AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

FOR AID USE ONLY
BATCH #14

1. SUBJECT CLASSIFICATION

A. PRIMARY
Serials

Y-AH10-0000-0000

B. SECONDARY
Agriculture--Pests of plants

2. TITLE AND SUBTITLE
Vertebrate damage control research,annual progress report,calendar year 1971

3. AUTHOR(S)
(101) Denver Wildlife Research Ctr.

4. DOCUMENT DATE
1972

5. NUMBER OF PAGES
82p.

6. ARC NUMBER
ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Interior

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(Research summary)

9. ABSTRACT

10. CONTROL NUMBER
PN-RAA-705

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Pest control

13. PROJECT NUMBER

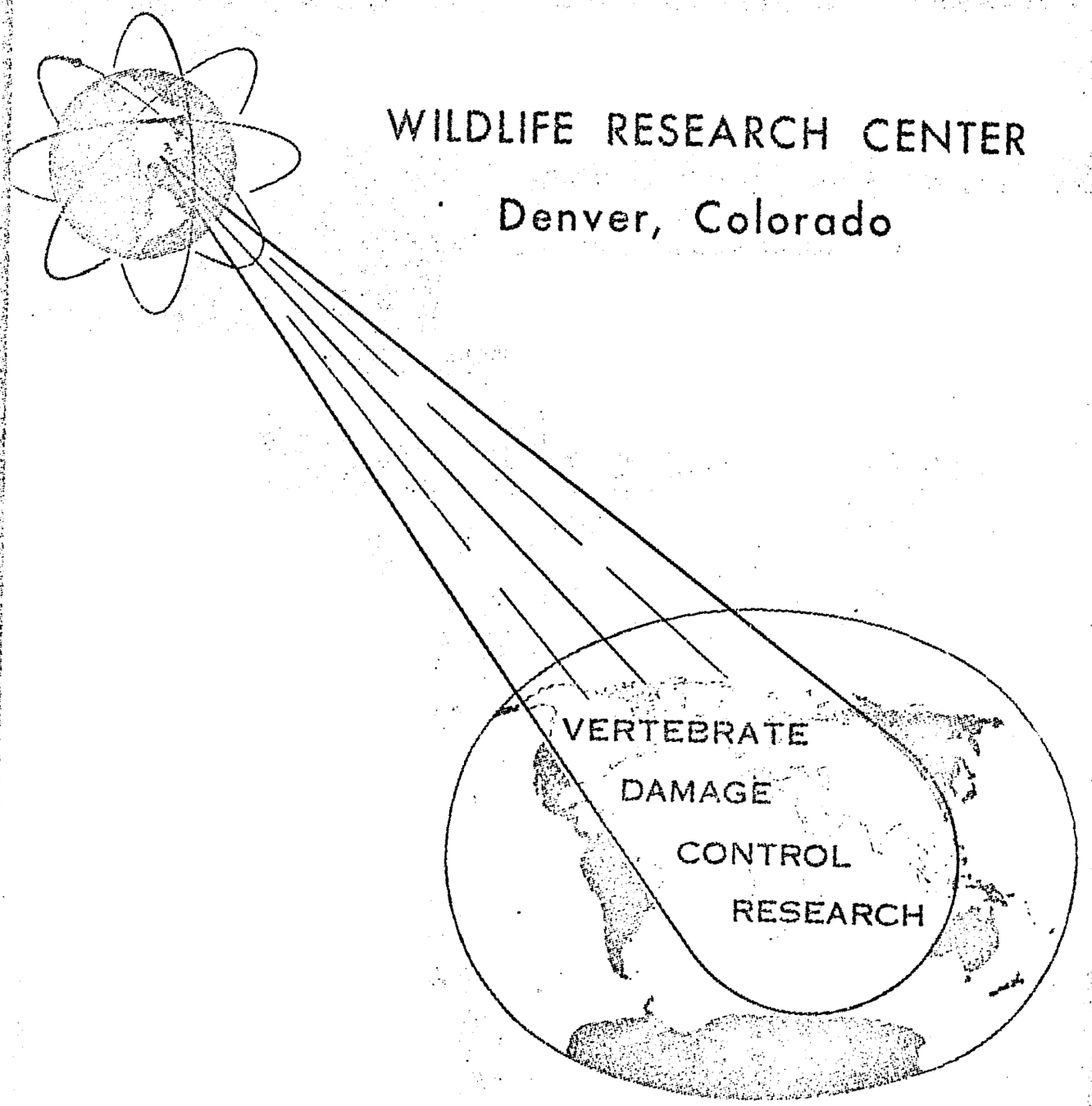
14. CONTRACT NUMBER
PASA RA(ID)1-67 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

WILDLIFE RESEARCH CENTER

Denver, Colorado



1971 ANNUAL REPORT

A Cooperative Program

U.S. DEPARTMENT OF
THE INTERIOR
Bureau of Sport Fisheries and Wildlife
Division of Wildlife Research
Denver Wildlife Research Center

U.S. AGENCY FOR
INTERNATIONAL DEVELOPMENT
Bureau of Technical Assistance
Office of Agriculture and Fisheries
Washington, D.C.

ANNUAL PROGRESS REPORT
Calendar Year 1971

Results Incomplete and Not for Publication,
Release, or Use without Authority of the
Director, Denver Wildlife Research Center

U.S. Department of the Interior
Bureau of Sport Fisheries and Wildlife
Division of Wildlife Research

U.S. Agency for
International Development
Bureau of Technical Assistance
Office of Agriculture and Fisheries

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ABSTRACTS

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

CHEMICALS

A class of nitrogen-containing chemicals from a manufacturer proved to be good repellents for deer mice when tested against wheat seeds, but were inactive as repellents when tested against Douglas-fir seeds. Only future work will ascertain if these chemicals can have application as repellents in other areas.

Preliminary results with an iodine chemical and a fluorescent dye indicated that they might be able to be used to mark animals for an extended period of time.

Chemicals tentatively identified as being in putrified fish had low toxicity and poor repellency in deer mice.

Structure activity relationship studies were performed with oxotremorine and some of its analogs. Good repellency appears to be associated with high toxicity in this series of chemicals. Only the di-pyrrolidinone analogs were active as repellents and/or toxicants.

Potentiation of the action of some rodenticides was investigated. Gophacide, strychnine, and norbormide could be potentiated by DRC-5999. DRC-5999 did not potentiate "1080," 6-aminonicotinamide, or zinc phosphide.

One new chemical, DRC-5976, was considered as a candidate rodenticide. Norway rats were found to be highly susceptible to the lethal effects of this chemical.

Additional laboratory equipment, bioassay supplies, and candidate control compounds were acquired this year through the continued support of the AID program.

The 4,000 compounds included in the rodent repellency study were encoded into the WLN cipher and added to the chemical structure and activity publication as an appendix.

Additional training and consultation in using the WLN to describe chemical compounds alphanumerically and converting our data bank to ADP formats was obtained.

PHYSIOLOGY AND BEHAVIOR

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

1. Visual Sensitivity of Vampire Bats. Operant conditioning techniques were used to establish a behavioral baseline for vampire bats. Each animal was trained to lick a drinking tube containing 20% blood to obtain small quantities of 75% whole blood reinforcement. A stimulus light was superimposed on this baseline and paired with electric shock. Detection of the light for various intensities was inferred by an examination of the suppression behavior to the warning light. The results are presented in a paper entitled "Conditioned Suppression as a Technique for Measuring Visual Sensitivity in Vampire Bats." The manuscript will be submitted to a technical journal for publication in 1972.

2. Attractancy Tests for Selected Odors of Nonbiological Origin in Ricefield Rats. A four-choice open-field odor test maze has been used to evaluate odor preference responses to over 50 compounds in ricefield rats. Food odor was used as a standard for assessing the compounds and deionized water served as a nonodor control. Several natural oils, amines, and aldehydes showed a high degree of attractant quality.

