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JOHN C. SEIDENSTICKER IV

B. A. University of Montana, 1966

UNIVERSITY OF MONTANA

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RESPONSE OF JUVENILE RAPTORS TO DDT IN THE DIET

2 • .

by

JOHN C. SEIDENSTICKER IV

B. A. University of Montana, 1966

Presented in partial fulfillment of the requirements for the degree of

Master of Science in Wildlife Technology

UNIVERSITY OF MONTANA

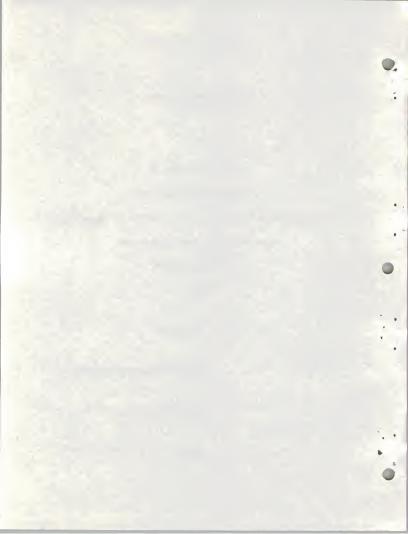
1968

Approved by:

Chairman, Board of Examiners

Dean, Graduate School

Date



Seidensticker, John C., IV, M.S., December 1968 Wildlife Technology Response of Juvenile Raptors to DDT in the Diet. (viii, 74 pp.)

Director: John J. Craighead

An investigation was conducted during the spring and summer of 1967 in south-central Montana to obtain information on the response of juvenile red-tailed hawks (Buteo jamaicensis) and golden eagles (<u>Aquila chrysaetos</u>) to DDT in their diet. The experiments were designed to: 1) measure the accumulation of DDT residues in nestling hawks and eagles, 2) measure storage and loss of DDT residues in post-fledging hawks, and 3) determine the effects of feeding DDT on their growth, development and behavior.

Nine red-tailed hawks and 1 golden eagle were fed 20 mg technical grade DDT per kg body weight as nestlings. At fledging, 3 hawks and the eagle were scarificed and the remaining hawks were retained in captivity for 40 additional days. Three of the post-fledging hawks were fed DDT at the nestling rate while the remaining hawks were fed no DDT. The chemical analysis of brains, breast muscles and livers from these birds showed:

- The nestling hawks and eagle accumulated substantial levels of DDT and its metabolites;
- Post-fledging hawks fed DDT as nestlings and for ¹O additional days contained the same levels of DDT and its metabolites as did nestlings;
- 3) Post-fledging hawks fed DDT as nestlings and DDT free diets for 40 additional days contained only one-fourth as much DDT and its metabolites as did nestlings.

These experiments indicated a difference in the accumulation of DDT and its metabolites in young raptors during different stages in their growth and development and that post-fledging juvenile hawks have the ability to eliminate DDT and its metabolites rather rapidly while on DDT free diets.

More DDT residues accumulated in the brains, breast muscles, and livers of diseased nestling hawks than in the brains, breast muscles and livers of healthy nestlings. DDT is retained in the tissue of juvenile red-tailed hawks longer than DDT+DDD. One of six red-tailed hawks fed DDT during the nestling period failed to learn to feed itself during a 40-day post-fledging period in captivity indicating that DDT might affect the behavior of developing hawks. DDT at the dosage used in these trials did not affect the growth of red-tailed hawks or golden eagles.

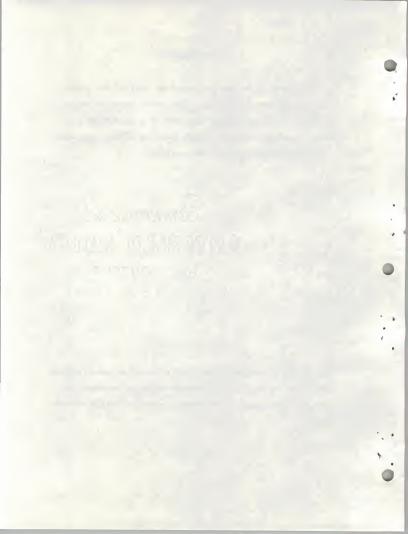


PROJECT FINANCING

Financial support for this project was supplied by U. S. Fish and Wildlife Service Contract No. 10-16-008-718 and by the Montana Cooperative Wildlife Research Unit: U. S. Fish and Wildlife Service, University of Montana, Montana State Fish and Game Department, and Wildlife Management Institute cooperating.

<u>PLEASE NOTE</u>: This dissertation is not a publication, and no portion herein may be quoted without express permission of the author, the Department of Zoology, and the Montana Cooperative Wildlife Research Unit.

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ACKNOWLEDGEMENTS

During the "eagle study" Harry Reynolds and I worked together in a team effort collecting data on our separate research projects. If this spirit of cooperation had not existed, my study would have been severely handicapped.

I deeply appreciate the assistance of Dr. John J. Craighead for his help and guidance throughout this study. I also wish to thank Drs. D. A. Jenni, P. L. Wright, and B. W. O'Gara for their helpful suggestions.

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Special thanks go to my wife, Sue, for her assistance in the field and her encouragement and support.

J. C. S.

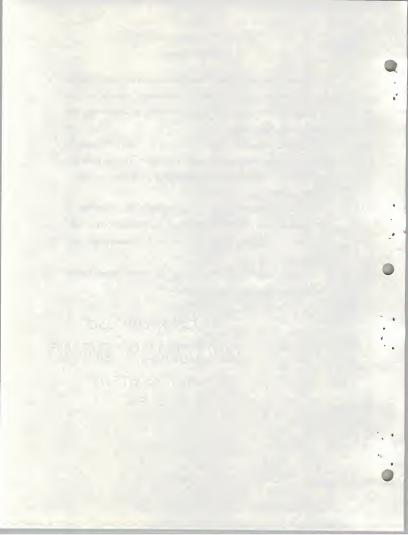
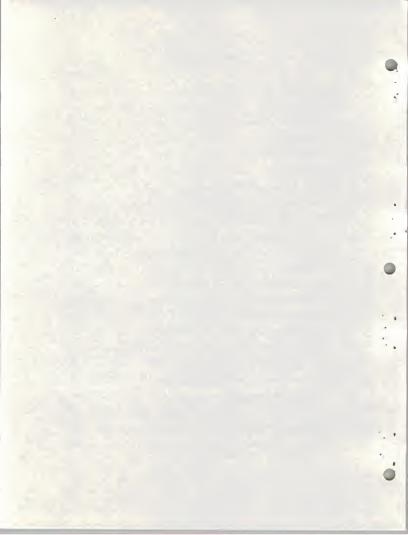


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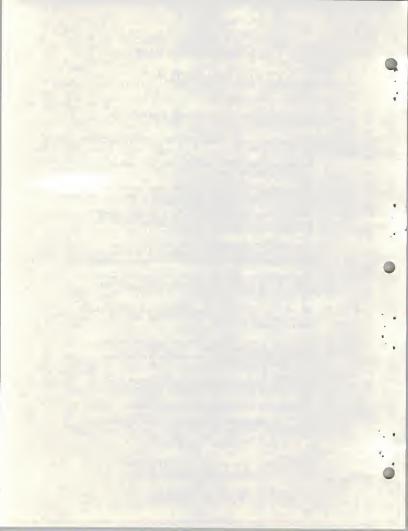


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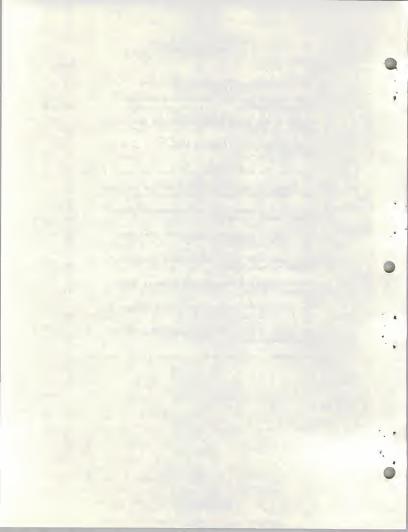
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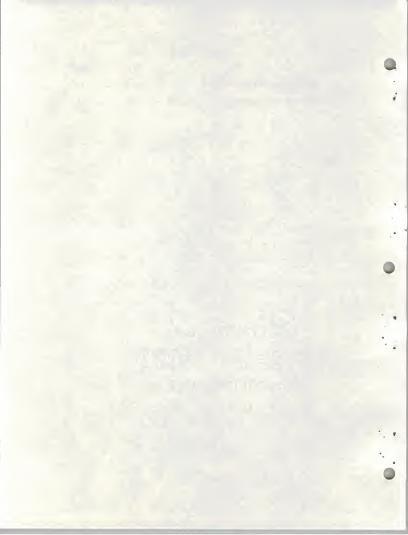
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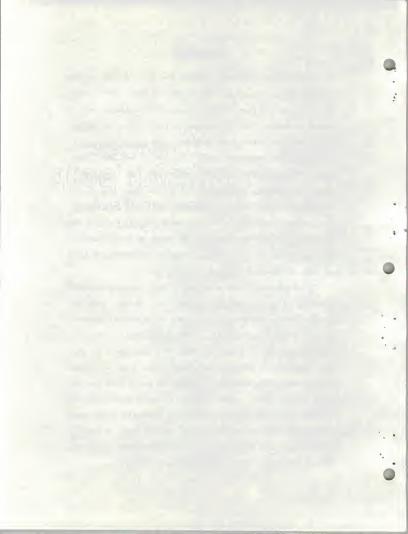


INTRODUCTION

In the United States and in Europe some raptor populations have recently suffered serious declines. Ratcliffe (1963, 1965) found that in the past 13 years there had been a rapid decline in both numbers and productivity of the peregrine falcon (Falco peregrinus). Similar declines in some Scottish golden eagle (Aquila chrysaetos) populations have also been reported. (Lockie and Ratcliffe, 1964). Cramp (1963) outlined the decline of the sparrow hawk (Accipiter nisus), merlin (Falco columbanius), and kestrel (Falco tinnunculus) in England. Frestt (1965, 1966) reported that the buzzard (<u>Buteo</u> <u>buteo</u>), sparrow hawk, merlin, kestrel, tawny owl (<u>Strix aluco</u>), and barn owl (<u>Tyto alba</u>) have declined. The number of sparrow hawks, kestrels, and barn owls has dwindled rapidly; the buzzard, merlin and tawny owl have been affected less severely.

Sprunt and Ligas (1963) have reported serious declines in bald eagle (<u>Haliae tus leucocephalus</u>) populations occurring in parts of the United States and Cottam <u>et al</u>. (1961) cited observations on the decline of the golden eagle. Breeding populations of osprey (<u>Pandion haliaetus</u>) on the eastern coast have diminished. For example, eight active nests were reported from nine areas in southern Cape May County, New Jersey, in 1963 where 28 active nests were reported in 1937 (Schmid, 1966). Ames and Mersereau (1964) found that the number of nesting pairs of osprey in a Connecticut River colony had decreased at a mean annual rate of 31% since 1960. In recent years the peregrine falcon has experienced catastrophic population declines over much of North America (Hickey, in press).

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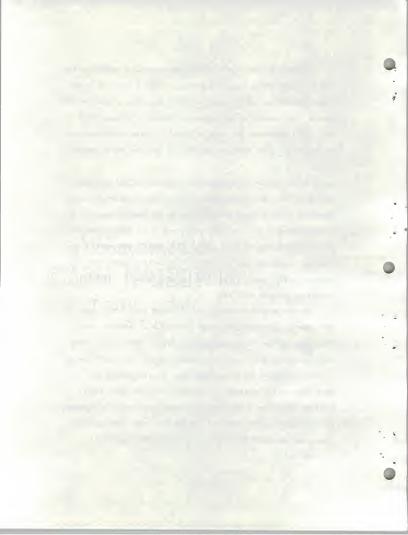


The rapid decline of some raptor populations has emphasized the need for establishing population norms. In 1963 a study was begun on the dynamics of a breeding golden eagle population in south-central Montana. In summarizing the results of the study, McGahan (1966, 1967, 1968) emphasized the value of ecological data in evaluating the effect of future environmental changes on golden eagle populations.

A second 3-year investigation was launched in 1965 (Reynolds, in prep.). This constituted Phase II of the long-term golden eagle population study directed and supported by the Montana Cooperative Wildlife Research Unit. A significant drop in the eagle productivity was detected during the first field season (1965); the next 2 years were spent gathering data on productivity, nesting density and food habits for comparison with similar data gathered earlier by McGahan on the same study area.

Declines of raptor populations have often been linked with environmental contamination by toxic chemicals. In Europe, where this condition has been investigated in detail, Cramp (1963) found pesticide residues in 13 different raptor species in Great Britain, Sweden, and Holland and he concluded that pesticides were the major cause of the declines. Lockie and Ratcliffe (1964) attributed the decline of a Scottish golden eagle population to increased use of persisting toxic chemicals on the landscape. Ames (1966) found a decreased hatchability of osprey eggs with increased

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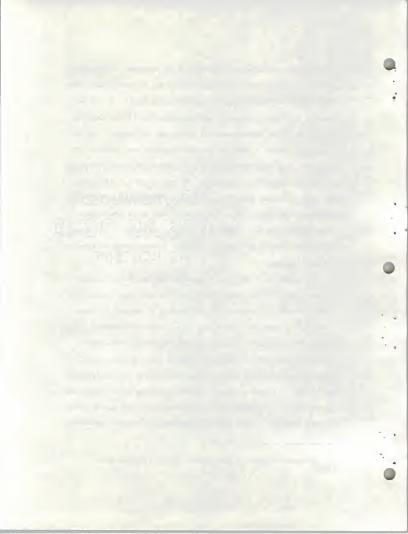


environmental contamination by DDT 1/. The peregrine falcon decline in Britain was analyzed by Ratcliffe (1963:86) who stated that "Circumstantial evidence pointed strongly to agricultural toxic chemicals as the cause of decline, through contaminating prey taken by peregrines, which then accumulated the poison indirectly." In the Netherlands, birds of prey and fish-eating birds were collected for analysis. Many of these specimens contained large amounts of chlorinated hydrocarbon residues (Koemen and van Genderen, 1966). Dustman (1966) reported that of the 68 bald and golden eagles collected throughout the United States, all but one bald eagle from Alaska contained DDT residues. Cade <u>et al</u>. (1968) and Enderson and Berger (1968) have found high levels of chlorinated hydrocarbon residues in arctic peregrines.

The presence of chlorinated hydrocarbon insecticide residues in the tissue of birds of prey, which are at the top of the biotic pyramid, is well documented but the build-up of residues in these birds is a biochemical process that is by no means understood (Hickey, 1966). The occurrence of organochlorine insecticide residues in predatory birds which do not normally have direct access to pesticides has resulted in a shift of research emphasis from toxicological studies on single species to studies involving entire ecosystems (Moore, 1967). Persistent, fat-soluble chemicals such as the organochlorine insecticides are transferred along food chains. Persisting

1/ The chemical names of insecticides can be found in Menzie (1966).

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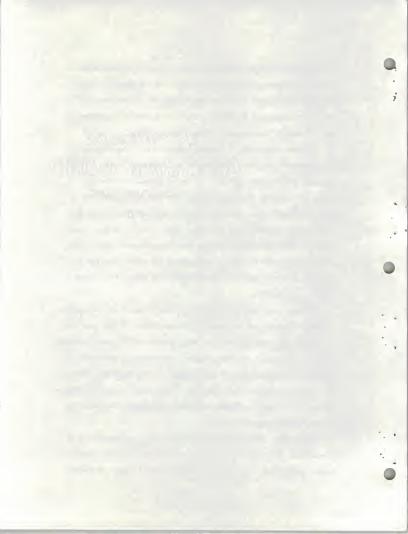
chemicals are sometimes concentrated at successive trophic levels in the biotic pyramid until they become toxic to the carrier. This phenomenon of delayed expression and biological concentration of persistent pesticides in both terrestrial and aquatic ecosystems is well documented (summarized by Rudd, 1964:250-267).

The vast quantities of organochlorine insecticides that have been dispersed over the landscape and the persistent nature of these insecticides have made them part of the geological and chemical cycles of the earth (Woodwell, 1967). The fact that persistent pesticides have become important new ecological factors in the environment was emphasized in the recent report by Wurster and Wingate (1968). These investigators found that organochlorine insecticides are widespread within oceanic organisms to the extent that the existence of pelagic species such as the Bermuda petrel (Pterodroma cahow) is threatened.

Despite considerable research, the mode of action of the organochlorine insecticides is incompletely understood. It has been postulated that continued ingestion of sublethal amounts of organochlorine insecticide residues by avian predators may result in reduction of clutch size, hatchability of eggs, number of young surviving, or a combination of these. Hickey (1966) believes that in some avian populations these result in fewer young birds to replace the adults that are gradually disappearing.

