1, 10 and 11 and five quality control extracts.

RESPONSE OF PLANT AND SMALL ANIMAL COMMUNITIES

The experimental design involved sampling three untreated and five treated plots in September 1978, before spraying, and again in June, July and August 1979 after spraying. Each plot was a 0.28 ha circular area (radius 30 meters) with 40 circula subplots or elements (1.8 m radius) distributed at intervals of 5 meters in a systematic, radial pattern along eight transects from the plot center (Figure 1). These elements or subplots were used to sample small mammals, animal signs, and vegetation structure and species composition.

Vegetation Structure and Composition

Vegetation structure and species composition were measured on the 20 even-numbered elements. Species or plant groups were identified and measurements of vegetation, height and percent cover were taken. These data were used to calculate frequency of occurrence and mean percent cover. Observations on the utilization of browse species by deer and cattle were made using standard procedures described in FSH 2209.21, R-5, Range Environmental Analysis Handbook.

Small Mammal

One Sherman live trap was placed in each of the 40 elements in each sampling grid. Traps were baited with peanut butter and rolled oats and set for three consecutive nights during each sampling period. Captured animals were marked by notching one ear, and released. Capture per unit effort was calculated based on 120 trap-nights. Animal sign such as male hills and trails were recorded to indicate the presence of certain species. A circular tracking plot (0.8 m radius) also was cleared within 15 to 30 meters from the plot center. These plots were checked every other day for 6 days before or after each trapping period.

Birds

Monitoring was not initiated early enough in 1978 to adequately sample birds and herpts fauna before treatment. Visual and acoustic detections of birds present within 10, 20, and 30 meters of the plot center were made during each of twenty, 10-minute periods, beginning one half hour after official sunrise. Only different individuals were recorded within a 10-minute period. Birds were sampled in June, July and August 1981 after spraying. Birds were not sampled in sprayed areas before spraying.

Reptile and Amphibian

Reptiles and amphibians were sampled by installing one 5-gallon, plastic bucket on each plot for pitfall traps. Traps were checked daily for 12 days after completion of each trapping period in June, July and August 1979.

Spray Deposit Sampling

Spray deposit sampling was performed to provide data on the quality of spray application and deposit of spray in terms of size and number of drops and volume deposited on the ground. Sampling was accomplished by use of a special paper (3M Premium Automatic 209 Caopy Type 658, white, measuring 8" x 10"), which detects the presence of spray droplets.

The established grid was followed to locate three sampling lines, which were oriented across the long axis (N-S) of the spray area; line C was located near the spray boundary adjacent to the unsprayed area. An additional sampling line (D), which extended diagonally through the soutth or untreated portion of the study area, was established to monitor spray deposit outside the sprayed area. Additional samplers were also placed at 100-foot intervals around the fenced perimeter of the entire study area to monitor the amount of 2,4,5-T that was deposited outside the spray boundary. These lines were designated E (east), S (south), W (west), and N (north). All samplers were placed away from shrubs and elevated above low vegetation, which would have shielded the samplers. They were also elevated to avoid ground moisture.

Deposit samplers were placed in position the morning of spraying. After spraying, the cards were allowed to remain in full sun for approximately 2 1/2 hous to permit the stains to "develop" on the white paper. The spray deposit samplers were Usually assessed by use ing a hand-held 7x measuring magnifier.

RESULTS AND DISCUSSION

SITE MANAGEMENT

Spray Deposit Assessment

Examination of spray deposit samplers showed that the spray application was contained within the spray block boundaries. Spray deposition on the boundary samplers from lines N, E, S, and W were, with only a few exceptions, light or negative.

Results of the spray deposit assessment for lines A, B, and C are shown in Table 1. There were too few positive samples from the other sampling lines (D, N, E, S, and W) to provide summary data. Drop size data appeared consistent with results from other herbicide spray projects. The volume recovered form the samplers was lower than expected but may reflect the small sample size used during the assessment.

Deer Behavior and Deer Tissue Sampling

Sixty-six visual observations of individual deer were made in the enclosure during the study; 25 before spraying and 9 immediately after spraying when all but 2 deer were in the unsprayed area after fleeing from the helicopter. An additional 32 observations were made 1 or more days after the spraying. Before spraying, 64 percent of the observations of deer were in the side that was sprayed compared with 66 percent after the application. The presence of 2,4,5-T apparently did not deter the deer from using the area, since the diurnal distribution observed was the same before and after spraying. Deer were observed feeding in both sides, although no attempt was made to measure feeding behavior because of the disturbance associated with the interruptive effect of locating the deer. Observations of feeding deer suggested that preferences of the captive deer changed from apparently indiscriminate browsing, which was observed when the deer were first put in the enclosure, to selective feeding on preferred deer foods (deer brush and snow brush) as they become acclimated.

With the exception of the control deer, 2,4,5-T was detected in all animals sacraificed over the 4-week period (Table 3). Analysis of variance indicated that time of sampling was not related to the detection of 2,4,5-T in tissues (Table 4). Different tissues contained significantly different levels of 2,4,5-T. The nonsignificant interaction term between time and tissue indicated that the concentration of 2,4,5-T in the deer tissues did not change with the time of collection after herbicide application. The coefficient of variation was extremely high, but this was anticipated because of the uncontrolled variation in the condition, age and health of the deer and the small number of animals used.

The stomach had significantly higher amount of 2,4,5-T than all other body parts (Table 5). The amounts of 2,4,5-T found in the feces and kidney were not different from each other but were higher than those of the liver, blood, lung, thyroid and muscle. There were no significant differences in the amount of 2,4,5-T in the latter tissues, however. Significantly, samples of stomach contents, feces, urine and kidney contained statistically higher levels of 2,4,5-T, which were an order of magnitude greater than the levels in other sampled tissues. Similar results were reported by Newton and Norris (1968) for blacktail deer and by Leng (1977) for calves. Muscle tissue, normally considered the most edible portion of wild deer, contained the least amount of 2,4,5-T detected (31 ppb).

