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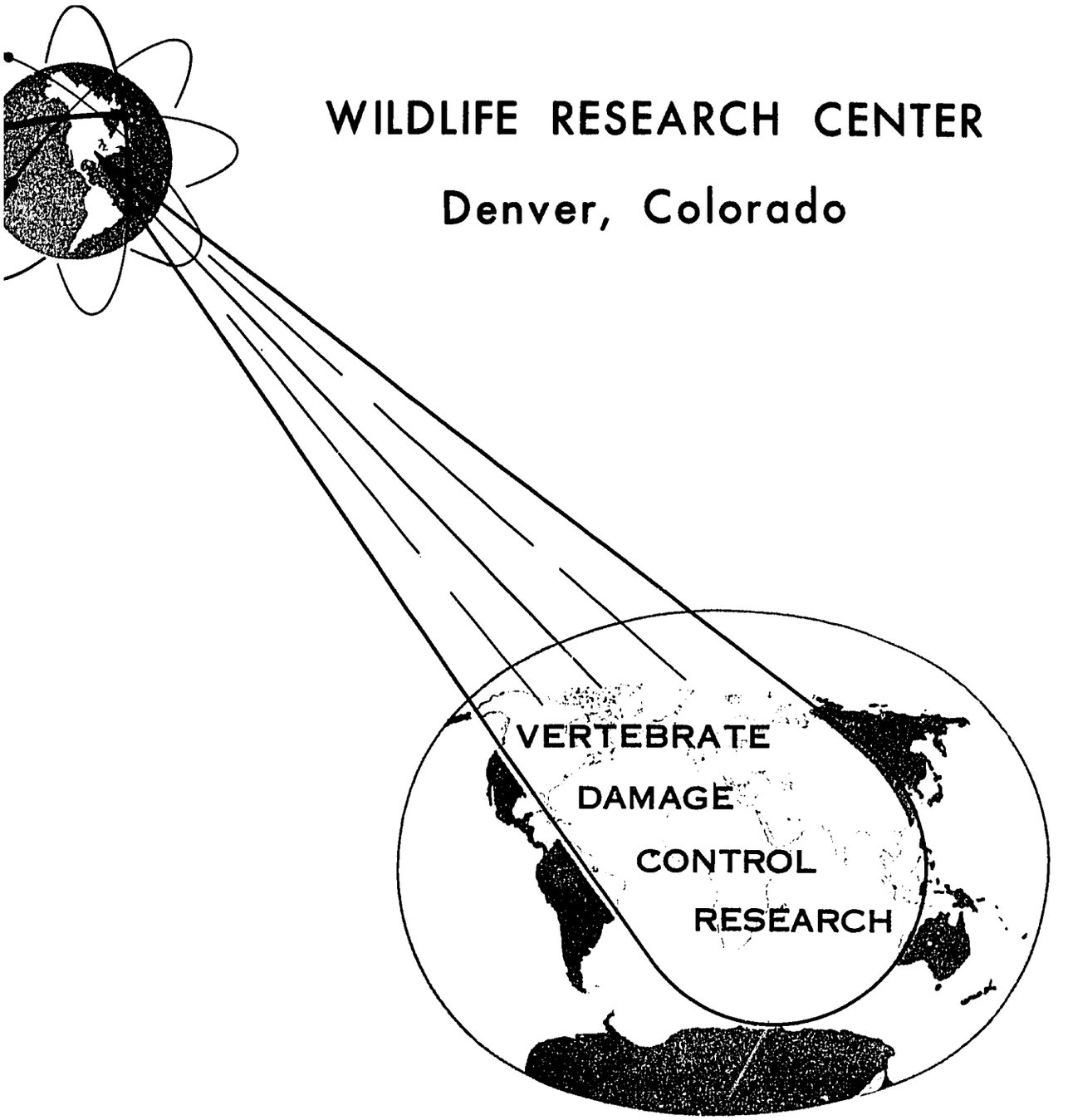

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Division of Wildlife Research
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ABSTRACTS

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

UNIT OF PHARMACOLOGY

Repellency, physiological marking, and rodenticide tests were conducted with candidate chemicals for use in controlling damage caused by vertebrate pests. Correlation of structure-activity relationships of chemicals potentially useful in damage control was advanced by encoding more chemicals into the WLN notation system. The subject areas are summarized below:

1. Initial bioassays. Several burning or bitter compounds were evaluated by the initial bioassay test as deer mouse repellents on seeds. Bitterness, per se, did not appear repellent, but one pungent chemical, DRC-5593, appeared promising, particularly since it did not show the high toxicity often associated with good repellency.

2. Physiological markers. Fifteen fluorescent pelage markers have been evaluated. To date only two, DRC-6082 and DRC-6090, appear useful, but several more will be evaluated. The best marker in terms of efficacy, toxicity, availability, and cost will then be selected for more detailed studies. At an oral dose of 1 mg/kg, one organo-iodine chemical was found to elevate the iodine content of the blood in coyotes for over 130 days; this chemical will be tested in rodents.

3. Rodenticide evaluations. Two chemicals were tested as rat toxicants by the EPA single-dose rodenticide protocol. DRC-3992 did not pass this test (less than 90% mortality for both sexes), and additional studies to increase its efficacy will be done. DRC-4575 did pass (100% mortality for both species), but company approval will be needed before future decisions can be made. Preliminary feeding studies with wild Norway and ricefield rats indicate that microencapsulation has greatly increased the efficacy of DRC-3901; tests with an improved microencapsulated form will be conducted as soon as it is available.

   Of three candidate chemicals, one, DRC-6027, was highly toxic to deer mice but was erratic in feeding tests with Norway rats. Preliminary toxicity tests with DRC-5976 have been concluded; of 12 mammal and bird species tested, only albino and wild Norway rats were susceptible to this compound.

4. Chemical data storage and retrieval. There are 6,000 chemicals now encoded into a computer notation system and are in the Center's file of biologically active compounds.

5. Structure activity relationships. The correlation study is undergoing editorial review and a continuing publication on compounds evaluated from 1960 to 1970 is in preparation.

PHYSIOLOGY AND BEHAVIOR

Physiological, behavioral, and analytical chemistry experiments were conducted pertaining to development of techniques and methodology for controlling damage caused by ricefield rats (*Rattus rattus mindanensis*) and vampire bats (*Desmodus rotundus*). The subject areas are listed along with a brief statement of accomplishments:

1. Ricefield rat pheromones. Female urine, preputial gland exudate, and vaginal washes for different stages of estrus were tested for attractant strength to male rats. The only material that was reliably preferred ($P < 0.05$) over deionized water was proestrus urine. Females showed no reliable preference for male urine, preputial gland exudate, or hair regardless of their stage of estrus when tested. It appears that for the ricefield rat, proestrus urine is the only potent olfactory attractant of the potential sources tested and its effect on toxic bait consumption needs further examination.

2. Constituents of rat preputial gland exudate. Investigations were continued to characterize rat preputial gland exudate, a possible source of pheromones. Twelve female rats were sampled twice in each of the four stages of estrus, and the resulting gas chromatograph "fingerprints" were compared for changes in component concentration. Two peaks changed more than the others with a maximum concentration occurring during proestrus. Duplicate fingerprints from twelve males did not reveal any significant inter-animal differences. Composite samples from male and female preputial gland exudate were analyzed by gas chromatography-mass spectrometry. Usable spectra were obtained on about 38 peaks from either sample, 25 of which were the same in both sexes.

3. Semifield preference test for ricefield rats. A standard bait consumption preference test intermediate between the laboratory and field situation has been developed. This semifield, closed colony test uses a converted grain storage tank sectioned into four enclosures, each containing five ricefield rats. When four rice bait preparations were evaluated simultaneously in the enclosures for

8 days, the preference pattern was stable and the sensitivity of the test compared favorably with laboratory results for individually caged animals. The usefulness of the semifield test for routine evaluation of other candidate attractants was thus demonstrated.

4. A ricefield rat attractant extracted from rice. The knowledge that prior sensory experience is important in food preference behavior provided the guidelines for developing a promising attractant for the ricefield rat. A volatile fraction, one of eight rice fractions tested, has been found to consistently enhance bait consumption both in a series of laboratory preference tests and in a semifield evaluation.

5. Visual sensitivity in vampire bats. To explore the possible usefulness of visual stimuli in controlling vampire bat attack, bats were conditioned to interrupt operant responding whenever a low-level light stimulus appeared. The bats had a low visual threshold (lowest light level reliably detected) and were thus comparable in sensitivity to other nocturnal predators that primarily depend on visual stimuli for prey detection. Therefore, extremely low-level moving visual stimuli may have potent attractive value to vampire bats.

6. Diphenadione residue studies in cattle and vampire bats. Studies were conducted to determine if there is a human or environmental hazard associated with treating cattle and vampire bats with diphenadione. Approximately 0.14 ppm of diphenadione was measured in livers and 0.08 ppm in kidneys of six beef cattle 90 days after treatment (1.0 mg/kg) but none was detected in heart, muscle, fat, brain, and blood plasma. Control beef liver and livers of treated cattle were fed to laboratory rats for 14 consecutive days. No mortality occurred and there was no significant difference in prothrombin clotting time of treated and control rats. No residue was detected in milk of three cows given an oral dose (2.75 mg/kg) of diphenadione and also in bat carcasses at 24, 72, and 120 hours after consuming 1.0 mg/kg of diphenadione. Results indicate that diphenadione presents no hazard when used as a livestock systemic for vampire bat control.

7. Livestock injection technique for control of vampire bats. In a cooperative field experiment with the Vampire Bat Research Station, Palo Alto, Mexico, 207 head of cattle on three adjoining ranches in Mexico were given single intraruminal injections of 1 mg/kg diphenadione. Fresh bites fell from 214 before treatment to 15 about 2 weeks after dosing. Chi-square analysis showed that the 93% reduction was highly significant ($P < 0.01$) and that the treatment was equally effective at the three ranches. The cattle showed no ill effects from the treatment. The technique is scheduled to be used throughout Latin America for reductional control of vampire bats.

REPRODUCTION

Reproductive physiology experiments were conducted with coyotes, rats, and birds to gain information that would lead to methods of controlling damage caused by the species.

Fecundity of a Norway Rat Colony with 85 Percent of the Males Sterile. A manuscript titled "Influence of Sterile Males on Fecundity of a Rat Colony" has been published. Eighty-five percent of the males in one of two colonies of Norway rats were surgically sterilized. The control and treated colonies produced 130 and 110 young, respectively. The greatest difference noted was in the number of pregnant juveniles at necropsy; nine were pregnant in the control colony but only three in the treated colony. The total number of pregnancies was 38 for the controls and 39 for the treated rats. The results suggest that an antifertility compound exerting an effect similar to vasectomy and affecting many, but not all, of the males in a rat colony would be ineffective in limiting population size.

Evaluation of an Antifertility Compound, DRC-4801 (SC-20775), in Norway and Polynesian Rats. A new synthetic estrogen (DRC-4801) was tested on Norway and Polynesian rats. Females of both species showed a significant ($P < 0.05$) increase in the interval between pairing with males and parturition. A high incidence of sterile offspring was observed among litters of Norway rats nursing on mothers treated at parturition with a single oral (gavage) dose of DRC-4801; the proportion of young so affected was dose dependent. Colony studies involving Polynesian rats offered DRC-4801 treated bait at 2-week intervals for 10 weeks showed a significant ($P < 0.05$) reduction in both the number of pregnancies and total number of implanted embryos. The rats showed increasing aversion at each subsequent baiting period to the treated bait.

Microencapsulation of Antifertility Compounds, Polynesian Rat. A filler, Avicel, and an encapsulating material, food grade shellac, were combined with two antifertility compounds, diethylstilbestrol and mestranol. Each encapsulated antifertility compound was mixed with rat food and fed to a group of 20 female Polynesian rats. There was no significant difference in acceptance between either compound and a control food. It is suspected that the encapsulation method was not coating all of the antifertility compound.

Observations on the Reproduction of Wild-Trapped Polynesian Rats. Female Polynesian rats were (1) examined daily by vaginal smears to determine the length of the estrous cycle, (2) placed in activity wheels to monitor activity as a function of changes in the estrous cycle, and (3) paired to determine if postpartum estrus or delayed implantation occurred. It was determined that the Polynesian rat has an average estrous cycle of 3.9 days, a postpartum estrus, and delayed implantation if more than one nursing young is present.