To evaluate possible effects of environmental contamination by persisting toxic chemicals on raptor populations, a survey of chlorinated hydrocarbon residues in the body tissues and eggs of golden

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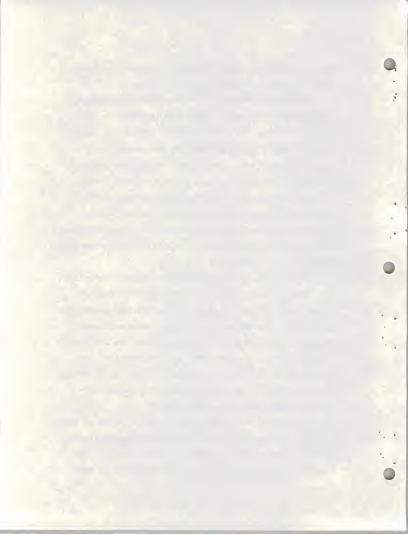
eagles and other large raptors and their prey species in southcentral Montana was conducted by Reynolds (in prep.).

The effect of sublethal levels of organochlorine insecticides on nestling and fledgling birds under field conditions has been studied very little. The nestling raptor is characterized during its development by rapid growth dependent upon the ingestion of large quantities of food. I postulated that during this period of development an altricial bird might be more susceptible to toxic chemicals than at any other time in its life. Lacking specific information on the ways sublethal environmental levels of organochlorine biocides might affect nestling raptors, a study was designed to investigate possible effects of DDT on nestling raptors from hatching until well after fledging.

Recently serious doubts have been raised concerning the projection of the results of laboratory pesticide studies to field conditions (Moore, 1965; Stickel <u>et al.</u>, 1965). With this in mind, preliminary field techniques were developed and time schedules were devised during the 1966 field season for conducting studies on the response of juvenile raptors to DDT under field conditions. With this preliminary field work as a background, a series of DDT feeding experiments in the field, using sublethal dietary levels of DDT, was conducted during the spring and summer of 1967.

The specific objectives of this study were: 1) to conduct feeding trials with nestling red-tailed hawks (<u>Buteo jamaicensis</u>) and golden eagles and measure accumulation of DDT residues, 2) to measure storage and loss of DDT residues in post-fledgling red-tailed

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hawks, and 3) to determine the possible effects of feeding DDT on growth, development, and behavior.



STUDY AREA

Geographic location

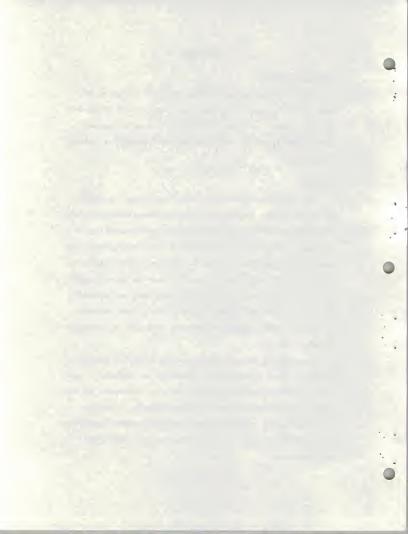
The intermontane valleys in the vicinity of Livingston, Montana, will be referred to throughout this report as the "study area" (Fig. 1). Nearly 80% of the 35-township golden eagle study area (Area A) used by McGahan (1968) and Reynolds (in prep.) is included in this area.

Topography

The Yellowstone River and its major tributaries (the Shields and Boulder Rivers) constitute the major watershed in the study area. The Yellowstone River flows from the southern mountainous regions northward through Paradise Valley, around the northwest flank of the Absaroka Range, and thence eastward. From the Crazy Mountains, the Sheilds River flows south and meets the Yellowstone near Livingston; the Boulder River forms in the Absaroka Range and flows northeast until it joins the Yellowstone River east of the study area near Big Timber. Numerous smaller tributaries enter these major streams throughout the area.

Elevations on the study area range from 4,000 feet along the Yellowstone River to more than 10,000 feet in the Absaroka, Bridger, Crazy, and Madison Ranges which bound the area. Throughout the area there is a transitional foothill zone between river bottoms and mountains which is characterized by buttes, escarpments, and ravines. McGahan (1966) calls this transition zone a typical golden eagle nesting habitat.

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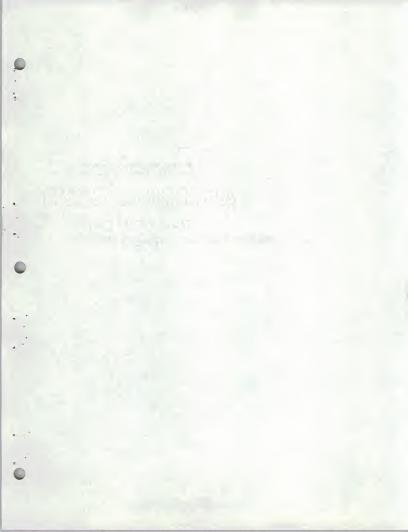
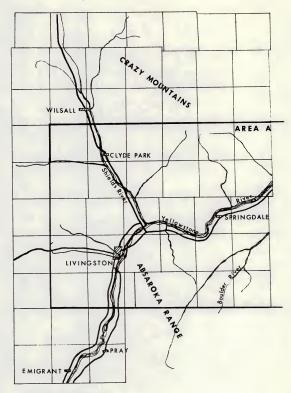


Figure 1. Map of the south-central Montana study area



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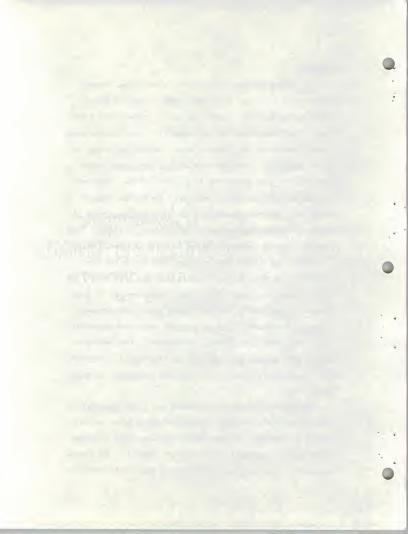
Vegetation

The complex pattern of vegetation in south-central Montana appears to be the result, as in other areas, of various environmental factors (moisture, soil, fire, etc.). At the lowest elevations in the intermontane valleys a majority of the flood plain and riparian communities are dominated by cottonwood (<u>Populus spp.</u>) and willow (<u>Salix spp</u>.). Quaking aspen (<u>Populus tremuloides</u>) groves occur on river flood plains only at higher elevations. Cottonwood and quaking aspen dominate the majority of the foothill riparian communities. However, Engelmann spruce (<u>Picea engelmanni</u>) and Douglas fir (<u>Pseudotsuga menziesii</u>) are prominent in foothill flood plain and riparian communities on the east side of Paradise Valley.

Much of the tillable area on lower slopes and in the creek and river flood plains has been planted to alfalfa, "wild hay", and grain. Vegetation on the remaining lower slopes consists of wheatgrasses (<u>Agropyron spp.</u>), fescues (<u>Festuca spp.</u>), needlegrasses (<u>Stipa spp.</u>), junegrass (<u>Koeleria cristata</u>), and other grasses and forbs. The grasses and forbs are interspersed in some areas with big sagebrush (<u>Artemisia tridentata</u>). In the foothill zone limber pine (<u>Pinus flexilis</u>) and juniper (<u>Juniperus scopulorum</u>) are found on some sites.

Douglas fir, together with lodgepole pine (<u>Pinus contorta</u>), is found above the foothill zone. Meadows of various sizes are interspersed in this zone. At these higher elevations aspen, Engelmann spruce, alder (<u>Alnus spp</u>.), and willows are dominant in the riparian communities. Lodgepole pine continues to be abundant to timber line.

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The alpine zone is characterized by boulder fields, rocky ledges, and scree slopes with little soil development. Small areas dominated by mat- and cushion-forming plants as well as some perennial grasses are present.

Climate

Altitude, latitude, mid-continent position, and mountain barriers all influence the climate in the study area (Shearer, 1958). Yearly maximum and minimum temperatures vary greatly. On the average, the coldest month is January and the warmest is July. The average January temperature (approximately 22°F.) in the upper Yellowstone River Valley is higher than that recorded for most other sections of Montana. Annual precipitation on the area is 13-14 inches.

A summary of climatological data for the 1967 study period is presented in Table 1. Mean temperatures on the study area during the study period for 1967 were cooler than normal, while precipitation ranged from below normal in February, May, and August to above normal in March, April, and July. More than twice the average rainfall was recorded during June.

Fauna

The species and general distribution of the mammals in southcentral Montana has been outlined by Hall and Kelson (1959). More recently Hoffmann and Pattie (1968) have compiled a detailed account of the status and distribution of Montana mammals. Richmond and Knowlton (1894) and Saunders (1921) have reported the status of

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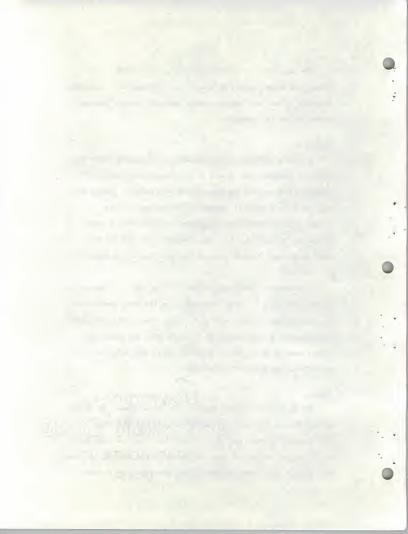
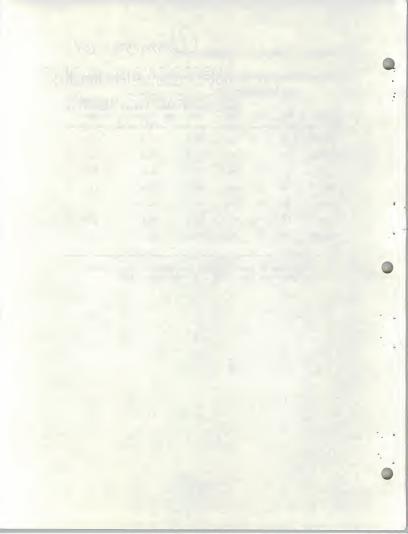


Table 1. Climatological Summary, 1967*

	ATT U	emperat	ure, oF	Marau		D
Month	Max.	Min.	Mean daily max.	Mean daily min.	Mean of daily max. and min.	Precipitation Total inches of water
February	58	5	40.6	24.0	32.3	.16
March	61	-7	38.5	17.3	27.9	2.21
April	60	11	49.3	27.9	38.6	1.60
May	82	16	60.8	37.0	48.9	1.90
June	82	34	67.0	46.0	56.5	5.30
July	89	46	81.8	51.5	66.7	2.98
August	93	40	84.85	48.9	66.7	.43

*Data taken at the Livingston Airport in the approximate center of the study area (U.S. Dept. Comm., 1967)



avian species in the area.

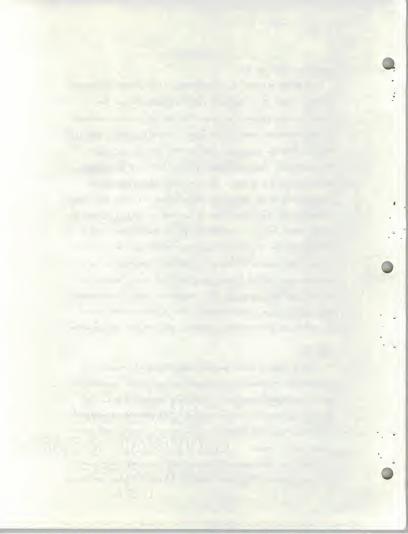
An effort was made during this study to determine the present breeding status of the birds of prev (Falconiformes and Strigiformes) present in the study area. Nesting records were obtained for the red-tailed hawk, golden eagle, goshawk (Accipiter gentilis), Cooper's hawk (A. cooperii), sharp-shinned hawk (A. striatus), Swainson's hawk (Buteo swainsoni), ferruginous hawk (B. regalis). marsh hawk (Circus cvaneus), prairie falcon (Falco mexicanus). American kestrel (F. spaverius), great horned owl (Bubo virginianus), long-eared owl (Asio otus), and short-eared owl (Asio flammeus) for a total of 13 species. In addition, Mr. J. Sumner (per. comm.) reports that the peregrine has nested on the study area in recent years. Other species which were occasionally observed in the area are the turkey vulture (Cathartes aura), bald eagle, osprey, and saw-whet owl (Aeolius acadicus). During early April rough-legged hawks (Buteo lagopus) were commonly seen in the northern part of the study area prior to their departure for northern nesting areas.

Land use

The economy of south-central Montana is based primarily on agriculture. The statistical analysis of agriculture prepared by various governmental agencies is based on the county unit. The following discussion is based, therefore, on the statistics from Park County where the majority of the raptor eyries used in this inquiry were located.

The U. S. Department of Commerce (1966) reports that during the 5-year period, 1959-1964, the total acreage in farms and ranches

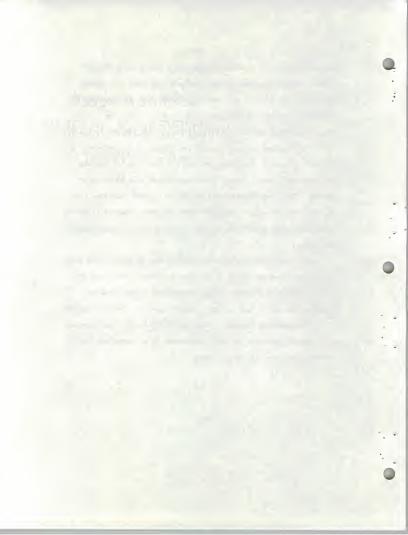
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remained relatively constant at 52% of the land area or 881,000 acres. During this same period, the number of farms and ranches has dropped from 458 to 420, and the average farm and ranch has increased in size from 1,926 acres to 2,073 acres. In 1959, 41,075 sheep (including lambs) were headquartered on 163 ranches, while in 1964, 19,636 sheep (including lambs) were based on 101 ranches. In 1959, 24,340 cattle (including calves) were based on 402 ranches while in 1946, 32,071 cattle (including calves) were based on 379 ranches. The cropland harvested during this period increased from 54,000 to 59,000 acres. The main source of farm income is livestock and livestock products with the sale of field crops being an important second.

While the above data do not cover the same period as this study, the general land use trends in the area are shown. The trend of land ownership in the area is toward fewer and larger farms and ranches, and cattle have become the most important livestock species. U. S. Department of Commerce (1966) data indicate that the increase in harvested croplands has been accompanied by an increased use of agricultural chemicals of all types.

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METHODS AND PROCEDURES

Locating nests

Formerly active golden eagle eyries in the vicinity of Livingston were checked early in the season. Two eyries were selected for use in the DDT trials. By following the procedures outlined by the Craigheads (1956: 196-199), 38 active red-tailed hawk nests were located prior to and during the incubation period. Thirteen hawk nests were selected for the experiments. To minimize nest loss through desertion and chilled eggs, disturbance of raptor nests prior to and during the incubation period was kept at a minimum.

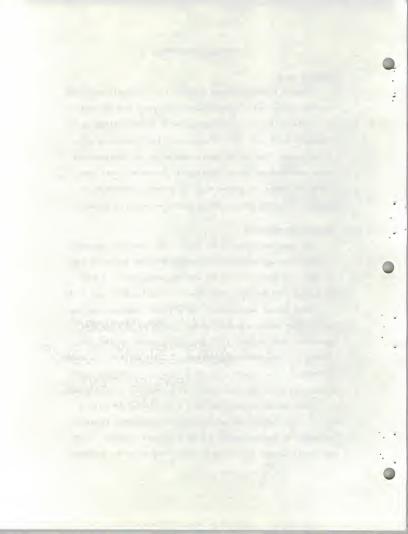
Experimental procedure

The experiments were of two types: those measuring the effect of DDT on nestling raptors and those measuring the storage or loss of DDT in the raptors during the nestling period and for a time after they left the nest. Both experiments utilized the same birds.

Newly hatched nestlings from 13 red-tailed hawk nests and two golden eagle eyries were selected for the feeding trials. From these nestlings, 11 hawks and 1 eaglet were placed on diets containing DDT. The remaining nestlings, 14 hawks and 2 eaglets acted as controls. Twelve hawk nests and 1 eagle eyrie contained 2 nestlings each; 1 hawk nest and 1 eagle eyrie contained 1 nestling each.

