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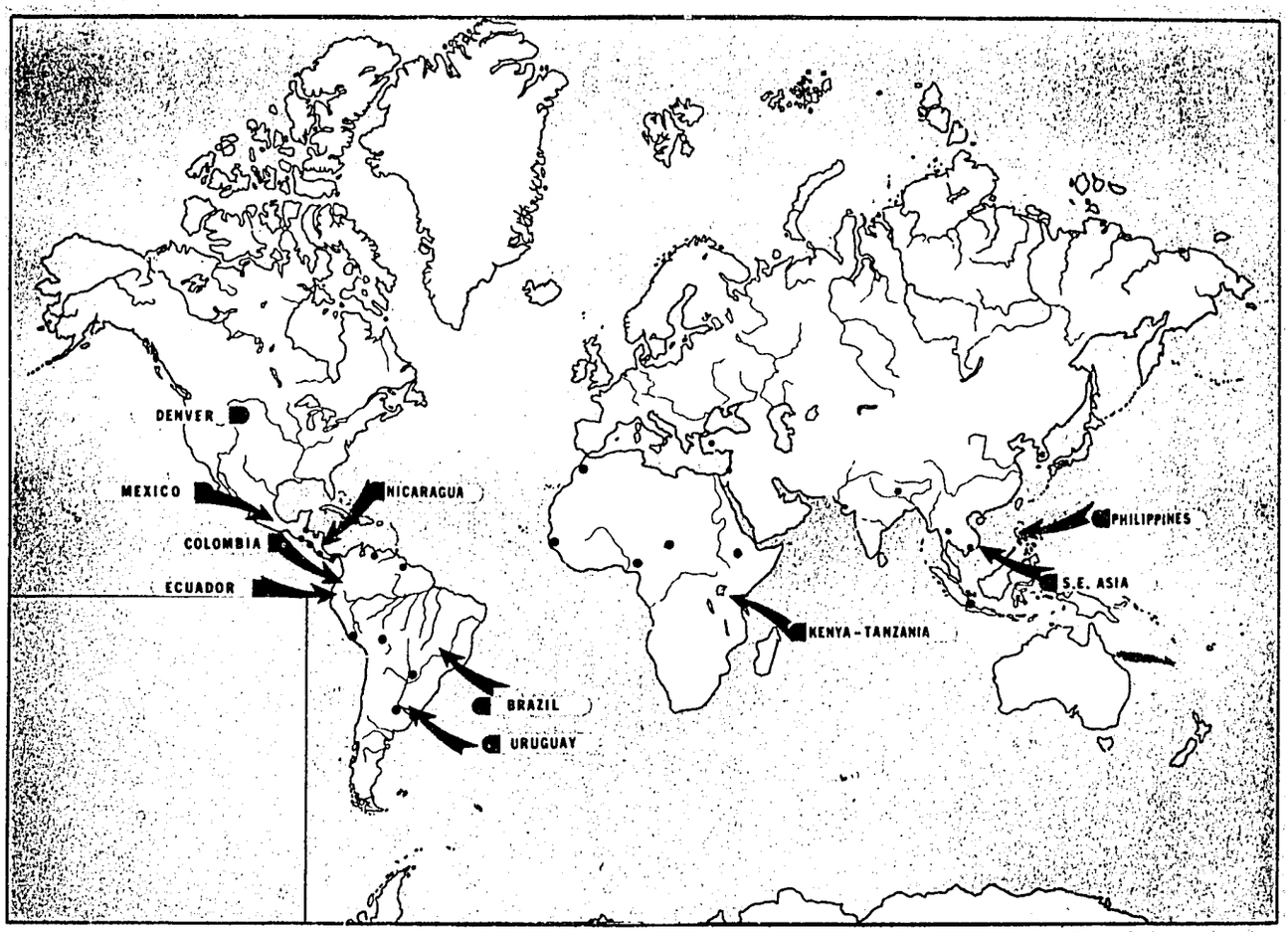

1974 ANNUAL REPORT

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Denver Wildlife Research Center

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ANNUAL PROGRESS REPORT*

Calendar Year 1974

SECTION OF SUPPORTING SCIENCES

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U. S. Department of the Interior
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Division of Wildlife Research
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ABSTRACTS

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

PHARMACOLOGY AND PHYSIOLOGY

Research activities were in the areas of physiological markers for animals, rodenticide evaluations, reproductive studies with rodents, bait formulations, coyote and vampire bat studies. Major accomplishments are summarized below:

I. Physiological Marking Agents for Birds and Mammals

A. Response of blackbirds to selected North American viruses. A contract was initiated with the University of Wisconsin, Madison, to study and test the feasibility of using selected viruses to induce antibodies in blackbirds. Antibody titers could then be used to identify birds that had been exposed to a specified virus. DWRC fulfilled its commitment to the project by supplying Wisconsin with 114 blood serum samples and 35 live blackbirds. An annual progress report is due on the anniversary of the contract (June 1975) and a final report is due after completion of the study (May 1976).

B. Preliminary iophenoxic acid studies in wild Norway rats. A single oral 1 mg/kg dose of iophenoxic acid to male wild Norway rats elevated the iodine content of the blood for 35-42 days posttreatment. Tests utilizing higher doses and additional animal species are planned. From the tests conducted to date, it would appear that iophenoxic acid could be a useful sensitive marker for mammals.

II. Rodenticide Evaluations

A. Testing of experimental rodenticides submitted by the World Health Organization (WHO). Through a cooperative agreement with the World Health Organization, Difenacoum, an anticoagulant, and Kayanex, an acute rodenticide, have been tested in rats. Results of these tests show that Difenacoum is a more effective rodenticide than Kayanex.

B. Potentiation of the rodenticidal properties of red squill. SKF 525A potentiated the rodenticidal effects of red squill in male albino rats, but was not an effective potentiator in female albino rats. In a free-choice feeding test with wild Norway rats, heliotropin did not potentiate the lethal effects of red squill.

C. Toxicological data on wild ginger (Asarum caudatum) in rats and deer mice. Samples of wild ginger gavaged at 1600 mg/kg in rats and deer mice, did not produce any toxic symptoms in the animals.

III. The Effect of DRC-4805 on the Fecundity of Wild Norway Rats

Female wild Norway rats were gavaged the day after littering with a single oral dose of DRC-4805 (1.0 mg/kg) to determine its effect on the young. The DRC-4805 that was transferred through the milk to the young made all but one of the treated males (16) and all of the treated females (16) selected for breeding trials infertile. All control males (18) and females (18) bred successfully.

IV. Bait Formulation Studies

A. Development of baits to deliver uniform dosage of 4-aminopyridine to red-winged blackbirds (Agelaius phoeniceus). Results from two preference feeding tests with red-winged blackbirds, indicated these birds would accept tableted corn. When 4-aminopyridine treated corn was tableted and coated with a moisture proofing compound, its acceptance was favorable when offered to caged birds.

B. Preliminary studies with single lethal baits for Polynesian rats (Rattus exulans). Four tableted baits, corn, Purina Laboratory Chow, oat groats, and corn plus oat groats were fed to individually caged Polynesian rats. Initial acceptance indicated rats would accept tablets even when regular diet of laboratory chow was present. Zinc phosphide treated tablets were offered to caged rats in a choice test with untreated tablets. The results indicated that this type of bait carrier could be effective in baiting programs in Hawaii.

V. Coyote Studies

A. Sublethal aversive chemicals for coyotes. Preliminary sublethal effects for 10 chemicals were obtained prior to a study designed to assess drug-induced predatory suppression in coyotes. The chemicals were chosen on their ability to alter various physiological systems. Emesis occurred with 4 out of 10 chemicals. Other sublethal effects were tranquilization, salivation, piloerection, mydriasis, and vocalization.

B. Acute oral testing of toxicants in coyotes. Coyote toxicant research was initiated to find acute oral toxicants for use in the toxic collar. The LD_{50}'s (mg/kg) of two potent chemicals are: nicotine, 7.1; and potassium cyanide, 4.0. The

highly toxic properties of two formulations of potassium cyanide were retained after 29 days of storage.

C. Sheep toxic collar tests in coyotes. Sheep toxic collar testing has been completed with toxic collar prototype 1. This collar is constructed of a plastic sheath which covers plastic packets that contain 33% sodium cyanide. Toxic collar prototype 1 was effective in killing 2 out of 4 coyotes. Tests with a single-layered toxic collar are being planned.

VI. Effects of Vampire Bat Control on Milk Production

The contention that vampire bat predation, even in the absence of disease, is debilitating and will result in lower milk production of dairy cattle, was investigated under field conditions. Milk production and several hemotological indicators were measured before and after controlling moderately severe vampire predation on 58 lactating Holstein cows near Quito, Ecuador. Single intraruminal doses (1 mg/kg) of Diphenadione (2-diphenylactyl-1,3,indandione) reduced vampire attacks by 98% from a pretreatment mean of approximately 4 bites/cow to 0.1 bites/cow posttreatment. Red blood cell count, packed cell volume, and percent butterfat increased significantly ($P<0.05$) but milk production remained virtually unchanged from a pretreatment mean of 28 lbs/cow/day.

BEHAVIORAL BIOLOGY

Research was conducted to enhance consumption of baits for rats, test preference responses to rice variety flavors, evaluate nonlethal electrical barrier designs for controlling ricefield rats, assess drug treatment effects on coyote predatory behavior, and study the development of coyote sheep killing behavior.

I. Bait Additive Development

Twelve commercially available food additives were each tested at three concentrations on ricefield rats with rice as the standard bait base. Although the animals showed increased variations in consumption at the higher concentration levels, none of these additives significantly increased rice consumption. This research will be completed in Fall 1975.

II. FK-178A Versus California Brown Rice Flavor

Ricefield rats showed a significant ($P<.05$) preference for FK-178A flavor treated rice over California Brown flavor treated rice. Rice variety preference shown by ricefield

rats appeared to be based on a flavor difference rather than other factors such as texture, nutritional value, or grain size. Results will be prepared for publication in 1975.

III. Nonlethal Electrical Barrier Development

Two series of tests were conducted under semifield conditions using eight 5 X 10 foot enclosures and three ricefield rats per enclosure. In the first test series, shock pulse rate from a commercial livestock electric fencer unit was varied from 1 to 5 pulses per second. Under conditions of half food ration and a reduced number of electrodes, the higher pulse rates appeared more effective. However, safety standards restricted any further evaluation of the pulse rate effect. We then conducted a second test with an unmodified fencer unit (0.9 pulses/second). Two barrier designs were evaluated for protection of a food source from rat infestation and reduction in rat numbers crossing the barriers. One design produced a 95 percent savings of food and a 97 percent reduction in rats. Under a half food ration condition, this design continued to show 85 percent food protection. Field trials for evaluating this design are planned for Spring 1975.