The absence of definitive human toxicity data for 2,4,5-T does not permit a precise estimate of potentially hazardous concentrations in animal tissues. The levels of 2,4,5-T that we found, especially those for the edible portions of the deer, are extremely low, however, and are not expected to be hazardous to human health.

The study design exposed the confined deer to greater amounts of 2,4,5-T than would normally occur in the wild. For example, the circular livestock watering tank placed in the enclosure was located in the sprayed area. No attempt was made to cover the tank during the application of 2,4,5-T or to replace the contaminated water with clean water after spraying.

Deer water use was not monitored, and no deer were observed drinking water

from either the tank or spring. Despite the high level of exposure to 2,4,5T, only minute amounts were found in muscle tissue. Most of the 2,4,5-T was found in the disgestive and urinary tracts from which it was being eliminated.

The deer were in poor to very poor condition when placed in the enclosure, except for the two deer from Chico that were in good condition. Because of the lack of body fat and our inability to obtain the quantity of fat needed for analyses (10 g) from any of the enclosure deer, the biopsy procedure to provide pre-spray samples was unsuccessful. Also, the biopsy operation is believed to have caused the weight loss observed the first two groups of deer collected (Table 1). Two of the three deer in the last collection had returned to their original weight.

All of the deer taken during the first two collections had varying amounts of lymph edema in the vicinity of the biopsy incision. Two deer had enough lymp. edema to cause visible swellings. Lymph edema was not present around the biopsy site of deer in the last collection.

MONITORING OF 2,4,5-T IN PLANT TISSUES, WATER, SOIL AND AIR

Foliage Samples

No TCDD was detected in any foliage samples of Arctostaphylos sp. [illegible] limit of — ppt.

All samples of Ceanothus foliage collected from the area of application were contaminated with 2,4,5-T on all sampling dates (Table ___). The detected levels of the herbicide 2,4,5-T 31 days after treatment represent a 98 percent reduction in the ester form, whereas the levels of the acid form present represent an approximate 61 percent loss. The 2,4,5-T levels on vegetation from the unsprayed section of the plantation were extremely low and virtually disappeared after 8 days (Table 1).

The Ceanothus sp. foliage samples from within the sprayed area contained significant levels of 2,4,5-T but no detectable TCDD throughout the 1-month post-application sampling period. Because Ceanothus sp. is a preferred food plant for deer and because the study animals were observed foraging on

Ceanothus within the sprayed area it can be assumed that the deer enclosed within the plantation ingested a significant amount of 2,4,5-T herbicide. Samples taken from the untreated end of the site show drift residues of 2,4,5-T that did not exceed 0.5 ppm. These sample stations were 65 feet (station 15) and 171 feet (station 16) from the nearest border of the treated portion of the site (Table 1).

Water Samples

Samples from the water tank showed the ester form of 2,4,5-T only during the first two periods of post-application sampling (Table ___). A sharp drop form the high value of 364 ppb ester to a level below detection limits occurred between 1 and 3 days after treatment. This decrease in the ester form of the herbicide occurred concurrently with an increase from 85 ppb to 249 ppb in the acid form. The acid was detected at high levels in the tank throughout the study period.

The high level of the ester form detected in the water tank samples on the date of application and following day seem reasonable considering the direct input of herbicide to the water. The sharp drop in ester levels and concurrent rise in acid form levels may be accounted for through the metabloic action of microorganisms. Because the tank provided a stagnant source of water a rapid growth in the population of microorganisms would be expected.

No ester form of the herbicide was detected in samples taken from the spring within the untreated portion of the area. Low levels of the acid form were detected in samples of spring water collected on the day of treatment and the following day. No herbicide was detected in the other samples.

The detected levels of herbicide from the spring in the untreated area were very low, indicating little contamination of this water source. Except for the 1.0 ppb acid form of the herbicide detected on the application date and the 0.3 ppb acid form on the next day, no other post-application sample produced detectable levels.

Soil Samples

No detectable levels of either ester or acid forms of 2,4,5-T were detected at the two sampling stations located within the untreated area (Table 3).

Detectable levels of both forms of the herbicide were recorded on all sampling dates at one or both stations within the treated area. Although no acid form was detected in samples taken 15 days after herbicide application.

Herbicide levels were detected in soil samples taken within the sprayed area during all sampling periods within 1 month after application. Levels of the ester form remained in the upper soil layer throughout the post-application period, with no indication of conversion to the acid form, except for samples taken 1 month after spraying at stations 8 and 11. Herbicide levels of the ester form decreased with time, however. - how much

Although detectable levels of herbicide were found in air and foliage samples from the untreated area, the soil samples from the same sampling stations did not produce detectable levels throughout the study period.

Air Samples

During the post-application air monitoring, widely separated morning and afternoon levels of 2,4,5-T ester were detected on all sampling dates, except in samples obtained 16 and 32 days after spraying (Table 5).

A gradient of decreasing amounts of 2,4,5-T ester was monitored at 1, 100, and 200 feet downwind of the sprayed area, and a total of 43 ug of material was collected just upwind at station 15 (Table 4). Similar results were obtained during an additional monitoring period from 1200 to 1400 in the afternoon despite no further aerial application. Low volume samplers were also used at stations 5 and 11 and produced detectable levels of 2,4,5-T. The high volume sampler at station 11 within the sprayed area did not produce the expected high levels of 2,4,5-T (Table 5).