Spermatogenic Cycle in Polynesian Rats. A study of the spermatogenic cycle and duration of epididymal sperm transport in the Polynesian rat has been completed. An estimation was made of (1) the duration of one cycle of the seminiferous epithelium: 14.1 days; (2) the mean duration of spermatogenic stages 1 to 14: 1.7, 0.9, 0.6, 0.4, 1.1, 0.7, 3.2, 1.3, 0.3, 0.5, 0.8, 0.9, 0.7, and 0.8 days, respectively; and (3) the time required for epididymal sperm transport: 18 days.

Sterilization of Male Red-winged Blackbirds. A cooperative study begun in 1971 with the Section of Birds was continued during the 1972 nesting season. Male red-winged blackbirds breeding in isolated cattail marshes were bilaterally surgically sterilized. When 100, 50, and 9 percent of the males in a marsh were sterilized, batches of eggs laid within the territories of the sterilized males were 12, 33, and 100 percent fertile, respectively.

ELECTRONICS

Areas of electronic instrumentation development are listed and described below.

1. Multiple-Use Wildlife Transmitter. A radio-frequency transmitter that contains digital integrated circuits has been developed into a versatile wildlife research tool. The transmitter has an electronic printed circuit board to facilitate fabrication and to allow for a convenient means of altering the operation such that parameters of temperature, mortality, location and diurnal activity may be monitored either individually or collectively. Packaging volume and weight of a transmitter designed to operate two years on a coyote are approximately 64 cubic centimeters and 265 grams, respectively.

2. Mobile, Elevating Platform with Antenna Rotor. An elevating platform which can be mounted on most types of vehicles was adapted to serve as a basic mechanism for a mobile tracking antenna system. The hydraulic elevating mechanism weighs about 200 pounds and raises to a height of 7 feet from a collapsed profile of 7 inches. A variable-speed motor-driven antenna rotor is mounted on the platform with a remote azimuth meter. The control panel cabled through a window of the vehicle allows the total operation of the platform and rotor to be conducted while seated in the vehicle.

3. Wildlife Transmitter Designs. Prototype designs for miniature transmitters were developed to increase battery life and improve reliability of the circuitry. These advancements are reflected by the manner in which the duty cycle (relationship between the pulse rate and pulse width) is controlled. Four designs are now available depending upon specific weight and volume limitations.

4. Transmitters for Detecting the Mortality of Sheep. A transmitter was developed to detect sheep mortality that utilizes digital-logic, integrated circuits. The transmitter emits rf signals when either of two conditions exist. First, if the logic circuit does not sense the animal's movement for approximately 3 hours, the mortality alarm is initiated. A second provision for turn-on is a delayed timing mechanism that can be programmed to switch at any time within an 8-month period. Thus, a stray live animal can be located by the delayed turn-on feature.

BIRD DAMAGE CONTROL RESEARCH

DEVELOPMENT OF CHEMICALS

Initial testing of compounds for repellent activity yielded no new compounds with activity greater than existing standards. Initial tests were completed on coturnix to determine the most effective and economical laboratory conditions for evaluating male avian chemosterilants. Twenty-two compounds were evaluated on coturnix using the recommended procedures; eight chemicals induced sterility for varying periods of time, two of which appeared to be permanent.

Advanced testing primarily involved gathering additional data on DRC-736, with numerous studies conducted to determine toxicity and repellency to additional bird species as well as preparing formulations for treating rice and pecan seeds. Some limited studies were conducted with chemosterilants on wild birds, primarily to develop techniques and to determine the comparability of laboratory results and small-scale field trials.

The comparative toxicity of 17 pesticides to three species of birds was investigated. Results indicated that toxicity to one species can often be used to indicate toxicity to another species.

Plant and soil studies with DRC-1327 continued. The results indicated that soil type, organic matter, and pH influenced degradation as did soil temperature and moisture content. Adsorption to soil colloids also makes DRC-1327 and its metabolites relatively unavailable for plant absorption. Plant studies indicated that DRC-1327, if available to sorghum roots, can be translocated to seeds; however, this appears highly unlikely, for the reasons as indicated above. This work concludes the DRC-1327 soil research.

Plant and soil studies with DRC-736 were initiated to provide additional data necessary for registration. A perfusion system was developed for studying the degradation of DRC-736 in soil and water. Results of a preliminary study using this system indicated rapid hydrolysis of the parent material in a slightly alkaline loamy sand.

A number of field support tests were conducted, principally involving the development of semiquantitative techniques for determining spray deposits of DRC-736 and its metabolites on fruit. Two germination tests of rice and marigold seeds treated with DRC-736 indicated no phytotoxicity. Two studies were also conducted to find an acceptable physiological marker that could be fed to wild birds to identify them 6 months to 1 year later. None of the three sudan dyes nor a protein-bound iodine appear to be suitable.

From studies conducted under this work unit, seven papers were published, four were submitted to journal editors or were in press, and eight others were in preparation in 1973.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

UNIT OF PHARMACOLOGY - Peter J. Savarie, Walter A. Bowles, Jr., Ralph W. Dutton, and Gary J. Dasch (January-June)

I. CHEMICAL EVALUATION ACTIVITIES

1. REPELLENTS

A. Introduction

The initial bioassay test using either wheat or Douglas-fir seeds as a food source for deer mice (Peromyscus maniculatus) is designed to give an indication of the repellency and/or toxicity of candidate chemicals which might have potential use in control of vertebrate pest species. Individually caged mice are offered 25 treated wheat seeds or 50 treated Douglas-fir seeds each night for 3 consecutive nights. Chronic effects are then observed for 4 days. Laboratory rat chow and water are available at all times. Mortality is noted daily and percent seed reduction is calculated.

B. Results, Discussion, and Conclusions

Fifty (50) chemicals were evaluated, but results and comments will be presented on only 12 in this section (Table 1). These 12 chemicals were selected because they have diverse chemical and biological characteristics.

It had been suggested that DRC-6094 and DRC-6095, which are registered organophosphorus insecticides, might have utility as vertebrate pest control agents. The known toxicology would have been helpful if these chemicals had been active. However, the results obtained indicated that neither chemical was sufficently active to warrant further studies as a rodenticide for bait preparations, or as a repellent.

DRC-6079 is a chlorinated thioamide and represents a new class of potentially active repellents. As compared to wheat seeds, its reduced repellency on Douglas-fir seeds was disappointing, but not unexpected. Generally speaking, this chemical has two good repellent characteristics (high repellency - wheat seeds, and low toxicity) and chemicals related to it will be tested as they are supplied by the contributor.

DRC-6077 is a fermented egg product and Center interest in it developed when it was found to be an effective compound for attracting coyotes. It did not deter deer mice from eating wheat seeds nor did it appear to pose any serious toxic effects. Toxicological data supplied by the manufacturer also indicates that its toxicity is minimal.

DRC-6068, ANTU, has been considered as a possible substitute for endrin as a seed protector. Based on the data in Table 1 and under the conditions this test was conducted, it would appear that ANTU is not a good candidate seed protector.

TABLE 1. Initial bioassay data in deer mice.

DRC	Wheat seeds, 2%		Douglas-fir seeds, 2%		Acute oral LD_{50}, mg/kg (95% confidence limits)
	% seed reduction	Mortality	% seed reduction	Mortality	
6094	86	1/5	nt	-	nt
6095	44	0/5	nt	-	891*
6079	94	0/5	17	0/5	> 400
6077	7	0/5	nt	-	> 1,600
6068	52	3/10	18	0/10	142(47-424) M
					142(20-1,006) F
5999	nt	-	0	0/5	nt
6081	nt	-	2	0/5	820(569-1,179)*
5592	86	0/5	43	0/5	> 480
5593	97	1/5**	49	0/5	> 480
6059	71	0/5	nt	-	> 480
6060	91	0/5	63	0/5	> 480
6076	77	0/5	nt	-	> 480

* Rat LD_{50}, data supplied by manufacturer.

** Death of animal probably not related to exposure to chemical.

nt - Not tested.

DRC-5999 and DRC-6081 are both bitter chemicals and DRC-6081 is reported to be at least 200 times as bitter as quinine. Toxicity of both compounds is minimal and so is their repellency on the initial bioassay test. These two chemicals point out that bitterness, as detected by man, does not produce a physiological insult strong enough to deter deer mice from eating seeds.

DRC numbers 5592, 5593, 6059, 6060, and 6076 are compounds which are capable of producing a sensation of burning. According to one source, the burning taste of DRC-6076 can be detected in 1 part per 100,000. The detection limits for the other compounds are not known. As a group, the chemicals capable of producing a burning taste are interesting because they have good repellency and low toxicity. Further tests will be conducted with them.

A preliminary potentiation study was conducted with DRC-5999 and DRC-5593 (Table 2). The combined effect of DRC-5999 and DRC-5593 was 17% greater than the algebraic sum of their individual effects. The practical significance of this data has not been ascertained. Additional tests will be conducted to determine if the increase in repellency of the combination is due to an increase in toxicity. Mortality data suggest that it is not.

TABLE 2. Initial bioassay test, Douglas-fir seed potentiation.*

Treatment	No. of animals	Seeds consumed		% seed reduction	Mortality
		Control	Test		
DRC-5999, 2%	10	1,496	1,184	21	0/10
DRC-5593, 2%	10	1,388	821	41	0/10
DRC-5999, 2% + DRC-5593, 2%	10	1,476	315	79	0/10

* Deer mice of either sex used. For 3 consecutive nights animals offered 50 seeds per night (control). The next 3 consecutive nights animals offered 50 treated seeds per night (test).

C. Literature Reviewed

Kverno, N. B., and G. A. Hood. 1963. Evaluation procedures and standards--chemical screening and development for forest wildlife damage. BSFW, 59 pp.

2. PHYSIOLOGICAL MARKERS

A. Introduction

Last year's report contained preliminary data on DRC-6058, a fluorescent marker, and DRC-6061, an iodinated compound. DRC-6058 is expensive ($5-$10/gram) and a search was made for inexpensive substitutes.

As indicated last year, one problem in the use of DRC-6061 was the lack of a method to determine the iodine content from microliter samples of blood. Mr. Ken Hawkins (Section of Mammals) has developed a microdetermination for iodine which has helped immensely in the study of iodinated chemicals.

B. Results and Discussion

Fluorescent markers:

Fifteen analogs of DRC-6058 have been tested for their marking ability on the pelage of deer mice. Only two (DRC-6082 and DRC-6090) were capable of producing a mark which could be identified 14-30 days post-exposure to 2% treated wheat seeds. Literature searches on the toxicity of these two chemicals suggest that they would not pose any serious toxicological effects to animals.

Iodinated chemicals:

In an initial probe conducted in cooperation with the Section of Mammals, 0.1 and 1.0 mg/kg of DRC-6061 were administered by gavage to eight coyotes (four animals per dose). Forty-nine days postgavage, three out of four coyotes administered 0.1 mg/kg had blood iodine levels approximately 2-4 times normal. At 1.0 mg/kg, the iodine content in three out of four coyotes was approximately 2-5 times normal after 133 days. More information on DRC-6061 is found in the Section of Mammals 1972 Annual Progress Report.

C. Conclusions

Separate study plans covering both fluorescent markers and iodinated chemicals are being initiated, and a full report on their uses as physiological markers should be issued next year.

3. RODENTICIDES

a. DRC-3992 and DRC-4575: EPA single-dose rodenticide protocol.

A. Introduction

The EPA single-dose rodenticide protocol is designed to test the efficacy of candidate chemicals. Individually caged wild Norway rats (*R. norvegicus*) were offered two food cups; one contained 50 g of treated bait, the other 50 g of untreated bait. At 24, 48, and 72 hours, bait consumption was calculated and cups replenished with fresh bait. Cup positions were alternated every 24 hours. After 72 hours, test baits were removed and animals given lab chow and observed for 5 days. A chemical "passes" the test when the total mortality is 90% or greater. The bait (EPA carrier) was 65% cracked corn, 25% rolled oats, 5% corn oil, 4.85% powdered sugar, and 0.15% cornstarch.

B. Results and Discussion

According to the criteria for this test, DRC-3992 did not pass, but DRC-4575 did (Table 3A). In an attempt to increase mortality with DRC-3992, 50 g of treated bait containing 3% DRC-3992 (0.15% active ingredient), 47% dried bread crumbs, and 50% hamburger was tried. The second cup contained 50 g of EPA carrier. Mortality was increased, but not enough to meet the EPA criteria (Table 3B). Presumably, the consumption of DRC-3992 was greater with the hamburger bait. However, accurate weigh backs could not be made because of severe dehydration of the hamburger.