IV. Coyote Studies

A. Predatory suppression in coyotes with drug-induced illness
Four drugs were evaluated to determine their suppressive effect on coyote predation involving a specific prey. Deer mice were used as the alternate prey and rabbits were used as prey to be associated with drug-induced illness. Only one of the drugs, digitoxin, showed both a wide safety margin and suppressive effects on rabbit-killing. Higher levels of this drug and related compounds will be evaluated before publication of the final results.

B. Some Preliminary Observations on the Sheep-Attack and Sheep-Kill Behaviors of Coyotes. A 100 X 110 ft. fenced test area was constructed to allow observation of coyote sheep kills and to evaluate a sheep toxic collar control method. Six live-trapped coyotes were adapted to killing rabbits and sheep in the test area. A total of 23 sheep kills were observed. Five of the six coyotes consistently killed sheep by attacking the neck region. A technique of tying bits of mutton to the sheep's neck area enhanced the sheep-kill training procedure. All coyotes appeared to undergo a gradual learning process in attacking sheep. However, actual time to kill sheep, once an attack had been made, was stable across sessions.

BIOCHEMICAL DEVELOPMENT

Research was conducted in the following areas: Analysis of milk of cattle treated with diphenadione for vampire bat control., Analysis and behavioral evaluation of fermented egg constituents. Analysis and synthetic duplication of rice aroma as a natural attractant. Development of an analytical procedure for warfarin.

I. Diphenadione Residue Studies in Milk

This is an expansion of a 1973 study of diphenadione residues in milk of cows which had been given a 2.75 mg/kg intraruminal dose of diphenadione. In this study two additional levels (1 and 5 mg/kg) were each tested on three lactating cows. Nursling calves were dosed at the same level as the cow. The prothrombin response to the 5 mg/kg dose reached a maximum 96 hours after dosing that was 4X normal for cows and 25X for their suckling calves. One of three calves dosed with 5 mg/kg of diphenadione died. Cows and calves dosed at the recommended 1 mg/kg level showed only slight changes in prothrombin times.

II. Properties of Volatile Fermented Egg Constituents

Tests have shown that a McLaughlin Gormley King (MGK) fermented egg product (FEP) is both attractive to coyotes and repellent to deer. Our objective is to determine the active components of FEP and prepare a synthetic formulation which produces responses similar to FEP. The volatile fraction has been isolated, characterized by gas chromatography - mass spectrometry and separated into 37 fractions for behavioral testing. We are able to test the human odor panel response on these fractions but have not successfully devised a behavioral test for coyotes.

III. The Analysis and Synthetic Duplication of Rice Aroma

The Philippine ricefield rat (*Rattus rattus mindanensis*) appears to acquire a "taste" for the flavor of rice volatiles. Consequently, knowledge of important volatile components could lead to the development of a synthetic flavor that would increase bait palatability. Rice aroma volatiles were collected and analyzed by combined gas chromatography - mass spectrometry. Seventy-three compounds were identified, 64 of which are being reported for the first time in either unprocessed or processed rice. Fifty-four of these have never been identified in any unprocessed cereal grain. Predicted identities were obtained on an additional 31 compounds. This is the most extensive work that has been reported for any of the uncooked cereal grains.

IV. Development of an Analytical Procedure for Warfarin

An analytical method was developed for measuring trace residues of warfarin in plants, water and soil. We have used nitrochromic acid oxidation to form a product that is detectable at levels equivalent to 10 nanograms of warfarin. A general cleanup procedure has also been developed that can be used with the oxidation process.

BIOELECTRONICS

Areas of electronic instrumentation development are listed and described below.

I. New Wildlife Transmitter Designs

Prototype mortality transmitters for grizzly bears and delayed turn-on transmitters for migrating birds have been designed and are ready for field evaluation. The Wyoming Game and Fish Commission is currently assisting with the testing of the bear transmitters. The bird transmitters will be evaluated in 1975.

II. Engineering Support and Production Services

An estimated 70 percent of the total electronics work effort during 1974 was related to satisfying specific requests for assistance. Electronics personnel aided several research programs by designing, constructing, and reworking specialized instrumentation. This assistance included work provided for two endangered species programs.

III. Evaluation of Cassette Tape Players

A contract was completed for the Air Force Weapons Laboratory and a final report was compiled on frequency response of selected mobile magnetic tape players. The Air Force plans to use the report as a basis to recommend a sound system for repelling birds from airport runways.

IV. Field Instrumentation for Recording Food Preference of Rats

A device is being developed to record food consumption data for ricefield rats in the Philippines. The device is unique in that it uses a coulometer component at the recording medium and requires only microwatts of battery power. This recording concept will be extended to many biological applications requiring the counting and timing of events.

V. Radio Transmitter for Tranquilizer Darts

A radio transmitter is being developed to attach to a tranquilizer dart so that immobilized animals may be quickly located. Transmitters have been constructed and used for several successful radio-tracked immobilizations.

VI. Automatic Wildlife Tracking System

Projected cost estimates for the completion of the automatic wildlife tracking system have exceeded any available funding. The development is being terminated.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

PHARMACOLOGY AND PHYSIOLOGY - APPLICATIONS IN WILDLIFE MANAGEMENT

Peter J. Savarie, Melvyn Garrison,
Brad Johns, and R. Dan Thompson
David J. Hayes

I. PHYSIOLOGICAL MARKING AGENTS FOR BIRDS AND MAMMALS

A. Responses of Blackbirds to Selected North American Viruses

Introduction, Results, and Discussion

A contract titled "Virologic and Serologic Responses of Blackbirds to Selected North American Viruses" was negotiated with Dr. Robert P. Hanson at the University of Wisconsin, Madison. Duration of the contract is from June 1974 through May 1976. This two year research project will investigate the feasibility of using a virus to induce serological marking in blackbirds. A selected virus would be considered for use as a possible marker if (1) antibodies to the virus were not present in natural blackbird populations, (2) the virus establishes a closed infection in the bird, and (3) the virus induces readily detectable antibodies. The ability of a virus to initiate a closed, antibody-inducing infection would be determined by exposing captive blackbirds to selected viruses, and by measuring the titer of antibody at various time intervals.

If a virus is found that meets the 3 primary criteria for a serologic marker, studies will be conducted on the safety and practicality of its use, including such matters as the hazard to associated avian populations and the methods required to develop a delivery system.

Under provisions of this contract, 114 blood serum samples and 35 live blackbirds were sent to Dr. Hanson. Collection sites for these samples were California, Colorado, South Dakota, Maryland, and Florida. Personnel from the Sections of Bird Damage Control (Schafer, Mott, Guarino) and Pesticide-Wildlife Ecology (Baer, Zinkl) have facilitated the Center's responsibilities for this project. Dr. Hanson is to provide an annual progress report on the anniversary of the contract and a final report is due after the completion of the contract.

B. Preliminary Iophenoxic Acid Studies in Wild Norway Rats

Introduction

Previous studies with coyotes, have shown that a 1 mg/kg single oral dose of iophenoxic acid elevates the protein-bound iodine (PBI) content of the blood for at least 130 days. Under practical situations an animal consuming iophenoxic acid would have elevated PBI values, and thus could be identified. Preliminary studies have been conducted in male wild Norway rats to determine if iophenoxic acid would be a feasible marker in these animals.

Methods

Iophenoxic acid was prepared in 0.5% gum tragacanth and administered in a single oral dose at 1 mg/kg and 0.1 mg/kg to adult male wild Norway rats that had been fasted for 17-19 hours. At specified times posttreatment rats were lightly anesthetized with ether and blood samples obtained by nipping off the end of the tail. PBI is analyzed in the plasma by a modification of an established procedure.

Results

PBI values at various time intervals after dosing with iophenoxic acid are shown in Table 1. When compared to previous data collected in coyotes, it is apparent that rats do not retain the iodine as the coyotes did. Differences in metabolic systems between the two species could account for this difference. However, the results in Table 1 do show that 1 mg/kg iophenoxic acid is a sensitive marker in rats for approximately 35-42 days.

Additional studies with higher doses of iophenoxic acid will be conducted with the expectation that the duration of iodine content of the blood will be extended.

Table 1. PBI content in male wild Norway rats after receiving a single oral dose of iophenoxic acid.

Days Posttreatment	Dose, mg/kg 1.0	0.1
0 (Control)	12 (11-16)[a](5)[b]	13 (9-16)(5)
1	820(790-933)(5)	92(50-110)(5)
7	293(255-337)(5)	47 (33-78)(5)
14	214(130-265)(5)	33 (26-44)(5)
21	114 (81-147)(4)	37 (28-47)(5)
28	74 (44-107)(4)	27 (18-36)(5)
35	57 (38-87)(4)	18 (13-29)(5)
42	60 (44-79)(3)	17 (13-24)(4)
49	31 (15-48)(3)	12 (8-18)(4)
56	14 (9-21)(3)	not tested
63	15 (12-20)(3)	not tested

a mean, (range) µg iodine/100 ml plasma
b (number) of animals tested

II. RODENTICIDE EVALUATIONS

A. Testing of Experimental Rodenticides Submitted by the World Health Organization (WHO)

Introduction

The Denver Center is cooperating with the WHO by evaluating candidate rodenticides that are in the advanced stages of development. Each rodenticide submitted by the WHO is accompanied by efficacy and toxicological data that help the Denver Center determine what value it may have in Fish and Wildlife Service programs. Through WHO contacts which the Denver Center does not have, experimental rodenticides can be obtained and tested on target species that are of mututal interest to both organizations.

Results and Discussion

Bioassay data have been obtained with two rodenticides, Difenacoum and Kayanex.

Difenacoum is an anticoagulant and results of feeding tests when it was tested at 0.01% in oatmeal bait were encouraging (Table 2). Mortalities observed in three species of rats were: wild Norway--95% (9/10 males, 10/10 females); ricefield--100% (5/5 males, 5/5 females); Polynesian--100% (5/5 males, 5/5 females). The male wild Norway rat that survived "cued" in on the treated bait each night. He ate 0.6 g. of treated bait the first night. On subsequent test nights, his consumption of treated bait ranged from 0.1 to 0.4 g.

The manufacturer of Difenacoum claims it is effective against warfarin-resistant rats. Arrangements have been made and the Denver Center will obtain anticoagulant-resistant wild Norway rats from the United States for testing with Difenacoum.

