Impact of 2,4,5-T

on

Blodgett Forest

II. Levels of 2,4,5-T in Tissues of

Deer Exposed to the Experimental

Aerial Application of 2,4,5-T

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ABSTRACT

Tissue samples from deer exposed to either direct aerial application of 2,4,5-T or to 2,4,5-T sprayed forage from Blodgett Forest were analyzed for the presence of the herbicide, Samples from the stomach, feces, kidney, thyroid, lung, liver, blood and muscle tissue were taken 2, 14, 28 days after the herbicide application.

Low levels of 2,4,5-T were detected in all tissues sampled. The stomach had significantly higher 2,4,5-T levels than any other tissue (4.98 ppm,wt/wt) and the muscle tissue the least (31.11 ppb,wt/wt). The time of sampling (days after the aerial application) did not influence the amount of 2,4,5-T detected in tissues and the relative level of the herbicide in tissues did not change over time. The muscle tissue, considered the most desirable portion of the deer for human consumption, contained extremely low levels. The likelihood that the muscle tissue might be hazardous to human health is extremely low.

ACKNOWLEDGMENTS' AND PARTICIPANTS

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2. Chemical Analysis: California Analytical Laboratories Inc.

3. Cooperators: H. Black, United States Forest Service
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DISCLAIMER

"The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products."

INTRODUCTION

The aerial application of the herbicide 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T) to large acreages of rangesand forest lands which are open to deer hunting has prompted major concern. This concern is centered on the possible bioaccumulation of 2,4,5-T and its contaminant 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in the deer and the subsequent possibility of human consumption.

The California Department of Food and Agriculture participated in a cooperative study titled "The Blodgett Forest Environmental Monitoring Project." This study was designed to answer questions about the environmental fate of 2,4,5-T and TCDD in forest and rangelands as well as the possible bioaccumulation of these compounds by deer.

The University of California at Berkeley provided the 11-acre site at the Blodgett Forest Research Station. Dr. Barrett of U.C. Berkeley studied the impact of 2,4,5-T on the small animal population. Dr. Hugh Black, United States Forest Service, supplied the funds necessary for fencing the site, applied the material to the application site, and coordinated the analysis for TCDD by Dr. Gross at the University of Nebraska. Dr. Black arranged for the preparation of the samples for TCDD analyses by the Environmental Protection Agency Laboratory at Bay St. Louis, Missouri and

an assessment of the spray distribution on the site using spray cards placed by the U.S.F.S. Forest Insect and Disease Management Unit in Davis. The California Department of Fish and Game participated in the study and was partly reimbursed by contractual funds supplied by the California Department of Food and Agriculture. They collected and transported the deer used in the project, studied their habits on the site, and collected the tissue samples at prescribed times. The Department of Food and Agriculture monitored the 2,4,5-T application using high volume air samplers; collected soil, water and vegetation samples; and supervised the analysis of herbicide levels in deer tissue. Additionally, the Department contracted with a private laboratory, California Analytical Laboratories, Inc. in Sacramento, California, to perform TCDD analyses on plant tissue, herbicide formulation, mylar fallout panels and 2,4,5-T analysis in the collected deer tissue. The mylar panels were used by Dr. Don Crosby of the University of California at Davis in a collateral study on photodegradation of TCDD in pesticide formulations when sprayed on a forest site.

This report will be limited to results of the analysis of 2,4,5-T in the deer tissue. A previous report discussed the results of the analysis of air, water, soil and vegetation by the Department's Chemistry Laboratory Services Unit and the methods used by the Department in collecting the samples (1).

METHODS AND MATERIALS

Site Description

An ll-acre site (Figure 1), previously described in detail (1), was enclosed by a nine foot hog wire fence. A circular livestock watering trough was placed in the enclosure to supply additional water since the flow from the spring located in the enclosure was judged to be inadequate for the confined deer population.

Herbicide Application (10/2/78)

An aerial application of 2,4,5-T was applied to the site on November 2, 1978 (0950-1020) at a rate of four pounds active ingredient in 10 gallons of water per acre to approximately eight acres at the north end of the enclosure.