Each nest was visited once every 4 days during the nestling period. Every effort was made to keep the experimental situation as natural as possible except for the experimental feeding of DDT. The birds remained undisturbed in their nests except for weighing.

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measuring, and feeding. The parent birds returned regularly with food. Thus, the natural diet was supplemented with a ration of DDT once every 4 days for those birds on DDT diets. Control birds were weighed and measured but not fed DDT.

At 40 days (the approximate fledging time for red-tailed hawks), one control hawk and 3 hawks on DDT diets were sacrificed. The remaining control hawks were biopsied, banded and released. Six hawks fed DDT in the nest were kept in captivity for an additional 40 days. Three of these were continued on DDT diets for the second period. The other three were placed on diets which contained no DDT. At the end of this second period all birds were sacrificed including one control bird that had been retained in captivity but never fed DDT.

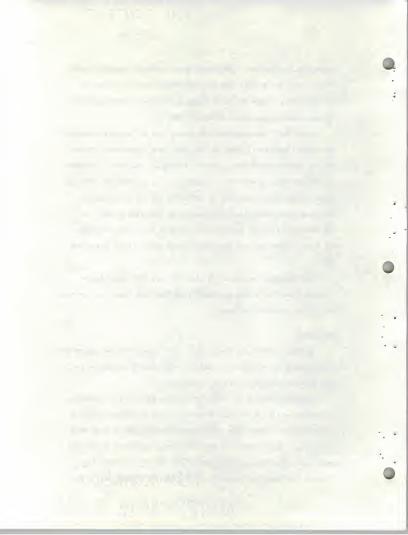
Two eaglets were sacrificed after 60 days (the approximate fledging time). A biopsy was taken from the third eaglet, a control, and it was banded and released.

DDT dosage

DDT was selected for use in this study because of its importance in environmental contamination and the considerable literature available from controlled laboratory experiments.

Technical grade p, p' isomer of DDT was dissolved in vegetable oil, inserted into no. 0 gelatin capsules, and administered orally to experimental birds. The dosage used was 20 mg DDT/kg body weight once every 4 days. Thus, the DDT application approximated periodic meals which were highly contaminated with DDT (<u>cf</u>. Stickel <u>et al.</u>, 1966a). The dosage was based on body weight to compensate for the

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weight change and increased food consumption by nestlings as they grew. It is not known to what extent the different metabolic rates of the two species affected the intake of DDT but for comparative purposes, the same dose was used for both the golden eagle and the red-tailed hawk.

A summary of total DDT intake is shown in Table 2.

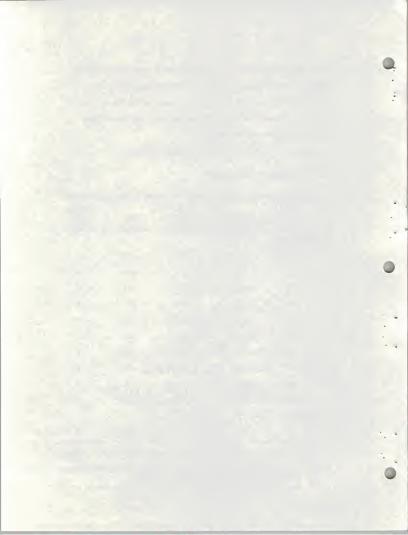
Table 2. Summary of DDT intake

		DDT dosage #			
Bird no.*	Days on test	Nestling (mg)	Post- nestling (mg)	Total (mg)	Killed or the died
Red-ta:	iled hawk:				
l	24	74		74	D
2	40	121		121	к
3	40	159		159	к
4	40	141		141	к
5	80	118	217	335	к
6	80	125	185	310	к
7	80	121	205	326	к
8	80	88	0**	88	к
9	80	174	0**	174	к
10	80	122	0**	122	к
Golden	eagle:				
11	60	758		758	к

The dosage used was 20 mg DDT/kg body wt once every 4 days

* Does not include 1 hawk which was fed DDT but was killed by a great horned owl early in the experiment

** Nestling kept in captivity for 40 days on diets devoid of DDT



Care of captive raptors

During the course of this study, the Craigheads' procedure (1956: 312-313) for maintaining captive raptors was followed. All birds were equipped with jesses, swivel, and leash and placed on perches. Every bird was well "manned" and they were exercised periodically by flying them from the perch to the gloved hand.

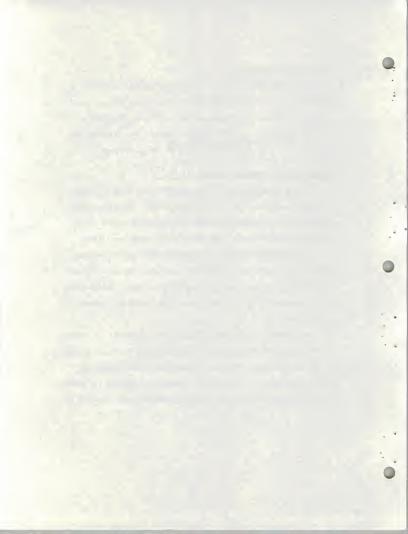
Collection and analysis of tissue

Tissues for pesticide analysis were obtained using a biopsy technique (Appendix A) or by killing the raptor. The biopsy technizue enabled the researcher to collect muscle and adipose tissue from living birds of prey, thus reducing the number sacrificed.

When collected, large tissue samples were placed in separate, double, polyethlene bags and labeled. The small samples collected by biopsy were placed in clean, screw-top 5 ml vials. All samples were frozen immediately after collection and stored in a freezer pending analysis.

The tissues collected for analysis were shipped via air express to Wisconsin Alumni Research Foundation, Madison, Wisconsin, and all residue levels reported here were determined in their laboratory under the direction of F. B. Coon. Samples were analyzed for organochlorine insecticide residues with a gas chromatograph (Appendix B).

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RESULTS AND DISCUSSION

Insecticide Residues and Background Levels

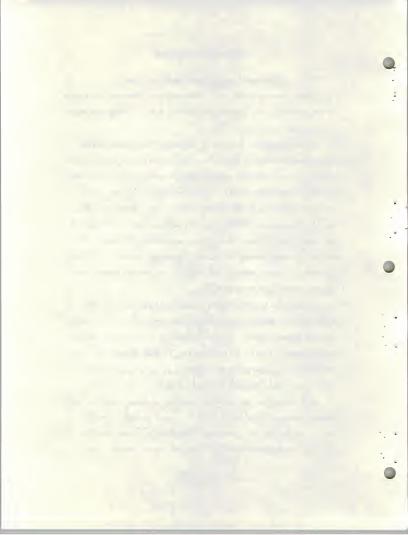
Before results of the tissue analyses are discussed, the source of the organochlorine insecticide residues found in hawks and eagles on the study area will be reviewed.

DDT is changed to a series of metabolites in animal tissues. While these metabolites differ in toxicity, they are similar to one another in their chemical and physical properties. Studies on the pathway of DDT metabolism in animal tissue indicates that DDT is converted primarily to DDE and DDD. DDD is not converted to DDE, nor is DDE converted to DDD. The only metabolites of DDT found in this study were DDE and DDD but other metabolites do exist. DDT and DDD are both present in technical grade DDT (Metcalf, 1955) and Reynolds (in prep.) reports that both DDT and DDD were marketed as insecticides in the study area.

In addition to DDT (and its metabolites) residues of other organochlorine insecticides (dieldrin and heptachlor epoxide) were found in raptor tissue. Aldrin is converted to dieldrin in animal tissue (Menzie, 1966). In south-central Montana dieldrin is used regularly as an insecticide but Reynolds (in prep.) reports that little aldrin has been used in recent years.

Both heptachlor and chlordane are used as insecticides in southcentral Montana (Reynolds, in prep.). Commercial chlordane is a mixture of at least five compounds including heptachlor which is converted to heptachlor epoxide in animal tissue (Menzie, 1966).

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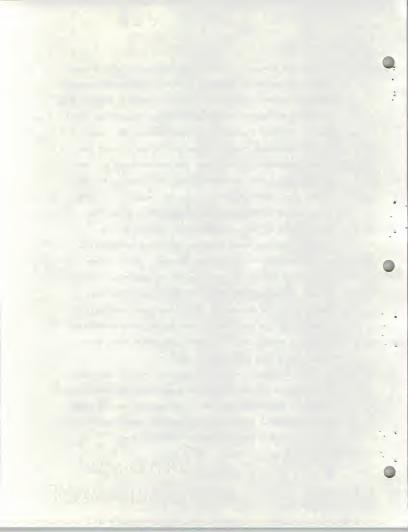


The hawk and eagle nestlings which were used in these trials remained in their nests throughout the nestling period and the nestlings were therefore subjected to background levels of organochlorine insecticide residues through the natural food. Reynolds (in prep.) measured the level of contamination by determining what organochlorine insecticide residues were present in the tissue of the raptors under study and their major prey species (Table 3). The average residual level of organochlorine insecticides in the muscle of prey species was less than 0.2 ppm. The average level in the breast muscles of fledling red-tailed hawks was 1.09 ppm. The breast muscles of fledgling golden eagles averaged 0.8 ppm.

These data indicate a generally low level of contamination, but contamination varied throughout the area. Levels in prey ranged from a high 0.9 ppm in the muscle of a Richardson's ground squirrel (<u>Citellus richardsonii</u>) to none in some of the muscle samples taken from white-tailed jackrabbits (<u>Lepus townsendii</u>). One red-tailed hawk egg contained over 12 ppm total organochlorine insecticide residues while the breast muscle taken from a newly hatched golden eagle contained 0.33 ppm.

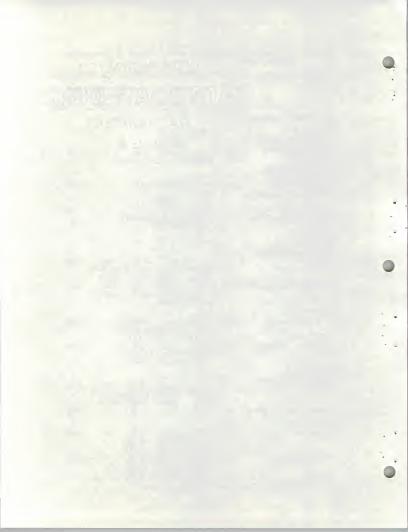
These background levels of contamination must be considered when results of feeding trials in the present study are interpreted. Undoubtedly they account for some of the variation observed in the chemical analyses. However, I do not feel that background levels were high enough to bias the conclusions of this study.

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	No. of speci- mens	Wet we DDE			nd range) Heptachlor epoxide	Mean Total Residue
RODENTS AND LAGOMORPHS	3					
Cottontail * (Sylvilagus audubonii and S. nuttallii)	11	0.02	0.03 (0.02- 0.04)	0	0	0.05
White-tailed jackrabbit * (<u>Lepus townsendi</u> i) Richardson ground	17	0.02 (0-0.03	0.04)(0-0.19	0.002 9)(0-0.02	o 2)	0.04
squirrel * (<u>Citellus</u> <u>richardsonii</u>	_) 10	0.15 (0.03- 0.88)	0.04 (0.02- 0.06)	0	0	0.19
Yellow-bellied marmot? (Marmota flauiventris)		0.02 (0.02- 0.03)	0.03 (0.02- 0.04)	0.01 (0-0.02)	0	0.06
RAPTORS						
Red-tailed hawks (<u>Buteo jamacicensis</u>)						
Eggs	5	2.90 (0.24- 10.30)	0.32 (0.05- 1.33)	0.35 (0.16- 0.63)	0.37 (0.09- 0.80)	3.94
Newly-hatched young	+ 2	1.26 (0.43- 2.10)	0.14 (0.09- 0.20)	0.12 (0.05- 0.19)	0,02 (0-0,04)	1.54
Fledglings						
whole muscle	2	0.93 (0.61- 1.41)	0.07 (0.04- 0.10)	0.09 (0.02- 0.16)	0	1.09
muscle (biopsy)	5	3.17 (1.27- 6.87)	1.33 (0.40- 2.30)	0.38 (0.10- 1.40)	0	5.88
Golden eagle (Aquila chrysaetos)						
Eggs	7	0.30 (0.13- 0.57)	0.04 (0.02- 0.09)	0.18 (0.06- 0.38)	0.07 (0.05- 0.08)	0.59
Newly-hatched young	+ 1	0.16	0.15	0.02	0	0.33
Fledglings						
muscle	2	0.45	0.31	0.04	0	0.80
fat (biopsy)	5	0.70) 3.67 (0.29 10.60)	0.44) 1.89 (0.30 7.35) 0.10	0.07) 0.29 (0.10-	0	5.85
Adult* *Muscle tissue	1	0.04	6:18	(0.45) 0.07	0	0.21

Table 3. Organochlorine insecticide residues found in south-central Montana, 1967



Red-tailed Hawk Feeding Trials

DDT accumulation in nestling hawks

Throughout this discussion hawks which were fed DDT will be referred to as experimental hawks and hawks which were not fed DDT will be called controls. Controls were not fed DDT but obtained organochlorine insecticide residues through natural foods.

Three experimental hawks and one control hawk were sacrificed at the end of the nestling period. The DDT residues (DDE, DDD, DDT) present in the tissue of these birds are compared in Table 4.

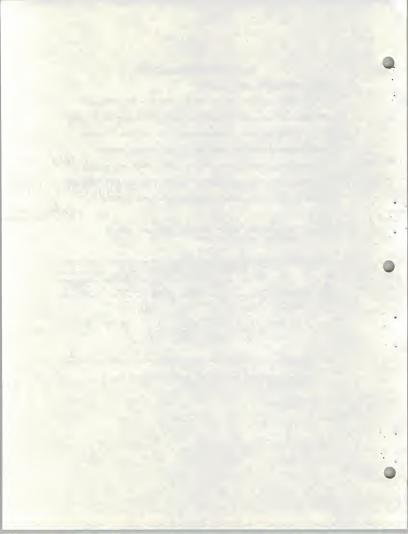
		Wet weig	ght ppm	(mean and	l range)		
No.		Brain		Breast n	nuscle	Liver	
of			DDD+		DDD+		DDD+
birds	Treatment	DDE	DDT	DDE	DDT	DDE	DDT
l	Control	.49	.47	.61	. 05		
3	Fed DDT*	5.80 (3.22- 7.60)	5.23 (3.85- 6.00)	10.17 (9.70- 10.60)	11.33 (9.14- 15.50)	16.30 **	6.90

Table 4.	Accumulation	of DDT	residues	(DDT,	DDD,	DDE)	in
	nestling red-	-tailed	hawks				

* The dosage was 20 mg DDT/kg body wt every 4 days for 40 days.

** One sample

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It can be seen in Table 4 that DDT residues (based on ppm wet weight) in experimental hawks were much higher than those in the control bird. It is apparent that nestling hawks were unable to completely metabolize or eliminate all the DDT which was fed and consequently DDT residues accumulated in their tissues.

Storage and loss of DDT by captive hawks

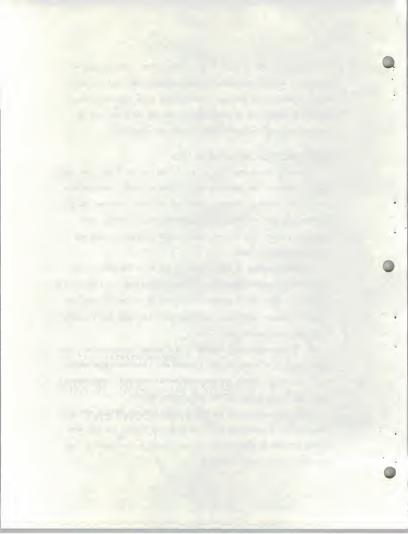
Six hawks which were fed DDT for 40 days while in the nest were kept in captivity for an additional 40 days at which time they were sacrificed. While in captivity three of the hawks were fed DDT at the nestling rate and the remaining three were given food which contained no DDT. One control was retained in captivity and was sacrificed after 40 days.

The DDT residues in the tissues of the birds fed DDT for 80 days were much higher than those in the tissues from the control bird (Table 5). (The residue analyses are based on ppm wet weight; the weight of captive hawks remained essentially the same from fledging until they were sacrificed.)

At 80 days total residual DDT levels in the brains, livers, and breast muscles of hawks fed DDT in captivity were about the same as the levels in the brains, livers, and breast muscles of experimental hawks sacrificed at the end of the nestling period.

Total DDT residues in the brains, breast muscles, and livers of hawks fed DDT as nestlings but food devoid of DDT as captives were only one-fourth as high as the residues found in experimental birds sacrificed as nestlings (Table 6).

