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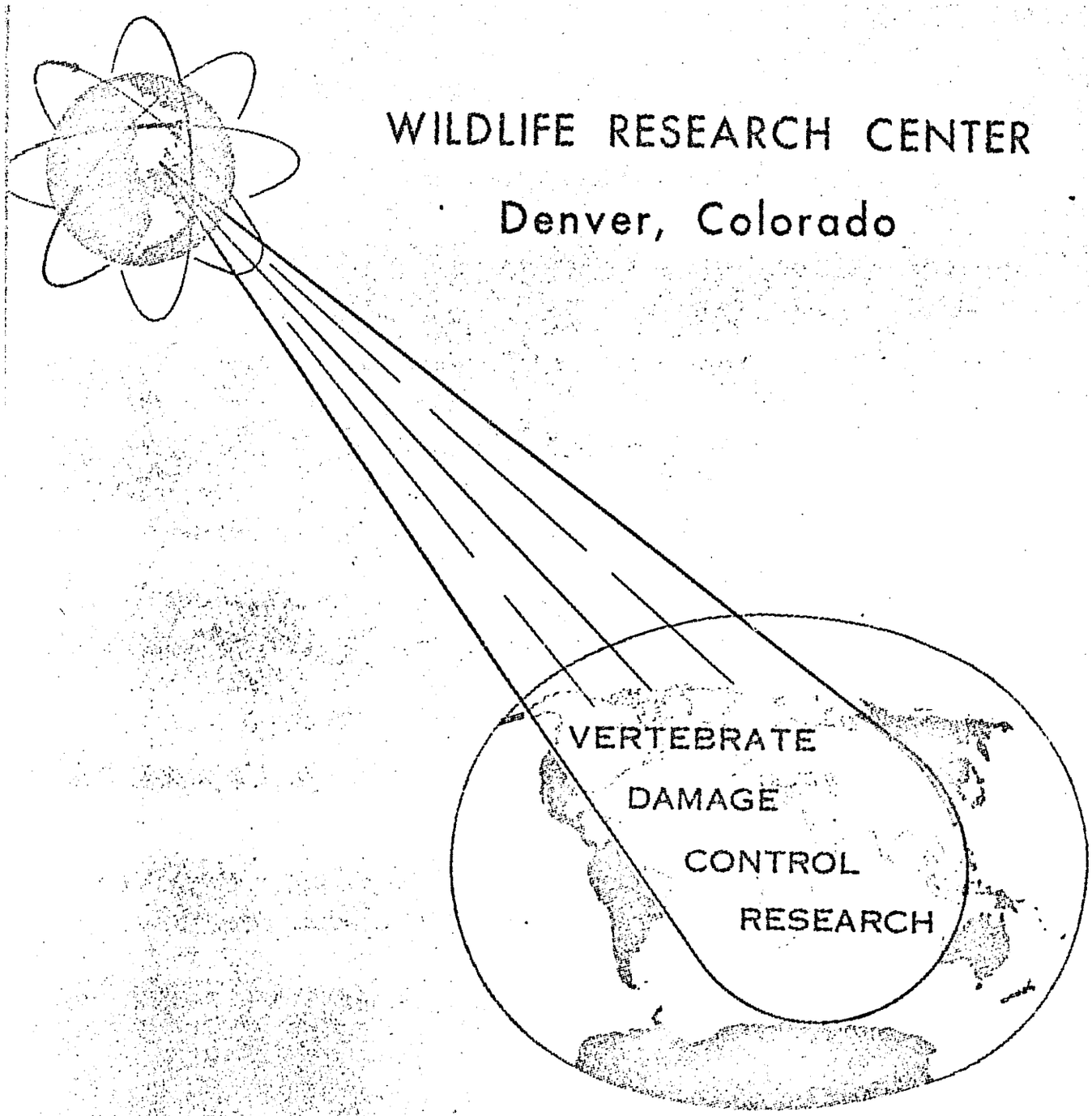

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CONTENTS

Page

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

ABSTRACTS

Pharmacology and Physiology 1
Behavioral Biology 4
Biochemical Development 5
Bioelectronics 7

REPORTS

Pharmacology and Physiology

I. Fluorescent Physiological Markers 8
II. Rodenticide Evaluations 8
III. Reproductive Studies 16
IV. Diphacinone Toxicity to Nontarget Insectivorous Bats 23
V. Comparative Nutritional and Feeding Studies with the Polynesian (*Rattus exulans*) and Laboratory Rats 25
VI. Coyote Studies 29

Behavioral Biology

I. Bait Additive Development 34
II. Rice Variety Preference 35
III. Effects of Rice Volatiles on Zinc Phosphide Bait Consumption 37
IV. Nonlethal Electrical Barrier Development 39

Biochemical Development

I. Diphenadione Residue Studies in Milk 42
II. The Analysis and Synthetic Duplication of Rice Aroma 44
III. Development of Analytical Procedures for Anticoagulants 45
IV. Controlled Release of Volatiles 46

Bioelectronics

I. Automatic Wildlife Tracking System 48
II. Field Instrumentation for Recording Food Preference of Rats 48

ABSTRACTS

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

PHARMACOLOGY AND PHYSIOLOGY

The primary areas of investigation were: fluorescent physiological marking; evaluations of rodenticides and reproductive inhibitors; toxicity studies of diphacinone to nontarget bats; and comparative nutrition and feeding studies in rats. The major accomplishments are summarized below:

1. Fluorescent physiological markers. Five candidate fluorescent pelage markers were evaluated, but none offered any advantage over DRC-6082 which was reported on last year. DRC-6082 was selected as the fluorescent marker of choice and was successfully used by personnel at the Rodent Research Center, Philippines. Advanced Studies are being conducted with DRC-6082, and a joint publication between the Denver Wildlife Research Center and the Rodent Research Center should be available in about 1 year.

2. Rodenticide evaluations. At the request of Mr. Young-Moo Shin, FAO Fellow-Korea, feeding tests in rats with two Korean zinc phosphide formulations and one of red squill were compared to zinc phosphide and red squill formulations prepared in the United States. There were no significant differences in mortality and amount of chemical consumed between the zinc phosphide or the red squill formulations. These bioassay data indicate that the potency of the respective formulations produced in the two countries is equal.

   Preliminary toxicology data were obtained with zinc phosphide. In one test, male albino rats were gavaged with 10 mg/kg of zinc phosphide for 3 consecutive days. On the fifth day, LD_{50} determinations were performed on this pretreated group and a control group. There were no differences in the LD_{50}'s between groups. This indicates that there is no cumulative or tolerance to the toxicity of zinc phosphide at the dose employed. In another test, a toxic dose of zinc phosphide was administered to fasted and nonfasted male albino rats. There was no difference in mortality between the two groups, but the time to death was significantly shorter in the nonfasted animals.

   Studies were initiated to find chemicals capable of potentiating the rodenticidal properties of red squill. Six chemicals were found which potentiate the mortality of red squill in male albino rats.

Tests will proceed with female albino rats and then into target rodents.

Laboratory results with the acute rodenticide, DRC-6091, have been encouraging. Its oral LD_{50} in Norway, Polynesian, and ricefield rats is less than 30 mg/kg for each species. In free-choice feeding studies, 80 to 100% mortality has been obtained in Polynesian rats and 100% in ricefield rats. In rat-to-rat secondary hazard tests, 50 to 60% of the rats have died, but no toxicity was observed in mongooses or raptors which consumed poisoned rat carcasses.

An anticoagulant bait containing diphacinone that was prepared in 1969 for the U.S. Army was found to be toxic to wild Norway rats, but not highly preferred. Thirty-five percent of the rats were "noneaters" and survived the test. All animals (65%) that consistently ate the bait died.

Benzothiazole, a candidate deer repellent, was found to be moderately toxic to rodents. Acute oral LD_{50}'s in mg/kg were: 305 (203-458) for male deer mice; 555 (437-705) for female deer mice; and 346 (237-505) for male albino rats.

3. Reproductive studies. Wild Norway, Polynesian, and ricefield female rats were gavaged with a single oral dose of DRC-4805 to determine its effect on the time interval from pairing to littering. Wild Norway rats treated at 10 mg/kg had a mean interval of 105 days from pairing to littering. Polynesian rats treated at 2.5 mg/kg had an interval of 64 days. Ricefield rats treated at 2.5 mg/kg did not show an effect because breeding of the control group was too erratic to provide a basis for comparison.

   A cooperative study was begun with the Section of Bird Damage Control to evaluate the effect of photoperiod stimulation and triethylene melamine (TEM) treatment on testicular growth and spermatogenesis in the red-winged blackbird. Control and TEM-treated birds were killed after exposure to various intervals of photoperiod stimulation and reproductive data were collected on presence of sperm in the seminal sac, testicular weight, and histology of the testes. A single oral dose of TEM at 1.78 mg/kg before the start of photoperiod stimulation produced the greatest effect in inhibiting testicular growth and spermatogenesis in the red-winged blackbird.

4. Diphacinone toxicity to nontarget insectivorous bats. The acute oral toxicity of diphacinone to big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifugus*) has been determined. The LD_{50}

with 95% confidence limits was found to be 2.8 (1.1 to 7.5) mg/kg for big brown bats and 2.8 (0.2 to 45.3) mg/kg for little brown bats. Females appeared to be more susceptible in both species. In big brown bats, two of five males and four of five females died. In little brown bats, only one of four males died, whereas five of six females succumbed. Low doses produced death in only the females.

5. Comparative nutrition and feeding studies between Polynesian and albino rats. Studies were performed to determine how increasing the level of acid detergent fiber (ADF-lignin, cellulose, and insoluble ash) in the diet affected the digestibility of dry matter, crude protein, acid detergent fiber (ADF), and gross energy. In both species, the digestibility of the above parameters, except ADF, decreased significantly with increasing levels of ADF in the ration. There were no differences between sexes, but species differences were significant. Digestibility of dry matter, nitrogen, and gross energy was higher for the albino rat, but the digestibility of ADF was higher for the Polynesian rat.