During the post-application air monitoring, widely separated morning and afternoon levels of 2,4,5-T ester were detected sampling periods in all but the (17 October 1978) sampling date (Table 5). Afternoon levels 16 days after

spraying of 2,4,5-T ester were consistently high, with one exception, than the mornings. Herbicide levels in samples generally decreased over time until no herbicide was detected in samples one month after treatment.

The discrepancy between high volume and low volume sampler results at station 11 on the morning of the application cannot be explained. A value of only 3 ug 2,4,5-T ester was detected on the high volume sampler versus a 115 ug level from the low volume sampler located at the same site. The herbicide had been applied directly overhead and the high volume sampler with a flow rate of about 70 cubic feet per minute should have contained a large amount of 2,4,5-T. Without data on herbicide recovery efficiency from the sorbent, and instrument calibration, it would be inappropriate to use the air monitoring samples obtained with the high volume samplers as accurate estimates of the levels of 2,4,5-T present. However, they can be used as indicators of relative changes in levels of 2,4,5-T ester over time.

The results show that significant levels of herbicide drifted at least 200 feet downwind of the sprayed area during the morning hours after herbicide application.

During the post-application period, the herbicide was detected in decreasing amounts at station 11 over time. After 1 month, no herbicide was detected in the air during either morning or afternoon samples.

In summary, the 2,4,5-T ester application produced appreciable levels of the ester over the exposed surfaces in the treated area. Foliage, soil, and water from the tank all contained detectable levels of the ester on the day of application. With the exception of the acid form in the tank, herbicide levels in the other substrates decreased with time. The 2,4,5-T ester conversion to acid was only documented in soil and in water from the tank.

Both foliage samples in the untreated area and downwind drift levels off the application site did not produce appreciable residues with an extended residence time. Only substrates within the application area appeared to retain significant levels of herbicide.

Table 1. Levels of 2,4,5-T on Ceanothus sp. Foliage, Blodgett Forest 1978.

Sampling Station	Sampling Location	Time of Sampling (Days after Spraying)	2,4,5-T Ester (ppm)[1]	2,4,5-T Acid (ppm)
5	Sprayed area	0[2]	105.60[3]	10.90[4]
		1	105.61	11.20
		3	79.30	2.00
		8	46.35	4.10
		15	5.80	3.45
		21	2.50	1.35
		31	2.40	4.35
		8 months	0.00	0.62
6a	Sprayed area	0	158.50	19.35
		1	179.30	12.50
		3	100.90	8.10
		8	55.82	4.00
		15	6.90	5.00
		21	3.10	2.20
		31	3.10	7.20
		8 months	0.00	1.20
15	Unsprayed area	0	0.45	0.20
		1	0.07	0.00
		3	0.03	0.03
		8	0.00	0.01
		15	0.00	0.00
		21	0.00	0.04
		31	0.00	0.00
		8 months	0.00	0.00

16	Unsprayed area	0	0.05	0.01
		1	0.04	0.01
		3	0.17	0.05
		8	0.00	0.01
		15	0.00	0.01
		21	0.00	0.00
		31	0.00	0.00
		8 months	0.00	0.00

[1]Parts per million calculated on a weight per weight basis.

[2]Day of application (October 2, 1978).

[3]Mean of two samples analyzed with an instrument sensitivity of .04 ppm 2,4,5-T ester.

[4]Mean of two samples analyzed with an instrument sensitivity of .01 ppm 2,4,5-T acid.

[5]May 18, 1979.

Table 2. Post-application levels of 2,4,5-T in Water Samples from Blodgett Forest.

Sampling Station	Location	Time of Sampling (Days after Spraying)	2,4,5-T Ester (ppb)[1]	2,4,5-T Acid (ppb)
Water Tank	Sprayed area	0[2]	26.0[3]	19.0[4]
		1	364.0	85.0
		3	0.0	249.0
		8	0.0	185.1
		15	0.0	128.9
		21	0.0	156.2
		31	0.0	132.7[5]
		8 months	0.0	2.2
Spring	Unsprayed area	0	0.0	1.0
		1	0.0	0.3
		3	0.0	0.0
		8	0.0	0.0
		15	0.0	0.0
		21	0.0	0.0
		31	0.0	0.0[5]
		8 months	0.0	0.0

[1]Parts per million calculated on a weight per volume basis.

[2]Application date, October 2, 1978 (0950 - 1020 PST).

[3]Detection limit = 1.3 ppb.

[4]Detection limit = 0.3 ppb.

[5]Mean of three samples, all others are single sample values.

[6]May 18, 1979.

Table 3. Levels of 2,4,5-T in Soil Samples from Blodgett Forest.

Station	Location	Time of Sampling (Days after Spraying)	2,4,5-T Ester (ppm)[1]	2,4,5-T Acid (ppm)
8	Sprayed area	0	9.20[2]	0.02[3]
11	Sprayed area	(10-2-78)	0.70	0.00
16	Unsprayed area		0.00	0.00
19	Unsprayed area		0.00	0.00
8		1	8.80	0.02
11			2.10	0.00
16			0.00	0.00
19			0.00	0.00
8		3	1.60	0.02
11			6.70	0.10
16			0.00	0.00
19			0.00	0.00
8		8	1.80	0.07
11			1.20	0.00
16			0.00	0.00
19			0.00	0.00
8		15	1.10	0.00
11			0.74	0.00
16			0.00	0.00
19			0.00	0.00
8		21	1.70	0.04
11			0.48	0.00

16		0.00	0.00
19		0.00	0.00
8	31	0.00	1.10
11		0.37	1.90
16		0.00	0.00
19		0.00	0.00
8	8 months	0.00	0.00
11	(5-18-79)	0.00	0.00
19		0.00	0.00

[1]Parts per million calculated on a weight per volume basis.