3. Pheromones and Odors of Biological Origin in the Ricefield Rat. The preputial gland secretions, urine, and vaginal secretions are being evaluated for attractant quality in ricefield rats. Currently, samples are being collected from females in various stages of estrus for future tests on males. Some females have been tested for attractant quality of male preputial gland extract. Current indications are that the females are most attracted to the male extract when they are in proestrus or estrus.

4. A Four-Choice In-Cage Odor Test for Ricefield Rats. Ricefield rats appear to avoid unfamiliar surroundings and often attempt to escape when confined to laboratory test chambers. To obviate this difficulty, a new testing device has been developed which is placed directly into the home cage. Uniformity, localization, and multiple odor test data on ricefield rats indicate that the device may be a more suitable instrument for measuring odor preference than the four-choice open-field odor test.

5. Analyses of Preputial Gland Exudate. Gas chromatographic "signatures" are being obtained from a single collection of ricefield rat preputial gland exudate. The material is extruded from a live rat (male or female) and collected onto the surface of an anhydrous sodium sulfate tablet. An apparatus and technique has been devised for extracting this collection and then concentrating the extract to 5 µl or less without loss of volatiles. The entire quantity can now be chromatographed, thereby producing an excellent signature showing even the minor components.

6. Chlorophacinone and Diphenadione Levels in Blood, Milk, and Meat. Chlorophacinone (2-[(p-chlorophenyl) phenylacetyl]-1,3-indandione) and diphenadione (2-diphenylacetyl-1,3-indandione) can be determined in blood plasma by either UV spectrophotometry or gas-liquid chromatography. Following acid hydrolysis, sample cleanup involves protein precipitation, centrifugation, and two liquid-liquid partitioning steps. Samples containing 5 µg or more can be analyzed by UV. Samples containing 0.05 to 5 µg can be carried through CrO_3 oxidation followed by glc analysis of the benzophenone or p-chlorobenzophenone oxidation product. Meat or milk samples may be analyzed by the same procedure with the only modification being in the sample preparation step.

7. Systemic Technique for Control of Vampire Bats--Laboratory Phase. Considerable progress was made in developing a livestock systemic technique for vampire bat control. Emphasis was centered on evaluating the effectiveness and safety of DRC-3776 and DRC-5652, both anticoagulants. Tests indicate that DRC-5652 has excellent potential as a livestock systemic for reductional control of vampire bats. It is more toxic to vampire bats (acute LD_{50} = 0.90 vs 3.75 mg/kg) but has much less effect on cattle prothrombin clotting time and therefore is much safer for cattle than DRC-3776. One blood feeding from calves dosed with either anticoagulant (single dose) as low as 0.5 mg/kg produces almost 50% mortality in bats up to 48 hours postdosing. Unlike DRC-3776, DRC-5652 appears not to be retained in the hosts' body for a prolonged period of time after treatment.

8. Systemic Technique for Control of Vampire Bats--Preliminary Field Phase. A preliminary experiment was conducted at two cattle ranches in Mexico to test the effectiveness of DRC-5652 for vampire bat control when used as a livestock systemic. Cattle being bitten by vampire bats were treated with 1.0 mg/kg of DRC-5652 in a single oral dose. The control treatment appeared to be effective at both ranches, but a larger scale testing program will be required to determine with certainty the effectiveness of this technique.

REPRODUCTION

A new synthetic estrogen (DRC-4801) was tested on Norway and Polynesian rats. Females of both species showed a significant ($P < .05$) increase in the interval between pairing with males and parturition following a single oral (gavage) dose. A high incidence of sterile offspring were observed among litters of Norway rats nursing mothers treated at parturition with a single oral (gavage) dose of DRC-4801; the proportion of young so affected was dose dependent.

Colony studies involving Polynesian rats offered DRC-4801 treated bait (0.005%) at 2-week intervals for 12 weeks showed a significant ($P < .05$) reduction in both the number of pregnancies and total number of implanted embryos. The rats showed increasing aversion at each subsequent baiting period to the treated bait. A bait taste enhancer (DRC-1813) reported to increase consumption of norbormide treated bait in Norway rats was ineffective when combined with DRC-4801 and presented to Polynesian rats.

A technique for the surgical sterilization of male redwings is described. A qualitative assessment of sperm in the tract of female redwings suggests that fertility begins to drop at the start of incubation. Spermatogenesis in adult male redwings requires 16-20 days.

ELECTRONICS

Electronic circuitry has been developed which will provide an indication of mortality based upon an animal's quiescence. A motion-activated switch provides the sensing function. The mortality indicator can be considered an accessory to the animal locator beacons so commonly used in tracking studies. The radio-frequency beacon operates as a normal pulsing transmitter until the animal's motion ceases for a prescribed time; the transmitter then changes its signal characteristic to a continuous signal.

Design and construction of miniature (less than 2 g) radio-frequency (rf), beacon transmitters required a great deal of time from the Electronics Unit. A new transmitter was designed for use on birds and bats which operates on our 164-megahertz (MHz) frequency assignment. A total of 167 rf beacons was constructed at the two frequencies of 30 and 164 MHz.

The Electronics Unit contracted for the construction of a mobile, collapsible antenna mast with funding provided by the Agency for International Development. This mast is now mounted on a vehicle and is being tested for mechanical reliability as well as receiving signal enhancement with various antenna systems. Antenna development in the last year has been limited to the construction of a portable, directional antenna for 164 MHz. Other types of receiving antennas previously developed were provided to field personnel on a routine basis.

The DWRC contracted with the Southwest Research Institute of San Antonio, Texas, in 1969 to develop an automatic animal tracking station. This equipment was accepted in September 1971. Further developmental work is now being performed in Denver to improve its compatibility to our field requirements. Funding for this system was provided by the Agency for International Development, and it is anticipated that the equipment shall be utilized in the Philippines for detailed investigations of rat populations.

Circuit modifications to the Johnson 350 DF receiver will convert this 30-Mhz receiver for use at 164 MHz. Tests have been conducted to work out the details of such a frequency conversion and measure the loss in receiver sensitivity.

BIRD DAMAGE CONTROL RESEARCH

DEVELOPMENT OF CHEMICALS

Acute oral toxicity and acceptance studies were conducted with Coturnix quail and redwings for six compounds having potential as reproduction suppressants. Of six compounds tested on Coturnix quail to determine effects on reproduction, isopropyl methane sulfonate and triethylene melamine (TEM) were most effective and resulted in high infertility in adult males. However, TEM produced excessive mortality. Nine of 17 compounds examined for toxicity on redwings showed activity at 100 mg/kg or less. None of 20 compounds tested in the laboratory for repellent activity on red-winged blackbirds was active below 1.000%.

Both cage and field studies showed the effectiveness of DRC-736 for repelling several species of birds when applied to lettuce seedlings, ripening rice and sorghum, and ripening fruits. DRC-736 had no effects on germination of lettuce seed, loblolly pine seed, or on rice seed treated while ripening. The acute toxicity of DRC-736 was obtained for robins and bobwhites; its acute chronic toxicity was determined for Coturnix; and an R_{50} for it was obtained on bobwhites.

Continued studies with ^{14}C-4-aminopyridine (DRC-1327) to gather data towards registration showed that there was very little movement of the radiolabeled compound through any of the alkaline and acidic soils tested; that DRC-1327 residues are more tightly bound to acidic than alkaline soils and they are less available for microbial and/or chemical degradation; that there were no apparent phytotoxic effects to sorghum seedlings grown in nutrient culture containing 10 and 100 ppm DRC-1327; and that there were detectable amounts of radio-activity in the shoots and heads of 1- and 3-month plants.

In a cooperative study at Ft. Lamy, Chad, under the auspices of USAID, 20 chemicals were tested for their toxicity to red-billed queleas; it was found that these birds were very sensitive to orally and dermally administered chemicals.