   Specific euphagia was studied by using phosphorus as the limiting essential nutrient. Control animals received basal ration with a normal supply of phosphorus. Treated animals received basal ration, but deficient in phosphorus. When the blood phosphorous level became significantly reduced in the treated animals, both groups were offered normal and phosphorous deficient rations. Both control and treated Polynesian groups and the albino rat control group consumed more of the phosphorous deficient ration than the normal phosphorous ration. Only the albino rat deficient group preferred the normal phosphorous ration over the deficient ration.

   Bait aversion in rats to a bait containing mestranol was evaluated to determine whether the aversion is due to taste perception or whether blood levels of estrogen might act as part of a feedback mechanism to inhibit food intake. Mestranol was administered to groups of rats in baits, by gavage, and by intraperitoneal injection. Consumption of treated bait was very poor, and in this group of animals there was no effect on posttreatment food consumption. Posttreatment food consumption was significantly reduced in both the gavage and intraperitoneal groups.

6. Coyote studies. A ruler-like measuring device was designed and produced for field use in aging coyote fetuses. By using the device to measure the crown-rump length of fresh coyote fetuses, the age of the fetus and the breeding date of the adult coyote can be determined.

   Coyote and beagle anal gland samples were collected for analysis by the Monell Chemical Senses Center. Improvements in collection

techniques were made and the analysis of the samples provided basic information as to the chemical identification of the odor-producing compounds in the anal glands.

Oral efficacy of five tranquilizers has been determined in the coyote. Acceptable levels of tranquilization were produced by Tranvet and Vetame. One compound, CI-744, was found to be a good intramuscular immobilizing agent.

Studies on chemicals to be used in coyote-conditioned aversion tests were initiated. Criteria for selection of chemicals include sublethal effects observed and onset and duration of action.

BEHAVIORAL BIOLOGY

Research was conducted to enhance consumption of treated and untreated baits for rats, testing for highly preferred varieties of rice, and evaluation of the effectiveness of nonlethal electrical barriers for repelling *Rattus rattus mindanensis*. Major accomplishments are summarized as follows:

1. Bait additive development. A three-phase bioassay procedure was developed and implemented for detecting and developing preferred bait additives to increase the effectiveness of ricefield rat control programs. Two additives, monosodium-L-glutamate and ribotide, have been tested thus far. Neither material showed promise as a bait enhancer. Other materials such as protein hydrolysates, flavor concentrates, and sweetness enhancers will be tested in this continuing program. The three phases in this program consist of: (1) a preference test for determining active concentrations and for classifying additives as repellents, enhancers, or inactive; (2) a brief-exposure test for reliability of the enhancing effects; and (3) a semifield test for assessing the potency and field potential of the candidate additives. All data are stored on magnetic tape for easy access and retrieval purposes.

2. Rice variety preference. Laboratory and semifield preference tests were conducted with ricefield rats on four varieties of rice. In both laboratory and semifield tests, significant preference ($P < 0.01$) was shown for one variety, FK-178A. Removal of rice hulls from each variety had no effect on the preference pattern, indicating that the preference was based on taste and nutritional factors rather than mechanical factors. Correlation analyses between preference data and biochemical data should lead to a rapid identification of important rice flavor compounds.

3. Effects of rice volatiles on zinc phosphide bait consumption. To demonstrate the usefulness of a rice volatiles bait additive in enhancing preference for toxic baits, two groups of male *Rattus rattus mindanensis* were compared in terms of toxic bait consumption and mortality. One group of 16 rats was allowed a choice between nontoxic rice and rice containing 0.2% zinc phosphide. The other group of 16 rats were allowed the same choice, but with rice volatiles added to the zinc phosphide bait. After two baiting days, mortality in the rice volatiles group was 86% compared to 50% in the control group ($P < 0.05$). During the two baiting days, the rice volatiles group consumed approximately twice as much toxic bait as control rats ($P < 0.05$). These data replicated previous pilot tests with 0.5% and 1.0% zinc phosphide rice baits with rice volatiles.

4. Nonlethal electrical barrier development. Pilot work was initiated with individual rats in a two-compartment shuttle box to demonstrate the feasibility of protecting rice-growing areas and high-value crops from damage by ricefield rats with nonlethal electrical barriers. It was determined that 1,200 volts at 300 microamperes were effective in deterring food-deprived rats from a food source. There was a 99% drop in food consumption and a 98% reduction in rat presence on the electrified side of the chamber ($P < 0.05$). Barrier tests were later conducted in 12 enclosures, each containing three animals under semi-field conditions. Tests established that an 18-inch barrier height, a 6-inch electrode spacing, and barrier angles between 45° and 90° produced the most consistent repellency. The conditions of free feed, one-half ration feed, and one-half barrier voltage indicated complete protection of a food source for up to 15 days. The prototype design is being evaluated with a storage-battery operated shock source. Field tests are planned for the Philippines.

BIOCHEMICAL DEVELOPMENT

Research was conducted pertaining to analysis of milk of cattle treated with diphenadione, analysis and synthetic duplication of rice aroma, development of analytical procedures for anticoagulants, and formulation for controlled release of volatiles. Major accomplishments in each area are as follows:

1. Diphenadione residue studies in milk. Studies were conducted to determine the human hazard associated with consuming milk from cattle that had been treated with diphenadione. Three cows, each nursing a young calf that had no other food source, were injected intraruminally with 2.75 mg/kg diphenadione (almost three times the 1 mg/kg recommended for vampire bat control). Approximately 0.02 ppm diphenadione

was present at 12, 24, and 48 hours after dosing, but this had decreased to less than detectable levels (0.005 ppm) at 72 hours. Prothrombin clotting time did not change in the nursing calves or in a control cow and calf (injected with physiological saline), and no diphenadione was detected in their blood plasma. Results indicate that diphenadione presents no hazard when used as a livestock systemic for vampire bat control.

2. The analysis and synthetic duplication of rice aroma. As part of the Center's effort in developing highly palatable baits for rat control, the volatile constituents of ground California brown rice were isolated and found to be attractant to *Rattus rattus mindanensis*. A "rice essence" of high quality was recovered from a rotary evaporator which had been modified for collecting volatiles. A 1,000-foot by 0.03-inch i.d. stainless steel capillary column was used for the subsequent gas chromatography-mass spectrometry analyses. Over 90 compounds were present and 55 of these were tentatively identified by mass spectra and retention times. The final objective is to prepare synthetic rice aroma from identified compounds that duplicates the behavioral attractancy of the natural product.

3. Development of analytical procedures for anticoagulants. Research was initiated to develop analytical methodology to measure diphenadione and warfarin residues in soil, rice plants, milled rice, and water. The methodology is required to fulfill the objectives of a cooperative study with the Philippines' Rodent Research Center entitled "Determination of Warfarin and Diphacinone Residues Following Sustained Baiting for Rodent Control in Tropical Lowland Rice." Methodology for analysis of diphenadione residues was almost completed. Methodology for warfarin and diphenadione analysis is scheduled to be completed in 1974.

4. Controlled release of volatiles. A formulation program for controlling release of a volatile ricefield rat attractant (rice aroma) into the environment was initiated. Literature searches and contacts with formulation laboratories were made for projecting the feasibility of meeting objectives in our laboratory as opposed to cooperative work elsewhere. A synthetic calcium silicate Micro-Cel E was investigated and found to be a promising material for short-term delayed release. Aqueous odorant solutions were mixed with Micro-Cel E and plaster of paris (used to add rigidity to molded forms) and cast into cubes. The odors were detected from the cubes for 72 hours. Results indicate that the most feasible means of meeting the objectives of this program would be to work cooperatively with industry.

BIOELECTRONICS

Areas of electronic instrumentation development are listed and described below.

1. Automatic tracking system. Efforts were made to correct defects in design of the automatic tracking system that was developed under contract by Southwest Research Institute for tracking and recording movement of ricefield rats in Southeast Asia. As part of this effort, a consultant was hired to determine the fastest and most economic plan to modify the design of the receiving system. Recommendations are forthcoming outlining cost and labor requirements to complete this development in the next 18 months.

2. Field instrumentation for recording food preference of rats. Research was initiated to design and construct reliable and economical instrumentation for measuring food preference of ricefield rats in rice paddy environments. A circuit design that employs components filled with an electrolytic solution facilitates recording of the average time and the number of rat visitations at bait stations. Test units should be available for field use in 1974.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

PHARMACOLOGY AND PHYSIOLOGY - Peter J. Savarie, Brad E. Johns, and Melvyn V. Garrison

I. FLUORESCENT PHYSIOLOGICAL MARKERS

Introduction

Fluorescent pelage markers offer a quick, easy method to identify animals. One fluorescent marker, DRC-6082, has been used by personnel at the Rodent Research Center, Philippines. Five candidate fluorescent markers have been evaluated in an effort to find more efficacious chemicals.

Results and Discussion

Only two of the five fluorescent markers tested in deer mice produced a desirable fluorescent marker. However, the intensity of the fluorescent mark for these two chemicals was not as great as that seen with DRC-6082. During the past 18 months, 20 candidate fluorescent pelage markers have been tested. Based on cost, acceptance, and quality and duration of the fluorescent mark, DRC-6082 is the best one that has been evaluated. In cooperation with the Rodent Research Center, additional studies will be conducted with DRC-6082 in target species of rodents.

II. RODENTICIDE EVALUATIONS

A. Zinc Phosphide and Red Squill Formulations

Introduction

Mr. Young-Moo Shin, FAO Fellow-Korea, during a training session at DWRC, requested feeding tests in rats for two Korean zinc-phosphide formulations and one of red squill, to be compared to formulations of the respective chemicals prepared in the United States. The purpose of his request was to determine the potency and acceptability of the Korean and United States formulations.