[2]Detection level = .04 ppm

[3]Detection level = .01 ppm.

Table 4. Levels of 2,4,5-T Ester from Air Samplers at Blodgett Forest on Day of Herbicide Application (10-2-78).

Station No.	Distance[1]	Time	Sampler Type	
	(ft.)	(PST)	HiVol[2] (ug)	LoVol[3] (ug)
5	0	100-1200	Not Sampled	71
11	0		3[4]	115
3	1		294	Not Sampled
4	100		122	"
6b	200		10	"
15	(upwind)		43	"
5	0	1200-1400	Not Sampled	21
11	0		163	Not Sampled
3	1		294	"
4	100		143	"
6b	200		33	"
15	(upwind)		0	"

[1]Distance downwind of application site.

[2]High Volume Air Sampler.

[3]Low Volume Air Sampler.

[4]A detection limit of 2 ug was documented for the 2,4,5-T ester analysis.

Table 5. Levels of 2,4,5-T Ester in Post-Application Air Monitoring at Station 11 within the Sprayed Area at Blodgett Forest.

Sampling Time		2,4,5-T Ester
Days after Spring	Hour (PST)	
1	1000-1200 (application period)	3[1]
	1200-1400	163
2	0700-0900	28
	1200-1330	161
4	0650-0850	8
	1200-1400	42
9	0700-0900	3
	1200-1400	7
16	0700-0900	4
	1200-1400	4
22	0700-0900	0
	1200-1400	13
32	0700-0900	0
	1200-1400	0

[1]Detection limit = 2 ug.

ANALYSIS OF TCDD IN DEER AND PLANT TISSUES

TCDD Analysis

1. Control Samples

Dr. Harless at the EPA Research Triangle Park Laboratory (RTP) reported TCDD in adipose and liver tissue from one control deer and in adipose tissue from the second control (Table 1). However, Dr. Gross at the University of Nebraska (UN) classified one of these samples as a "possible positive" and two as "analytical discrepancies" (DFS-26 and DFS027). Dr. Gross detected no TCDD in two portions of DFS-27, which he reextracted and analyzed (Table 3). He, therefore, concluded that DFS-27 was a "false positive." He further concluded that none of the control samples contained TCDD.

It should be noted that the levels that were found in these samples were very low and close to the detection limit.

Dr. Gross did not detect TCDD in deer liver from animal No. 1 (UN 229), after reextraction and reanalysis (Table 3). He observed no hint of a signal in the analysis (this was the only control reexamined). This was in accord with his first analysis.

He tentatively concluded, therefore, that the detection at RTP was a "false positive."

2. Deer Removed after 48 Hours

RTP reported TCDD in adipose tissue (DFS-21) and liver tissue (DFS-40) from deer No. 8 (Table 1). Dr. Gross considered that DFS-21 was a "possible positive," as was his first analysis of adipose tissue from this deer (FS-19). He initially classified DFS-40 as an "analytical discrepancy." But after finding TCDD in one liver tissue sample (UN 228) from deer No. 8, after reextraction and analysis (Table 3), he changed his original assignment from "analytical discrepancy" to "possible positive," this was the only sample from this group that was reexamined, and the only one from the group that Dr. Gross

considered positive. Again, the detections are at a very low level.

3. Deer Removed after Two Weeks of Exposure

TRP and UN both found TCDD in one sample of muscle tissue (DFS-11, FS-4) at a level between 1.6 and 12 parts per trillion (Table 1). TRP also reported TCDD in three samples of adipose tissue (DFS-25, DFS-16, and DFS-17), albeit at levels below 5 parts per trillion. However, after reextraction and reanalysis of portions of the samples corresponding to DFS-16 and DFS-17, Dr. GRoss considered that DFS-16 (and DFS-25) were "possible positive" and that DFS-17 was an "analytical discrepancy." In both cases (UN 225 and UN 230) (Table 3), the extracts were found to contain no TCDD at detection limits of 3 and 4 parts per trillion, in accord with the original assignments made at the University of Nebraska. Thus, Dr. Gross concluded that the detection of 7 parts per trillion (DFS-17) reported by TRP was a "false positive."

4. Deer Removed after Four Weeks of Exposure

TRP and UN both found TCDD in muscle, adipose and liver tissue samples from Animal No. 6 at levels that ranged from 2.5 to 24 parts per trillion; TRP reported TCDD in adipose tissue (DFS-20) of Animal No. 9; and TRP and UN found TCDD in muscle and liver tissue of Animal No. 10; UN also reported TCDD in adipose tissue of this animal (Table 1). However, Dr. Gross classified levels of TCDD in adipose tissues of deer No. 9 (DFS-20) and deer No. 10 (FS-34) and in liver tissue of deer No. 9 (FS-34) as "possible positives." After reextraction and reanalysis of two additional fractions of the adipose tissue sample corresponding to FS-24 (DFS-18), UN-L detected TCDD in each analysis at 3 and 2 parts per trillion, with detection limits of 3 and 1 parts per trillion, respectively (Table 3). These observations support Dr. Gross' assignments at "possible positives." Results of reextraction and reanalysis by UN-L of adipose tissue (UN 226, Table 3) from deer No. 9 showed no detectable TCDD, although Dr. Gross indicated that his laboratory should have been able to detect 10 ppt, the level found at RTP. Therefore, Dr. Gross classified the TRP result (sample DFS-20) as an "analytical discrepancy." However, the detection of TCDD in the liver of this animal after reanalysis (UN 227, Table 3) affirms the original detection by UN-L and strengthens their

conclusion that the liver sample was a "possible positive."

