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Effects of Diethylstilbestrol on Reproduction in the Black-Tailed Prairie Dog

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EFFECTS OF DIETHYLSTILBESTROL ON REPRODUCTION IN THE BLACK-TAILED PRAIRIE DOG

by

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EFFECTS OF DIETHYLSTILBESTROL ON REPRODUCTION

IN THE BLACK-TAILED PRAIRIE DOG

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

EFFECTS OF DIETHYLSTILBESTROL ON REPRODUCTION IN THE BLACK-TAILED PRAIRIE DOG

Abstract

DONALD G. PFEIFFER

Research was conducted in southwestern South Dakota in 1970 and 1971 to obtain information on the reproductive cycle of the black-tailed prairie dog (*Cynomys ludovicianus*) and to test effectiveness of diethylstilbestrol as a reproductive inhibitor. Embryo body lengths from 10 pregnant females were compared to body lengths of 14 known day-old young to learn the peak breeding period when an estrogenic chemosterilant would be most effective. Conception dates were obtained for three females by backdating the gestation period. The breeding peak was found to occur in late February and early March in South Dakota. Nine of 11 prairie dogs aged by maturation in the humerus were pregnant and were 2 years old or younger indicating that yearling prairie dogs were an important segment of the breeding population. No successful breeding occurred among 13 pairs of captive prairie dogs, however, five females treated with 100 mg DES over a 6-week period showed anatomical and physiological differences from the eight control females. Weight losses among treated females were significantly different ($P < 0.01$) from untreated animals held in cages. No significant weight differences were detected between females collected from DES-treated and untreated towns. Sixty-three females were collected from experimental prairie dog towns. Nine of 31 (29 percent) females from DES-treated

towns were pregnant while 27 of 32 females (84 percent) from untreated towns were pregnant. This difference was significant ($P < 0.01$). Differences were significant between both live embryo ($P < 0.01$) and total embryo counts ($P < 0.05$) from treated and untreated females. Age ratio counts showed significantly fewer young per adult ($P < 0.05$) on treated portions of two divided towns, but counts were not significantly different ($P > 0.05$) when two treated towns were compared to a control town.

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INTRODUCTION

Many species of animals compete with man for their livelihood and existence. These animals are often termed pests and are therefore subjected to control. The black-tailed prairie dog (*Cynomys ludovicianus*)[1] is such a species.

Nelson (1919) estimated over 100 million acres of land in the United States were occupied by prairie dogs in 1919. A 1961 inventory indicated 1.5 million acres remained occupied by four species of prairie dogs (U. S. Dept. Interior 1963). Effective poisoning campaigns were widespread. However, widespread poisoning programs, particularly on public lands, are now frowned upon by environmentalists. The objective of such control should be the alleviation of damage and not the destruction of the species (Howard 1967).

The black-footed ferret (*Mustela nigripes*) is endangered by prairie dog control since ferrets live with prairie dogs and prey readily upon them (Sheets 1970). The use of poisons also presents the possibility of secondary poisoning of the ferret (Hillman 1968).

One method of vertebrate control is the use of chemosterilants. Kilgore (1967) defined these as chemicals which have the unique property of sexually sterilizing but not killing the pest species. Howard (1967) stated that chemosterilants could equalize some of man's conflicting interactions with the ecological biota and that antifertility agents will probably prove to be helpful in maintaining populations of vertebrates at

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1 Scientific names for mammals are after Hall and Kelson (1959).

a reduced level.

In order to evaluate the effectiveness of an antifertility agent on prairie dogs, this study was initiated to (1) gain more information on reproduction in the prairie dog, and (2) evaluate the effects of a chemosterilant on the reproduction of the prairie dog.

DESCRIPTION OF STUDY AREAS

Field studies were conducted on prairie dog towns located on the Buffalo Gap National Grasslands, the Rapid City Aerial Gunnery Range, and at Wind Cave National Park in southwestern South Dakota (Fig. 1).

Buffalo Gap National Grasslands are administered by the Forest Service, U. S. Dept. of Agriculture. Soils are of the Badlands association developing from the White River Beds (Westin 1971). They are thin and derived from unconsolidated silts and clays on rolling topography (Westin 1951).

The area is primarily used for grazing, but some small grains and hay are produced. The potential natural vegetation of the area is a wheatgrass-grama-buffalo grass association. Cultivation during the homestead period of 1910-30 was later abandoned and Japanese brome (*Bromus tectorum*)[2] invaded quickly, remaining a cool season dominant. Western wheatgrass (*Agropyron smithii*), blue grama (*Bouteloua gracilis*), and buffalo grass (*Buchloe dactyloides*) are the climax dominant species (Küchler 1964).

The Rapid City Aerial Gunnery Range is a 42,000-acre area used by the United States Air Force for summer military training. It is located in northeastern Shannon County, with 3,500 acres of the area lying

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2 Scientific names for grasses are according to Fernald (1950).

