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RADIOECOLOGY OF NATURAL SYSTEMS

FOURTEENTH ANNUAL PROGRESS REPORT

for the period
May 1, 1975 – July 31, 1976

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DISCLAIMER

FOURTEENTH TECHNICAL PROGRESS REPORT

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RADIOECOLOGY OF NATURAL SYSTEMS IN COLORADO

Department of Radiology and Radiation Biology
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Fort Collins, Colorado 80523

for the period
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I. ABSTRACT

I. ABSTRACT

This report summarizes project activities during the period May 1, 1975 through July 31, 1976 on ERDA Contract E(11-1)-1156 with Colorado State University. During the reporting period, significant progress was made on most of the sub-projects. The major study on the distribution and levels of plutonium in major components of the terrestrial ecosystem at Rocky Flats was completed. Supportive studies on the ecology and pathology of small mammals and their role in plutonium transport were essentially completed as well. Detailed studies on mule deer food habits, population dynamics, and movements at Rocky Flats are progressing very well. These studies are designed to measure the potential of mule deer in transporting plutonium to uncontrolled areas. Alpha autoradiographic studies designed to measure plutonium particle size and distribution and spatial patterns in soil were initiated. Field and greenhouse transport pathways from soil to vegetation are in progress and some early results reported. The status of studies on seasonal kinetics of cesium in a montane lake and stable lead geochemistry in an alpine lake watershed are also reported.

II. SUMMARY

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Studies on the radioecology of plutonium made good progress during the reporting period. A major study on distribution and levels of plutonium in ecosystem compartments was completed by C. A. Little. The results of this work are presently in the form of Little's Ph.D. dissertation. The data are presently being prepared for publication in the open literature. Some major findings were: (1) over 99 percent of the plutonium is contained in soil, (2) predictable relationships of soil plutonium to location, depth and particle size exist, (3) detectable, but relatively low plutonium concentrations exist in plants and animals, (4) sampling variability in all ecosystem components was extremely large and frequency distributions highly skewed, and (5) substantial evidence that ^{238}Pu is more mobile in the ecosystem than ^{239}Pu was found.

Substantial data on small mammal ecology were obtained. Eight species were documented, however, most population data were obtained for the deer mouse, the most abundant rodent in the study area. Specific information on seasonal density, sex ratio, age class ratio and home range was gathered for the deer mouse.

Food habit trials utilizing tame deer were successfully conducted in the field at Rocky Flats. This technique involves recording the species ingested with each bite taken by a tame deer that is allowed to roam and feed at will in specified study areas. In addition, soil ingestion has been documented in deer by observation and quantitatively estimated by a titanium tracer method. Preliminary data indicate that deer consume of the order of 40 g of soil daily in winter.

Mule deer density by season and study area were studied by observation and by pellet group plots. The deer population at Rocky Flats appears to vary between 100 and 150 animals. The specific areas used and social behavior seem to vary seasonally. Some 24 animals were live-trapped in November, December and January and marked with numbered neck collars. In addition, 11 newborn fawns were marked with ear tags in June. Observations are made on these animals to improve our understanding of population dynamics and movements. To estimate plutonium levels in deer at Rocky Flats, we have collected tissue samples from six deer and these have been submitted for assay.

One experiment was conducted in which PuO_2 particles were fed to a deer in a metabolic cage to study possible particle size changes during transit through the gastro-intestinal tract. The material was unexpectedly found to be polydisperse in the oral dose and it was therefore not possible to accurately distinguish its distribution from that in the fecal material.

The study on the role of pocket gophers in turning up plutonium-contaminated soil was essentially completed. Quantitative estimates of mound densities, appearance rate, size, and plutonium content are reported. This study is currently being prepared for publication.

The search for pathological effects of plutonium in small mammals from Rocky Flats was continued and is now essentially complete. Of the 149 microscopic lung examinations, 152 skeletal radiographs, and 217 necropsies, all were negative. These data are being prepared for publication. A separate report on the incidence of besnoitiosis in rodents is ready for submission to a journal.

The study on the relative importance of root uptake and aerial deposition utilizing transplanted sod blocks is continuing. Seasonal sampling was conducted through the year and to date, it appears that root uptake and aerial deposition are approximately equal in importance. This finding is surprising and suggests the need for continued study and verification. A related greenhouse study on plutonium uptake by cheatgrass from contaminated soil cores is in progress. No results are available to date.

In an effort to understand the large sampling variability for plutonium in environmental samples at Rocky Flats, a study was initiated to measure PuO_2 particle size distribution in Macroplot 1 soil. This work uses alpha autoradiographic methods, which shows single tracks and stars on nuclear emulsion plates. To date, only sub-micron particles have been verified. Scanning of larger quantities of soil using 8x10" sheets of industrial x-ray film for micron-sized particles is underway.

Two small-scale studies on plutonium levels in snakes and mourning doves at Rocky Flats were completed during the year. Samples ranged from non-detectable activity to a few tenths of a dpm/g. No samples exceeded 1 dpm/g. Nine of 27 snake tissue samples were below detection limits, while 17 of 24 dove tissue samples were non-detectable for plutonium.

The study on seasonal kinetics of cesium in East Twin Lake is still in progress. Neutron activation for stable cesium in samples taken periodically from the Lake to measure concentration factors through time is essentially complete. Many of the activated samples have not been analyzed. Considerable effort during the year was devoted to kinetic studies in the laboratory and ingrowth curve fitting routines.

Only minimal progress was made during the year on the geochemical studies of stable lead in alpine watersheds. Continued collections were made to estimate fallout input and loss rates from Snow Lake. Analyses are in progress. Data analysis has not yet begun due to other committments of the principal investigator.

III. PLUTONIUM IN THE TERRESTRIAL ENVIRONS OF ROCKY FLATS

This investigation is presently the major effort under Contract E(11-1)-1156. Individual projects are summarized in this section. These projects involve a range of topics which all contribute to our understanding of the terrestrial radioecology of plutonium. The projects involve measurements of plutonium in ecosystem components, basic ecological investigations, pathology, quantification of Pu transport mechanisms, and physical characterization of Pu.

III. PLUTONIUM IN THE TERRESTRIAL ENVIRONS OF ROCKY FLATS

A. Distribution and levels of plutonium in ecosystem compartments (C. A. Little)

This project recently culminated in a Ph.D. dissertation by C. A. Little entitled, "Plutonium in a Grassland Ecosystem." Since copies accompany this report and are separately available, only the abstract is presented here:

"This study was concerned with plutonium contamination of grassland at the U. S. Energy Research and Development Administration Rocky Flats plant northwest of Denver, Colorado. Of interest were: the definition of major plutonium-containing ecosystem compartments; the relative amounts in those compartments; how those values related to studies done in other geographical areas; whether or not the predominant isotopes, ^{238}Pu and ^{239}Pu, behaved differently; and what mechanisms might have allowed for the observed patterns of contamination.

Samples of soil, litter, vegetation, arthropods, and small mammals were collected for plutonium analysis and mass determination from each of two macroplots. Small aliquots (5 g or less) were analyzed by a rapid liquid scintillation technique and by alpha spectrometry.

Of the compartments sampled, greater than 99% of the total plutonium was contained in the soil. The concentrations of plutonium in soil were significantly inversely correlated with distance from the contamination source, depth of the sample, and particle size of the sieved soil samples. The soil data suggested that the distribution of contamination largely resulted from physical transport processes. A mechanism of agglomerated submicron plutonium oxide particles and larger (1-500 μm) host soil particles was proposed.

Concentrations of Pu in litter and vegetation were inversely correlated to distance from the source and directly correlated to soil concentrations at the same location. Comparatively high concentration ratios of vegetation to soil suggested wind resuspension of contamination as an important transport mechanism.

Arthropod and small mammal samples were highly skewed, kurtotic, and quite variable, having coefficients of variation (standard deviation/mean) as high as 600%. Bone Pu concentrations were lower than other tissues. Hide, GI, and lung were generally not higher in Pu than kindey, liver and muscle.

In all compartments sampled, the expressed variation relative to the mean was quite high; coefficients of variation (standard deviation/mean) of up to 600% were found. The variation and the presence of zero or less than detectable values required the use of nonparametric statistical tests in many cases.

Isotopic ratios of ^{238}Pu to ^{239}Pu by activity concentration in surface soil were about 65. Litter and vegetation isotopic ratios were similar to

soil suggesting that the contamination was largely surficial. Arthropod and small mammal tissue isotopic ratios were much lower than soil which implies the possibility that the isotopes were incorporated into the animal bodies and ^{238}Pu taken up at a higher rate. The isotopic ratio of bone was lower than for other tissues further suggesting different uptake rates for the two isotopes."

B. Small Mammal Ecology (T. F. Winsor)

Field studies of small mammals were initiated during June, 1973. These studies have consisted primarily of a live-trapping program conducted to estimate small mammal species biomass and to collect animals for plutonium analysis. The program also enabled us to trace the population dynamics of the most abundant rodent, the deer mouse, *Peromyscus maniculatus*, and to observe the habits of associated species. Our objectives included observations of fluctuation in relative abundance; measurements of density, reproductive performance, and movements; and a comparison of our data with those from the literature.

The program was concluded in July, 1976. We are now in the process of final data analysis and preparation for publication. This report summarizes some of the data collected through October, 1975. Trapping methods have been described previously.[1]

Species captured included the deer mouse, thirteen-lined ground squirrel, northern pocket gopher, hispid pocket mouse, silky pocket mouse, harvest mouse, meadow vole, and house mouse. The latter six species have been discussed in previous reports.[1]

Thirteen-lined ground squirrels were not caught in abundance until August, 1974, and generally reached highest population levels during the summer of 1975 (Fig. 1). The low numbers or occasional failure to trap ground squirrels during colder months should be interpreted as a reflection of life habits rather than periodic extinction from the community. We have never trapped these animals in numbers sufficient to allow a detailed population analysis, but their mass occasionally approached the highest levels attained by deer mice.

With respect to deer mice, there were large seasonal fluctuations in relative abundance in trappable individuals (Fig. 2). The data indicate that populations are high November through April, while lows come during the hot summer months. Lechleitner[2] reported that deer mice in Colorado "breed in the warmer months of the year with pregnancies occurring as early as April and as late as September." Our summer data may reflect the results of heavy spring and summer predation and low trappability,

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[1]Whicker, et al. 1975. Radioecology of natural systems in Colorado. 13th Annual Report to ERDA on Contract AT(11-1)-1156 (COO-1156-80). Dept. Radiology and Radiation Biology, Colo. State Univ. 104 p.

[2]Lechleitner, R. R. 1969. Wild Mammals of Colorado. Pruett Publishing Co. Boulder, Colorado. 254 pp.