Amount of bait consumed for the data depicted in Table 3A is shown in Table 4. With DRC-3992, animals consistently ate more of the untreated bait; but after the first 24-hr interval, some animals were so sick that neither of the baits were consumed. There was little aversion to the DRC-4575 treated bait and animals consumed enough to produce 95% mortality during the first 24-hr test interval.

C. Conclusions

On the EPA single-dose rodenticide test, DRC-4575 was found to be more effective than DRC-3992. Company approval has to be obtained before additional tests are conducted with DRC-4575. Additional tests will be conducted with DRC-3992 in an attempt to increase its effectiveness.

TABLE 3. EPA rodenticide protocol test results for DRC-3992 and DRC-4575.

DRC	% active ingredient	Male mortality	Female mortality	Total mortality
		A.		
3992	0.15	0/10	5/10	5/20 - 25%
4575	1.0	10/10	10/10	20/20 - 100%
		B.		
3992	0.15	4/10	6/10	10/20 - 50%

b. Microencapsulated DRC-3901.

A. Introduction

DRC-3901 is an acute rodenticide specific to members of the genus Rattus. From an environmental standpoint, it is probably the safest chemical to use. However, it produces bait shyness and for that reason has never achieved widespread use. To circumvent bait shyness, DRC-3901 has been microencapsulated by its manufacturer. Acute oral LD_{50}'s and preliminary feeding bioassays have been conducted with microencapsulated DRC-3901 against wild Norway (R. norvegicus) and ricefield rats (R. r. mindanensis).

B. Results and Discussion

The microencapsulated DRC-3901 was very toxic to both species of rats tested (Table 5). There was no difference in LD_{50} values between the microencapsulated chemical and the technical grade material that was tested in previous years. It would appear that all of the enteric coating on the chemical breaks down, thereby making the chemical readily available for absorption.

TABLE 4. Amount of bait consumed by *R. norvegicus* on EPA test.

		Test interval					
		0-24 hr		24-48 hr		48-72 hr	
DRC	Sex	T	U	T	U	T	U
3992	F	1.57 (0.1-4.5)* (10)**	4.84 (0.0-16.0)	0.18 (0.0-0.4) (6)	2.3 (0.0-13.4)	0.38 (0.3-0.5) (6)	4.7 (0.0-15.0)
	M	2.24 (0.4-5.1) (10)	7.3 (0.2-18.3)	0.14 (0.0-0.4) (10)	5.35 (0.0-19.8)	0.26 (0.0-0.4) (10)	8.25 (0.0-19.0)
4575	F	2.35 (0.8-3.5) (10)	2.4 (0.2- 7.9)	0.9 (1)	1.7	—	—
	M	3.59 (1.5-8.2) (10)	2.18 (0.0- 6.6)	—	—	—	—

* Mean (range), grams.

** Number of animals per test interval.

T = treated bait; U = untreated bait.

TABLE 5. Acute oral toxicity of microencapsulated DRC-3901*.

Species	Sex	Lot No.	Animals per dose x doses tested	LD_{50}, mg/kg (95% C.L.)
R. norvegicus	F	AC 675-4:1	2 x 3	12.5(6.3-25.0)
R. r. mindanensis	F	676-10:1	3 x 4	35.4(18.4-68.0)
R. r. mindanensis	F	AC 675-4:1	3 x 4	21.0(12.5-35.4)

* Protocol: Adult animals fasted 4-6 hr before dosing. Preparations of DRC-3901 suspended in 0.5% gum tragacanth. Animals observed 7 days after dosing.

Feeding test results are shown in Table 6. Basically, the results are encouraging, although it is difficult to logically account for the exceedingly high LD_{50}'s consumed by the two surviving ricefield rats.

C. Conclusions

Initial test results with microencapsulated DRC-3901 appear very promising and with the cooperation of its manufacturer, future testing will be done to aid in the development of this product.

c. New class of rodenticides: Structurally related carbamates.

A. Introduction

Three chemicals submitted by a contributor were claimed to be relatively selective acute rodenticides. Acute oral toxicity to target and nontarget species, and efficacy to target species have been the primary tests conducted with these three chemicals.

TABLE 6. Feeding test results with microencapsulated DRC-3901*.

Species	No. tested and sex	Lot No.	% A.I.	LD_{50}'s consumed	Mortality
R. norvegicus	5 F	676-10:1	1.0	7.9**	5/5
R. norvegicus	5 F	AC 675-4:1	1.0	6.3	4/5
R. r. mindanensis	5 F	676-10:1	1.0	5.2	4/5
R. r. mindanensis	5 F	AC 675-4:1	1.0	22.6***	3/5

* Protocol: Adult animals were singly caged and had free access to lab chow and water at all times. For 3 consecutive nights animals prebaited with 10 g of bait (90% oatmeal, 5% vegetable oil, 4.85% powdered sugar, 0.15% cornstarch). Consumption of this bait averaged 9.6-9.8 g per rat per night. Animals then offered 10 g of bait containing 1% DRC-3901 for 3 consecutive nights. Animals observed for 7 days after last exposure to DRC-3901.

** Acute oral LD_{50} not determined for R. norvegicus with Lot No. 676-10:1. LD_{50} for Lot No. AC 675-4:1 used in calculating the LD_{50}'s consumed.

*** LD_{50}'s consumed for three rats that died = 8.0.
LD_{50}'s consumed for two rats that survived = 44.6.

B. Results and Discussion

The oral toxicity of these chemicals to various animals is shown in Table 7. When compared to the data reported by the company, there is a big discrepancy between our results on Norway rats and albino mice with DRC-6027. Acute oral LD_{50}'s (mg/kg) submitted by the company for Norway rats range from 16-21, and for albino mice the LD_{50} was 13. No apparent reason(s) for this big discrepancy are known. The deer mouse LD_{50} of 35 reported by the company compares very favorably with our results. The LD_{50} of DRC-6027 to birds is greater than 500, and the company reported LD_{50}'s in chickens and pigeons being greater than 4,000.

TABLE 7. Acute oral toxicity of three candidate rodenticides to animals.*

Common name	Sex	Acute oral LD_{50} (mg/kg)**		
		DRC-6027	DRC-6092	DRC-6091
Albino rat	M	> 480	nt	nt
Wild Norway rat	F	> 120	nt	nt
Albino mouse	M	> 960	nt	nt
Deer mouse	M	30.0(24.0-37.5)	22.3(-)	87.2(-)
	F	26.0(15.3-44.2)	31.7(15.9-63.3)	87.2(-)
Gerbil	M	nt	3.8(1.9-7.5)	43.7(-)
Bobwhite quail	M,F	> 500	90***	> 500
Starling	M,F	> 500	> 500	male died, female survived
Red-winged blackbird	F	> 500	nt	nt
House sparrow	M,F	> 500	F 356*** (M > 500)	> 500
Brown-headed cowbird	M,F	> 500	M 178***	> 500
Coturnix quail	M,F	nt	90***	> 500

* Dosing in birds conducted with cooperation of Edward Schafer, Section of Birds.

** All animals observed for 14 days.

*** Estimated LD_{50}.

nt - Not tested.

The toxicity of DRC-6092 was found to be broader than originally thought, and the company has dropped it from their rodenticide development program.

Our results for DRC-6091 are in agreement with the company and additional toxicity studies will be conducted with it.

Efficacy of the three chemicals on the initial bioassay test against deer mice is shown in Table 8.

TABLE 8. Initial bioassay results of three candidate rodenticides in deer mice.

	Wheat seeds			Douglas-fir seeds		
DRC	Conc.	% seed reduction	% mortality	Conc.	% seed reduction	% mortality
6027	1	55	100	2	2	20
	0.5	39	90	1	1	20
	0.25	77	60	-	-	-
	0.1	2	10	-	-	-
6092	0.5	67	100	nt	-	-
	0.1	20	80	-	-	-
	0.05	4	0	-	-	-
6091	1.0	58	100	nt	-	-
	0.5	32	60	-	-	-
	0.25	4	20	-	-	-
	0.1	0	0	-	-	-

nt - Not tested.

Efficacy of DRC-6027 in two free-choice feeding tests with female wild Norway rats is shown in Table 9. No explanation is available to account for the difference in mortality between the two tests. Both tests have produced good mortality with other chemicals. Differences in feeding behavior may have been a factor, but in-depth studies would be required to find the actual explanation.

TABLE 9. Mortality results of DRC-6027 in two free-choice feeding tests with female wild Norway rats.

	Test 1 - tableted lab chow*		Test 2 - oatmeal**
mg/kg chemical eaten	First night	Second night	First night
Mean (range)	253 (7.1-550)	161 (0-402)	135 (59-200)
Mortality***	1/5		5/5

* For 2 consecutive nights, five individually caged rats were offered two cups each containing 20 g of tableted rat chow. Consumption from each cup was recorded daily, and on the third night, 20 g of tablets containing 2% DRC-6027 were placed in the "preferred" cup and 20 g of untreated tablets in the second cup. Positions of the treated and untreated cups were alternated the second test night.

** Animals were singly caged and had free access to lab chow and water at all times. For 3 consecutive nights animals prebaited with 10 g of untreated bait (90% oatmeal, 5% vegetable oil, 5% glycerol). Consumption of this bait averaged 9.8 g per rat per night. Rats then offered 10 g of bait containing 1% DRC-6027.

*** All deaths occurred after first night exposure. Survivors observed for 14 days.

Center interest in DRC-6027 was drastically diminished when the Twin Falls field station reported that it was secondarily toxic to raptors. This secondary toxicity is detailed in the Section of Mammals 1972 Annual Progress Report.

C. Conclusions

Of three new rodenticides submitted by a company, only one, DRC-6091, is still being evaluated as a candidate rodenticide. Because one chemical in this series produced secondary poisoning, tests with DRC-6091 will proceed with cautious optimism.

d. DRC-5976: Highly specific rodenticide.

A. Introduction

In the 1971 Annual Progress Report, some initial test data were given for DRC-5976, a chemical which was being investigated as a rodenticide. Acute oral toxicity tests with 12 species of birds and mammals, and feeding tests with three rat species have been concluded.

B. Results and Discussion

The acute oral LD_{50} of DRC-5976 was determined in the following animals: bobwhite quail, pigeon, starling, blackbird, sparrow, domestic rabbit, deer mouse, gerbil, ricefield rat, wild Norway rat, albino Norway rat, Polynesian rat, and albino mouse. Only wild and albino Norway rats were susceptible, with mean LD_{50}'s (mg/kg) of 8.4 (male), 8.9 (female) for wild, and 11.9 (male) for albino. The LD_{50} was 354 for male Polynesian rats, 205 for male albino mice, and greater than 120 in female ricefield rats. In all other animals tested, the LD_{50} was greater than 100, usually greater than 500.

The efficacy of DRC-5976 was determined in wild Norway, Polynesian, and ricefield rats in bait concentrations of 0.125-2%. No mortality occurred in Polynesian and ricefield rats. There was 80% mortality in wild Norway rats at the 2% level.

C. Conclusions

DRC-5976 appears to have desirable properties as a raticide for Norway rats. However, to develop one chemical for use against a single species would probably be an uneconomical venture, and tests with DRC-5976 have been suspended. Preliminary tests with this chemical were reported in Nature 241:551, 1973.

II. INFORMATION STORAGE AND RETRIEVAL DEVELOPMENTS

4. CHEMICAL DATA STORAGE AND RETRIEVAL

A. Introduction

The introduction is documented in the 1968 Annual Progress Report.

B. Results and Discussion

Progress was made in converting the present data storage retrieval system--which uses the chemical fragmentation concept in describing chemical compounds by citing functional groups, their location, and extent in a molecule--to the Wiswesser Line-Formula Notation (WLN) system.

This is the notation scheme the Unit selected to encode all chemical structures for the Denver Wildlife Research Center's data storage and retrieval system. It is a method for expressing the molecular structure of a chemical compound by an alphanumeric notation.

We participated in a cooperative effort between this Center, the U.S. Army Industrial Liaison Office, the U.S. Army Vegetation Control Laboratories and the Chemical Notation Society. As a result of this effort, we received a card deck of the 4,000 compounds screened as repellents by Patuxent plus a printout of the results. The results will be used for Appendix II in the structure correlation publication that is being sponsored by the U.S. Army Natick Laboratories.