Kayanex, a thiosemicarbazide derivative, is an acute rodenticide. It was supplied as a granular bait containing 2% Kayanex, and as the technical product which could be formulated into any bait desired. Bioassay data for Kayanex are shown in Table 3. Mortalities observed in feeding tests with the 2% granules were: wild Norway--80% (5/5 males, 3/5 females); ricefield--70% (4/5 males, 3/5 females); Polynesian--20% (1/3 males, 1/7 females).

It was thought that oatmeal treated bait would provide more efficacy for Kayanex. However, although the wild Norway rats did eat more of the 2% treated oatmeal bait than they did the 2% granular bait, mortality did not increase. These data are clearly shown in the female wild Norway rats.

All mortalities in the Kayanex feeding test occurred after the first test night. On subsequent test nights, survivors developed "bait shyness" and only negligible amounts of treated bait were eaten. This feeding behavior is not unlike that observed with other acute rodenticides, such as red squill.

Table 2. Nine (9) day free-choice feeding test results in rats fed oatmeal bait containing 0.01% Difenacoum.[a]

Species	Sex	Mortality	Time to death (days)	Bait eaten, mean (range) g Trested	Untreated	Chemical consumed mean, (range) mg/kg
wild Norway (Rattus norvegicus)	M	9/10	5 -8	20.8 (7.6-43.2)[b]	42.4(15.5-72.3)[b]	6.4(2.3-11.6)[b]
wild Norway (Rattus norvegicus)	F	10/10	4-11	19.5 (7.7-34.2)	38.3(23.2-79.7)	7.7(3.2-12.9)
Ricefield (R. r. mindanensis)	M	5/5	5 -8	33.4(21.9-43.2)	45.4(35.0-54.0)	15.1(9.9-18.1)
Ricefield (R. r. mindanensis)	F	5/5	6-14	18.7 (3.8-36.7)	46.1(38.3-60.5)	11.2(1.9-23.2)
Polynesian (R. exulans)	M	5/5	4 -9	10.5 (5.1-16.3)	17.7(16.7-18.9)	11.9(4.3-20.0)
Polynesian (R. exulans)	F	5/5	4-13	10.9 (6,0-17.3)	16.2 (6.9-25.3)	15.1(6.6-25.9)

a Testing protocol: Individually caged adult rats were offered two separate food cups for 9 consecutive nights. No Purina Laboratory Chow was available during this interval. The untreated cup contained 50 g. of oatmeal bait (90% oatmeal, 5% glycerol, 5% Alcolec S). The treated cup contained 50 g. oatmeal bait containing 0.01% Difenacoum. Position of the untreated and treated cups were reversed each night to overcome any position preference by the test animals. After 9 nights of treatment, survivors were given lab chow ad lib and observed for 12 days.

b Figures do not include amount eaten by survivor. Survivor ate 2.4 g. treated bait, 132.3 g. untreated bait, and consumed 0.7 mg/kg of Difenacoum.

Table 3. Free-choice feeding test results in rats with 2% Kayanex.[ab]

Species	Sex	Bait	Mortality	Treated bait eaten mean, (range) g	Chemical consumed mean, (range) mg/kg
wild Norway (Rattus norvegicus)	M	Granules	5/5	0.6(0.3-0.9)	32.3(13.1-65.5)
wild Norway (Rattus norvegicus)	F	Granules	3/5	0.4(0.1-0.7)	31.4 (5.5-59.5)
Ricefield (R. r. mindanensis)	M	Granules	4/5	0.4(0.1-1.1)	36.6 (8.0-83.0)
Ricefield (R. r. mindanensis)	F	Granules	3/5	0.4(0.1-0.8)	41.2 (6.6-82.0)
Polynesian (R. exulans)	M	Granules	1/3	0.2(0.0-0.5)	59.4(0.0-131.0)
Polynesian (R. exulans)	F	Granules	1/7	0.1(0.0-0.2)	31.8 (0.0-74.0)
wild Norway (Rattus norvegicus)	M	Oatmeal	5/5	2.3(0.9-4.8)	114.2(42.0-294.0)
wild Norway (Rattus norvegicus)	F	Oatmeal	1/5	1.0(0.1-2.2)	64.2 (9.0-133.0)

a Testing protocol: Individually caged adult rats had free access to Purina Laboratory Chow and water at all times. A cup containing either 10 g of 2% granules or 10 g of 2% Kayanex in oatmeal bait (90% oatmeal, 5% glycerol, 5% Alcolec S) was placed in each cage for 1 to 3 consecutive nights. Survivors were observed for 4 to 6 days.

b All figures represent the results from the first test night.

B. Potentiation of the Rodenticidal Properties of Red Squill

Introduction

The purpose of the red squill potentiation study is to increase rat mortality when red squill is gavaged simultaneously with a potentiator. The ultimate goal is to incorporate a chemical into red squill baits which will enhance the toxicity of red squill to rodents in free-choice feeding situations.

Results and Discussion

Acute oral dosing

In previous tests with male albino rats, five chemicals were found that potentiated the lethal effects of red squill. These five chemicals have been tested in female albino rats (Table 4). Satisfactory potentiation was observed only with tropital. Contrary to the potentiation observed in male rats with SKF 525A, initial tests indicated that SKF 525A antagonized the lethal effects of red squill in female rats (Table 4).

To confirm if a sex difference with SKF 525A exists, male and female albino rats were dosed the same day with SKF 525A and tropital (Table 5). Results in Table 5 show that SKF 525A produced potentiation in male rats, but not females. However, tropital produced potentiation in both sexes.

Four additional potentiators have been tested in male albino rats (Table 6). Two chemicals, bis (butyl carbityl methyl) methylene dioxybenzene and bis (butyl carbityl methyl) propyl methylene dioxybenzene, resulted in favorable potentiating results and will soon be tested in female albino rats.

Free-choice feeding with red squill plus potentiator

Heliotropin produced potentiation in male albino rats but not in females. Despite the lack of potentiation in females, it was decided to test heliotropin in a free-choice feeding test. Wild Norway rats, 10 males and 10 females, were offered untreated and treated baits simultaneously for 3 consecutive nights. EPA test protocol and bait (65% cracked corn, 25% rolled oats, 5% corn oil, 5% powdered sugar) were used. The treated bait contained 3% MGK F-1901 (0.015% scilliroside), plus 1.5% heliotropin. None of the males died and only 1 of 10 females died. When compared to previous free-choice feeding tests with red squill, there was reduced consumption of the bait. Based on previous gavage studies, 1.5% heliotropin would be about the minimum needed in a bait to achieve potentiation. It would appear, however, that heliotropin caused bait shyness. The problem of finding a potentiator which is acceptable to rats is one that plagues the whole field of potentiator investigation.

Table 4. Potentiation of red squill in female albino rats.

Red Squill* mg/kg	Potentiator	Dose of Potentiator mg/kg	14-Day Mortality
0.18 (control)	---	---	0/9
0.18	SKF 525A	25	2/8
0.18	SKF 525A	12.5	3/9
0.2 (control)	---	---	6/10
0.2	SKF 525A	100	1/10
0.2	SKF 525A	25	3/9
0.2	MGK-264	100	1/10
0.2	MGK-264	25	3/10
0.2	Tropital	100	10/10
0.2	Tropital	25	9/10
0.2	Heliotropin	100	7/10
0.2	Heliotropin	25	7/10
0.2	Sexamex	100	5/10

* For all studies, red squill was administered by gavage simultaneously with the indicated dose of potentiator. The red squill dose is calculated in terms of the principle active ingredient, scilliroside.

Table 5. Potentiation of red squill in female and male albino rats.

Red Squill* mg/kg	Sex	Potentiator	Dose of Potentiator mg/kg	14-Day Mortality
0.19 (control)	F	---	--	0/10*
0.19	F	SKF 525A	100	0/10**
0.19	F	SKF 525A	25	0/10*
0.19	F	Tropital	100	6/10***
0.19	F	Tropital	25	4/10***
0.45 (control)	M	---	--	0/10*
0.45	M	SKF 525A	100	6/10!
0.45	M	SKF 525A	25	3/10!!
0.45	M	Tropital	100	9/10***
0.45	M	Tropital	25	1/10!!!

* 3 Animals became very ill, but recovered.

** 1 Animal became slightly ill, but recovered.

*** All animals became very ill.

! 3/4 survivors became very ill.

!! 5/7 survivors became very ill.

!!! 1/9 survivors became very ill.

Table 6. Potentiation of red squill in male albino rats.

Red Squill mg/kg	Potentiator	Dose of Potentiator mg/kg	14-Day Mortality
0.45 (control)	---	---	0/10
0.45	bis (butyl carbityl methyl) methylene dioxybenzene	100	8/10
0.45	bis (butyl carbityl methyl) methylene dioxybenzene	25	5/9
0.45	bis (butyl carbityl methyl) propyl methylene dioxybenzene	100	9/10
0.45	bis (butyl carbityl methyl) propyl methylene dioxybenzene	25	1/10
0.45	butyl carbityl piperonyl ether	100	4/10
0.45	butyl carbityl piperonyl ether	25	0/10
0.45	1, 2-methylene dioxybenzene	100	2/10
0.45	1, 2-methylene dioxybenzene	25	0/10

C. Toxicological Data on Wild Ginger (*Asarum caudatum*) in Rats and Deer Mice.

Introduction

Tests conducted by the Section of Mammal Damage Control, Olympia field station, have shown that wild ginger is highly repellent to elk. Little is known about the toxicity of wild ginger to animals. With cooperation from the Olympia field station, some toxicological data have been obtained in rats and deer mice on wild ginger.

Results and Discussion

Five samples of wild ginger consisting of rhizomes and leaf petioles ground to #30 mesh have been tested. Samples were prepared in distilled water and formulated at 53.3 mg/ml for rats

and at 40 mg/ml for deer mice. Formulations were gavaged at 3 ml/100 g body weight for rats, and 1 ml/25 g body weight for deer mice.

Albino rats

Eight (8) male albino rats were dosed at 1600 mg/kg for each of the 5 wild ginger samples. Eight (8) rats treated in an identical manner received an equivalent volume of distilled water and served as controls. Rats were checked daily and individually weighed at 7 and 15 days after dosing. The test was terminated after 15 days.

None of the rats died from the 1600 mg/kg oral dose of wild ginger. No toxic or bizarre gross behavioral symptoms were observed. Analysis of variance of weights using a 2-factor factorial with repeated measures on the second factor showed that there was no significant difference between weight gains in the control and wild ginger treated animals.