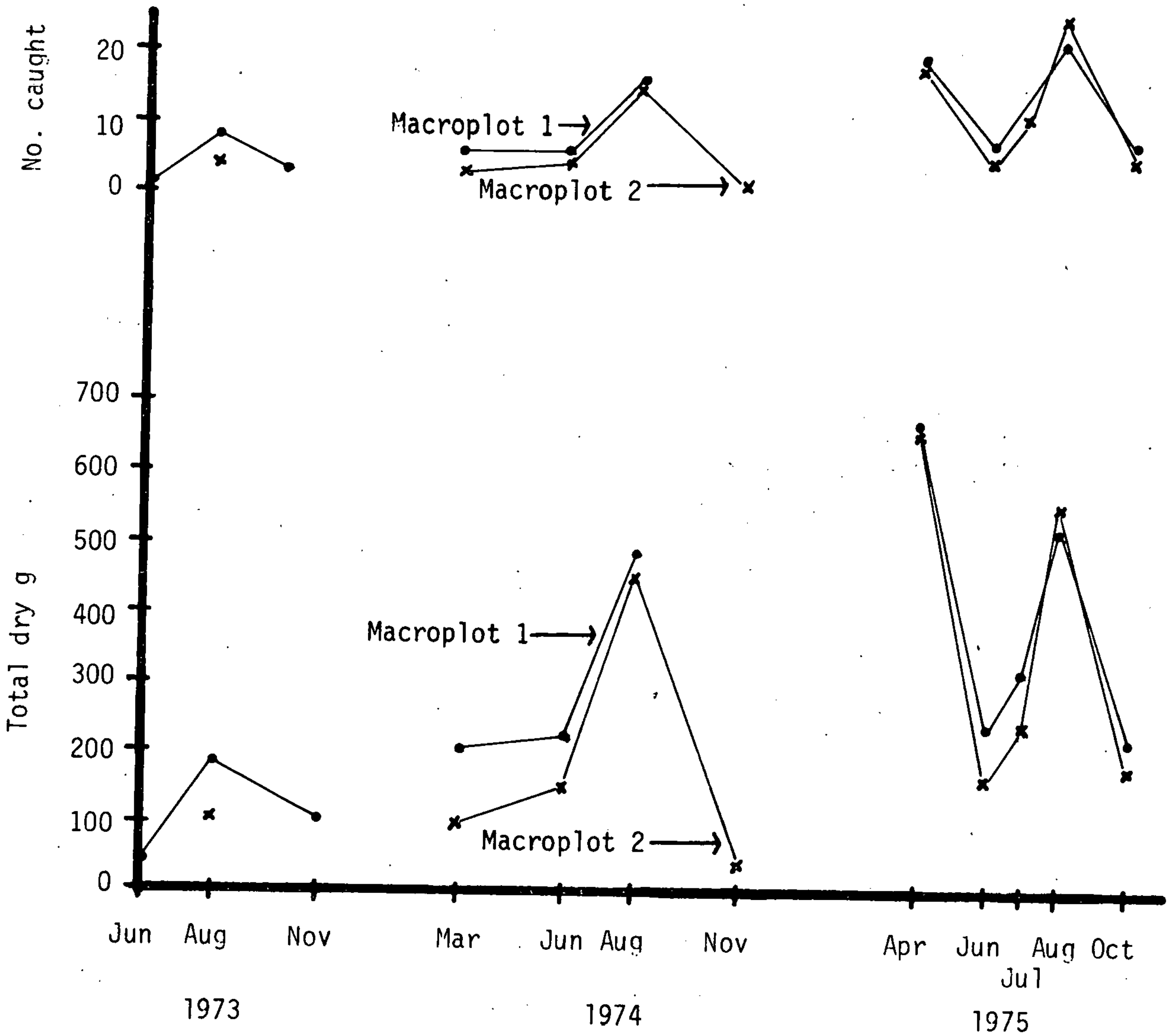

Figure 1. Total number and estimated dry mass of live-trapped *Spermophilus tridecemlineatus* at Rocky Flats.

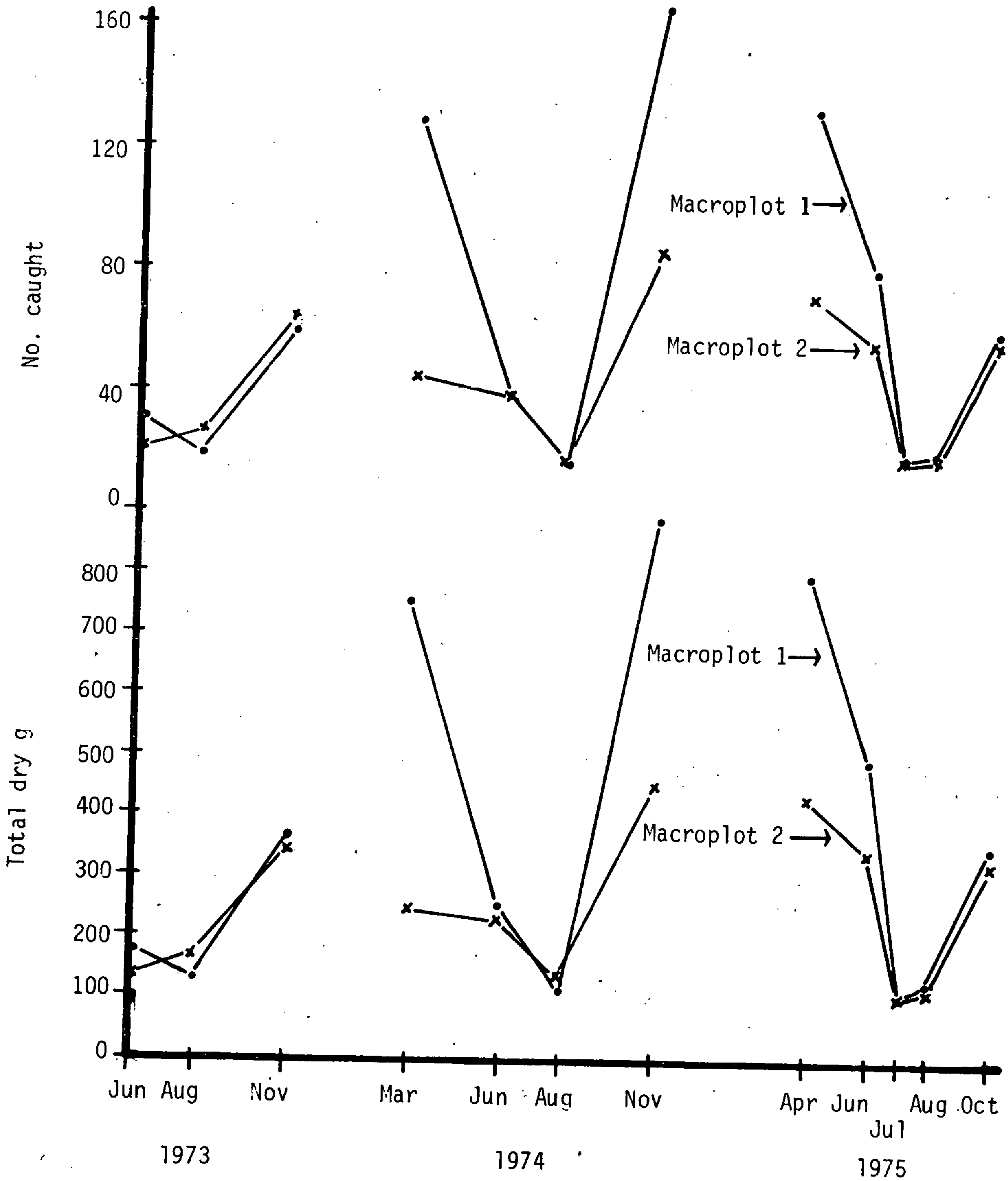

Figure 2. Total number and estimated dry mass of live-trapped *Peromyscus maniculatus* at Rocky Flats.

as well as other, undetermined causes of mortality. November data indicate successful reproduction and March data seem to indicate high overwinter survival.

Evidence exists that few deer mice survive longer than 1 year in field conditions. Longevity in the field is difficult to resolve because of emigration. Grid recapture of identifiable animals were arranged according to date of first capture to estimate gross survival (Table 1). We will later strip out age classes as a measure to estimate survival as a function of birth period. Numbers in the table do not include individuals collected for plutonium analysis, or those which died by trap stress. The data imply that survival during the winter months exceeds that during the summer. Some lost individuals may actually have emigrated or become unresponsive to trapping. An analysis of assessment line data may assist us in determining whether emigration is substantial.

Our data point to successful late year reproduction and high winter carryover followed by heavy summer mortality or emigration. The balance of environmental resources and hazards may therefore be most favorable to Peromyscus during the winter.

Table 2 shows sex ratio data, and the percentage of each sex that were reproductively active during each trapping period. These data are not weighted by age class. Sex ratios generally favored the males, especially in March or April. The ratios were most nearly equivalent in October or November with no clear trend occurring during the summer breeding months. The low percentage of reproductively active deer mice during March, 1974, may have represented the breeding season onset. Generally, a large proportion were reproductively active during summer months. We cannot explain precisely why reproductively active mice maintain low population numbers through the summer, followed by high populations in October and November. Predation and the presence of increased food resources probably account for much of the poor summer trapping success. During November, 1973, a moderately sized, reproductively active population was succeeded the following March by a higher population. Animals were reproductively less active on Macroplot 2 the same November, and the population decreased by March. During November, 1974, large reproductively inactive populations on both macroplots preceded lower numbers during April, 1975.

Age class ratios are given in Table 3. Subadult and juvenile Peromyscus are classified in a single group for these comparisons. The ratios for June and August, 1973, reflect that we were catching young animals. The low ratio during November, 1973, indicated many trappable recruits prior to the high March, 1974, populations. The high ratios during 1974 may have resulted partly from the high winter carryover. November, 1974, was a time of high population, but few subadults were trapped. Many of these latter animals were of subadult size but could not be distinguished from mature animals on the basis of pelage. The combination of many animals and few in reproductive condition during November, 1974, implies that the preponderance of the population matured after August and before November. A high winter population again carried over from 1974 to 1975 and few subadults were captured until July.

Table 1. Survival of *Peromyscus maniculatus* live-trapped at Rocky Flats.

Trapping period animal first caught	Trapping period after initial capture																					
	Aug 73		Nov 73		Mar 74		Jun 74		Aug 74		Nov 74		Apr 75		Jun 75		Jul 75		Aug 75		Oct 75	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Jun 73	6	25	3	13	2	9	1	4	0		0		0		0		0		0		0	
Aug 73			6	54	5	50	1	11	0		0		0		0		0		0		0	
Nov 73					34	65	13	28	7	16	5	11	1	2	0		0		0		0	
Mar 74							23	26	12	14	11	13	4	5	3	3	1	1	1	1	1	1
Jun 74									3	23	2	15	1	8	0		0		0		0	
Aug 74											4	57	3	43	2	29	1	14	1	14	1	14
Nov 74													90	60	52	35	16	11	12	8	7	5
Apr 75															9	14	3	5	3	5	3	5
Jun 75																	4	20	4	20	4	20
Jul 75																			4	67	3	50
Aug 75																					5	100

Table 2. Number of males and females and percentage of reproductively active *Peromyscus maniculatus* live-trapped at Rocky Flats.

Time	Macroplot 1 No.male	(% active)	No.female	(% active)	Macroplot 2 No.male	(% active)	No.female	(% active)
1973								
Jun	15	(67)	15	(80)	8	(88)	12	(100)
Aug	13	(69)	6	(100)	19	(63)	7	(71)
Nov	29	(52)	30	(63)	30	(23)	34	(44)
1974								
Mar	80	(6)	49	(4)	27	(11)	18	(0)
Jun	24	(50)	14	(50)	19	(24)	19	(21)
Aug	8	(50)	8	(38)	6	(100)	11	(55)
Nov	84	(1)	83	(0)	41	(2)	45	(0)
1975								
Apr	82	(43)	50	(52)	51	(65)	20	(85)
Jun	35	(91)	45	(80)	33	(82)	23	(91)
Jul	12	(67)	6	(67)	11	(64)	6	(67)
Aug	11	(73)	9	(56)	13	(92)	6	(67)
Oct	28	(46)	32	(63)	33	(48)	23	(70)

Table 3. Age class ratios of Peromyscus maniculatus live-trapped at Rocky Flats.

	Age Class Ratios					
	Macroplot 1			Macroplot 2		
Time	No.adult	Ratio	No.subadult	No.adult	Ratio	No.subadult
1973						
Jun	23	3.3	7	17	5.7	3
Aug	15	3.8	4	19	2.7	7
Nov	39	2.0	20	34	1.1	30
1974						
Mar	129	--	0	45	--	0
Jun	34	8.5	4	33	6.6	5
Aug	15	15.0	1	16	16.0	1
Nov	154	11.9	13	79	11.3	7
1975						
Apr	132	--	0	71	--	0
Jun	78	39.0	2	55	55.0	1
Jul	14	3.5	4	14	4.7	3
Aug	16	4.0	4	14	2.8	5
Oct	48	4.0	12	42	3.0	14

Criteria for estimating areas of activity of deer mice were: the animal must have been captured in at least three different locations during one trapping session, the captures could not all be on a line, and only one capture on the grid edge was allowed. For immediate purposes we have treated all the animals that met these criteria as one sample from each macroplot. Results listed in Table 4 indicate that areas of movement during the trapping sessions were quite small (about 0.025 acres) compared to values reported in the literature. Home ranges of P. maniculatus have been reported as being from 0.31 acres[3] to 0.74 acres,[4] and differ in relation to such variables as habitat type, density, sex, and breeding condition. It should not be surprising that our data produced smaller estimates than those cited, as we required only a minimum of three captures over a 5-day span. Seven individuals caught during previous trapping sessions were captured on the assessment lines, but not the grids, during the November, 1974, session. One of these mice was caught 172.5 m from the grid edge, a substantial emigration distance.