FIELD METHODOLOGY

In Venezuela, wintering dickcissels fed on natural food sources in 1971 in preference to maturing sorghum. Initial phases of study plans for work on bird damage problems in Colombia were formulated, and problem species in several crops were ascertained. Quelea were found to prefer millet in a feeding trial in Africa and consumed 4 g/day. They responded well to 4-aminopyridine and were repelled by low levels of methiocarb on millet.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

CHEMICALS - Peter J. Savarie, Walter A. Bowles, Jr., Gary J. Dasch, and Ralph W. Dutton

I. CHEMICAL EVALUATION ACTIVITIES

A. Overview

During the past year, less emphasis was placed on accepting compounds from chemical suppliers. The emphasis has been on literature searches to provide leads which might be used to help solve vertebrate pest problems. Edgewood Arsenal, Fort Detrick, and the Toxicology Information Program at the National Library of Medicine are three organizations, among others, who have contributed to our literature search efforts. These searches provide toxicological, pharmacological, and other data which are analyzed to determine if a particular compound warrants additional work as a candidate vertebrate pest control agent. In the area of repellency, only those chemicals which are efficacious and have low toxicity are considered for further tests. Additional tests on a toxic compound for rodents are considered only if that compound demonstrates a high degree of selectivity.

The Initial Bioassay test with deer mice (Peromyscus maniculatus) is one test by which initial evaluation of chemicals is achieved. This allows all chemicals to be compared on a single test and is one of the tests by which repellent chemicals evolve from for use in forest animal damage control. In addition to the standard Initial Bioassay test, other areas were investigated and are reported below.

A portion of the AID allotment was used to purchase unique chemicals that are analogs or homologs of known biologically active chemicals. Chemicals procured in this manner are those not normally offered by contractural or solicited sources, and they are needed to advance knowledge at DWRC concerning active chemicals whose identities are known. The remaining allotment was used to purchase animals, equipment, and supplies necessary to support and maintain chemical evaluation activities.

B. Literature Reviewed

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

Kverno, N.B., G.A. Hood, and W.E. Dodge. 1965. Development of chemicals to control forest wildlife damage. Proc., Soc. of Amer. Forest., Detroit, Mich. p. 222-226.

1. REPELLENT CLASS OF CHEMICALS

A. Introduction

A series of 59 chemicals from one chemical company were evaluated on the Initial Bioassay test. Twenty (20) chemicals resulted in 76% or greater food reduction on wheat seeds and were selected for acute oral LD_{50} determinations and tests on Douglas-fir seeds.

B. Results and Discussion

Initial Bioassay and LD_{50} data for the 20 chemicals are presented in Table 1.

TABLE 1. Bioassay data in deer mice.

	Wheat seeds, 2% (w/w)		Douglas-fir seeds, 2% (w/w)		
DRC	% food reduction	Mortality	% food reduction	Mortality	Acute oral LD_{50} (95% confidence limits) mg/kg
5353	95	0/5	20	1/5	473(315-710)
5355	94	0/5	14	1/4	706(173-2877)
5357	92	0/5	4	0/5	1059(706-1589)
5362	89	0/5	31	0/4	173(173-173)
5365	99	0/5	4	0/5	482(213-1082)
5371	81	0/5	8	0/5	865(865-865)
5375	76	0/5	42	0/5	1059(706-1589)
5379	90	0/5	44	0/5	706(470-1059)
5862	87	0/5	20	0/5	153(62-382)
5866	88	0/5	31	0/5	415(218-790)
5867	99	0/5	15	0/5	415(218-790)
5868	96	0/5	30	0/4	315(77-1283)
5869	98	0/5	13	0/5	63(28-141)
5870	92	0/5	20	0/5	93(62-139)
5873	89	0/5	22	0/5	863(486-1532)
5874	81	0/5	14	0/5	1600 ALD*
5875	93	0/5	2	0/5	210(140-315)
5876	84	0/5	16	0/5	587(331-1043)
5885	93	0/5	29	0/5	93(62-139)
5887	83	0/5	4	0/5	705(470-1057)

*Approximate lethal dose, 1/2 dead at 1600 mg/kg.

One of the most dramatic aspects about these chemicals is the loss of food reduction activity from the wheat seeds to the Douglas-fir seeds. It is common knowledge that it is extremely unpredictable to extrapolate results of one test to another. The results of these chemicals on two different bioassay tests is a good example.

C. Conclusions

Twenty chemicals found to be active repellents in deer mice when tested against wheat seeds were found to be inactive when tested against Douglas-fir seeds.

2. PHYSIOLOGICAL MARKERS

A. Introduction

Pilot studies were initiated for the purpose of developing chemicals with the ability to mark animals for extended periods of time.

B. Results and Discussion

Iodinated compounds:

A series of iodinated compounds were investigated for their potential use as marking agents in animals. Iodinated compounds have been used as radiopaque diagnostic agents in man for many years. One of these chemicals, dl-3-hydroxy-2,4,6-triiodo-α-ethylhydrocinnamic acid (iophenoxic acid, DRC-6061), binds to proteins of the serum and will astronomically elevate the serum protein-bound iodine (PBI). In man, its half-life is about 2.5 years and it has been calculated that it would remain in the blood for about 30 years. This chemical can be transferred across the placenta in man and hamsters, and its metabolism has been studied in dogs. The rationale for using iodinated compounds is thus: if an animal ingests these compounds and if they become bound to blood proteins, then an animal could be identified by its elevated PBI. The tables below indicate the effect that DRC-6061 has on the PBI of wild Norway rats, domestic sheep and bobwhite quail. Animals of either sex were used.

TABLE 2. DRC-6061 effect in wild Norway rats.

Treatment	PBI* µg/100 ml	# tested
Control	1-15	5
24 hr postfeeding**	3200-4150	5
7 days postfeeding	3250	1
10 days postfeeding	2750	1
6 weeks postfeeding	180	1

*All PBI values were determined by DCL Health Laboratories, Inc., Denver, Colorado.
**Animals offered 5 g of bait containing 0.5% DRC-6061, plus 5 g of untreated bait in two separate cups. Acceptance of DRC-6061 under these conditions was excellent. Dose of DRC-6061 consumed ranged from 58-101 mg/kg.

TABLE 3. DRC-6061 effect on PBI in sheep.

Treatment	PBI µg/100 ml	# tested
Control	4.5,5.0	2
24 hr post 25 mg/kg*, intramuscularly	5300	1
5 weeks post 25 mg/kg*, intramuscularly	7260,7380+	1
24 hr postfeeding**	550,3500+	1
5 weeks postfeeding	1720	1

*Approximate dose administered; +duplicate samples analyzed.
**Animal ate 61 g of feed containing 1% DRC-6061.

TABLE 4. DRC-6061 effect on PBI in bobwhite quail.

Treatment	PBI µg/100 ml	# tested
Control	2.4-15.2	3
24 hr post 50 mg/kg, intraperitoneally	245-275	3
24 hr postfeeding*	375-550	3
2 weeks postfeeding	50,125	2
4 weeks postfeeding	14	3

*Birds offered 6.5 g of food containing 0.75% DRC-6061. Exact dose of chemical not calculated.

In addition to DRC-6061, five other iodinated compounds were administered by gavage at 100 mg/kg to Simonsen/Sprague Dawley male albino rats. Effects on the PBI for these chemicals are listed in Table 5.

TABLE 5. Effects on PBI by iodine compounds in albino rats.

DRC no.	PBI* µg/100 ml, postgavage	
	24 hr	23 days
6062	>2500,>5000	18,18
6063	340,995	8,9
6064	90,>1500	7,8
6065	50	7,8
6066	80,130	10,91
	Control PBI = 9	

*Data represents the value for each animal tested.

Of the six iodinated compounds tested, DRC-6061 appears to offer promise as a long-term marking agent. This chemical has low toxicity and does not appear to adversely affect physiological functions. At the present time, 3-4 ml of blood is needed to obtain enough serum for the PBI analysis. This volume can easily be obtained from rats or other animals of equivalent size. A microdetermination would have to be developed for PBI analysis in smaller animals.