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No.		Wet we Brain	eight pp	m (mean an Breast n	nd range)	Liver	
of	Treatment	DIT	DDD+ DDT	DDE	DDD+ DDT	DDE	DDD+ DDT
l	Control	.16	. 04	.07	• O ¹ 4	.12	. 04
3	Fed DDT*	5.57 (5.00- 6.14)	3.30 (2.59- 4.09)	16.47 (14.60- 20.00)	10.72 (7.10- 17.80)	10.00#	4.55
3	"Clean" food**	2.38 (1.19- 4.25)	.38 (.29- .46)	4.54 (1.19 8.60)	.63 (.30- .91)	2.00#	• 55

Table 5. Storage and loss of DDT residues (DDT, DDD, DDE) in captive red-tailed hawks

* Fed DDT during a 40-day nestling period and during a 40-day post-fledgling period in captivity. The dosage was 20 mg DDT/kg body wt every 4 days for 80 days.

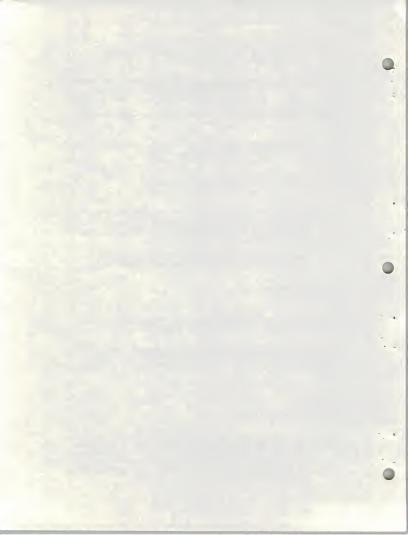
** Fed DDT during a 40-day nestling period but were fed food devoid of DDT during a 40-day post-fledgling period in captivity.

One sample

Table 6. Loss of DDT residues (DDT, DDD, DDE) by post-nestling, captive red-tailed hawks on diets devoid of DDT

Tissues	Total DDT residues Nestlings	(ppm wet weight) Captives	% Change	
Brain	11.03	2.76	75	
Breast muscle	21.50	5.17	76	
Liver	23.20	2.55	89	

Note: Residues reported are means of three birds. Nestlings were fed 20 mg DDT/kg body wt every 4 days for 40 days. Captives were fed DDT at the same rate as nestlings for 40 days but were retained in captivity for 40 additional days and fed diets devoid of DDT.



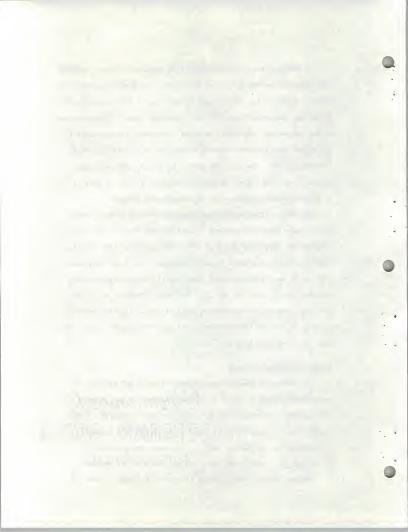
In summary, the tissue analyses from experimental hawks indicate that DDT residues did not accumulate in the breast muscle, liver, and brain of captives but DDT residues accumulated in nestlings when fed DDT at the same rate (Fig. 2). It is possible that the rapid growth of the red-tailed hawk nestlings taxes their metabolic processes to the extent that they are relatively inefficient in metabolizing and eliminating DDT. However, once growth is essentially completed, juvenile red-tailed hawks in captivity appear to have the ability to metabolize and eliminate DDT at the employed dosages.

The basic differences between captive and wild juvenile redtailed hawks must be considered. Captive hawks acquired very little exercise and were never short of food. The post-nest life of wild, juvenile, red-tailed hawks is more demanding: the young bird must learn to be an effective hunter, and when it acquires independence from the parent, fend for itself. Thus, the ability of wild, postnestling juvenile hawks to handle loads of organochlorine insecticides could be less than that exhibited by captive hawks of the same age. More research is needed.

Changes in DDT metabolites

In addition to the storage and loss of total DDT residues in experimental hawks, a change in relative amounts of each metabolite was observed. Recent studies have reported that conversion of DDT to DDD occurs in animal tissue after death (Barker and Morrison, 1964; Jefferies and Walker, 1966). I followed the procedure of Stickel <u>et al</u>. (1966<u>b</u>) and added DDT and DDD residues together. DDE and DDD+DDT are expressed as percentages of the total DDT

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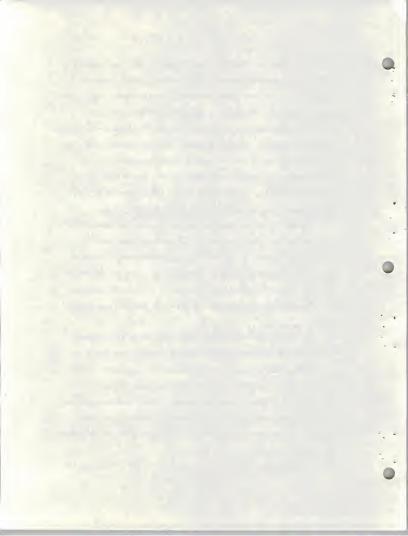
residues in Table 7. Data in Table 7 indicate that there are differences in the relative amounts of DDE in the different groups of experimental hawks and in the different organs of hawks within each group. Generally, the relative amounts of DDE are greater in experimental captive hawks than in experimental hawks which were sacrificed as nestlings. In captive hawks, total DDT residues (DDT, DDD, DDE) decreased while relative amounts of DDE increased (Fig. 2). This supports the contention by other workers (<u>cf. Stickel et al.</u>, 1966<u>b</u>) that DDE is retained in the tissue longer than DDD or DDT. DDT residues in hawk nestlings dying from "natural" causes

Information in the above section was derived from hawks which were sacrificed for study according to a predetermined schedule, but during the nestling period 6 red-tailed hawks died. Two hawk nestlings from 1 nest were lost through great horned owl predation and only fragmented remains were recovered. Post-mortem examinations and chemical analyses are available for the remaining 4 nestlings.

All post-mortem examinations were conducted by veterinarians of the Montana Livestock Sanitary Board. Pneumonia was listed by the veterinarians as the cause of death of all nestlings. These nestlings were recovered shortly after an extended period of wet, cold weather. The adult male was present at each hawk eyrie where nestlings were found dead but circumstantial evidence indicated that the adult female at each nest was shot just prior to the period of inclement weather.

In Table 8 the results of the analyses for DDT residues in the

-25-



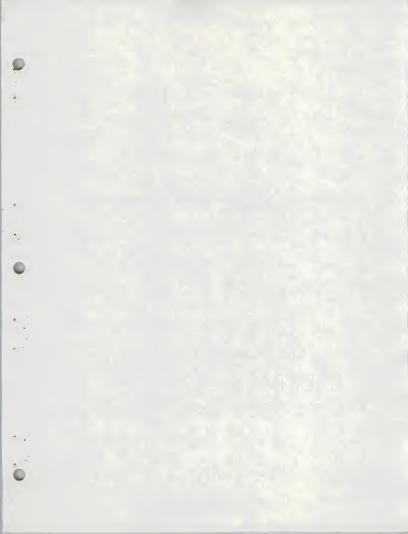


Figure 2. Total DDT residues (DDT, DDD, DDE) in experimental red-tailed hawks

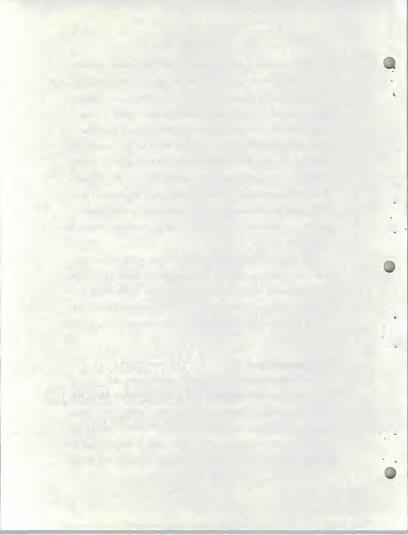
brains of nestlings dying of pneumonia are compared with analyses of brain tissue from hawks sacrificed at the end of the nestling period. Total DDT residue levels (DDE, DDD, DDT) in the experimental hawk nestling that died of pneumonia were almost 5 times higher than those in the experimental nestlings which were sacrificed. The relative amounts of DDT residues (DDE and DDD+DDT) in the brains of hawks dying from pneumonia and hawks sacrificed at the end of the nestling period are compared in Table 8. The ratio of DDE to DDD+DDT was 1:1 in the brains of both experimental and control hawks which were sacrificed according to a predetermined schedule; a ratio of 1:4 was found in the brains of nestlings dying of pneumonia.

Variations in experimental conditions and small sample size make it difficult to interpret the observations which are outlined above. Different ratios of the DDT metabolites in the brains of nestling hawks indicate that more DDT residues accumulated in the tissues of diseased nestlings than in the tissues of nestlings which were sacrificed.

Growth and development

The nestling period in the life of an altricial bird is a period which is characterized by high growth efficiency dependent upon the ingestion of large quantities of food (Kahl, 1962). Gross morphological changes occur in the young bird during a relatively short time. It has been postulated that a raptor might be more susceptible to toxic chemicals during the nestling period than at any

-29-

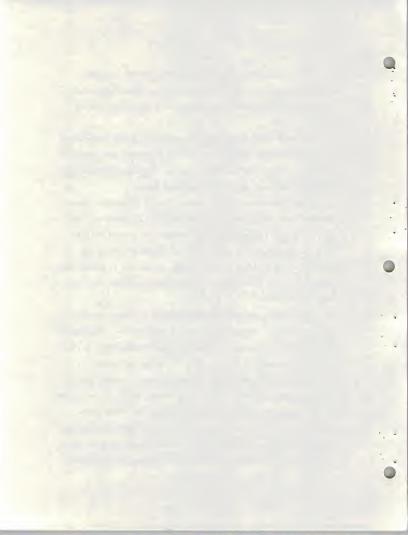


The average nestling period for red-tailed hawks in southcentral Montana is 41 days. The gross morphological changes that occur in red-tailed hawks from hatching to fledging are shown in Plate 1.

Growth of birds in experimental and control groups was measured to determine if any changes resulted from the induced DDT. The indicies which were used to assess growth in nestlings included weight, length of right foot pad, and plumage development.

There were several sources of error in the growth data. Measurements were taken from living birds under field conditions. The growth analysis is based on the age of nestlings in days but only in a few cases were ages known. When the age of a nestling was unknown, it was established by comparing the nestling with known-age individuals. The established ages are no more accurate than \pm one day.

Body weight: The growth of experimental and control red-tailed hawks is shown by changes in weight in Figures 3 and 4. Three main stages of nestling growth as shown by changes in weight can be recognized in altricial birds. There is (1) an initial period of slow gain in weight, (2) a period of maximum increase in weight, and (3) a final interval of minor fluctuations (Summer, 1933). In red-tailed hawks, the initial growth period lasts 5 days; the second period lasts about 30 days; and the third period lasts approximately 6 days. The growth curves of nestling, male red-tailed hawks reach the final stage at a lower weight than females. The average maximum weights



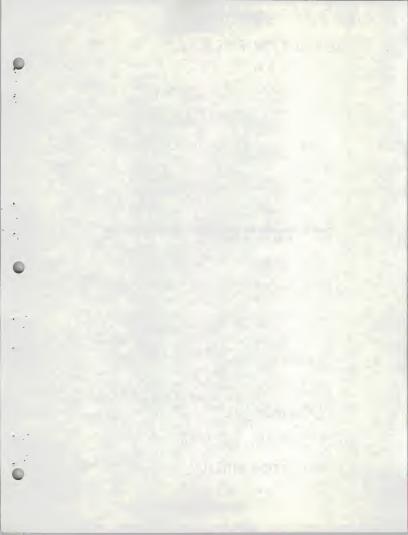
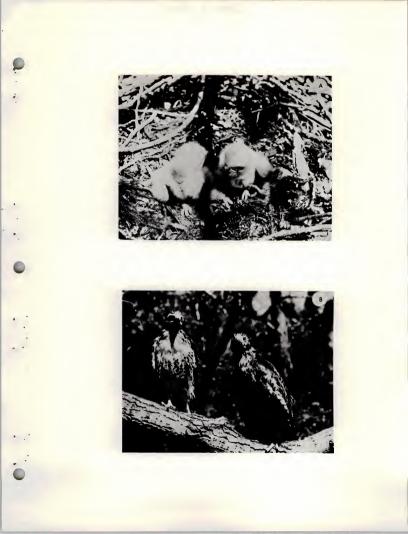


Plate 1. Nestling red-tailed hawks: A, 1 and 2 days old; B, 43 and 45 days old



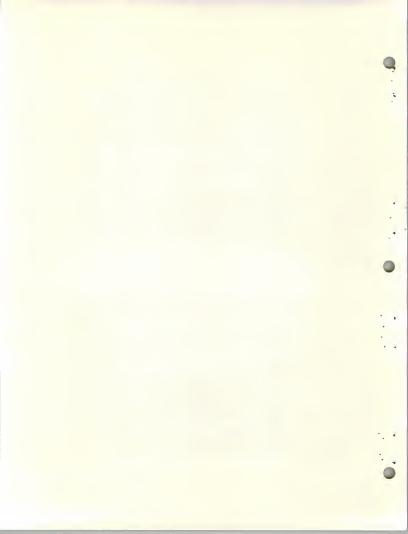
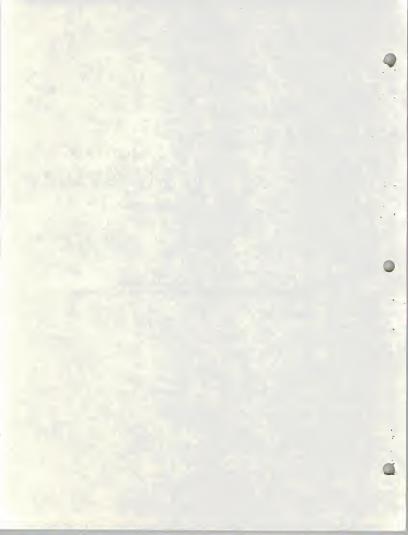
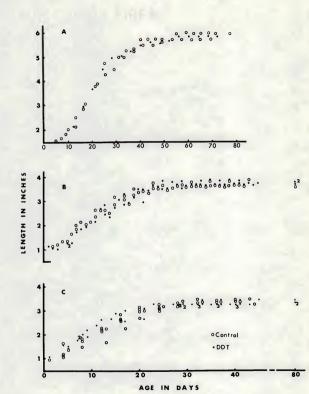
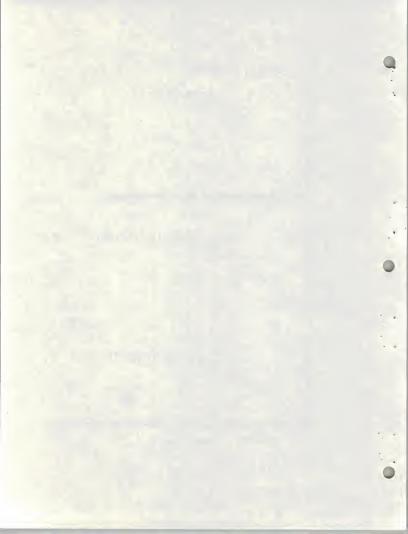


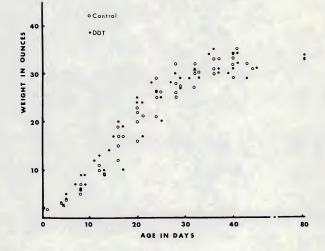
Figure 3. Growth of male red-tailed hawk nestlings shown by changes in weight



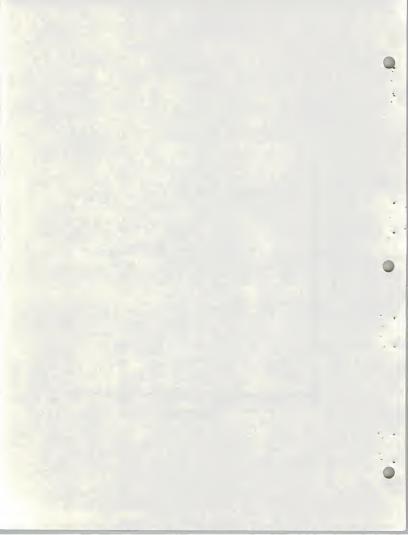


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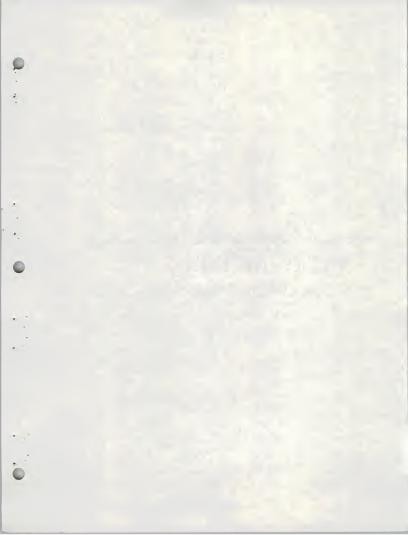
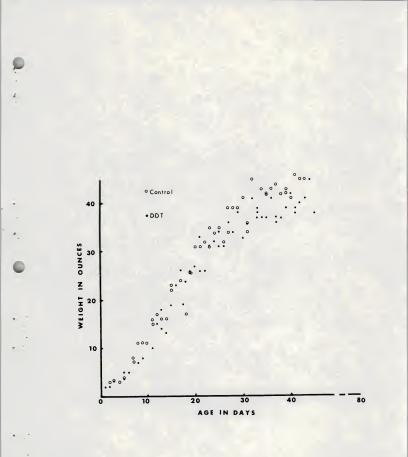
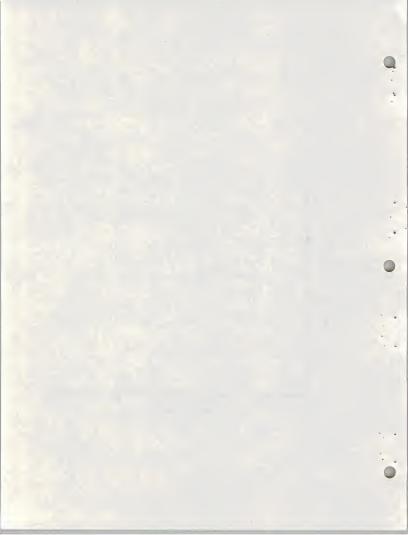


Figure 4. Growth of female red-tailed hawk nestlings shown by changes in weight

.





of control and experimental hawks were not significantly different. Male control and experimental hawks weighed 33 oz (935 g) and 34 oz (964 g) respectively and females weighed 41 oz (1162 g) and 44 oz (1247 g) respectively. Control, nestling, male and female redtailed hawks achieved more than 91 and 102% respectively of their adult weights.