We have not yet determined the optimum correction factors based on assessment line information, applicable to all our data. Because of this, we have not yet reported biomass and population density estimates.

C. Mule Deer Studies (A. W. Alldredge, W. J. Arthur, and G. S. Hiatt)

This sub-project was designed to determine what, if any, function mule deer might have in transport of plutonium in the environs of Rocky Flats. Deer are numerous at Rocky Flats and because of their feeding habits, they can ingest a variety of environmental pollutants. The mobility of these large ungulates could serve to spread an ingested pollutant such as plutonium from a contaminated area. In addition, data presented by Paine[5] suggest that bacteria may decrease plutonium particle size. Microbes in the deer rumen break down ingested forage and make energy available to the deer. If these same microbes were to break down plutonium particles, then deer could conceivably enhance the biological availability of this radionuclide over periods of time.

To determine potential plutonium intake by mule deer feeding in the more contaminated areas of Rocky Flats, we have employed the "tame deer" technique reported by Wallmo and Neff.[6] Briefly, this technique involves hand-rearing and training deer to allow an observer to walk with them and record the plants they consume while feeding. During field observation

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[3]Williams, O. 1955. Home range of Peromyscus maniculatus rufinus in a Colorado ponderosa pine community. J. Mammal. 36: 42-45.

[4]Fitch, H. S. 1958. Home ranges, territories, and seasonal movements of vertebrates of the natural history reservation. Univ. Kans. Publ. Mus. Nat. Hist., 11: 63-326.

[5]Paine, D. 1974. Plutonium in aquatic systems. Ph.D. Thesis, Colorado State University, Ft. Collins. 164 p.

[6]Wallmo, O. C. and D. J. Neff. 1968. Direct observations of tame deer to measure their consumption of natural forage. pp. 105-110 In Range and Wildlife Habitat Evaluation. U. S. Dep. Agric. Misc. Publ. 1147, 220 p.

Table 4. Calculated mean areas of activity of *Peromyscus maniculatus* live-trapped at Rocky Flats.

Macroplot	Area $\bar{X}(m^2)$	Area SE	n
1	102.3	15.9	56
2	126.8	16.9	54

periods, deer are unrestrained and thus must have a positive association with the observer. To compare plants eaten by tame deer with those of wild deer we have spent time observing, with a spotting scope, feeding wild deer, and as the opportunity arises, rumen contents from deceased wild animals have been analyzed. Thus far, rumen analysis and observations appear to correlate with data obtained from observation of tame deer. In addition to determining plutonium intake from vegetation we have also begun evaluating intake via soil ingestion utilizing the titanium analysis method of Healy.[7] The plutonium intake segment of this project is being conducted by W. J. Arthur as partial requirement for a Master of Science degree in the Department of Radiology and Radiation Biology.

G. S. Hiatt, also a Master of Science degree candidate in the Department of Radiology and Radiation Biology, is investigating the population dynamics and movements of mule deer inhabiting Rocky Flats. This work is aimed at answering the questions: 1. How many deer use the Rocky Flats area and what are current population trends? 2. Are the deer migratory and what are the major seasons of use? 3. How many deer use the contaminated area and how long do they reside in this area? 4. Where do they move and where might we expect to find them excreting any ingested plutonium? 5. What levels of plutonium do these deer retain? To ascertain deer use of the area, 500 pellet group plots have been established in a stratified random sample design. Plots are evaluated for deer use on a seasonal basis. Observations of wild deer will help answer questions with regard to herd size, residence time, and deer movements. During winter of 1975-76, and again in June 1976, indigenous deer were captured, marked, and released to facilitate these observations. Tissue samples from deer killed in the Rocky Flats area are being analyzed to determine plutonium content. In addition to providing data to evaluate the function of deer in plutonium transport, information we collect should also be of value in managing deer on a protected island of habitat in juxtaposition to a metropolitan area. The remaining pages of this section of the report will discuss methods and preliminary results of investigations into the deer and their role in plutonium movement in the environs of Rocky Flats.

Because potential intake of plutonium was the major thrust of this project, the area of Macroplot 1 was selected as the site for conducting food habits trials. Deer were allowed to forage over an area of approximately 200 hectares (S 1/2, SE 1/4, Sec 11, T 2 S, R 70 W, Louisville Quadrangle). Two vegetation types dominated here: 1. Meadow with wheatgrass (*Agropyron* sp.), bluegrass (*Poa compressa*), salsify (*Tragopogon dubius*), nodding thistle (*Cardus nutans*) and sage (*Artemesia ludoviciana*), and 2. Stream bottom with willow (*Salix* sp.), lead plant (*Amorpha fruticosa*), snowberry (*Symphoricarpus occidentalis*), plains cottonwood (*Populus sargentii*), and sedge (*Carex* sp.). On May 1, 1976, the study area was moved 1.6 km W-SW due to conflicts with the Rocky Flats shooting range. The new area is similar in vegetation type and physical characteristics, thus changing areas should not greatly influence deer food habits.

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[7]Healy, W. B. 1967. Ingestion of soil by sheep. New Zealand Soc. Anim. Prod. 27: 109-120.

During the summer of 1975, five mule deer fawns were raised and trained for food habits research. An additional nine fawns are currently being reared for the same purpose. It is imperative that the deer tolerate close association of persons conducting field observations and thus nearly constant contact is maintained during rearing and training.

Food trials are conducted seasonally with the objective of obtaining 15,000 bites of vegetation per season. A bite is defined for our purposes as any complete severence of a plant part with subsequent ingestion by the deer. The selection of 15,000 bites was based strictly on time constraints imposed by the study. Preceding a trial, one tame deer is picked randomly by each observer and then taken to the study area. The animal is released and followed by the observer as long as it productively forages (usually 1.5 to 3 hours). Deer are allowed to roam freely over the area and are not disturbed unless they either wander along a road or cease feeding.

Bite count data are summarized with the aid of a computer and expressed seasonally as percent species consumed. Sampling is being initiated to estimate the average weight of a bite for the major plant species consumed. During fall and winter plants are weighed and offered to the deer while observers count the number of bites taken. The average weight per bite is obtained by dividing pre and post weight difference by the number of bites taken. Loss of moisture from collected vegetation during spring and summer necessitated that we attempt to estimate bite size by observing how much of a plant the deer consumed in one bite and then plucking by hand a similar portion of the plants. From data we collect, an estimate of seasonal food habits can be obtained on a weight basis.

Each season plants grouped according to forbs, shrubs, and grasses are sampled and analyzed for Pu-239. Composition of plant groups is based upon the proportion each plant in the group contributed to the total diet. For example, if yarrow was 35 percent of the spring diet, then 35 percent of the forb sample collected for analysis in spring would be composed of yarrow. Plants will be sampled by random clipping in the Macroplot 1 area, washed to eliminate surface contamination, oven dried, homogenized, and a 5 gram aliquot sent for analysis.

Preliminary food habits research with tame deer and observation of wild deer indicates that soil is ingested in varying amounts by these animals. Consumption of soil occurs through (1) soil attached to plant roots, (2) soil adhered to above ground portions of plants, and (3) direct eating of soil. Soil has been found to contain far more plutonium than vegetation,[8] thus soil intake must be evaluated as a component of plutonium intake by deer.

To estimate the amount of soil consumed, an atomic absorption spectrometry analysis is being made for titanium in deer feces. Titanium is a trace element in soil (about 2000 ppm) and is not taken up by plants

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[8] Little, C. A. 1976. Plutonium in a grassland ecosystem. Ph.D. Thesis. Colorado State University, Fort Collins. 170 p.

to any extent (1 ppm measured in plants). As no titanium is retained in animal tissue, essentially all titanium found in fecal material must come from ingestion of soil.[9]

Five random soil samples were taken to ascertain titanium levels in Rocky Flats soil. Each season four plant samples and twenty fecal samples are collected. Care is taken to assure that pellet groups sampled were deposited during the season of interest and external soil contamination is removed from plant and fecal samples. Samples are oven dried for 48 hours at 100 C and ground in a Wiley mill. Calculation of the soil ingestion rate (R) is:

$$R = \frac{F\ C}{S}$$

where: R = soil ingestion rate (g/day)
F = fecal output rate (g/day)
C = titanium concentration in feces (μg/g)
S = titanium concentration in soil (μg/g)

A plutonium contamination factor for soil will be derived by integration of data taken by Little[8] and Krey and Hardy[10] to give a mean dpm/g for soil over the entire study area. This value will be applied to soil ingestion data to give an estimate of the plutonium intake by deer via soil.

A complete list of the major plant species consumed and the percentage by bites for winter 1975-76 is given in Table 5. Note that green grass (primarily Bromus tectorum) comprised 36.1 percent of the diet. All of this was consumed during the last third of winter.

Based on 15 hours of observation, a list of plant species consumed by wild deer was compiled (Table 6). The major difference between the tame and wild deer diets were in sandbar willow (Salix interior) and plains cottonwood (Populus sargentii) leaves. This is probably because few wild deer observations were made in the stream bottom where these species dominate. Observations were made on large groups of deer when possible (fall, winter, early spring). Thereafter due to herd dispersal, individual animals were observed. In spring and summer with tall vegetation it was often impossible to distinguish what plants were being consumed. At these times, observations were made to see where deer were foraging and then a thorough search of the area of use made for evidence of browsed plants. Most observations were made close to the study area, but in spring and summer minimal deer use occurred on the study area and observations were expanded to cover most of the Rocky Flats property.

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[9]Mayland, H. F., A. R. Florence, R. C. Rosenau, V. A. Lazar and N. A. Turner. 1975. Soil ingestion by cattle on semiarid range as reflected by titanium analysis of feces. J. Range Manage. 28(6): 448-452.

[10]Krey, P. W. and E. P. Hardy. 1970. Plutonium in soil around the Rocky Flats plant. USAEC Report HASL-235.

Table 5. Percent species by bite for winter food trial data.

Species	Percent Total
Green grass*	36.08%
Salix interior	9.64%
Populus sargentii	6.83%
Bromus tectorum	5.87%
Cardus nutans	5.76%
Amorpha fruticosa	5.29%
Symphoricarpos occidentalis	3.45%
Mixed grass	3.03%
Cirsium arvense	2.74%
Poa sp.	2.65%
Tragopogon dubius	2.06%
Artemesia ludoviciana	1.52%
Unknown forb	1.12%
Total	86.04%

*Mixture of new grass (*Agropyron* sp., *Poa* sp., and *Bromus tectorum*) growing in late winter.

Table 6. Plant species occurrence in the Rocky Flats deer diet based on direct observations for Fall, Winter, and Spring.

	Season		
Species	Fall	Winter	Spring
Agoseris glauca			X
Agropyron sp.		X	X
Andropogon gerardii		X	
Artemesia ludoviciana	X	X	X
Bromus tectorum		X	X
Cardus nutans		X	
Carex nebraskensis			X
Carex sp.			X
Cruciferae family			X
Equisetum laevigatum			X
Erigonium alatum			X
Gutierrezia sarothrae		X	
Heterotheca villosa	X	X	
Iris missouriensis			X
Koeleria gracilis	X		
Lactuca serriola			X
Lomatium orientale			X
Mertensia lanceolata			X
Poa sp.	X	X	X
Podospermum laciniatum			X
Populus sargentii			X
Rosa arkansana			X
Solidago missouriensis			X
Taraxacum officinale			X
Tragopogon dubius	X	X	
Yucca glauca		X	
Zygadensus venenosus			X

Table 7 gives a summary of all plant species found in deer rumen contents. All animals obtained for analysis were killed between 11-10-75 and 3-15-76, the winter season of our study. Five rumens were collected shortly after the deer were killed by automobile collisions. The other three animals were coyote (Canis latrans) or dog kills. Similarity exists between plants found in the rumen analysis of deceased wild deer and those consumed by tame deer during food habits trials. The major species found were plains cottonwood, sandbar willow, nodding thistle, cheatgrass, salsify, and sage.