We also arranged to obtain a deck of 2,000 compounds encoded into the WLN and an alphabetized printout of the U.S. Food and Drug Administration's list of the Generally Recognized As Safe (GRAS) compounds. This deck combined with the one we have from the cooperative activity described above, represents 6,000 compounds which are in this Center's data bank.

Several organizations handling data at both the disseminating and producing levels, contacted us asking for information on our procedures, results, and programs. All were interested in engaging in joint actions for mutual benefits.

C. Conclusions

Some preliminary discussions were held with the Center Biometrician to explore the methodology required to achieve the conversion to the WLN and adopt a data bank in-put format useful for Center-wide applications.

A general conclusion is that representatives from all disciplines that are potential users of and contributors to a Center data storage and information retrieval system should be selected to review, modify, and eventually approve it for use. In this manner, the system, as it is developed, should have optimum accession and usage with minimum details not essential to those engaged in wildlife research problems.

III. BIOLOGICAL ACTIVITY AND CHEMICAL STRUCTURE CORRELATION

5. STRUCTURE ACTIVITY RELATIONSHIPS

A. Introduction

The introduction is documented in the 1968 Annual Progress Report.

B. Results and Discussion

The final phases of the correlation study on those compounds screened at the Patuxent Wildlife Research Center using the food acceptance bioassay method have been completed. The publication is scheduled for printing by the U.S. Army Natick Laboratories by mid-1973.

Dr. John J. Pratt, Jr., sponsor from the Natick Laboratories, visited us during June this year. At this time he expressed his willingness to continue support of this project's activity dealing with preparation for publication of the results on those compounds evaluated by the concentration-effect method as developed by this Center for measuring rodent repellency.

As a result of his continued interest in completing the second publication, some of the companies that had supplied candidate compounds were contacted verbally to obtain an approval for listing their compounds in the publication. All indicated that they would have to take such a request under advisement and would have to seek an opinion from their legal, chemical, and public relations departments. This represents a reversal of cooperation and a change in authorization attitudes since 1964 when permission was solicited for 4,600 compounds and granted on 4,000.

C. Conclusions

Before too much time and effort is devoted to the concentration-effect publication, the absolute number of compounds authorized by the manufacturers for publication must be established. The results on the compounds appraised in this testing procedure are qualitative for evaluating chemicals as rodent repellents for packaging materials. Their effectiveness in terms of concentration estimated to repel any given percentage of a rodent population is developed by a regression line in addition to deriving an R_{50} value.

Therefore, if the number of compounds released for publication is restricted to the point where there are not sufficient compounds with representative functional groups available for comparisons, then there can be no significance to any conclusions made on a limited group used to correlate chemical structure and repellency. The initial effort will be made to contact all suppliers for approval and then review the total number and types of compounds released before preparing the publication.

PHYSIOLOGY AND BEHAVIOR - R. D. Thompson, S. A. Shumake, R. W. Bullard, S. E. Gaddis, G. Holguin, and K. Crane

1. RICEFIELD RAT PHEROMONES

Introduction

Odors of biological origin that have pheromone activity offer a potential means of increasing baiting effectiveness in rodent control programs by attracting rats to the baits and increasing bait consumption. This study was conducted to compare attractant strength of four odors that have potential pheromonal activity.

Samples of urine, vaginal washes, and preputial gland exudate were collected from female ricefield rats in each of the four estrus stages and pooled into 12 samples for testing with male rats. Each pooled sample was divided into 20 aliquots and frozen for testing. Each odor sample was tested on 20 male ricefield rats in individual cages with a four-choice, in-cage preference tester. Position of odors and a deionized water control were systematically rotated and the sequence of testing samples was counter-balanced.

This same procedure was used to test the response of female rats to male odors, with the exception that hair taken from the base of the tail of male rats was substituted for the vaginal wash. Female rats were each tested four times, once at each stage of estrus. Elapsed time and visitation frequency were recorded for 30 minutes after exposure to odors.

Results and Discussion

Results of both studies are shown in Tables 1 and 2. There was a large amount of variability in elapsed time response of male rats to female odors. Over 80% of the male rats chose the odor of proestrus urine and it was significantly preferred ($P < 0.05$) to the control. Female rats showed no preference for any of the odors over the control. If more females were tested, significant differences might be detected; however, it is obvious that none of the odors tested had much potency for attracting females.

2. CONSTITUENTS OF RAT PREPUTIAL GLAND EXUDATE

Introduction

This study was concerned with the chemical composition of preputial gland exudate. Females were compared for gas chromatogram differences in

TABLE 1. Elapsed time response (seconds) of male ricefield rats exposed to female odors (Mean ± SE)

Stage of estrus of female sample	n	Odor source			
		Preputial gland	Urine	Vaginal wash	Water (control)
Diestrus	20	19.8 ± 6.5	10.6 ± 4.7	9.2 ± 3.0	23.7 ± 8.1
Proestrus	20	19.3 ± 8.4	100.5 ± 80.9*	49.5 ± 43.4	13.2 ± 8.1
Estrus	20	12.2 ± 3.2	59.5 ± 44.3	24.5 ± 10.1	29.9 ± 13.7
Metestrus	20	30.7 ± 11.8	7.6 ± 2.3	18.2 ± 5.9	7.5 ± 3.1

* P(test sample > water control) < 0.05.

TABLE 2. Elapsed time response (seconds) of female ricefield rats exposed to male odors (Mean ± SE)

Stage of estrus of females tested	n	Odor source			
		Preputial gland	Urine	Hair	Water (control)
Diestrus	16	27.4 ± 8.9	26.8 ± 14.8	76.8 ± 55.3	65.9 ± 44.8
Proestrus	14	27.2 ± 7.1	45.0 ± 24.0	28.9 ± 9.4	28.2 ± 8.3
Estrus	13	18.3 ± 5.2	17.9 ± 3.2	12.2 ± 3.6	28.9 ± 11.8
Metestrus	16	21.3 ± 5.1	54.2 ± 42.7	22.1 ± 4.7	20.7 ± 5.7

relation to stage of estrus. Male chromatograms were examined for among animal differences. Constituents from composite male and female samples were also compared.

Results and Discussion

A total of 96 chromatograms (two from each of 12 animals for the four stages of estrus) of female preputial gland exudate were compared for estral changes in component concentration. Two peaks were found to vary in peak height, being lowest in diestrus and highest in proestrus and estrus. An incidental observation was that these components changed with the light cycle from being minor peaks under continuous lighting to major peaks under a 14 hour light-10 hour dark cycle.

Male preputial chromatograms revealed a substantial among animal variation. The higher molecular weight compounds were more constant and a three-peak series (same retention time as a three-peak series in female) was constant enough to serve as an internal standard for comparison of relative peak heights in the chromatogram.

Usable spectra were obtained for about 38 peaks in the gas chromatography-mass spectrometry analysis of the male and female composite samples. Retention time and mass spectral correlation confirmed that 25 peaks were the same in both male and female preputial gland exudate. The results of the experiment have provided background information that will make any future attempts at isolation and identification a much easier task.

3. SEMIFIELD PREFERENCE TESTS OF POTENTIAL BAIT ENHANCERS

Introduction

Semifield preference tests were conducted on four rice formulations with five ricefield rats in each of four 10- by 10-ft enclosures. Each enclosure contained four feeding stations designed to allow simultaneous feeding by all rats; to prevent contamination from urine and feces; and to prevent spillage of food. A feeder containing one of the four formulations was located in each corner of each enclosure. Water and "cover" were placed in the center of the enclosures. Each day, the feeders were systematically rotated to counteract position habits.

Results and Discussion

The results are summarized in Table 3. Consumption of rice treated with rice flavor averaged almost six times that of whole grain rice. Consumption

measures of granulated rice and soybean-oil-treated rice were two and three times greater, respectively, than that of whole grain rice. The reliability of the preference profiles indicates that the method has utility for routine screening of other candidate bait enhancers.

TABLE 3. Rice bait consumption of five ricefield rats each in four enclosures (Mean ± SE)

	Bait			
Day	(A) Whole grain rice (g)	(B) Granulated whole grain rice (g)	(C) Granulated whole grain rice treated with trapped rice volatiles (g)	(D) Granulated whole grain rice treated with 1.0% soybean oil (g)
1	8.1 ± 1.7	12.9 ± 2.7	35.1 ± 3.2**	22.8 ± 2.9
2	6.6 ± 2.1	8.9 ± 1.6	35.1 ± 3.2*	21.9 ± 2.3
3	5.3 ± 2.0	15.5 ± 2.7	35.9 ± 3.3**	25.9 ± 2.5
4	6.4 ± 2.4	14.6 ± 2.9	34.4 ± 2.8**	16.4 ± 3.0
5	5.8 ± 2.0	12.0 ± 2.3	34.6 ± 2.6**	19.8 ± 3.0
6	4.5 ± 1.7	15.6 ± 2.6	36.8 ± 3.1***	21.6 ± 2.6
7	5.5 ± 2.6	18.9 ± 2.4	35.8 ± 2.7**	15.0 ± 2.2
8	7.0 ± 2.8	19.1 ± 3.3	32.4 ± 1.7**	21.9 ± 3.0

* $P(C > A) < 0.10$
** $P(C > A) < 0.05$
*** $P(C > A) < 0.01$

4. A RICEFIELD RAT ATTRACTANT EXTRACTED FROM RICE

Introduction

There is a need for development of highly palatable baits to achieve greater effectiveness in baiting programs for the control of urban and agricultural rodents. In this context, one of the major goals of the Center's AID research program is to develop highly preferred bait carriers for reductional control of ricefield rats (*Rattus rattus mindanensis*). Most bait materials previously tested have been novel to the animal and have often produced poor results. Knowing that prior experience influences preference behavior, we attempted to determine if certain constituents from a familiar food (rice) have attractant qualities.

Eight constituents of rice (bran oil*, acetonitrile extract of bran oil*, ether extract of brown rice*, endosperm, polish, bran, volatiles of bran and volatiles of ground rice) were formulated on granulated ground brown rice. Preference was determined between the treatment and a granulated rice control by an apparatus and procedure developed at this laboratory for brief-exposure, foods-together testing. The volatiles fraction of ground rice (rice volatiles) was the preferred treatment.

A two-choice overnight consumption test was then conducted for comparison of preference results for ground rice volatiles with the brief-exposure foods-together test. This procedure was then used to test the ability of rice volatiles to improve acceptability of a marginally palatable ground rice-cellulose formulation. In a fourth test, we tested the olfactory preference response for the rice volatile odor. Finally, a semifield evaluation was conducted to test the colony preference behavior for a choice of rice volatiles trapped on granulated rice, whole grain rice, untreated granulated rice, and granulated rice treated with 1% soybean oil.

Results and Discussion

The results of the initial test are presented in Table 4. In data analysis by the "t" test, only the whole grain rice volatiles treatment was better than the control ($P < 0.01$) for both eating time and consumption measurements. This distinction provided the justification for subsequent testing.

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* Two concentrations were tested.

TABLE 4. Ricefield rat preference data for a choice between the treatment and granulated ground brown rice containing 25% Avicel

Treatment[1]	Percent total eating time	Percent total food consumption
2% Rice Bran Oil	39.7 ± 12.7	38.8 ± 11.4
0.2% Rice Bran Oil	46.7 ± 6.6	48.8 ± 2.9
2% Rice Bran Oil Acetonitrile Extract	54.0 ± 8.8	49.7 ± 5.4
0.2% Rice Bran Oil Acetonitrile Extract	46.1 ± 2.4	48.2 ± 4.7
2% Ground Brown Rice Ether Extract	52.9 ± 5.6	45.3 ± 3.7
0.2% Ground Brown Rice Ether Extract	52.4 ± 5.7	48.9 ± 4.3
50% Endosperm	51.7 ± 6.0	56.0 ± 5.1
50% Rice Polish	56.7 ± 4.02	52.1 ± 13.5
50% Rice Bran	61.8 ± 10.9[2]	55.8 ± 8.2
Trapped Rice Bran Volatiles	51.1 ± 8.9	47.1 ± 3.6
Trapped Whole Rice Volatiles	68.5 ± 19.0[2]	65.3 ± 20.0[2]

[1] Remainder of formulation is ground brown rice.

[2] Significant difference obtained at the 0.01 level of probability.