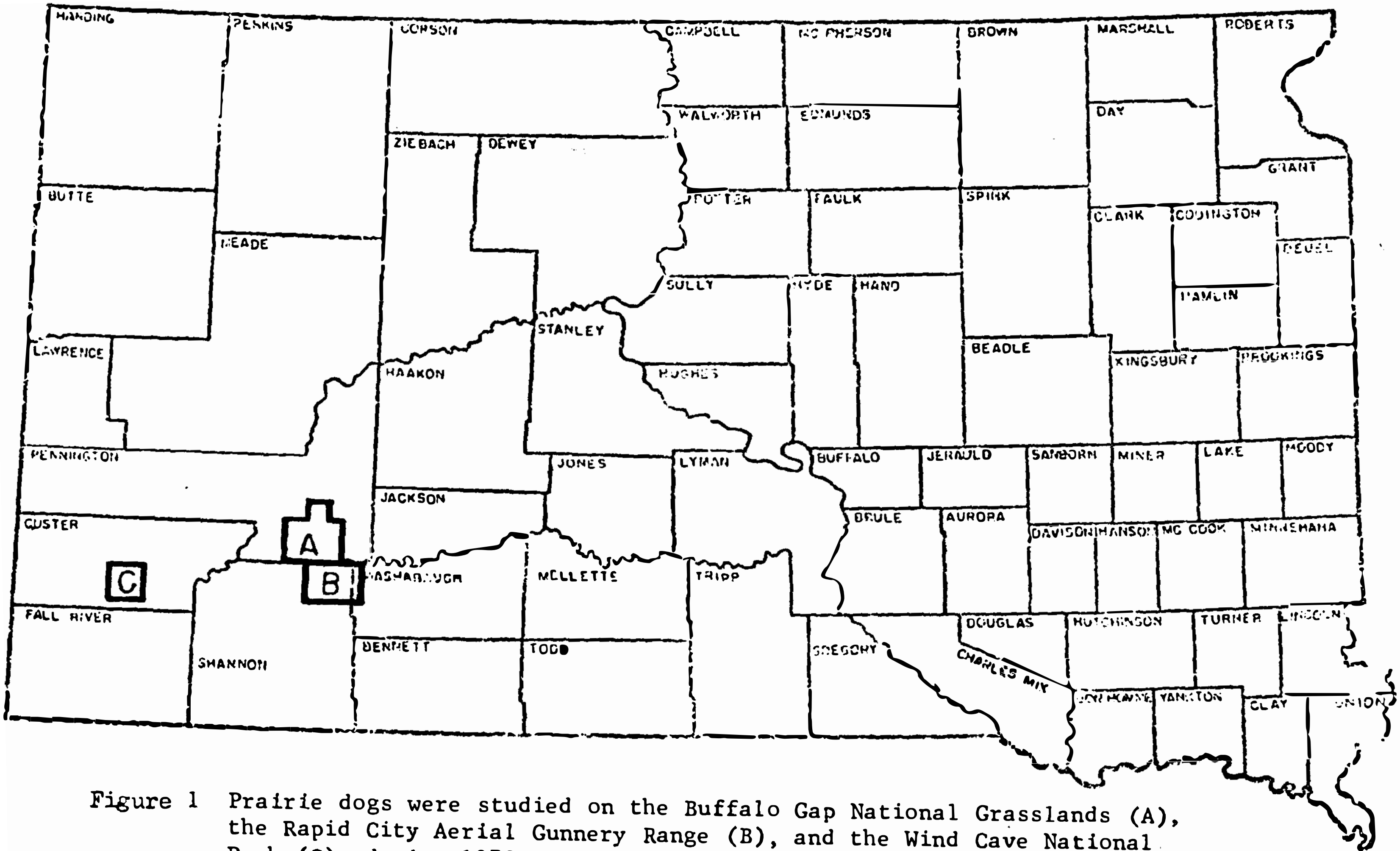

Figure 1 Prairie dogs were studied on the Buffalo Gap National Grasslands (A), the Rapid City Aerial Gunnery Range (B), and the Wind Cave National Park (C), during 1970 and 1971.

immediately east in northwestern Washabaugh County.

Soils are mainly a Badland association and Tuthill-Richfield association in the eastern half and a Valentine-Anselmo association in the western one-half. The flood plain of the White River and intermittent streams which dissect the gunnery range consists of an Alluvial-Haverson association (U. S. Dept. Agr. and U. S. Dept. Interior 1971).

The vegetation is typical of mixed prairie, although short grasses dominate the overgrazed areas. Much of the bottomland and silty upland was farmed until the U. S. Air Force acquired the land in 1942, and vegetation remains in successional stages of subclimax. The principal invader, Japanese brome, dominates the areas once farmed. Küchler (1964) lists this area as climaxed by a wheatgrass-needlegrass community. Mid- to tall-prairie grass species dominate the Valentine-Anselmo, Richfield-Tuthill and Alluvial-Haverson soil associations, while shortgrass species dominate the Badlands association.

The climate of southwestern South Dakota, in which the above national grasslands and gunnery range are located, is semiarid and continental. It is characterized by cold winters and hot summers. Precipitation is light in winter and marginal during the growing season. Average precipitation is 15.1 inches, of which 11.9 inches (79 percent) fall during the growing season. The main source of rainfall is thundershowers, which produce a wide range of amounts and intensities of rain. Seasonal snowfall averages 24 inches. Snow cover of 1 inch or more is found, on the average, 36 days per year. The average annual wind speed is 11 mph with prevailing direction from the northwest during winter and from the south during the summer.

Annual temperature averages 47 F, but usually temperatures rise to 100 F in summer and drop to -20 F or lower in winter. A temperature of 100 F or higher may occur, on the average, 11 days per year. A reading of -20 F or lower has been recorded an average of 4 days annually. The average date for the last frost is May 19 and the first frost is September 22, with an average growing season of 126 days. These weather data were compiled by National Weather Service records at Cottonwood, South Dakota, 30 miles northeast of the study areas.

Two prairie dog towns (Rankin and Sanctuary) were studied in Wind Cave National Park, which is located in Custer County in the southern Black Hills. Topography is a gently rolling limestone plateau. Dominant grasses include western wheatgrass, green needlegrass (_Stipa viridula_), needle and thread (_Stipa comata_), blue grama, buffalo grass, Kentucky bluegrass (_Poa pratensis_), and Canada bluegrass (_Poa compressa_).

Climatological data were taken from National Weather Service records compiled at Hot Springs, South Dakota, 9 miles south of the prairie dog towns studied. Average annual temperature is 47 F, with large variations from winter to summer and from day to day. The continental climate is shielded from cold outbreaks during the winter by the Black Hills. Temperature extremes include an average of 2 days above 100 F, and 19 days below zero annually. The average date for the last frost is May 16, and the first fall frost occurs on September 25, giving an effective growing season of 132 days.

The average annual precipitation is 17.5 inches, of which 13.6 inches (77 percent) fall during the growing season. Thundershowers with a wide range of intensities and amounts are the main source of precipitation during the growing season. Seasonal snowfall averages 36.7 inches, with 1 inch or more on the ground an average of 23 days per year. Average annual wind speed is about 11 mph, with the prevailing direction from the northwest during the winter and from the southeast during the summer.