Error in Analyses, University of Nebraska

The intial data obtained from quality assurance samples were low by a factor of three or four. After reexamination of their analytical procedures, UN-L determined that the error probably occurred when the gain (or sensitivity) of the mass spectrometer was set at a maximum under the assumption that the levels of TCDD that might be detected would be below 5-10 parts per trillion. Extracts containing greater amounts of TCDD produced signals that led to saturation of the detector and produced results that were too low (Table 1). UN-L verified this by repeating the analysis of some samples (Table 2). The results obtained after reanalysis showed levels considerably higher than reported originally, in accord with the explanation given above. Because of this systematic error in the initial UN-L data, Dr. Gross suggested that more confidence be placed in the actual concentrations of TCDD reported by TRP than in values for the same samples from UN-L.

Dr. Gross, UN-L, considered a sample to contain TCDD only if it was found positive in both mass spectrometry laboratories. He considered other samples "possible positives" if TCDD was detected in one of the laboratories bot not in the other because the detection limit in that laboratory was comparable or higher than the level determined by the first laboratory. Therefore, the second laboratory was simply not able to verify the detection. Dr. Gross considered a few detections " analytical discrepancies" because the capabilities of the second laboratory were adequate to verify the detection (i.e. their detection limit was lower than the level found by the other workers), but TCDD was not detected.

The systematic error in the UN-L data does not discredit the analyses. There were no false positive or false negatives in the analyses of the controls provided to UN-L. No positives were found in the control samples. An excellent correlation exists between the fortified amounts of TCDD and the actual amounts, although the UN-L values are systematically low by a factor of 3 to 3.5. The initial Un-L data (Table 1) register a binary result: "positive" or "not detected." Secondarily, the data can be used to establish

a concentration level. The 95 percent confidence interval obtained by UN-L at the 5 parts per trillion level (ppt) is about ± 7 parts per trillion. Therefore, there may be no significant difference between 4 or 5 ppt, for example, and 12 or 13 ppt.

The results are in accord with the low TCDD uptake as a function of time. The correlation appears excellent.

One explanation for the results reported above, as proposed by Dr. Gross, is that the TCDD concentrations in the tissue of the exposed deer are distributed about an average; some samples have concentrations lower than an average value, and some contain TCDD at levels greater than the average. Furthermore, Dr. Gross observed that the average TCDD level slowly increases with time of exposure. Thus for deer collected 48 hours after spraying, the presence of TCDD was not detectable and there are only hints of the possible presence of the contaminant. For deer collected after living on the study are for 2 weeks after spraying, there was one definite detection and further hints of the possible presence of TCDD. Finally, for deer collected after living on the study for 4 weeks after spraying, the average level exceeds the detection limit, and five tissue samples are found to contain TCDD up to a level of 27 parts per trillion. Four samples are found to be definitely "not detectable" in accord with a distribution concept.

RESPONSE OF PLANT AND SMALL ANIMAL COMMUNITIES

Vegetation Structure and Composition

Vegetation on the treated and untreated portions of the site were similar, although there were minor differences in structure and species composition of the plant community on the smaller untreated portion of the area (Table 2). Cover of willows and total live canopy cover was greater on the untreated portion, and the untreated area contained a small planting of sequoia saplings—the only sequoia on the site.

The data in Table 2 indicate that Ceanothus integerrimus was effectively suppressed, although other shrubs were affected to a lesser degree. Even C.

integerrimus was resprouting to some extent. There was no evidence that the treatment affected conifer growth up to 1 year after spraying.

There was no measureable shrub utilization by ungulates as they were excluded from the area by the fence. The nine deer held within the enclosure in September - October 1978 had no apparent impact on the 1979 browse production.

The form class of the shrub species in 1978 suggests the relative preference for each species by deer and cattle (Table 3). Ceanothus integerrimus and Salix appeared the most desirable browse Arctostaphylos and Ribes appeared less desirable. Thus the most palatable browse species appear to be most affected by herbicide treatment.

Mammal Community

The herbicide treatment produced no statistically significant changes in the occurrence or abundance of any small mammal species observed (Table 4). The methods of assessing short term vertebrate response to the herbicide treatment, the small size of the study site in relation to home range size of most vertebrates, and the aritificial exclosure of many animals from the area after treatment preclude a rigorous analysis.

Bird Community

Because breeding birds were not sampled before treatment, no cause and effect conclusions about the influence of spraying on breeding bird occurrence or abundance are possible. However, the post-treatment survey suggests that of nine bird species common in the brush habitat before herbicide application, three species (dusky flycatcher, MacGillivray's warbler, red-breasted sapsucker) may be benefited, four species (fox sparrow, yellow warbler, Anna's hummingbird, wrentit) may not be affected, and two species (robin, junco) may find the habitat less favorable as a result of (Table 5). The treated site had 26 percent fewer bird species observed than the control site.

Reptile and Amphibian Community

A post-treatment sampling of reptiles by means of pitfall traps provided data on two lizard species. Western fence lizards were caught nearly twice as frequently in the control area as in the sprayed area but the difference was not significant. The sagebrush lizard was caught only in the sprayed area. Western terrestrial garter snakes and Pacific tree frogs were observed in the study area.

CONCLUSIONS

TCDD Analysis

Therefore, we conclude with high certainty that TCDD can exist in the environment after a normal dosage of 2,4,5-T for a sufficiently long time to accumulate in the tissue of animals confined to the sprayed area, and that the levels of TCDD in the tissues appears to increase with increased time of exposure. However both levels of 2,4,5-T and TCDD and the below levels report to cause effects in humans, other animals.

One year after the herbicide application, vegetation monitoring showed conclusively only that the treatment suppressed most Ceanothus intergerrimus, an important deer and cattle forage, but had little noticeable impact on other vegetation. It was too early to determine whether the treatment enhanced conifer gorwth or whether it was cost effective.