Fluorescent marker:

Dansyl chloride (DRC-6058) reacts with chemicals in the body to form fluorescent derivatives which can be detected in amounts ranging down to 1 millimicromole. Preliminary results with deer mice have shown that DRC-6058 is capable of marking the hair around the feet and lips up to 30 days after animals consumed wheat seeds coated with 1% (w/w) of the chemical. Fluorescence can be detected in the body (gastrointestinal tract, urine, feces) up to 24 hrs after gavaging.

C. Conclusions

Additional studies are planned for both DRC-6061 and 6058. These chemicals might serve as adjuvants with other methods used for long-term identification of animals.

D. Literature Reviewed

Astwood, E.B. 1957. Occurrence in the sera of certain patients of large amounts of a newly isolated iodine compound. Trans. Ass. Amer. Physns. 70:183-191.

Hall, R.R., and W.P. Vanderlaan. 1961. Transplacental transmission of "Teridax", a new cause of elevated PBI. Clin. Res. 9:71.

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Wade, D.N., N. Desbiens, G.J. Strewler, Jr., W.O. Berndt, and G.H. Mudge. 1971. Metabolism of iophenoxic acid in the dog. J. Pharmacol. Exp. Ther. 178:173-179.

3. ACUTE TOXICITY OF TENTATIVELY IDENTIFIED PUTRIFIED FISH CHEMICALS

A. Introduction

Putrified fish fractions are promising candidates for use as repellents against big game mammals in reforestation projects. Previous data collected at DWRC have shown that these fractions are poor repellents against deer mice and they have low mammalian toxicity. Roger Bullard, chemist, Section of Basic Studies, has tentatively identified 37 chemicals as being in putrified fish. Initial Bioassay and LD_{50} tests were performed on commercially obtained samples of these chemicals to determine if they possessed any serious, acute toxicological effects.

B. Results and Discussion

Deer mice of either sex were used for testing. For the acute toxicity tests, chemicals were prepared in 1% gum tragacanth and administered by gavage at 470 mg/kg to two animals. If deaths occurred at 470 mg/kg, an LD_{50} using two animals per dose was calculated. For the Initial Bioassay, chemicals were applied at a level of 2% (w/w) on wheat seeds.

Data in Table 6 and data obtained for nine of the chemicals (DRC-5835, 3481, 5468, 5847, 5848, 5849, 3482, 5856 and 5901) from the literature indicate that these chemicals have low toxicity.

C. Conclusions

From an acute toxicity standpoint, the 37 tentatively identified chemicals from putrified fish do not appear to constitute a serious hazard.

D. Literature Reviewed

Spector, W.S. 1956. Handbook of Toxicology. Vol. 1. Acute toxicities of solids, liquids and gases to laboratory animals. W.B. Saunders, Philadelphia.

The Merck Index. 1968. 8th Edition. Merck and Company, Rahway, N.J.

Thompson, W.R., and C.S. Weil. 1952. On the construction of tables for moving average interpolation. Biometrics 8:51-54.

TABLE 6. Bioassay data of tentatively identified putrified fish chemicals.

DRC no.	Chemical name	LD_{50}, mg/kg with 95% confidence limits	Initial Bioassay % food reduction	Initial Bioassay % mortality
5835	Quinaldine (2-methylquinoliine)	>470	6	0
5836	2-N-Propyl furan	472.5(315.0-708.7)	0	0
5837	Dihydropyran	>470	0	0
5838	o-Hydroxyacetophenone	>470	2	0
5839	2,5-Dimethylbenzothiazole	>470	33	0
5840	1,2,3-Trimethylbenzene	>470	5	0
3481	Indole	>470	19	0
2811	2-Furaldehyde (furfural)	>470	2	0
5841	3,5-Dimethylpyrazole	>470	16	0
5842	Diphenylacetylene	>470	1	0
5843	Ethyl Stearate	>470	1	0
5844	Ethyl Myristate	>470	7	0
5845	Didecyl Ether	>470	4	0
5846	N-Octylamine	>470	14	0
5847	Benzaldehyde	>470	0	0
5848	Dibutyllamine	385.9(244.0-684.4)	0	0
5849	2-Methylbenzothiazole	>470	4	0
5840	Pentadecanenitrile	>470	6	0
5851	1,3,5-Trimethylbenzene	>470	0	0
5852	N-Nonylamine	479.9(213.3-1080.0)	35	0
3482	Skatole	>470	64	0
5853	p-Hydroxyacetophenone	>470	7	0
5854	m-Hydroxyacetophenone	>470	1	0
5855	2,3-Dimethylindole	>470	54	0
5856	Benzimidazole	>470	0	0
5857	N-Phenethylacetamide	955.7(701.1-1302.0)*	0	0
5858	N-Tetracosane	>470	0	0
5859	Hexadecyl Acetamide	>470	2	0
5860	Ethyl Hexadecanoate	>470	0	0
5889	N-Hexyl sulfide	>470	0	0
5901	Diphenyl Guanidine	>470	5	0
5902	N-Hexylacetamide	>470	28	0
5903	Benzylacetamide	>470	15	0
5904	1-Phenyldecane	>470	0	0
5905	Octadecane	470	0	0
5906	1-Octadecene	470	0	0
5907	N-Ethyl Cyclohexylamine	470	0	0

*Four animals per dose.

4. STRUCTURE ACTIVITY RELATIONSHIPS (SAR) WITH OXOTREMORINE

A. Introduction

Oxotremorine 1-[4-(1-pyrrolidinyl)-2-butynyl]-2-pyrrolidinone (DRC-5826), is a potent cholinergic compound. Its LD_{50} is 10-15 mg/kg by gavage in deer mice. On the Initial Bioassay test at 2% (w/w) it gave 87% food reduction and 40% mortality; at 0.1% (w/w) it gave 86% food reduction with no mortality. The above results, plus other observations, indicate that DRC-5826 is a poorly accepted toxicant. It is also nonselective in that cats, dogs and monkeys show signs of severe toxicity when doses well below 100 mg/kg are administered by various routes. Through SAR studies an attempt was made to find related chemicals which would have more desirable toxicant or repellent activity.

B. Results and Discussion

Information on 41 analogs and homologs of the pyrrolidine nucleus have been obtained. Initial Bioassays were conducted on nine of these chemicals, which represented various modifications of the oxotremorine nucleus. Eight of these chemicals were inactive (0% mortality, 0%-28% food reduction) when tested at 2%. One chemical, tremorine (DRC-3832), gave results similar to that of oxotremorine (Table 7).

TABLE 7. Initial Bioassay data: DRC-3832 and 5826.

DRC no.	Initial Bioassay - 2% (w/w)	
	% food reduction	% mortality
3832	87	40
5826	94	60

Tremorine is metabolized to oxotremorine in rodents; this probably accounts for its effects being almost identical to oxotremorine. Toxicity data for some of these analogs were obtained from Edgewood Arsenal and Bayer. All eight of the inactive compounds on the Initial Bioassay had low toxicity. Seven of the inactive compounds were mono-pyrrolidinones; one was a mono-pyrrolidine. All had various substituents on the nitrogen. Future studies will be done on dipyrrolidine derivatives.

C. Conclusions

The good repellent activity of tremorine and oxotremorine on the Initial Bioassay test is most likely related to their high toxicity. Derivatives of these two compounds with low toxicity were poor repellents.

D. Literature Reviewed

Hammer, W., B. Karlën, A. Rane, and F. Sjöqvist. 1968. Rate of metabolism of tremorine and oxotremorine in rats and mice. Life Sci. 7:197-204.

Holmstedt, B. 1967. Mobilization of acetylcholine by cholinergic agents. Ann. N.Y. Acad. Sci. 144:433-458.

5. POTENTIATION OF RODENTICIDES

A. Introduction

Potentiation of the actions of insecticides, particularly the pyrethroids, has had practical significance for many years. However, potentiation in regards to practical application of rodenticides has not received a great deal of attention. One of the goals of rodenticide potentiation is to develop combinations of chemicals which selectively control target species without harm to man and nontarget species. Potentiation would also result in less of a toxicant being applied in the environment. The ability of DRC-5999 to potentiate the action of other chemicals is well known, and its action has been studied on many of the rodenticides currently registered for use by the Bureau.