Analysis of soil samples for titanium indicated little variability in the presence of this trace element (mean = 2800 μg Ti/g soil S.D. = 192.0). Low concentrations of titanium were found in plants (8.75 μg Ti/g vegetation S.D. = 2.86). A range of 89 to 430 g Ti per gram of feces indicates variability in the consumption of soil by deer ($\bar{x}$ = 200, S.D. = 89.9). More samples will be taken next winter to refine estimates. Below is an estimate of the mean daily soil intake per deer during winter.

$$\frac{\text{g-soil}}{2800\ \mu\text{g Ti}} \times \frac{191.25\ \mu\text{g Ti}}{\text{g feces}} \times \frac{600\ \text{g feces}}{\text{deer-day}} = \frac{40.9\ \text{g soil}}{\text{deer-day}}$$

Based on our observations, we believe that the majority of this soil ingestion is associated with consumption of plant root material, principally roots of sage and cheatgrass. However, we have observed tame deer directly consuming soil from pocket gopher mounds and disturbed areas.

Table 8 gives an estimate of the total plutonium intake for a deer feeding in Macroplot 1 based on daily plant consumption of 1400 g dry plant material. The best estimates currently available for vegetation and soil plutonium contamination are 63.4 dpm/g and 1853 dpm/g respectively.[8]

In order to fully understand the function of mule deer in transport of plutonium at Rocky Flats, it is necessary to understand the characteristics and behavior of the deer herd utilizing the area. We have already briefly mentioned the questions we hope to answer with regard to the deer herd, and the following pages will discuss methods and preliminary results obtained to date.

The 500 circular pellet group plots, each with an area of 10 m^2 (0.0025 acre), were established in June and July of 1975. Plots are located in five, 35 ha (86 acre) study areas within the A-zone of the ERDA property, with 100 plots allotted to each area. The Southeast (SE) area covers much of the Woman Creek drainage southeast of the asphalt pad. The Southwest (SW) area is moister habitat, south of the Rocky Flats Plant. The Flats (FL) area is located on the prairie northwest of the plant. The Northwest (NW) location consists of shrub-filled valleys farther northwest, and in the grass covered hills of the northeast section of the Rocky Flats property is located the Northeast area (NE). Plots within each of the five areas are arranged in ten parallel transects with ten plots on each transect. Transects are set at random distances along a central axis and run perpendicular to it. This results, for the most part, in transects running perpendicular to the contour of the land and

Table 7. Winter plant species consumed based on analysis of eight rumens from deer killed between 11-10-75 and 3-15-76. Key (ST = stem, HD = flower, LV = leaf, FT = fruit, ENT = entire plant consumed).

Species	Part	Number of rumens containing	Percent rumens found
Forbs			
Artemesia ludoviciana	LV,ST	6	75.0%
Tragopogon dubius	HD,ST	5	62.5%
Opuntia compressa	FT	4	50.0%
Cardus nutans - *Cirsium arvense**	LV,ST	4	50.0%
Artemesia frigida	ENT	1	12,5%
Artemesia campestris	ENT	1	12.5%
Heterotheca villosa	LV	1	12.5%
Hypericum perforatum	HD,ST	1	12.5%
Erigeron sp.	HD	1	12.5%
Linum lewisii	FT	1	12.5%
Shrubs			
Salix interior	LV,ST	6	75.0%
Symphoricarpos occidentalis	LV,ST	5	62.5%
Populus sargentii	LV	4	50.0%
Grasses			
Bromus tectorum	ENT	3	37.5%
Agropyron sp.	ENT	3	37.5%
Carex nebraskensis	ENT	2	25.0%
Poa sp.	ENT	1	12.5%
Carex sp.	ENT	1	12.5%

*Hard to differentiate leaves due to partial digestion.

Table 8. Annual plutonium-239 intake for one standard weight (55 kg) deer feeding on Macroplot 1.

	Days feeding on Macroplot 1	Soil (grams/day)	Vegetation (grams/day)	Annual Plutonium Intake (dpm)
Maximum possible	365	76.1	1400	8.4×10^7
Maximum credible	365	40.9	1400	6.0×10^7
Probable	30*	40.9	1400	4.9×10^6

*Estimate based on preliminary deer use observations thus each transect randomly samples terrain of varying slope, aspect, and vegetation type reducing the effects of these factors on the variability between transects.

As plots were established, all fecal material was removed and at the end of each season any new pellet groups are recorded and removed. The number of groups found in the ten plots on each transect is divided by the elapsed time (in days) since the plots were last cleared, yielding an average deposition rate in pellet groups per day per 100 m^2. Multiplying this by a factor of 100 expresses data in pellet groups per day per hectare. Data are analyzed using analysis of variance techniques and tests for difference, such as Duncan's New Multiple Range Test.

Table 9 summarizes the first year's data. The overall deposition rates for summer, fall, and winter are not significantly different, suggesting there was no major change in deer utilization of the plant environs sampled. The deposition rate for spring is significantly ($\alpha = 0.01$) lower than the other seasons. Field observations indicate this probably was due to deer moving almost exclusively into the flats of the newly acquired buffer zone properties, and not to a large proportion of the population moving out of the Rocky Flats region. Seasonal data from the SE area, which contains soils with relatively high plutonium levels, follow the same pattern; deer use of this area in spring was one-tenth of that of the rest of the year.

Over 500 observations of wild deer in the Rocky Flats area have been made, with date, time, observer, location, number of deer, and when possible, the age and sex of the deer being recorded. Data are collected from walked transects, helicopter surveys, or incidental observations. R. L. Shipley, a programming consultant, is currently working with us to develop a computerized storage-retrieval system to aid in analyzing this information. A cartesian coordinate system is used in recording observation locations. The origin of this system is 3 km southwest of the plant at the SW corner of Section 16, R 70 W, T 25 N. Coordinates are 200 m apart, thus each point represents 4 ha (10 acres).

To facilitate identification of individuals in the population, 24 deer were trapped between 5 November 1975, and 14 January 1976, marked with numbered blue neck collars and aluminum ear tags, and released. Table 10 lists trapping information on these animals as well as their present status, number of subsequent sitings, and the date they were last observed. Eleven newborn fawns were captured and marked with red or yellow numbered ear tags during June 1976 (Table 11). Observations on these 35 marked animals provide data for estimating population size, natality, mortality, emigration, immigration, seasonal movements, and home ranges, as well as the number of deer using the contaminated area and their residence time.

No estimates of population size are available for summer, 1975, due to initiation of our study at this time. A walked transect in late October yielded a count of at least 107 deer within or near the A-zone. Estimates made after many of the collared deer were returned to the population placed the herd size at 130-150 deer in early winter, this

Table 9. Average pellet group deposition rates (groups per day per hectare).

Study Area	Season S	Season F	Season W	Season Sp	Year Average
SE	1.9	2.6	2.1	0.2	1.7
SW	3.0	3.8	1.9	1.0	2.4
FL	1.7	1.4	1.9	1.7	1.7
NW	5.3	3.3	5.3	1.5	3.9
NE	2.5	1.1	1.2	0.8	1.4
All areas	2.9	2.4	2.5	1.0	2.2

Table 10. Collared deer trapping and sitings summary.

Eartag#	Collar#	Date Trapped	Sex[1]	Age Class[2]	Present Status[3]	# Sitings[4]	Latest Siting
43000	Blank	5 Nov 75	F	Y	L	27	20 June 76
43001	0	6 Nov 75	F	A	D	10	13 Jan 76
43002	30	6 Nov 75	F	A	L	27	20 June 76
43003	25	7 Nov 75	M	F	D[5]	8	22 Jan 76
43004	1	7 Nov 75	F	F	U	2	2 Dec 75
43005	23	10 Nov 75	F	F	U	14	15 Feb 76
43006	29	10 Nov 75	F	A	L	28	12 June 76
43007	27	10 Nov 75	F	F	D	13	15 Feb 76
43009	24	13 Nov 75	F	F	D	5	13 Jan 76
43010	21	13 Nov 75	F	A	L	27	20 June 76
43011	5	13 Nov 75	F	A	U	28	21 May 76
43012	34	14 Nov 75	M	F	D	3	11 Dec 75
43013	40	14 Nov 75	F	A	L	30	19 June 76
43014	32	25 Nov 75	M	F	D	10	3 Feb 76
43015	7	25 Nov 75	F	A	L	32	20 June 76
43016	28	27 Nov 75	F	F	D[5]	3	9 Jan 76
43017	8	25 Nov 75	F	A	L	32	20 June 76
43018	9	26 Nov 75	M	Y	U	18	21 May 76
43019	11	27 Nov 75	F	A	L	25	19 June 76
43020	26	17 Dec 75	F	F	L	28	19 June 76
43021	31	17 Dec 75	M	F	L	25	17 June 76
43022	36	17 Dec 75	M	F	D	0	17 Dec 75
43023	13	7 Jan 76	M	F	L	25	18 June 76
43024	35	14 Jan 76	M	F	D	0	14 Jan 76

[1]F = female, M = male
[2]F = fawn, Y = yearling, A = adult
[3]As of 20 June 76 L = living, U = unknown, D = dead
[4]As of 20 June 76
[5]Exact fate unknown, but presumed dead

Table 11. Fawn tagging records.

Eartag	Date Marked	Estimated Birth Date	Sex	Single or Twin	Doe
Yellow 1	8 June 76	5-6 June 76	F	S	unmarked
Yellow 2	10 June 76	6-7 June 76	F	T	unknown
Yellow 3	10 June 76	6-7 June 76	F	T	unknown
Yellow 4	12 June 76	6-7 June 76	F	T	#7
Red 1	12 June 76	6-7 June 76	M	T	#7
Red 2	12 June 76	9-10 June 76	M	T	#21
Red 3	12 June 76	9-10 June 76	M	T	#21
Yellow 5	15 June 76	8-10 June 76	F	S	unmarked
Red 4	18 June 76	17 June 76	M	S	#8
Red 5	18 June 76	15-16 June 76	M	T	unmarked
Red 6	18 June 76	15-16 June 76	M	T	unmarked

declining to 100-120 animals by early spring. By mid-May 1976, 90-100 deer were using the area. Does comprised 50 percent of the winter population, fawns 30 percent, and bucks 20 percent.

Recorded deaths of collared and unmarked deer indicate that the population decline in winter and spring may be attributed to mortality not emigration, with the main causes of death being predation and auto-deer collisions. Three collared fawns (numbers 24, 25, 28) slipped one of their forelegs through their collars in early January and were probably later killed by canids. Three deer, two of which were collared, are known to have been killed by motor vehicles during this period, and at least six deer, including four collared fawns, are believed to have been killed by coyotes or dogs. Fawn mortality was highest, around 50 percent for marked deer with a similar proportion of unmarked fawns disappearing from the population. Three adults and one yearling were also known to have been killed in this period. Four collared deer are unaccounted for: number 1 is presumed dead or permanently emigrated, while the other three may have moved temporarily offsite.

The peak fawning period was the second week of June. During that month we observed a fawn-to-doe ratio of 1.5:1. With 40-50 does producing fawns, we estimate 60-75 fawns were added to the population.

During summer, bucks are grouped into bachelor herds of 5 to 15 animals, and are found usually on the periphery of the ERDA property, or on adjacent land. Does are originally isolated with their fawns in spring, although they still move over large areas and later join together with other does and fawns, usually meeting along creek bottoms. Yearlings are often seen alone during this season. In fall these groups break up, rejoin, and intermingle. During this dispersal period, single animals, possibly transients, are commonly seen. Deer appear to use all areas of the plant site more during fall. By early winter they were grouped into two distinct herds, one of about 45 deer on the south side of the plant, the other on about the same size in the northwest region. Approximately six to twenty animals were continually moving between these two herds, or in small groups along Walnut Creek. During winter these herds were quite mobile, moving down the valleys after snowstorms, otherwise they are found on the southeast facing slopes. In spring they moved up onto the flat prairies to take advantage of new vegetation there. These herds broke up in May.