The results of the two-choice overnight consumption test agreed with the initial test. In four replications with 12 animals (switching treatment positions each test) the "t" test indicated that the rice volatiles were significantly preferred ($P < 0.01$) over the granulated rice control. In the third test it was demonstrable that rice volatiles significantly increase acceptance of a marginally palatable food ($P < 0.01$). In the odor test, rats showed a consistent preference for the odor of trapped volatiles over the odor of untreated granulated whole grain rice. This occurred whether or not the rats had been recently fed rice.

The semifield evaluation of the rice volatiles treatment indicated that consumption was increased six-fold over that of whole grain rice, and was approximately doubled over that of untreated granulated rice or granulated rice treated with 1% soybean oil. This preference pattern remained highly stable throughout the 8 test days, indicating that novelty effects were not operating to any extent.

Both the earlier observations on individually caged animals and the semifield evaluation indicated that trapped rice volatiles markedly and consistently enhance bait consumption by ricefield rats. The same principle and procedure is potentially useful in developing attractants for other species and/or habitats.

5. VISUAL SENSITIVITY OF VAMPIRE BATS (*DESMODUS ROTUNDUS*)

Introduction

The visual sensitivity of two vampire bats was measured by an operant conditioning technique in an effort to gain insight into the importance of vision in prey detection. The bats were conditioned to suppress operant licking for blood reinforcer when a brief light stimulus flash was paired with electric foot shock. Different light intensities above and below visual threshold were randomly presented according to the method of constant stimuli.

Results and Discussion

Mean threshold values for two replications were 6.58×10^{-7} and 3.49×10^{-7} millilamberts. These values are close to the range of visual sensitivity of other nocturnal predators. It appears, therefore, that vampire bats are capable of using visual information for prey location and general orientation.

6. DIPHENADIONE RESIDUE STUDIES IN CATTLE AND VAMPIRE BATS

Introduction

In the 1971 Annual Progress Report we reported the development of a highly effective livestock systemic method for reductional control of vampire bats (*Desmodus rotundus*) in Latin America. Three residue studies were conducted in 1972 to (1) determine if there is any safety hazard involved in consuming meat and milk of cattle dosed with diphenadione and (2) conduct whole body analysis of bats dosed with diphenadione and sacrificed at various time intervals after dosing.

For meat residue analysis, six beef steers were dosed with diphenadione (1.0 mg/kg, intraruminal) and slaughtered 30, 60, and 90 days postdosing. Samples of blood plasma, liver, kidney, brain, muscle, and fat were analyzed for diphenadione and the livers were bioassayed with laboratory rats. In a second experiment three cows, each nursing a newborn calf that had no other food source, were treated with diphenadione (2.75 mg/kg). Milk or blood samples were collected at various time intervals for diphenadione and prothrombin time determinations.

In the third study, whole body residue analysis was conducted on six vampire bats--two at 24, two at 72, and two at 120 hours after consuming 1.0 mg/kg of diphenadione (approximately three times the LD_{50} dose).

Results and Discussion

Diphenadione content of liver, kidney, heart, muscle, fat, brain, and blood plasma of treated beef yearlings is shown in Table 5 and bioassay data on beef liver is summarized in Table 6. Prothrombin response of nursing calves consuming milk of cows treated with diphenadione is shown in Table 7.

Detectable levels of diphenadione were found in kidney ($\approx$ 0.08 ppm) and liver ($\approx$ 0.14 ppm) but none was detected in heart, brain, muscle, or fat. The fact that laboratory rats are extremely sensitive to diphenadione (chronic LD_{50} = 0.2 mg/kg) and that rats consumed the equivalent of 1.4 kg of liver per 70 kg human for 14 consecutive days without mortality or significant change in prothrombin time is strong evidence that no safety hazard is involved in consuming meat of cattle treated with diphenadione.

TABLE 5. Mean diphenadione (ppm) of liver of treated beef yearlings

Days posttreatment	Liver	Kidney	Heart	Muscle	Fat	Brain	Blood plasma
30	0.14	0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
60	0.14	0.10	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
90	0.15	0.08	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

TABLE 6. Bioassay of beef liver with rats

Description	No. of rats	No. of feeding days	$\bar{X}$ liver consumption (kg/70 kg human per day)	Rat mortality	Rat prothrombin clotting time (sec) at end of feeding period *
Control liver	15	14	1.37	0/10	15.4
Liver, 30 days postdosing	10	14	1.37	0/10	25.3
Liver, 60 days postdosing	10	14	1.37	0/10	16.8
Liver, 90 days postdosing	10	14	1.37	0/10	15.6

* Value based on 5 rats.

TABLE 7. Residue analysis and prothrombin response of lactating cows treated with diphenadione and prothrombin response of their nursling calves

Hours postdosing	Diphenadione in milk (ppm)	Mean ± SE (seconds)	
		Treated cows	Nursling calves
0	< 0.01	13.5 ± 1.5	14.4 ± 2.4
12	< 0.01	13.5 ± 1.5	14.4 ± 2.4
24	< 0.01	15.2 ± 0.73	14.2 ± 1.0
48	< 0.01	17.1 ± 2.48	14.1 ± 1.4
72	< 0.01	21.1 ± 0.83	14.1 ± 1.2
96	< 0.01	21.8 ± 2.45	13.4 ± 1.3
120	< 0.01	22.6 ± 3.10	13.8 ± 1.4
144	< 0.01	19.2 ± 1.79	13.8 ± 1.6

Diphenadione was not detected in milk of treated cows, and prothrombin time of calves feeding exclusively on milk of treated cows did not change. This indicates an insignificant intermammary transfer of the anticoagulant. The gas chromatographic resolution, however, was not satisfactory and interpretation of chromatograms was difficult. A more comprehensive study is being planned. Diphenadione was not detected in bats 24, 72, or 120 hours after they had consumed 1.0 mg/kg of diphenadione. Therefore, there appears to be no environmental hazard involved in using diphenadione as a livestock systemic for vampire bat control.

7. LIVESTOCK INJECTION TECHNIQUE FOR CONTROL OF VAMPIRE BATS (*DESMODUS ROTUNDUS*)

Introduction

An experiment was conducted at three adjoining ranches in the state of San Luis Potosi, Mexico, in February 1972 to test the effectiveness of injecting cattle with diphenadione to reduce vampire bat biting. In

the experiment 207 head of cattle (cows, bulls, and calves) were treated with 1.0 mg/kg of diphenadione by intraruminal injection. The number of fresh vampire bat bites were counted on each animal 30 days prior to treatment, immediately prior to, and approximately 2 weeks after treatment.

Results and Discussion

Diphenadione was very effective in reducing the number of bites. A total of 214 fresh bites were counted on 207 head of cattle prior to treatment, but only 15 on 215 head after treatment, which is a 93% reduction in biting. Chi-square analysis showed that the magnitude of bite reduction is highly significant ($P < 0.01$) and that the treatment was equally effective at the three ranches. No ill effects were exhibited by the cattle following the treatment. Details of the experiment are published in Science, 1 September 1972, Volume 177, pp 806-808.

REPRODUCTION - James J. Kennelly, Melvyn V. Garrison, Brad E. Johns, and Jerry D. Roberts

Personnel Changes

In August 1972, Mr. Melvyn V. Garrison began a period of leave for additional education at West Virginia University. He will return to the Denver Center in May 1973.

Dr. James J. Kennelly, Leader, Unit of Reproductive Studies, transferred in January 1973 to the position of Assistant Unit Leader with the Massachusetts Wildlife Cooperative Unit, University of Massachusetts, Amherst. At the date of this transfer, the Unit of Reproductive Studies will be terminated and the remaining personnel reassigned.

RODENT REPRODUCTIVE STUDIES

A. Fecundity of a Norway Rat Colony with 85 Percent of the Males Sterile

Published:

Kennelly, J. J., B. E. Johns, and M. V. Garrison. 1972. Influence of sterile males on fecundity of a rat colony. J. Wildl. Mgmt. 36(1):161-165.

B. Evaluation of an Antifertility Compound, DRC-4801 (SC-20775), in Norway and Polynesian Rats

Introduction

The work with DRC-4801 was reported in the 1971 Annual Report. This report deals with the effect of DRC-4801 in regards to: (1) delaying the onset of pregnancy, (2) the induction of sterility in offspring nursing on treated mothers, and (3) controlling the ultimate size of closed colony populations.

Results and Discussion

A manuscript titled "Antifertility Effects of SC-20775 in Norway and Polynesian Rats" has been approved by Washington for publication. It is expected that this manuscript will be published in the Journal of Mammalogy in 1973.

C. Microencapsulation of Antifertility Compounds, Polynesian Rat

Introduction

Bait aversion is a major problem in attempting to control rodent populations. Microencapsulation of the control agent contained in the bait is a potential means of reducing or eliminating bait aversion. One of the many available methods of microencapsulation was used by Abbott Laboratories, a cooperator in the study, to encapsulate two female reproductive inhibitors that were known to be poorly accepted by Polynesian rats (*Rattus exulans*).

Methods

The method used to microencapsulate the reproductive inhibitors required that a filler be mixed with them so that the final microencapsulated particle would be composed of 1 percent active ingredient. A preliminary test determined the acceptance by the Polynesian rat of various types of fillers and encapsulating materials. The fillers and encapsulating materials were fed in the normal ration of the rat, and there was no significant difference in consumption between the normal diet and those containing fillers coated with encapsulating materials.

The filler Avicel (Microcrystalline cellulose) and an encapsulating material, food grade shellac, were selected for the second part of the study. The estrogenic reproductive inhibitors diethylstilbestrol

and mestranol were combined with Avicel and food grade shellac to produce a microencapsulated material containing 1 percent estrogen and having a particle size of approximately 180 microns.

Twenty forest-trapped female Polynesian rats were used with each estrogen to test bait acceptance. Two methods of combining the estrogens with the bait were used. In Method 1, the microencapsulated estrogen was mixed uniformly with the bait (ground lab chow containing 20% dried molasses, granulated to approximately 800 microns). In Method 2, the reproductive inhibitor was dissolved in ether and mixed with the bait. The ether was then removed from the bait with a flash evaporator.

Five treatments (Table 3) of each reproductive inhibitor were tested with a switchback experimental design (Lucas, 1956) (Table 4). The rats were fed control ground lab chow for 2 days before the start of the study and for 5 days between each of the three feeding periods. Daily food weighbacks were taken.

TABLE 3. Bait treatments

Treatment	Percent of estrogen in bait	Form of estrogen
1	0.0	-
2	0.005	Microencapsulated
3	0.01	Microencapsulated
4	0.005	Ether coated
5	0.01	Ether coated

TABLE 4. Experimental Design[a]

Rat No.	1	2	3	4	5	6	7	8	9	10
Feeding Period One[b]	1[c]	2	3	4	5	1	2	3	4	5
Feeding Period Two	2	3	4	5	1	3	4	5	1	2
Feeding Period Three	1	2	3	4	5	1	2	3	4	5

[a] The design was replicated with rats numbered 11-20.
[b] Each feeding period was 2 days.
[c] Treatments offered to a rat.

Results and Discussion

With each estrogen, the control treatments were significantly different from the other four treatments containing estrogen (Table 5). However, there were no significant differences between any of the four treatments containing estrogen in either study. It is possible that the estrogens were not completely coated with the encapsulating agent and therefore the rats were able to taste them. However, the rats may have rejected the encapsulated estrogen treated bait for physiological reasons other than taste, and further studies will be needed to answer this.

D. Observations on the Reproduction of Wild-Trapped Polynesian Rats

Introduction

Basic reproductive information was obtained on the Polynesian rat (*Rattus exulans*) to aid in control of this pest species by reproductive means.

Methods

Female Polynesian rats were checked by vaginal smear daily to identify the length of the estrous cycle. Another group of females were placed in activity wheels, and the number of revolutions were

TABLE 5. Bait consumption

Percent of estrogen in the bait	Mean rat consumption per feeding period (gms)	
	Diethylstilbestrol	Mestranol
0.0	9.15 [a]	11.12
0.005 microencapsulated	0.26	0.34
0.01 microencapsulated	0.93	1.86
0.005 ether coated	1.73	1.80
0.01 ether coated	1.27	0.81

[a] Means connected by a line are not significantly different.

recorded daily. Day-to-day variations in the recordings are known to correlate with a rat's estrous cycle. Additional females were paired with males, and data was gathered to determine whether the rats had a postpartum estrus and delayed implantation of ova.