Dr. Gross, UN-L, concluded that 2,3,7,8-TCDD can be detected in the tissue of deer living in a forest area sprayed with 2,4,5-T herbicide.

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Table 1. Origin, Sex, Weight and other Data for Deer Used in Study

Deer No.	Origin	Sex	Time Of Removal From Site	Days In Enclosure	Weight (lbs.) In	Weight (lbs.) Out	Carcass Fat Condition
1	Kook	F[a]	Control				
2	El Camino	M	Control				
3	Growlersburg	F	2 weeks	35	NA	90	Poor
4	Growlersbury	F	48 hrs.	21	105	102	No fat
5	Growlersbury	F	48 hrs.	13	90	85	Poor
6	Placer E. C.	M[b]	4 weeks	39	90	82	Poor
7	Placer E. C.	M[b]	Died	2			
8	Placer E. C.	F	48 hrs.	13	95	90	Poor
9	Placer E. C.	M	4 weeks	39	95	96	Good
10	Placer E. C.	F	4 weeks	39	80	80	Good
11	Bidwell Park, Chico	F	2 weeks	20	112	106	Very Good
12	Bidwell Park, Chico	M	2 weeks	20	87	80	Good

a) M = Male; F = Female; C = Castrated Male

b) Castrated

Table 2. Recovery of 2,4,5-T from Spiked Deer Tissues

Tissue	Concentration 2,4,5-T (ug)	2,4,5-T Added[a] (ug)	Percent Recovery
Kidney	2.3	4.0	59.0
Liver	0.8	1.0	83.0
Blood	0.9	1.0	90.0
Urine	252.0	300.0	84.0
Lung	1.2	1.0	117.0
Feces	4.9	6.0	82.0
Stomach Contents	6.9	10.0	69.0
Muscle	0.9	1.0	81.0
Thyroid[b]	----	----	----
		Average recovery	83.1

a) The spike level corresponds to the average level of 2,4,5-T found in each organ in the deer tissue samples.

b) Insufficient sample for analysis.

Table 3. Results for Analysis of Deer Tissues for 2,4,5-T

	Time After 2,4,5-T Application									
	2 days			2 weeks			4 weeks			Control
Deer No.	5	4	8	3	11	12	6	9	10	2
Tissue	Concentration 2,4,5-T (ppm)[a]									
Kindey	1.0	4.5	5.5	2.1	0.6	2.2	0.7	1.3	1.6	<0.1
Liver	<0.1[b]	0.4	1.0	0.4	NA[c](0.2)[d]	0.1	<0.1	<0.1	0.1	<0.1
Blood	<0.1	0.6	0.5	0.1	NA (0.2)	0.4	<0.1	0.1	0.1	<0.1
Urine	NA	330.0	NA	82.0	185.0	80.0	64.0	160.0	NA	<0.1
Lung	<0.1	0.7	1.0	0.3	0.5	0.1	<0.1	0.1	<0.1	<0.1
Thyroid	<0.1	2.2	0.4	0.4	<0.1	<0.1	<0.1	0.2	NA (0.1)	<0.1
Feces	1.1	8.5	5.0	2.1	5.7	1.6	0.9	0.8	0.5	<0.1
Stomach	1.9	2.4	8.5	9.0	7.5	3.0	1.5	4.0	7.0	<0.1
Muscle	<0.1	<0.1	0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1

a) Values are uncorrected for 83.1% average recovery. See Table.
b) Although some samples had a lower limit of detectability, 0.1 ppm was adopted for uniformity.
c) NA = Not available.
d) Values calculated from average of available data and used in ANOVA calculations are enclosed in a parenthesis.

Table 4. Calculated Means and Analysis of Variance of 2,4,5-T in Deer Tissue from Blodgett Forest, 1978. The Following Symbols are Used: T = Time; P = Tissue

Combination	Count Per Mean	Subclass T	Subclass P	Means
T	24	1	0	1.902[a]
2 Days		2	0	1.543
14 Days		3	0	0.802
28 Days				
P	9			
Kidney		0	1	2.167
Liver		0	2	0.265
Blood		0	3	0.254
Lung		0	4	0.332
Thyroid		0	5	0.384
Feces		0	6	2.914
Stomach		0	7	4.978
Muscle		0	8	0.031

Analysis of Variance of Variable 1 2,4,5-T (PPM)

Source of Variation	DF	SS	MS	F	CV
T	2	0.151E-02	0.754E-01	1.45	
ERROR A	6	0.311E-02	0.519E-01		160.9%
P	7	0.200E-03	0.287E-02	13.06xxxb	
TXP	14	0.334E-02	0.239E-01	1.09	
ERROR	39	0.857E-02	0.220E-01		104.7%
TOTAL	68	0.366E-03			

a) Data are presented in parts 2,4,5-T per-million-parts deer tissue (wt/wt).
b) Denotes significance at the .01 level.

Table 5. Duncan's Multiple Range Tests of 2,4,5-T Concentrations in Deer Tissue Samples Collected at Blodgett Forest, 1978.

Deer No.	Name	Mean	Homogeneous Subgroups		
		Significance at 5 Percent, Ranked Means			
7	Stomach Contents	4.978[a]	X[b]		
6	Feces	2.914		Y	
1	Kidney	2.167		Y	
5	Thyroid	0.384			Z
4	Lung	0.332			Z
2	Liver	0.265			Z
3	Blood	0.254			Z
8	Muscle	0.031			Z
LSD	1.413				

a) Data is in parts 2,4,5-T per-million-parts deer tissue (wt/wt).
b) Means that have letters under the same subgroup are not significantly different.

