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AGENT IN WILD NORWAY RATS.

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**ACCEPTANCE AND EFFICACY OF AN ANTIFERTILITY AGENT
IN WILD NORWAY RATS**

by

Gerald Wayne Gwynn

**A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Wildlife Management)
in The University of Michigan
1971**

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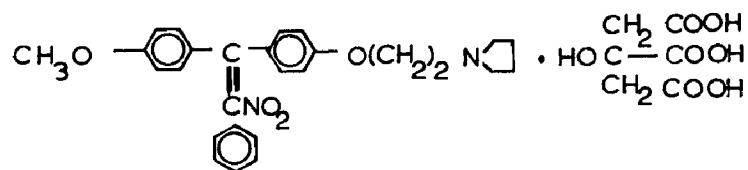
INTRODUCTION

The antifertility approach to rodent control has received much emphasis over the past decade. Davis (1961) conducted one of the earliest such studies in rats with promising, though inconclusive, results. More recently Balser (1964), Brooks and Bowerman (1969, 1971), Howard (1967a, 1967b), Howard and Marsh (1969), Kennelly et al. (1970), Marsh and Howard (1969), Pingale et al. (1967), Skinner (1968), Srivastava (1966), and Wetherbee (1964) have investigated antifertility agents as a means of controlling natality in wild rodents. Of particular significance is the study by Brooks and Bowerman with W3566 (17 α -ethynylestradiol-3-cyclopentyl ether), in which fecundity of garbage-dump rats was dramatically reduced, even though a decline in bait acceptance, particularly by females occurred.

A recent addition to the list of promising drug candidates is the non-steroidal estrogen antagonist CN-55,945-27¹, one of several active stilbene homologs synthesized by pharmaceutical chemists in the search for chemicals to control human fertility (Callantine 1967, DeWald et al. 1966). The compound is chemically described as (1-[2-[ρ -[α -(ρ -methoxyphenyl)- β -nitrostyryl]phenoxy]ethyl]pyrrolidine-monocitrate and has the following structural

¹U. S. patent number 3,272,841; example 15.

formula.



The minimum one-hundred percent effective antifertility dose (MED_{100}) of CN-55,945-27 in laboratory rats is 25 mcg/kg body wt/day (DeWald et al. 1966).

Studies (Gwynn and Kurtz 1970) have indicated that caged female wild Norway rats (Rattus norvegicus) will, under laboratory conditions, readily accept a diet containing the drug at concentrations that provide up to 125 mcg/kg body wt/day, even when offered unrestricted access to untreated food. This is approximately 5 times the MED_{100} for the laboratory rat.

Two distinct advantages of CN-55,945-27 are its relatively low toxicity to all species studied to date (studies at Parke-Davis on rats, dogs, guinea pigs, monkeys, rabbits and, to a limited extent, man) as compared to currently-used lethal toxicants and its low cost.

In general, members of this class of compounds have been demonstrated to antagonize certain actions of estrogen and, in the presence of low endogenous estrogen levels, to exert a mild intrinsic estrogenicity (Callantine 1967:83). By virtue of its strong affinity for estrogen receptor sites, CN-55,945-27 is thought to exert its antagonism to the effects of estrogen, if endogenous estrogen levels are high, either by blocking uptake or

causing displacement of 17 β -estradiol (Callantine, Clemens and Shih 1968:384). Conversely, if endogenous estrogen levels are low, CN-55,945-27 can bind to unoccupied receptors and consequently exert its own intrinsic mild estrogenicity (Callantine et al. 1969).

Estrogen receptor sites are known to exist in the hypothalamus, adenohypophysis, uterus, and vagina (Eisenfeld and Axelrod 1966). Predicated on the hypothesis that CN-55,945-27 should influence the complex of estrogen-dependent events, experiments have shown that it interferes with such mechanisms as the decidual response of the endometrium (Callantine et al. 1966:165) and the contractility of the uterus and fallopian tubes (Callantine et al. 1966:166) with consequent effects upon fertility. The extent of these and other antifertility effects are dependent on the dosage, duration of treatment, and the hormonal activity in the animal at the time of treatment (Callantine et al. 1966:164).

The objective of this study was to assess, under field conditions, the effect of CN-55,945-27 on fecundity and recruitment of wild Norway rat populations. This was done in two parts: Part I was designed to determine whether, under nearly natural conditions, wild Norway rats living in large outdoor enclosures, would accept water treated with the drug in various concentrations when untreated water was freely available. Part II was a field trial to test efficacy of the drug under natural conditions.

PART I. ACCEPTANCE AND EFFICACY STUDIES

METHODS

Choice of Site for the Experimental Enclosures

A rectangular area approximately 80' x 200' located behind a livestock feed manufacturing plant in Washtenaw County, Michigan, was chosen for the site of the experimental rat enclosures. The long axis of the site was oriented in a general north-south direction. The site was in an area that had been used for the dumping of corn cobs over a 30-year period. Ground consisted of a 2'-deep layer of highly organic soil layered on a sandy clay base. The organic layer was held together by the highly organized root systems of quack grass (Agropogon repens).

The site was bounded on the east side by a drainage ditch and on the west by a large corn cob pile. The two ends were bounded by a large metal shed on the south and an oil-polluted pond on the north.

Construction of Experimental Enclosures

Construction of the enclosures was done during April, May, and June of 1970. The general design of the enclosures was similar to that of Calhoun (1962:5). Two 40' square enclosures, each divided into 20' x 20' cells, were constructed from hardware cloth (Figure 1). Figure 2 is a photograph of the North enclosure. The cloth extended 3' above ground and 2' underground. To prevent the rats from burrowing their way out of their cells, a 2' wide shelf

Figure 1. Diagram of the experimental enclosures as viewed from above. Pallets are shown as rectangles, watering trays with waterers as circles with two dots, and feed hoppers as solid circles.

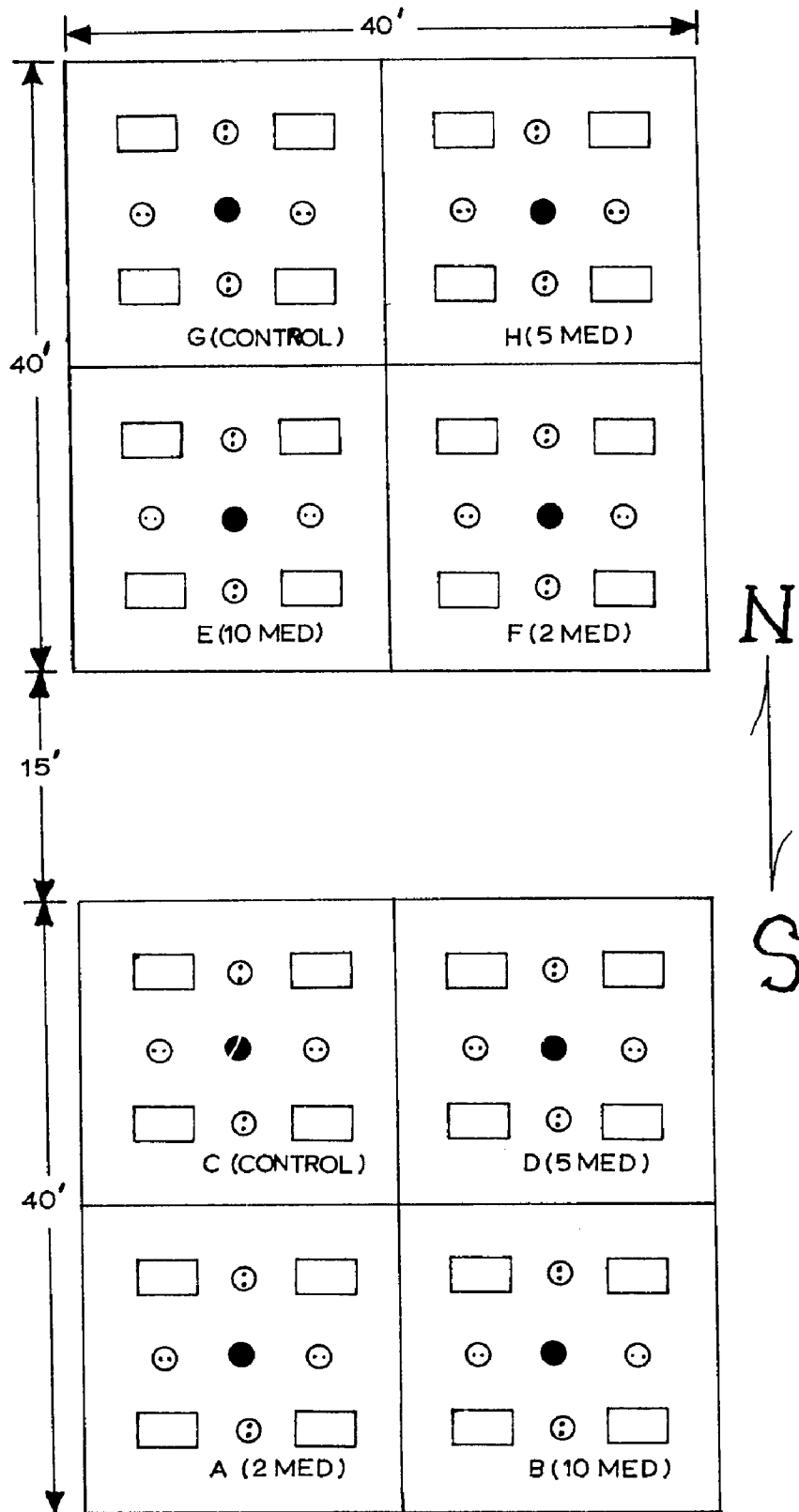


Figure 2. Photograph of the North enclosure. Cell E is in the foreground. The corner of the South enclosure is on the right.