Results and Discussion

Zinc phosphide formulations were tested against female albino rats (*Rattus norvegicus*) and red squill formulations against male ricefield rats (*R. r. mindanensis*). Rats were individually caged and water and rat chow were available at all times. The bait for both species was 90% oatmeal, 5% vegetable oil, and 5% glycerol. Albino rats were prebaited for 3 consecutive nights with 10 g and ricefield rats for 2 nights with 5 g. Prebait consumption was excellent. The poisoned bait was offered for 3 consecutive nights. Albino rats received 10 g of bait per night which contained 1% active ingredient (a.i.) zinc phozphide. Ricefield rats received 5 g of bait per night and it contained 0.015% a.i. red squill. Animals were observed for 7 days after exposure to treated bait. Results for the zinc phosphide formulations are in Table 1. Table 2 contains the results of the red squill formulations. Only the first night consumption data are shown because surviving animals consumed only negligible amounts on the second and third nights.

Statistical analysis ($P > 0.05$) showed that there were no differences in mortality, amount of bait consumed, or mg/kg of rodenticide consumed between the respective Korean and United States formulations of zinc phosphide and red squill.

Table 1. Bioassay results of three zinc phosphide formulations in female albino rats.

		First night data	
Formulation	Mortality	1% a.i. zinc phosphide bait eaten Mean, range (g)	mg/kg zinc phosphide bait eaten Mean, range
Korea - 25%	5/5	2.1 (1.3-3.0)	107 (68-159)
Korea - 83%	4/5	2.5 (1.2-6.0)	136 (67-345)
U.S. - 94%	5/5	2.3 (1.8-3.6)	125 (73-161)

Table 2. Bioassay results of two red squill formulations in male rice-field rats.

Formulation	Mortality	First night data: 0.015% red squill bait eaten Mean, range (g)	First night data: mg/kg red squill eaten Mean, range
Korea - 0.7%	6/9	3.8 (0.8-4.9)	2.8 (0.6-3.8)
U.S. - 0.5%	5/9	2.8 (0.9-5.0)	2.2 (0.6-4.1)

B. Zinc Phosphide Toxicology

Introduction

There are many comparative LD_{50} and field reports on zinc phosphide, but relatively few published papers on its toxicology. It is known that in the presence of acid, zinc phosphide liberates phosphine gas which is the toxic agent. Factors affecting the release of zinc phosphide within the gastrointestinal tract of rodents have not been studied. The present studies were initiated to gain some basic information on the toxicology of zinc phosphide in rodents.

Results and Discussion

1. Tolerance Studies

Eight groups each containing six male albino rats were used. Four groups of rats received zinc phosphide (10 mg/kg) daily for 3 days. The other four groups (control) received an equivalent volume of corn oil (0.1 ml/100 g). All animals were fasted 16-17 hours prior to acute oral zinc phosphide determinations, which were performed on the fifth day. The control rats had an LD_{50} of 40.9 (30.5 to 54.8). The LD_{50} for the rats pretreated with 10 mg/kg of zinc phosphide had an LD_{50} of 52.0 (37.7 to 71.8). There was no difference between the LD_{50}'s. These data suggest that no tolerance or cumulative effects were induced by pretreatment of the rats with 10 mg/kg zinc phosphide.

2. Fasting and Nonfasting Studies

Two groups of rats were fasted approximately 17 hours before oral dosing with zinc phosphide. An equal number of rats had rat chow available to the time of dosing. Water was available to both groups all the time. Animals were observed for 7 days posttreatment. Results are shown in Table 3.

Table 3. Effect of nonfasting and fasting on the mortality of zinc phosphide in male albino rats.

Dose*		Posttreatment mortality (hours)									Total
mg/kg	Condition	0-2	2-4	4-6	6-8	8-11	11-14	14-24	24-48	48-96	mortality
45	Nonfasted	0	4	3	5	-	-	-	-	-	12/12
45	Fasted	0	1	0	4	←	3	→	2	0	10/12
35	Nonfasted	0	2	1	2	3	1	0	0	1	10/15
35	Fasted	0	0	0	0	1	1	4	3	0	9/15

* Zinc phosphide prepared in corn oil and administered 0.1 ml/100 g body weight.

For a given dose of zinc phosphide, there were no differences in mortality between the nonfasted and fasted animals. However, there was a big difference in time to death between the nonfasted and fasted animals. At the 45-mg/kg level, 100% of the nonfasted animals died within 8 hours. In the fasted group, only 50% of the total mortality occurred within 8 hours. Results at 35 mg/kg showed that 50% of total mortality for nonfasted rats occurred within 8 hours, whereas there was 0% mortality in the fasted rats for the same time period.

It is relatively easy to rationalize the effects observed in the nonfasted rat. A stomach containing food retains the dose of zinc phosphide in an acid environment which quickly releases phosphine gas that induces rapid toxic effects. The delayed toxic manifestations in fasted rats are harder to explain. The time intervals are long enough to allow passage of zinc phosphide into the intestine, but being

alkaline, one might not expect liberation of phosphine gas. Total mortality results indicate that enough phosphine is being produced in fasted rats, but its site of evolution within the gastrointestinal tract is uncertain.

C. Red Squill Potentiation

Introduction

A study has been initiated to find chemicals capable of potentiating the rodenticidal properties of red squill. In the first phase of the study, a candidate chemical is gavaged simultaneously with a dose of red squill that, by itself, produces 10 to 20% mortality in albino rats of either sex. In the second phase, chemicals found to be active against albino rats will be gavaged simultaneously with the appropriate dose of red squill to both sexes of wild Norway rats. In the third phase, chemicals active by gavage in wild Norway rats will be incorporated into red squill baits and offered to wild Norway rats in free-choice feeding experiments.

Results and Discussion

Expressed as mg a.i./kg, with 95% confidence limits, the acute oral LD_{50} of red squill was found to be 0.65 (0.52 to 0.82) in male albino rats and 1.04 (0.84 to 1.29) in male wild Norway rats. Inspection of the dose response curves indicated that 0.45 mg/kg (albino) and 0.75 mg/kg (wild Norway) would be satisfactory doses to potentiate with candidate chemicals. At least two doses of the candidate potentiator were tested with the appropriate dose of red squill. Ten (10) animals receiving only red squill were routinely dosed each time a group of potentiators were tested.

Twelve (12) candidate potentiators have been tested in male albino rats (Table 4). DRC numbers 5999, 6230, 6244, 6250, and 6251 produced favorable potentiating effects. Only two of the five potentiators found to be active in male albino rats have been tested in male wild Norways (Table 5). Results in Table 5 indicate that DRC-6230 and DRC-6250 at the doses used are not effective potentiators of red squill in male Norway rats. Additional testing will be performed with active potentiators in the various phases of this study.

Table 4. Effect of candidate potentiators on the mortality of red squill in male albino rats.

Red squill mg/kg a.i.	DRC No. of potentiator	Dose of potentiator mg/kg a.i.	14-day mortality
	Test 1		
0.45 (control)	-	-	0/10
0.45	5999	100	10/10
0.45	5999	25	6/10
0.45	6245	100	1/10
0.45	6245	25	0/10
0.45	6229	100	5/10
0.45	6229	25	0/10
0.45	6230	100	9/10
0.45	6230	25	5/10
	Test 2		
0.45 (control)		-	3/10
0.45	6247	100	6/10
0.45	6247	25	3/10
0.45	6219	100	0/10
0.45	6219	25	0/10
0.45	6248	50	3/10
0.45	6248	12.5	1/10
0.45	6249	100	0/10
0.45	6249	25	0/10
0.45	6067	100	4/10
0.45	6067	25	2/10
	Test 3		
0.45 (control)	-	-	0/10
0.45	6250	100	6/10
0.45	6250	25	2/10
0.45	6251	100	4/10
0.45	6251	25	6/10
0.45	5999	12.5	10/10
0.45	5999	6.25	6/10
0.45	6230	12.5	8/10
0.45	6230	6.25	4/10
0.45	6244	100	7/10
0.45	6244	25	1/8

Table 5. Effect of candidate potentiators on the mortality of red squill in male wild Norway rats.

Red squill mg/kg a.i.	DRC No. of potentiator	Dose of potentiator mg/kg a.i.	14-day mortality
0.75 (control)	-	-	4/10
0.75	6230	12.5	5/10
0.75	6230	3.125	6/10
0.75	6250	100	5/9
0.75	6250	50	5/9

D. DRC-6091 (RH-787)

Introduction

Last year's report contained some data on the acute rodenticide, DRC-6091. Additional efficacy data in target rodents and secondary hazard tests in rats have been obtained.

Results and Discussion

In prebait feeding tests its rating index (percent chemical/acute oral LD_{50}/number of LD_{50}'s consumed/percent mortality) was 1/22.3/6.1/100 in female F_1 ricefield rats, and 1/17.7/14.8/100 and 1/28.1/10.8/100, respectively, for female and male F_1 Polynesian rats (R. exulans).

The rating index in feeding tests without prebaiting in F_1 female and male Polynesian rats was 1/17.7/14.1/100 and 1/28.1/3.2/100, respectively. In a second test with male F_1 Polynesian rats, the rating index was 1/28.1/6.0/80.

In the first of two secondary hazard tests, carcasses of Polynesian rats killed in a feeding study were prepared and offered to four F_1

male wild Norway rats. Theoretically, these rats consumed 92 to 178 mg/kg of the chemical. Two of the four rats died. In the second test, carcasses of 10 F_1 male Norway rats that were gavaged at 10 times their LD_{50} were prepared and offered to five F_1 male Norway rats. Theoretical consumption of the chemical by these rats was 97 to 124 mg/kg. Three of the five rats died. In both secondary tests, all rats died 5 to 8 days after starting the test. Personnel from the Section of Mammal Damage Control reported no observance of toxicity in mongooses or raptors which consumed rat carcasses killed with DRC-6091.