Deer mice (Peromyscus maniculatus)

Eight (8) deer mice (4 males, 4 females) were dosed at 1600 mg/kg for each of the 5 wild ginger samples. Four (4) males and four (4) female deer mice treated in an identical manner received an equivalent volume of distilled water and served as controls.

Deer mice were checked daily and the test was terminated after 15 days. None of the deer mice died and no toxic or bizarre gross behavioral symptoms were observed.

A report detailing the test protocols and results were sent to personnel at the Olympia field station. Depending upon priority demand, additional toxicity tests might be conducted with purified fractions of wild ginger.

III. THE EFFECT OF DRC-4805 ON THE FECUNDITY OF WILD NORWAY RATS

Introduction

With reproductive inhibitors, an important effect results when a female rat passes a compound through the milk to her young and the young are permently sterilized. This study determined if DRC-4805 significantly affected the fertility of F_1 wild Norway rats (*Rattus norvegicus*) that have nursed on treated dams.

Methods

On the day after littering, female Norway rats were gavaged with a single oral dose of DRC-4805 dissolved in corn oil with a dose

volume of 0.1 cc of liquid per 100 grams of body weight. Two treatment levels were used: 0.0 (corn oil only) and 1.0 mg/kg with 16 females on each treatment. The light cycle of the animal room was 10 hours dark, 14 hours light. Food and water were provided ad libitum with no pre-dose fasting.

All F_1 litters were weaned at 27 days of age. When the F_1 young were at least 120 days of age, 18 males and 18 females from the control litters and 16 males and 16 females from the treated litters were paired with untreated mates of proven fertility and of the same age class to determine F_1 fertility.

Results and Discussion

At the time the F_1 rats were paired to determine fertility, 10 of 16 control litters and 10 of 16 treated litters were surviving. Within 45 days after pairing, all control males (18) and females (18) had sired or produced litters. By 65 days, when the study was ended, only 1 of 16 treated males had sired a litter and 0 of 16 treated females had littered. Necropsy showed that all treated rats except the male that sired a litter had reproductive tract abnormalities.

The results of this study and a previous study, reported in last years report, demonstrate the success of DRC-4805 in affecting the fertility of the wild Norway rat. This chemical is a potentially good rodent chemosterilant and additional studies with the compound are planned.

IV. BAIT FORMULATION STUDIES

A. Development of Baits to Deliver Uniform Dosage of 4-Aminopyridine to Red-winged Blackbirds (*Agelaius phoeniceus*)

Introduction

One major problem in efforts to control damage by birds to agricultural crops, has been the variability of chemical concentrations applied on the baits. This is particularly true when cracked corn is used as the bait. The chemical concentration variability among cracked corn particles frequently results in birds receiving too much or too little of the chemical, thereby preventing the bird from emitting an effective distress behavior. In these situations, the resultant effect is less than favorable; therefore, there is a need for a better uniform baiting system than the present cracked corn method.

Methods

A commercial cracked corn was passed through a Willey Mill, using a 1-mm size sieve. The ground corn was then ether extracted for

3 hours to remove some of the oil content. After extraction, the corn was air dried in a fume hood, and a 100 g aliquot of the dried corn was thoroughly mixed with a 25% plasdone-alcohol solution. After mixing, the mixture was dried in a force-draft oven. After all alcohol had been removed, the dried corn was passed through a Wiley Mill, using a 2-mm sieve. Using a Stokes single-punch tablet machine, the granulated corn was tableted to a 1/8 inch diameter and 24 mg weight.

Two preference feeding studies with the tablets were conducted using red-winged blackbirds. Study 1 consisted of six individually caged birds. Each bird was offered two dishes of food; one dish contained 100 pieces of screened cracked corn (approximately 23 mg in weight) and the other 100 tablets (approximately 24 mg in weight). The amount consumed was recorded to determine the acceptability of the tablets. Study 2 was a group feeding test. Since baiting in the field is usually conducted at either a 1:50 or 1:100 dilution rate of treated to non treated grain particles, one group of birds (6) were offered a dilution of 1 tablet to 50 pieces of cracked corn. The second group of birds (6) were fed a dilution of 1 tablet to 100 pieces of cracked corn.

Since the future sites for utilization of the treated tablets will be in areas of heavy morning dew and frequent heavy rains, moisture proofing of tablets is deemed necessary. Several water repellants, such as latex, carboset, various waxes, lipids and a protein binder were tested. The effectiveness of the coating materials was proven in a weatherometer. Acceptance of coated tablets was determined on caged birds.

Results and Discussion

The results of the preference feeding studies are shown in Tables 7 and 8.

Table 7. Feeding of corn tablets to individually caged red-winged blackbirds

Bird No.	Offered				Refused	
	No. cracked corn pieces	Wt. of corn (gm)	No. corn tablets	Wt. of tablets (gm)	No. cracked corn	No. tablets
1	100	2.09	100	2.49	0	7
2	100	1.88	100	2.49	0	1
3	100	1.98	100	2.49	0	0
4	100	2.02	100	2.48	2	8
5	100	1.94	100	2.48	0	2
6	100	1.92	100	2.48	3	0

Table 8. Acceptance of tableted corn when fed at a dilution rate of 1 to 50 and 1 to 100 to six red-winged blackbirds caged in a group.

Dilution Rate	Amount Offered	Weight in grams	Amount Refused
1:50	600 cracked corn 12 tablets	12.3	0
1:100	600 cracked corn 6 tablets	12.2	0

As can be seen in Tables 7 and 8 individually caged birds consumed on the average of 99 pieces of cracked corn and 97 corn tablets, while grouped birds on both dilution rates consumed all the baits. From these studies it appears there will be little rejection of the tableted corn.

The following chemicals were tested for their moisture proofing ability and deemed unacceptable for use on the tableted corn: stearic acid, household parafin, carboset 225, latex, rhoplex, shellac, lard, myristic acid and lauric acid. An embedding parafin was tested and proved to be effective when the coated tablets were placed in water. Granulated corn was formulated with 3% 4-aminopyridine, tableted and coated with parafin. When

two cages of 3 birds each were offered uncoated treated tablets and coat treated tablets, four of the six birds were affected; 1 from uncoated tablets and 3 from parafin coated tablets.

B. Preliminary Studies With Single Lethal Baits for Polynesian Rats (*Rattus exulans*).

Introduction

Baiting programs in sugarcane fields in Hawaii use crimped oat groats as the bait carrier. When 2% zinc phosphide bait is used, 3 to 5 seeds are the lethal dose for an average weight Polynesian rat. In a baiting system where a singles particle could carry several LD_{50}'s of zinc phosphide, the potential lethal effects would greatly be enhanced over oat groat baiting. The purpose of these preliminary studies was to determine if a tableted bait containing several LD_{50}'s of an acute toxicant will be accepted by caged Polynesian rats.

Methods

Four carrier baits, laboratory chow (with 20% dried molasses), oat groats, corn, and corn plus oat groats (50/50) were granulated using a 25% solution of plasdone and tableted in a Stokes' single punch machine. A 1/4 inch punch was used, which yielded a 150 mg tablet. Initial feeding results indicated rats would accept tablets even when their regular diet of laboratoy chow was present in the cage. Zinc phosphide then was formulated at 7% with each of the four granulated baits (approximately 6 LD_{50}'s). Each bait was fed to three individually caged adult male Polynesian rats. Each animal was offered a choice between the treated tablet and untreated tablets (control).

Results and Discussion

The results of feeding zinc phosphide tablets is shown in Table 9.

Table 9. Consumption of zinc phosphide treated tablets by male Polynesian rats.

Animal No.	Type Tablet	Tablets Offered		Tablets Consumed		Effects
		Control	Treated	Control	Treated	
1	Lab Chow	20	1	0	0	----
2	Lab Chow	20	1	16	1	Died
3	Lab Chow	20	1	15	1	Died
4	Corn	20	1	17	1	Died
5	Corn	20	1	0	0	----
6	Corn	20	1	16	1	Died
7	Oat Groats	20	1	4	1	Died
8	Oat Groats	20	1	0	0	----
9	Oat Groats	20	1	20	1	Died
10	Corn/Oats	20	1	0	0	----
11	Corn/Oats	20	1	4	1	Died
12	Corn/Oats	20	1	17	1	Died

The tablets were offered for only 1 night with no pre-baiting. There was one non-eater in each group. These results suggest that this type of bait carrier could be effective in a zinc phosphide baiting program in Hawaii.

V. COYOTE STUDIES

A. Sublethal Aversive Chemicals for Coyotes

Introduction

Prior to a cooperative study with the Behavioral Biology Project on drug-induced predatory suppression in coyotes, coyotes were orally dosed with ten candidate chemicals and observed for up to seven days posttreatment. Observations were recorded for onset and duration of sublethal effects, and prominent side effects such as

emesis and ataxia. The purpose for this preliminary testing was to select chemicals that displayed definite signs of sublethal effects which had a short onset of action and relatively long duration (up to 12 hours).

Results and Discussion

The chemicals tested and major effects observed are listed in Table 10.

Table 10. Sublethal aversive chemical testing in coyotes.

Chemical	Dose, mg*	Observation
Ergotamine tartrate	200,400	Emesis within 30 minutes; otherwise normal
Tremorine di-HCL	25,50,100,200	Mortalities at 100,200; Salivation at 50; no gross effects at 25
Amidephrine mesylate	25,50,100,200	Mortalities at 50,100,200; coyotes at 25 survived and displayed emesis and piloerection
Nicotine (free base)	15,50,200,400	Mortalities at 200,400; Vocalization, salivation and emesis at 25,50
Reserpine	100,200	Heavily sedated within 4 hours; some signs of tranquilization after 4 days at 200
Nicotice sulfate	160,400,520	Salivation and ataxia within 1 minute; retching at 400,520
Cantharidin	100,200	Salivation within 2 minutes; coyotes at 200 salivated for 7 days
Digitoxin	200	Emesis in 30-45 minutes
Atropine sulfate	100,200	Decreased alertness, mydriasis
Podophyllin	100,200	Salivation, signs of mucous irritation

*Total dose administered. Based on an average weight of 10 kg the approximate mg/kg can be calculated.

From these ten chemicals four were chosen for evaluation of predatory suppression in coyotes. The four chemicals and their predominate cite of action and/or effect were: (1) reserpine - (peripheral and central nervous system, sedation and tranquilization); (2) nicotine sulfate - (peripheral nervous system ganglionic stimulant, stimulation); (3) digitoxin - (cardiac glycoside which can cause irregular heart rhythms); and (4) cantharidin - (highly potent irritant and vesicant to mucous membranes). The modes of action and effects that each chemical produces are not similar. Since sublethal effects and predatory suppression are not well documented in coyotes, it was decided that a test regime which employed different sublethal effects would be the best approach. A summary of the test results for these chemicals in suppressing coyote predatory behavior can be found in the Behavioral Biology section of this annual report.