Home ranges of marked deer are estimated using the cartesian coordinates and calculating the area of a convex polygon which contains all points where the animals were sited. In the future, this will be done using computer programs, but estimates have been hand calculated for two deer. Number 11, a doe from the south herd, covered an area at least 3.25 km^2. The estimated range of number 30, another doe but from the transient herd, was 8.5 km^2. Both of these animals have been in the vicinity of Macroplot 1. A similar program will be used to determine the rate of movement for these deer. We have records of #11 leaving the Macroplot 1 area and traveling 2.6 km in two days and of #30 covering 2.25 km in one day. We have no reason to consider such movements unique or uncommon.

Judging from the ranges of deer herds at Rocky Flats, we estimate 30 to 70 deer may be using the more contaminated area around Macroplot 1. When our data storage-retrieval system is functioning, we hope to refine this estimate by analyzing siting frequencies. Similar methods will be applied to estimate the proportion of a season or year that deer may reside on plutonium contaminated areas. Our current estimate is 10 to 50 days per year. Residence times may vary between one hour and one month. Increased intensity of field observations will be used to improve estimates of mean residence time in areas of concern.

To estimate plutonium content of deer around Rocky Flats, we have collected tissue samples from six deer killed in the area and these are currently being analyzed. To date five lung, four liver, and five metacarpal samples have been collected from Rocky Flats, as well as one lung, two liver, and five metacarpal samples as controls.

During 1974-75 some preliminary work was begun to examine the potential for plutonium particle size change as particles passed through the deer gut. A number of analytical problems were encountered in this work and it was concluded that particles fed to the deer were not monodisperse and thus no statements could be made with respect to change. We are currently preparing to once again feed a deer plutonium of known particle size and attempt to ascertain any changes that might occur. Results of this work should be available during the coming year.

In addition to work with mule deer at Rocky Flats we have also recorded observations on coyotes in the area. The wintering coyote population at Rocky Flats is estimated to be between 7 and 12 individuals. They are commonly sighted, with winter packs containing 3 to 7 animals. Two active dens have been discovered, and also one badger (*Taxidea taxus*) den with young was discovered. We have been collecting fresh coyote scat which will be analyzed by C. A. Ribic, a student in wildlife biology, in an attempt to ascertain seasonal food habits of coyotes.

D. Role of Pocket Gophers in Plutonium Transport (T. F. Winsor)

During the course of our studies it has become apparent that only a small portion of the total plutonium inventory actually resides in the mammalian compartment. The notion that metabolic movement through mammals may be of little importance relative to other forces driving plutonium led us to other considerations. We decided to include an assessment of rodent behavioral activity. Burrowing and mound building exposes quantities of loose soil to erosive forces and aerial resuspension. Pocket gophers move visibly greater amounts of soil from below to above the ground surface than other mammals resident to the site, thus they were obvious subjects for investigation.[11] The objectives of this study include: (1) estimating the quantity and origin of loose soil upcast by gophers, (2) determining the plutonium content of the exposed soil, and (3) comparing the amount

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[11]Miller, M. A. 1948. Seasonal trends in burrowing of pocket gophers (*Thomomys*). J. Mammal. 29: 38-44.

of plutonium in upcast soil to the plutonium content of relatively undisturbed soil. We describe here the current findings about pocket gopher effects on soil plutonium distribution at Rocky Flats. All work was done on a 2.6 ha plot overlaying our Macroplot 1 trapping grid (Fig. 3).

Three types of surface sign are left by pocket gophers: (1) concentric mounds or mound clusters, (2) surface plugs (soil plugs used to block temporary exits from burrow), and (3) winter casts (soil used to fill burrows made in snow). Only mounds comprised enough soil to be used in this investigation. Between February 1975 and July 1976, we took measurements of effects of gopher soil remodeling activity on the grid overlaying Macroplot 1. "Fresh" mounds (judged to be a week or less old) were measured to the nearest cm along the greatest and least axes. The average of the two values was used to compute a plane circle which represented a rough estimate of mound surface area. We scooped mound soil by hand and trowel from the top downward until approximate ground level was reached, and weighed it to the nearest 10 g. The soil was mixed until reasonably homogeneous and samples of about 100 g were oven dried for calculation of mound dry weight and for plutonium analysis. We then sampled the soil profile adjacent to the mound by collecting, and separately homogenizing samples from 0-10, 10-20, and 20-30 cm depths, also for plutonium analysis. Finally, we measured the depth of the burrow leading from the mound to a distance of 1 m away to determine the origin of the mound soil.

We estimated pocket gopher activity on the plot by periodically counting fresh mounds constructed over 2 to 25 day spans. We were also able to estimate the total number of mounds constructed, from June 4, 1975, to May 21, 1976, on a 0.9 ha area enclosed by a single stranded wire fence (Fig. 3).

Table 12 depicts average mass and surface area of mounds upcast by pocket gophers on Macroplot 1. We feel that 2910 g acceptably represents the average mound mass on Macroplot 1, but point to the broad range as an example of variable gopher activity. Field experience during the past 3-1/2 years also leads us to strongly suspect that younger, smaller pocket gophers tend to build narrower burrows and smaller mounds. The calculated surface area, as a plane surface, is an obligatory underestimate of the erodible mound surface; but serves as a useful guide to the area of former topsoil now covered by soil from depth. We attempted to measure mound depth with the intention of calculating mound volume as a predictor of mass, but the results were generally ambiguous.

Data concerning mound construction rate are shown in Table 13. Notably, data covering the winter months November through February are missing. We know from observation that mound building is not stopped during the winter, but were unable to obtain accurate counts during that period. Mound building rates did not vary greatly between sampling periods, with the possible exception of March 1975. This data must be taken as representative only of the stated period. During June 1974, through October 1974, we attempted to trap pocket gophers. We estimate that during this earlier period, mound building rates did not exceed 20% of those attained in 1975 and 1976. Also, between March 1973 and June 1975, no more than 30 mounds were constructed in the 0.9 ha subplot. All the

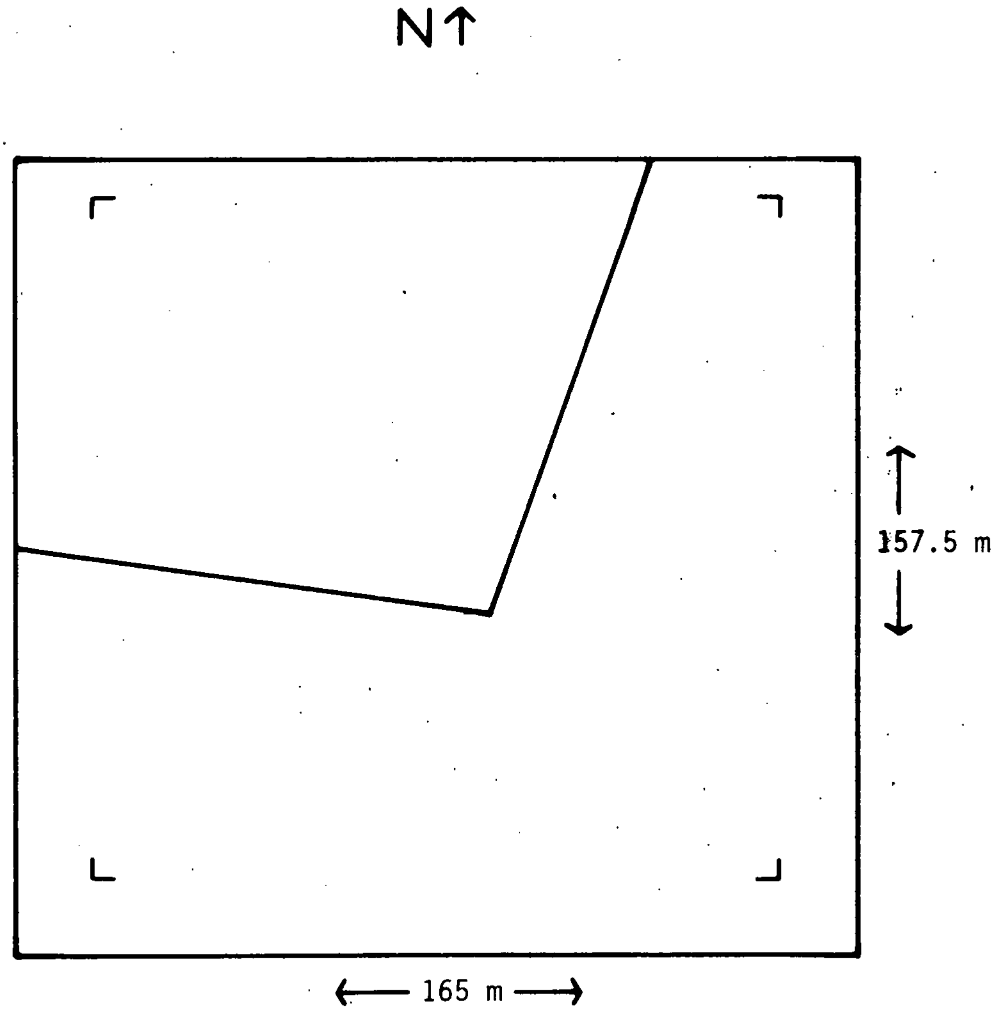

Figure 3. Schematic of area surveyed for gopher mound construction activity. Enclosed subplot represents a 0.9 ha area wherein total mounds were estimated during 1 year. Corners of Macroplot 1 trapping grid indicated by L.

Table 12. Mass and estimated surface area of mounds upcast by pocket gophers.

	Mean	SE	Range	n
Dry wt. (g)	2910	291	460-7580	43
Surface area (cm^2)	1235	104	315-3170	44

Table 13. Mound construction rates by pocket gophers.

Sampling period	No. mounds	Mounds/day
March 11 - March 18, 1975*	55	7.9
April 21 - April 23, 1975	6	3.0
June 4 - June 11, 1975	25	3.6
July 2 - July 8, 1975	30	5.0
July 14 - July 22, 1975	22	2.8
August 16 - August 21, 1975	25	5.0
October 15 - October 22, 1975	24	3.4
March 11 - October 22, 1975**		4.5
June 10 - July 5, 1976	111	4.5
0.9 ha subplot		
June 4, 1975 - May 21, 1976*	190	0.5

*Estimate: believed to be within 10 of true value

**Mean computed using number of mounds counted during listed sampling periods above shows the sporadic nature of total pocket gopher activity, from year to year, and is largely a result of population fluctuation. We probably artificially dampened total activity during the sampling period, as we removed 9 pocket gophers from the plot.

Analytical results appear in Table 14 along with measured burrow depths. A more detailed statistical analysis will follow the receipt of data concerning 12 final mound and profile sets. The data are evidence that mound soil is comprised mostly of material pushed up from depths of generally 10 cm or greater, but seldom more than 30 cm.

Average mound building activity data imply that roughly 1000 mounds were constructed between March 11, 1975 and October 22, 1975. The application of mass, surface area, and plutonium concentration averages leads to an approximation of 3 metric tons of subsurface soil exposed by pocket gophers over an area of 125 m^2 during that period, containing 50 μCi plutonium. We recognize the hazards of extrapolated data, but believe our final overall estimates are easily within one order of magnitude of the true situation. While it is true that mound soil is lying exposed to erosive forces, it is clear that sampled mound soil contains less plutonium than undisturbed surface soil. We cannot evaluate whether exposed mound soil or surface soil bound by vegetation presents the greater resuspension hazard. Erosive forces tend to flatten and spread mound soil over a greater area, and eventually to render the mound unrecognizable as such. Also, vegetation regrowth occurs and assists in stabilization.

E. Rodent Pathology (T. F. Winsor)

The overall plutonium study has included a search for pathologic effects of plutonium alpha particles on small mammals, and conditions not seen as related to plutonium, such as parasitic infestations and other diseases. This report updates results. The scope of the investigation has not been broadened, but more animals have been analyzed.

Prior to sacrifice for plutonium analysis, some mammals were radiographed by the clinical veterinary staff of the Department of Radiology and Radiation Biology at Colorado State University. We have taken no new radiographs during the last year, but hope to obtain 30 to 40 more shortly. These anticipated radiographs will mostly include animals trapped near Fort Collins.