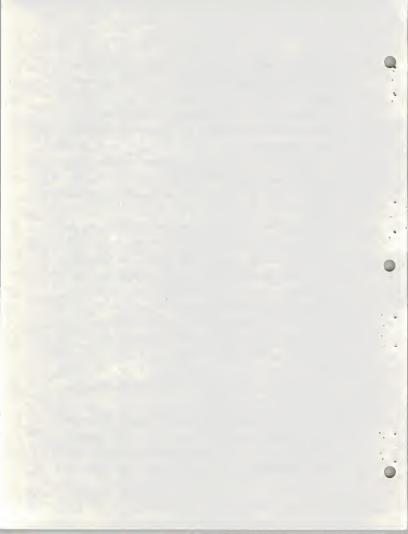
Weights of nestling hawks vary a great deal but Figures 3 and 4 show that the DDT dosage which was used in these trials did not affect the growth of experimental nestlings as shown by changes in weight.

Foot pad length: The increase in length of the right foot pad (from the tip of the hallux to the tip of the middle toe) of redtailed hawks is shown in Figure 5. The foot pad grows rapidly from hatching until about day 25 and at fledging the growth of the pad is essentially complete. Sexual dimorphism in the length of the foot pad in red-tailed hawks appeared between day 10 and 15. The foot of the female grows at a faster rate than that of the male until day 25. The mean foot pad length at fledging in known sex birds (both experimental and control) is 3.31 in. (3.25 - 3.45) in males and 3.64 in. (3.62 - 3.69) in females.

The growth of the foot pad of experimental birds was not different from that of control birds.

Development of the feather coat: The development of the feather coat of nestling hawks was studied in two ways: 1) the appearance of contour feathers in the various pterylae was recorded and 2) the seventh right primary and the sixth right rectrix were measured.

-34-



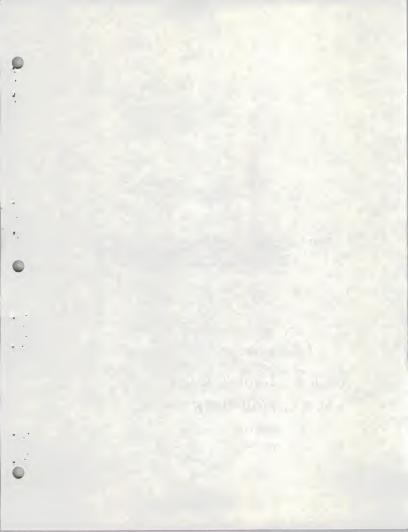


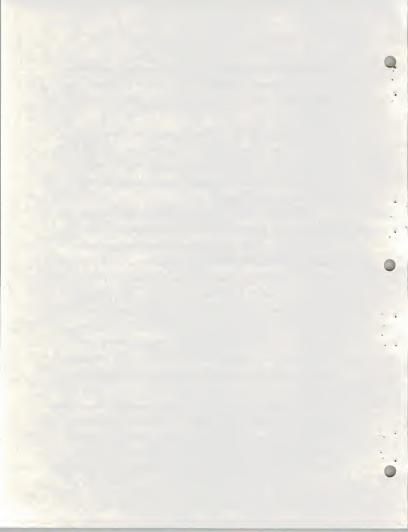
Figure 5. Growth of the right foot pad (from the tip of the hallux to the tip of the middle toe): A, female, nestling golden eagles; B, female, nestling redtailed hawks; and C, male, nestling red-tailed hawks The development of the juvenal plumage and other main points in the physical development of red-tailed hawks is shown in Table 9. The nomenclature follows that of Compton (1938) and Humphrey and Parks (1959).

Because observations on the appearance of the juvenal plumage in the various feather tracts were made at 4-day intervals, it was necessary to arrange the data in a general form. No differences in the development of juvenal plumage of control and experimental hawks were detected.

The growth of the seventh right primary and the sixth right rectrix in both experimental and control nestlings is shown in Figures 6-9. These flight feathers were measured from their tips to the points where the shafts emerged from the skin. Quills of flight feathers are the first to appear and these feathers are still in a state of rapid growth at fledging. The seventh right primary achieves approximately three-fourths its total length and the sixth right rectrix achieves two-thirds its total length at fledging.

There is a great deal of individual variation between birds in the growth of the primary and rectrix but there were apparently no significant differences between control and experimental birds. Behavior

Warner <u>et al</u>. (1966) pointed out that the behavior of any organism represents the integrated result of a diversity of biochemical and physical processes. Moreover, "Behavior patterns are known to be highly sensitive to change in the steady state of an organism. This sensitivity is one of the key values for their use in exploring



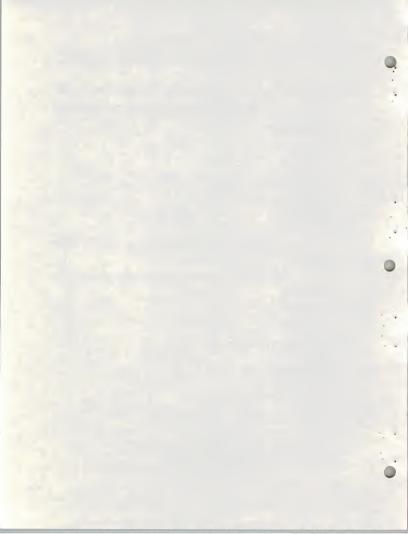
Tract	0-7	in Da; 8-14	15-21	22-28	29-35	36-42	43-49	50-56	80
Red-tailed hawk									
Alar tract									
Primaries		S	U						
Secondaries		S	U						
Caudal tract									
Rectrices		S	U U						
Humeral tract		s s	UU						
Spinal tract Ventral tract		5	SU						
Capital tract			s	U					
Crural tract			ŝ	SU	U				
Femoral tract			ŝ	s	Ŭ	U			
						-			
Eye color	Brown			Gray					Yello
Ear opening	v				NV	NV			
Egg tooth	Pres.	Abs.	Abs.						
Toe nails	Pink-								
	Black								
Golden eagle									
outon oupao									
Alar tract									
Primaries			S	U					
Secondaries			S	U					
Caudal tract									
Rectrices			S	U					
Humeral tract			S	U					
Spinal tract				S	U				
Ventral tract				S	U S	U	U		
Capital tract Crural tract					S	U	U		
Femoral tract					SU	U			
remotar diaco					50	v			
Ear openings	v							NV	
Egg tooth	Pres.				Abs.	Abs.	Abs.	Abs.	
	Pink		Black						

Table 9. The development of the juvenal plumage and other main points in the physical development of red-tailed hawks and golden eagles.

tes: S indicates feather tips breaking the skin;
U feather tips breaking their sheaths;
V ear openings are visable;

NV ear openings are obscured by bristles

-37-



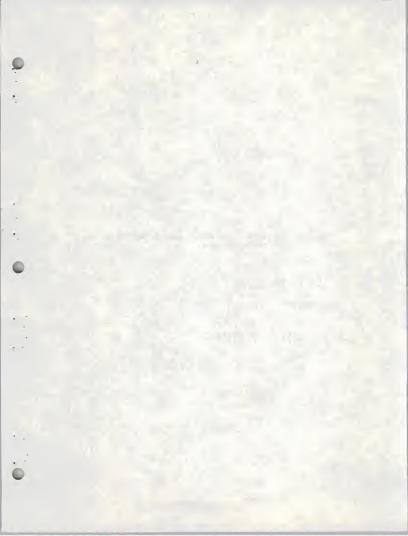
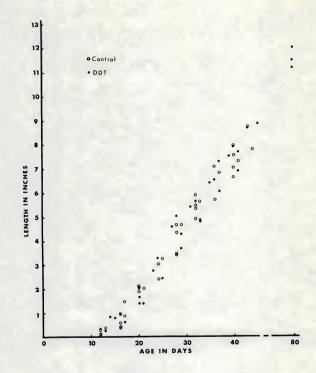
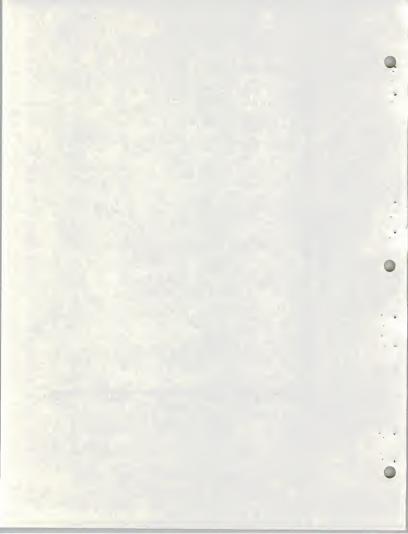


Figure 6. The length of the seventh right primary of nestling, male red-tailed hawks





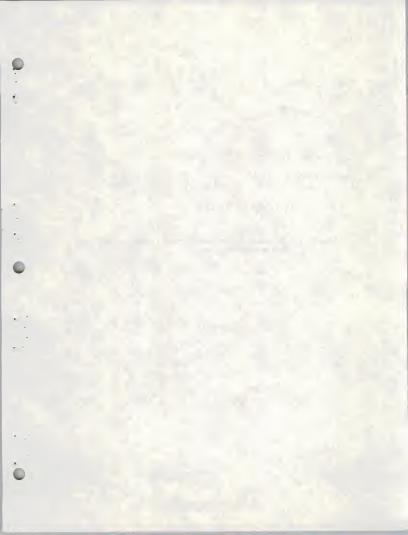
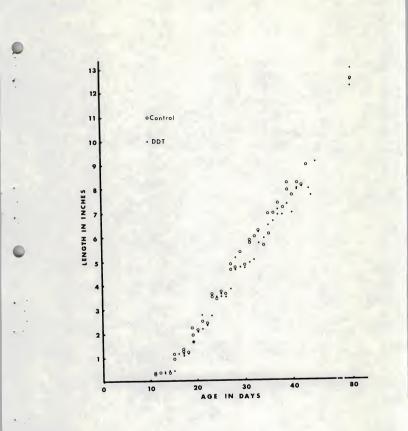


Figure 7. The length of the seventh right primary of nestling, female red-tailed hawks





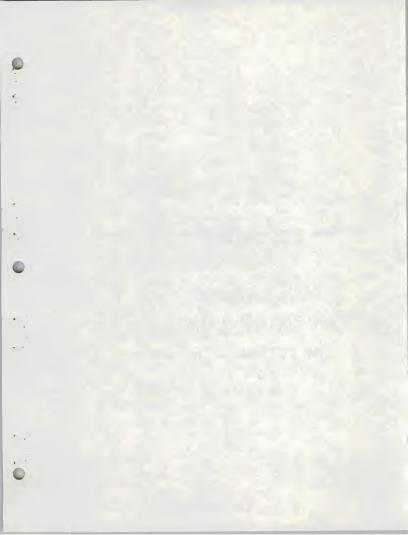
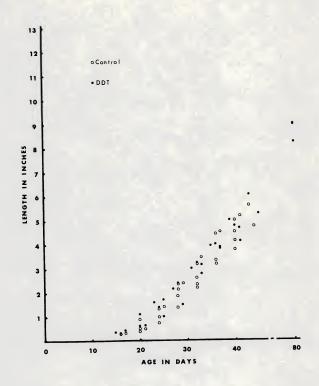
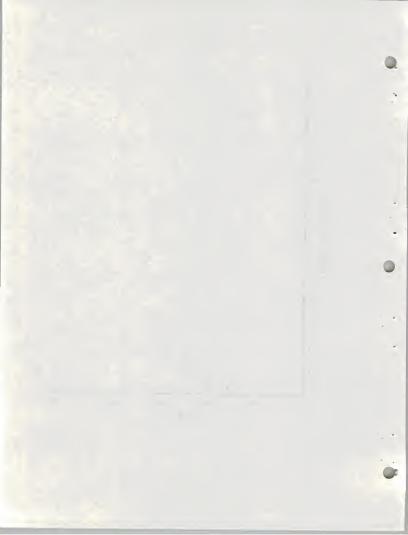


Figure 8. The length of the sixth right rectrix of nestling, male red-tailed hawks

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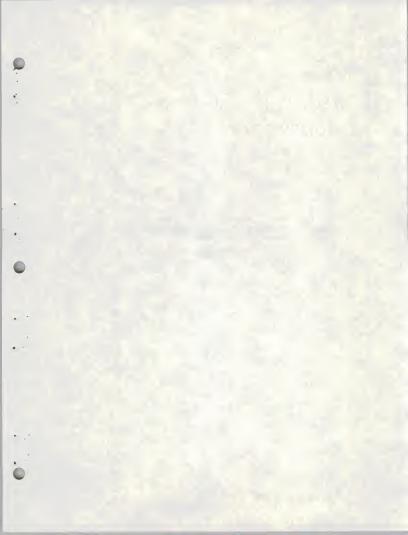
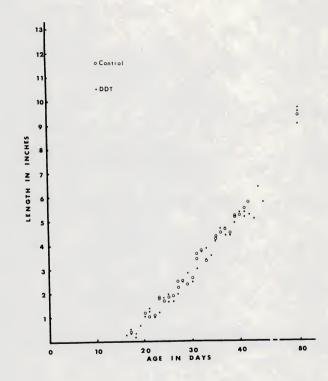


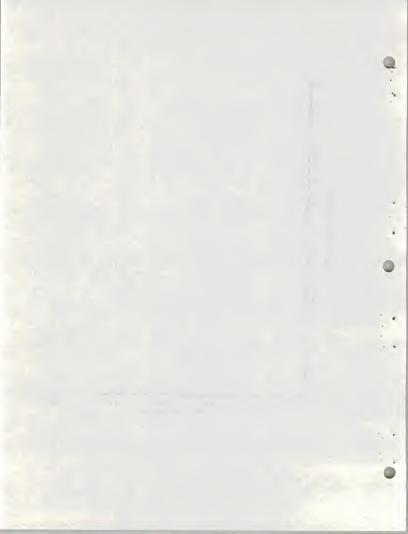
Figure 9. The length of the sixth right rectrix of nestling, female red-tailed hawks

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sublethal toxication (Warner et al., 1966: 224-225)."

Quantitative measurements of behavior patterns were not undertaken during this inquiry; however, casual observations were made. Seven red-tailed hawk nestlings were taken into captivity at the end of the nestling period: 6 of the hawks were on DDT diets and 1 hawk acted as a control during the nestling period. All hawks which were taken into captivity were handled similarly.