of hardware cloth was extended into each cell at a depth of 2'. A tractor-mounted back-hoe was used to dig the required trench for the placement of the below-ground portion of the cells. The corner formed by the vertical wall of the fence and the shelf was made escape proof by sewing the two together tightly with galvanized wire. No rats ever solved the underground baffle although they occasionally dug down that far.

At the top of the 3' above-ground portion of the walls an 18" overhang of screen glass extending into each cell prevented above-ground escape.

Predators were kept out of the cells by the placement of a chicken-wire canopy over each of the two main enclosures at a height of 5' above ground. The 2' vertical section between the canopy edge and the top of the hardware cloth was filled with chicken wire and sewn in place with galvanized wire. Human entrance to the cells was obtained by "unsewing" a portion of the enclosure canopy. Upon entering an enclosure, access to the 4 cells of the enclosure was gained by using a specially constructed portable ladder to climb over the screen glass baffles.

Thirty-two old wooden pallets approximately 4' x 6' x 6" were obtained from a nearby livestock warehouse and were used in constructing four shelters in each cell as follows: A 4' x 6' x 10" excavation was made in the corner of each cell and recessed 2' from the 2 walls forming the corner. A 2" thick layer of corn husks and

ground corn cobs was spread in the bottom of each excavation. A 4' x 6' x 6" pallet was then placed on the corn husk layer, then more shucks were layered on top of the pallet. Finally, 2" of dirt was layered over the husks. The result was an underground labyrinth suitable for rat habitat.

Midway between each of the buried pallets a metal lid from a 55-gallon drum was placed and leveled (4 lids/cell). Each of these lids served as a tray for a pair of 1 gallon plastic poultry waterers¹. Each pair of waterers was placed in the center of the trays, oriented such that a line drawn through the centers of their bases was perpendicular to the nearest cell wall (Figure 1).

Three bricks were then placed at equidistant points on the circumference of the drum lid, and served as supports for a #3 galvanized washtub which was inverted and placed over the waterers. This protected the waterers from debris and rainfall; at the same time a secluded, darkened environment was provided around the waterers.

Feed hoppers were fashioned from 20-gallon galvanized trash cans by cutting 4 semi-circles 3" in diameter in each quadrant of the can bottoms. By pressing from the inside of the can on the centers of each semicircle, four openings were formed; the openings could be varied to best dispense whatever feed type chosen. With their lids in place, the trash cans proved to be good weather-proof hoppers that minimized feed wastage. One hopper was placed

¹"Little Giant" brand, Muller Mfg. Co., Wisconsin.

on a base of 4 bricks in the center of each cell.

Capture and Preliminary Treatment of Animals

A dumping area adjacent to the experimental enclosures where old unmarketable livestock feeds and other refuse were dumped provided a source of wild Norway rats. Forty live traps¹, baited with horse sweet-feed and peanut butter, were used to capture a total of 55 female and 55 male rats during the period from May 4 through June 6, 1970. Covering of the traps with feed sacks to provide a tunnel effect greatly enhanced success in trapping. Of the 55 males captured only the last 24 were kept, since only 16 were required for the experiment. The 55 females and 24 males were individually caged in 1 sq. ft. wire mesh cages, each containing a coffee can to provide a degree of privacy for the animals. The cages were kept in racks in a large outdoor three-sided shed. Large sheets of brown cardboard were hung on all four sides of the cage racks to provide a seclusion effect, a factor which previous experience had indicated to be of particular importance to the survival of caged wild rats. The animals were maintained for a minimum of 22 days to allow any females which were pregnant at the time of capture to bear their young. Purina Rat Chow and water were supplied ad libitum.

By June 26, 6 females and 5 males had died--probably due to stress effects of captivity. For 3 days prior to distributing the captive rats to their respective cells,

¹Manufactured by Tomahawk Live Trap Co., Tomahawk, Wisc.

the antifertility agent was added to the drinking water of the females at a concentration of 0.125 mg/100 cc. This regimen would provide approximately 5 times the MED_{100} known for the albino laboratory rat (Callantine et al. 1966:153), and thus protect against pregnancy for approximately 3 days after the third dose. This was done to preclude the possibility that females might become impregnated during that period of time between their introduction to the cells and their subsequent consumption of treated water.

Experimental Design and Application

The experiment was designed to offer all rats a free choice between treated and non-treated water in a semi-natural habitat where predation, immigration, and emigration were eliminated.

Three drug levels were chosen for study--2, 5, and 10 times the MED_{100} . This range was necessary to derive information on possible rejection based upon concentration and possibly to estimate some arbitrarily high dose that might be necessary under actual field conditions. Dosage calculations were based on a theoretical total daily water consumption of 30 cc/300 gm rat (Gwynn, unpublished data from caged wild rats in the laboratory) of which half would be drug-treated water and half untreated water, assuming random consumption. Thus, the three concentrations of drug used were 1.0, 2.5, and 5.0 mcg/cc.

Duplicate cells for control and each treatment group were established in the enclosures. Assignment of treatment levels to the 4 cells of each enclosure (North, South) was such that each of the study treatments and control (no drug) could be replicated (Figure 1). Assignment of treatments within each enclosure was made using random numbers. To provide a free choice of treated and untreated water, one of the two waterers at all but the control watering stations contained an appropriate solution of the drug--the other contained plain water. In the cells containing treated water, the waterers with drug were marked "T" (treated), while those with plain water were marked "S" (standard). The 2 waterers at each site in the control cells were marked either "s" or "t" for positioning purposes; neither contained drug.

The relative initial positioning of the containers at each watering site was decided by a coin toss. During the entire 106-day study the 2 waterers at each site were reversed in position every 3 days to help equalize the effects on consumption of any positional preference the rats may have had.

The feed hoppers were kept filled with a mixture of Purina Rat Chow, horse sweet-feed, and beef fattener.

Forty-eight females were weighed and then equally divided among 6 descending weight categories while 16 males were weighed and then divided equally among 2 categories.

One animal from each category was then randomly assigned to each cell, thus allocating 6 females and 2 males to each cell. The mean weights of the animals are tabulated in Table 1. The females were not marked, but males were marked by using a toe clipping code (one front and one back toe) under ether anesthesia. All releasing was done in the late evening of July 2.

Upon release, the majority of the animals immediately went under the pallets. Approximately 1 hr. later, several were seen climbing the cell walls and crawling along the juncture of the screen-glass baffle and the cell walls. Inspection of the cells early the next morning indicated that all animals had taken shelter under the pallets. Several burrows had been started at the edges of the cells--particularly in the corners.

During the course of the experiment the outside perimeters of the enclosures were checked every 2 days to be certain that no animals were attempting to burrow into the cells from outside. Grasses and weeds were periodically cut back to facilitate better observation of rat activity. Two Great Horned Owls (Bubo virginianus) were frequently observed in the late evening and night flying near the enclosures. They sometimes perched in a dead elm tree within 30 yards of the enclosures, and probably would have taken rats were it not for the chicken-wire canopy.

Evening and night observation of rat activity in the cells from a concealed viewpoint approximately 20 yards

Table 1.--Mean weights (+ standard deviations) of animals at the time of admission to the cells

TREATMENT LEVEL AND CELL	MEAN WEIGHT (GMS)	
	FEMALES	MALES
Control (C)	205 $\pm$ 65	198 $\pm$ 17
Control (G)	206 $\pm$ 77	187 $\pm$ 109
2MED ₁₀₀ (A)	209 $\pm$ 67	204 $\pm$ 100
2MED ₁₀₀ (F)	178 $\pm$ 87	215 $\pm$ 148
5MED ₁₀₀ (D)	188 $\pm$ 58	177 $\pm$ 86
5MED ₁₀₀ (H)	198 $\pm$ 64	184 $\pm$ 45
10MED ₁₀₀ (B)	213 $\pm$ 66	194 $\pm$ 72
10MED ₁₀₀ (G)	194 $\pm$ 76	288 $\pm$ 152

from the cells was done at approximately 3 week intervals. Flashlights and binoculars were used. Mature males could be distinguished by the ventral protrusion of the scrotum. Although observations were hampered by vegetation growth, at least one of the original colonizing males was seen in every cell on August 3, which was 32 days after their admission to the cells.