Lung sections were preserved in buffered formalin for shipment to Dr. G. E. Dagle at Battelle Northwest Laboratories, Richland, Washington. Dagle microscopically examined the lung sections for evidence of cancerous conditions, and submitted a few of the lung tissues to 8-week autoradiography. We also searched for pathologic conditions during routine necropsy. Numbers of samples examined are ordered according to location and examination type in Table 15. Of the 149 microscopic lung examinations performed by Dagle, 152 skeletal x-rays taken, and 217 necropsies, all were negative. Six autoradiographs of lung tissue from Macroplot 1, and four from Macroplot 2 revealed no detectable alpha activity.

Table 14. Plutonium concentration of mounds and adjacent soil profiles, and depths of burrows leading to the mounds.

	Mean	SE	Range	n
Mound (dpm Pu/g)	36.4	7.6	4.7-133.5	21
0-10 cm (dpm Pu/g)	151.6	24.8	14.6-404.5	20
10-20 cm (dpm Pu/g)	24.0	5.7	1.1-108.6	20
20-30 cm (dpm Pu/g)	11.5	3.7	0.2- 71.4	21
Burrow depth (cm)*	15	1.0	9 - 27	17

*Depth from soil surface to center of burrow. The angled shaft leading from the mound to the horizontal burrow was not included in the calculations.

Table 15. Numbers of small mammals examined for abnormalities similar to those caused by plutonium.

Location	Method of Analysis * **		
	Skeletal X-ray	Microscopy	Necropsy
Macroplot 1	68	80	108
Macroplot 2	61	51	55
Asphalt pad area	19	18	28
Fort Collins area	4	0	26

*We performed follow-up necropsy and microscopic lung examinations on most animals which were X-rayed.

**No abnormality similar to those induced by plutonium was discovered in any animal.

Evidence of nonradiogenic disease, as revealed by necropsy and Dagle's efforts, is shown in Table 16. Because we committed tissues to plutonium analysis, we avoided destructive necroscopic techniques which might have uncovered some pathologic conditions. Nevertheless, a small number of animals were found to have suffered disease. Microscopic analysis indicated 12% of submitted lung sections were damaged or parasitized. Necropsy revealed internal parasites or cysts in 7% of the small mammals examined.

Childs and Cosgrove[12] studied pathology of wild rodents in areas of high and low radioactivity (principally ^{106}Ru, ^{60}Co, and ^{90}Sr), and saw no differences. Our results seem in accordance. Cosgrove (personal communication) reported that 29 of 79 P. maniculatus from the Pacific Northwest suffered a variety of internal pathologic conditions, a higher proportion than we have found, but again, we have examined only the lungs microscopically.

If any small mammals do suffer from lesions similar to those induced by plutonium, we may never observe them. It is possible that individuals suffering from disease, advanced sufficiently to be perceived by our methods, may not be trappable. We have held some animals in captivity in an effort to check the above possibility. Fourteen from Rocky Flats and 24 from the Fort Collins area have been examined with negative results. Approximately 30 from Rocky Flats and 30 from Fort Collins remain to be examined. It seems obvious that these data, and related data comparing lifespans in the laboratory will be far too few to detect differences.

We are currently awaiting microscopic results from Dr. Dagle concerning 16 animals from Macroplot 1, 5 from the asphalt pad area, and 19 from the Fort Collins area. Possibly 60 to 100 more necropsies will be performed. Our pathologic examinations are being phased out with the conclusion that our current methods fail to show a plutonium hazard for small mammals at Rocky Flats.

Finally, we have appended a manuscript, to be submitted to the Journal of Wildlife Diseases, entitled "Besnoitiosis in rodents from Colorado".

F. Root Uptake Versus Aerial Deposition of Plutonium (M. Miller and L. Fraley, Jr.)

During the summer of 1974, an experiment was initiated to measure concentration ratios for plutonium between soil and plants and to evaluate the relative importance of root uptake and aerial deposition. The method,

12Childs, H. E., Jr. and G. E. Cosgrove. 1966. A study of pathological conditions in wild rodents in radioactive areas. Amer. Midl. Nat. 76:309-324.

Table 16. General pathology and parasitic infestation of mammals caught at Rocky Flats.

	Location		
	Macroplot 1	Macroplot 2	Asphalt pad area
Microscopic analysis of lung*			
parasitic	6/80	4/51	0/18
emphysema	1/80	1/51	0/18
pleural fibrosis	0/80	2/51	0/18
alveolar edema	3/80	1/51	0/18
Necropsy			
internal parasites or cysts	1/80	4/51	5/18

*Each instance of pathology or parasitism revealed by microscopic analysis was discovered in a different animal.

in brief, was to transfer contaminated soil blocks (about 575 d/m·g dry soil in the top 3 cm) to an uncontaminated locale--Macroplot NW, two miles northwest of the plant site, and inversely, transfer uncontaminated soil blocks to a contaminated locale--Macroplot 1, adjacent to the southeast plant boundary.[13] Any activity detected in vegetation samples from Macroplot NW is due to root uptake and that from Macroplot 1 is due to resuspension and aerial deposition.[14] Sampling through March of 1975 served to establish background information such as Pu isotopic ratios and soil block Pu concentration for later determination of concentration ratios.

Data from this year's sampling (June, 1975 - February, 1976) in Table 17 and Figure 4 indicate that mean values for Macroplot NW (root uptake) are generally greater than the means of Macroplot 1 for the same sampling dates. This means that root uptake may be a more important pathway than aerial deposition, which is in direct contrast to what is generally believed concerning Pu contamination of vegetation.[15] However, since both macroplot means follow approximately the same temporal pattern, one cannot say with certainty that root uptake (with the given soil Pu concentration) as a pathway in Macroplot NW is significantly different from the aerial deposition pathway in Macroplot 1. A two-way analysis of variance in Table 18 confirms this.

The F-Value for macroplot difference is not large anough to be termed significant, as is the case for the interaction of time and macroplot. However, time is a significant factor affecting the level of Pu concentration. A Tukey's Q Test using the Studentized Range[16] indicated that for the month of August, Pu concentrations in both macroplots were significantly higher than for the June, July, and October, though not for the February sample dates.

Although present data indicate there is no difference in the pathways being studied, it is noteworthy that each pathway appears to contribute approximately equally to the transfer of plutonium to the plant. The concentration ratios observed in Macroplot NW ranged from 3.6×10^{-4} to 6.3×10^{-3}. Although the conditions of this study were much different than other studies done in this area, they still compare favorably.

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[13] Baker, S. J. 1975. Soil-Plant Transfer of Plutonium. Master's Report. Dept. of Radiology & Radiation Biology. Colo. State Univ., Ft. Collins, CO 80523.

[14] Baker, S. J. and L. Fraley, Jr. 1975. Soil-Plant Transfer of Plutonium, in Whicker, F. W. 1975. Thirteenth Annual Progress Report on U.S. Energy & Development Administration Contract E(11-1)-1156. COO-1156-80.

[15] Little, C. A. 1976. Plutonium in a Grassland Ecosystem. Ph. D. Dissertation, Colo. State Univ., Ft. Collins, CO 80523. COO-1156-83.

[16] Snedecor, G. W. and W. G. Cochran. 1967. Statistical Methods, 6th Edition. Iowa State Univ. Press. Ames, Iowa.

Table 17. Pu-239 Concentration in Rocky Flats vegetation.

Sampling Date	Time elapsed since start of study (d)	Pu Concentration (d/m·g)*	Ratio MPNW/MP1	Concentration Ratio MPNW**
6 June 1975	305	MP1 .28 ± .20 MPNW .55 ± .25	2.0	9.6×10^{-4}
3 July 1975	336	MP1 .08 ± .09 MPNW .64 ± .56	8.0	1.1×10^{-3}
19 August 1975	379	MP1 1.7 ± 2.0 MPNW 3.6 ± 3.5	2.1	6.3×10^{-3}
20 September 1975	441	MP1 .50 ± .35 MPNW .21 ± .12	.42	3.6×10^{-4}
3 February 1976	547	MP1 1.3 ± .39 MPNW 2.0 ± 2.7	1.5	3.5×10^{-3}

*mean ± 1 S.D. (n = 4 in each case)
**Concentration ratio is defined as conc. in dry vegetation/conc. in dry soil.

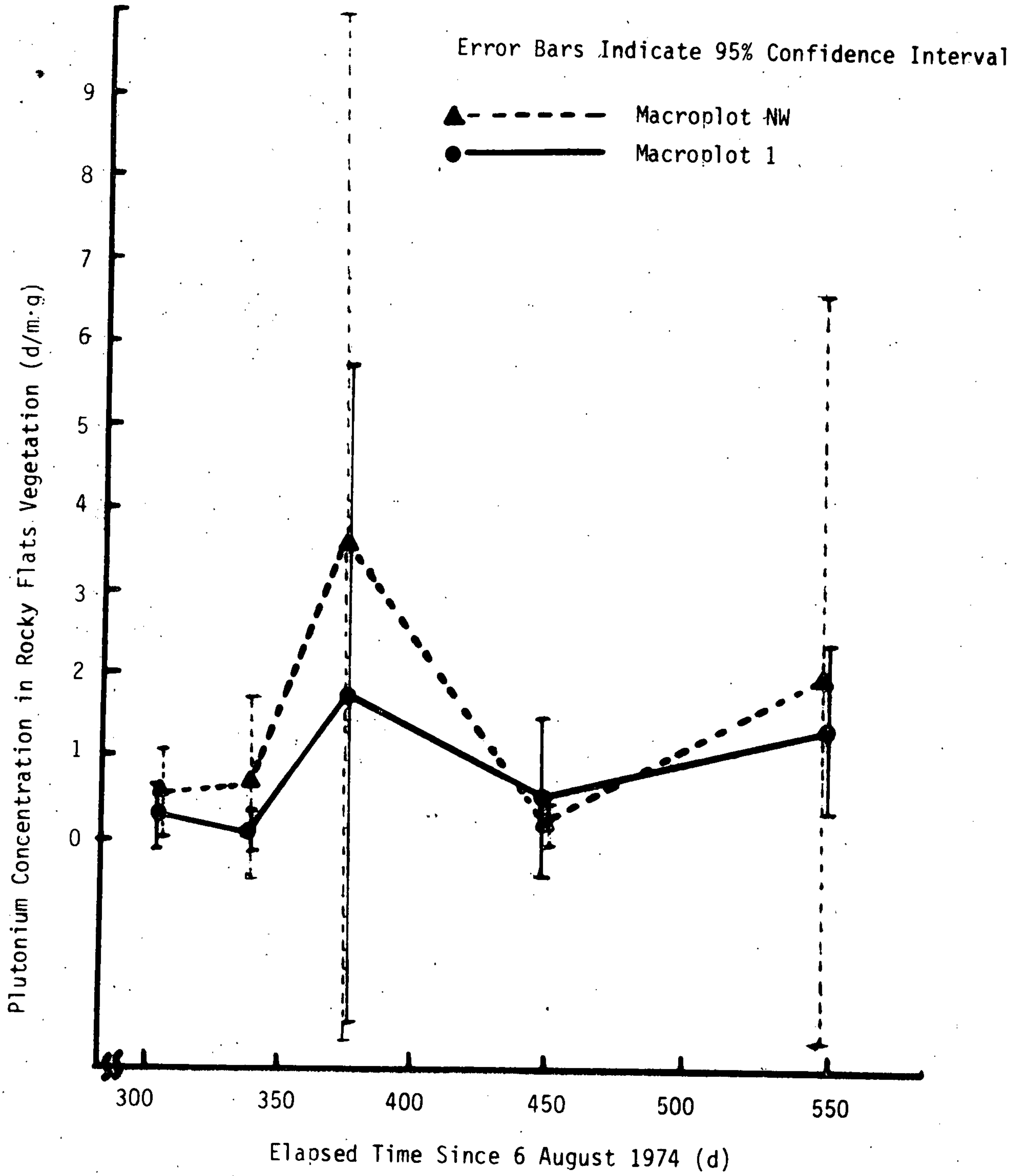

Figure 4. Pu concentration in vegetation at Rocky Flats as a function of elapsed time since start of study.