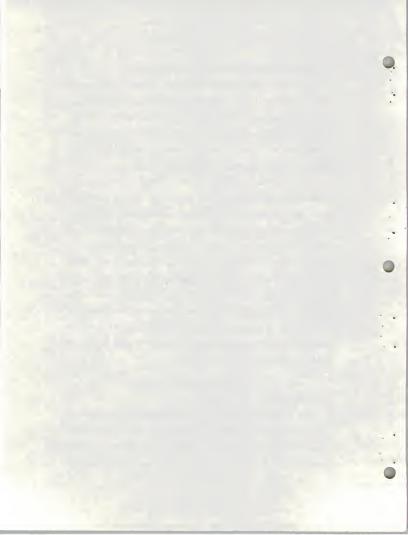
One captive hawk that had been on a DDT diet during the nestling period never learned to feed itself when food was placed on its perch and food had to be lifted to its beak before it would eat. All other captive hawks readily learned to feed themselves. Organochlorine insecticide residues in the tissue of the hawk which failed to learn to eat were no higher than those which were found in other hawks under similar experimental conditions. Ludwig (1965) and Warner <u>et al</u>. (1966) found that sublethal levels of both organophosphorus and organochlorine insecticides affect the learning ability of fish. However, the effect of sublethal residues of organochlorine insecticides on the learning ability of birds has not been measured under controlled conditions.

Golden Eagle Feeding Trials

DDT accumulation in nestling eaglets

The DDT residues (DDT, DDD, DDE) found in nestling golden eagles and red-tailed hawks sacrificed at the end of the nestling period are compared in Table 10. DDT residues found in the control eaglet were lower than those found in the control red-tailed hawk.

-42-



Residues in experimental hawks and eagle were much higher than those found in the control birds. Differences among the amounts of DDT residues stores in the various tissues of experimental birds are apparent. However, the residual levels in experimental birds of both species are of the same general magnitude. This indicates that nestling eagles are similar to nestling hawks in their inability to completely metabolize and eliminate all the DDT which was fed and consequently DDT residues accumulate in their tissue.

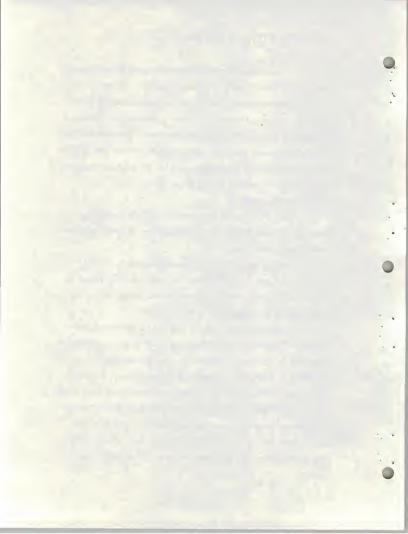
Growth and development

Nestling golden eagles were handled similarly to nestling hawks and the same sources of error are present in the growth data obtained from eagles.

The average nestling period for golden eagles is 75 days (Reynolds, in prep.) as compared to the 41-day nestling period of the red-tailed hawk. The gross morphological changes that occur from hatching to fledging are shown in Plate 2.

Body weight: All the eaglets used in this experiment were females. Changes in weight of both experimental and control eaglets are shown in Figure 10. The initial growth period which is characterized by slow gains in weight lasts about 10 days; the period of maximum increase in weight extends over 55 days; the third interval of minor fluctuations in weight lasts about 10 days. The average maximum weight of control and experimental female nestling golden eagles was 147 oz (4166 g) and 150 oz (4251 g) respectively. Female nestling golden eagles achieve about 77% of their adult weight.

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Species	No. of birds	Treatment	Wet weight ppm Brain		Breast muscle		Liver	
			DDE	DDD+ DDT	DDE	DDD+ DDT	DDE	DDD+ DDT
Golden eagle	1	Control	.15	.04	.20	.19	.19	.13
Red-tailed hawk	1	Control	.49	.47	.61	.05		
Golden eagle	1	Fed DDT*	2.84	3,80	9.12	22.97	11.90	12.22
Red-tailed hawk	3	Fed DDT*	5.80	5.23	16.30	6.92	10.17	11.33

Table 10. DDT residues (DDE, DDD, DDT) in control and experimental golden eagles and red-tailed hawks at the end of the nestling period

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* The dosage was 20 mg DDT/kg body wt every 4 days



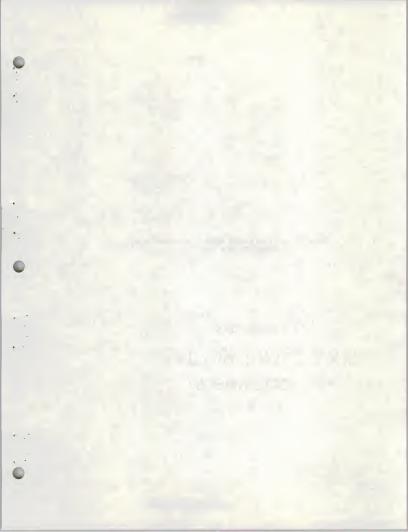
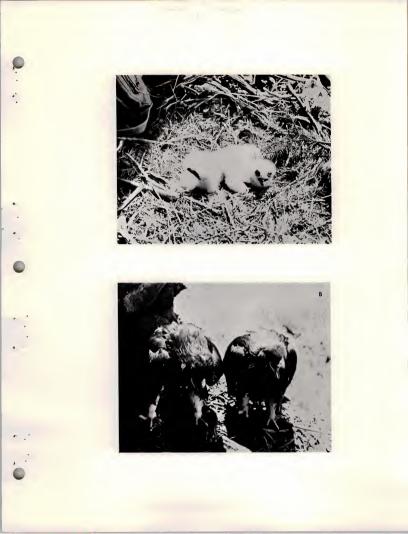
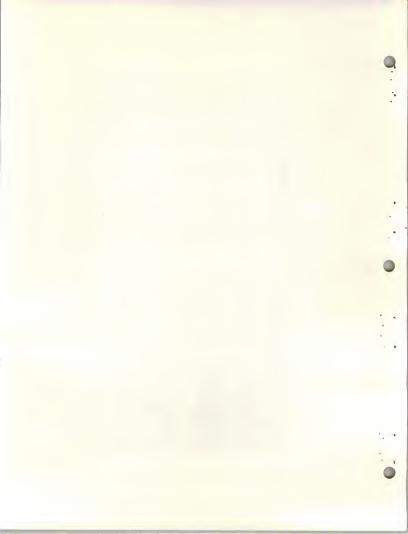


Plate 2. Nestling golden eagles: A, 9 and 12 days old; B, 69 and 72 days old





The weights of nestling eagles vary a great deal but the data in Figure 10 show that the DDT dosage which was used in these trials did not affect the growth of the experimental nestling as shown by changes in weight.

Foot pad length: The increase in the length of the right foot pad of golden eagle nestlings is shown in Figure 5. The foot pad grows rapidly from hatching until about day 55. At fledging the growth of the foot pad is essentially complete. The average length of the foot pad at fledging was 5.87 in. (5.75-6.00) in the female eagles used in this study.

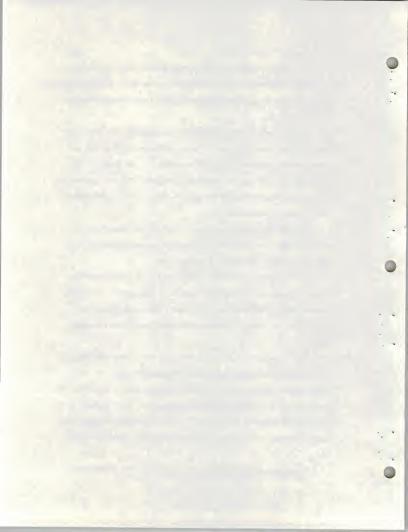
From the data shown in Figure 5, it does not appear that the growth of the foot pad of the eaglet which was experimentally fed DDT was different than that of control birds.

Development of the feather coat: Quills of flight feathers began to appear during the third week in golden eagles. At fledging all flight feathers were well along in development and the majority of the contour feathers were unsheathing in all pterylae (Table 8).

There were some discrepancies between the plumage data which I collected and that which were gathered by Summer (1933) for the golden eagle. Summer (1933: 281-282) reported that in a male golden eagle the quill of the sixth primary appeared at day 7 and the sixth left rectrix appeared at day 9. I did not observe quills appearing in the caudal or alar tracts until the third week (between days 15-21).

The growth of the seventh right primary and the sixth right

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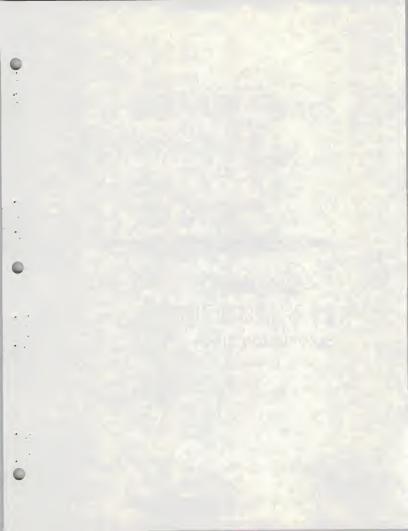
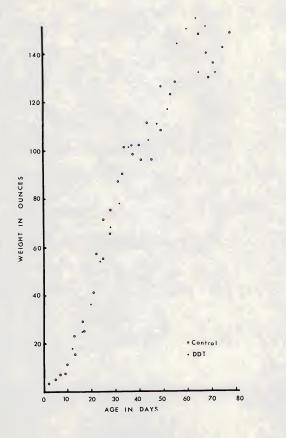
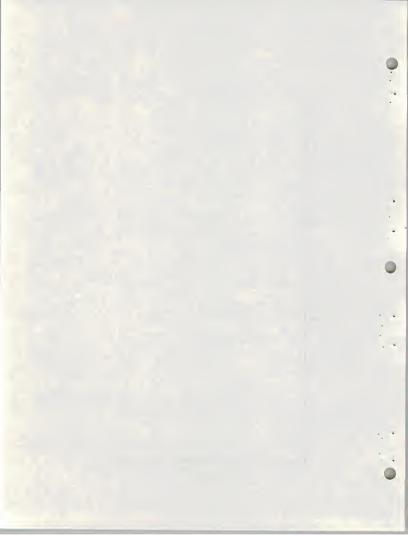


Figure 10. Growth of female golden eagle nestlings shown by changes in weight





rectrix in both experimental and control eaglets is shown in Figures 11 and 12. There is a great deal of individual variation in the growth of these feathers but there were apparently no differences between the control eaglets and the eaglet fed DDT.

General Discussion

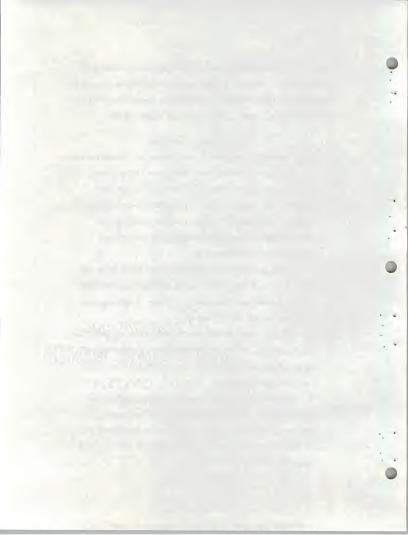
The observations outlined in this section are summarized below: 1. Low levels of DDT and its metabolites (DDD and DDE), dieldrin and in some instances heptachlor epoxide were found in the tissue of control red-tailed hawks and golden eagles. The presence of these residues indicated that organochlorine insecticide residues were being transferred through the natural food.

2. At the dosage used in the feeding trials (20 mg DDT/kg body wt every 4 days), DDT and its metabolites accumulated in the brains, breast muscles, and livers of nestling redtailed hawks and golden eagles.

3. Residual levels of DDT and its metabolites were similar in the brains, livers, and breast muscles of hawks fed DDT as nestlings and hawks fed DDT as nestlings and for a 40day post-nestling period.

4. Total DDT residues in the brains, breast muscles, and livers of hawks fed DDT as nestlings and food devoid of DDT for a 40-day post-nestling period were only one-fourth as high as the residues found in the same tissue taken from hawks fed DDT during the nestling period.

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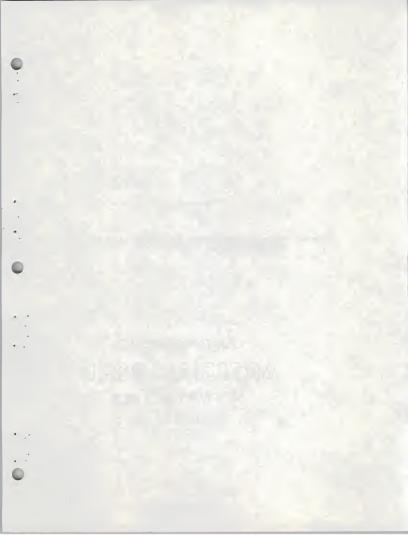
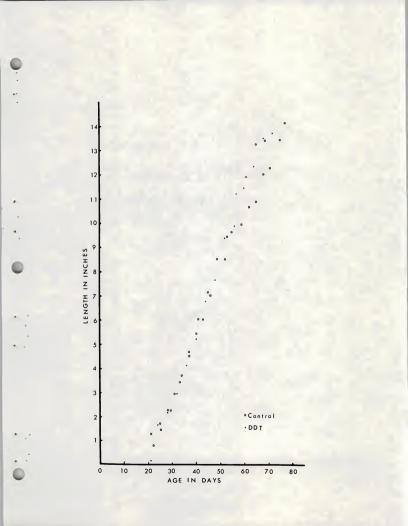
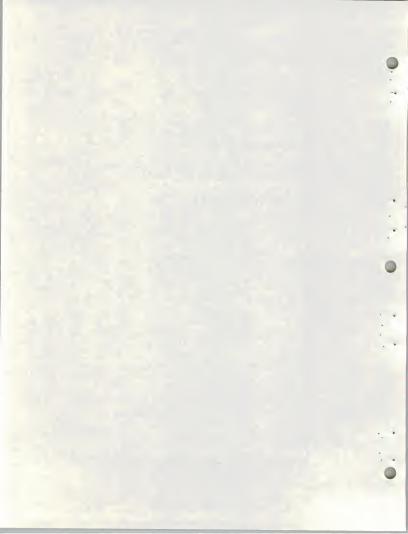


Figure 11. The length of the seventh right primary of nestling, female golden eagles





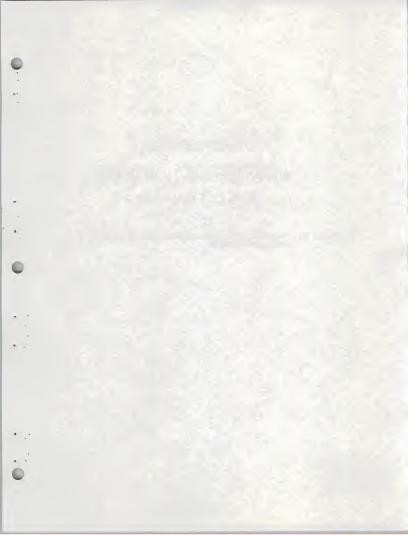
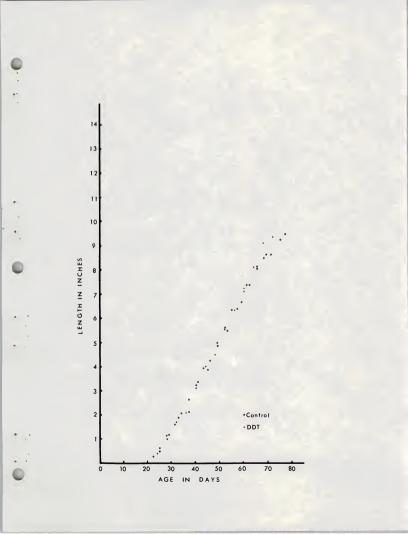
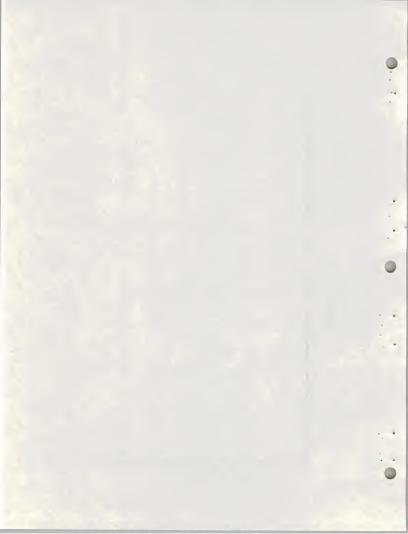


Figure 12. The length of the sixth right rectrix of nestling female golden eagles





5. The data suggest that more DDT residues accumulated in the brains, breast muscles, and livers of diseased nestlings than in the brains, breast muscles, and livers of healthy nestlings.