During the course of the experiment 10 rats were found dead in the cells (Table 2). There probably were others which either died underground or were cannibalized and therefore, never found. Any of the original colonizing males which were found dead were replaced immediately with fresh-caught males and given the same identification number (toe clip). Females were not replaced due to the lack of pretreated replacements.

The rats consumed and cached approximately 1500 lbs. of feed from the hoppers during the course of the study, including 300 lbs. of Purina Rat Chow, 800 lbs. of horse sweet-feed, and 400 lbs. of beef fattener.

During the first 5 weeks after introduction of rats to the cells (July 2 through August 6) water consumption was determined by weighing each waterer on July 9, July 23, and August 6. After weighing, each waterer was emptied and then refilled with appropriate fresh solution and weighed again. An evaporation standard was set up near the enclosures, adequately protected by metal screen to prevent losses due to animals or insects. The 2 waterers on this site were

Table 2.--Rats found dead in the cells

CELL	DATE	SEX	WEIGHT(GMS)	NOTES
A	7/10	F	125	Not pregnant.
	8/29	F	100	Hole in abdomen.
	10/6	M	90	Exudate from left ear. Right ear chewed off.
B				No rats found dead.
C	9/12	M#55	180	Lower incisors maloccluded. Replaced with 140 gm male.
D	8/3	M#58	210	Wounds on back. Replaced with 216 gm male.
E	7/23	F	250	No external lesions. Not pregnant.
F				No rats found dead.
G	7/6	M#65	100	Replaced with 270 gm male on 7/21.
	10/6	M	110	Wounds on back of head.
	7/23	F	280	Blind in one eye. Not pregnant.
H	9/3	F	150	Lesions on back.

filled and weighed concurrently with the schedule used in the cells.

During the first 4 weeks of the study it was noted that a thin oily film was forming on the surface of the water in the containers with drug, but not in the untreated waterers. Analysis of this film indicated that it was the free-base of the drug. Since the water supply was quite hard (well-water) it was surmised that the citrate anion was chelating calcium, thus allowing the free-base to come out of solution. At the 5 and 10 MED₁₀₀ concentrations the film was abundant enough to almost completely cover the surface of the water in the troughs. Therefore, in the course of drinking, the rats probably received an effective dose in the process of dipping their tongues through the film. The film was scant in the 2 MED₁₀₀ cells and it is conceivable that the animals did not receive a MED₁₀₀ dose during this four week period.

Because aliquots were being taken from the waterers for analyses during the period from August 6 through 14 the water consumption data was subject to considerable error, and this data was therefore discarded.

A pilot study on drug solubility in a series of citrate buffered systems prepared from well-water indicated that a 0.003M citric acid buffer preserved with 0.033 percent benzoic acid was sufficient to keep the drug in solution. Two stock solutions were used in the preparation of the buffered system: (A) a 15 percent aqueous citric acid

solution and (B) a 26.5 percent benzoic acid-in-95 percent ethanol solution. For a given batch, 56.8 cc of A and 18.9 cc of B were added to 4 gallons of well-water. The pH of this system was approximately 4.1. Then, the appropriate volume of a 2 percent aqueous solution of the drug (0.76, 1.88, or 3.78 cc) was added to the buffered system to make the final solution.

On August 24 all "T" waterers received fresh drug solutions made with the buffered system and the "S" waterers received fresh plain water. The "t" waterers in the control cells were also filled with the buffered system, but without drug, and the "s" waterers received fresh plain water. These regimens were used for the remainder of the study.

By August 14 the water consumption rate had risen to the point that some waterers in both control and treated cells were in danger of going dry during the 2-week periods. Therefore, for the next 60 days waterers were weighed and refilled every 10 days. Thus, during the course of the experiment the consumption data covered 9 successive periods of 7, 14, 14, 10, 10, 10, 10, 10, and 10 days.

On the evening of October 16, 106 days after admission of rats, the feed hoppers were removed from the cells. Five live traps were placed in each cell and baited with peanut butter. In addition, 25 Victor snap-traps baited with peanut butter were set in cells A, C, and G which, based on the high water consumption rate, were presumed to have high rat densities. All females and male recruits were

assigned an identification number and were weighed as they were captured. The secretory state of the teats of all females was noted according to the method of Calhoun (1962:158).

The uterus or an epididymis was excised from each animal, placed in an embedding bag, and fixed in 1:10 formalin for histological examination.

Blood, liver, and body fat samples were taken from the first three live-trapped females taken from each drug treatment level (i.e., a total of 3 females from each cell-pair). Liver and fat were frozen to await analysis. Blood was heparinized, centrifuged, and the plasma pipetted off for analysis. Blood and tissue samples were analysed for the free-base of the drug using a fluorometric method for the assay of organic bases described by Glazko et al. (1962).

Starting on the fifth trapping night all trapping was done using 5 live-traps and 15 snap-traps in each cell. All waterers were removed and small paper cups containing water were placed in the 5 live-traps in each cell. Traps were set through November 9, but no rats were caught after October 29.

After termination of trapping some fresh digging was noted in cell E which appeared to have been done by a mature rat. This animal was never captured.

RESULTS

Drug Acceptability

For each of the 9 periods the consumptions from the four watering sites in each cell were pooled for like water types (T, S, t, s). The mean daily water consumption of the two water types per period for each cell is shown in Figure 3. A two-way completely crossed analysis of variance of this data for each of the 3 treatment levels and control was done using the BMD08V canned program written by the Health Sciences Computing Facility at UCLA, revised and adapted by the Statistical Research Laboratory, University of Michigan. The IBM 360/67 computer was used. The main hypothesis tested was that there was no difference in the mean consumption of the two water types in each treatment level.

The formula for the model is:

$$Y_{ijk} = \mu + T_i + P_j + R_k + TP_{ij} + TR_{ik} + PR_{jk} + TPR_{ijk},$$

μ = consumption,

T_i = the effect due to water type (S and T in the drug-treatment cells, s and t in the control cells),