Deer Collection

The seasonal constraint of the study made the collection of wild deer uncertain, but the use of transmitter darts to collect deer was believed to have the greatest potential to collect deer during the late summer when trapping is not effective. Deer are considered the "problem species" of wildlife for use of remote chemical restraint techniques* Presently, there are no drugs that have proven totally satisfactory for deer. However, some experimental success has been achieved with a mixture of 3.8 mg M-99 and 30 mg Rompun with 150 IU hyaluronidase.

Six transmitter darts were purchased from Wildlife Materials, Carbondale, Illinois, to be used with Palmer Cap-Chur syringes and gun to attempt to capture nine wild Columbian black-tailed deer (*Odocoileus hemionuscol-umbianus*). These darts, when used with a 3cc syringe, weighted 30+ grams and measured 16.4 cm in length plus a 19 cm trailing wire antenna. Practice with a 30 gram dummy dart of similar length demonstrated the very limited effective range of these darts.

At 35 yards, aiming one foot above the deer's back, the dart would be driven three to four inches into the upper leg muscle using the .22 projector. Trials with the CO_2 projector were too inconsistent to use this gun. Through experimentation it was determined that satisfactory delivery of a transmitter dart could be accomplished using the .22 projector and pushing the dart half way down the barrel of the gun. The maximum range using this technique was approximately 40 feet.

The 40-foot maximum range of the darts dictated that a source of very tame free-roaming deer be located. Such a herd was reported at the State Correctional Facility at Growlersburg Conservation Camp near Georgetown, El Dorado County, and less than 15 miles from the deer enclosure on the Blodgett Forest. The inmates at the camp had raised some of the deer from fawns. These deer ran free with the wild deer of the area, which in turn came into the camp for handouts from the inmates. The first attempt to collect one of the Growlersburg deer was a textbook classic. After being darted, the adult female deer ran approximately 50 yards into the forest where the drugs took effect. Utilizing a radio receiver leased from

Wildlife Materials, Inc. the deer was easily located after waiting approximately six minutes for the drug to take effect. A fat biopsy was taken and the deer was transported and released in the enclosure one hour and thirty minutes after being darted. The next day two deer were hit with darts but neither was immobilized. An attempt was also made to trap deer on the Conservation Camp. There was good bait acceptance (bread, apples and watermelon). One female deer was caught and used in the study. Another was released when two small fawns were found waiting near the trap. Two other deer managed to get out of the traps before collection. Several other attempts to dart deer resulted in one additional animal being taken and fewer numbers coming into the area where they were fed by the inmates. Additional attempts were made to collect deer at a site near Kool, El Dorado County, where a fair number of relatively tame deer were reported. However, these deer could not be approached close enough for use of transmitter darts and none were collected.

The decision was made to conclude the effort to dart additional wild deer when the deer hunting season opened in the Sierras. The remainder of the deer used in the study were captive animals obtained from the Placer Ecology Center and Bidwell Park in Chico. The deer at the Ecology Center were contained in 6-foot by 15-foot cyclone fence pens with concrete floors and the Chico deer were in a two-to-three acre pen containing grass and shrubs. One of the Chico deer was darted and immobilization took 35 to 45 minutes using the same quantities of drug used at Growlersburg. The second deer at Chico was restrained by hand.

Deer Preparation and Data Collection

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 two-to-three inch incision made over the cranial aspect of the thigh in the area of the prefemoral lymph node. Cadaver dissection had revealed this area to contain 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 two to three inch 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 Penicillin and Streptomcycin were injected intramuscularly.

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 browsed and what areas of the enclosure utilized.. Observations were intensified around the application date with the deer being located the day before, during, immediately after, and the day following application.

Deer Tissue Collection'

Forty-eight hours after application three deer were collected by shooting. One deer had been in the enclosure 21 days, while the other two had only 13 days to acclimate in the enclosure. Two of the deer taken at this time were wild deer and the other was from the Placer Ecology Center. Initially all three wild deer were to be collected first; however, one of the Ecology Center deer developed a swelling of unknown cause under the biopsy site. It was decided to take this animal rather than run the chance of it dying from possible infection. 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 to 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, fecal material, 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.