REVIEW OF LITERATURE

Reproduction

The breeding period of the black-tailed prairie dog ranges over 2 to 3 weeks (Anthony and Foreman 1951; Tileston and Lechleitner 1966; Smith 1958). Smith (1958) found this period to begin the last week in January in Kansas prairie dogs and Tileston and Lechleitner (1966) after measuring embryos believed that conceptions occurred in late February in Colorado. King (1955) surmised that mating occurs shortly after the vagina opens in late February and early March in South Dakota. In Oklahoma the breeding season occurred the last week of January to early February (Anthony and Foreman 1951). Wade (1928) observed breeding activity to begin 2 weeks later in prairie dogs taken from north central Kansas than in collections from the Kansas-Oklahoma border 100 miles south. Latitude and altitude differences were predicted by Merriam (1901) to alter the onset of the breeding season in prairie dogs.

The peak of male sexual activity occurs during the first week in February in Oklahoma (Anthony 1953). Ninety percent of the males had scrotal testes at that time. Anthony and Foreman (1951) reported that motile sperm in the epididymis during the last week in January in Oklahoma prairie dogs persisted throughout the month of February in both captive and wild forms. Smith (1958) found testes of Kansas prairie dogs to be largest during the first 10 days of February.

The breeding season apparently ends abruptly as Anthony (1953) found that testes regressed very rapidly, and that by late March, the testes were abdominal and their weight was reduced 90 percent from the peak breeding weight.

Foreman (1962) noted that during estrus in the female black-tailed prairie dog, the vulva swells and becomes pigmented, and the ventral opening gradually enlarges to a wide slit along the entire surface. She observed these conditions in her laboratory colony as early as January 20. Involution of the vulva signaled the end of the breeding period and began by March 1.

King (1955) observed that the female black-tailed prairie dog did not breed until the second spring after birth. Smith (1958) also noted that few juveniles bred. Foreman (1962) found coming year-old females exhibiting estrus, but their estrus was usually later than in older females. Bakko and Brown (1967) found that pregnant females collected later than usual represented juveniles of the previous year in their studies on the white-tailed prairie dog (Cynomys leucurus).

Further evidence that many of the juvenile females present in the colony will breed is seen in observations by Tileston and Lechleitner (1966) and Wade (1928). Wade observed that over 78 percent of 64 females collected during the breeding season in Kansas were pregnant. Tileston and Lechleitner collected 32 females in Colorado and all were pregnant except for 3. One of these was a yearling, and the other two were adults. It seems reasonable to assume that juveniles (almost 1 year old) make up more of the population than the portion of non-pregnant females collected in these two studies.

Anthony and Foreman (1951) believed the gestation period to be 32 days with a range of 30-35 days. Smith (1958) observed the length of pregnancy to be 28-32 days.

Resorption and abortion of young were common in the laboratory colony of prairie dogs maintained by Foreman (1962). Bakko and Brown (1967) found resorption taking place in 4 percent of wild white-tailed prairie dog populations.

Litter size is also important in the growth of a prairie dog population. Table 1 lists the number of embryos found by different investigators. The fewest embryos reported in the wild were 2 while the most embryos present were 10, with the common litter size being 4 or 5 young.

Chemosterilants

If reproduction processes of a specific pest species are known, a chemosterilant might be used as a realistic method of control. For example, Balser (1964) pointed out that applying antifertility agents in a bait to species having one litter per year is much simpler than application to those which produce several litters a year. Prairie dogs, being monestrous, is a species to which chemosterilant can be easily administered as they are gregarious and the colonies are sedentary.

Although many chemical compounds have been tested for suppressing mammalian reproduction, diethylstilbestrol (DES) was found to have desirable estrogenic properties. Estrogens have been used on rats (*Rattus norvegicus*)(Marsh and Howard 1969; Dreisbach 1959; and Brooks and Bowerman 1971), rabbits (*Sylvilagus floridanus*)(Greenwald 1957), and voles (*Microtus pennsylvanicus*)(Storm and Sanderson 1970) to interrupt normal reproduction and terminate pregnancy (Saunders 1968).

Table 1. Prairie dog reproductive data found by other investigators which include average embryos found per female and the range observed.

Investigator	State	Average Embryos Per Female	Range
Smith (1958)	Kansas	4.00	? - 5.0
Tileston and Lechleitner (1966)	Colorado	4.75	2.0 - 7.0
King (1955)	South Dakota	4.90	? - 6.0
Wade (1928)	Oklahoma	5.02	2.0 - 10.0
Pfeiffer (This Study)	South Dakota	5.20	3.0 - 9.0
Tyler (1968)	Oklahoma	5.20	3.0 - 7.0

A chemosterilant which operates during the female's estrous cycle must be available to the target species during the complete range of the breeding period. A steroid sex hormone such as DES interferes with implantation, delays parturition, and causes fetal death and resorption of fetuses through day 10 following mating in rats (Dreisbach 1959). Linhart and Enders (1964) prevented production of offspring in foxes (*Vulpes fulva*), using DES from 9 days prior to and until 10 days after mating. Resorption of embryos has been reported by a number of authors after administering a chemosterilant (Storm and Sanderson 1969; Storm and Sanderson 1970; Linhart and Enders 1964). Travis and Schaible (1962) found lower embryo counts, but complete termination of pregnancy did not necessarily occur in their DES-treated mink (*Mustela vison*).

Treatment with steroid hormones can cause physiological differences in treated animals compared to control animals. Internal differences in the reproductive organs of DES-treated voles included heavier uteri and lighter ovaries (Storm and Sanderson 1970). Brooks and Bowerman (1971) found that distended uteri and inactive ovaries were indicative of high levels of estrogen intake.

METHODS

Studies of Reproduction

Prairie dogs were collected from the field for reproductive information by shooting and live trapping, and reproductive organs were removed and preserved in formaldehyde. Front legs were also preserved to determine if maturation of the long bones could be used as an aging technique, which would provide the age structure of the breeding females. Muscle was removed from the humerus, and each bone was X-rayed. X-rays were examined to determine if the epiphyseal cartilage was present. This technique has been used to age cottontail rabbits (Hale 1949).