Table 2. Influence of Aerially Spraying 2,4,5-T on the Structure and Species Composition of a Shrub Community as Measured by the Change in Live Canopy Cover and Live Canopy Height One Year Post-Treatment.

Variable	Untreated (Plots 1,2,3)	Treated (Plots 4,5,7,8)[a]	Change from Untreated to Treated	Statistical Significance[b]
Plant species (per 100 m^2)				
1978 (pre-spray)	23.7	23.3		
1979 (post-spray)	15.0	15.3		
Difference	-8.7	-8.0	+0.7	NS
Canopy height (m)				
1978	3.0	3.2		
1979	2.0	1.2		
Difference	-1.0	-2.0	-1.0	***
Total live canopy cover (%)				
1978	96.7	86.0		
1979	116.0	56.3		
Difference	+19.3	-29.7	-49.0	**
C. integerrims cover (%)				
1978	26.0	19.5		
1979	39.0	4.0		
Difference	+13.0	-15.5	-28.5	**
C. cordullatus cover (%)				
1978	2.0	9.0		
1979	1.3	2.3		
Difference	-0.7	-6.7	-6.0	NS
Arctostaphylos sp. cover (%)				
1978	25.0	19.5		
1979	25.7	17.3		
Difference	+0.7	-2.2	-2.9	NS
Abies concolor cover (%)				
1978	2.3	4.8		
1979	0.6	3.0		
Difference	-1.7	-1.8	-0.1	NS

[a]The vegetation data for plot number 6 were lost.

[b]Statistical significance of the observed changes were tested by Student's t test (5 degrees of freedom) for unequal sample size (Simpson et al. 1960:176) Symbols used include: NS = P > 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

Table 3. Condition of Browse Species in Study Area, Blodgett Experimental Forest in September 1978. Species are Listed in Order of Their Apparent Preference by Deeer and Cattle

Browse species	Sample Size	Form Class[a]							
		1	2	3	4	5	6	7	8
		Percent							
Salix lemmonii	12	0	33	5	5	57	0	0	0
C. integerrimus	211	2	28	10	45	12	3	0	0
C. cordlatus	60	23	72	1	3	1	0	0	0
A. patula	179	23	69	5	2	0	1	0	0
A. viscida	59	40	37	10	4	3	0	6	0
Ribes roezlii	136	86	8	1	0	0	0	5	0

[a]Form Class 1 = available, unhedged
2 = partially available, unhedged
3 = available, moderately hedged
4 = part available, moderately hedges
5 = available, severely hedged
6 = partially available, severely hedged
7 = unabailable
8 = dead

Table 4. Influence of Aerially Spraying 2,4,5-T on the Occurrence or Abundance of Selected Mammal Species One Year Post-Treatment

Variable	Untreated (Plots 1-3)	Treated (Plots 4-8)	Change from Untreated to Treated	Statistical Significance[a]
Beechy ground squirrel[b]				
1978	1.0	0.2		
1979	2.0	0.2		
Difference	+1.0	0.0	-1.0	NS
Deer mouse				
1978	3.7	3.8		
1979	10.0	9.0		
Difference	+6.3	+5.2	-1.1	NS
Trowbridge shrew[c]				
1978	0.0	0.2		
1979	0.3	0.6		
Difference	+0.3	+0.4	+0.1	NS
Broad-footed mole[d]				
1978	8.3	21.0		
1979	15.0	33.0		
Difference	+6.7	+12.0	+5.3	NS
Brush mouse				
1978	2.0	2.0		
1979	0.0	1.6		
Difference	-2.0	-0.4	+1.6	NS
Pinyon mouse				
1978	2.3	1.6		
1979	0.3	3.6		
Difference	-2.0	+2.0	-4.0	NS
Long-eared chipmunk				
1978	2.6	0.0		
1979	0.3	0.0		
Difference	-2.3	0.0	+2.3	NS

[a]Statistical significance of the observed changes was tested by Student's t test (6 degress of freedon) for unequal sample size (Simpson et al. 1960:17). No changes were

significant: NS = $p > 0.05$.

[b]Mean percent frequency of sign per six track plot nights on each sample plot using one 10 ca track station per plot.

[c]Mean new captures per 120 trap nights (corrected for trap failure) on each sample plot using 40 Sherman live traps per plot.

[d]Mean percent frequency of sign per 20 10 ca circular elements on each sample plot.

Table 5. Comparison of Mean Standardized Abundance of Birds Found in Untreated and Treated Portions of Study Area in June 1979.

Species	Untreated (Plots 1-3)	Treated (Plots 4-8)	Percent Difference
MacGillivray's warbler	1.6	5.2	+225
Red-breasted sapsucker	0.3	0.6	+100
Dusky flycatcher	3.8	6.6	+74
Wrentit	0.4	0.5	+25
Fox sparrow	8.7	8.9	+2
Anna's hummingbird	0.7	0.6	-14
Yellow warbler	5.4	4.6	-15
Dark-eyed junco	1.7	0.7	-59
American robin	3.1	0.9	-71
Total abundance	28.4	30.3	+7
Bird species	23	17	-26

Table 1. Spray Deposit Data Summary by Sampling Line, of Aerial Herbicide Application, Blodgett Forest, October 2, 1978

Sampling Line	Drop Diameters (μm)[a] Mass Median[b]	Mass Mean[c]	All Drops Median	All Drops Mean	Drops/cm^2	Recovery Rate (gal./A) Range	Average
A	954	990	170	282	5.99	0.71 to 10.6	3.54
B	953	983	189	308	5.47	0.43 to 7.54	3.80
C	865	921	244	327	3.95	0.01 to 6.18	2.71

[a]Micron = 0.001 mm.