The feeding tests in rats and the absence of secondary toxic effects in mongooses and raptors are encouraging results for DRC-6091. The secondary toxic effects observed for rats probably would not constitute a major handicap towards the use of it as an economic poison. The chemical is currently being petitioned to EPA by its manufacturer. The status of this chemical as a rodenticide should be known within a few months.

E. Bioassay of Military Rodenticide - Diphacin

Introduction

At the request of the U.S. Army, a diphacinone anticoagulant bait prepared in 1969 for military use was bioassayed against wild Norway rats.

Results and Discussion

Twenty rats were individually caged and had free access to laboratory chow and water. For 15 consecutive nights, 20 g of bait were offered to each rat. Bait consumption was calculated daily. Rats were then observed 6 days for delayed toxic effects.

Eight of the 10 females and 5 of the 10 males died. The survivors were "noneaters"; one of the two females and one of the five males consumed sublethal amounts of bait only on the 15th night. Rats that consistently ate bait died within 6 to 10 days.

These results indicate that the bait is toxic but not highly preferred when tested against laboratory chow (35% of the rats were classified as "noneaters"). It would probably be an effective bait when the source of normal rat food is at a minimum.

F. Acute Oral Toxicity of Benzothiazole, a Candidate Deer Repellent

Introduction

Toxicity studies were requested for benzothiazole, a chemical which has been undergoing evaluation as a deer repellent.

Results and Discussion

Deer mice (*Peromyscus maniculatus*) were fasted 6 to 7-1/2 hours and albino rats 17 hours before dosing. All animals were observed for 14 days posttreatment. The acute oral LD_{50}, with 95% confidence limits, was 305 mg/kg (203 to 458) in male deer mice and 555 mg/kg (437 to 705) in female deer mice. The LD_{50} in male albino rats was 346 mg/kg (237 to 505). Symptoms of toxicity included deep sedation, loss of righting reflex, decreased sensitivity to pain, and finally, deep narcosis from which most of the animals did not recover. LD_{50} data indicate that benzothiazole is a moderately toxic chemical.

III. REPRODUCTIVE STUDIES

A. The Effect of DRC-4805 on the Fecundity of Wild Norway, Polynesian, and Ricefield Rats

Introduction

Three species of rats were gavaged with a single dose of DRC-4805, a long-acting estrogen. The objective of the studies was to determine if the time to littering of treated rats differed significantly from normal control rats. The Polynesian rat (*Rattus exulans*), and the ricefield rat (*Rattus rattus mindanensis*) are discussed in Study A. The wild Norway rat (*Rattus norvegicus*) is discussed in Study B.

STUDY A

Methods

Fourteen pairs of ricefield rats and 20 pairs of Polynesian rats were assigned to each of two treatments, 0.0 mg/kg and 2.5 mg/kg SC-24674. The rats were gavaged on the day of pairing with a single oral dose of DRC-4805 in corn oil, 1.0 cc/100 g body weight. Animals were paired on a ranked weight basis, heaviest male with heaviest female, etc.,

housed on a 14-hour light:10-hour dark cycle, and fed lab chow and water ad libitum with no predose fast. Recordings were made on vaginal plugs, the date of littering and the number of young per litter. The study was terminated 125 days after pairing and all pairs that did not litter were killed and autopsied to determine their reproductive status and general health.

Results and Discussion

The vaginal plugs indicated that often a female Polynesian rat dosed with DRC-4805 would breed many times (Table 6). Six of the 10 Polynesian estrogen-treated females that produced vaginal plugs never conceived. However, only two of the nine control females that produced vaginal plugs failed to conceive. This difference in conception when compared with vaginal plug production is not significantly different but may indicate a trend. The breeding behavior of the rice-field rat was erratic with no apparent differences noted between control and estrogen-treated rats.

Table 6. Frequency of Polynesian rat breeding indicated by vaginal plugs.

Frequency of breeding[a]	No. of control females	No. of treated females
1 breeding	7	3
2 breedings	2	2
3 breedings	0	3
4 breedings	0	2
Total females	9	10

[a] When a female bred more than once, it occurred on a different date. The difference between the control and treated groups is significant ($P < 0.01$); 9 control females produced 11 dates with vaginal plugs and 10 treated females produced 24 dates with vaginal plugs.

Autopsies at the end of the study provided data (Table 7) concerning the reproductive status of pairs that did not litter during the study. The data in Tables 8 and 9 are self-explanatory.

Table 7. Reproductive status of nonlittering pairs at autopsy.

	Ricefield rat		Polynesian rat	
	Control	Treated	Control	Treated
Pairs with poor health or partially impaired reproductive system	1	2	3	3
Pairs with nonfunctional reproductive system	2	1	0	2
Subtotal	3	3	3	5
Pairs with no apparent abnormalities	5	8	7	11
Total	8	11	10	16

STUDY B

Methods

A single oral dose of DRC-4805 in Mazola corn oil was gavaged at the rate of 0.1 cc of liquid per 100 g of body weight. Five treatment levels were used: 0.0 (corn oil only), 1.0, 2.5, 5.0, and 10.0 mg/kg. Twenty pairs of wild-trapped Norway rats were used on each treatment. Rats were paired on the day of dosing with pairs chosen on a ranked weight basis, heaviest male paired with heaviest female, etc. Males were not rotated during the study and were left with the female until littering occurred.

The light cycle of the animal room was 10 hours dark:14 hours light. Food and water were provided ad libitum with no predose fasting. The rats were checked daily for littering for 190 days after pairing.

Table 8. Number of litters, young, and deaths.

	Ricefield rat		Polynesian rat	
	Control	Treated	Control	Treated
Pairs	14	14	20	20
Litters	6	3 (4)[a]	10	4
Mean No. of young per litter	7.2	7.3	3.5	3.2
Dam deaths[b]	0	1 (7)	2 (16,43)	4 (2, 9.16,21)

[a] One female was pregnant at autopsy on the 125th day.

[b] Deaths that occurred before littering; the parenthesis indicate the days past pairing.

Neither the number of litters born or the number of dam deaths differ significantly ($P > 0.05$) in either species of rat.

Table 9. Days from pairing to littering for each dam.

	Ricefield rat		Polynesian rat	
	Control	Treated	Control	Treated
	26	33	25	45
	28	44	25	53
	34	48	26	54
	41	(136)[a]	26	105
	70		26	
	103		26	
			27	
			28	
			31	
			44	
Total litters	6	3 (4)	10	4
Mean days to littering	50.3	41.7 (65.2)	28.4	64.2

[a] One female was pregnant with a litter estimated to be 11 days old when autopsied at the end of the study (125th day).

The ricefield rat control and treated groups do not differ significantly ($P > 0.05$). This is true with or without the litter mentioned under footnote a. The Polynesian rat control and treated groups are significantly different ($P < 0.01$), a mean difference of 35.8 days.

Results and Discussion

Table 10 shows that DRC-4805 had a significant effect on the fecundity of the wild Norway rat. The delay in littering of 79.5 days at the 10.0 mg/kg level is long enough to make the time between baitings practical for field use. Table 11 shows a toxic effect DRC-4805 may have on the Norway rat. Even though the effect cannot be demonstrated with statistical significance, a trend seems to be present in the number of deaths and uterine infections among the treated groups.

An additional study is in progress to determine the effect of DRC-4805 on F_1 rats' reproductive ability after they have suckled on DRC-4805 treated dams.

Table 10. DRC-4805 effect on fecundity of female wild Norway rats.

	Treatment (mg/kg) (20 pairs per treatment)				
	0.0	1.0	2.5	5.0	10.0
Litters[a]	17	13	12	6	5
Days pairing to littering					
Mean[b]	25.1	54.5	71.7	82.5	104.6
S.D.	3.5	17.7	14.0	15.7	20.6
Range	22-38	40-107	40-99	60-99	73-128
Delay (Control mean minus treatment mean)		29.4	46.6	57.4	79.5

Table 11. DRC-4805 effect on the mortality of wild Norway rat females.

	Treatment (mg/kg)				
	0.0	1.0	2.5	5.0	10.0
Female deaths[a] (days post-treatment)	2 (5,87)	3 (3,84,84)	4 (6,28,82 101)	8[b] (12,12,13, 13,14,19, 83,126)	6 (11,27, 74,92, 95,108)
Number with uterine infection		1	2	3	2

[a] The deaths can be compared statistically only during the period from the pairing of all the rats to the littering of the first pair. The first littering occurred at 22 days. The death ratio at that time was 1:1:1:6:1 for 0.0, 1.0, 2.5, 5.0, and 10.0 treatments, respectively. If the ratios 1:19 and 6:14 are compared with a chi-square analysis (2 x 2 with Yates correction factor) there is no significant difference ($P > 0.05$).

[b] The female that died at 126 days was pregnant and within 3 days of term. Hemorrhaging produced by uterine rupture was probably the cause of death.

B. The Effect of TEM on Testicular Morphology of Red-winged Blackbirds (*Agelaius phoeniceus*)

Introduction

Histological assistance was provided the Section of Bird Damage Control in evaluating the effect of photoperiod stimulation and triethylene melamine (TEM) treatment on testicular growth and spermatogenesis in the red-winged blackbird.

Results and Discussion

After testes weights were obtained, birds were examined for the presence of sperm in the seminal sac. Histological examination of testicular tissue and correlation of testicular activity with presence

or absence of sperm in the seminal sac was begun in 1973 and will be completed in 1974. A single oral dose of TEM at 1.78 mg/kg before the start of photoperiod stimulation produced the greatest effect in inhibiting testicular growth and spermatogenesis.