$i = 1, 2$ (fixed),

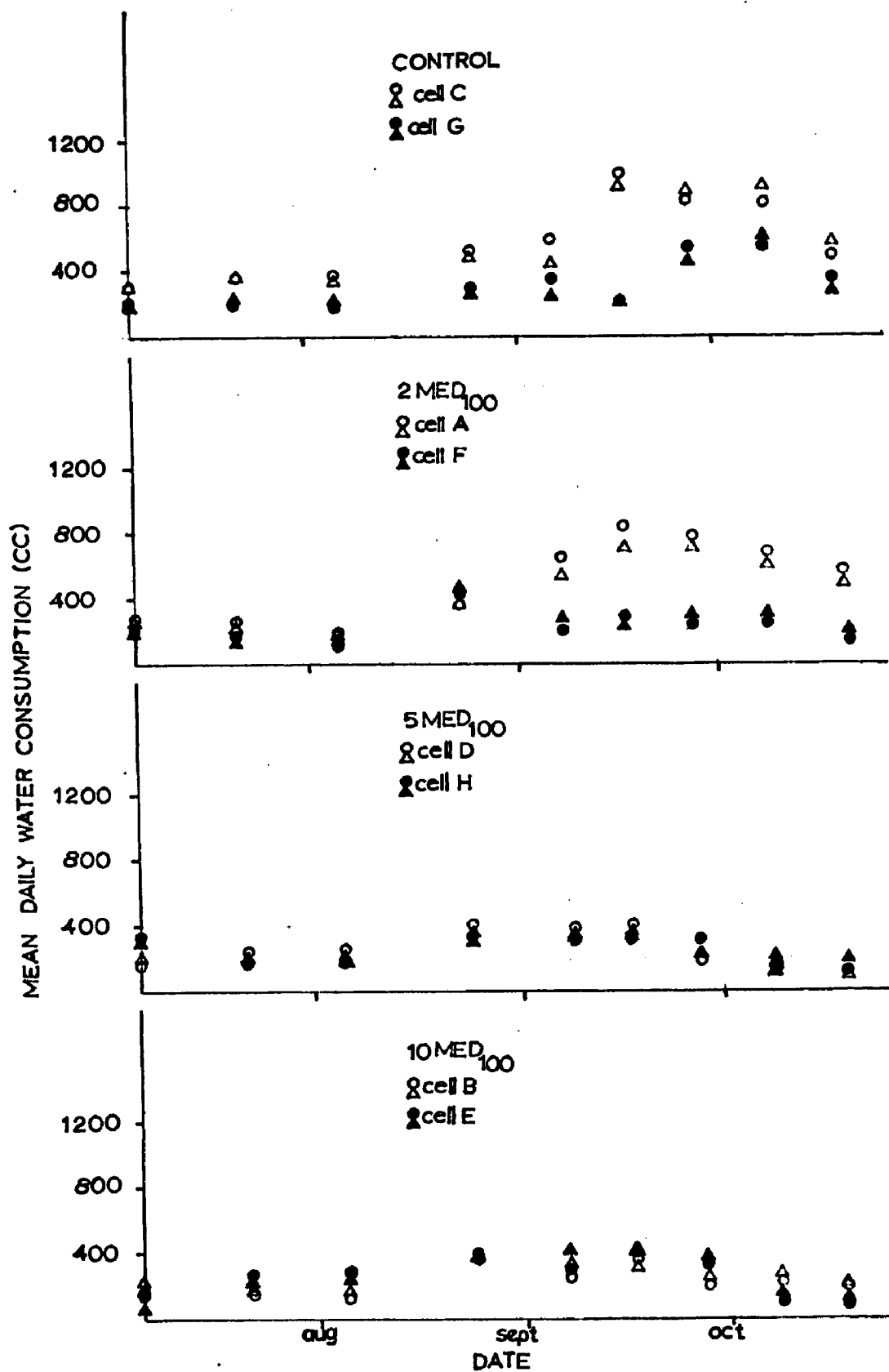
P_j = the effect due to periods,

$j = 1, 9$ (fixed),

R_k = the effect due to replicates (cells)

$k = 1, 2$ (random).

Figure 3. Mean daily water consumption per period. Triangles represent standard water and circles represent treated water.



The ANOVA table is shown in Table 3.

The downward trend in consumption during October was probably due to the fact that the weather was wet and cool during this time. Simple linear regression of mean daily drug-treated water consumption (T) on mean daily standard water consumption (S) for each drug-treated cell over the 9 periods was done using the BMD05R polynomial regression program from UCLA, revised by the University of Michigan Statistical Research Laboratory. Similarly, t was regressed on s. The regression line for each cell was generated (9 obs.), and in every case the F statistic for regression was highly significant ($P < 0.001$). Comparisons of the two lines for cell-pairs (e.g., cell C and cell G) were done using the method of Rao (1966:236) for the comparison of 2 or more regression equations.

The general formula used was:

$$F_{(J+1)(I-1), n_1+n_2-I(J+1)} = \frac{\frac{SSE_p - (SSE_1 + SSE_2 + \dots + SSE_I)}{(J+1)(I-1)}}{\frac{SSE_1 + SSE_2 + \dots + SSE_I}{n_1 + n_2 - I(J+1)}}$$

J = number of independent variables (1 in all cases),

I = number of population equations to be pooled,

n_1 = number of observations in population 1,

n_2 = number of observations in population 2,

SSE_p = sum of squares of the error term of the equation for the pooled population,

SSE_1 = sum of squares of the error term of the equation for population 1,

Table 3.--ANOVA tables for the four cell-pairs

SOURCE		SS	df	MS	$F_{1,8}^*$
CONTROL CELLS	Treatments(T)	16,486.6	1	16,486.6	0.09
	Periods(P)	145,760,168.0	8	18,220,021.0	
	Replicates(R)	34,110,272.2	1	34,110,272.2	
	Error(TPR)	1,404,200.6	8	175,525.1	
2MED ₁₀₀ CELLS	Treatments(T)	308,321.1	1	308,321.1	4.42
	Periods(P)	26,720,678.3	8	3,340,084.8	
	Replicates(R)	50,435,563.2	1	50,435,563.2	
	Error(TPR)	560,488.0	8	70,061.0	
5MED ₁₀₀ CELLS	Treatments(T)	12,829.3	1	12,829.3	0.13
	Periods(P)	25,260,822.4	8	3,157,602.8	
	Replicates(R)	277,869.6	1	277,869.6	
	Error(TPR)	783,436.2	8	97,929.5	
10MED ₁₀₀ CELLS	Treatments(T)	34,225.0	1	34,225.0	1.16
	Periods(P)	25,309,204.1	8	3,163,650.5	
	Replicates(R)	230,144.1	1	230,144.1	
	Error(TPR)	235,472.1	8	29,434.0	

* $F_{1,8;0.05} = 5.32$

SSE_2 = sum of squares of the error term of the
equation for population 2,

SSE_1 = sum of squares of the error term of the
equation for population 1.

In all cases the hypothesis that the two lines for the cell-pairs were alike in slope and level was accepted ($P > 0.10$). (Table 4, first 4 line entries).

The data for each cell-pair were then pooled (18 obs.) and a regression line generated.

The equations for these cell-pair lines were as follows:

Control, $Y = 9.940 + 0.973 X$, $S_p = 0.045$;

2MED₁₀₀, $Y = -33.165 + 1.137 X$, $S_p = 0.045$

5MED₁₀₀, $Y = 5.540 + 0.962 X$, $S_p = 0.103$;

10MED₁₀₀, $Y = 16.080 + 0.897 X$, $S_p = 0.081$.

Using Rao's method, comparisons of each of the MED₁₀₀ lines with the control line were done.

These comparisons test the hypothesis that the regression line for each of the 3 drug treatment cell-pairs was not different in slope and level from the line for the control cell-pair. The results of these comparisons are shown in the last 3 line entries in Table 4. Note that there was significance in the 2MED₁₀₀:Control comparison (line 5 in Table 4).

The total consumption of treated and untreated water in each cell is shown in Table 5.

A paired t test (Steel and Torrie 1960:78) of the hypothesis that equal amounts of treated and untreated water were consumed in each cell-pair over the course of the

Table 4.--Comparisons of regression lines for treated (T) on standard (S) water consumption. The null hypothesis is that the compared lines are the same in slope and level.

CELL COMPARISON	d.f.	F
Control(C):Control(G)	2,14	0.12 ^a
2MED ₁₀₀ (A):2MED ₁₀₀ (F)	2,14	2.44 ^a
5MED ₁₀₀ (D):5MED ₁₀₀ (H)	2,14	1.33 ^a
10MED ₁₀₀ (B):10MED ₁₀₀ (E)	2,14	0.61 ^a
2MED ₁₀₀ ~~Control~~ Cells pooled ~~Cells pooled~~	2,50	6.35 ^b
5MED ₁₀₀ ~~Control~~ Cells pooled ~~Cells pooled~~	2,50	0.17 ^b
10MED ₁₀₀ ~~Control~~ Cells pooled ~~Cells pooled~~	2,50	0.84 ^b

$$^a F_{2,14}^{0.05} = 3.74$$

$$^b F_{2,50}^{0.05} = 3.20$$

Table 5.--Total consumption (liters) of treated and untreated water in each cell.

TREATMENT LEVEL AND CELL	WATER CONSUMPTION		t TEST (8d.f.)
	S	T	
Control (C)	56.97	55.84	
Control (G)	38.70	39.06	
Total	95.67	94.90	0.63 ^a
2MED ₁₀₀ (A)	45.39	49.26	
2MED ₁₀₀ (F)	26.29	25.76	
Total	71.68	75.02	0.97 ^a
5MED ₁₀₀ (D)	27.68	26.44	
5MED ₁₀₀ (H)	28.36	28.93	
Total	56.04	55.37	0.64 ^a
10MED ₁₀₀ (B)	22.77	21.29	
10MED ₁₀₀ (E)	23.28	23.65	
Total	46.05	44.94	0.40 ^a

^a No significant difference in consumption of standard and treated water ($P>0.3$).

experiment was insignificant ($P > 0.30$).

An estimate of the mean daily drug intake, based on total weight of rats trapped in each cell-pair and their total water consumption over the final 10-day period indicated that the rats were consuming about fifty percent more water than predicted (about 46 cc/300 gm rat/day). Therefore the estimated drug consumption rate was about fifty percent more than predicted (Table 6).

Drug Efficacy

An estimate of the degree of recruitment in the cells can be obtained from Table 7. Since the oldest recruits (approximately 80 days old) would rarely weigh more than 250 grams (Calhoun 1962:218), one may assume that the animals in the five weight-class groups covering the span from 0 through 250 grams in Table 7 are recruits. Those animals in the three upper weight-class groups may be considered survivors of the original colonizing groups. Note (Table 7) that there were a total of 81 and 41 animals judged to be recruits in the control and 2MED₁₀₀ cell-pairs respectively, and no recruits in the two high treatment level cell-pairs. In one control cell (C) neither of the two colonizing males survived to the termination of the study. At least one of them, however, was seen on the evening of August 3.