Most potentiation studies with DRC-5999 are conducted by pretreating the animal with DRC-5999 and after a 40-60 min delay the test compound is administered. This procedure is well suited for laboratory investigations, but is not practical for field use. To have practical field application potentiation should be evident when both chemicals are simultaneously ingested by the rodent. Simultaneous administration and the feeding of chemical combinations to rodents has been investigated.

B. Results and Discussion

Some initial testing was carried out by gavaging male albino mice with DRC-714 at 22.5 mg/kg, followed immediately by intraperitoneal injection of saline (control) or DRC-5999, 20 mg/kg. The potentiating effect of DRC-5999 is most pronounced after 24 hours and less at the end of 14 days (Table 8).

TABLE 8. Effect of DRC-5999 on the toxicity of DRC-714 in male albino mice.

Treatment	% mortality	
	24 hr	14 days
714, 22.5 mg/kg + saline	30 - 6/20	70 - 14/20
714, 22.5 mg/kg + 5999, 20 mg/kg	85 - 17/20	95 - 19/20

Results of feeding studies with 714 and 5999 indicated that potentiation could be obtained when these chemicals were simultaneously ingested (Table 9). However, an apparent antagonistic effect was also observed when the level of 5999 was high. This dual action, potentiation and antagonism, has been observed by other investigators for 5999.

TABLE 9. Bioassay results of 714 and 5999 in deer mice.*

Concentration, % (w/w) 714	+ 5999	% mortality	% food reduction
0.2	0	90 - 9/10	60
0.2	0.1	80 - 8/10	60
0.2	0.5	30 - 3/10	42
0.2	1.0	33 - 3/9	58
0.15	0	10 - 1/10	28
0.15	0.1	70 - 7/10	36
0.15	0.2	30 - 3/10	46

*Animals of either sex were offered 25 wheat seeds treated with either 714 or 714 plus 5999 for 1 night. Animals were observed for 7 days and lab chow was available as an alternate source of food.

DRC-5999 also potentiated the lethal effects of strychnine (DRC-4007) when both chemicals were simultaneously administered by gavage (Table 10).

TABLE 10. Effect of 5999 on the toxicity of 4007 in male albino mice.

Treatment	24 hr mortality (%)
4007, 2.5 mg/kg	20 - 2/10
4007, 2.5 mg/kg + 5999, 100 mg/kg	80 - 8/10

The combination of 5999 and 4007 in baits also indicated that potentiation could be obtained in feeding tests (Table 11).

TABLE 11. Bioassay results of 4007 and 5999 in deer mice.*

Concentration, % (w/w)				
4007	+	5999	24 hr mortality (%)**	Cumulative results
0.2		0	30 - 3/10 40 - 4/10	35% - 7/20
0.2		0.2	90 - 9/10 30 - 3/10	60% - 12/20
0.1		0	0 - 0/10 0 - 0/10	0% - 0/20
0.1		0.2	80 - 8/10 30 - 3/10	55% - 11/20

*Animals of either sex were offered 24 wheat seeds treated with either 4007 or 4007 + 5999 for 1 night. Lab chow was available as an alternate source of food.
**Interval of 1 week between two separate tests.

Some of the other feeding studies of 5999 with 714 or 4007 were inconclusive and the status of 5999 as being a practical potentiating agent for these chemicals is uncertain.

In feeding studies, 5999 was not effective in potentiating the action of "1080", DRC-3492, or zinc phosphide. Histamine, 2-deoxy-D-glucose, and aminotriazole did not prove to be potentiators of zinc phospide either.

Norbormide (DRC-3901) is probably the most specific raticide available at the present time. However, rats develop a "bait shyness" to it and this precludes it from being an effective raticide. Many chemicals were tested for their ability to potentiate norbormide's action with the goal in mind that perhaps a rat could be "tricked" into consuming a

lethal amount before "shyness" developed. Table 12 depicts the biphasic action of 5999 on the toxicity of norbormide. No studies have been performed in an attempt to explain this biphasic effect. The delay in time to death with certain doses of 5999 was unexpected. Thus, depending upon the dosage, 5999 imparts two desirable qualities to the action of norbormide: potentiation and a delay in its lethal effects.

TABLE 12. Effect of 5999 on the toxicity of norbormide to male albino rats.*

Treatment		% mortality	Average time to death (min)
Norbormide, mg/kg	+ 5999 mg/kg, base		
5	0 (Control)	20 - 4/20	53.3
5	1.5	15 - 3/20	53.3
5	3.125	45 - 9/20	52.6
5	6.25	50 - 10/20	53.9
5	12.5	85 - 17/20	52.0
5	25	90 - 18/20	52.4
5	50	85 - 17/20	179.8
5	100	50 - 10/20	275.7
5	200	15 - 3/20	283.0

*Both chemicals simultaneously administered by gavage.

A preliminary study with wild Norway rats indicated that 5999 potentiated norbormide when animals ingested bait containing both chemicals (Table 13).

TABLE 13. Bioassay results of norbormide and 5999 in Norway rats.*

Concentration, % (w/w)		
Norbormide	+ 5999	% mortality
0.5	0	43 - 6/14
0.5	0.5	71 - 10/14

*Animals of either sex used. Chemicals were prepared in lab chow tablets and offered for 1 night. No alternate source of food was available.

A major, serious drawback to the use of 5999 for potentiating norbormide is that Norway rats have an aversion to baits containing 5999. Perhaps a "preferred food" would be the best bait to help disguise the 5999 and this aspect will be looked into further.

Twelve other chemicals were tested for potentiation of norbormide, but none were effective.

C. Conclusions

Potentiation of the action of rodenticides has been demonstrated in the laboratory, but this technique has an uncertain future for some chemicals. Because they appear to have more desirable properties than other chemicals, future studies will be conducted mainly with norbormide and zinc phosphide. Potentiation need not be concerned entirely with lethal agents for the control of pest species. In situations where the repellent action of a chemical is due to postingestational effects, potentiation might be useful to prolong or intensify these effects. Potentiators might also be useful to augment the action of chemosterilants.

D. Literature Reviewed

O'Brien, R.D. 1967. Insecticides—action and metabolism. Academic Press, New York.

Roszkowski, A.P. 1965. The pharmacological properties of norbormide, a selective rat toxicant. J. Pharmacol. Exp. Ther. 149:288-299.

6. RODENTICIDE STUDIES

A. Introduction

During the past year only one new chemical, DRC-5976, was considered for additional testing as a rodenticide.

B. Results and Discussion

Preliminary data indicate that DRC-5976 is highly lethal to Norway rats (Table 14).

TABLE 14. Acute oral toxicity of DRC-5976 in animals.

Animal tested	Sex	Dose, mg/kg	Comments
Red-winged blackbird*	Male	100	0/2 dead
Rabbit, Dutch-belted	Male	500	1/3 dead
Rabbit, California	Male	100	0/2 dead
Albino mouse	Male	205 (85-492)	LD_{50}
Albino rat	Male	11.9 (5.7-24.5)	LD_{50}
Wild Norway rat	Female	10.6 (10.6-10.6)	LD_{50}
Ricefield rat	Female	120	0/2 dead
Polynesian rat	Male	250-500	2/2 dead at 500; 0/2 dead at 250

*Tested by Ed Schafer, Section of Birds—7-day observation; 14-day observation for all other animals.

In the Initial Bioassay test with deer mice, 5976 was not repellent nor did it produce mortality when tested at a level of 2%.

Mortality results of 5976 in wild Norway rats in voluntary acceptance tests are shown in Table 15.