B. Acute Oral Testing of Toxicants in Coyotes

Introduction

This phase of research is designed to aid in the development of selective coyote toxicants. In particular, there is a need for quick-acting, low-dose toxicants for use with the sheep toxic collar.

In an effort to find species-specific toxicants for coyotes, many sources have been queried. A literature search entitled "Poisons for Canines" with 768 citations was received from the Toxicology Information Response Center at Oak Ridge National Laboratory. There was no indication of a chemical which could be classified as being species-specific for coyotes. Of the chemicals that are available for use, it seems more appropriate to describe them as being selective for coyotes only because they can be selectively administered. Efforts will be made to find toxicants that are species-specific for coyotes. Until such a chemical is found, available chemicals will be evaluated.

Results and Discussion

The LD_{50} - with 95% confidence limits, of nicotine (free base) in adult male coyotes was found to be 7.1(2.1-18.8) mg/kg. Coyotes succumbing to nicotine had toxic and clonic convulsions in 15-30 seconds and were dead within 60-90 seconds. For practical considerations, the onset of convulsions "immobilized" the animals and most animals died at the site at which they first experienced the tonic convulsion.

The oral LD_{50} of freshly prepared (0-day-old) potassium cyanide (KCN), formulated in 56% ethylene glycol- 44% water, in adult female coyotes was 4 mg/kg. Two 29-day-old formulations of KCN

were tested in adult male coyotes. Both of the 29-day-old formulations were prepared by Creative Biology Laboratory, Inc. One was prepared in water and contained 33% KCN. The other one contained 28% KCN in a gel. Comparative LD_{50} results for all three KCN formulations are shown in Table 11.

Table 11. KCN LD_{50}'s in coyotes.

Formulation	Sex	LD_{50}(95% C.L.) mg/kg
56% ethylene glycol- 44% water, 0-day-old	F	4.0(2.0-8.0)
28% in gel, 29-day-old	M	4.0(1.0-16.0)
33% in water, 29-day-old	M	2.8(2.8-2.8)

Coyotes succumbing to KCN became ataxic in 2-5 minutes, had convulsions in 5-7 minutes, and died within 8-15 minutes.

Because of its high dermal toxicity and associated safety hazards to personnel, tests with nicotine were discontinued. Advanced testing was also discontinued with KCN. Through conversations with EPA personnel and other research investigators, it was decided that it would be more "logical" that sodium cyanide (NaCN) be the chemical of choice for advanced testing in the sheep toxic collar. Toxic properties of both NaCN and KCN are very similar. As soon as the appropriate NaCN formulation for the toxic collar is decided upon, an oral LD_{50} for it will be obtained in coyotes.

C. Sheep Toxic Collar Tests in Coyotes

Introduction

One primary advantage of the sheep toxic collar technique is that it would be a selective method for delivering a toxicant to coyotes that preyed upon sheep. The toxicant would pose a primary hazard to only those animals which attacked sheep. A toxicant that kills quickly is desirable and would increase the chances of finding the coyote near the site of attack on the sheep. The dead coyote, and if necessary the sheep contaminated with the toxicant, could then be properly disposed.

The chemical chosen for studies in the toxic collar was sodium cyanide (NaCN). For many years NaCN was used in the coyote getter, and more recently in the M-44.

Methods

A detailed narrative of the methods used in this study can be found in the Behavioral Biology section of this annual report. Briefly, the procedures involve the training or "shaping" of wild-trapped coyotes to kill lambs in a 110 X 100 foot fenced enclosure. After each coyote has made at least three lamb kills by attacking the neck, it is classified as a sheep killer and is ready to be tested on a lamb wearing a toxic collar.

The toxic collars for this project are prepared by Creative Biology Laboratories, Inc. They consist of an outer plastic sleeve with straps for attachment to the lamb's neck. The inside of the plastic sleeve contains 10-14 polyethylene packets, each containing about 30 ml of 33% sodium cyanide in water. The collars are camouflaged by gluing wool on the outside of the plastic sleeve. This collar has been designated toxic collar prototype 1.

Results and Discussion

The testing of toxic collar prototype 1 has been completed (Table 12). Four adult male coyotes, each weighing approximately 10 kg were used. All coyotes punctured the toxic collar on the initial trial but only coyotes #1 and #6 received lethal doses of sodium cyanide. Coyotes #2 and #5 were each re-tested twice at 16 day intervals after their first exposure to toxic collared lambs. Each coyote attacked toxic collared lambs during re-test trials, but received only sub-lethal amounts of cyanide. Coyote #2, Test 11, did not puncture toxic packets, but he may have received some sodium cyanide which leaked from the packet.

Based on the coyotes first attack on toxic collared lambs, 2 out of 4, or 50% of the coyotes were killed. When computed on the total number of trials, (8), in which the toxic collar was bitten, the efficacy of the toxic collar drops to 25% (coyotes killed in 2 out of 8 trials).

These data suggest that modifications are needed in the toxic collar design. Current plans are to develop a single-layered collar (i.e., not a sheath-type). A single-layered collar will hopefully aid delivery of lethal quantities of toxicant into the attacking coyote's mouth.

Table 12. Summary of tests with toxic collar prototype 1.

Test	Coyote	No. Toxic Packets in Collar	No. Toxic Packets Punctured	Remarks
1	6	10	1	Coyote died within 23 min.
2	1	14	4	Coyote died within 10 min.
3	5	13	7	Sublethal dosing of coyote
4	5	11	3	Sublethal dosing of coyote
5	2	10	1	Sublethal dosing of coyote
6	5	11	0	Coyote did not attack
7	5	11	0	Coyote did not attack
8	5	11	0	Coyote did not attack
9	5	11	0	Coyote did not attack
10	5	11	2	Sublethal dosing of coyote
11	2	11	0	Toxic packet heat seal broke
12	2	12	6	Sublethal dosing of coyote

VI THE EFFECTS OF VAMPIRE BAT CONTROL ON MILK PRODUCTION

Introduction

Vampire bats are a common predator of cattle in many areas of Latin America. Though the main problem is that of bat-transmitted rabies, there is widespread belief that blood letting and harassment of dairy cattle will lower milk production. Numerous statements to this effect can be found in the literature, but there is an apparent lack of documentation to support them. To test this contention, a study was conducted on a dairy farm near Quito, Ecuador.

Methods

Fresh vampire bite counts were made prior to treatment for vampire control and at 30 and 60-day intervals following treatment. Control was achieved using the systemic method in which 1 mg/kg of diphenadione is injected into the rumen of the cow. Milk and blood samples were collected for measurement of various parameters, and milk production records were obtained for each animal both pre and post treatment.

Results and Discussion

Vampire predation on the herd was dramatically reduced from an average of 3.95 bites/cow to 0.12 bites/cow at the end of the 60-day posttreatment period, but milk production remained virtually unchanged from a pretreatment mean of 28 lbs/cow/day.

BEHAVIORAL BIOLOGY - APPLICATIONS IN WILDLIFE MANAGEMENT

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I. BAIT ADDITIVE DEVELOPMENT

Introduction

Bait additives offer a potential means of improving effectiveness of rodent baiting programs by attracting larger numbers of rats to the baits and increasing toxic bait consumption. Twelve bait additives were evaluated for use in damage-control programs aimed at the Philippine ricefield rat (*Rattus rattus mindanensis*).

Methods

Thirty-two wild ricefield rats were randomly assigned to four groups each consisting of eight animals. Each group was preference-tested with a different bait additive concentration. Lowest concentrations tested were those recommended for human consumption. The other concentrations were two-fold and four-fold increases over the initial level. If repellency effects were observed at the first concentration, we then used one-half strength and double strength concentrations. Ten protein flavor enhancers, Veltol Plus and sucrose were tested at three concentrations in ground rice against untreated ground rice. Two overnight preference test replications were conducted for each additive.

Results and Discussion

The ten protein flavor enhancers did not significantly increase ground rice consumption. Preference values for the protein enhancers, Veltol Plus and sucrose are shown in Table 1. No preference for treated rice is represented by values close to 50 percent. Only 6 of the 36 tested concentrations led to even slight preference increses relative to control preference values for ground rice. The only significant ($P<.05$) difference in preference was for Veltol Plus at .4% (C_3), but this was a repellent effect. Even sucrose did not produce reliable preference increases at the levels tested (10-40%). This finding is somewhat unusual but resulted from different rats preferring different levels of sweetener.

Table 1. Mean percent preference ± S.E.M. for twelve bait additives each tested at three concentrations.

No.	Additive[a] Name	Concentration[b] Control	C_1	C_2	C_3
1	V-50	47.4±1.0	47.0±2.7	45.3±4.2	29.7±6.5
2	Dried Humen	49.2±1.0	45.1±5.6	39.9±5.3	48.6±4.8
3	Zymino	50.3±1.1	42.0±4.8	47.3±6.8	43.1±3.8
4	Canned Food Flavor	51.7±1.8	42.1±4.2	30.6±7.8	38.6±4.8
5	Cereal Flavor	50.0±0.8	51.1±1.7	39.4±5.0	37.7±5.2
6	Vegamine #1	48.7±2.0	46.3±2.5	50.1±2.9	38.3±6.6
7	Vegamine #28	50.7±1.2	43.8±4.7	41.5±6.3	49.6±5.2
8	Vegamine #69	52.1±0.8	43.1±6.3	41.4±4.9	44.6±6.1
9	V 84T	48.2±0.8	44.1±2.1	38.0±4.0	42.1±3.1
10	Soy Sugar	50.1±1.0	46.1±5.1	52.9±2.1	49.8±4.0
11	Veltol Plus	51.9±1.7	51.8±0.7	46.1±0.8	44.7±1.7[c]
12	Sugar	51.7±0.8	57.6±3.2	51.9±2.6	60.2±3.7

a. Additive numbers 1-10 are protein hydrolysates; number 11 is a sweetness enhancer; number 12 is a nutritive sweetener.
b. Additive numbers 1-8 were tested at: C_1=0.3%, C_2=0.6%, C_3=1.2%; number 9 tested at: C_1=.15%, C_2=0.3%, C_3=0.75%; number 10 and 11 tested at: C_1=.10%, C_2=0.2%, C_3=0.4%; number 12 at: C_1=10% C_2=20%, C_3=40%
c. $P(C_3 > \text{Control}) < .05$

II. FK-178A VERSUS CALIFORNIA BROWN RICE FLAVOR

Introduction

A volatile rice flavor derived from California Brown (CB) rice has been shown to improve toxic bait acceptance by ricefield rats (*R.r. mindanensis*). More specifically, laboratory tests showed that the CB flavor produced a 75% increase in mortality and a 70% increase in consumption over the same bait without the flavor additive when used with 0.2% zinc phosphide on ground rice.