Histological examination of the epididymides of the nine original colonizing males that were ultimately trapped revealed that all had abundant spermatozoa. At the termination of the study only 2 rats were pregnant (Table 7).

Table 6.--Estimate of mean daily intake of drug (mcg). Calculations are based on total weight (grams) of rats trapped in each cell-pair and the mean daily water consumption (cc) in the last 10-day period. The rats were assumed to have consumed equal amounts of treated and untreated water daily.

TREATMENT LEVEL AND CELL-PAIR	DAILY WATER CONSUMPTION cc/kg	DAILY DRUG CONSUMPTION mcg/kg
Control (C,G)	152	0.0
2MED ₁₀₀ (A,F)	148	74.0 ^a
5MED ₁₀₀ (D,H)	165	206.2 ^b
10MED ₁₀₀ (B,E)	147	367.0 ^c

^aequivalent to 2.96 times the MED₁₀₀ for the laboratory rat.

^bequivalent to 8.28 times the MED₁₀₀ for the laboratory rat.

^cequivalent to 14.70 times the MED₁₀₀ for the laboratory rat.

Table 7. --Weight-class distribution (grams) of rats trapped in each cell at termination of the study with notes on the reproductive state of females. All rats weighing less than 250 grams are assumed to be recruits. Numbers in parentheses are mean weights (standard deviations) of animals in the class.

TREATMENT LEVEL AND CELL	WEIGHT-CLASS (grams)												MALES FEMALES	
	0-50	51-100	101-150	151-200	201-250	251-300	301-350	351-400					17	24
CONTROL(C)	1 (45)	1 (65)	10 (138±15)	20 (173±14)	8 (217±12)	1 ^f (281)	0	0					17	24
CONTROL(G)	0	2 (74±12)	12 (132±12)	22 (173±15)	5 (214±9)	2 ^b (283±25)	3 ^c (324±19)	0					26	20
2MED ₁₀₀ (A)	0	0	7 (136±11)	14 (176±15)	7 (227±14)	3 ^{b,d} (294±4)	2 ^{c,d} (335±8)	0					16	17
2MED ₁₀₀ (F)	0	0	9 (134±15)	3 (169±14)	1 (245)	1 (272)	3 ^c (319±10)	1 ^c (392)					4	14
5MED ₁₀₀ (D)	0	0	0	0	0	2 (266±17)	4 ^c (324±20)	1 (395)					1	6
5MED ₁₀₀ (H)	0	0	0	0	0	2 (253±2)	4 ^e (327±15)	0					2	4
10MED ₁₀₀ (B)	0	0	0	0	0	5 (277±19)	1 ^c (348)	0					1	5
10MED ₁₀₀ (E)	0	0	0	0	0	1 (265)	3 ^c (317±16)	0					1	3

^aFemale with elongated cylindrical teats.

^bOne female with milk expressable from teats by hand.

^cIncludes one original colonizing male.

^dOne female was pregnant.

^eIncludes two original colonizing males.

The ovaries of approximately half of the females were examined histologically. All appeared to be normal and functional. All non-pregnant females of breeding age, which is approximately 100 days, or 210 gm (Calhoun 1962: 218), had normal uteri either in the secretory or estrogenic stages.

The results of the fluorometric tissue analyses are given in Table 8.

Table 8.--Analysed drug concentration (nanograms) per gram of female rat tissue.

TREATMENT LEVEL	CELL PAIR	RAT WT. (GMS)	DRUG CONCENTRATION/GM. TISSUE		
			PLASMA	BODY FAT	LIVER
CONTROL	C&G	172	3	11	12
		192	1	57	49
		240	1	12	11
2MED ₁₀₀	A&F	185	2	146	65
		298	2	12	2
		315	2	67	149
5MED ₁₀₀	D&H	245	2	122	Lost
		310	2	24	42
		310	1	129	84
10MED ₁₀₀	B&E	258	2	9	83
		298	2	24	92
		310	4	225	34

DISCUSSION

Drug Acceptance

The ANOVA, regression analyses, and t test (Tables 3, 4, and 5) indicate that no rejection of the drug-treated water occurred over the 106-day length of the study. There was a significant preference for the drug-treated water in the 2MED₁₀₀ cells, as indicated by the significance of the F test in Table 4 and the regression coefficient of 1.137 in the 2MED₁₀₀ equation on page 27. A possible explanation for this is that the drug may have had a preferred taste at this low concentration, but less preferred at the higher concentrations encountered in the higher treatment-level cells.

Drug Efficacy

The presence of spermatozoa in the epididymides of males, while not prima facie evidence of functional fertility, is good evidence that they were fertile.

The pregnancies in the 2MED₁₀₀ cells may have occurred during the period from July 2 through August 24 when problems were encountered with drug solubility. Even so, it appears that reproduction was attenuated in these cells (Table 7). A chi-square test (1 d.f.) indicated that the 2MED₁₀₀ cells had a significantly ($P < 0.05$) lower incidence of recruits at the termination of the study than the control cells.

The absence of reproduction in the 5 and 10MED₁₀₀ treatment level cells (Table 7) established that complete effectiveness can be obtained with water containing between 3 and 8MED₁₀₀ when untreated water is available.

The normal appearance of the ovaries and uteri of females in all cells confirms that the antifertility activity is not related to gross dysfunction of the reproductive organs, but is probably related to interference with implantation mechanisms. Studies by Callantine et al. (1966:160) indicated that the drug has no effect on the blastocyst per se.

The low incidence of pregnancy in control group females at the termination of the study is not surprising. Calhoun reported (1962:160) that due to the influence of photoperiodicity upon mating behavior wild Norway rats cease to reproduce when the period between sunrise and sunset is less than approximately 13 hours. All rats born in the cells could not have been older than approximately 80 days at the time of trapping. Since wild Norways normally do not exhibit adult sexual behavior until approximately 80 days of age (Calhoun 1962:152) it is not surprising that no pregnancies were seen in these animals.

The data of Table 8 show that the tissue analyses were not a dependable means of determining whether, under field conditions, rats had taken the drug offering. Hippuric acid, a metabolite of benzoic acid, was possibly being extracted along with the drug in the fluorometric

procedures. This would account for the high values for the control tissue analyses seen in Table 8. Further modifications of the extraction procedures might make the fluorometric procedure specific for the drug. Thus, the fluorometric technique should be re-evaluated before final judgement is passed on its value. Studies by Dr. A. J. Glazko et al. (unpublished) at the Parke-Davis Research Laboratories at Ann Arbor, Michigan, have indicated that, in laboratory rats dosed peroral in single doses of 25 mg base/kg body wt, the highest drug concentration was found in lung tissue (94 nanograms/gm tissue), with progressively lower concentrations in spleen, liver, body fat, kidney, heart, skeletal muscle, brain, and blood plasma. The biological half-life was essentially the same in all tissues--approximately 4 hrs. Approximately 4 percent of the dose was recovered in 24-hour urine.

Thus, after the rats entered the live-traps, they could not have ingested drug during the time spent in the traps prior to sacrifice--as long as 16 hours. Some animals may have drunk treated water just before entering the traps while others may have entered without drinking. Those that entered traps without drinking may not have taken water during the previous period of daylight either (the rats were not active above ground during daylight hours); thus, some animals that were sacrificed may not have taken drug for the previous day and night--a total of approximately 28 hrs, or 7 half-lives. This is undoubtedly another reason

for the wide variation of tissue levels within drug treatment groups.

PART II. FIELD TRIAL

METHODS

Choice of Treatment Areas

The data from the study in Part I indicated that a field trial was justified. Therefore, it was decided that the livestock feed plant and the dumping area behind it, where the rats were trapped for the enclosure studies, would be treated with the drug. Another feed plant of similar size with a dumping area adjacent to it was chosen as a control (untreated) site. It was located near the town of Williamston, Michigan, approximately 50 miles north of the Chelsea (treated) plant.

Experimental Design and Application

This phase of the study was designed to compare recruitment and fecundity in two rat populations, one being offered the drug in a continuously available water supply, and the other not offered the drug. Figures 4 and 5 are photos of the Chelsea (treated) and Williamston (control) plants and dumping areas.

A preliminary trapping was done to determine relative population sizes at the two areas. On May 1, 1971, one-hundred Victor snap-traps were set on each area. Traps, baited with peanut butter, were placed at all visible burrow entrances and in any runs which appeared to be highly used. Traps were checked daily for 5 days. Thus

Figure 4. Photographs of the Chelsea plant and adjacent dumping area. Note the two 5-gallon reservoirs on the wall of the plant and the watering sites (inverted tubs) in the dumping area.

The Chelsea plant.



The Chelsea dumping area.



Figure 5. Photographs of the Williamston plant and adjacent dumping area.

The Williamston plant.