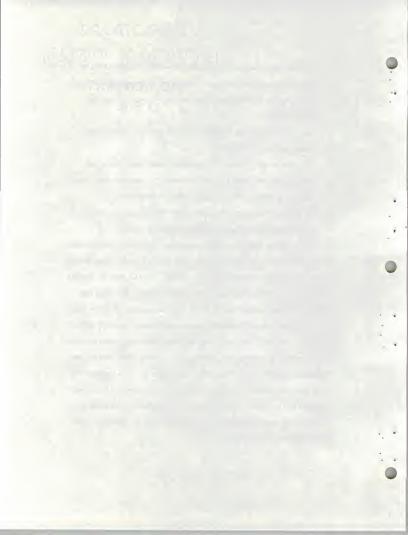
 DDE is retained in the tissue of juvenile red-tailed hawks longer than DDD+DDT.

7. One of six fledgling red-tailed hawks which were fed DDT during the nestling period failed to learn to feed itself during a 40-day post-fledging period in captivity.

 DDT at the dosage used in these trials did not affect the growth of red-tailed hawks and golden eagles.

Even though nestling hawks and eagles accumulated substantial levels of DDT in vital body organs (brain and liver), these levels had no measurable affect on growth. These results gave no insight into other possible effects of the induced DDT on the nestlings. Burlington and Lindman (1950) found that the growth of white leghorn cockerels was not affected by sublethal injections of DDT but the development of the comb, wattles, and testes was inhibited as a result of the treatment. Warner <u>et al.</u> (1966: 245) stated that "Empirical research and experience are teaching us, sometimes the hard way, the folly of assuming that lack of evidence is the same as negative evidence." Unfortunately, our present knowledge permits only an imperfect estimate of the effects of organochlorine accumulation on any species.

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SUMMARY

1. An investigation was conducted during the spring and summer of 1967 in south-central Montana to obtain information on the response of juvenile red-tailed hawks and golden eagles to DDT in the diet. The experiments were designed to: 1) measure the accumulation of DDT residues in nestling hawks and eagles, 2) measure storage and loss of DDT residues in post-nestling hawks and eagles, and 3) determine the effects of feeding DDT on their growth, development, and behavior.

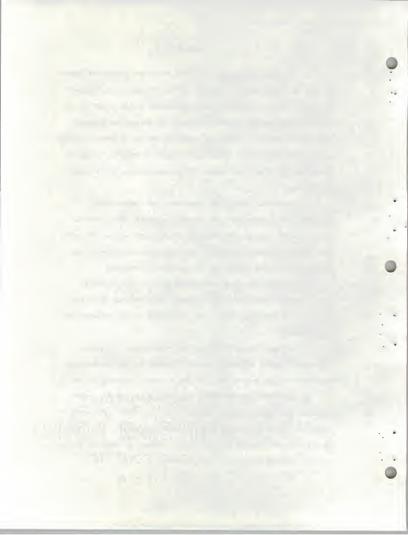
2. Low levels of DDT and its metabolites (DDD and DDE), dieldrin, and in some instances heptachlor epoxide were found in the tissue of control red-tailed hawks and golden eagles. The presence of these residues indicated that organochlorine insecticide residues were being transferred through the natural food.

3. At the dosage used in the feeding trials (20 mg DDT/kg body wt every 4 days), DDT and its metabolites accumulated in the brains, breast muscles, and livers of nestling red-tailed hawks and golden eagles.

4. Residual levels of DDT and its metabolites were similar in the brains, livers, and breast muscles of hawks fed DDT as nestlings and hawks fed DDT as nestlings and for a 40-day post-nestling period.

5. Total DDT residues (DDT, DDD, DDE) in the brains, breast muscles, and livers of hawks fed DDT as nestlings but food devoid of DDT for a 40-day post-nestling period were only one-fourth as high as the residues found in the same tissue taken from hawks fed DDT during the nestling period.

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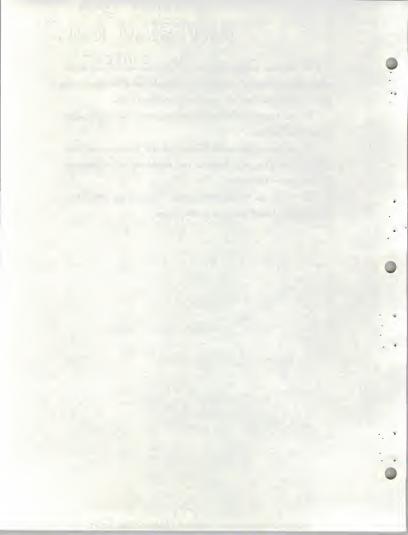


6. The data suggest that more DDT residues accumulated in the brains, breast muscles, and livers of diseased nestlings than in the brains, breast muscles, and livers of healthynestlings.

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 One of six red-tailed hawks which were fed DDT during the nestling period failed to learn to feed itself during a 40-day postfledging period in captivity.

 DDT at the dosage used in these trials did not affect the growth of red-tailed hawks and golden eagles.

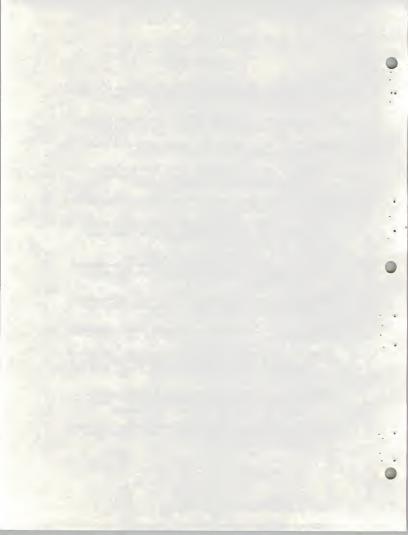


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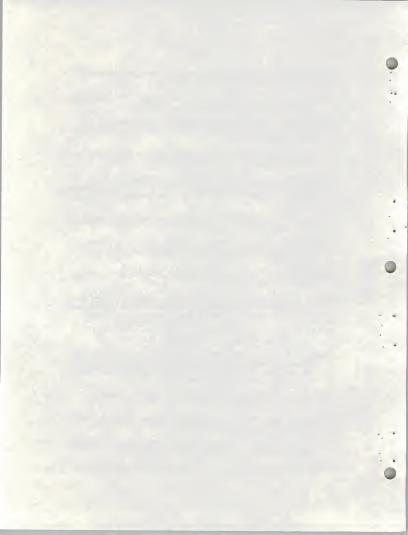
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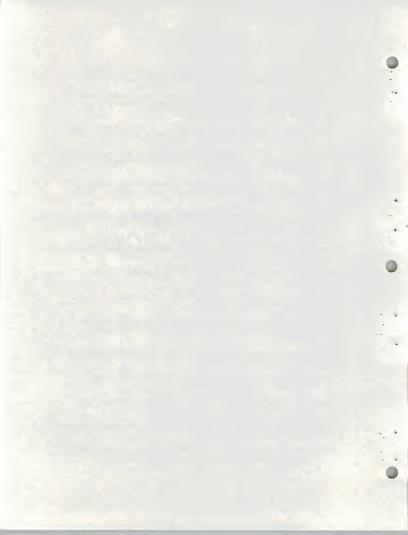
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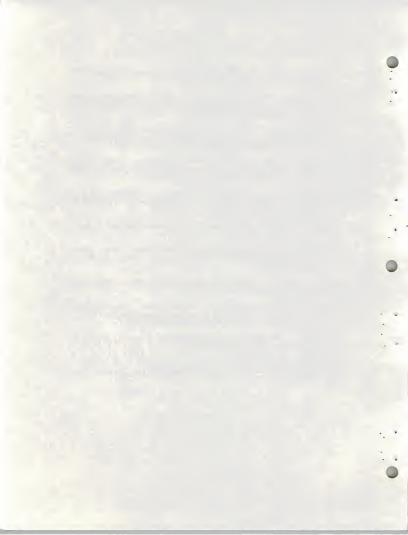
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APPENDIX A THE BIOPSIC PROCEDURE



THE BIOPSIC PROCEDURE

The Biopsy

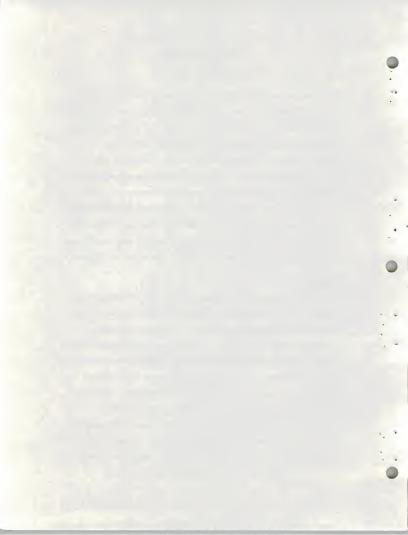
Biopsies have been utilized in collecting tissue for the study of insecticide kenetics in monkeys (Durham, <u>et al.</u>, 1963) and cattle (Radeleff, 1950). Enderson (1968) used biopsies to collect adipose tissue from adult peregrine falcons captured in the Arctic. A biopsic technique for collecting tissue from raptors was used in this study for two reasons: first, it enabled the investigator to sample the persistant pesticide residue level present in various raptor species on a study area without sacrificing birds and, thus, altering the composition of the populations under observation; and second, the technique increased the amount of data that could be obtained from the limited number of raptors used in the DDT trials.

Procedure

After experimenting with magpies (<u>Pica pica</u>) and captive raptors, a method of taking small samples of muscle from a bird's pectoralis miscle was developed. During the initial experiments, I found it was possible to carry out the biopsy without first anesthetizing nestling hawks and eagles, and they seemingly experienced little pain. Once a bird was sufficiently developed to leave the nest, an anesthesia was necessary. The recommended anesthetic, dosage, and anesthetizing procedure for older birds is outlined below.

To collect a small sample of muscle the raptor is anesthetized or in the case of a nestling firmly restrained. A large cloth is placed over the nestling's head and the wings are folded aginst the

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body. After the wings are secure, the legs are held and the feet fitted into a small leather sack. A young raptor's talons can inflict serious wounds if the handler is unwary. Once the talons are rendered useless, the bird is placed on its back. The cloth should remain over the nestling's head as it helps to keep it calm.

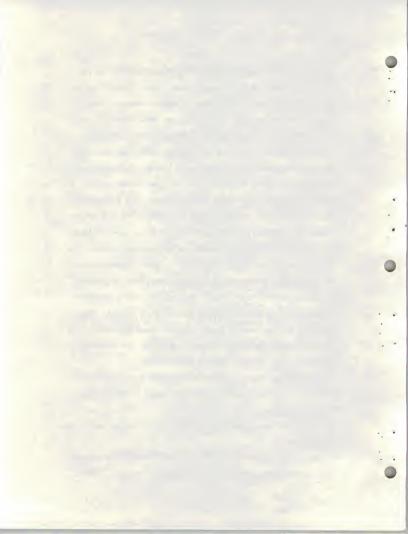
In preparing for surgery, the feathers of the keel region are wetted with alcohol from a small squeeze bottle. The feathers are then spread to expose the median apterium. The exposed skin is cleaned with a small disposable alcohol sponge. With a sharp scalpel a 25 mm anterion-posterior incision is made in the skin to one side of the keel ridge just posterior to the apex. A second incision 4 mm deep and 20 mm long is made in the pectoralis muscle. The muscle tissue at the anterior end of the incision is grasped with pointed forceps. A third incision of the same length is made in the muscle parallel to the first. This second incision is angled so that a small triangular strip of muscle can be removed. This procedure is illustrated in Figure 17. The length and depth of the incision must be adapted to the size of the bird. The measurements given here are for a juvenile golden eagle.

The average weight of tissue samples taken from red-tailed hawks was .05 g, and .3 g was the average weight of samples taken from golden eagles.

After the tissue sample is obtained, it is inserted into a clean vial and frozen.

The wound is not sutured, but left open to drain. It is, however, sprayed with an aerosol topical calloidin dressing. During

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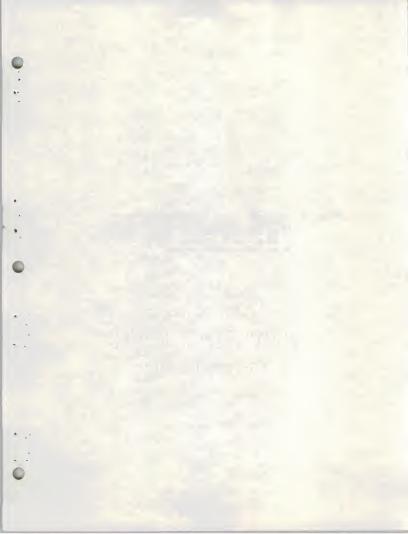
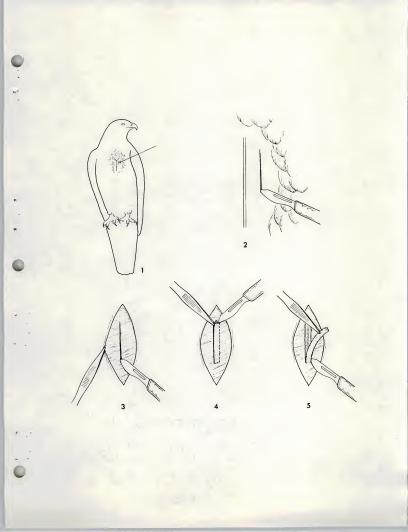


Figure 13. The biopsy: 1) the site where the blopsy is performed; 2) the initial incision in the skin; 3) the first incision in the pectoralis muscle; 4) and 5) a small triangular strip of muscle is removed for residue analysis





experimental tests unsutured wounds healed faster and with less infection than birds with sutured wounds. Complete healing requires about ten days. When called, manned experimental hawks would readily fly to the gloved fist the next day.

If bleeding occurs, it can be stopped by placing a small disposable alcohol sponge on the wound and applying fingertip pressure for a few minutes.

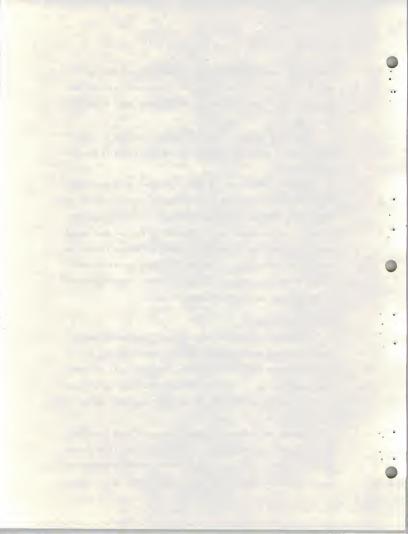
No adipose tissue that can readily be taken, using this technique, was found on nestling red-tailed hawks, Swainson's hawks, or marsh hawks. Adipose tissue can be obtained from subcutaneous deposits in nestling golden eagles; however, the incision must be made near the posterior end of the keel. Samples of adipose tissue from subcutaneous deposits can be obtained from older red-tailed hawks if they are in good condition. These fat samples can be taken without cutting into the pectoralis muscle.

Results and discussion

During the development of the technique, more than 50 small samples of muscle were collected from four raptor species: golden eagle, red-tailed hawk, Swainson's hawk, and marsh hawk. Biopsies were repeated at various intervals on some individuals and a series of tissue samples was obtained. In all cases the wounds healed without complication.

No sample can represent in every respect the whole from which it is drawn and it was necessary to determine discrepancies in water content, liquid content, and organochlorine insecticide content that exist between the entire breast muscle and the small sample of muscle

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taken. Chemical analysis data from breast muscle biopsies and entire breast muscles which were taken from fledgling red-tailed hawks fed DDT as nestlings at the rate of 20 mg DDT/kg body wt every 4 days are compared in Table 11.

Table 11. Water, lipid and organochlorine insecticide contents in whole pectoralis muscles and small muscle samples (biopsies) which were taken from pectoralis muscles of fledgling red-talled hawks

Tissue collections		Mean and range				
	No. of speci- mens			Wet weight (ppm)		
		% water	% lipid	DDE	DDD+ DDT	
Whole muscle	3	72.00 (70.30- 73.12)	1.09 (.65- 1.65)	10.17 (9.70- 10.60)	11.33 (9.14- 15.40)	
Biopsy	5	74.41 (71.42- 77.73)	3.47 (1.13- 8.16)	9.19 (1.04- 14.00)	8.55 (6.34- 11.45)	

Note: All birds were fed 20 mg DDT/kg body wt every 4 days for 40 days during the nestling period

The water content was higher in muscle samples than in whole muscles and on the average, samples contained more than twice the extractable lipids found in whole muscles. The mean total DDT content (wet weight basis) in biopsies is 31% below the mean amounts found in the whole muscle. However, the relative amount of DDT metabolites (DDT+DDD and DDE) are nearly the same in both groups.