Recently, preference tests have shown that FK-178A, a rice variety grown in the Philippines, is greatly preferred to CB rice. All 16 preference-tested rats ate more FK-178A than CB rice, showing an average fourfold increase in consumption. However, this preference for FK-178A could have been related to differences in nutritive value, texture, rice grain size, or flavor. We therefore eliminated all but the flavor variable by collecting the flavor constituents of FK-178A and adding this material to ground CB rice.

Methods

Fifteen ricefield rats were preference tested with CB flavor-treated versus untreated CB rice. A second group of 16 ricefield rats was preference tested with FK-178A flavor-treated versus untreated CB rice. Mean percent preference scores for two replications of each test were statistically analyzed using the Wilcoxon Ranked Sum procedure.

Results and Discussion

The mean preference for FK-178A-flavored CB rice was 64.3%, and the mean preference for CB-flavored CB rice was 58.5% ± 3.0%. This preference comparison was statistically significant ($P<.05$). Preference levels for the two flavor treatments for each animal are shown in Table 2. Whereas 12 of the 15 CB group animals showed preference for treated rice, all 16 FK-178A group animals showed some preference for the treated rice.

Table 2. A comparison of preference for CB versus FK-178A flavor-treated rice.

Preference for Volatiles Trapped from C.B. Rice			Preference for Volatiles Trapped from FK-178A Rice		
Rat No.	$\bar{X}$ % Preference	Rank	Rat No.	$\bar{X}$ % Preference	Rank
1	49.4	3	16	60.2	18.5
2	52.3	6	17	61.7	20
3	54.4	9	18	55.3	11.5
4	52.7	7	19	57.5	16
5	48.5	2	20	70.7	25
6	44.4	1	21	84.2	29
7	60.2	18.5	22	56.2	15
8	50.5	4	23	60.3	20
9	51.4	5	24	57.6	17
10	68.3	24	25	55.3	11.5
11	65.2	22	26	75.4	27
12	55.6	13	27	92.7	31
13	65.6	23	28	56.0	14
14	70.8	26	29	54.8	10
15	88.9	30	30	78.0	28
			31	53.6	8
$\bar{X}$	58.5			64.3	
S.E.	3.0			3.0	
Rank Sum		193.5			301.5
Rank Difference		108			

P(FK-178A>CB)<.05

III. NONLETHAL ELECTRICAL BARRIER DEVELOPMENT

Introduction

For several years, lethal electrical barriers have been used on a limited scale in the Philippines to prevent crop damage by ricefield rats. These barriers, when properly maintained, are effective in greatly reducing damage. However, there are two important problems associated with lethal barriers: the labor cost involved in maintaining these barriers is too high to allow full-scale application and the electric shock levels necessary to kill rats are also dangerous to humans and livestock. To alleviate these problems, we have tested several nonlethal electrical barrier designs.

Methods

An 18-inch high, 3 electrode wire, nonlethal barrier was evaluated with a battery-operated commercial electric fence charger. Shock pulse rates were varied from 1/second to 5/second to measure the effects on barrier efficiency. All tests were conducted in eight 5 X 10 foot enclosures each containing three ricefield rats (*R. r. mindanensis*). Each day, rat counts, water and food consumption were measured on each side of the eight barriers. Baseline levels for these measures were taken for seven days before the barriers were electrically charged. These basal measures allowed us to measure barrier efficiency by calculating percent protection of rice and percent reduction in rats observed on the protected side of each barrier.

Results and Discussion

As shown in Table 3, the unmodified fencer unit at 1 pulse/second afforded 94.5% protection of a food source under the full feed condition. When the food available to the rats on the unprotected side of each barrier was reduced by one-half (16 g/rat/day to 8 g/rat/day), protection levels dropped to 79.7%. At higher pulse frequencies (3/second to 5/second), barrier protection remained high (95.1% and 88.2%) under the half food ration conditions. With the number of electrodes reduced from 3 to 1, the 5/second pulse rate continued to show 88.5% rice protection. However, we learned later that safety standards for livestock fence chargers do not permit alteration of any electrical parameter including pulse frequency. To increase the chance that each rat climbing a barrier will get shocked, we had to change our barrier design to show the rat's climbing speed. We therefore evaluated two barrier designs with a 110-volt unmodified commercial fence charger under the same conditions of food deprivation with a new group of rats. Barrier A was vertical, 18 inches in height and contained two active electrodes. Barrier B was slanted at 45 degrees toward the protected side of each enclosure, 13 inches

in vertical height, contained three active electrodes and had an added segment to discourage the rats from jumping clear of electrodes. As shown in Table 4, at the fixed pulse frequency of .9/second, barrier A afforded 94.5% protection and barrier B yielded 82.1% protection. Under the one-half feed ration condition, barrier A effiency dropped to 85.4% and barrier B fell to 65.8% Other barrier designs which discourage the rats from rapid running across electrodes and jumping the barrier will be evaluated under similar test conditions before field evaluation in the Philippines.

Table 3. Shock pulse rate effect on barrier efficiency.

Condition	Pulse Frequency					
	1/second		3/second		5/second	
	% rice protection	% reduction in rats	% rice protection	% reduction in rats	% rice protection	% reduction in rats
Full Feed	94.5	83.3	----	----	----	----
One-half Feed	79.7	71.5	95.1	82.2	88.2	84.1
One-half Feed and 1 electrode	----	----	----	----	88.5	84.1

Table 4. Comparison of two barrier designs, both charged with an unmodified livestock electric fencer unit.

Condition	Barrier Type			
	A		B	
	% rice protection	% reduction in rats	% rice protection	% reduction in rats
Full Feed	94.5	97.1	82.1	91.6
One-half Feed	85.4	97.1	65.8	76.5

IV. COYOTE STUDIES

A. Predatory Suppression in Coyotes with Drug-induced Illness

Introduction

Conditioning coyotes to avoid specific-prey by means of sub-lethal aversion to drugs would satisfy the demands and needs of both livestock producers and concerned environmentalists. Both conditioning studies with aversive levels of electric shock and bait shyness research with coyotes have suggested the potential field application of these principles to the predator problem. Our major concerns in this research area have been selection of an appropriate aversive drug and development of a practical field dosing method.

Methods

Four drugs with different modes of action were evaluated. Fifteen live-trapped coyotes (9-13 kg) adapted to killing and consuming deer mice were presented with live white rabbits during 1- to 2-hour daily sessions. Within 5-10 minutes after a given rabbit-kill, each coyote was restrained and orally dosed. Drug treatments were administered between one to four times to each of three coyotes. Specifically, these treatments were: 200 mg cantharidin (vesicant), 200 mg digitoxic (cardiac glycoside), 600 mg nicotine sulfate (ganglionic stimulant), 200 mg reserpine (tranquilizer), and 800 mg cornstarch (control). Each animal was then presented with live white rabbits after oral dosing, and predatory behavior was observed.

Results and Discussion

Cantharidin and nicotine sulfate proved unacceptable because of narrow safety margins that resulted in the death of some coyotes. Nicotine sulfate did, however, suppress rabbit killing in one of the animals for three test sessions. Table 5 shows the change in attack and kill times compared to a baseline period before dosing with either cornstarch (control), digitoxin or reserpine. Up to three doses of reserpine and cornstarch were not aversive; coyotes in these groups killed rabbits faster than before treatment. Only digitoxin produced consistent effects on rabbit-killing. Digitoxin suppressed rabbit-killing in one coyote for two successive 90-minute test sessions. Further tests with other drugs and higher dose levels of digitoxin are planned.

Table 5. Drug effects on predatory attack and kill time.

Drug	Animal Number	Total Doses	Mean Attack Latency Change (minutes)	Mean Kill Latency Change (minutes)
Cornstarch (control)	1	4	+ 2.5	+13.3
	2	3	-14.3	-13.3
	3	3	+ .4	- 4.3
Digitoxin	4	3	+ 6.3	+19.3
	5	3	+ 5.3	+11.3
	6	3	+ 1.3	+25.3
Reserpine	10	3	-22.2	-20.3
	11	3	-43.3	-47.7
	12	1	-22.0	-21.0

B. Some Preliminary Observations on the Sheep-attack and -kill Behaviors of Coyotes

Introduction

Recent research conducted by the Section of Supporting Sciences to develop a sheep toxic collar provided us the opportunity to examine coyote (*Canis latrans*) sheep-attack and sheep-kill behaviors under penned conditions. Specifically, two procedures for inducing sheep-attack and kill behavior were compared: a direct sheep-pairing and a sequential rabbit-to-sheep-pairing procedure. We predicted that the initial killing of rabbit prey would facilitate sheep attacks and kills by coyotes. These observations were important for our selection of neck-attacking coyotes in order to evaluate toxic sheep collars.

Methods

Observations were made in a coyote-test enclosure located on the Denver Federal Center. Briefly, this facility consists of: (1) a 100 ft by 110 ft fenced test area, (2) two 8 ft by 8 ft by 12 ft buildings which serve as observation booths, (3) an 8-pen coyote-housing kennel with a connecting runway between the kennel and test enclosure, and (4) a 3,500 sq. ft. sheep-housing area with shelter.

Basically, the procedure involved the daily pairing of coyote and prey for 60 minutes. Six male, live-trapped coyotes (20-26 lbs.) were used for the reported observations. Prey were 40-50 lb. sheep and 5-7 lb. albino rabbits. Five adaptation sessions, without prey, preceded testing. Three coyotes were paired directly with sheep and a 3-4 lb. portion of mutton. The remaining coyotes were initially paired with a live and dead rabbit followed by the sheep and mutton pairing. Two successive mutton-feedings or a single sheep kill was the criterion used for discontinuance of mutton presentation; whereas, after 2 successive feedings on the dead rabbit we presented the live rabbit alone. After 2 successive rabbit kills we used the sheep-pairing procedure previously cited. Additionally, it should be noted that after 12 days exposure to this procedure the coyotes not having killed sheep were presented a lamb with bits of mutton tied around the neck. Although this somewhat confounded the treatments, it was done in an effort to attract coyotes to bite and attack the lamb's neck.