Studies of Populations

To obtain estimates of current densities of prairie dog populations in South Dakota, the Lincoln Index technique (ratio of marked to unmarked animals) of population estimation was employed. Counting of the number of marked and unmarked animals seen above ground at any one time gave an estimation of the population. Prairie dogs were live-trapped and the pelage dyed yellow with picric acid in an alcohol solution. Age classes (young and adult) were distinguished by applying the dye to either side of the body. Dye was applied to the head and chin of all prairie dogs to enable observation of a marked animal if its marked side was turned away from the observer. These animals were easily observed from a point outside the town.

Counts of those prairie dogs above ground at any one time were made at 15-minute intervals to obtain estimates of age ratios on treated and control towns. A spotting scope was used in counting from a point near the town.

Studies of Bait Preferences

Food preferences of caged prairie dogs were tested, offering whole oats, hulled oats, barley and rolled oats. A field experiment was also conducted to evaluate bait preference on Wind Cave National Park on August 18, 1971. One cup each of seven different baits was placed on paper plates in random order on a prairie dog town. Bait was placed before sunrise, and the unconsumed bait was collected at sunset, approximately 12.5 hours later. Two bait stations were placed on activity areas newly pioneered; these were expansion areas of the town that had been occupied for no more than 2 years. Vegetation on these two areas was comparable to that immediately adjacent to the town, although it was somewhat shorter. Vegetation at these two stations had not undergone successional regression, as was seen on the baiting areas in the main town.

Studies of Caged Prairie Dogs

Thirteen pairs of prairie dogs were trapped on the study areas in August, 1970, and placed in individual cages at the Wildlife Research Farm in Brookings, South Dakota to observe effects of chemical reproductive inhibitors. These animals were housed in a building with controlled heat and light. Each prairie dog was individually marked with a numbered metal ear-tag.

The cages (18 x 24 x 48 inches) consisted of two levels (Fig. 2). The lower compartment was filled with hay for the nesting area, and the upper level was used for feeding. A sheet metal floor in which an

Figure 2 Thirteen cages held pairs of prairie dogs for reproductive experiments. The prairie dogs are in the lower nesting compartment with the floor to the upper feeding level removed.

Figure 3 A prairie dog town on the Buffalo Gap National Grassland spring, 1971.

opening was cut enabled the prairie dogs to move from the nesting area to the feeding area. The floor separating the two levels was easily removed for cleaning. Feeding and cage cleaning occurred at intervals of 5 days to minimize disturbance.

The prairie dogs were paired at random in October, 1970, and assigned for control or treatment in January, 1971. Five of the pairs were selected to receive the chemosterilant. Eight pairs served as controls, as information on prairie dog reproduction in captivity is scarce.

Each animal was weighed to the nearest gram in October, February, and April. They were quieted by placing them in a burlap bag while on the balance scale.

Tablets containing 25 mg DES were administered singly to each treatment female on February 8 and 24 and March 4 and 11. A prairie dog will average about 1 kg during the breeding season and 25 mg/kg is a commonly-used dosage in chemosterilant studies.

Field Studies of Chemosterilant

DES was mixed with hulled oats at the Bait Mixing Station, Division of Wildlife Services, Bureau of Sport Fisheries and Wildlife, Mitchell, South Dakota by Steve V. Moro, District Supervisor. The grain mix was similar to that for preparation of poisoned oats. Six ounces of lipoidal were used per 50 pounds of grain as a carrier for the DES. A yellow dye to serve as a bird deterrent and also to show the thoroughness of mixing was included in the mixture. Three hundred pounds of grain were mixed with 150 grams of DES, while another 300 pounds were mixed in the same manner except no DES was added.

Prairie dog towns on national grasslands and on the gunnery range were selected for field experiments (Fig. 3). Two of the towns had a natural division (intermittent stream and rocky soil out-cropping), and a buffer zone was established on either side of this dividing line. These towns were named Homestead and Bomb Range. Two other towns on national grassland (National Grassland Nos. 41 and 41A) served as treatment towns and were paired with a town less than a mile away for control (National Grassland No. 40). DES-treated bait was also placed on parts of two towns (Conata and Crew) other than treated or control towns for the purpose of collecting animals to study effects of DES on embryos.

Baits were distributed on the prairie dog towns in three applications; one each February 27, March 6 and 20, 1971. A cupful of the oats was scattered at each active burrow. A burrow was judged active if it was not filled with snow and numerous tracks indicated recent prairie dog use. Approximately 30 doses of 25 mg DES per dose were applied per acre on the treated town and the same number of doses without DES on untreated towns. Snow was present on the towns during each application, but the depth of 1-2 inches was packed around the active burrows. Although the overnight temperatures dropped below 0 F, the prairie dogs were active during the day when the temperature reached 15-28 F.

Prairie dogs can still be separated into two age classes by size in June. Age ratio counts were made at that time to evaluate relative success of prairie dog reproduction on untreated towns compared to DES-treated towns. Counts of animals were made from the same vantage point for each census. The prairie dog towns used in the field experiments were censused in 1970 to serve as pretreatment indices, and posttreatment counts were obtained in 1971. Female prairie dogs were also collected from treated and untreated towns during late June to estimate reproductive success.

RESULTS AND DISCUSSION

Breeding Seasons

Measurements were made on testes from four prairie dogs collected February 27, 1971, and from six prairie dogs collected April 2, 1971, to obtain data on gonadal regression (Table 2). On February 27, average length of the testes was 26 mm (24 - 28 mm) and average width was 15 mm (15 - 16 mm). Eleven of 13 captive prairie dogs had testes which were palpable from February 10 through March 8. Anthony (1953) observed palpable testes during the peak breeding period for male prairie dogs. Smith (1958) found a range of 28 - 40 mm for length and 13 - 16 mm for width of testes in the peak of the breeding season in Kansas.

Following the breeding season the testes regress and become abdominal (Fig. 4). Lengths and widths of abdominal testes were 11 - 16 mm and 5 - 10 mm respectively on April 2. These measurements agree with Smith's (1958) findings of 10 - 16 mm length and 5 - 8 mm width for abdominal testes. Anthony (1953) found testes were abdominal within 6 weeks following the peak of breeding.