The Williamston dumping area.



there were 500 trap-nights of effort on the two populations.

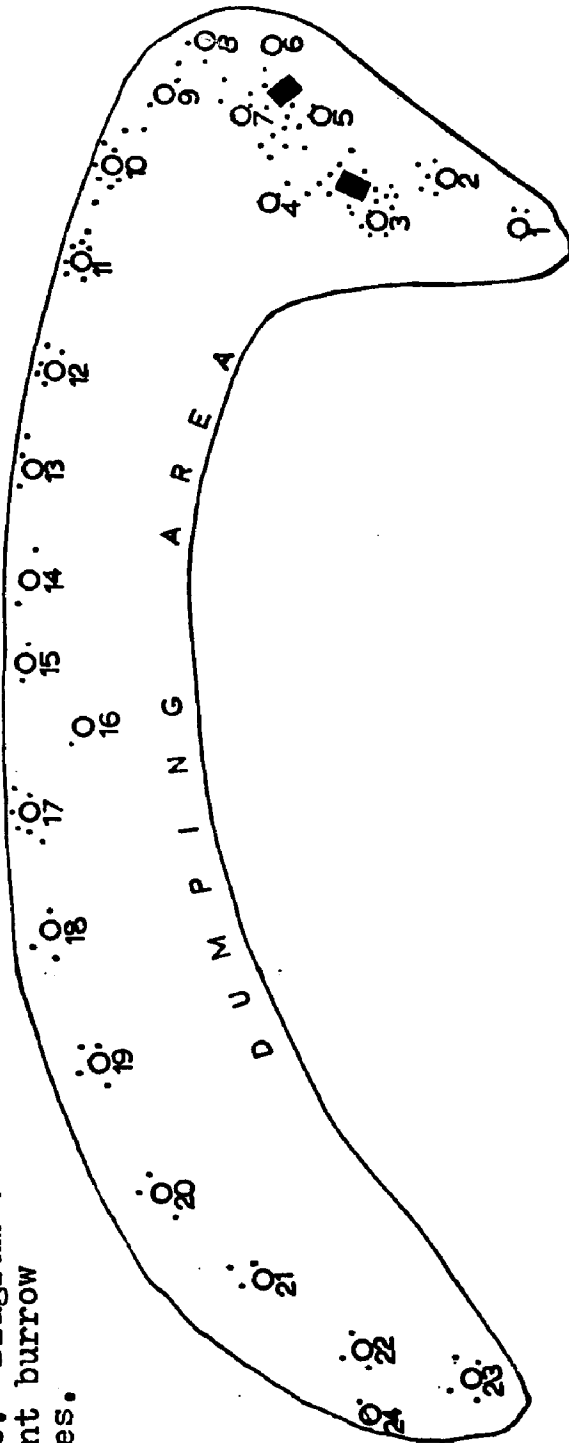
All rats were weighed and their length from nose to base of tail were recorded. The uterus and ovaries were excised from all females and fixed in 1:10 formalin.

On May 7, 1971, a total of 31 watering sites were established at the Chelsea plant area (Figure 6). Five sites were located in the plant warehouse; these were fashioned from automatic gravity-feed poultry fountains¹. Five-gallon capacity polyethylene carboys served as reservoirs for each fountain. The waterers were placed in areas where rats were assumed to be most likely to harbor; that is, in areas where the turnover rate of feed stockpiles was low.

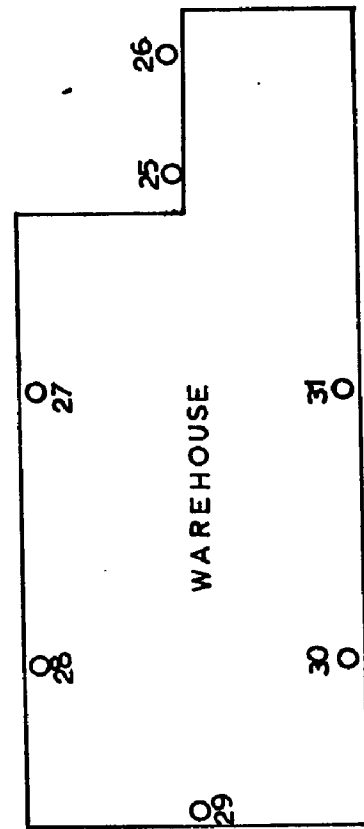
Twenty-six outdoor watering sites, fashioned from pairs of 1-gallon poultry-waterers placed on a 55-gallon steel-drum lid and covered with a #3 galvanized washtub supported by 3 bricks, as in Part I, were established in the dumping area. These were placed at approximately 20-foot intervals around the periphery of the dump, which was the prime harboring area. Dumping of waste feed and grain was done at the periphery of the area, thus the watering sites were in the midst of the great majority of the food available to the rats. Two open 55-gallon drums of rancid peanut butter, lying on their sides, were located near watering sites 3 and 6. These were prime

¹Manufactured by Miller Manufacturing Co., Milwaukee, Wis.

Figure 6. Diagram of the Chelsea area. Numbered circles represent watering sites. Dots represent burrow entrances.



COB
SHED



20'

sources of food for rats in the immediate area. Raccoons, skunks, and birds also fed regularly from this food. Two sites (25 and 26) were placed near the warehouse.

On May 7, 1971, waterers at all sites were filled with aqueous drug solution at a concentration of 9.5 mg per gallon which was made 0.003 M with citric acid and preserved with 0.033 percent benzoic acid. This was the same dosage form used in the 5MED₁₀₀ cells in Part I. The watering sites were maintained through September 24.

Consumption of this treated water was measured by weighing the waterers at each site at 14-day intervals. After each 14-day weighing the waterers were emptied and refilled with fresh solution and weighed again. An evaporation standard was set up and protected with screen wire. This standard was filled and weighed concurrently with the treatment waterers.

No water was put out at the Williamston (control) area since water was available from a drainage ditch under the plant and a pond near the dumping area.

Post-treatment trapping was started on the evening of September 17, 1971, one hundred and thirty-three days after initiation of treatment; this was carried out in the same way as the pretreatment trapping, using 100 snap-traps at each area (Chelsea and Williamston) for a period of 5 nights (500 trap-nights). Traps were baited with fresh peanut butter. Rats trapped were weighed, their lengths from nose to base of tail recorded, and an epididymis or

uterus with ovaries was excised and fixed in 1:10 formalin.

RESULTS

Drug Efficacy

The 500 trap-nights of pretreatment trapping done in May netted only 19 and 11 rats at the Chelsea and Williamston areas respectively. This observed difference in trapping rate was not significant ($\chi^2 = 2.2; P > 0.1$). Four of 5 adult females (>180 mm) trapped at Williamston were pregnant as were 2 of 5 trapped at Chelsea. Three and five deer mice (Peromyscus maniculatus) were trapped at Williamston and Chelsea respectively during the pretreatment trapping.

Table 9 shows the mean treated water consumption (cc) per 14-day period from each watering site at Chelsea over the course of the experiment, and Table 10 shows the consumption from all sites combined for each 14-day period.

Using the total water consumption during the last 14-day period and an estimated consumption of 153 cc water/kg rat/day (calculated from data in Table 6) the rat biomass in the Chelsea area was estimated at approximately 18 kg, or approximately sixty-eight 265 gm rats (265 gm was the mean weight of post treatment rats trapped at Chelsea).

Post treatment trapping (September 17 through 21) yielded 19 rats at Chelsea and 45 at Williamston (Figure 7). This difference in trapping success indicated a larger