IV. DIPHACINONE TOXICITY TO NONTARGET INSECTIVOROUS BATS

Introduction

The anticoagulant, diphacinone, is being used successfully in the AID vampire bat program, but its effects on nontarget bats are not known. An assessment of its effects in two species of insectivorous bats, big brown bat (*Eptesicus fuscus*) and little brown bat (*Myotis lucifugus*) has been completed.

Bats were acclimated to laboratory conditions for a minimum of 21 days before dosing. They were housed in cardboard quart-size ice cream containers fitted with a plastic watering tube and wire mesh at the top. Bats were fed mealworms daily, except Sunday, and water was available at all times.

Diphacinone in 0.5% gum tragacanth was administered at a rate of 0.1 ml/10 g body weight. Big brown bats were gavaged. With little brown bats, the chemical solution was placed directly into the mouth and they "drank" the dose. Bats had not eaten for 24 hours prior to dosing. Approximately 15 minutes after the last bat was dosed, all bats were fed their daily ration of mealworms.

Five dose levels with one male and one female per dose (except the lowest dose in little brown bats, which had two females) were tested. Bats were observed for 28 days posttreatment.

Results and Discussion

The LD_{50} with 95% confidence limits was found to be 2.8 (1.1 to 7.5) mg/kg for the big brown bat and 2.8 (0.2 to 45.3) mg/kg for the little brown bat. Table 12 depicts the time course of mortality produced by diphacinone in the two species.

Females appeared to be more susceptible than males in both species. In big brown bats, two of five males and four of five females died; in little brown bats, one of four males and five of six females died.

Table 12. Acute oral toxicity of diphacinone in two species of insectivorous bats.

Dose mg/kg	Sex	Number of deaths (and sex) days posttreatment 0-7 days	8-14 days	15-21 days	22-28 days	28-day total
		Big Brown Bat (*Eptesicus fuscus*)				
16	1M, 1F	0	1 (M)	1 (F)	-	2/2
8	1M, 1F	0	1 (M)	1 (F)	-	2/2
4	1M, 1F	0	0	1 (F)	0	1/2
2	1M, 1F	0	0	1 (F)	0	1/2
1	1M, 1F	0	0	0	0	0/2

LD_{50} = 2.8 (1.1-7.5) mg/kg

Two of five males died.
Four of 5 females died.

		Little Brown Bat (*Myotis lucifugus*)				
16	1M, 1F	0	0	1 (F)	1 (M)	2/2
8	1M, 1F	0	0	0	1 (F)	1/2
4	1M, 1F	0	0	1 (F)	0	1/2
2	1M, 1F	0	1 (F)	0	0	1/2
1	2F	0	0	0	1 (F)	1/2

LD_{50} = 2.8 (0.2-45.3) mg/kg)

One of four males died.
Five of six females died.

These preliminary results indicate that diphacinone would be highly toxic to nontarget bats if they became contaminated, and point out the need to make accurate field identification before applying topical treatments for vampire bat control. The intraruminal injection of diphacinone offers a more selective delivery method to vampire bats, and would circumvent the hazards to nontarget bats.

V. COMPARATIVE NUTRITIONAL AND FEEDING STUDIES WITH THE POLYNESIAN (*RATTUS EXULANS*) AND LABORATORY RATS

A. Digestibility of Fiber by Polynesian and Laboratory Rats

Introduction

Little is known of the comparative ability of laboratory and wild species of rat to digest natural fibers, nor of the effect of fiber components on digestive utilization and intake of the feed. A capacity of the gut flora to utilize dietary cellulose and hemicellulose may be important in determining feeding behavior under natural conditions in wild species.

Methods

This study measured intake, weight gains, and the digestibility of nutrients when the two species were fed a defined, semipurified diet. Digestible dry matter, nitrogen, and energy were measured. The digestibility of cellulose was evaluated by using different levels of bagasse as the source of cellulose. Four treatments containing 0, 5, 10, and 15 percent cellulose were tested. Each treatment contained three males and three females of each species.

All animals were fed their respective diets for 10 days prior to the collection periods. Feces and urine were collected for a 7-day period. All animals were fed at the *ad libitum* level and effects on intake were recorded.

Results and Discussion

In both species, the digestibility of dry matter, nitrogen, and gross energy decreased significantly with increasing levels of ADF in the ration. There were no differences between sexes, but species differences were significant. The digestibility of dry matter, nitrogen, and gross energy were higher for the laboratory rat, but the digestibility of ADF was higher for the Polynesian rat. A manuscript entitled "Digestibility of Acid Detergent Fiber by Polynesian and Laboratory Rats" is now being prepared for publication.

B. The Role of Specific Euphagia in the Polynesian and Laboratory Rat

Introduction

A factor of relatively undefined significance in determining the feeding behavior of animals is that of "specific euphagia" or "nutritional wisdom." Experiments have been conducted to examine the concept of specific euphagia in both monogastric and ruminant species. The importance of specific euphagia in rodents has not been clearly determined.

Methods

Eight animals from each species were assigned to a control group (normal phosphorus), and eight animals to a treated group (low phosphorus). Normal inorganic blood phosphorus levels were determined for each species before the beginning of the study. Animals were then fed their respective diet (offered in two feeding cups) for 28 days, at which time blood analysis indicated that the test animals were near a depleted blood phosphorous state. Both diets (phosphorus adequate and phosphorus depleted) were offered to all animals for a period of 10 days. Cup location was alternated each day and the feeding behavior of the rats was recorded. Blood phosphorous levels were determined at the end of the feeding period.

Results and Discussion

Both of the groups of Polynesian rats (normal and low phosphorous) and the laboratory rat normal phosphorous group consumed more of the phosphorous-deficient diet than the normal phosphorous diet. Only the laboratory rat deficient group preferred the normal phosphorous diet over the deficient diet (Table 13). Although three of the four groups showed a preference for the deficient diet, they did consume adequate amounts of the normal diet to maintain a normal blood phosphorous level or to increase the blood phosphorous level to normal concentrations.

C. The Effect of Estrogen on Food Consumption with the Polynesian Rat

Introduction

This study was conducted to determine whether the previously determined phenomenon of an aversion by rats to a diet containing an estrogen is due simply to taste perception, or whether physiological levels of estrogenic compounds in the blood may act as part of a feedback mechanism inhibiting feed intake.

Table 13. Results from specific euphagia study.

Species	Treatments (phosphorous)	Diet consumed (g)	
		Normal phosphorous	Deficient phosphorous
Polynesian rat	Normal	101.0	160.5
	Deficient	64.5	178.5
Laboratory rat	Normal	352.0	526.0
	Deficient	698.0	260.5

Methods

The study was divided into two phases. In the initial phase, rats (both the Polynesian and the laboratory rat) were offered four levels of estrogen incorporated into the basal ration. The purpose of this phase was to determine at what level (percent) of estrogen there will be a pronounced rejection of the diet. This level will then be used in the second phase. The design is as follows:

	Treatments/Percent Estrogens			
Animals	0.0	0.001	0.01	0.1
Polynesian	5	5	5	5
Laboratory	5	5	5	5

The second phase consisted of six treatments in which the estrogenic compound was delivered to castrated male and female rats by gavage, feeding, and injection. The basal ration, which all rats received for 4 days prior to dosing, was then offered again for 4 days. The purpose was to determine the effect of estrogens and modes of administration of the estrogen on subsequent food intake. The experimental design was as follows:

	Treatments					
	Feeding		Gavage		I.P. injection	
Animals	Basal	Basal + estrogen[a]	Vehicle only	Vehicle + estrogen[a]	Vehicle only	Vehicle + estrogen[a]
Polynesian	6	6	6	6	6	6
Laboratory	6	6	6	6	6	6

[a] Percent of estrogen would be determined in Study A.

Results and Discussion

In two of the three modes of administering the estrogen, significant reduction in food was noted. The acceptance of treated bait was very low and the level of mestranol was not sufficient to cause a significant reduction in food intake (Table 14). Also, mestranol had a greater effect on food consumption in the Polynesian rat than in the laboratory rat.

Table 14. Effect of estrogen treatment on food consumption.

Species	Treatment	No. of animals	Average body weight	Amount mestranol	Food consumption g/kg body weight	
					Pre-treatment	Post-treatment
Polynesian	Feeding	6	79	0.51 mg/kg	98.82	91.57
Laboratory	Feeding	6	376	0.24 mg/kg	63.53	60.41
Polynesian	Gavage	6	70	1.00 mg/kg	90.72	71.05
Laboratory	Gavage	6	378	1.00 mg/kg	58.57	47.93
Polynesian	I.P.	6	78	1.00 mg/kg	93.08	69.64
Laboratory	I.P.	6	370	1.00 mg/kg	61.08	49.03

VI. COYOTE STUDIES

A. Coyote Embryo Development

Introduction

Using data collected during a study of the development of known-age coyote fetuses (1972 Annual Report), a ruler-like measuring device was designed and produced for field use in aging fetuses.

Results and Discussion

The linear regression equation generated by the comparison of crown-rump lengths of fresh fetuses with their known age proved to be the best means of estimating fetal age. Starting at 30 days of fetal age and at 2-day intervals up to 64 days, the day intervals were inserted into the linear regression equation. The measurements for each age interval were obtained and plotted on a model of the ruler-like scale (Fig. 1). A diagram showing how to measure the crown-rump distance on a fresh fetus was added. The average day of birth and a formula showing how to obtain the date the female was bred were also presented on the model. The model was manufactured in plastic, 2 x 8 inches, to provide a durable, waterproof measuring device. Figures 1 and 2 illustrate the report form that was designed to accompany the use of the ruler-like scale.