Testes data indicated the peak breeding season occurred the last 2 weeks of February through early March for male prairie dogs in South Dakota. However, in the prairie dog as in other mammals, the male breeding season is less precise than the female breeding season (Foreman 1962).

Prairie dogs were collected during the spring of 1971 to determine time of breeding season. Three female prairie dogs were collected on February 27; two were not pregnant. Five more females were collected

Table 2. Measurements of testes from prairie dogs collected in the field, South Dakota, 1971.

Date Collected		Length (mm)	Width (mm)
February 27			
1		24	15
		25	15
2		27	16
		28	16
3		24	15
		26	16
4		26	15
		26	15
	Average	25.8	15.4
April 2			
1		13	6
		11	7
2		11	5
		11	6
3		16	10
		16	9
4		14	7
		14	7
5		12	6
		13	7
6		13	7
		15	9
	Average	13.3	7.2

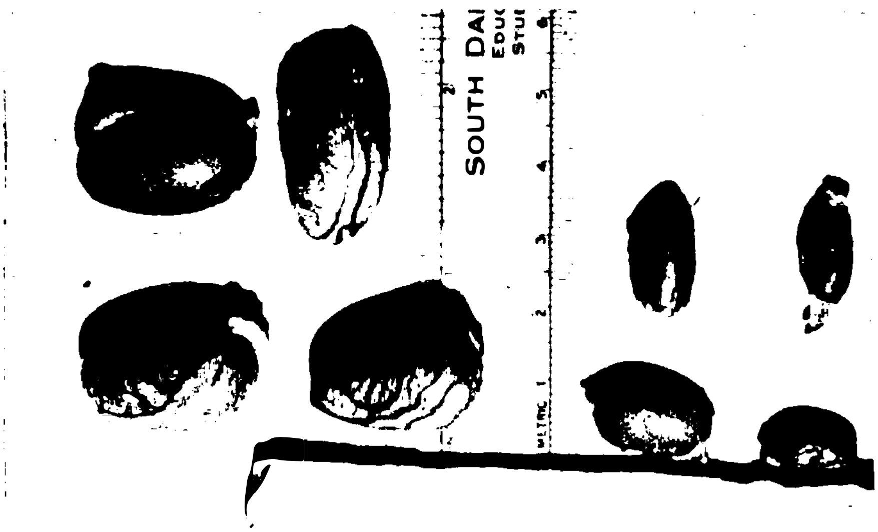

Figure 4 Gonadal regression occurs rapidly following the peak of breeding as seen in size differences of testes collected in February (Left) and in April (Right).

Figure 5 Resorption of dead embryos is in progress in this prairie dog uterus. Five of the embryos are dead and are being resorbed. The single live embryo is half-dollar size.

Figure 4 Gonadal regression occurs rapidly following the peak of breeding as seen in size differences of testes collected in February (Left) and in April (Right).

Figure 5 Resorption of dead embryos is in progress in this prairie dog uterus. Five of the embryos are dead and are being resorbed. The single live embryo is half-dollar size.

March 6; four contained embryos. King (1955) found open vaginas in two of six female prairie dogs examined on February 25 in South Dakota and in all females examined the first week in March.

Fourteen of 18 female prairie dogs collected from untreated towns on April 2 were pregnant. Two were not pregnant, and two had previously given birth and were suckling young. Body lengths of embryos from 10 of the 14 pregnant females were measured (Table 3); one reproductive tract was lost and three females were held in captivity until they gave birth. Body lengths of embryos from each female collected on April 2 were averaged and compared to the average body lengths of 14 one-day-old young born to three females in captivity (Table 4). Five females collected April 2 (Nos. 6, 7, 8, 9, 10) contained embryos with body lengths ranging from 62 to 80 mm. Birth would have occurred shortly as these embryo lengths are close to or overlap the range (65 - 83 mm) of body lengths of the known day-old young. These measurements are similar to those made by Johnson (1927) and Smith (1958) who found average body lengths at birth to be 69 mm (average for four young) and 65 - 70 mm respectively in their studies.

Conception dates for the three females giving birth in captivity were obtained by backdating the gestation period of 32 days. One female gave birth to eight young on April 6 and the other two had three young each on April 9. The conception dates were March 5 and 8. Because of the embryo sizes of females Numbers 6, 7, 8, 9, and 10 relative to the sizes of known day-old body lengths, conception in these females is believed to have occurred after February 28 and before March 10. The

Table 3. Average body lengths of embryos from 15 pregnant female prairie dogs collected in spring, 1971. Presence or absence of the epiphyseal cartilage is listed for the April 2 collections.

Number of Female	Date Collected	Epiphyseal Cartilage	Number of Embryos	Average Body Length (mm)
1	February 27	-------	9	11
2	March 6	-------	8	8
3		-------	6	8
4		-------	6	7
5		-------	6	5
6	April 2	Absent	4	80
7		Absent	4	74
8		Absent	4	65
9		Absent	7	64
10		Present	6	62
11		Absent	4	41
12		Present	3	29
13		Present	8	24
14		Present	4	15
15		Present	5	11

Table 4. Body lengths and weights of 14 one-day-old prairie dogs born in captivity to wild-captured pregnant females, 1971.

Date of Birth		Body Length (mm)	Weight (grams)
April 6		70	17.8
		70	15.0
		69	14.7
		69	14.2
		65	14.0
		70	15.0
		68	14.2
		66	13.5
	Average	68	14.9
April 9		73	17.1
		83	20.4
		83	25.7
	Average	80	21.1
April 9		81	21.9
		76	21.7
		73	17.9
	Average	77	20.6

other five females (Nos. 11,12,13,14,15) bred later in March as seen by shorter body lengths of embryos.

Two females of the 18 which had young when collected April 2 had bred before February 28, and parturition had occurred less than a week previous judging from uterine tissue and vascularization at placental scars.

An estrogenic chemosterilant need only be available during the peak breeding period; if this period is prolonged for any reason more bait must be available. Foreman (1962) found that young prairie dogs entering their first breeding period exhibited estrus later than mature animals. Humeri from 26 prairie dogs were X-rayed to evaluate use of the epiphyseal cartilage as an aging technique to determine if young prairie dogs breed later than adults. A cartilage line was present in three yearlings of known age (Fig. 6,A) and absent in one prairie dog known to be over 2 years old (Fig. 6,C). No animals known to be 2 years old were examined; however, the line was faintly evident in one female that was known to be 18 months old (Fig. 6,B).