Table 18. Two-way Analysis of Variance and Tukey's Q Test (Studentized Range) of plutonium in vegetation (dpm/g)

Source	d.f.	S.S.	M.S.	F	Significance
Total (corr)	39	115.09			
Macroplot	1	3.60	3.60	1.50	F(.05,1,30)=4.17
Time	4	33.92	8.48	3.53	F(.05,4,30)=2.69
Interaction	4	5.51	1.38	.58	F(.05,4,30)=2.69
Error	30	72.06	2.40		

Ranked Means

July	October	June	February	August
.35	.36	.41	1.65	2.65

$s_{\bar{y}}$ = .548 Q(.05,5,30) = 4.11 $Qs_{\bar{y}}$ = 2.25

For example, Jacobson and Overstreet in 1948[17] obtained values from 4.5×10^{-6} to 1×10^{-4}; Price in 1972[18] got 1.7×10^{-5} and Cline in 1968[19] saw a concentration ratio of 1×10^{-4}. Further sampling of our soil blocks through time should provide a broader data base upon which to base meaningful conclusions about Pu behavior in the Rocky Flats ecosystem.

A greenhouse study initiated and presently being conducted at CSU of cheatgrass grown in Rocky Flats soil profiles (from Macroplots 1, 2, and NW) should provide additional comparative information for this study. This project is described in the next section of this report.

G. Uptake of Plutonium by Cheatgrass from Contaminated Soil Cores
(M. P. Carson and L. Fraley, Jr.)

The purpose of this study is to investigate the possible transport pathway for plutonium through the root system of cheatgrass (*Bromus tectorum*) to the aerial portion of the plant. Soil cores were taken from Macroplots 1, 2, and NW (northwest) for uptake studies in greenhouse conditions. Macroplot 1 provided a relatively high concentration of plutonium in soil, Macroplot 2 provided soil concentrations about 2 orders of magnitude lower, and Macroplot NW concentrations were on the order of background.[20]

Plutonium in plant tissues sampled from Rocky Flats is believed to be from two sources; through the root system, and/or from airborne deposition on plant surfaces. By sampling plants from the study area and determining plutonium content, it has not been possible to isolate these transport sources. Removal of intact, undisturbed soil samples from each of the three macroplots at Rocky Flats to greenhouses located on the Colorado State University Foothills Campus at Fort Collins, Colorado, and subsequent analysis of cheatgrass grown in the soil for plutonium could provide a solid basis for isolation of the two transport pathways by removing the possibility of aerial deposition. With the separation of transport pathways, two objectives of this study are facilitated; determination of the concentration of plutonium caused by root uptake in cheatgrass over a growing season, and determination of the rate of uptake of plutonium through the root system in cheatgrass.

[17] Jacobson, L. and R. Overstreet. 1948. The Uptake by Plants of Pu and some Products of Nuclear Fission Adsorbed on Soil Colloids. Soil Science. 65. pp. 129-143.

[18] Price, K. R. 1972. Uptake of Np-237, Pu-239, Am-241, and Cm-244 from Soil by Tumbleweeds and Cheatgrass. BNWL-1688.

[19] Cline, J. F. 1968. Uptake of Am-241 and Pu-239 by Plants. BNWL-714. p. 8.24.

[20] Little, C. A. 1976. Plutonium in a grassland ecosystem. Ph.D. Dissertation. Colo. State Univ., Ft. Collins, CO 170 p.

To extract soil "cores" which are relatively undisturbed, sections of plastic (polyvinylchloride, PVC) pipe 10 cm in diameter by 20 cm in length were driven into the soil to a depth of approximately 18 cm at the Rocky Flats study areas. The bottom of the soil sample was sliced off flush with the bottom of the pipe and the soil sample removed intact with the pipe. Twelve soil cores were removed from each of the 3 macroplots in the same manner.

After the core was removed, a plastic square, large enough to cover the bottom of the soil core tube, and with a single hole in its center (1 cm diameter), was glued to the bottom of the PVC pipe with plastic cement. Soil cores from Macroplots 1 and 2 were enclosed in separate plastic bags, placed in a steel locker, and transported to a greenhouse on the CSU Foothills Campus. Uncontaminated soil cores from Macroplot NW were transported to the same greenhouse in a cardboard container.

After inherent vegetation had been removed from the soil cores by desiccation, an attempt was made to germinate cheatgrass seed in the pots, but this failed due to high peak temperatures in the greenhouse.

After the initial failure to obtain germination in the greenhouse, all seeds were germinated on moistened filter paper at room temperature in the laboratory. As soon as germination was noted, the plants were transferred to pots in the greenhouse in a random manner to keep the average age of plants as close to the same as possible. In a period of 16 days a minimum of 5 viable plants in each pot was achieved using this method.

While the seedlings were becoming established in the pots (about 3 weeks), surface irrigation was added on an average of every other day. After this period the equivalent of 1.3 cm of rain was added about every seven days.

Sampling by clipping all cheatgrass aerial portions at ground level will be done at three phenological stages; at start of stem production, early bloom, and at seed production.

Experimental design has allowed for the performance of a two way analysis of variance, using two observations per cell. This method allows comparisons to be made between macroplots in time, plutonium soil concentrations, and interactions of time and plutonium soil concentrations. Analysis of the individual macroplot data should also provide a method to estimate the uptake rates of Pu by the plants.

A total of 6 vegetation samples have been submitted for analysis with no results returned to date. Two more vegetation croppings are planned when the plants attain the desired phenological state, and will contribute a total of 12 additional data points.

H. <u>Physical Characterization of Plutonium in Soil</u> (L. M. McDowell)

Initiation of this study was prompted by previous observations of great variability in plutonium concentrations among soil samples collected from Macroplots 1 and 2. It seems probable that heterogeneous distribution of plutonium particles may contribute to the sampling variability. Therefore plutonium particle size and plutonium association with soil host particles are being studied with autoradiographic techniques.

The procedure for sizing plutonium particles has been modified from that previously used in the summer of 1975, involving alpha track etching in cellulose nitrate. The modification was necessary because commercial-grade cellulose nitrate is not as uniform in sensitivity or thickness as is desired (personal communication, Dr. Klaus Becker, 1975). The current autoradiographic procedure, developed with the aid of LFE Environmental Analysis Laboratory, provides preservation of sample orientation, such that the exact same soil or plutonium particles may be repeatedly assayed, which was not yet possible with previous techniques.

The autoradiographic technique being employed involves the use of Kodak Type NTA nuclear emulsion plates (1"x3", 25 micron thick emulsion). The principle underlying this procedure is identical to that on which photography is based. Ionizing radiations, such as the alpha particles emitted by Plutonium atoms, produce latent images in the sensitized emulsion, which contains silver bromide crystals. These latent images may be made visible in an optical microscope after development.[21] The images produced by alpha particle interactions in the emulsion appear as straight tracks, signifying the loss of energy by the particle as it traverses the medium. The NTA emulsion is sensitive to moderate energy alpha particles, but not to beta particles, or any other less densely ionizing radiations, resulting in a reduction in background.

The soil samples to be analyzed are sieved to 40 microns, then mounted on 1" x 3" microscope slides with 3% collodian in amyl acetate. Approximately one milligram of soil is then exposed to an NTA plate for a pre-selected amount of time: usually one or two weeks. Through optical microscopy, after development, particle tracks can be seen by scanning the plates. Theoretically, "stars", or tracks from Plutonium-containing particles emitting alphas in an isotropic array, "clumps" of tracks suggesting a single host soil particle, or single tracks, i.e., tracks with no apparent affiliation with other tracks in the microscope field, could be seen. However, only the existence of stars and single tracks has been ascertained at this point. Illustrations of a star located on nuclear emulsion plates exposed to Macroplot H soil with different exposure times are shown in Fig. 5. Soil samples mounted on slides may be reexposed to succeeding plates, via alignment jigs, such that exposure times may be adjusted to facilitate track counting of the larger stars, while the position of the star on each plate is maintained.

Particle sizes may be inferred from the number of tracks counted per star. Calibration of this sizing technique is being accomplished with microspheres of PuO_2 of known sizes.

Limitations inherently imposed on this technique include particle size detection limits and small sample sizes. The detection limits are inversely proportional to the exposure times used. However, for practical reasons, the exposure times must be somewhat limited. A two week exposure allows detection down to approximately .15 micron particle sizes. Therefore,

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21 Baserga, R., Malamud, D., Autoradiography, pp. 2,41, Harper and Row (1969).

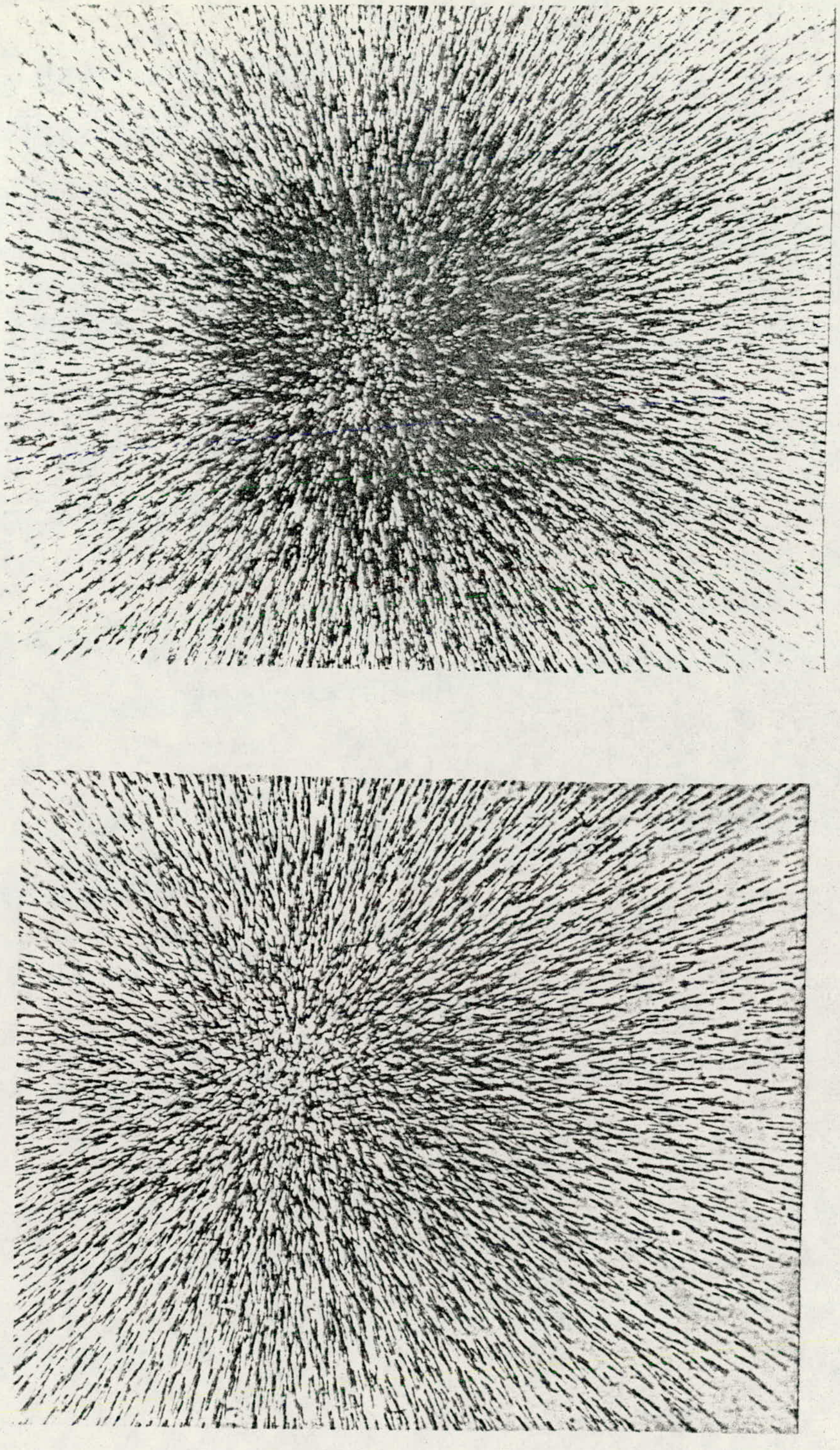

Figure 5. Pu particle tracks on NTA plates: (top) "star" from a relatively large Pu particle in Macroplot H soil, exposed to emulsion for one week and (bottom) "star" from same particle, exposed for one day.

the discrimination against smaller particle detection must be taken into account when considering size distributions from data obtained.