Results and Discussion

Vaginal smears were unreliable in determining the length of the estrous cycle. The rats are very nervous, and the daily handling seemed to disrupt the rats so that they would not evidence an estrous cycle. The activity wheel provided better data with six females showing an average estrous cycle of 3.9 days with a range of 2-6 days.

Postpartum estrus occurs in the Polynesian rat within 48 hours of littering. Delayed implantation of ova occurs if there are two or more young nursing the female. The length of gestation, determined from 46 females, was 22.1 days, and the mean number of young per litter was 3.8.

This study is being prepared for publication as a note in 1973.

E. Spermatogenic Cycle in Polynesian Rats

Introduction

A study of the spermotogenic cycle and duration of epididymal sperm transport was undertaken as part of a major study of the reproductive patterns of Polynesian rats. The general objective was to establish criteria whereby the selection and evaluation of male antifertility compounds could be accomplished. The specific objectives of this phase of the work were (1) to define the duration of one cycle of the seminiferous epithelium, (2) to define and estimate the duration of the stages of the spermatogenic cycle, and (3) to establish the time required for epididymal sperm transport.

Results and Discussion

The duration of one cycle of the seminiferous epithelium was calculated to be 14.1 days. The seminiferous epithelium travels through four cycles of 14.1 days, or a total of 56.4 days, to produce a spermatozoa that is ready to be released from the testis into the epididymis for further maturation. The mean duration of each of the 14 spermatogenic stages within one 14.1-day cycle of the seminiferous epithelium are: 1.7, 0.9, 0.6, 0.4, 1.1, 0.7, 3.2, 1.3, 0.3, 0.5, 0.8, 0.9, 0.7, and 0.8 days, respectively. The time required for epididymal sperm transport is 18 days.

Adding the 56.4 days needed for sperm development in the testis to the 18 days required for epididymal sperm transport equals a total of 74.4 days required from the start of sperm development until their availability for fertilization. These values, when combined with the data on the length of each spermatogenic stage, will enable (1) evaluation of where and when a particular reproductive inhibator exerts its action and (2) prediction of when a rat will become infertile following treatment.

II. AVIAN REPRODUCTIVE STUDIES

A. Sterilization of Male Red-Winged Blackbirds

Introduction

The cooperative study begun in 1971 with the Section of Birds was continued. The potential of using male reproductive inhibitors to control red-winged blackbird populations was studied by

sterilizing male red-winged blackbirds via bilateral vasectomy and observing whether any fertile clutches were produced on the males' territories. The 1971 Annual Progress Report for the Unit of Reproductive Physiology contains the methods used in this study.

Results and Discussion

The results of the study conducted in the breeding season of 1972 are contained in the Annual Progress Report of the Section of Birds for 1972. The 2-year study is being prepared for publication in 1973.

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ELECTRONICS - A. L. Kolz, G. W. Corner, and R. E. Johnson

I. MULTIPLE-USE WILDLIFE TRANSMITTER

A. Introduction

Recent developments in low-voltage, integrated circuits (IC's) have been adapted for use with radio-frequency wildlife transmitters. In particular, the use of complementary-symmetry/metal-oxide-semiconductors (COS/MOS) are advantageous because of their low power requirements and cheaper costs. Transmitter production time has been reduced and the reliability increased through the use of IC's.

B. Results and Discussion

Results of this work are described in the Bureau of Sport Fisheries and Wildlife Special Scientific Report - Wildlife No. 163 in 1973 (in press).

II. MOBILE, ELEVATING PLATFORM WITH ANTENNA ROTOR

A. Introduction

Radio-tracking a highly mobile bird or animal requires a refined antenna receiving system. The antenna height in marginal reception areas is critical and every effort must be made for an optimum elevation. Our mobile, elevating platform supplemented with an accurately controlled antenna rotor now provides a system capable of quickly obtaining fixes on a moving transmitter.

B. Results and Discussion

The commercially available elevating platform that was used as the basic mechanism for the mobile antenna system is manufactured by the Weaver Division of the Dura Corporation, Springfield, Illinois. The system is designed to elevate warning lights above an emergency vehicle with a total lifting capacity of 60 pounds. Maximum current ratings are 40 amperes during elevation and 31 amperes when collapsing.

The lifting capacity of the platform is more than adequate for antenna systems used for wildlife tracking at 164 megahertz. We could, therefore, add the weight required for a motor-driven antenna rotor and associated control. A reversible, variable-speed, d-c motor rotates the

antenna mast with torque derived through a 400:1 gear reducer. The antenna mast is a 1-inch-diameter aluminum pipe mounted vertically in two bearing plates above the raising platform. The mast is easily removed so that different antenna configurations can be affixed.

The control panel for the system is cabled through a window of the vehicle and provides a complete remote control. The operator can elevate, collapse, or stop the platform at any point. The rotor is designed to sweep continuously through an arc of 450° or it can be put in a single sweep mode where the operator controls the start and stop. The antenna azimuth is read on a 270° meter movement mounted on the control panel. The radio-frequency signals are cabled into the vehicle separately from the control system through coaxial cables.

III. WILDLIFE TRANSMITTER DESIGNS

A. Introduction

Miniature radiotransmitters built with Colpitts oscillators have higher energy requirements because the pulse forming circuitry is not independent of the radio-frequency (rf) circuit. Therefore, additional circuitry was added to a transmitter to control the pulse duty cycle.

B. Results and Discussion

The ideal design for a pulsing wildlife transmitter is to have the pulse forming circuitry completely independent of the radio-frequency (rf) circuit. Unfortunately, to conserve weight and volume, this isolation is not possible with simple transmitter designs.

Three methods have been developed to improve the pulse generator isolation. The first method actually utilizes a modified multivibrator to drive the rf circuit. Two additional components are required for this change plus an increase in size for some other component parts. The second type of circuit utilizes a feedback loop between the rf circuitry and the pulse generator. Again, only two additional components are required for this feedback. The third circuit development applies a COS/MOS integrated circuit (IC) and provides a nearly optimum isolation. The disadvantages of this IC circuit are that it requires a minimum of

3 volts and weighs about four times (6 grams) more than our miniature transmitters. A 65-gram transmitter with a radiating output power of about 2 milliwatts (including potting, attachment, and a battery having a 90-day life expectancy) has been developed with this IC pulse generator for use on pelicans.

IV. TRANSMITTERS FOR DETECTING THE MORTALITY OF SHEEP

A. Introduction

Research scheduled in 1973 will require a means for detecting and classifying the causes of death in a sheep flock. Therefore, a transmitter design was developed for production in 1973 which will provide the field biologist with a remote mortality sensor. The transmitter is designed to turn on whenever the logic circuit senses a prolonged cessation in the instrumented animal's movements.

B. Results and Discussion

Two COS/MOS, integrated circuits each containing 4-dual input NOR gates form the basis of the mortality sensing instrumentation. The actual motion sensor is a miniature mercury switch that is activated through the normal movements of an animal. The transmitter is held inoperative as long as the switch is activated within every 3-hour period. Should this time interval be lapsed without a switch closure, the rf transmitter will turn on either pulsing or continuous depending upon the logic circuit configuration. Normally, the pulsing mode is used since it conserves the battery energy.

A second timing mechanism designed into these transmitters overrides the mortality circuit and can activate the pulsing transmitter after a programmed delay. This delay can be set for any time extending to about 8 months, and can be reset on a transmitter so that it can be used repeatedly.

The sheep mortality transmitters are designed for fabrication with a printed circuit board in order to decrease the production time and maintain uniformity. The electronics circuit and antenna will be enclosed in a plastic case containing a watertight access port for battery replacement. The weight of the completed package will be about 150 grams with a volume of approximately 130 cubic centimeters.

BIRD DAMAGE CONTROL RESEARCH

DEVELOPMENT OF CHEMICALS - Edward W. Schafer, Jr., Robert I. Starr, Ronald B. Brunton, Donald J. Cunningham, Norman F. Lockyer, and other personnel in the Section of Birds

INTRODUCTION

Birds cause major damage to agricultural crops in the United States, create hazards to military and civilian aircraft, and create health and nuisance problems in many urban areas. Although a variety of methods have been tried to reduce some of these problems, little use of effective, safe, and economical chemicals has been made. Research is therefore needed to develop the chemical approach, especially in the field of nontoxic repellents, reproductive suppressants, and fright-producing agents.

Although considerable information has been published on bird repellents, few, if any, have proven to be effective when used in damage situations covering large areas. Recent breakthroughs in field test design and the continued success with DRC-736 has allowed an expanded program in this area. Temporary permits are expected for seed corn and feed lots during 1973.

The use of reproductive suppressants is becoming widely accepted as a potential technique for managing animal populations. Reproductive suppressants have been extensively evaluated on insects and mammals and a number of compounds have also been examined for domestic fowl; however, only a few studies have been conducted on wild birds. In programs of other researchers, the major emphasis to date has been on finding compounds that inhibit some phase of the reproductive cycle in females. Perhaps a more logical approach in many strongly territorial migratory species is to direct efforts at discovering compounds that would be effective on males.

The use of chemical fright-producing agents is a recent approach to controlling damage by birds. These compounds either produce unique behavioral actions in birds, which elicit a fright response from other members of their flock and, as a result, treated areas are evacuated; or immobilize birds, allowing their removal from damage or nuisance situations. Our most promising compound (DRC-1327) was registered in April 1972 for use on ripening corn, and temporary permits or registration are expected in 1973 for sweet corn, grain sorghum, and pecans.

Toxicant research has been de-emphasized.

A four-step development program is being used to discover and register compounds for reducing damage by birds: (1) initial testing on red-winged blackbirds (reproductive suppressant studies use coturnix initially); (2) advanced testing on target and related species; (3) preliminary field studies with candidate materials; and (4) plant translocation and degradation, and leaching-degradation studies in soils of

compounds of proven utility that are pending registration. Due to the increasing concern of the Bureau and the new public awareness regarding chemical contamination of the environment, the fourth step in our development program has been expanded to provide answers on the safe use of potential bird damage control compounds on our lands and waters. Procedures used in conducting these four steps are reported in Annual Progress Reports F-9.1, F-9.2, F-9.3 from 1961 through 1967, and in DF-102.2 from 1968 to present.

RESULTS, DISCUSSION, AND CONCLUSIONS

I. PHARMACOLOGY, TOXICOLOGY, AND FORMULATION CHEMISTRY

A. Initial Testing

1. Repellents

Of 109 chemicals evaluated for repellency using red-winged blackbirds, 12 compounds had R_{50}'s* of between 0.100% and 1.000% and 2 compounds had R_{50}'s below 0.100% (Table 1). One compound, DRC-5586, was partially repellent at all concentrations tested from 0.100% to 1.000% but an R_{50} could not be determined. Ninety-four compounds were inactive at 1.000%. The two chemicals that were repellent below 0.100% were also highly toxic to redwings, and therefore neither was considered for further evaluation. It is interesting to note that the most repellent compounds continue to be the most highly toxic. This reinforces our belief that some form of intoxication is usually necessary to produce acceptable repellency in birds.

2. Reproductive Suppressants

All four phases of the study described in Study Plan Outline DF-102.2-23 have been completed. A brief summary of the Report of this study is as follows:

Six chemicals having known chemosterilant activity were evaluated on male coturnix quail with testes in various stages of development using single acute oral treatments. Of the six compounds, DRC-3225, DRC-4800, and DRC-5537 were essentially inactive at the levels tested (Table 2). The

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* The calculated level at which 50% of the birds are repelled.

remaining three compounds (DRC-1806, DRC-5533, and DRC-5535) all alklylating agents, produced transient infertility or sterility in one or more of the test groups representing four different phases of testicular development (Table 2). As a result of these studies, we feel that initial evaluation of potential chemosterilants on adult breeding male coturnix, followed by a secondary test (if necessary) on adult male coturnix with testes artificially regressed by photoperiod regulation will provide the most economical and effective evaluation of compounds proposed for use on wild avian populations during the period of testicular recrudescence. Evaluations using 12-day-old male chicks and 6-week-old males with testes undeveloped (due to photoperiod deprivation) were more variable and did not approximate the results obtained on adult males with artificially regressed testes.

During the year, 16 additional compounds were evaluated on adult-breeding male coturnix as a preliminary test for potential chemosterilization activity when given in single acute doses (at 50%-75% of an LD_{50}). The compounds selected included many that have shown acute chemosterilization activity in male insects or mammals. Of the 16 chemicals, none produced permanent sterility at the levels tested although five induced partial or complete sterility for 1 to 3 weeks (Table 3).