For current purposes, release of the attack bite is equated with killing; even though some lamb's were not dead at this time, feeding usually started soon after bite release occurred and death invariably occurred within several minutes.

With the exception of the aforementioned food presentations, all animals were kept on continuous food deprivation from the start of pairing with prey until their initial sheep kill. Coyotes were allowed 20 minutes feeding time immediately following release of the attack bite (i.e., rabbit or sheep), with alternate-day feedings of 3-4 lb. portions of mutton provided after each animal's second kill.

Results and Discussion

Table 6 presents the number of sheep kills and number of 60-minute sheep-pairing sessions which occurred before the first kill by each coyote. As shown, a total of 23 sheep kills were observed. Moreover, with the exception of one animal (Coyote #4), all sheep kills involved neck or throat wounds with death probably due to suffocation and/or loss of blood.

Regarding the data presented in Table 6, the total number of sessions required for an initial sheep kill was generally longer for coyotes undergoing the rabbit-sheep versus sheep-direct pairing procedure. Although these data were confounded by the presentation of a sheep having mutton tied around the neck, the fact that all coyotes in the sheep-direct treatment killed in the same or fewer sessions than the rabbit-sheep treatment indicates no facilitation in killing was produced by prior exposure to rabbit prey.

Table 6. Summary of select test-session data characterizing onset of sheep killing.

Treatment	Coyote	Number of Sheep Kills Observed	Session of Initial Sheep Kill
Rabbit-to-Sheep	1	3	15th
	2	3	13th
	3	3	26th
Sheep-Direct	4	3	13th
	5	5	13th
	6	6	3rd

Table 7 shows the respective times-to-attack and times-to-release-of-attack-bite for each coyote's sheep kills. As can be seen, times-to-attack decrease sharply from the initial to final kill for 5 of the 6 animals; whereas, times-to-release-of-attack-bite are somewhat stable across kills except for coyotes #4 and #5. These data suggest that while coyotes which kill sheep under penned conditions display shortened delays in times to onset of attack, no decrease in the time actually spent killing was observed, even for animals having killed 5-6 sheep.

Table 7. Times-to-attack (minutes) and times-to-release-of-attack-bite (minutes) for respective sheep kills of each coyote.

Coyote	Times-to-Attack						Times-to-Release-of-Attack-Bite					
	Sheep-Kill						Sheep-Kill					
	1	2	3	4	5	6	1	2	3	4	5	6
1	8	44	21				8	14	13			
2	10	< 1	< 1				10	11	14			
3	1	2	< 1				4	6	5			
4	25	5	< 1				10	5	13			
5	15	30	17	< 1			*	10	5	14		
6	58	8	< 1	2	1	< 1	8	6	7	10	5	7

* No time estimate computed; coyote sporadically bit and mauled face of sheep for approximately 45 minutes.

BIOCHEMICAL DEVELOPMENT ON WILDLIFE MANAGEMENT RESEARCH

Roger W. Bullard, R. Dan Thompson, and
Stephen Kilburn

I. DIPHENADIONE RESIDUE STUDIES IN MILK

Introduction

In 1973 we reported an initial study of mammilary transfer of diphenadione in lactating cows. The objective of this research was to determine if humans can safely consume the milk of cows that have been systemically treated for vampire bat control. Milk from cows dosed at 2.75 times the recommended dosage contained small quantities at 12, 24 and 48 hours after treatment. Since then we have added two additional levels to obtain a greater understanding of mammilary transfer of diphenadione.

Three lactating cows were dosed with 1 mg/kg diphenadione and three with 5 mg/kg. Each cow had a nursling calf and the calves of the high dose cows were also given 5 mg/kg. Milk and blood samples were collected from each cow and blood from each calf immediately posttreatment, at 12 hours, and then every 24 hours thereafter. Samples aliquots are to be analyzed by a glc method developed earlier for diphenadione analysis. Prothrombin clotting times were determined by the Sera-Tek Prothrombin Reaction Chamber Technique.

Results and Discussion

The prothrombin clotting times in cows dosed at 1 mg/kg were much faster than those for the 2.75 and 5 mg/kg doses (Table 1). The maximum prothrombin clotting time of 25.3 seconds was reached at 48 hours compared to 42.0 seconds and 66.7 seconds at 96 hours for 2.75 and 5 mg/kg doses, respectively. Prothrombin readings in the untreated calves remained normal (14-18 seconds) as reported in 1973 for the 2.75 mg/kg dosage.

The prothrombin response to the 5 mg/kg dosage was far greater than to the 1.0 mg/kg dose. The maximum response at 96 hours was nearly 4X normal for cows and over 25X for their suckling calves. The return to normal levels for cows appears to be dose related; at 144 hours the readings were 16.7, 23.2 and 25.3 seconds for 1, 2.75 and 5 mg/kg respectively. One calf dosed at 5.0 mg/kg died 123 hours after treatment. An autopsy of the carcass reveled that the animal had died of internal hemoraging. The Prothrombin value was over 4X normal for the two surviving calves. The calf that died was the youngest (about 2 weeks old) test animal. This indicates that overdosing cows and their young suckling calves at

5.0 mg/kg may introduce considerable risk in vampire bat control programs. The prothrombin data at 1.0 mg/kg demonstrates the safety of dosing cows with suckling calves at the recommended level or even overdosing at 2.75 times that level.

Table 1. Prothrombin clotting time of cows and calves dosed with diphenadione.

	Hours Posttreatment					
	0	12	24	48	72	96
$\bar{X}$ 1 mg/kg cows	15.3	18.7	19.0	25.3	24.6	21.2
$\bar{X}$ 1 mg/kg calves	14.8	16.7	15.8	18.5	15.2	15.7
$\bar{X}$ 5 mg/kg cows	17.8	17.6	19.5	31.5	48.2	66.7
$\bar{X}$ 5 mg/kg calves	14.3	18.8	30.0	93.0	171.3	365.7

	Hours Posttreatment						
	120	144	168	192	216	240	264
$\bar{X}$ 1 mg/kg cows	20.3	16.7	----	----	----	----	----
$\bar{X}$ 1 mg/kg calves	16.3	14.4	----	----	----	----	----
$\bar{X}$ 5 mg/kg cows	53.7	25.3	28.8	22.7	20.1	----	----
$\bar{X}$ 5 mg/kg calves	96.0	75.8	96.5	56.5	34.8	21.6	19.8

II. PROPERTIES OF VOLATILE FERMENTED EGG CONSTITUENTS

Introduction

Researchers of the Denver Wildlife Research Center have observed that the decomposition of biological materials can yield chemical products that are both attractive to coyotes and repellent to deer. Development of attractants and repellents has been a major objective in pest management research for years. A McLaughlin Gormley King Company (MGK) fermented egg product (FEP) appears to satisfy both objectives.

The problem has been that air innoculation of organisms produces the most active product. Unfortunately, this method is subject to changing conditions, and batch to batch variation in quality is common. These subtle changes can influence the behavioral response of animals and cause variable results. Our objective is to determine the active componenets of FEP and prepare synthetic formulations which produce behavioral responses analogous to those of the natural product.

Since volatile fatty acids have recently been reported to have pheromonal properties in other species, these compounds were of special interest. An air innoculated ("old") sample of FEP had been compared with a sample ("new") that had been innoculated with a single organism and the "old" was over twice as effective. We determined the quantities of 10 volatile fatty acids in both samples to see if they could contribute to the relative attractancy.

The remainder of our effort was with the volatile fraction of FEP. This was collected via a modified rotary evaporator (discussed p. 44 of the 1973 Annual Progress Report) and analyzed by combined gas chromatography - mass spectrometry. This qualitative evaluation was to provide information on the chemical composition that would help in subsequent behavioral evaluation of that fraction.

Volatiles for behavioral testing were separated on a 500-ft. X 0.03-in capillary gas chromatograph column and 37 fractions were collected via a specially designed cryogenic system. We collected the major peaks individually and the minor ones in combination. The objective of the behavioral tests was to classify fractions by human odor panel response into qualitative categories. We would then test the various categories on coyotes to see which are most attractive. Individual compounds belonging to the attractive categories would then be identified and formulatied in a synthetic preparation.

Results and Discussion

The quantities of 10 volatile fatty acids in "old" and "new" FEP are given in Table 2. The concentration in the "old" was high for most of the fatty acids whereas only trace quantities were present

in the "new." These results indicate that fatty acids may have a role in the attractancy of FEP to coyotes.

Eight human subjects participated in tests to establish a program for odor panel evaluation of FEP volatiles. They used 24 different odor quality descriptions for the 37 FEP volatile fractions. Twelve of these descriptions which had been used most frequently, or which had been synonymous with other terms, were chosen as categories for descriptions in subsequent odor panel tests. They were as follows: flower, fruit, sweet, juicy fruit, earthy, cheese, sour, musty-moldy-mildew, rotten, fermented, other, not recognizable or not detected. Several of the subjects tested under these criteria gave responses of satisfactory consistency.

We felt that this program could be used satisfactorily for human odor panel evaluation. However, efforts to develop a behavioral test for coyotes had lagged. This called for a change in emphasis if the two programs were to be coordinated.

The first test was a modification of one used by Doty and Dunbar (Doty, R. L., and I. Dunbar. 1974. Physiology and Behavior 12:825-833) for beagles. We modified a coyote nest box by adding two 3-inch odor ports in the back pane. Photocells sensed the investigation frequency and time when an animal sampled the two odor ports. This system proved too confining for a test on wild coyotes.

We then tried two procedures in the 5 X 10-ft. coyote pens. In the first, we placed FEP under pyrex glass odor stations on the floor of the cages and observed coyote responses by closed circuit television. The responses ranged from none to highly variable. We then tested a drinkometer system consisting of wire mesh on the floor and stainless steel odor cups attached to the chain link cage walls. The coyotes avoided these "strange" additions to their cage.

The three behavioral tests convinced us that live-trapped coyotes could not be realistically tested in confined quarters. A coyote field event counter and timer is being developed for remote monitoring of scent stations in a 100 X 110-ft. semifield test area. If we are able to use this test, then it will be coordinated with the human odor panel testing. A similar coordinated effort with the Forest Animal Damage Field Station at Olympia, Washington, is planned. They have an appropriate behavioral test for deer. Hopefully, this coordinated effort will remove the need to chemically identify volatile componenets other than the most active ones.

Table 2. Fatty acid content of fermented egg.