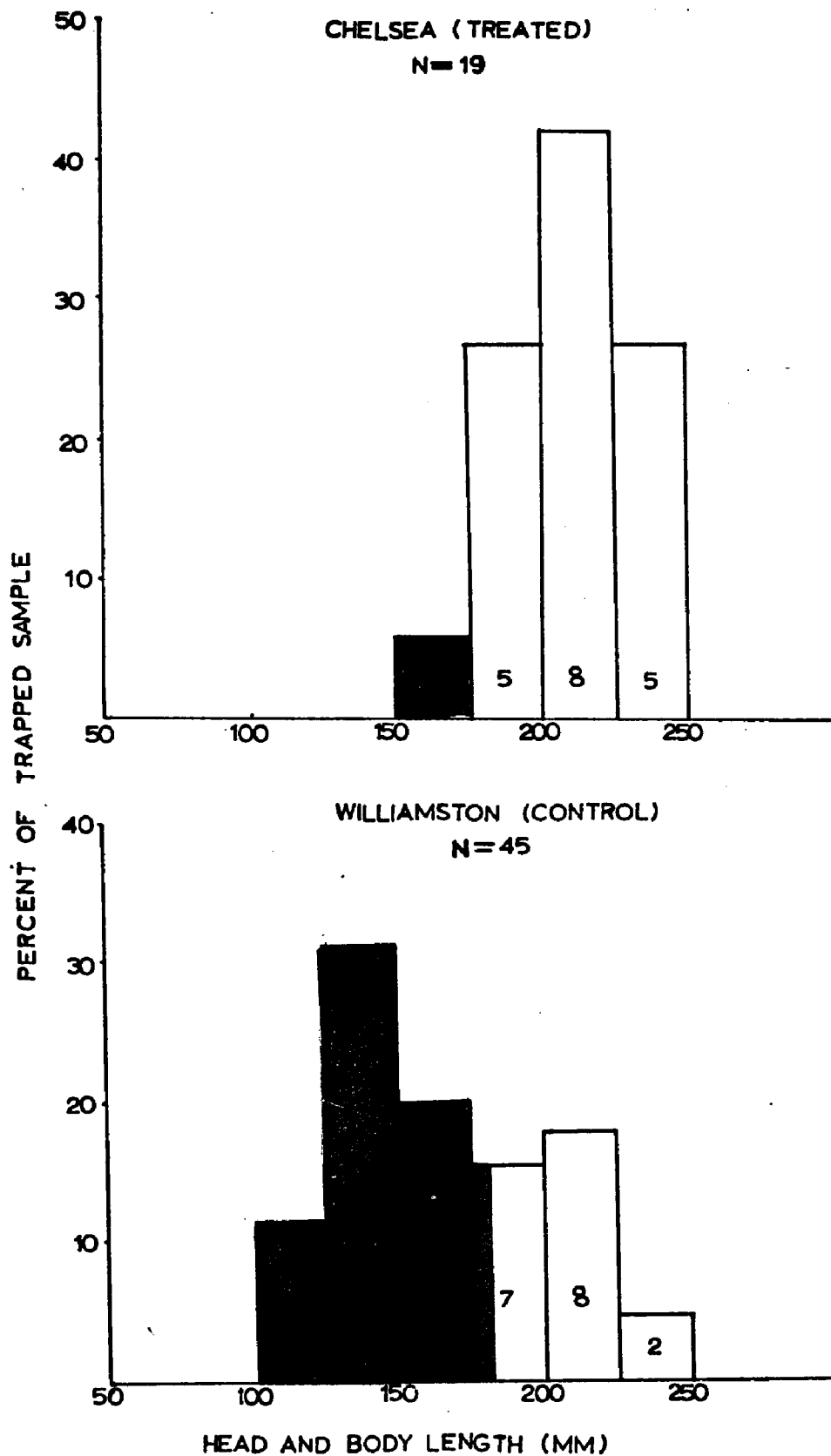
Table 9.--Mean consumption ($\pm$ standard deviation) of water (cc) from watering sites per 14-day period.

SITE	MEAN CONSUMPTION	SITE	MEAN CONSUMPTION
1	2340 $\pm$ 286	17	1992 $\pm$ 224
2	2832 $\pm$ 350	18	316 $\pm$ 82
3	2616 $\pm$ 128	19	1832 $\pm$ 142
4	916 $\pm$ 90	20	788 $\pm$ 90
5	1300 $\pm$ 127	21	712 $\pm$ 117
6	1860 $\pm$ 144	22	1920 $\pm$ 134
7	1032 $\pm$ 157	23	2260 $\pm$ 298
8	2040 $\pm$ 278	24	888 $\pm$ 204
9	2292 $\pm$ 336	25	592 $\pm$ 92
10	1832 $\pm$ 188	26	772 $\pm$ 152
11	3008 $\pm$ 342	27	72 $\pm$ 16
12	1496 $\pm$ 197	28	168 $\pm$ 65
13	260 $\pm$ 64	29	1008 $\pm$ 153
14	796 $\pm$ 101	30	516 $\pm$ 140
15	604 $\pm$ 94	31	308 $\pm$ 56
16	1016 $\pm$ 108		

Table 10.--Total consumption (liters) of water from all sites combined per 14-day period.

DATE	TOTAL CONSUMPTION
5/21	33.23
6/4	37.88
6/18	40.53
7/2	42.22
7/16	43.74
7/30	43.42
8/13	41.77
8/27	40.96
9/10	38.45

Figure 7. Length-class distribution of rats trapped in September (post-treatment). Length-classes judged to be recruits (<175 mm) are shaded. Two Williamston males in the 176-200 mm class were also judged to be recruits.



population at Williamston ($\chi^2 = 16.0; P < 0.005$).

Length (mm), rather than weight, was chosen as the criterion of age since the variance of length with age is less than that of weight (Calhoun 1962:216).

Any males and females under 100 days old, which is equivalent to 195 mm or 180 mm total nose-to-base-of-tail length for males and females respectively (Calhoun 1962:219), were judged to be recruits. The 100 day figure was based on a total drug-exposure time of 133 days at Chelsea. Allowing 10 days for the rats to become familiar with and begin to consume the treated water and 23 days for females which became pregnant during these 10 days to bear their litters, this leaves 100 days of treatment period during which no litters should have been born in the treated population.

The most significant feature of the post-treatment trapping was that only 1 rat of a total of 19 trapped at Chelsea was a recruit, while 30 of 45 trapped at Williamston were recruits. This difference in recruitment was highly significant ($\chi^2 = 20.0; P < 0.001$).

A t test (Snedecor and Cochran 1967:105) indicated a highly significant ($t = 5.26; P < 0.001$) difference in the mean lengths of rats from the Chelsea and Williamston populations ($\bar{x}_{\text{Chelsea}} = 210.9 \pm 21.7$ mm; $\bar{x}_{\text{Williamston}} = 167.1 \pm 33.4$ mm).

Of the 19 rats trapped at Chelsea, 9 were adult females (>180 mm). None of these females were pregnant or lactating. Of the 45 rats trapped at Williamston, 9 were adult females,

of which 6 were either pregnant or lactating. A chi-square test indicated that this observed difference in fecundity was highly significant ($\chi^2 = 9.0; P < 0.005$).

The ovaries and uteri of all adult females appeared histologically to be normal and functional. The epididymides of all adult males contained abundant sperm.

During the post-treatment trapping, 23 deer mice, including 10 females and 13 males, were caught at Chelsea, while only 6 mice (3 males and 3 females) were trapped at Williamston. This difference in trapping rate was significant ($\chi^2 = 13.7; P < 0.005$). Seven of the Chelsea females were macroscopically judged to be pregnant or lactating, while 2 of the 3 Williamston females were found to be pregnant or lactating. The majority of Chelsea mice were trapped in the vicinity of watering sites 13 through 16, which were areas with low rat burrow densities (Figure 6).

DISCUSSION

This experiment establishes that water offers a practical vehicle for delivering an antifertility agent having the characteristics of CN-55,945-27. Since the rats do not reject relatively high concentrations of this drug, it is not necessary that they receive their total daily water requirements from treated water supplies in order to maintain infertility.

Associated with acute poisoning programs, reduction of fertility should substantially diminish indigenous Norway rat population levels. The disadvantage occasioned by the need for more-or-less constant availability of CN-55,945-27 for adequate control of fertility is more than off-set by its success, relatively low cost of application, and low toxicity. The temporary antifertility effect of the drug, as opposed to a permanent effect, may be an asset for those ecological applications where the risk of sterilization of non-target species is high.

Reproductively unimpeded populations would have had a high incidence of recruits and pregnant females, as demonstrated by the Williamston population. The lack of recruits and pregnant females in the post-treatment sample from Chelsea indicate that there was essentially no ingression of rats from the surrounding untreated territory.

This is very encouraging, since a high ingression rate would nullify the effects of a localized rat control program.

Pre- and post-treatment trapping at Chelsea both yielded 19 rats. This suggests that recruitment of rats born or conceived in the spring prior to initiation of treatment was sufficient to balance natural mortality. Had the treatment been started early in the breeding season, recruitment probably could have been inhibited. Calculations, based on the post-treatment capture rate and the estimated post-treatment population size at Chelsea indicated that the Williamston population grew from an estimated 39 rats in May to approximately 161 rats in September.

Since there was no open fresh-water source, the Chelsea area was ideal for treatment with the drug in water. In addition, the summer was one of the driest in Michigan history. It is important to note the clustering of burrow entrances around the watering sites in Figure 6. The conclusion can be drawn that, especially in localities where water is sharply limited, watering sites would be sought out by wild rats, particularly if they were convenient to harboring or feeding areas.

Placement of watering sites at 20' intervals, such as was done in the Chelsea dumping area, is not economically feasible. Therefore, further studies should be done to determine the minimum density of watering sites which will provide the desired degree of effectiveness.

The apparent increase in the mouse population at

Chelsea points out a possible side-effect of the treatment. If mice were competitively excluded from the area by rats, then a decline in the rat population may have opened the habitat to invasion by mice. Further studies of the effect of the agent on mouse fecundity are needed.