The deer collection, tissue sampling and handling process was repeated two weeks after application. The wild deer taken in this collection had been in the enclosure for 35 days; the other two deer obtained from Bidwell Park were in the best fat condition when placed in the enclosure 20 days prior to this collection. The final collection was made four weeks after application. These animals were all from the Placer Ecology Center and had been in the enclosure for 39 days before removal.

Two additional deer were collected by shooting from areas not known to have 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

Tissue samples were received frozen and stored at -10°C; the samples(2-10g) were thawed, homogenized with a Virtis Homogenizer, and a 2.0 g (+ 0.05 g) sub-sample 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 hydroxide, and 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 2mm (id) glass column 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.

Sample peak heights in the 50-200 pg range were compared to 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% 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 sub-samples 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%, the level 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.

RESULTS AND DISCUSSION

A total of 66 visual observations of individual deer were made in the enclosure during the study, 25 before spraying and nine immediately after spraying when all but two deer were in the unsprayed area after fleeing from the helicopter. Thirty-two observations were made one or more days after the spraying. Before spraying, 64 percent of the deer observed were in the side that was sprayed compared to 66 percent after the application. The presence of 2,4,5-T apparently did not deter the deer from using the area. All observations were made during the period from 10:00 a.m. to 4:00 p.m. Deer were observed feeding in both sides, although no attempt was made to measure feeding behavior because of the interruptive effect of locating the deer. When observed feeding, the preferences for captive deer changed from seemingly indiscriminate browsing when first put in the enclosure to selecting the preferred deer foods (deer brush and snow brush) as they became acclimated.

The 2,4,5-T analysis of deer tissues is presented in Table 3. With the exception of the control deer, 2,4,5-T was detected in all animals sacrificed over the four-week period. The ANOVA 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 within tissues did not change with the time of deer tissue sample

collection after the aerial 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 amounts of 2,4,5-T than all other body parts (Table 5). The amount of 2,4,5-T found in the feces and kidney was not different from each other but was higher than those of the liver, blood, lung, thyroid and muscle. The latter tissues were found to contain a statistically equivalent amount of 2,4,5-T. It was interesting to note that those portions of the digestive and excretory systems contained statistically higher levels of 2,4,5-T which were an order of magnitude greater than the other sampled tissues. This is in good agreement with 2,4,5-T analyses previously reported by Newton and Norris (3) for blacktail deer and are also comparable to results reported by Leng (2) for calves. Muscle tissue, normally considered the most edible portion of wild deer, contained the least amount of 2,4,5-T detected (x-31 ppb).

The absence of definitive human toxicity data for 2,4,5-T does not allow a firm prediction of potentially hazardous concentrations in animal tissues. The reported tissue concentrations for this study, especially those for the edible portions of the deer, are however, extremely low and one would not expect them to be hazardous to human health.

The study design inadvertently exposed the confined deer to higher levels of 2,4,5-T than would probably have been encountered in the wild. The circular livestock watering trough placed in the enclosure was located in the area sprayed, No attempt was made to cover the open water during the application of 2,4,5-T. The use patterns of the trough were not monitored

so no data was collected on how many deer used the trough instead of the spring located in the unsprayed area of the enclosure. Despite the higher level of exposure to 2,4,5-T very little was found in muscle tissue. Most of the 2,4,5-T was found in the digestive and urinary tracts from which it was being eliminated.

The fat condition of the deer when placed in the enclosure was generally poor except for the two deer from Chico which were in good condition. The animals from the Placer Ecology Center were all in very poor condition. The poor fat condition of the deer made the biopsy for a control unsuccessful. The quantity of fat necessary for analysis (10 grams) was not obtained from any of the deer. Also, the biopsy operation is believed to have been the probable cause in the weight loss observed in all the deer measured before the last collection (Table 1). Two of the three deer in the final collection had returned to their original weight. This improvement in their condition was noticeable in the observations made during the two weeks prior to their collection. It was also noted during this time that the third deer seemed to be declining in condition. However, this deer had more fat tissue than expected when necropsied and rated fifth overall in fat condition at the time collected.