Acid	"old" (ppm)	"new" (ppm)	Acid	"old" (ppm)	"new" (ppm)
acetic	n.d.*	>0.5	valeric	2,020	>0.5
propionic	20	>0.5	caprylic	8,270	>0.5
isobutyric	255	0.58	caproic	5,600	>0.5
butyric	3,970	>0.5	pelargonic	340	>0.5
isovaleric	970	>0.5	capric	310	>0.5

* Unable to achieve resolution needed for analysis under these conditions.

III. THE ANALYSIS AND SYNTHETIC DUPLICATION OF RICE AROMA

Introduction

Development of better baits and baiting procedures has been a major goal in our effort to find improved methods for reductional control of ricefield rats (*Rattus rattus mindanensis*). Previously we have reported that rice volatiles are highly effective in increasing the consumption of a rice bait. The consistently high acceptance of these formulations in several advanced tests provided justification for pursuing the isolation and identification of the important volatile constituents. The procedure and tentative identification of fifty-five compounds was reported in the 1973 Annual Progress Report. Continuation of the work has led to positive identification of an even larger number of compounds.

Results and Discussion

Over 174 compounds were recognizable when a 500-ft. X 0.03-in. capillary column was temperature programed at 1°/minute from 40° to 185°C. Seventy-three of these were positively identified and are listed in Table 3. The predicted identity was obtained on an additional 31 compounds.

Sixty-three compounds were reported for the first time in either unprocessed or processed rice. Fifty-four of these have never been identified in any unprocessed cereal grain. This work constitutes the most extensive isolation and identification of volatiles in any of the uncooked cereal grains.

Table 3. Positively identified rice volatiles in order of glc retention time.

acetaldehyde	benzaldehyde	2-decanone
acetone	1,3,5-trimethyl benzene	napthalene
ethanol	6-methyl-5-hepten-2-one	decanal
isobutanal	2-octanone	2,4-nonadienal
n-propanol	2-n-pentyl furan	2-decenal
2-butanone	octanal	2-undecanone
butanal	1,2,4-trimethyl benzene	2-methyl napthalene
isopentanal	2,4-heptadienal	2,4-decadienal
2-methyl butanal	Δ3-carene	1-methyl napthalene
benzene	1,2,3-trimethyl benzene	phenylacetaldehyde
3-penten-2-one	p-cymene	2-dodecanone
2-pentanone	trans B-methyl styrene	2-ethyl napthalene
pentanal	p-diethyl benzene	2-dodecenal
2, 5-dimethyl furan	2-octenal	
2-methyl pentanal	m-diethyl benzene	
toluene	1,3-dimethyl-5-ethyl benzene	
2-hexanone	o-diethyl benzene	
hexanal	p-methyl benzaldehyde	
2-hexenal	1,3-dimethyl-4-ethyl benzene	
ethyl benzene	1,4-dimethyl-2-ethyl benzene	
p-xylene	2-nonanone	
m-xylene	nonal	
2-heptanone	2,4-octadienal	
heptanal	1,2-dimethyl-3-ethyl benzene	
2-butyl furan	1,2,4,5-tetramethyl benzene	
2,4-hexadienal	1,2,3,5-tetramethyl benzene	
o-xylene	2-nonenal	
2-acetyl furan	1,2,3,4-tetramethyl benzene	
-pinene		
2-heptenal		
n-propyl benzene		
1-ethyl-4-methyl benzene		

IV. DEVELOPMENT OF AN ANALYTICAL PROCEDURE FOR WARFARIN

Introduction

Because of increased emphasis on the use of anticoagulant rodenticides in tropical lowland rice, there is need to determine the residue levels in plants, water and soil. Analytical procedures are needed for such determinations. In the 1973 Annual Report we reported that the procedure had been developed for diphenadione but more work was needed for warfarin. Various techniques, such as flourometry, high pressure liquid chromatography, thin layer chromatography, spectrophotometry and gas chromatography were subsequently tested.

Results and Discussion

Flourometry, high pressure liquid chromatography, thin layer chromatography and spectrophotometry techniques can all be used at concentrations above 1 ppm. Unfortunately, none of these meet the 0.1 ppm sensitivity that is required for residue analysis.

Gas chromatography was potentially the answer, provided that warfarin could be converted into a compound having sufficient volatility. Several different derivitization techniques were tested without success. After exploring numerous oxidation techniques, a nitrochromic acid procedure was used successfully in forming a degredation product that is detectable at levels equivalent to 10 nanograms of warfarin. A general cleanup method has been developed that can be used with this oxidation process. Most of the remaining effort will be in finding the optimum conditions for all steps of the method.

ELECTRONIC DEVELOPMENT FOR WILDLIFE RESEARCH AND MANAGEMENT

A. Lawrence Kolz, George W. Corner,
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I. NEW WILDLIFE TRANSMITTER DESIGNS

Introduction

Two wildlife transmitter designs have been developed for field evaluation: a 1.8-kg mortality transmitter for grizzly bears and a 5-g delayed turn-on transmitter for birds. Ten prototype bear transmitters were loaned to the Wyoming Game and Fish Commission for a cooperative study. The bird transmitters will be field tested in 1975.

Results and Discussion

Five of ten mortality transmitters sent to Wyoming were used on grizzly bears. Some problems were encountered in fitting the collar design to small bears (200 to 300 lbs.). One bear lost it's transmitter, and we now plan to modify some of the collars to fit smaller animals. The general field results with the mortality transmitters have been favorable. The radio tracking reception has been adequate in spite of the rugged terrain, and the transmitter packaging has been proven durable. A continuation of this telemetry study is planned for 1975.

The delayed turn-on transmitter was designed as a minimum size instrumentation package for monitoring migrating birds. This transmitter turns-on after a predicted time delay and then transmits until its battery fails. For example, a transmitter might be designed for a 2-month delay followed by 1-month of transmitting life. The advantages of having a delayed turn-on over a conventional wildlife transmitter are that more time is available for the initial trapping and instrumenting, the instrumented birds have time to adapt to the transmitter, there is a net weight savings over the life of the test, and the battery power is conserved until needed.

II. ENGINEERING SUPPORT AND PRODUCTION SERVICES

Introduction

Each year the Bioelectronics Project serves research programs by constructing specialized telemetry equipment. This year the Section of Predator Damage Research received the major work

commitment with lesser time being devoted to programs for the Wyoming and Montana Game and Fish Departments, Wildlife Services, the Sections of Mammal Damage Control, and Pesticide-Wildlife Ecology.

Results and Discussion

Approximately 280 sheep mortality transmitters and 80 small rodent transmitters were reworked for the Section of Predator Damage. The mortality transmitters were used to instrument 234 lambs in southeast New Mexico to help biologists locate dead animals, and the rodent transmitters were planned for a predator damage assessment study in Idaho. In addition to this work, 60 coyote mortality transmitters, having a new design, were delivered to the Unit of Predator Ecology and Behavior and more of these units are now under construction.

The work completed for the Wyoming and Montana Game and Fish Departments included the construction of ten prototype grizzly bear transmitters plus some smaller tracking transmitters for use on orphaned cubs. We supplied small bird transmitters to Wildlife Services in Minnesota for studies relevant to the survival of the Kirtland Warbler and mortality transmitters to Texas for use on Red Wolves. The Section of Mammal Damage Control requested telemetry equipment support for a pesticide study. Preparation for this work has necessitated development of transmitters specific to the need and working out the attachment problems.

III. EVALUATION OF CASSETTE TAPE PLAYERS

Introduction

In June, 1973 the Air Force Weapons Laboratory requested the Wildlife Research Center to measure the frequency response of selected 12 V-dc magnetic tape players. The work was completed in 1974.

Results and Discussion

A final report was submitted to the Air Force Weapons Laboratory summarizing our frequency response measurements for cassette tape players. This report was subsequently published: Kolz, A. L. and Johnson, R. E. "Frequency Response Measurements for Cassette Tape Players," Air Force Weapons Laboratory, AFWL-TR-74-147 (October, 1974), pp. 52.

IV. FIELD INSTRUMENTATION FOR RECORDING FOOD PREFERENCE OF RATS

Introduction

The concept of using a coulometer component (E-Cell) as a recording mechanism for counting and timing biological events is being developed as a food preference device for ricefield rats. These devices are primarily designed for field operation and must be inexpensive, battery operated, and long-lived. The data recorded in the field are read-out with a laboratory instrument which can support several of the field recorders. Prototype units are now ready for testing.

Results and Discussion

Six prototype food preference devices have been built for food consumption studies using ricefield rats. These devices record the number of rat visitations to a food and total the feeding time. Preliminary design data indicates a measurement error of about ±6 percent. It is planned to test the prototypes in Denver with ricefield rats before finalizing a design for a field device to be sent to the Rodent Research Center, Los Banos, Philippines.

V. RADIO TRANSMITTER FOR TRANQUILIZER DARTS

Introduction

The techniques and expertise developed in designing miniature wildlife transmitters were extended to other uses. In late 1973, we started to develop a radio equipped tranquilizer dart for a liquid syringe projectile to locate drugged animals. The technical aspects of this work have not presented any major problems, but the work has been stopped until a registered liquid tranquilizer becomes available.

Results and Discussion

Progress has been made toward developing a radio transmitter for tranquilizer darts including a number of successful radio-tracked immobilizations. The miniature transmitters have withstood the dart's accelerations and impacts, but the added weight of the radio does alter the trajectory. However, we do not consider this a serious design limitation. The major problem to be solved is finding a registered animal tranquilizer. Our design uses a liquid dispensing syringe, and at this time, the only registered animal tranquilizer is in powder form. A decision must be made on how to handle this situation: either design for a different delivery system or wait for a liquid tranquilizer.

VI. AUTOMATIC WILDLIFE TRACKING SYSTEM

Introduction

Two electronic companies were contacted and an engineering consultant hired in an attempt to complete development of the automatic wildlife tracking system. The problem being that the field data collected with the present equipment have indicated a serious design deficiency in receiving sensitivity and stability. However, the projected costs for completing the design exceed the available funding. The development is being terminated.

Results and Discussion

A consultant engineer was hired to review the receiver design of the 30 MHz automatic wildlife tracking system. This work is summarized in a report submitted to the Denver Center and outlines some alternate design approaches.

Following a review of the consultant's recommendations, two companies were requested to submit cost estimates for redesigning the system's receivers. Southwest Research Institute of San Antonio, Texas, estimated a cost greater than $30,000; and Ocean Applied Research of San Diego, California, informally stated that the cost would exceed $20,000. The cost projections for redesigning the system were twice the original development. Such cost is not justified at the present time. We are therefore, terminating this development.