B. Coyote and Beagle Pheromones

Introduction

A cooperative study with the Monell Chemical Senses Center, Philadelphia, Pennsylvania, was continued. The method of collecting anal gland samples from coyotes and dogs (beagles) was improved and additional samples were collected and sent to Monell.

Results and Discussion

The 1972 Annual Report describes the study in detail. The main improvements in methods involved (1) the injection of 0.15 ml of saline into the opening of each anal sac, allowing the saline to remain for 10 minutes

before removal and (2) the freezing of the samples in liquid nitrogen immediately after collection. An additional 64 samples were collected in 1973 and sent to Monell for evaluation. A manuscript discussing the evaluation of these samples has been submitted by Monell for publication in The Proceedings of the Society of Experimental Biology and Medicine. An additional manuscript is being prepared by Monell for publication. It will discuss other aspects of the samples collected in 1973 and also the work done in 1972.

C. Laboratory Evaluation of Tranquilizers for Capturing Wild Carnivores

Introduction

The use of a tranquilizer with the steel trap for the capture of wild carnivores is needed. A tranquilizer has the desired effects of reducing self-inflicted injuries, escape, and aggression, and more easily permits the release of nontarget species. Such effects are beneficial, particularly in trapping areas where domestic and endangered species might be caught. Diazepam has been used successfully as a tranquilizer in a trap tab, but the manufacturer has curtailed its use for the capture of wild animals.

Oral efficacy and toxicity of candidate tranquilizers have been tested in penned coyotes with the cooperation of Mr. Jerry Roberts from the Section of Predator Damage.

Results and Discussion

Two desirable characteristics of a tranquilizer for the trap tab is that it have a short onset and long duration of action. All five of the tranquilizers tested produced various degrees of tranquilization and sedative effects (Table 15). The most promising results were obtained with Tranvet and Vetame. Ro-53448 also produced good results, but further studies with it were not emphasized because of company reluctance to supply adequate amounts for field tests.

We were interested in chemicals that could produce immobilization in animals, but none of them produced anesthesia or immobilization by the oral route. However, intramuscular (i.m.) doses of CI-744 produced anesthesia in coyotes in less than 2 minutes and were judged as a very good agent when administered i.m. (Table 16).

Table 15. Laboratory testing of tranquilizers in coyotes.

Chemical	No. tested	Total dose* (mg)	Behavioral effects**	
			Onset (min)	Duration (hr)
Tranvet	2	100	<15	18-24
Tranvet	4	500	2-10	30-36
Tranvet	2	1,000	<10	36-42†
Vetame	2	100	<15	24-30
Vetame	4	500	5-15	36-42
Vetame	2	1,000	<10	36-42†
Ro-53448	2	500***	30-60	24-30
Ro-53448	2	500	15-30	24-30
Ro-53448	2	1,000	15-30	36-48
Lorazepam	1	750	45	6-12
Lorazepam	1	1,000	30	12-18
CI-744	2	250	5	3-4

* Dose administered directly into mouth with M-44.

** Ataxia was first symptom noted. Parameters used to judge duration of effect included disinterest of coyote to observer, sedative or tranquilized appearance, and ease by which animal could be snared.

*** Administered with gelatin capsule.

† One-half of coyotes died.

Tranvet and Vetame were both field tested in trap tabs by personnel of the Section of Predator Damage. Field test results for both chemicals were good. Company approval will have to be obtained before Ro-53448 can be field tested. The manufacturer of CI-744 is attempting to obtain registration, and if this agent becomes readily available, it has the possibility of becoming an immobilization agent for wildlife.

D. Evaluation of Physiological Aversive Agents for Coyotes

Introduction

Physiological aversive agents are chemicals that produce sublethal effects which could be of value in suppressing coyote predation in sheep.

Table 16. Intramuscular testing of CI-744 in penned coyotes.

Coyote No.	Sex	Weight (kg)	Dose (mg/kg)	Time of observation	Observation
293	M	12.4	20.8	90 sec	Ataxic
				135 sec	Deep anesthetic sleep
				2 hr	Deep anesthetic sleep
				4 hr	Signs of recovery, crawling and some paddling
				8-1/2 hr	Appeared normal
279	M	11.4	22.6	30 sec	Ataxic
				45 sec	Deep anesthetic sleep
				2 hr	Deep anesthetic sleep
				4 hr	Signs of recovery, crawling and some paddling
				8-1/2 hr	Appeared normal
277	M	9.9	30.3	60 sec	Deep anesthetic sleep
				4-1/2 hr	Standing, ataxic, vomitus in pen
				6 hr	Slight ataxia, salivating
280	M	11.9	25.2	15 sec	Ataxic
				60 sec	Deep anesthetic sleep
				5 hr	Standing, ataxic
				6 hr	Slight ataxia

These chemicals will be used in conditioned aversion tests in cooperation with Stephen A. Shumake, Unit of Behavioral Biology. Before recommending chemicals for such tests, acute oral toxicity and sublethal symptoms have to be known.

Results and Discussion

Preliminary evaluations have been conducted with five chemicals (Table 17.)

Table 17. Effects elicited in coyotes by candidate physiological aversive agents.

Chemical	No. tested	Dose (mg)	Gross symptoms
Cantharidin	1	200	Salivation, vomiting, animal salivated for 7 days
Cantharidin	1	50	Immediate salivation and lasted about 2-1/2 hours
Podophyllin	1	100	Immediate salivation and lasted about 2-1/2 hours
Atropine So_4	1	200	Immediate salivation, dilated pupils, stupor appearance
Emetine	1	200	Emesis within 45 minutes
Digitoxin	1	200	Emesis within 45 minutes

Additional chemicals that produce sublethal effects by altering various physiological systems will be tested. A complete report will be available next year.

BEHAVIORAL BIOLOGY - Applications in Wildlife Management

Stephen A. Shumake, Kenneth A. Crane, Stanley E. Gaddis

I. BAIT ADDITIVE DEVELOPMENT

Introduction

Bait additives offer a potential means of increasing baiting effectiveness in rodent control programs by attracting rats to the baits and increasing toxic bait consumption. A three-phase bioassay for evaluating bait additives has been designed to provide a method of developing highly preferred rodent bait additives.

Methods

Phase 1 is a preliminary preference evaluation for classifying additives as repellents, attractants, or inactive. This first phase involves the use of four groups of eight ricefield rats each tested for preference with three concentrations of a given additive. Additives that lead to a statistically significant increase in preference for treated rice bait are then tested in the next two phases. The second phase consists of a brief-exposure, foods together preference test using 12 ricefield rats. In this phase, the relative rank of attractant activity for a given additive is established along with 95% confidence limits for the mean percent preference values. The third phase consists of a semifield preference test using eight 5- by 10-foot enclosures each containing three ricefield rats. Treated and untreated baits were offered to rats for 4 days. This phase indicates the potency of the attractant effect under semifield conditions. Summary statistics for all three phases are stored on magnetic tape.

Later, final field data for promising additives are entered on the tape.

Results and Discussion

Two additives tested during the calendar year did not show promise as bait enhancers. Test results are shown in Table 1. No concentration of monosodium-L-glutamate or ribotide was significantly different from preference values of the control.

Table 1. Bioassay results of two potential bait enhancers tested at three concentrations.

Concentration*	Enhancer Type			
	Monosodium-L-glutamate		Ribotide	
	% preference $\bar{X} \pm$ S.D.	C.V.	% preference $\bar{X} \pm$ S.D.	C.V.
Control	48.52 ± 4.23	0.09	47.12 ± 8.23	0.17
C_1	49.01 ± 5.30	0.11	45.14 ± 12.48	0.28
C_2	46.68 ± 3.58	0.08	50.40 ± 3.52	0.07
C_3	47.83 ± 5.40	0.11	41.07 ± 17.27	0.42

* C_1 is the recommended concentration; C_2 and C_3 are two- and four-fold increases over C_1, respectively.

II. RICE VARIETY PREFERENCE

Introduction

An experiment was conducted to answer two basic questions concerning rice variety preference in ricefield rats. The first question is whether or not this species shows varietal preference under semi-field conditions. Such information is needed to predict the intensity of damage to certain rice varieties. The second question concerns the reliability of varietal preference shown by individual ricefield rats. This profile will facilitate correlation with biochemical flavor analyses and should lead to identification of the most active compounds for synthesis of a preferred rice flavor bait additive.

Methods

Preference evaluations were conducted in two experiments. In one study, 16 individually caged ricefield rats were preference tested 6 nights in a long-exposure, four-choice laboratory preference test. A semifield evaluation was conducted using eight 10- by 10-foot enclosures each

housing five ricefield rats. Animals were presented with a choice of four different unhulled rice varieties (Milagrosa, FK-178A, IR-20, and C-4) for 7 hours daily for 8 days.

Results and Discussion

Laboratory and semifield preference data are summarized in Tables 2 and 3, respectively.

Table 2. Daily consumption of four Filipino varieties of rice by 16 ricefield rats given six long-exposure, foods together preference tests.

Variety	Consumption (g) $\bar{X} \pm$ S.E.
Milagrosa*	5.63 ± 0.51
FK-178A**	7.49 ± 0.48
IR-20	2.25 ± 0.51
C-4	2.19 ± 0.42

* Different from IR-20 and C-4 at $P < 0.05$.
** Different from IR-20, C-4, and Milagrosa at $P < 0.01$.

Table 3. Rice bait consumption (g) of eight rat colonies for four varieties of rice (Mean ± SE).