TABLE 15. Bioassay results of DRC-5976 in wild Norway rats.*

Concentration, % (w/w)	Sex	No. tested	Mortality**	No. nights offered
0.25	F	5	1/5	4
0.25	M	5	0/5	4
0.5	F	5	3/5	4
0.5	M	5	2/5	4
1.0	F	5	2/5	2
2.0	F	5	5/5	2
2.0	M	9	7/9	2

*Animals were individually caged and for 2 consecutive nights offered 2 cups each containing approximately 20 g of tableted rat chow. The amount eaten from each cup was recorded and on the third night 20 g of treated tablets were placed in the "preferred" cup. The other cup contained 20 g of control tablets. On subsequent test nights, positions of the treated and control cups were alternated.

**Mortality that occurred after first night that the treated tablets offered. No mortality occurred on subsequent nights. In general, animals almost entirely avoided the treated tablets on subsequent nights.

Feeding tests with ricefield rats at concentrations of 0.125%, 0.25% and 0.5% were not lethal. At these concentrations animals were consuming approximately 80-90 mg/kg. Higher concentrations were not tested in ricefield rats.

Seven derivatives of DRC-5976 were not lethal to male albino rats at 100 mg/kg when administered by gavage.

C. Conclusions

Preliminary studies indicate that DRC-5976 might be useful as a rodenticide. Studies on its mechanism of action are being initiated.

II. BIOLOGICAL ACTIVITY AND CHEMICAL STRUCTURE CORRELATION

A. Introduction

The problems of designing compounds eliciting predictable, controllable, and desirable effects on target biomedia have long challenged investigators. Screening programs usually embrace the classic approach to isolating effective compounds by empirically evaluating large numbers of chemicals with standardized bioassay procedures. These programs have generated large volumes of data and developed useful supportive measures in controlling animal damage, but there have been relatively few acceptable chemical control agents discovered from these efforts.

Part of this Unit's work load is concerned with correlating biological activity to chemical structure as one approach to "tailor-making" compounds for specificity of action. Any correlative proof or activity rational established through such an approach will intensify cooperation between industrial, academic, and government agencies mutually engaged in the objectives of modifying molecules to potentiate protective abilities.

Fundamental knowledge now exists on the activity of several thousand compounds appraised for repellency properties by the Denver and Patuxent Wildlife Research Centers. A publication listing 4,600 compounds evaluated as repellents by the Patuxent food acceptance test method was authorized by the U.S. Army Natick Laboratories. They have also expressed a willingness to support a publication describing this Center's method to measure repellency by a concentration-effect test method and the results for 1,500 compounds assayed by this procedure. Also available for supportive evidence are the results for a small number of identified compounds that were evaluated as both toxicants and repellents. Data from all sources will be reviewed with particular emphasis placed on gross structural considerations and stressing the presence and location of functional groups within an active molecule.

B. Results and Discussion

The manufacturer of the group of tin compounds that were the most effective of all chemicals tested did not release their chemical identities for listing, nor did he authorize including them by a company code number in Table III; therefore, they do not appear in Part B, the Barrier Study Section, of the publication.

Dr. John J. Pratt, Jr., sponsor from the U.S. Army Natick Laboratories, visited us during July at which time he requested that the final publication include a cross-referencing or indexing of the chemical name and its corresponding assigned sample code number by page number(s). In order to meet this requirement, an appendix was developed listing the code number, the pagination and the structural formula, as expressed by the Wiswesser line-formula notation (WLN), for all compounds appearing in Table I.

Cooperative arrangements were made with Mr. William J. Wiswesser, Research Chemist, U.S. Army, Ft. Detrick, Md., developer of the WLN, to encode the more than 4,000 compounds appearing in Table I into WLN cipher. The following is an example of the Appendix format developed to fulfill Dr. Pratt's request:

Code No.	Page No.	Wiswesser Line-Formula Notation
2701	38,69	T66 B6 A B- C 1B I ASWN DN FN HNSWTJ
2702	23,81	T56 BNYSJ B2 CU2U2U1-CT56 BK DSJ B2
	139	&I &29/39
2703	72	GR B1K2&2&2&2MV 2 &G &G &6/18 &6/21

A list of compounds screened by the concentration-effect test method for measuring rodent repellency as developed by the Denver Center is in preparation. The compounds are listed sequentially by DRC number, chemical name, the WLN cipher and test results. This information will be published following completion of the correlation study publication.

C. Conclusions

With the completion of both publications, the study concerning biological activity and chemical structure relationships can be emphasized. Combining the knowledge gained from the previous bioassay methods with that resulting from more refined techniques now being developed, will enable selecting compounds for specificity of action instead of fortuitous discoveries derived through "blind" screening programs.

The data made available in the Natick-supported publications also merit inclusion in the chemical information storage and retrieval system. The investigations and steps taken to accomplish these objectives are described in Part III of this report, which details those responsibilities of this Unit concerned with data processing.

D. Literature Reviewed

Bellack, E., J.B. DeWitt, and R. Trerchler. 1953. Relationship between chemical structure and rat repellency. Chemical-Biological Coordination Center Review No. 5:48-156.

III. INFORMATION STORAGE AND RETRIEVAL DEVELOPMENTS

A. Introduction

The storage and retrieval of technical information on more than 3,500,000 organic and 50,000 inorganic compounds, in addition to new compounds synthesized each year, is one of the major problems of research groups concerned with chemical-biological relationships. Before this project was activated in 1961, no information storage and retrieval system had been devoted exclusively to recording chemical and physical characteristics and test data for compounds to control animal damage. The system that was developed and the procedures for encoding data are outlined in the 1961-64 Annual Progress Reports.

The primary objective of this service is adapting and maintaining an information storage and retrieval system on compounds studied at the Denver Wildlife Research Center for use by research units responsible for investigating and developing control agents. Any modification of recording techniques, utilization of new storage media, or application of more effective alerting services are concurrent responsibilities.

B. Results and Discussion

A major portion of the total time spent on project-related activities was devoted to adapting and modifying the present data storage and retrieval system for conversion to ADP procedures. The initial change entails eliminating the chemical fragmentation concept used to describe chemical compounds by degree of substitution and citation of functional groups, and replacing it with the Wiswesser Line-Formula Notation (WLN) system.

The WLN has been described as being a relatively new chemical information tool that enables one to describe the structure of chemical compounds with unique, unambiguous, linear sequences of letters and numbers. It is believed by many to be the most efficient and concise method providing chemical structure input for both manual and computer-based chemical data storage and retrieval systems. Already in use by more than 100 organizations in the United States and abroad, well over 1 million compounds have been encoded to WLN, indicating that the notations are particularly suitable for indexing and computer applications.

As part of this conversion mission, I attended a 2-day WLN Tutorial Session given during the Spring Meeting of the American Chemical Society held in Los Angeles. The session was taught by Dr. E. Smith, Mills College, San Francisco, and Mr. K. Horner, Horner Associates, Palo Alto. Both are information scientists, and they presented a general introduction to WLN encoding and decoding procedures.

Additional assistance and experience was obtained through the cooperation of Mr. W.J. Wiswesser and several other members of the Chemical Notation Society who encoded into WLN the more than 4,000 compounds tested as repellents at Patuxent under Dr. J.B. DeWitt. They supplied us with a printout listing the corresponding cipher for each compound evaluated; this list will serve a dual purpose in that it will be included in the structure/activity correlation publication and will be used as the initial input material for the Center's data bank.

Three members of the American Society for Information Services made separate visits to this facility to discuss and evaluate our data program. These people are all engaged in the information handling field and are well qualified to give opinions on our operations. Dr. Ralph O'Dette is a Staff Specialist from the American Chemical Society, Chemical Abstracts, in Columbus, Ohio; Mrs. Joan Burnside is an Information Scientist from the Toxicological Information Program, National Library of Medicine, HEW, in Bethesda, Maryland; Mr. Charles Granito is Director, Chemical Information Services, Institute for Scientific Information, in Philadelphia, Pennsylvania.

Collectively, they felt that this Center's research programs generate data that should be made available and these data can be easily entered into existing information repositories. They also stressed the fact that the key to useful and acceptable data systems, based on chemical structures, is dependent upon codification schemes that permit entry into data banks by chemical descriptors precisely defining spatial relationships and molecular configurations. They agreed that the WLN satisfies this prerequisite and recommended we should direct our efforts in establishing this system.