[b]Diameter associated with the average, drop value.

[c]Diameter associated with the median drop volume.

	Volume median diameter[a] (μm)
A	954
B	953
C	865

a] Volume median diameter is the drop diameter that divides the spray volume into two equal parts: 50 percent of the volume is in drops smaller than the mind and 50 percent is above.

I would include "limit of detection."

Table 1. Results for Analysis of Deer Tissue for TCDD Obtained at the University of Nebraska (FS-code) and Research Triangle Park (DFS-code)

Time of Removal From Site	Animal No.	Muscle		Adipose		Liver		Bone Marrow	
		Code	TCDD (ppt)	Code	TCDD (ppt)	Code	TCDD (ppt)	Code	TCDD (ppt)
Control	1	FS-1	ND(1)	FS-15	2[a]	FS-39	ND(2)	———	———
		DFS-14	ND	DFS-27	5	DFS-31	2	———	———
	2	FS-2	ND	FS-16	ND(.9)[b]	FS-38	ND	FS-43	ND
		DFS-13	ND	DFS-26	3	DFS-32	ND	DFS-48	ND
48 Hours	4	FS-5	ND	———	———	FS-32	ND	———	———
		DFS-10	ND	———	———	DFS-38	ND	———	———
	5	FS-6	ND	FS-19	2	FS-37	ND	———	———
		DFS-9	ND	DFS-23	ND(2)	DFS-33	ND	———	———
	8	FS-9	ND	FS-21	3[a]	FS-30	ND(2)	———	———
		DFS-6	ND	DFS-21	3	DFS-40	5	———	———
2 Weeks	3	FS-4	1.6(1)	FS-17	1.9[a]	FS-33	ND	———	———
		DFS-11	12 (d)	DFS-25	5	DFS-37	ND	———	———
	11	FS-12	ND	FS-25	ND(1)	FS-35	ND	FS-48	ND
		DFS-3	ND	DFS-17	7	DFS-42	ND	———	———
	12	FS-13	ND	FS-26	2.3[c]	FS-28	ND	———	———
		DFS-2	ND	DFS-16	4	DFS-42	ND	———	———
4 Weeks	6	FS-8	3.4	FS-20	2.9	FS-31	2.5	FS-44	2.0[b]
		DFS-7	24	DFS-22	12	DFS-39	3	DFS-47	ND
	9	FS-10	ND	FS-22	1.6[d]	FS-34	2.1	FS-46	ND
		DFS-5	ND	DFS-20	10	DFS-36	ND(2)	DFS-47	ND
	10	FS-11	4.6	FS-24	2.2	FS-42	3	FS-47	ND
		DFS-4	27	DFS-18	ND(3)	DFS-28	2	DFS-44	ND

[a]Incorrect isotope intensity ratio observed for the signals at m/z 320 and m/z 322 in the second analysis at UN-L. Considered "ND" by Dr. Gross.

[b]Detection limit in parentheses, ppt.

[c]No signals detected at both m/z 320 and m/z 322 at UN-L in the UN validation study. Considered "ND."

[d]Signals below the detection limit observed at m/z 320 and 322 in the UN validation study. Considered "ND."

Table 2. Results for Reanalysis of Deer Tissue Extracts for TCDD Obtained at the University of Nebraska (FS-code) September 17, 1980

Animal No.	Code	Tissue Type	TCDD (ppt)[a]	Initial Results University of Nebraska	True Value of spikes	RTP[b] Value
1	FS-3	Muscle (quality control)	16	TCDD ppt 3	TCDD ppt 15	TCDD ppt -
3	FS-4	Muscle	9	1.6	-	12
4	FS-5	Muscle	ND	ND(0.9)	-	ND
1	FS-7	Muscle (quality control)	28	7.4	30	-
6	FS-8	Muscle	14	3.4	-	24
10	FS-11	Muscle	19	4.6	-	27
1	FS-14	(Quality control)	ND	ND	0	-
1	FS-18	Adipose (quality control)	34	12	43	-
1	FS-23	Adipose (quality control)	17	4.9	16	-
8	FS-30	Liver		ND(2)	-	5
9	FS-34	Liver		2.1	-	ND(2)

[a]Concentrations corrected for trace (ca. 0.2%) native TCDD in the TCDD cl-37 internal standard.

[b] TCDD analysis values obtained from EPA, Research Triangle Park.

Table 3. Results for Reextraction and Reanalysis of Deer Tissues for TCDD at the University of Nebraska December 1980

Time of Removal from Site	TCDD Levels are Compared with Initial Results At UN-L and RTP: Animal No.	Tissue Type	Reextraction and Reanalysis UN-L: TCDD (ppt)		Initial Results UN-L/RTP: TCDD (ppt)	
Control	1	Liver	UN 229	ND	FS-39	ND(2)[b]
					DFS-31	2
	1	Adipose	UN 1	ND	FS-15	2[a]
					DFS-27	5
48 Hours	8	Liver	UN 228	3	FS-30	ND(2)
				3	DFS-40	5
2 Weeks	11	Adipose	UN 225	ND	FS-25	ND(1)
					DFS-17	7
	12	Adipose	UN 230	ND	FS-26	2, 3
				ND	DFS-16	4
4 Weeks	9	Adipose	UN 226	ND	FS-22	1.6
					DFS-20	10
	9	Liver	UN 227	3	FS-34	2.1
				ND	DFS-36	ND(2)
	10	Adipose	UN 10	3	FS-24	2.2
				2	DFS-18	ND(3)

[a]Incorrect isotope intensity ratio observed for the signals at m/z 320 and m/z 322 in the second analysis at UN-L. Considered "ND" by Dr. Gross.

[b]Value in parenthesis is detection limit in ppt.