A complete discussion of the activity of 8 of these 16 compounds is contained in Technical Report DF-102.3-40.

3. Fright-producing Agents

No new chemicals were tested as fright-producing agents on redwings during the reporting year.

B. Advanced Testing

1. Repellents

a. DRC-736.--Laboratory studies continue to indicate that DRC-736 is highly repellent to most species of birds. Studies this year have been directed at determining acute toxicity to species not previously tested, comparing the learned behavior of six species and conducting formulation studies to aid field applications.

(1) Acute oral toxicity.--The acute oral toxicity of DRC-736 was determined on the following species; starling, 24.2 mg/kg; house finch, 2.42 mg/kg, white-crowned sparrow, 4.21 mg/kg; and golden-crowned sparrow, 3.16 mg/kg.

(2) Comparative repellency.--A study of the comparative repellency of DRC-736 and DRC-156 (thiram) was completed on six species of birds involved in agricultural damage. The test species were starlings, robins, grackles, cowbirds, house sparrows, and white-crowned sparrows. Treatments of 0.075% DRC-736 and 0.178% DRC-156 were compared during a 7-week period using standard bird-repellency bioassay procedures. The test was divided into 3 periods; 2 weeks pretreatment, 3 weeks treatment, and 2 weeks posttreatment. Each test week consisted of two 18-hour testing periods separated by 3- or 4-day rest periods.

DRC-736 treatments of 0.075% reduced food consumption by the six species by an average of 84% during the treatment period. Reductions ranged from 70.4% for starlings to 92.6% for house sparrows. Reduction in food consumption among all species during the treatment period was progressive with food intake in the third week about 50% of that of the 3-week average. Intake of untreated food during posttreatment period indicated a considerable difference in the response of the six species. Starlings returned to pretreatment levels almost immediately; house sparrows and cowbirds to 35-50% of pretreatment levels; and grackles and robins continued to feed at less than 10% of pretreatment levels. White-crowned sparrows were severely stressed by test conditions and only 1 of 20 birds survived the treatment period.

Food intake during the treatment period for the six species exposed to 0.178% DRC-156 was reduced by an average of only 23%, but varied greatly among species. Food consumption by white-crowned sparrows and grackles was not lowered by the treatment. Food consumption by house sparrows, starlings, and robins was reduced 25-50% during treatment, but immediately returned to pretreatment levels in the posttreatment period. Only cowbirds were markedly repelled by DRC-156 treatments,

food consumption during treatment decreased by 66% and recovered to about 50% of pretreatment levels during posttreatment.

This study showed that DRC-736 was superior to DRC-156 as a repellent when birds were repeatedly exposed to treated food. It also showed that various species apparently differ in their abilities to detect and identify contaminants in palatable foods, or in their abilities to break learned behavior patterns.

A detailed report of this study, including some yet unfinished portions will be available in mid-1973 as Technical Report DF-102.2-44.

(3) Formulation for seed rice.--A formulation containing 0.5% DRC-736-75% WP and 0.25% Dow Latex was found to provide an effective coating for seed rice and was not adversely affected by soaking in water. The treatment had no effect on germination and provided adequate repellency when tested on caged redwings. The details of this study can be found in Technical Report DF-102.2-37.

(4) Formulation for pecans.--Dipping pecans in 5% and 10% DRC-736 - Rhoplex-methocel solutions provided concentrations of 0.30 and 1.77% DRC-736 on individual pecans. Extrapolation of these data indicated that dipping in a 6% DRC-736 solution should provide an approximate treatment level of 0.5%. Although only a small sample of seeds were available for germination studies, it did not appear that a treatment of 0.30% or 1.77% DRC-736 reduced germination. The details of this study can be found in Technical Report DF-102.2-36.

2. Reproductive Suppressants

a. Development of male chemosterilants.--Advanced programs in developing reproductive suppressants have been limited to methodology development. During the reporting year, two small studies were completed.

(1) Effects of testosterone proprionate on redwings.--Aviary held male red-winged blackbirds dosed with 6.8 to 22.1 mg/kg testosterone proprionate during April showed no change in the rate of testicular development when compared to sham-treated birds.

The details of this study appears in Technical Report DF-102.2-43. This was the first of a number of studies planned to investigate laboratory methods of inducing testicular development in redwings during the nonbreeding season.

(2) Photoperiod stimulation of testicular growth.--Studies were initiated to determine the effects of photoperiod stimulation on redwings and starlings testes growth during the December-April period and will be reported in Technical Report DF-102.2-46 in mid-1973.

(3) Acute toxicity of DRC-5535 to starlings.--The acute oral LD_{50} of DRC-5535 to starlings was 287 mg/kg (130-632 mg/kg, $p = 0.05$). On the basis of these data, the dose selected to sterilize male starlings was approximately 150 mg/kg. The details of this study are contained in Technical Report DF-102.2-39.

(4) Effects of DRC-5535 on starlings in nest boxes.--Subsequent to the collection of data above, a small-scale field trial of DRC-5535 was initiated on male starlings using nest boxes on the Denver Federal Center. Males were captured during March and April, orally dosed with 150 mg/kg DRC-5535 and allowed to nest and renest under close observation. No behavioral effects were noted, and reproduction was minimally impaired; however, the evaluation technique proved to be feasible. More detailed information on this study can be found in Study Plan Report DF-102.3-31.

C. Comparative Toxicity Studies

During the past decade, this unit has amassed a great mass of data on the toxicity of chemicals to birds. During many of our investigations, we have noted a consistent tendency for some species to show more susceptibility to chemical intoxication than others. Much of this information is being made available through publications; however, there appears to be limitations as to how far one should go in predicting toxicity to one species from data on another species. The following study was conducted to better delineate sensitivity of several species involved in damage on several continents.

1. The Comparative Toxicity of 17 Pesticides to Quelea, House Sparrows, Red-winged Blackbirds, and Rats

The results of these tests are summarized in Table 4. The average po (per os) LD_{50} for the 17 pesticides was 2.3 mg/kg for quelea, 4.4 for house sparrows, 1.9 for redwings, and > 231 for rats. Six of the chemicals had been selected for testing on quelea because they showed considerably higher toxicity to birds than to mammals (> 100x). The average po LD_{50} in mg/kg for these six was 2.5 for quelea, 5.4 for sparrows, 2.2 for redwings, and > 604 for rats. The toxicity of the remaining 11 chemicals averaged 2.1 mg/kg for quelea, 3.7 for sparrows, 1.8 for redwings, and 28 for rats. Thus, even if chemicals selected for differential toxicity are eliminated, the three species of birds, on the average, were 8 to 16 times more easily poisoned than rats.

Ratios between the quelea LD_{50} and the LD_{50}'s of the other three species indicated that quelea were approximately as sensitive as house sparrows, and about 12.5 times as sensitive as rats to po administration of the 11 nonselected chemicals, and about 3 times as sensitive as sparrows to dermal administration. Quelea LD_{50}'s for the 17 pesticides were compared with those of the other species by regression analysis. Only two comparisons had correlation coefficients significantly different from zero ($P < 0.01$): quelea po with redwing po, and quelea dermal with house sparrow dermal. For both relationships, the correlation coefficient was 0.98. The details of this study are presented in Report of Study DF-102.2-34, and the results have been prepared for publication.

I. PLANT PHYSIOLOGY AND SOIL CHEMISTRY

A. Soil and Plant Studies

1. DRC-1327 (4-aminopyridine)

Previous soil leaching studies with 4-aminopyridine (1971 Annual Progress Report DF-102.2) have shown the chemical to be adsorbed strongly to alkaline and acidic soils, and therefore resistant to leaching. These residues, in instances of repeated bait application or misuse treatment levels, could accumulate in certain soils and become absorbed by future crops planted in the treated areas. Thus, the degradation of 4-aminopyridine in soils is a key factor as to whether the compound and/or metabolites

would be present in sufficient quantities to be absorbed and translocated by plants.

This work concludes the 4-aminopyridine soil research, the results of which have been incorporated into a manuscript which is presently awaiting publication.

Anticipating future submission of a registration petition for 4-aminopyridine in grain sorghum, translocation and degradation studies were completed during this reporting period, and manuscripts have been prepared for publication.

a. Degradation in soil systems.--Metabolism studies concerning the effects of clay content, organic matter, and pH on the extent of breakdown of ^{14}C-4-aminopyridine, demonstrated that increasing the organic matter by a factor of 1.7 in two acidic loam soils (both containing 16% clay), increased the rate of degradation of the soil-applied chemical to radiolabeled carbon dioxide by a factor of 2.8. Conversely, an increase in the clay colloidal content increased the surface area available for adsorption of the chemical, which, in turn, reduced the rate of degradation.

There was essentially no breakdown (0.35%) of the chemical to radiolabeled carbon dioxide in a highly acidic (pH 4.1) loam soil.

An increased rate of metabolism of the chemical occurred in two loam-loamy sand soils as the temperature and moisture were increased, with the extent of breakdown being less in the soil containing the larger amount of clay.

Half-life values were determined for the chemical in six different soils maintained at 30° C and 50% moisture for a 3-month period. These values are shown in Table 5.

The distribution of radioactivity in six soils after a 3-month incubation period is illustrated in Table 6. This work further demonstrates the high degree of adsorption of 4-aminopyridine to acidic soils, with only 0.5% of the applied radioactivity being extracted from a highly acidic loam soil.

In view of these metabolism studies, a scheme is suggested for the breakdown of this compound in soil systems. The

first metabolite produced, i.e., 4-hydroxypyridine, would likely be adsorbed rather strongly to soils and would in turn be relatively unavailable for plant absorption. Once the heterocyclic ring is ruptured, the aliphatic compounds formed would be closely related to those normally present in a biological system.

b. Absorption and translocation in 3-month-old sorghum.--Previous studies (1971 Annual Progress Report) regarding the absorption and translocation of ^{14}C-4-aminopyridine in older sorghum plants showed trace amounts of the radiolabeled material (0.01 to 0.09 ppm) were detected in the intact heads of 3-month-old sorghum plants nurtured 1 week in solutions containing 5 ppm of the labeled compound.

An additional study was conducted in which the seeds from treated 3-month sorghum heads were removed from the head and analyzed separately. Vegetative tissues were also combusted and analyzed for translocated radioactivity by liquid scintillation spectrometry. Generally a higher ^{14}C activity was detected in the seeds than in the stripped head itself, i.e., rachis, branches, and racemes (Table 7). The amount of radiolabeled 4-aminopyridine translocated into the vegetative tissues of 3-month-old sorghum was found to be inversely proportional to plant age, with the majority of radioactivity present in the lower 8-inch sections of the plants (Table 8).

Although this work demonstrated that, if available to sorghum roots, the chemical and/or metaboliten could be translocated to the individual seeds, it is very unlikely that the compound would be detected in sorghum heads under field conditions due to the adsorptive characteristics of soils.

2. DRC-736 (methiocarb)

In view of the interest in methiocarb as a corn seed treatment and as a protectant for fruit crops, it is important to determine the fate of the parent compound and certain of its sulfoxide and sulfone metabolites in soil, water, and fruit tissues to ascertain its safety in the environment.

a. Development of a perfusion system for soil-water studies.--In "tooling up" for a study involving the metabolism of ^{14}C-methiocarb and its primary metabolites in soil and water systems, an all-glass perfusion or percolation system was developed to enable the ^{14}C-labeled parent material and metabolites (including radiolabeled carbon dioxide) to be collected and measured under both aerobic and anaerobic conditions under controlled environmental situations. Using these all-glass closed perfusion units, the metabolism of a given chemical can be followed in either soil or water. Aqueous solution perfused through a treated soil falls back into a collection reservoir for recycling, thus permitting air or nitrogen to be bubbled through an ethanolamine scrubber for removal of carbon dioxide.

b. Degradation in soils.--A pilot study was conducted to evaluate the rate of breakdown of methiocarb in soil under aerobic conditions using the perfusion system.