C. Conclusions

In view of the recommendations made during the consultations described above, it is obvious that a maximum effort program is indicated to adopt the WLN as the method of handling chemical-biological information for this Center's data bank. Thus, not only will we be in a mutually cooperative position to request information on a "short time turn around expectancy" from others, but also will be able to contribute meaningful data retrievable through random searches or subject scans initiated by the more than 100 users of the WLN. Often the sources of bioassay data are as helpful as the test results, and in light of the current popular phrase "environmental impact and wildlife effects," this Center is destined to become a major information source on chemical actions on biosystems.

There are two approaches to the developmental phase of encoding the 200,000-odd biological and chemical data bits we have presently in our data bank to the WLN storage format. One entails attending a week-long WLN workshop sponsored by the Institute of Scientific Information in Philadelphia, Pa.; however, the $600 estimated expenses eliminates this possibility in view of current project funds available. A second choice, which is preferable, involves attending a special course taught by Dr. David Renaurd, Information Officer, U.S. Army Office of Industrial Liaison, Edgewood Arsenal, Maryland. Every effort should be made to implement and effect the proposed changeover to the WLN system as soon as possible.

PHYSIOLOGY AND BEHAVIOR - R.D. Thompson, S.A. Shumake, and R.W. Bullard

1. VISUAL SENSITIVITY OF VAMPIRE BATS

Introduction

The contribution of the visual sense to prey detection by the vampire bat is relatively unknown. Many species of cave-dwelling bats use their echolocating auditory apparatus for orientation and prey detection. However, evidence for visual, as well as auditory, cues has been uncovered in many species of bats including the vampire. Three vampire bats have been trained individually to lick a drinking tube containing 20% whole blood to intermittently receive .25 cc of 75% whole blood reinforcements. When stabilized licking baselines were observed, a 15-sec light stimulus was administered to the bat and terminated with a .2 ma electric shock to the feet. The light intensity was systematically lowered until suppression of licking no longer occurred at the visual threshold.

Results and Discussion

Absolute visual thresholds were obtained in three vampire bats which ranged from -3.00 to -4.80 log foot Lamberts. These values are not distinctly different from human thresholds obtained under these conditions. It appears that the technique is suitable for measuring sensitivity to other forms of stimulation such as sound or odors. Representative data from the tests are shown in Table 1.

TABLE 1. Absolute thresholds, above and below threshold suppression ratios to light, and above and below threshold suppression ratios to shutter control for three vampire bats.

Subject No.	Absolute threshold range (Log Foot Lamberts)	Mean above threshold S.R. to light	Mean below threshold S.R. to light	Mean above threshold S.R. to shutter	Mean below threshold S.R. to shutter
1	-4.75 to -4.80	.86	.06	.03	-.10
2	-2.70 to -2.75	.92	.27	.03	-.02
3	-3.00 to -3.05	.90	.26	-.08	-.17

Note: Abbreviation--S.R. = Suppression Ratio.

2. ATTRACTANCY TESTS FOR SELECTED ODORS OF NONBIOLOGICAL ORIGIN IN RICEFIELD RATS

Introduction

Many compounds have been tested for attractant quality in various species of rodents. The ricefield rat, however, has not been subjected to these tests for attractancy. A four-choice open-field test was used to evaluate over 50 compounds. Each pair of compounds was tested against a food-odor standard and a deionized water control. The number of visitations made by each animal for each odor and the elapsed time spent in the presence of each odor were recorded.

Results and Discussion

Attractant quality of each compound was scaled by calculating the mean percent of the food odor response for each animal. In cases where the responding to the deionized water was equal or greater than 20% of total responding, the data for the test were rejected. Close to 40% of the data had to be discarded due to excessively high water control responding. Each test for paired compounds was subjected to a Wilcoxon Rank-Sum statistical test and, with six animals per treatment, very few significant effects were found. Twenty of the compounds are scaled for attractant quality in Table 2.

For practical purposes, all but the top 20% of these compounds may be discarded from further testing. The remaining compounds may be added to a base bait material for further odor and flavor preference testing.

3. PHEROMONES AND ODORS OF BIOLOGICAL ORIGIN IN THE RICEFIELD RAT

Introduction

Three potential sources of pheromones (preputial gland secretions, urine, and vaginal secretions) are currently being investigated for attractant function in ricefield rats. Twenty females have been tested initially in a four-choice open-field test for responsiveness to male preputial gland extract. Samples are currently being collected from both males and females for future tests. The objective in this program is to determine the source of attractant odors, and then to eventually isolate and identify the active compounds.

TABLE 2. Twenty compounds scaled for attractant quality for ricefield rats.

Compound	Percent of food odor response	Standard error	Coefficient of variation
Soybean Oil	94.6	±22.96	.47
Isoraleric Aldehyde	94.3	±21.64	.47
N-Butyldiethanolamine	87.5	±38.58	.74
N-Propylamine	80.0	±56.36	1.05
Peanut Oil	80.0	±18.33	.51
2-Furaldehyde	77.5	±31.32	.72
Linseed Oil	76.2	±24.11	.64
Ethyldiethanolamine	75.6	±11.51	.34
Sassafrass Oil	74.4	±23.93	.61
Dihydroxyethylaniline	72.5	±58.87	1.14
Wintergreen Oil	72.2	±16.91	.46
Corn Oil	67.5	±16.27	.51
Hexanoic Acid	65.5	±25.87	.75
N-Octylamine	61.9	±26.65	.81
N-Pentylamine	60.0	±42.90	1.08
Isobutylamine	52.4	±19.66	.74
Cod Liver Oil	50.0	±19.29	.73
N-(n-propyl) benzylamine	40.0	±28.13	1.05
Valerone	36.4	± 4.56	.27
N-Hexylamine	35.6	± 3.55	.22

Results and Discussion

For preputial gland secretions, preliminary data indicate greatest activity when female ricefield rats are in proestrus or estrus. Since reduction of the female population would have a greater impact on population growth rate than would a comparable male rat reduction, response of the female to the male odor is being examined initially. However, male response to the female odor may be more effective. No male ricefield rats have been tested on the secretions from this gland since the open-field odor test may be an inadequate tool for assessing closely related odors of biological origin. An in-cage odor test device has been developed and will be used for tests on males for response to female preputial gland secretions, vaginal secretions, and urine.

4. A FOUR-CHOICE IN-CAGE ODOR TEST FOR RICEFIELD RATS

Introduction

When the wild ricefield rat is transferred from home cage to a test chamber, there is often a long delay before behavioral testing may proceed. Ricefield rats appear to be highly emotional and exhibit strong escape-avoidance response tendencies which often interfere with experimental behavioral measurement of odor preference in a previously used four-choice open-field odor test apparatus. To circumvent these difficulties, a four-choice in-cage test has been developed which allows for measurement of approach responses without confounding of avoidance responses unrelated to odor stimulus presentation.

Results and Discussion

Since the animals are tested in the familiar surroundings of their home cages, few, if any, escape responses occur. The in-cage odor test device appears to show no significant position bias as indicated by the uniformity data shown in Table 3. In a food-odor localization test, the animals exhibited an ability to localize the attractant food odor over water. Currently, multiple odor tests are being evaluated with ricefield rats. It appears that the animals can discriminate various odors in the in-cage test. The device may be removed from the cage after testing and thoroughly cleaned to remove odor residues.

TABLE 3. Visitation frequency measure of odor preference for the four-choice in-cage odor preference test.

Test	Odor type	Mean number of visitations		Standard error	Significance level
Uniformity (n = 18)	F	51.22	±	14.69	n.s.
	F	46.00	±	26.58	n.s.
	F	48.72	±	11.49	n.s.
	F	47.11	±	14.50	n.s.
Localization (n = 16)	F	136.63	±	36.92	*
	$W_{(1)}$	9.38	±	1.62	n.s.
	$W_{(2)}$	9.63	±	3.37	n.s.
	$W_{(3)}$	8.94	±	2.65	n.s.