Day	Rice variety			
	Milagrosa	FK-178A	IR-20	C-4
1	18.7 ± 4.8	26.4 ± 5.7	7.4 ± 2.4	7.4 ± 2.5
2	15.1 ± 4.2	34.6 ± 5.0	5.9 ± 1.7	9.4 ± 1.9
3	14.4 ± 3.3	33.9 ± 7.3	10.1 ± 2.6	7.0 ± 1.5
4	8.9 ± 1.5	38.7 ± 4.2	7.8 ± 1.6	8.6 ± 1.4
5	15.6 ± 5.2	37.4 ± 5.1	10.5 ± 4.2	8.8 ± 1.4
6	6.6 ± 2.3	49.9 ± 6.7	4.6 ± 1.3	9.9 ± 3.2
7	4.9 ± 2.1	49.1 ± 7.5	9.1 ± 3.1	6.6 ± 2.2
8	4.0 ± 1.4	49.4 ± 8.6	8.3 ± 3.6	10.2 ± 3.3
9	10.1 ± 3.9	23.5 ± 3.0	9.8 ± 4.0	8.5 ± 2.0
10	6.9 ± 2.7	36.6 ± 4.8	5.6 ± 1.3	12.5 ± 5.5
11	5.0 ± 2.6	34.9 ± 3.4	11.1 ± 3.0	2.5 ± 1.0
12	3.9 ± 0.9	36.6 ± 5.7	8.1 ± 1.1	6.6 ± 2.5

III. EFFECTS OF RICE VOLATILES ON ZINC PHOSPHIDE BAIT CONSUMPTION

Introduction

Treating bait with zinc phosphide for control of ricefield rats is often not effective in field situations since growing rice plants appear to be the most palatable and plentiful food available. Reductional control by baiting must be carried out within a restricted time period during the growing season to effectively reduce rat populations and alleviate damage. In order to increase baiting efficiency during this critical time period, and to extend the effective baiting interval, a rice flavor bait additive is needed to enhance bait consumption. The purpose of this study was to demonstrate the effects of rice flavor additive on bait consumption and subsequent mortality of ricefield rats.

Methods

Two groups of 16 male live-trapped ricefield rats were allowed continuous access to whole grain unpolished rice for 7 days prior to prebaiting. During the 3-day prebait period, both groups were allowed a choice between 20 g granulated whole grain rice and 20 g of granulated whole grain rice treated with rice volatiles for a period of 7 hours per day. During the 3-day baiting period, 0.2% zinc phosphide was added to the rice volatiles-treated rice for one group (A), and 0.2% zinc phosphide was added to the untreated rice for the control group (B). Both rice flavor and control groups had an alternate choice of 20 g untreated granulated rice. Bait consumption, untreated rice consumption, and mortality data were collected for both groups on each of the 2 baiting days.

Results and Discussion

Mortality data for both groups were analyzed with the 2 x 2 chi square contingency test. Table 4 depicts the statistical significance levels between the two groups for the 2 test days. At the end of the 2-day period, the mortality was 86% in the rice flavor group and 50% in the control group.

Consumption of toxic and nontoxic rice bait of both groups is shown in Table 5. There was a significant increase ($P < 0.05$) in consumption of bait treated with rice volatiles (A). The few survivors in group A beyond the first baiting day precluded further statistical analysis. Percent preference for the zinc phosphide-treated rice in group A did not decrease over a 3-day baiting period indicating that

this material does not provide a taste cue for sublethal aversion. In fact, the rice volatiles material may lead to less aversion than that produced with an untreated rice bait base.

Table 4. Mortality data analyses for 2 days of baiting using either rice volatiles or untreated zinc phosphide rice bait.

	Dead	Alive	Mortality
Day 1			
Rice Flavor (A)	9	7	56%
Control (B)	4	12	25%
$x^2 = 3.12$; $P < 0.10$			
Day 2			
Rice Flavor (A)	14	2	86%
Control (B)	8	8	50%
$x^2 = 7.62$; $P < 0.05$			

Table 5. Consumption levels and percent preference for toxic rice for rice volatiles versus untreated zinc phosphide rice bait.

Day	Zinc phosphide bait consumption (g) ($\bar{X} \pm$ S.E.)		Mean percent preference for toxic rice bait	
	Group A	Group B	Group A	Group B
1*	2.96 ± 0.69	1.66 ± 0.38	50.5	36.0
2	2.79 ± 0.73	2.23 ± 1.20	67.2	38.2
3	0.17 ± 0.25	0.30 ± 0.69	66.7	17.1

* P (A > B) < 0.05.

IV. NONLETHAL ELECTRICAL BARRIER DEVELOPMENT

Introduction

Improved farming practices have led to a trend toward multiple cropping of high-value crops (corn, soybeans, and vegetables) in the Philippines. The trend toward multiple cropping places even greater emphasis on the need for development of improved methods of protecting crops from rat damage. As such, research was initiated to design and develop effective nonlethal electrical barriers for control of ricefield rats.

Methods

A three-phase barrier design test was conducted after preliminary laboratory evaluation of different shock intensities with individual food-deprived ricefield rats. Barrier height, angle, and electrode spacing were evaluated. All tests were conducted in eight 5- by 10-foot enclosures each containing three laboratory-born F_1 generation male ricefield rats. The procedures for evaluating each parameter were essentially identical. For example, with barrier heights of 12, 18, and 24 inches, there were four enclosures each holding three animals and a barrier of a given height. A 7-day baseline period was used to determine the number of rats, rice consumed, and water consumed on both sides of the nonelectrified barrier. The shock source was activated at 1,600 volts for the following 5 days and at 800 volts for the final 5 days. With the parameters of height and electrode spacing, a third 5-day test followed with the voltage at 800 and the rats allowed only one-half their normal food ration (16 g/rat/day reduced to 8 g/rat/day).

Results and Discussion

At the high-voltage level, barrier height did not appear to be a significant variable in controlling barrier efficiency (Table 6). The lower voltage level with the 12-inch barrier was ineffective, producing only a 37.4% savings of protected rice. The 18-inch barrier, however, remained at 95.4% efficiency at half voltage. Because the extra 6 inches of height for a 24-inch barrier would add substantially to cost, the 18-inch barrier height was chosen in subsequent tests.

With barrier angle as the parameter, the lower 12-inch section of each barrier was vertical and the rest was slanted at various angles (0°, 45°, 60°, and 90° from the horizontal). Again, at 1,600 volts, the parameter had an insignificant effect on barrier efficiency. At

800 volts, however, the 90° barrier continued to operate at 100% efficiency with deviations of barrier angle dropping efficiency to as low as 86.7% at 60°. The data indicated that the one-way effect produced by slanting the top portion of the barrier would probably not justify the increased construction costs or loss in efficiency.

Data for the three electrode spacings indicated that the 6-inch spacing remained at 100% efficiency regardless of voltage level or food deprivation level. The 9-inch spacing level produced more barrier efficiency than the 3-inch spacing; however, in a field situation the lowest electrode would have to be well above ground to prevent short circuiting with weeds.

The advanced barrier design is being evaluated with a battery-operated commercial livestock shock source. Electrical parameters such as electric shock pulse rate and voltage will be evaluated before field tests are conducted.

Table 6. Effects of barrier height and angle and electrode spacing on electric fence efficiency for repelling ricefield rats. Rice consumed and number of rats are means of 5 test days. Numbers in parentheses represent mean percent protection over baseline levels.

A. Effect of Barrier Height on Electrical Repellency

	Height (inches)					
	12		18		24	
Condition	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats
Baseline	17.90	2.20	19.95	1.30	23.35	1.55
1,600 volts	0.45 (97.5)	0.00 (100.0)	0.10 (99.5)	0.05 (96.2)	0.00 (100.0)	0.00 (100.0)
800 volts	11.20 (37.4)	0.70 (68.2)	0.91 (95.4)	0.16 (87.7)	6.75 (71.1)	1.00 (35.5)
800 volts and one-half food ration	16.05 (9.3)	0.45 (79.5)	- -	- -	6.25 (73.2)	0.35 (77.4)

Table 6. - Continued

B. Effect of Barrier Angle on Electrical Repellency

Condition	Angle (degrees from horizontal) 90		60		45		0	
	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats
Baseline	27.25	2.00	15.05	1.60	19.95	1.30	8.60	1.85
1,600 volts	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.10 (99.5)	0.05 (96.2)	0.00 (100.0)	0.00 (100.0)
800 volts	0.00 (100.0)	0.00 (100.0)	2.00 (86.7)	0.31 (80.6)	0.92 (95.4)	0.17 (86.9)	0.35 (95.9)	0.00 (100.0)

- -

C. Effects of Electrode Spacing on Electrical Repellency

Condition	Spacing (inches) 3		6		9	
	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats	Rice consumed (g)	No. of rats
Baseline	17.95	1.30	10.25	1.25	12.30	1.70
1,600 volts	0.10 (99.4)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
800 volts	0.91 (94.9)	0.10 (92.3)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
800 volts and one half food ration	- -	- -	0.00 (100.0)	0.00 (100.0)	0.15 (98.8)	0.05 (97.1)

BIOCHEMICAL DEVELOPMENT - Roger W. Bullard, Walter A. Bowles, and Gilbert Holguin

I. DIPHENADIONE RESIDUE STUDIES IN MILK

Introduction

It is important to determine if any human safety hazard is associated with consuming meat or milk of cattle systemically treated with diphenadione to control vampire bats in Latin America. Prior to this study there were no literature reports concerning mammilary transfer of diphenadione.

Three cows, each nursing a 3-month-old calf that had no other food source, received a 2.75-mg/kg intraruminal injection of diphenadione. A control cow (also with nursing 3-month-old calf) received a "sham" intraruminal injection of physiological saline. Milk and blood from each of the four cows and blood from each calf were collected at 0, 12, 24, 48, 72, 96, 120, and 144 hours. Sample aliquots were analyzed by a glc method developed earlier for diphenadione analysis. Prothrombin was determined by the Sera-Tek Prothrombin Reaction Chamber Technique.