Soil samples were restricted to the top one centimeter of soil in the northwest quadrant of Macroplot 1, due to sample size limitations. An attempt is being made to overcome these limitations, at least with respect to detection of relatively large plutonium-containing particles. Kodak type AA Industrial X-Ray film, a high resolution film, is being used to scan larger amounts of soil mounted on 8" x 10" glass plates. Particles with an activity greater than .5 dpm, or approximately .7 microns in diameter, can be located with this technique after a two week exposure.

Isolation of plutonium-containing particles, or host particles, is a potential application of the results obtained from this autoradiographic technique. The feasibility of this relies on the use of the alignment jigs, such that orientation of the plates exposed to slides containing soil may be reproduced after stars have been located in the transparent, developed emulsion. Conceivably, isolated particles may then undergo further analysis via chemical or electron microscopy techniques.

The ultimate significance of this study lies in its contribution to the overall study of plutonium kinetics and characteristics in the soil at the Rocky Flats facility. Immediate results will consist of data on ratios of numbers of single tracks (atoms or very small particles) to stars or clumps of tracks, particle size distributions, and occurrence probabilities of particles per unit mass of surface soil. To date, feasibility of the technique involving both the nuclear track plates and X-Ray film has been verified, but the amount of soil scanned for track analysis is too small to generate reliable statistics on the data obtained.

I. Plutonium in Nestling Mourning Doves (L. E. Alexander)

The main purpose of this study was to measure contamination levels of plutonium in mourning doves. Specific objectives of the study were (1) to measure amounts of plutonium in doves; and (2) to describe population density, breeding and nesting characteristics.

Mourning doves are a migratory species and the most important game bird in the United States.[22] It is a resident in Colorado, abundant in summer and casual in winter. At Rocky Flats, many doves were seen along the roads in early May but none after mid-October. They feed mainly on cultivated crops and weed seeds but will consume a variety of foods, depending upon seasonal availability.[23] Soil particles and insects are also ingested. Because plutonium at Rocky Flats exists mainly in the soil, doves could pick up substantial amounts of plutonium in their grit and food.

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22 Reeves, H. M., A. D. Geis, and F. C. Kniffin. 1968. Mourning doves capture and banding. U. S. Fish and Wildl. Special Sci. Rept. Wildl. No. 117, 63pp.

23 Carpenter, J. W. 1971. Food habits of the mourning dove in northwest Oklahoma. J. Wildl. Manage. 35(2):327-331.

The study area included about 50 hectares directly south and southwest of the plant. Originally I planned to capture adults and nestlings to determine levels of plutonium contamination in each and to measure any possible transference from adults to young. Five modified Kniffin traps were placed along Woman Creek in spots where frequently doves had been observed. Unfortunately, trapping was unsuccessful and no adults were caught.

Capturing nestlings proved more successful. By periodically walking the study area fifteen nests were found throughout the summer (Fig. 6). However, only 6 of the 15 nests were successful in producing young. Nestlings were collected at about 8 days of age. This allowed them maximum time on the nest before being able to fly. Nestlings collected were transferred live to Fort Collins in a cage, immediately killed, and frozen prior to dissection. Lung, liver, and skeleton were dissected from the birds. Samples were sent to LFE Environmental Analysis Laboratories of Richmond, California for analysis.

Results from lab analysis of the tissue samples are in listed in Table 19. Fifteen of the 24 samples showed no detectable plutonium contamination. Detectable tissue burdens were all less than 1 dpm/sample. Statistical analysis showed no correlation between levels of contamination and distance from the contamination source. Differences between amounts of Pu-238 and Pu-239 were significant. Ratios of Pu-239 to Pu-238 were approximately 3 to 1.5. In comparison, ratios of 40 to 1 have been found in soil.

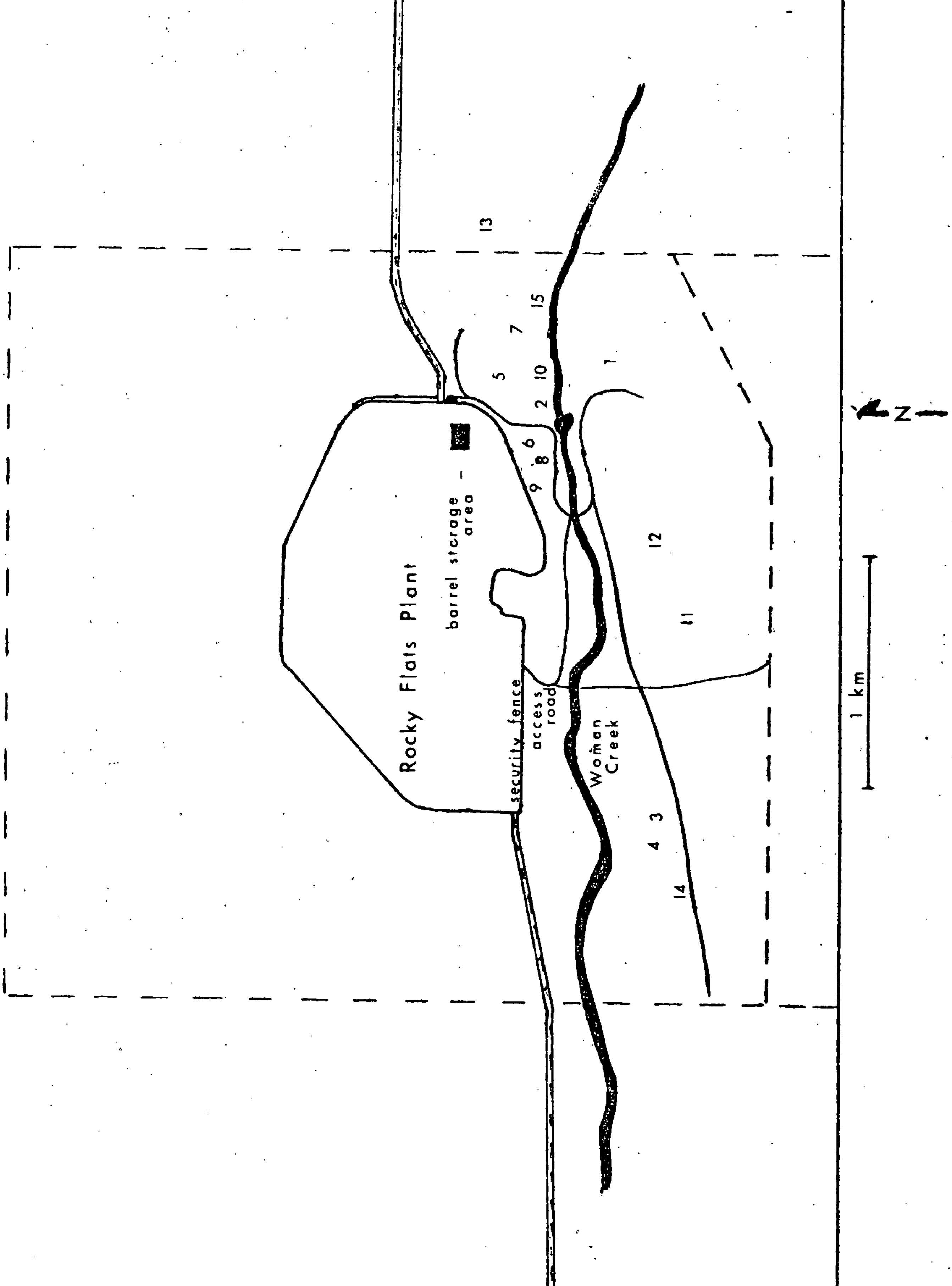

Figure 6. Location of nests.

Table 19. Results of mourning dove tissue samples.

Nest No. & Tissue	Pu-238 dpm/sample (w)[a]	Pu-239 dpm/sample (w)
8-1 Liver	ND[b]	ND
8-1 Lung	ND	ND
8-1 Skeleton	ND	ND
8-2 Liver	6.42×10^{-2} (0.38)	9.44×10^{-2} (0.38)
8-2 Lung	1.74×10^{-1} (0.08)	6.10×10^{-1} (0.08)
8-2 Skeleton	ND	ND
12-1 Liver	ND	ND
12-1 Lung	ND	ND
12-1 Skeleton	ND	ND
12-2 Liver	ND	3.76×10^{-2} (0.18)
12-2 Lung	ND	9.04×10^{-2} (0.54)
12-2 Skeleton	ND	ND
14-1 Liver	ND	ND
14-1 Lung	ND	5.68×10^{-2} (0.50)
14-1 Skeleton	ND	1.44×10^{-1} (1.31)
14-2 Liver	5.95×10^{-2} (0.47)	ND
14-2 Lung	ND	ND
14-2 Skeleton	ND	ND
15-1 Liver	ND	ND
15-1 Lung	7.35×10^{-2} (0.18)	ND
15-1 Skeleton	ND	ND
15-2 Liver	ND	ND
15-2 Lung	6.67×10^{-2} (0.13)	2.17×10^{-1} (0.13)
15-2 Skeleton	ND	ND

[a] (w) = sample dry wt in grams
[b] ND = Detection limit was 0.04 dpm/sample

IV. SEASONAL KINETICS OF CESIUM IN EAST TWIN LAKE

Seasonal Kinetics of Cesium in East Twin Lake (K. L. Weaver)

Seasonal variation of concentration factors in East Twin Lake aquatic invertebrates continues under study. East Twin Lake (ETL) is a montane semi-drainage lake in the Colorado Front Range with interesting and well-studied cesium kinetics.[24,25]

Neutron activation of samples taken at East Twin Lake is essentially complete. Early samples have already been counted for induced ^{134}Cs activity as a measure of stable ^{133}Cs levels and, time having been allowed for interfering shorter-lived activation products to decay out, assay of the remainder is now underway.

Several rounds of aquarium microcosm ^{134}Cs uptake experiments with the amphipod Gammarus lacustris in ETL water of varied water temperature and specific conductance have been completed. Retention experiments are in progress.

Programming of an existing ETL ecosystem compartment model to incorporate seasonal variation in invertebrate fish-food cesium transfer rate constants is begun. Testing model sensitivity to variation in the rate constants associated with variation in lake temperature and specific conductance is still in progress.

Data analysis is not complete for either field or laboratory work. Completed analysis and discussion will be presented in a forthcoming dissertation.

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[24] Gallegos, A. F. 1969. Radiocesium kinetics in the components of a montane lake ecosystem. Ph.D. Dissertation, Colorado State University, Fort Collins, CO, 342 p.

[25] Hakonson, T. E. 1971. Cesium kinetics in a montane lake ecosystem. Ph.D. Dissertation, Colorado State University, Fort Collins, CO, 152 p.

V. LIST OF PUBLICATIONS

The following reports on work wholly or partially supported under ERDA Contract AT(11-1)-1156 have been published or prepared for publication (the recent reprints and pre-prints accompany this report):

Hanson, W. C., F. W. Whicker, and A. H. Dahl. 1963. Iodine-131 in the thyroids of North American deer and caribou: comparison after nuclear tests. Science 140: 801-802. (COO-1156-2)

Hanson, W. C., A. H. Dahl, F. W. Whicker, W. M. Longhurst, V. Flyger, S. P. Davey, and K. R. Greer. 1963. Thyroidal radioiodine concentrations in North American deer following 1961-1963 nuclear weapons tests. Health Physics 9: 1235-1239. (COO-1156-4)

Whicker, F. W., E. E. Remmenga, and A. H. Dahl. 1965. Factors influencing the accumulation of I-131 in Colorado deer thyroids following 1961-1962 nuclear weapons tests. Health Physics 11: 293-296. (COO-1156-8)

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Farris, G. C., F. W. Whicker, and A. H. Dahl. 1969. Strontium-90 levels in mule deer and forage plants, p. 602-608. In D. J. Nelson and F. C. Evans (Ed.) Symposium on Radioecology. CONF-670503. U. S. Atomic Energy Commission, Division of Technical Information Extension, Oak Ridge, Tennessee. (COO-1156-23).

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VI. LIST OF ORAL PRESENTATIONS

The following papers representing work conducted under ERDA Contract AT(11-1)-1156 have been presented orally at scientific meetings or formal seminars at other institutions:

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