Twenty-one females of unknown age were X-rayed to examine evidence of the epiphyseal line with regard to the age of breeding animals (Fig. 6,D,E). The line could be seen in 11 of these animals (Fig. 6,D), nine of which were pregnant. These animals were probably no older than 2 years old. Evidently some of the pregnant females were yearlings because it seems unlikely that only 2 of the 11 collected were yearlings.

Other evidence also indicates younger females may exhibit estrus later in the breeding season than older animals. Of the ten females

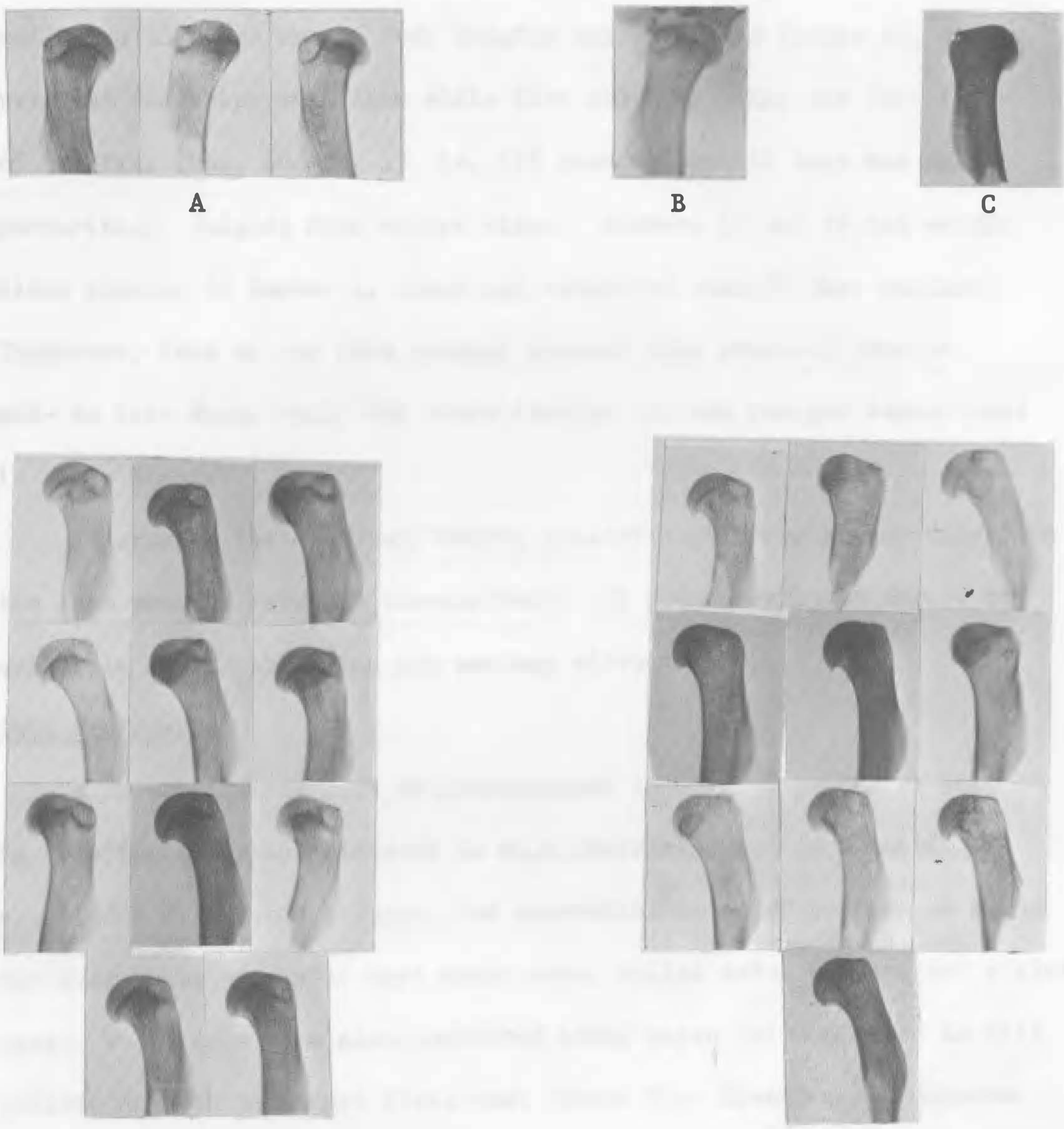

Figure 6 X-ray photos of prairie dog humeri showing presence or absence of epiphyseal cartilage in known and unknown age animals. Epiphyseal cartilage can be seen in A, B, and D.

A 12 months old
B 18 months old
C More than 24 months old
D Unknown age
E Unknown age

collected in which embryo body lengths were averaged (Table 3), five retained the epiphyseal line while five did not. Only one (No. 10) of the five (Nos. 10, 12, 13, 14, 15) younger prairie dogs was near parturition, judging from embryo sizes. Numbers 14 and 15 had embryo sizes similar to Number 1, which had conceived some 30 days earlier. Therefore, four of the five younger prairie dogs probably bred in mid- to late March while the older females and one younger female bred in early March.

It appears that in South Dakota prairie dogs breed at any time from the last week in February through March and chemosterilants should be available during that time for maximum effectiveness.

Bait Preference

A chemosterilant must be incorporated in or on a preferred bait to be effectively administered to wild prairie dogs. In a baiting experiment with caged animals, the descending order of preference among the four baits available was: whole oats, hulled oats, barley, and rolled oats. Whole oats were also preferred among seven baits offered to wild prairie dogs in an August field test (Table 5). However, DES adheres to the outside of the grain and prairie dogs do not eat the hull of whole oats. For this reason hulled oats were used as the DES carrier in the field experiments since the kernel, coated with DES, is entirely consumed. Observations on treated towns showed that the baits were consumed following distribution.

Table 5. The percentage of bait consumed after being available to prairie dogs for 12.5 hours.