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 lymph edema to cause swellings readily seen before they were collected. One swelling was about four inches in diameter and the others at least twice this size. By the third and final collection the presence of lymph edema no longer was present around the biopsy site. In future studies if fat controls, are needed, other sources of fat on the deer should be tried and, if possible,

the biopsy should be made a minimum of one and one-half months before the deer are to be collected. The data collected on the deer used in this study is summarized in Table 1.

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2. Leng, M.L. "Comparative Metabolism of Phenoxy Herbicides in Animals," Fate of Pesticides in the Large Animal, Academic Press, Inc., New York, N.Y., pp53-76 (1977).

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Figure 1. Experimental Site Used in the Blodgett Forest 2,4,5-T Study

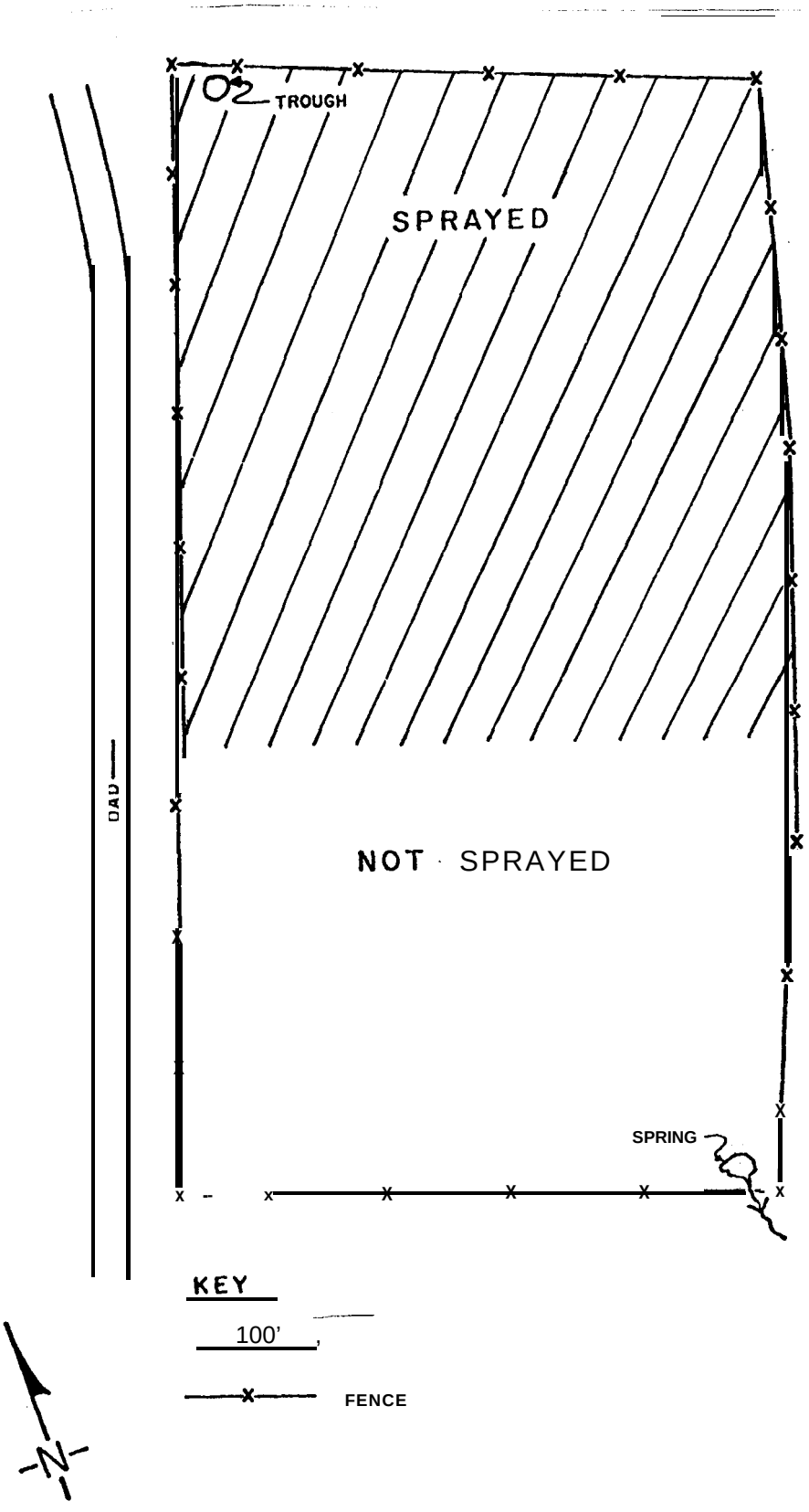

Table 1. O&in, Sex, Age, Weight and other Data for Deer Used in Study

Deer No.	Origin	Sex	Removal After Application	Days In Enclosure	Weight (lbs.) In Out	Relative Carcass Fat Condition	Age
1	Kool	F[a]	Control				Adult
2	El Camino	M	Control				YRLG
3	Growlersburg	F	2 wks.	35	NA 90	4[b] (poor)	Adult
4	Growlersburg	F	48 hrs.	21	105 102	1 (no fat)	Adult
5	Growlersburg	F	48 hrs.	13	90 85	3 (poor)	Adult
6	Placer E. C.	C	4 wks.	39	90 82	5 (poor)	YRLG
7	Placer E. C.	C	Died	2			
8	Placer E. C.	F	48 hrs.	13	95 90	2 (poor)	YRLG
9	Placer E. C.	M	4 wks.	39	95 96	8 (good)	YRLG
10	Placer E. C.	F	4 wks.	39	80 80	7 (good)	YRLG
11	Bidwell Park	F	2 wks.	20	112 106	9 (fat)	Adult
12	Bidwell Park	M	2 wks.	20	87 80	6 (good)	YLRG

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

b) Arbitary index where larger numbers relate to greater amount of fat