The metabolites of CN-55,945-27 are unknown at present. Indeed, it is not known whether the active moiety is the drug or a biotransformed product. Metabolic studies on target and non-target species will be needed in the ultimate evaluation of CN-55,945-27 for rodent control.

Ideally, an antifertility agent should be specific for the target species and cause permanent sterility in both males and females, yet not cause side effects which would lead to rejection of the drug. Specificity for the target species may be achieved by considering the ecology of the species, then judiciously selecting a mode of treatment that will most likely exclude non-target species.

In animals which are polygamous or promiscuous breeders, such as rats, it would seem more effective to treat females than males, in the absence of a drug that diminishes fertility of both sexes, since a single fertile male could impregnate many females.

A chemosterilant with the characteristics of CN-55,945-27 will have to be more-or-less constantly used to prevent a resurgence of a population after treatment with rodenticides. Cities, with their complicated interconnection of sewer systems, garbage heaps, and harboring areas would be difficult

to treat successfully under this limitation. The most ideal situations for overcoming this shortcoming would be in treating granaries, dumps, croplands, and rangelands where quantitative treatment of the target species may be attained, either through cachable baits or water. In areas where raw garbage is dumped, a spraying technique might provide quick and inexpensive treatment.

There is a wealth of published data on bait acceptance by wild rats that could be used eclectically to design baits best suited for specific treatment problems. It remains to be demonstrated whether, in areas where food and water are abundant, baits can be designed which will be preferentially taken relative to the indigenous food supply.

Due to the high potency of CN-55,945-27 it is possible that it could be applied in powder form, or perhaps in a sticky vehicle such as molasses, in rat runways and consequently adhere to the feet of rats. Effective amounts of the drug then might be ingested during licking of the feet associated with washing and grooming activities.

Agents such as CN-55,945-27 should not be used on a widespread basis until the effects of the drug and its metabolites on non-target species, including those animals which prey on the target species, are known. Ecological half-lives of the drug and possible active metabolites must be considered in predicting long-term effects of the drug.

It is important that guidelines for ecological applications of pharmacological agents be formulated as soon

as possible, with their thrust toward minimizing the ecological consequences of the treatment, particularly the effects on non-target species which may come into contact with the drug or its metabolites.

As noted by Marsh and Howard (1970:58) destruction of rats by acute poisoning techniques, where the habitat remains unchanged, and particularly where a sizable portion of the population is not destroyed, will have only a temporary effect. Recent evidence of population resurgence following acute poisoning campaigns is contained in reports by Batcheler (1968) and Rowley (1968). It is of little value to reduce rat populations with rodenticides unless there are some means of inhibiting recovery. Antifertility agents could exert a strong influence on such recovery.

There may be many other advantages in controlling fertility of wild animal populations. For example, antifertility agents may be useful in suppressing populations of ground squirrels (Spermophilus spp.) in which sylvatic plague is endemic, and where its proximity to human populations poses a health hazard. Controlling the squirrel population through decreased natality, rather than by destroying large numbers of rodents with toxicants, should diminish the occurrence in the environment of fleas, mites, and ticks that abandon the carcasses of poisoned rodents.

The prime advantage of CN-55,945-27 over rodenticides would appear to be in its ready acceptance, which is probably related to the subtle way in which it inhibits

fertility. There apparently are no side-effects which cause rejection of the drug. Conversely, rodenticides, if ingested in sub-lethal amounts on the first offering, are frequently rejected on repeated offerings. The use of CN-55,945-27 may reduce the need for lethal rodenticides, a worthy goal in its own right, since accidental ingestion by non-target species would result in far less serious consequences than would the ingestion of acute toxicants.

LITERATURE CITED

- Balser, D. S. 1964. Antifertility agents in vertebrate pest control. Proc. Second Vertebrate Pest Control Conf., Anaheim, Calif., (J. W. Koehler, ed.) pp. 133-137.
- Batcheler, C. L. 1968. Compensatory response of artificially controlled mammal populations. Proc. N. Z. Ecol. Soc. 15:25-30.
- Brooks, J. E., and A. M. Bowerman. 1971. Estrogenic steroid used to inhibit reproduction in wild Norway rats. J. Wildl. Mgmt. 35:444-449.
- _____ and _____. 1969. Chemosterilants in rodent control. Soap and Chem. Specialties. Oct. 1969. pp. 58, 60, 62, 64, and 82.
- Calhoun, J. B. 1962. The ecology and sociology of the Norway rat. U. S. Dept. Health, Educ., and Welfare Public Health Service Publ. 1008. 288 pp.
- Callantine, M. R., R. R. Humphrey, S. L. Lee, B. L. Windsor, N. H. Schottin, and O. P. O'Brien. 1966. Action of an estrogen antagonist on reproductive mechanisms in the rat. Endocrinology 79:153-167.
- _____. 1967. Nonsteroidal estrogen antagonists. Clin. Obstet. Gynecol. 10:74-87.
- _____, E. Clemens, and Y. Shih. 1968. Displacement of 17β -estradiol from uterine receptor sites by an estrogen antagonist. Proc. Soc. Exptl. Biol. Med. 128:382-386.
- _____, _____, and _____. 1969. Displacement of 17β -estradiol from the rabbit fallopian tube. Am. J. Physiol. 216:1241-1245.
- Davis, D. E. 1961. Principles for population control by gametocides. Trans. N. Am. Wildl. Conf. 26:160-167
- DeWald, H. A., O. D. Bird, G. Rodney, D. H. Kaump, and M. L. Black. 1966. A new class of orally effective antifertility agents with hypocholesteremia activity. Nature 211(5048):538-539.
- Eisenfeld, A. J., and J. Axelrod. 1966. Effect of steroid hormones, ovariectomy, estrogen pretreatment, sex and immaturity on the distribution of H^3 -estradiol. Endocrinology 79:38-42.

- Glazko, A. J., W. A. Dill, and R. L. Fransway. 1962. Determination of diphenhydramine blood levels using a new fluorescent dye-salt procedure generally applicable to basic organic compounds. *Fed. Proc.* 21:269.
- Gwynn, G., and S. M. Kurtz. 1970. Acceptability and efficacy of an antifertility agent in wild Norway rats. *J. Wildl. Mgmt.* 34:514-519.
- Howard, W. E. 1967a. Biological control of vertebrate pests. *Proc. Third Vertebrate Pest Conf., San Francisco, Calif., (M. Cummings, ed.)* pp. 137-157.
- _____. 1967b. Vertebrate pests: biocontrol and chemosterilants. *In* *Pest Control: Biological, Physical, and Selected Chemical Methods* (W. W. Kilgore and R. L. Doutt, eds.) pp. 343-386. Academic Press, New York, 477 pp..
- _____, and R. E. Marsh. 1969. Mestranol as a reproductive inhibitor in rats and voles. *J. Wildl. Mgmt.* 33:403-408.
- Kennelly, J. L., M. V. Garrison, and B. E. Johns. 1970. Laboratory studies of the effect of U-5897 on the reproduction of wild male rats. *J. Wildl. Mgmt.* 34:508-513.
- Marsh, R. E. and W. E. Howard. 1969. Evaluation of mestranol as a reproductive inhibitor of Norway rats in garbage dumps. *J. Wildl. Mgmt.* 33:133-138.
- _____ and _____. 1970. Chemosterilants as an approach to rodent control. *Proc. Fourth Vertebrate Pest Conference, West Sacramento, California, March 3, 4, and 5, pp. 55-63.*
- Pingale, S. V., K. Krishnamurthy, and T. Ramasivan. 1967. Rats. *Bull. of Grain Tech. Foodgrain Tech. Res. Assoc. of India, Hapur (U.P.)*, 91 pp.
- Rao, C. R. 1966. Linear statistical inference and its application. John Wiley and Sons, Inc: New York, London, Sydney. 522 pp.
- Rowley, I. 1968. Studies on the resurgence of rabbit populations after poisoning. *CSIRO Wildl. Res.* 13:59-69.
- Skinner, W. A. 1968. Chemosterilants in rodent control. *In* *Asia-Pacific interchange, Proc. Rodents as Factors in Disease and Economic Loss, Honolulu, Hawaii, June 17-27, pp. 192-201.*

- Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods. Iowa State Univ. Press: Ames, Iowa. 593 pp.
- Srivastava, A. S. 1966. Mixture of furadantin and colchicine acts as effective chemosterilants against male or female field rat. Labdev. J. Sci. Tech. 4:178-180.
- Steele, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill: New York, Toronto, Sydney. 481 pp.
- Wetherbee, D. K. 1964. Vertebrate pest control by biological means. Proc. AAAS Symp. Pest Control, Montreal, Canada, December, 1964, 19 pp.