Bait	Station					Average Consumed
	1	2	3	4	5	
Whole Oats	100.0	100.0	100.0	5.1	75.9	78.0
Hulled Oats	100.0	100.0	80.0	1.2	14.4	59.1
Barley	69.3	100.0	33.2	3.2	41.8	49.1
Poultry Pellets	23.7	27.0	12.3	2.0	52.9	23.6
Corn	16.2	66.0	8.0	1.4	11.8	20.7
Wheat	17.1	21.8	7.2	2.9	11.7	12.1
Sorghum	12.0	11.4	13.2	0.9	9.5	9.4

Bait Application Rates and Costs

An estimate of the number of animals on a prairie dog town that may consume the bait must be known to determine the amount of bait to apply. Maximum densities of animals on prairie dog towns in the central United States occurred following pup emergence and ranged from 3.6 to 35.0 dogs per acre. Minimum densities occurred during the breeding season and ranged from 2.6 to 20.0 per acre (Table 6). Smith (1958) found 20 prairie dogs per acre present during the breeding season in Kansas, higher than any density observed by the other investigators. This density was used as a minimum figure in treating the experimental towns with chemosterilant.

Based on the population density of 20 dogs per acre during the breeding season, 30 doses per acre were considered sufficient to reach all the animals present. This would be sufficient to reach the majority of the population, even if males consumed some of the bait before the females had eaten a full dose.

The cost per acre of the treated bait would vary with the number of applications administered during the breeding season. Using three applications at 1.5 pounds of bait per acre per application (30 doses per acre), the cost was $1.03 per acre for the breeding season. The cost breakdown for 100 pounds of treated grain is shown in Table 7; mixing costs are not included.

The Lincoln Index technique was used on the Wind Cave study area in July and August, 1971 (Table 8). Although these results were too late to use in my study, they will be available for further field tests.

Table 6. Various prairie dog density levels as found by other investigators.

Investigator	State	Prairie Dogs Per Acre	
		Maximum	Minimum
King (1955)	South Dakota	15.0	4.0
Smith (1958)	Kansas	33.0	20.0
Tyler (1968)	Oklahoma	3.6	?
Tileston and Lechleitner (1966)	Colorado	12.9	2.6

Table 7. Cost of products used in chemosterilant bait mixing; 100 pounds was used to treat 20 acres of active prairie dog town during the breeding season.

Amount used per 100 pounds	Item	Cost per 100 pounds
12 ounces	Lipoidal	$00.26
1 ounce	Yellow Dye	$00.21
2 bags	Sacks	$00.22
50 grams	DES	$15.00
	Hulled Oats	$ 5.00
	Total	$20.69

Table 8. Estimated density of prairie dogs per acre using the Lincoln Index on three prairie dog towns on the Wind Cave study area, July and August, 1971.

	Rankin (6.5)[a]			Sanctuary I (0.9)			Sanctuary II (0.8)		
	Total	Adult	Young	Total	Adult	Young	Total	Adult	Young
Number of counts	235	235	230	151	150	150	151	151	146
Number of marked animals	42	22	20	24	15	9	12	7	5
Average actual count	8.1	3.4	4.7	26.9	11.2	15.9	19.8	14.1	5.7
Average estimate	36.0	13.6	28.5	50.6	22.8	28.6	30.2	18.9	11.9

[a]Total acres of town shown in parenthesis.

The 6.5-acre Rankin town had a total population estimate of 36 prairie dogs per acre with estimates of 14 adults and 29 young in July. On the 1-acre acreas of Sanctuary towns I and II, totals were estimated to be 51 prairie dogs per acre on Sanctuary I and 30 on Sanctuary II. Smith (1958) found that the summer population declined 40 percent to the breeding season minimum. Tileston and Lechleitner (1966) observed a 75 percent decline from the summer prairie dog density to the breeding season population.

Effects of DES on Caged Prairie Dogs

Even though there was no successful breeding among the caged females, anatomical and physiological differences between the treated and untreated females were seen. Anatomical differences in DES-treated females included an enlarged vulva which gave the appearance that the females were in estrus. The females had enlarged teats and appeared to be suckling young. However, lactation was not present. These external conditions persisted through June 1, 10 weeks after the fourth and final 25 mg dose of DES was administered. These differences are believed to be due to the high doage of chemosterilant administered.

Uteri measured on April 13 (33 days following the fourth 25 mg treatment of DES) had diameters of 2 mm and 9 mm in a control and a treated female respectively. Ovaries and uteri were larger in treated females than in controls. The uteri were shortened and thickened in the treated animals following the breeding season.

All females treated with DES showed weight loss (Table 9). The weight of one treated female decreased from 805 grams on February 8

Table 9. Average weights of DES-treated female prairie dogs compared to untreated animals in caged experiments.

Date Weighed	Average Weight in Grams	
	Treated	Untreated
February 24	718 (5)[a]	824 (8)
April 6	614 (4)**	815 (8)

[a] Sample size in parenthesis
** Significant weight loss ($P < 0.01$) using t-test.

to 312 grams at her death March 25. The weight loss ($P < 0.01$) of treated females and the death of one animal were attributed to the high dosage of DES administered. Weight loss was not different ($P > 0.05$) on treated towns compared to untreated towns on the study areas. The females collected from treated towns weighed 960 grams (average for 6) while those females from untreated towns weighed 867 grams (average for 10).

Effects of DES on Wild Prairie Dogs

Collections were made in the spring on DES-treated towns (Conata and Crew) and untreated towns for the purpose of comparing pregnancy rate (Table 10). Of 19 females collected in the spring on untreated towns 17 were pregnant, and of 9 from treated towns only 2 were pregnant. This difference is significant ($P < 0.01$) tested by Chi-square analysis. Only females with live embryos were considered pregnant.

The number of live embryos in pregnant females was lower (1.5 versus 5.2) on DES-treated towns (Table 11); this difference was significant ($P < 0.01$). Total embryos per pregnant female from treated towns was also significantly lower ($P < 0.05$) than from untreated towns (4.5 versus 5.2) (Table 11). These embryo differences probably resulted from the DES causing embryo death and resorption and interfering with implantation as other investigators have found to occur in DES-treated animals (Travis and Schaible 1962).