A slightly alkaline loamy sand soil was treated in triplicate with 100 ppm of ^{14}C-methiocarb labeled in the carbonyl carbon position (specific activity 1.7 mc/mM). Distilled water was added to each collection reservoir and air was bubbled through the systems for 3 days. The aqueous reservoir fluids and ethanolamine solutions were monitored daily for radioactivity. Under these conditions, there was a rapid loss (46%) of radioactivity from the reservoir fluids within 1 day, with 36% of this ^{14}C-activity collected as radiolabeled carbon dioxide, indicating a rapid hydrolysis of the parent compound. After 2 and 3 days, 18% and 9% of the initially-applied ^{14}C was present in the aqueous solutions, respectively, with the loss of radioactivity being typical of a first order reaction. Essentially all of the radioactivity remaining in the aqueous reservoir solutions 2 and 3 days following treatment was present in chloroform-soluble fractions. Thin layer chromatographic-autoradiographic analysis of these fractions revealed only the presence of the parent compound. Additional metabolism studies are planned using radiolabeled methiocarb in water and soil systems under controlled climatic conditions.

III. MISCELLANEOUS STUDIES

A. Field Support Analytical Methodology

1. DR-736

a. Development of a technique for estimating aerially-applied spray deposits.--A need to evaluate the dispersion and deposition of the avian repellent, methiocarb, from aerial applications to crops prompted the development of a semiquantitative method to detect aqueous sprays of the compound. In lieu of using such techniques as water-soluble dyes incorporated in the spray preparations, etc., a somewhat different approach was taken. The method consists of placing filter papers with cardboard backing at selected sites in the spray path; a few silica gel or alumina-containing thin-layer plates with fluorescent indicator are also placed in close proximity to the filter papers. A rapid visual assessment of the dispersion and deposition of the methiocarb spray is accomplished by viewing the thin-layer plates under ultraviolet light (254 nm). If a permanent record of the spray distribution is desired, the filter papers are developed using a previously reported ferric chloride-potassium ferricyanide chromogrnic reagent, which results in a series of blue spots on a white background.

For more accurate information, a portion of the filter paper spray sheet is removed, and following an acetone extraction, the solution is evaporated under nitrogen; the concentrated solution is then applied to a silica gel thin-layer plate. After hydrolysis of the methiocarb using a potassium hydroxide spray, the sample application is developed using the above-mentioned reagent, and the intensity of the resulting blue spots compared visually with methiocarb standards. Preliminary work has shown the lower limit of detectability to be approximately 1 microgram.

A recent field trial in which the effectiveness of helicopter-applied methiocarb (3 pounds of chemical per 10 gallons water per acre) for protection of blueberries from bird damage, has shown that concentrations ranging from 0.3 to 3.5 (average 1.4) pounds per acre of the chemical reached the indicator papers.

b. Semiquantitative method for residues in fruit.--In view of the development of methiocarb for protection of fruit crops (blueberries, cherries, grapes, etc.) from bird damage, a need was expressed for a rapid semiquantitative method for the analysis of methiocarb and its sulfoxide and sulfone metabolites. Such a method would be beneficial in correlating surface residues to bird activity. Work was initiated during this reporting period to develop a TLC method of sufficient sensitivity to detect the initial methiocarb application and also the parent compound and metabolites remaining 1 to 2 weeks following application.

The methodology as developed to date for analysis of this chemical in blueberries involves a benzene surface stripping; the fruit is then extracted with acetone, followed by a single liquid-liquid partitioning with chloroform; a precipitation step follows which removes remaining plant pigments and waxes. The resulting purified extract is then concentrated under vacuum and nitrogen and aliquots applied to a silica gel TLC plate containing a fluorescent indicator. After development of the plate in one of several satisfactory mobile phase systems, the plate is viewed under ultraviolet light at 254 nm. This methodology is a modification of previously reported techniques.

B. Germination and Phytotoxicity

1. DRC-736

a. Rice seed.--Rice seed from fields aerially treated with with 3.0 lb/acre DRC-736 had the same germination rate as untreated seed. All seeds in both lots germinated. Details of this study can be found in Technical Report DF-102.2-42.

b. Marigold seed.--Marigold seed treated with a 3.0% concentration of DRC-736 germinated at a slower rate than untreated marigold seed. At the conclusion of a 7-day germination test, however, there was no significant difference in the total germination of treated (DRC-736) and untreated seeds. Details of this study can be found in Technical Report DF-102.2-41.

C. Physiological Marking Agents

1. Sudan Dyes as Physiological Markers for Redwings.--Sudan dyes stain neutral fat when given in physiological doses. Studies in quail have indicated that this method had little applicability; however, the effects on wild birds such as red-winged blackbirds was unknown. In this study DRC-5581, DRC-5582, and DRC-5583, all sudan dyes, failed to stain body fat or internal organs of redwings when given in single acute oral doses. More detail on this study can be found in Technical Report DF-102.2-34.

2. Protein Bound Iodine (PBI) as a Physiological Marker in Wild Birds

   A number of iodinated radio-opaque compounds, when given to animals, combine with albumin and remain in the body for varying periods of time, up to 3 years. Initial studies conducted with bobwhite quail indicated that one compound, DRC-6061, might also be residual in birds. Studies are currently under way to determine the utility of various radio-opaque compounds in elevating avian PBI for extended periods of time. Preliminary information in red-winged blackbirds and coturnix indicate an extremely short period of elevation, and a short period of detectability. More detail will be found in Technical Report DF-102.2-47, available in mid-1973.

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Schafer, E. W., Jr., J. L. Guarino, and R. B. Brunton. 1974. An evaluation of the male coturnix as a laboratory test species for developing avian chemosterilants. Intended for J. Reprod. Fertil.

Schafer, E. W., Jr., R. B. Brunton, and N. F. Lockyer. 1974. The effects of 25 chemicals on reproductive performance in male coturnix. Intended for J. Reprod. Fertil.

Table 1. Chemicals active as repellents in initial evaluation on red-winged blackbirds.

DRC No.	R_{50}(%)	LD_{50}(mg/kg)
4917	0.028	0.42
6019	0.056	1.00
5569	0.128	2.42
5375	0.316	> 100
5570	0.421	13.3
5571	0.561	10.0
5379	0.650	100
5885	0.650	100
5545	0.750	> 100
5557	0.750	> 100
5547	0.824	> 100
5576	0.824	24.2
5868	0.824	-
5597	0.866	> 100

Table 2. Significant effects of six chemosterilants on male coturnix quail dosed with testes in various development stages.

Chemical and Level	Adult-breeding		6 Week Old		12-day Chicks		Adult Regressed	
	Fertility	Testes Weight	Fertility	Testes Weight	Fertility	Testes Weight	Fertility	Testes Weight
Propylene glycol (Control)	0	NA	0	0	0	0	0	0
DRC-5537 100 mg/kg	0	NA	0	0	0	0	0	0
DRC-5533 31.6 mg/kg	+	NA	0	+	0	0	++	+
DRC-4800 100 mg/kg	0	NA	0	0	0	+	0	+
DRC-5535 316 mg/kg	++	NA	+	++	++	+	0	0
DRC-3225 316 mg/kg	0	NA	0	-	0	0	0	0
DRC-1806 31.6 mg/kg	++	NA	0	0	++	++	++	++

Fertility: 0 = No effect, + = < 50% sterility, ++ = > 50% sterility.
Testes weight: - = > Control, 0 = No effect, + = < 50% reduction in weight, ++ = > 50% reduction in weight.
NA = Not available.

Table 3. The effects of single acute doses of five chemosterilants on male coturnix fertility.

DRC No.	Dose (mg/kg)	LD_{50} (mg/kg)	Duration of sub-fertility (days)	Sub-fertility Range (%)	Fertility at end of 35 days	Mortality (%)	Testes Weight (mg)
5584	562	750	5	(50.0)	96.6	0.00	3.913
	316		5	(26.1)	66.7	0.00	2.6[illegible]4
5585	56.2	100	15	(0.00-52.6)	95.5	28.6	2.798
5586	56.2	1.33	10	(19.0-52.2)	100	57.2	2.909
5589	31.6	42.1	10	(48.3-56.5)	90.9	0.00	2.770
5590	562	1000	10	(38.1)	90.9	0.00	3.805
Control	-	-	0		91.7	0.00	2.832

Table 4. Acute oral and dermal toxicity of 17 chemicals to the quelea, house sparrow, red-winged blackbird, and rat.

DRC No.	Acute LD_{50} (mg/kg)						
	Quelea		House Sparrow		Red-winged Blackbird	Rat[a]	Rat[b]
	Oral	Dermal	Oral	Dermal	Oral	Oral	Oral
1327	5.6	> 100	7.5	> 100	2.4	-	21
2538	1.3	4.2	1.3	18	1.0	-	23
5781	4.2	5.6	2.4	3.2	2.4	-	4
4226	2.4	> 100	3.2	> 100	2.4	1,750	-
4241	0.75	1.3	1.0	3.2	0.56	75	-
5527	2.4	2.4	5.6	7.5	2.4	375	-
5528	0.75	2.4	1.8	7.5	1.3	175	-
5529	5.6	75	3.2	> 100	2.4	> 1,000	-
142	1.3	1.3	4.2	1.8	1.0	-	22
4917	0.42	100	1.3	100	0.42	-	5
1324	3.2	7.5	10	75	3.2	-	84
5569	1.8	10	2.4	18	2.4	-	30
1329	0.24	0.42	0.32	1.0	0.32	-	6
1143	1.3	1.8	5.6	13	2.4	-	7
632	1.3	1.8	5.6	2.4	1.8	250	-
736	4.2	100	18	> 100	4.6	-	100
1150	1.8	1.8	1.3	1.8	2.4	-	5
Average LD_{50} (mg/kg)	2.3	30	4.4	38	1.9	604	28

[a] Toxicity of compounds selected for differential toxicity (> 100) between birds and mammals.

[b] Toxicity of compounds not selected for differential toxicity between birds and mammals.

Table 5. Rate of breakdown of ^{14}C-4-aminopyridine to radiolabeled carbon dioxide in six soils maintained at 30° C and 50% moisture for 3 months.

Soil Texture	Clay (%)	O.M. (%)	pH	Half life[a] (months)
Alkaline soils:				
Loamy sand	7	2.9	7.8	3
Loam	20	4.0	7.7	11
Sandy clay loam	26	1.9	7.6	32
Acidic soils:				
Loam	16	5.0	5.6	8
Loam	16	2.9	5.8	22
Loam	25	1.4	4.1	>> 22

[a] Approximate time required for 50% of parent compound to be degraded to carbon dioxide.

Table 6. Distribution of radioactivity in six soils after a 3-month incubation period at 30° C and 50% moisture.[a]

Soil Type	pH	Percent Radioactivity			Total ^{14}C Recovery (%)
		Degraded to $^{14}CO_2$	Residual		
			Acetone Extractable	Combustible	
Loam	4.1	0.35	0.46	42.00	42.81
Loam	5.6	16.52	3.83	46.84	67.19
Loam	5.8	5.95	1.87	51.78	59.60
Loamy sand	7.8	54.16	4.54	26.60	85.30
Loam	7.7	10.74	5.66	47.15	63.55
Sandy clay loam	7.6	4.88	11.23	45.32	61.43

[a] Moisture expressed as 50% of the maximum capillary capacity.

Table 7. Distribution of radioactivity translocated into sorghum heads of 3-month plants cultured 1 week in solutions containing 5 ppm ^{14}C-4-aminopyridine.

Head	Radioactivity Recovered (dpm x 10^3)		Radioactivity in Seeds (% of total in hood)
	Seeds	Stripped Head	
1	1.5	0.2	88
2	1.3	not detected	100
3	4.5	0.3	94
4	0.5	not detected	100
5	0.3	0.2	60
6	0.3	0.5	38
7	11.8	1.3	90
8	7.3	0.4	95

Table 8. Radioactivity translocated into the vegetative tissues of 3-month-old sorghum grown 1 week in nutrient cultures containing 5 ppm of ^{14}C-4-aminopyridine.

		Radioactivity					
		Uppermost Internode of Stalk		Upper 8-inch Stalk-leaf Section		Lower 8-inch Stalk-leaf Section	
Culture	Plant	dpm (x 10^4)	ppm[a]	dpm (x 10^4)	ppm	dpm (x 10^4)	ppm
A	1	ND[b]	--	0.07	0.01	12.30	2.26
A	2	ND	--	0.30	0.02	16.00	0.74
B	1	0.03	0.01	1.68	0.11	25.60	1.82
B	2	ND	--	0.61	0.10	5.34	0.87
C	1	ND	--	ND	--	123.65	14.20
C	2	ND	--	ND	--	170.43	10.47

[a] Calculations based on fresh weight and assumed that all radioactivity present as the parent chemical.

[b] Not detected.