Results and Discussion

Diphenadione content in milk and blood of test animals is shown in Table 1. Small quantities of diphenadione were found at 12, 24, and 48 hours, but the level had dropped below the detection limit at 72 hours. Milk samples from the control cow were blank and unchanging. Several packed gas chromatographic columns were tried and all had inadequate resolution for milk samples. A 100-foot by 3-inch OV-101 capillary column provided the solution. The gas chromatographic peak identified as benzophenone (the oxidation product formed in the analytical procedure for diphenadione) in 12-, 24-, and 48-hour samples was collected and confirmed by gas chromatography-mass spectrometry.

The plasma levels reach maximum in approximately 24 hours then decline slowly to nondetectable levels by 144 hours. Conversely, the prothrombin clotting time (Table 2) increases gradually to reach a maximum at 96 hours then falls rapidly back to normal. Prothrombin readings in the treatment calves and control animals did not change and no diphenadione was detected in their blood plasma.

Diphenadione is used as a prothrombinopenic agent in human therapy; Remington's Pharmaceutical Sciences (1970, pp 832-833) lists the range

Table 1. Diphenadione residues in milk and plasma of test cows (ppm)

Hours posttreatment	Milk		Blood plasma			
	Dosed[a] cows	Control cow	Dosed[a] cows	Treatment[b] calves	Control cow	Control calf
0	<0.005	<0.005	<0.01	<0.01	<0.01	<0.01
12	0.019	<0.005	3.51	<0.01	<0.01	<0.01
24	0.021	<0.005	4.09	<0.01	<0.01	<0.01
48	0.020	<0.005	2.17	<0.01	<0.01	<0.01
72	<0.005	<0.005	1.47	<0.01	<0.01	<0.01
96	<0.005	<0.005	0.93	<0.01	<0.01	<0.01
120	<0.005	<0.005	0.34	<0.01	<0.01	<0.01
144	<0.005	<0.005	<0.01	<0.01	<0.01	<0.01

[a] Represents mean value from three cows dosed at 2.75 mg/kg.

[b] Represents mean value from three calves, each nursing one of the dosed cows.

Table 2. Prothrombin clotting time following dosing with 2.75 mg diphenadione

	Hours posttreatment							
	0	12	24	48	72	96	120	144
$\bar{X}$ Treatment cows	16.7	16.7	22.0	29.2	39.8	42.0	30.5	23.2
$\bar{X}$ Treatment calves	17.3	15.5	15.8	16.0	15.3	15.7	15.2	14.3
Control cow	19.0	17.0	16.5	19.0	15.8	16.1	16.8	15.0
Control calf	18.0	15.7	15.4	17.0	15.4	15.5	16.0	15.5

of daily dosages as 2.5 to 30 mg. Therefore, even if milk contained the 0.02-ppm maximum resulting from the high dosage given in this study, a person would have to drink 31 gallons of it to obtain the 2.5-mg minimum daily dose of diphenadione used in human anticoagulant therapy. The fact that there was neither a detectable plasma level nor observable prothrombin response in the nursing calves confirmed this proposition.

II. THE ANALYSIS AND SYNTHETIC DUPLICATION OF RICE AROMA

Introduction

One of the major goals of the Center's AID research program is the development of highly preferred bait carriers for reductional control of ricefield rats (Rattus rattus mindanensis). In the 1972 Annual Progress Report we reported that the volatiles of California brown rice "markedly and consistently enhance bait consumption by ricefield rats." Additional behavioral tests since that time have confirmed this attractancy, thereby justifying the isolation and identification of important rice aroma constituents.

A rotary evaporator was modified for trapping the volatiles. A 30 ml/min stream of helium sweeps the volatiles from a rotating beveled flask (providing tumbling action to enhance volatile release) into a liquid nitrogen trap. Dibutyl phthalate (0.2 ml) serves as "keeper" in the trap. The trap contents are then analyzed by gas chromatography-mass spectrometry. A 0.03-inch OV-101 capillary column provided the required high resolution separation of the volatiles.

Volatile constituents are then tentatively identified by interpretation of the mass spectra. Positive identification is realized through acquiring the tentatively identified compound and matching the mass spectrum and retention time.

Results and Discussion

Over 90 compounds were resolved when the capillary column was temperature programmed at 2°/min from 65° to 185° C. Fifty-five of these were tentatively identified. The compound classes represented are listed in Table 3.

We are attempting to gather and store mass spectral data and glc retention times for several common food volatiles.

Table 3. Compounds tentatively identified in rice volatiles

Class	No. identified
Alcohols	6
Aldehydes (saturated)	12
Aldehydes (unsaturated)	9
Alkyl Aromatics	12
Furans	5
Ketones	7
Miscellaneous	4

Behavioral tests have been initiated to determine which compounds contribute to the natural rice aroma. The final objective is to duplicate the behavioral attractancy of the natural product.

III. DEVELOPMENT OF ANALYTICAL PROCEDURES FOR ANTICOAGULANTS

Introduction

Because of the potential for widespread, intensive use of anticoagulant rodenticides in tropical lowland rice, the development of basic information on the occurrence of anticoagulants is needed. This has not been studied in temperate countries. Therefore, a study was proposed by the Philippine Rodent Research Center involving warfarin and diphenadione, each representing a major class of anitcoagulants. The objective was to develop appropriate analytical procedures and conduct analyses of samples.

An analytical procedure existed for determining diphenadione residues in meat, milk, and blood but modifications were needed for plant analysis. Both plant and grain samples were analyzed under various technique modifications. The modification was tested with fortified samples.

Methods development was initiated for analysis of warfarin in plant material. The major emphasis was on modifying a fluorometric procedure designed for analysis of blood plasma.

Results and Discussion

A diphenadione procedure developed earlier in this laboratory for analysis of meat, milk, and blood plasma was successfully modified to include plant tissues and grains. The existing cleanup procedure was adequate and emphasis, therefore, was on sample preparation and extraction. Recovery of fortified diphenadione in pilot trials was consistent with that for animal materials.

Some progress was made in cleanup of plant samples for warfarin analysis. A portion beteen 1N NaOH and methylene chloride places the sodium salt of warfarin in the water layer with most plant interferences remaining in methylene chloride. Considerable work remains before a method can be developed.

IV. CONTROLLED RELEASE OF VOLATILES

Introduction

An important goal for the Section is to provide a formulation program that will meet the many needs that arise in animal damage control research. An immediate need is the development of a method for controlling the release of volatile chemicals into the environment. The chemical and physical complexities of such a task are enormous. A preliminary literature search followed by contacts with numerous industrial and institutional laboratories helped in the research planning.

Results and Discussion

One of the most promising materials for retarding rates of volatilization appears to be a group of synthetic calcium silicates manufactured by Johns-Manville Corporation. One, Micro-Cel E, retains up to six times its weight of water and is available in particle sizes less than 0.1 μ. Volatile materials, ammonium hydroxide and peppermint water, were added to a dry mixture of Micro-Cel E and plaster of paris (used to supply rigidity to molded forms) and cast into cubes. The formulation did retard the rate of release of both volatile components. Ammonium odors were detected at the end of 3 days and peppermint odors were noted at the end of 2 days.

Through the literature search and personal contacts we have learned of sources and requirements for having future formulation work conducted elsewhere. We are not staffed or equipped to handle the more difficult problems. This field is highly specialized and most of the corporate expertise is confidential and therefore not published. Outside contacts appear to be the best answer to our needs at this time.

BIOELECTRONICS - A. Lawrence Kolz, George W. Corner, and Richard E. Johnson

I. AUTOMATIC WILDLIFE TRACKING SYSTEM

Introduction

Field tests conducted with the automatic wildlife tracking system developed by Southwest Research Institute have revealed serious design deficiencies in the interferometer receiving circuitry. Basic performance incompatibilities exist between our wildlife transmitter designs and the receiver specifications. It is therefore necessary to resolve these differences, and a consultant has been hired to review and recommend changes in the system's design.

Results and Discussion

Three 30-MHz interferometer receivers are used with the automatic wildlife tracking system. Laboratory tests indicate that, in order to operate with state-of-the-art miniature wildlife transmitters, these receivers must be modified for increased sensitivity, greater signal dynamic range, automatic frequency tuning, and an improved automatic gain control. There is also the possibility that a single receiver system could be developed by using a different component arrangement.

Another modification alternative is to use improved wildlife transmitters designed at 164 MHz and frequency convert the tracking system. These transmitters exhibit greater frequency stability and increased radiation over the 30-MHz designs. However, the higher frequency will possibly introduce larger measurement errors caused by propagation bounce phenomena.

The engineering alternatives will be carefully considered before continuing work on this tracking system, and the recommendations forthcoming from the consultant engineer are intended to provide the necessary guidelines.

II. FIELD INSTRUMENTATION FOR RECORDING FOOD PREFERENCE OF RATS

Introduction

A recording apparatus is being designed to measure food consumption of ricefield rats under field conditions. The apparatus will augment

present food consumption measurement techniques by counting the number of rat visitations and recording feeding time. The recording mechanism is an electrolytic cell which operates with a simple, easily maintained electronic circuit.

Results and Discussion

A need exists for timing devices that are inexpensive, mechanically rugged, and easily maintained under laboratory and field conditions. A commercially available electrolytic coulometer (E-Cell) has the potential for satisfying these requirements.

The E-Cell is now being used in the design of a food preference tester that can be used under field conditions. As conceived, only a minimum of electronic circuitry would be required in the field for storing data into the E-Cell, and the playback mechanism would be located at a central laboratory. E-Cells are reusable, and small plug-in modules are being made for maintenance of the equipment. In this manner, a field technician can service and collect the data from a number of bait stations simply by inserting E-Cell modules into the field devices and returning the recorded modules to the laboratory for readout. The playback equipment will be designed to ensure an instrumentation error of less than 5%.