A laporotomy was performed on two prairie dogs which had access to DES-treated grain. The uterus of one held seven pea-sized embryos which were apparently being resorbed. The second female had five dead embryos

Table 10. Pregnancy in prairie dogs collected from DES-treated and untreated towns.

Time of Collection	Number in Sample	Number Pregnant	Number not Pregnant	Percent Pregnant
Spring[a]				
Treated	9	2	7	22*
Untreated	19	17	2	90
Summer[b]				
<u>National Grassland</u>				
Treated				
Number 41	5	2	3	40**
Number 41A	3	1	2	33**
Untreated				
Number 40	8	7	1	88
<u>Bomb Range</u>				
Treated	14	4	10	29**
Untreated	5	3	2	60
Spring and Summer Combined				
Treated	31	9	22	29**
Untreated	32	27	5	84

a Pregnancy based on embryo counts.
b Pregnancy based on evidence of suckling.
* Significant difference ($P < 0.05$) between treated and untreated towns using Chi-square.
** Significant difference ($P < 0.01$) between treated and untreated towns using Chi-square.

Table 11. The number of live and dead embryos in pregnant female prairie dogs collected from treated and untreated town, spring, 1971.

Type of Town	Number	Embryos		
		Live	Dead	Total
Treated	1	0	7	7
	2	1	5	6
	3	6	0	6
	4	0	2	2
	5	0	7	7
	6	1	0	1
	7	0	3	3
	8	4	0	4
Average		1.5	3.0	4.5
Untreated	1	5	0	5
	2	7	0	7
	3	6	0	6
	4	4	0	4
	5	5	0	5
	6	6	0	6
	7	7	0	7
	8	3	0	3
	9	4	0	4
	10	6	0	6
	11	4	0	4
	12	5	0	5
	13	3	0	3
	14	9	0	9
	15	8	0	8
	16	3	0	3
	17	3	0	3
Average		5.2	0	5.2

	Treated	Untreated
Live embryos per pregnant female	1.5 (8)[a]**	5.2 (17)
Total embryos per pregnant female	4.5 (8)*	5.2 (17)

a Sample size in parenthesis
* Significant difference ($P < 0.05$).
** Significant difference ($P < 0.01$).

being resorbed and one live fetus that was half-dollar size (Fig. 5). The live fetus was not born. It is not known whether the operation caused eventual resorption of this fetus.

In late June, females were also collected from the towns on which age-ratio counts were made. Reproductive success of adult females was determined by noting evidence of suckling by young. Thirty-five females were collected from the five towns. Of the 22 from treated towns, 7 were suckling young while 10 of 13 had suckled young from the untreated towns (Table 10). Differences in number of successful females between treated and untreated towns were significant using Chi-square analysis ($P < 0.01$).

Sixty-three females were collected during the two collection periods, 32 from untreated towns and 31 from treated areas (Table 12). Twenty-seven were pregnant on untreated towns compared to 9 from treated towns; this difference is also significant using Chi-square analysis ($P < 0.01$).

In 1970, 24 pretreatment counts were made on the three towns on national grassland. Counts were made again in 1971 following distribution of the chemosterilant bait on two towns while the third served as a control. On both treated and untreated towns age ratios were lower in 1971 (Table 12). Although the decreases in age ratios were proportionally greater in the treated towns, these decreases were not significantly different ($P > 0.05$) between the treated and untreated towns. Even with a total of 90 doses of a preferred bait available, it is possible that some females did not receive sufficient chemosterilant to inhibit reproduction.

About half of the Homestead town and two-thirds of the Bomb Range

Table 12. Counts of young and adult prairie dogs on treated and untreated towns, June, 1970 and 1971.

Town	1970			1971			Age Ratio[a]	
	Number of Counts	Young	Adults	Number of Counts	Young	Adults	1970	1971
National Grassland								
Number 40 Untreated	5	234	134	33	2325	1513	2.033	1.733
Number 41 Treated	14	490	270	23	1068	655	1.875	1.619
Number 41A Treated	5	41	29	12	67	96	1.465	0.719
Homestead								
Untreated	--	--	--	5	170	89	--	1.905
Treated	--	--	--	5	14	96	--	0.119**
Bomb Range								
Untreated	--	--	--	48	864	842	--	1.187
Treated	--	--	--	48	560	1299	--	0.444**

[a]The age ratio is the average of ratios observed in each count.

**Significant difference ($P < 0.05$) using paired t-test between treated and untreated towns.

town were treated with DES and the remaining portion of the towns served as controls. No pretreatment counts were made. The age ratio on the treated portion of the Homestead town was 0.1 young per adult compared to 1.9 young per adult on the untreated portion. The Bomb Range town had an age ratio of 0.4 young per adult on the treated portion and 1.2 young per adult on the untreated portion (Table 12). The DES-treated sections of both towns had significantly ($P < 0.05$) lower young per adult ratios than the untreated sections using the paired t-test.

CONCLUSIONS

The breeding season of female prairie dogs in South Dakota begins February 20 and may last until the end of March. The peak of breeding occurs the first week in March, with the majority of those females breeding after March 10 being yearling females. Male prairie dogs are also at their breeding peak in late February. This is the period when an estrogenic chemosterilant would be most effective.

Diethylstilbestrol was shown to decrease reproduction significantly in prairie dogs in field experiments. This offers a potential control alternative to poisoning, especially where it is feasible to maintain prairie dogs at a population level which does not encourage acreage expansion.

Chemosterilants may be important in lowering prairie dog reproduction on federal lands, specifically grasslands under control of the U. S. Forest Service, Bureau of Land Management and National Park Service. Private landowners interspersed among federally owned lands and grazing lease-holders pressure these federal agencies to control expanding prairie dog populations which, as herbivores, compete with domestic livestock. The use of chemosterilants would not force the extermination of the towns on public lands but would retain them as habitat for the rare and endangered black-footed ferret as well as for the survival of the prairie dog.

If control of animal populations is warranted, chemosterilants would permit animals to live while poison, not only kills the target species, but also presents possible secondary poisoning to any predator or scavenger utilizing the target species as a food source.

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