In summary, the chlorinated hydrocarbon residues determined from muscle samples, which were taken via the biopsy, should be viewed as estimates of the contents of the entire tissue rather

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than as measures of the residues present in the whole muscle. Apparently, there is a variation in the storage of insecticide residues in different parts of the muscle. This warrants further investigation.

The Anesthetic

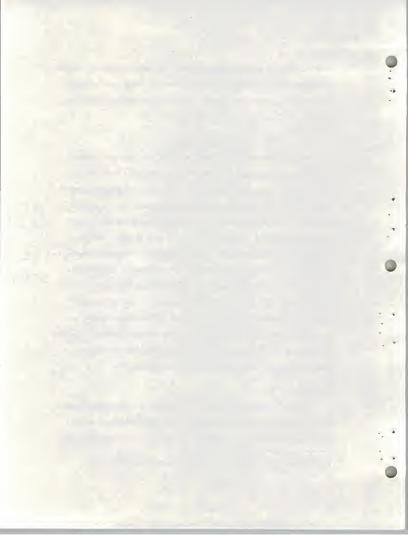
Many of the anesthetic agents commonly used for small mammals have been unsatisfactory for anesthetizing various avian species. Gandal (1956) found Equi-thesin 1/ to be a suitable general anesthetic for birds. Each 500 cc of Equi-thesin contains 21.1 g chloral hydrate, 4.8 g pentobarbital, and 10.6 g magnesium sulfate in an aqueous solution of propylene glycol with 9.5% alcohol. The manufacturer's literature states: "The combination of chloral hydrate, magnesium sulfate and pentobarbital provides some of the desirable depressant action of each compound without the pronounced toxicities of any drug." The literature further reports: "One of the most important advantages of this combination is the absence of the excitement stage. This is accomplished with the small amount of magnesium sulfate, measured so that toxic effects and the narrow margin of safety characterized by larger doses do not appear."

Procedure

Because of the difficulties in attaining vein puncture, Gandal (1956) recommended the intramuscular route (via the breast muscle) of drug administration. Sanger and Smith (1957) reported some local

1/ Equi-thesin is a product of Jensen-Salsbery Laboratories.

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inflammation in the muscle after Equi-thesin was injected in their experimental birds. Instead, I followed Lumb's (1963: 277) recommendation and injected the drug into the leg muscle to eliminate the effects of inflammation upon the bird's flight.

After preliminary experiments, the basic procedure in this study was to weigh each bird with a spring scale or beam balance, compute a dose, inject the prescribed amount into the leg muscle, release the bird on the floor in a quiet room. The bird was periodically checked to determine its reactions to the drug. One cc, disposable, plastic, tuberculin syringes with 25-gage needles were used for administering the drug to smaller raptors; correspondingly larger needles and syringes were used for larger raptors.

Results and discussion

Before anesthetizing raptors, I tested Equi-thesin at different dosages on a group of captive magpies. I successfully anesthetized 5 magpies in 5 trials using Gandal's (1956) recommended dosage. Four other attempts with higher doses than recommended resulted in three birds being successfully anesthetized. The fourth bird died when more than twice the recommended dose was given. These trials are outlined in Table 12.

Three raptors (two red-tailed hawks and one sharp-shinned hawk) died when Equi-thesin was administered at the rates recommended by Gandal and others for birds. Therefore, I found it necessary to establish a correct dose for falconiform birds. To establish the correct dose, I administered Equi-thesin to a series of raptors; I

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started with relatively low levels and continued until I found a satisfactory dosage. No multiple doses were given. A total of 46 experimental injections were administered to 21 individual raptors representing seven species: 1 prairie falcon, 1 sparrow hawk, 1 sharp-shinned hawk, 1 marsh hawk, 2 Swainson's hawks, 7 red-tailed hawks, and 8 golden eagles. Three other raptors (1 golden eagle and 2 red-tailed hawks) were sacrificed with overdoses of the drug. These data are presented in Table 12.

On the basis of these tests, minimum doses that would consistently produce surgical anesthesia (Lumb, 1963:10) were determined for four species: prairie falcon, red-tailed hawk, Swainson's hawk, and marsh hawk. An insufficient number of trials were run to establish the correct dose for sparrow hawks or sharp-shinned hawks. Successful anesthesia was obtained with golden eagles but results were so variable that no conclusions could be made (Table 13).

A wide variation in reaction to the drug has been observed in nestling golden eagles and golden eagles that have been retained in captivity for some time. Surgical anesthesia and satisfactory recovery were achieved in nestling golden eagles with doses ranging from 2.0 - 2.5 cc/kg body weight. A dose of 1.45 cc/kg body weight achieved surgical anesthesia in two captive golden eagles 1.5 years old or older. The duration of the anesthesia was more than 3 hours for the first and the second eagle was revived from the anesthesia (after 24 hours) when a 1.5 cc dose of Mikedimide <u>2</u>/ (a barbiturate antagonist) was administered. Older wild eagles have not been anesthetized

2/ Mikedimide is a product of the Paralem Corporation

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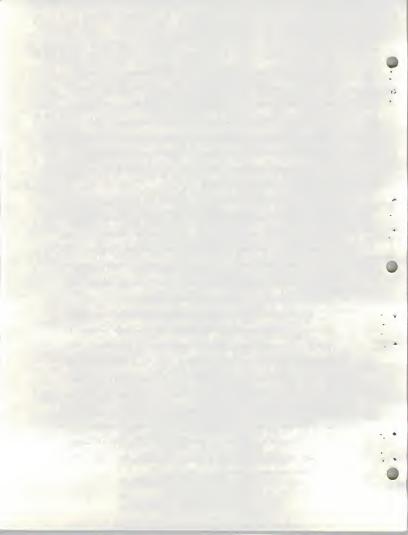
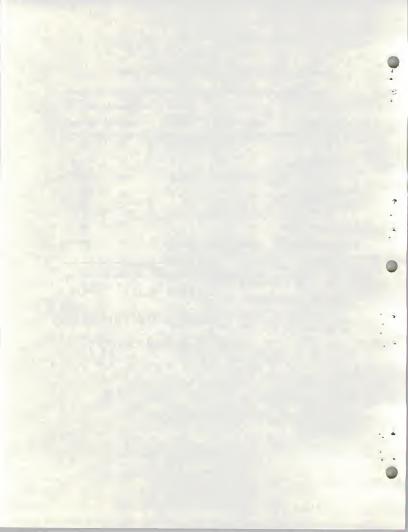


Table 13. Dosage of Equi-thesin for some avian species

Species	Age	No. of birds	Dosage (cc/kg body wt)	time	Duration* (minutes)
Magpie	J	5	2.5	11	91
Prairie falcon	J	l	1.5	17	67
Swainson's hawk	J	2	1.6	23	192
Red-tailed hawk	J	5	1.5	35	322
Marsh hawk	J	2	1.53	23	270
Golden eagle	N	l	1.9	10	90
Golden eagle**	1늘 yr.+	+ 2	1.45	52	360+

* Interval between light anesthesia and regaining the ability to lift the head

** Not recommended - see text

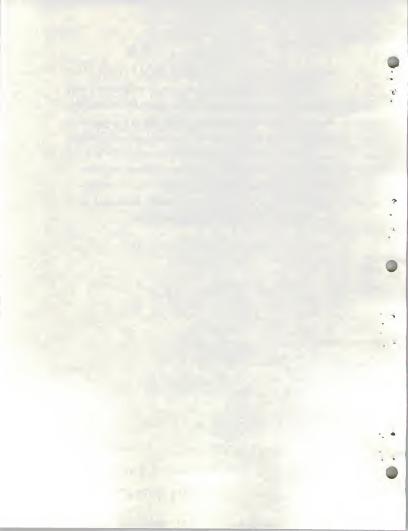


for comparison.

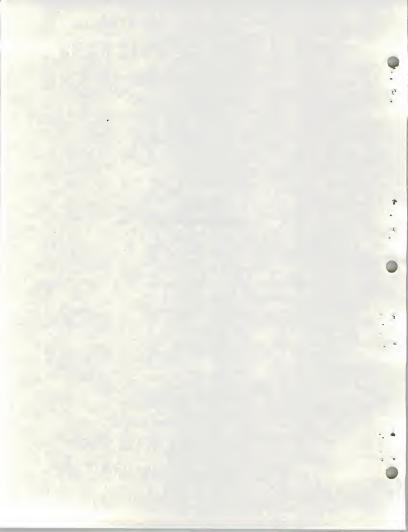
In addition to the three raptors which died during the initial experiments and the three which died from deliberate overdoses of drug, one juvenile red-tailed hawk, 82 days old, died unexpectedly when the drug was administered at a rate of 1.6 cc/kg body weight.

Although successful anesthesia was achieved 24 times in 5 raptor species, the unexpected death of the juvenile red-tailed hawk and the extended duration of anesthesia in one golden eagle renders Equi-thesin unsatisfactory for valuable experimental and trained birds of prey except in case of emergency.

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APPENDIX B ANALYTICAL METHODOLOGY



ANALYTICAL METHODOLOGY

All chemical determinations reported in this study were made by the Wisconsin Alumni Research Foundation. The following description of procedures was provided by the Chemical Department (in litt.).

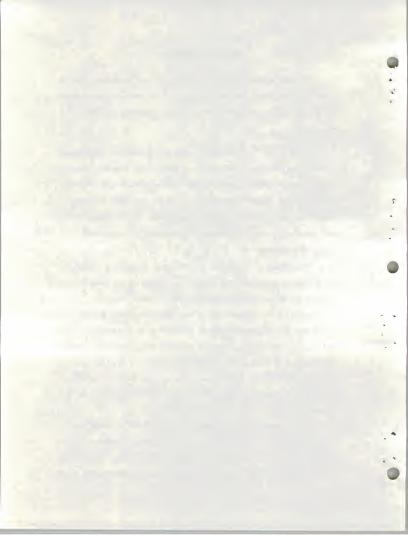
"Sample preparation:

"Total weights are originally taken on all samples. The samples are then weighed into tared beakers. If the total weight is less than 20 gm the entire sample is used. If the total weight is greater than 20 gm the sample is homogenized and a 20 gm portion taken for analysis. The beakers and samples are dried in an air oven at 40-45° C for 36-48 hours. The beakers are weighed and the percent moisture determined.

"The samples are then ground with sodium sulfate and transferred to extraction thimbles. The thimbles are placed in the Soxhlet apparatus and extracted with a mixture of 70 ml ethyl ether and 170 pet. ether for 8 hours. After extraction the ethers are removed from the erlenmyer flasks by evaporation on a steam bath. The fat is dissolved in pet. ether and transferred to a volumetric flask. After making to volume, one half of the solution is pipetted into a tared beaker. The solvent is removed on a steam bath and the beaker dried in an air oven at 4_{0} - 4_{5} °C for three hours. The beaker is reweighed and the percent of fat calculated.

"The remaining solvent in the volumetric flask is washed onto a florisil column. Pesticides are eluted from the column with 5%-95% (ethyl ether-pet. ether) and 15%-85% (ethyl ether - pet. ether) solutions. The respective solutions are taken to near dryness on a steam

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bath and then transferred to a volumetric flask. After making to volume, a known volume of each is injected into the gas chromatagraph.

"Instrumental Conditions

"Barber-Colman Pesticide Analyzer, Model 5360 equipped with a Sr-90 electron capture dector.

Column - 1/4" Pyrex. 5% DC200 on Chromport XXX

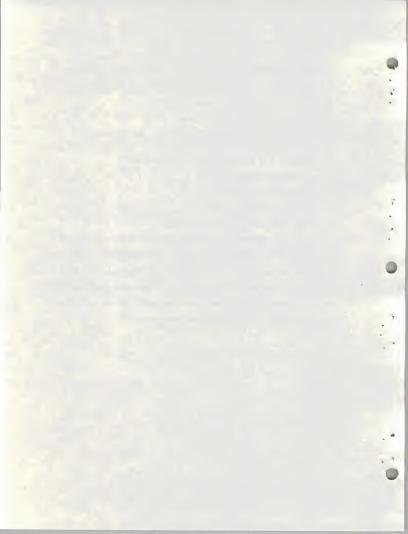
Column Temperature - 182°C

Injector temperature - 235°C

Detector temperature - 240°C

Gas - Nitrogen, flow rate 100cc/min."

A check on analytical results was conducted by determining residual levels in the same tissue samples by both gas chromatography and thin-layer chromatography. These data are shown in Table 14. The variations in residual levels in the same tissue sample as determined by two different analytical methods indicate that the analytical data must be viewed as approximate rather than absolute.

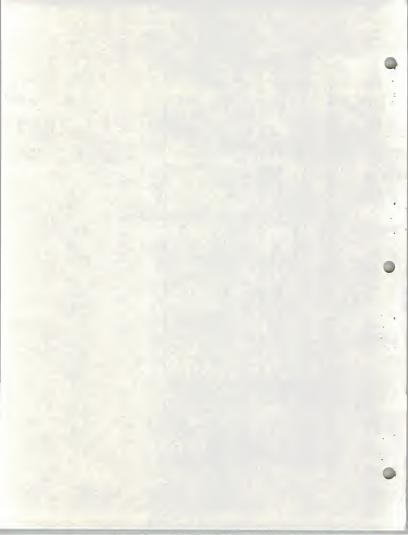


	Brain			Breast mus	scle		Liver		
Treatment	Total DDT residues (ppm)	<u>% of re</u> DDE	sidue as DDD+ DDT	Total DDT residues (ppm)	<u>% of re</u> DDE	sidue as DDD+ DDT	Total DDT residues (ppm)	<u>% of</u> DDE	residue as DDD+ DDT
Fed DDT as nestlings	11.03	53	47	21.50	47	53	16.30	70	30
Fed DDT as nestlings and captives	8.83	63	37	27.14	61	39	14.55	69	31
Fed DDT as nestlings but not as captives	2.76	86	14	5.17	88	12	2.55	78	22

Table 7. Total DDT residues (DDE, DDD, DDT) in tissue of experimental hawks with the proportion of DDE and DDD+DDT expressed as a percentage of the total.

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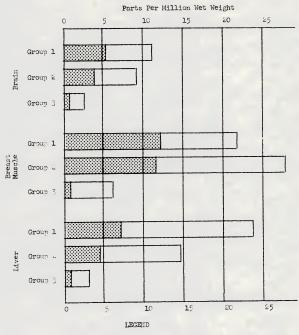
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Table 14. Analytical results when the same tissue was analyzed by two different methods

Tissue	Gas chromatograph					Thin layer chromatograph				
	DDE	DDD	DDT	Dieldrin	Heptachlor epoxide	DDE	DDD	DDT	Dieldrin	Heptachlor epoxide
Egg	10.30	. 58	.75	。63	- 80	10.0	.50	.50	.50	,50
Brain	40.40	3.00	6.23	1.53	. 05	35.00	4.30	8.60	1.80	0
Muscle	.20	.13	.062	.017	2.01	.25	.13	.25	0	0

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DDE

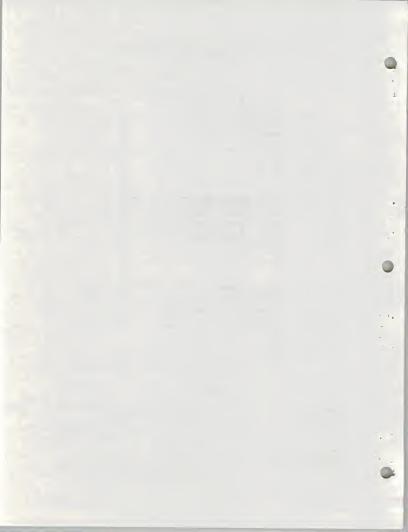
DDT + DDD

TOTAL DDT RESIDUES (DDT, DDD, DDE) IN EXPERIMENTAL RED-TAILED HAWKS

Group 1. Fed DDT during the nestling period and sacrificed as fledglings.

Group 2. Fed DDT during the nestling period and during a 40-day post-fledgling period in captivity.

Group 3. Fed DDT during the nestling period but were fed "clean" food during a 40-day post-fledgling period in captivity.



No. of birds	Treatment	Total DDT residues (ppm)	<u>% of resi</u> DDE	Ratio	
1	Control (sacrificed)	• 96	51	49	1:1
3	Control (died)	• 54*	83	17	4:1
3	Fed DDT** (sacrificed)	11.03*	53	47	1:1
1	Fed DDT** (died)	49.63	81	19	4:1

Table 8. Comparisons of DDT residues (DDE, DDD, DDT) in the brains of red-tailed hawk nestlings dying of pneumonia and those sacrificed at the end of the nestling period

* Means

** The dosage was 20 mg DDT/kg body wt every 4 days