Table 2. Recovery of 2,4,5-T from Spiked Deer Tissues'

Tissue	2,4,5-T µg found	2,4,5-T µg[b] added	% Recovered
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[c]	--a.--	-----	-----
		Average Recovery	83.1

a) 2.0 g samples processed as described under procedure.

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

c) Insufficient sample received.

Table 3. Summary of 2,4,5-T Analyses in Deer Tissue from Blodgett Forest, 1978.

	Time After 2,4,5-T Application									
	2 days			2 weeks			4 weeks			Control
DFA Deer #	#5	#4	#8	#3	#11	#12	#6	#9	#10	#13
CAL Tag #	10236	10235	10238	10196	10241	10242	10237	10239	10240	10243
Tissue	2,4,5-Trichlorophenoxyacetic acid found (ppm)[a]									
Kidney	1.0	4.5	5.5	2.1	0.6	2.2	0.7	1.3	1.6	CO.1
Liver	<0.1[b]	0.4	1.0	0.4	NA[c](0.2)[d]	0.1	<0.1	co.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	co.1	0.1	<0.1	<0.1
Thyroid	<0.1	2.2	0.4	0.4	co.1	co.1	co.1	0.2 NA	(0.1)	co.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	co.1
Muscle	<0.1	CO.1	0.1	0.1	<0.1	<0.1	<0.1	co.1	<0.1	co.1

a) Values are uncorrected for 83.1% average recovery. See Table 2.

b) While some samples actually 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		1609%
P	7	0.200E-03	0.287E-02	13.06xxxb	
TXP	14	0.3343-02	0.2393-01	1.09	
ERROR	39	0.8573-02	0.220E-01		104.7%
TOTAL	68	0.3663-03			

a) Data is presented in parts 2,4,5-T per-million-parts deer tissue (wt/wt)

b) Denotes significance at the .001 level

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

			Significance at 5 Percent, Ranked Means		
No.	Name	Mean	Homogeneous Sub-Groups		
7	Stomach	4.978[1]	X[2]		
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	0.311			Z
LSD	1.413				

1) Data is in parts 2,4,5-T per-million-parts deer tissue (wt/wt)

2) Means which have letters under the same sub-group are not significantly different.