*$P(F>W_{(1)}, W_{(2)}, \text{ or } W_{(3)})<.01$.

Note: Abbreviations--F = food odor; W = deionized water.

5. OBTAINING GAS CHROMATOGRAPHIC "SIGNATURES" OF PREPUTIAL GLAND EXUDATE FROM LIVE RICEFIELD RATS (*Rattus rattus mindanensis*)

Introduction

The preputial gland in rats appears to be the source of the kind of pheromonal activity that is potentially exploitable for rat control objectives. This study is primarily concerned with the chemical composition of preputial gland exudate and for females, the changes that occur during various stages of estrus. Gas chromatograms provide us with the "signature" for a particular collection of preputial gland exudate. The signatures are examined and interpreted in terms of individual, sex, and estral differences.

Results and Discussion

The reportable results at this time are mainly the development of the capability to do such an experiment. It has been difficult to find a way to collect the material from a live animal, extract it, and then concentrate the extract (without losing volatiles) to a small volume (∿5 µl) so that enough of the extract can be injected into a gas chromatograph to give a good quality chromatogram.

Preputial gland exudate is extruded from the live animal by gentle application of pressure in the area of the preputials. It is collected onto the surface of 1/8-inch anhydrous sodium sulfate tablets (which remove water). These are placed in a 1.5 ml syringe, crushed and tared. A specially designed rack holds the syringe in a vertical position and the needle extends into a 0.3 microvial. Diethyl ether applied to the syringe barrel flows through the sodium sulfate-exudate and the solvent extract drips into the microvial which is bathed continuously with a flow of nitrogen to remove the solvent. The syringe plunger can be used to adjust the solvent flow.

Prior to extraction, 4 µl of dioctyl phthalate is added to the vial. This traps the extract volatiles and prevents their loss during solvent removal. The dioctyl phthalate elutes from the chromatograph later than any of the preputial gland components and thus does not interfere with the chromatogram. The syringe is reweighed to determine the amount of material extracted.

An internal standard (n-pentadecane) is added in a quantity proportional to the amount of material extracted from the sodium sulfate. The entire quantity is chromatographed on a 1/8-inch x 5-ft s.s. column containing 3% QF-1 on Gas Chrom Q 100/120 mesh with the temperature programmed from 65°C to 165°C. Peaks are measured and assigned an index value based on the peak height ratio to the internal standard. This indexing provides a means of statistical interpretation of the signature which is correlated with the physiological state of the animal at collection time.

6. DETERMINING CHLOROPHACINONE AND DIPHENADIONE LEVELS IN BLOOD, MILK, AND MEAT

Introduction

The anticoagulant rodenticides chlorophacinone (1,3-indandione,2-[(p-chlorophenyl) phenylacetyl]) and diphenadione (2-diphenylacetyl 1,3-indandione) have been investigated for potential use as control agents for the vampire bat (*Desmodus rotundus*). In conjunction with this program, an analytical procedure was developed for determining chlorophacinone levels in bovine plasma. The procedure was expanded later to accommodate milk and meat tissue samples. The same procedure is applicable for diphacinone analysis with the only change being in the column over temperature during gas-liquid chromatography analysis (glc).

Results and Discussion

Most of the analyses have been conducted on bovine plasma. Plasma is first subjected to mild acid hydrolysis to release chlorophacinone or diphenadione from protein binding. Ascorbic acid is added to prevent heme complexes from forming. Plasma proteins are precipitated with acetone and removed by centrifugation. Acetone is removed by rotary evaporation and the extract is partitioned between 0.625 N NaOH and methylene chloride. The methylene chloride phase (containing the indandione) is reduced to dryness by rotary evaporation. Further cleanup is accomplished by partitioning the residue between acetonetrile and n-hexane. The acetonitrile phase may then be analyzed by UV spectrophotometry if the sample contains 5 μg or more of the anticoagulant.

If the sample contains less than this, the residue from the acetonitrile phase is oxidized by refluxing 30 min with CrO_3 solution. Chlorophacinone is oxidized to p-chlorobenzophene and diphenadione to benzophenone. Both products are readily detectable by glc, utilizing electron capture detection. The lower detection limit for the procedure is 0.05 ppm if 1 ml of plasma is used.

Meat tissue and milk may be analyzed by the same procedure with the only modification being in the sample preparation. Milk is air-dried overnight under a hood. The residue is mixed with anhydrous sodium sulfate and then extracted with acetone. Meat tissue is ground with anhydrous sodium sulfate before acetone extraction. The extract from both sources is handled as described above for plasma.

Part of this work was reported at the 27th Southwest Regional Meeting of the American Chemical Society held December 1-3, 1971, at San Antonio, Texas.

7. LIVESTOCK SYSTEMIC TECHNIQUE FOR CONTROL OF VAMPIRE BATS (*Desmodus rotundus*)--LABORATORY PHASE

Introduction

Since the vampire bat depends solely on blood of vertebrates for food, it appears that an ideal method of applying vampiricidal agents is through the blood of cattle undergoing predation. Preliminary laboratory results were presented in the 1971 Annual Progress Report which demonstrated that the principle of administering a systemic to cattle for vampire bat control was feasible and possibly had good

potential for reductional control. The research was continued and expanded in 1971. Emphasis was centered on determining the effectiveness of two anticoagulants (DRC-3776 and DRC-5652) as

Results and Discussion

DRC-3776

Initial tests to develop a livestock systemic technique to control vampire bats were conducted with DRC-3776, using sheep and cattle. DRC-3776 was administered orally in gelatin capsules by balling gun at several dose levels. Blood samples were obtained prior to dosing, at 12 and 24 hours postdosing, and at 24-hour intervals thereafter until prothrombin clotting time returned to baseline. Plasma prothrombin time and plasma DRC-3776 were determined at each bleeding interval and an aliquot of the 24, 48, and 72 hour sample defibrinated and fed to vampire bats. For toxicity determination, two beef calves were slaughtered at 15 and at 90 days after being treated with DRC-3776. Liver tissue was analyzed for DRC-3776 and bioassayed with rats.

Results are summarized in tables 4 to 7:

TABLE 4. Prothrombin clotting time (sec) response of cattle to DRC-3776.

Dose level (mg/kg)	No. animals	Baseline prothrombin clotting time (mean)	Peak prothrombin clotting time (mean)	Hours after dosing to peak prothrombin time	No. hours for prothrombin to return to normal
0.5	4	17.1	97.6	96	192
1.0	4	19.0	140.6	144	240
3.0	4	18.6	224.2	144	240

TABLE 5. Plasma DRC-3776 (mg/ml) in cattle at three dose levels.

Dose level (mg/kg)	Hours after dosing			Hours after dosing to peak level
	12	120	Mean	
0.5	0.239	0.109	0.253	24
1.0	1.240	0.950	1.210	48
3.0	4.920	3.800	4.230	24

TABLE 6. Mortality data from bats fed blood of cattle dosed with DRC-3776.

Dose level (mg/kg)	No. bats fed at each bleeding interval	Total n	Mean amt. blood consumed (gm)	% mortality (hours after dosing)		
				24	48	72
0.5	2	6	26.0	50	100	100
1.0	2	6	25.4	50	100	100
3.0	3	9	26.4	100	100	100

TABLE 7. Bioassay and chemical analyses of cattle liver for DRC-3776.

Description	No. rats	No. feeding days	Mean liver consumed (g/kg B.W.)	Mortality	DRC-3776 contents (µg/gm)
Uncooked control liver	5	5	751.9	0/5	0.0
Uncooked liver, 15 days postdosing (0.5 mg/kg)	5	5	311.2	5/5	approx. 1.0
Uncooked liver, 90 days postdosing (0.5 mg/kg)	5	5	496.9	5/5	approx. 0.5
Cooked liver, 90 days postdosing (0.5 mg/kg)	5	5	741.2	1/5	---

The results show that one feeding of blood from cattle dosed as low as 0.5 mg/kg is lethal to vampire bats for at least 3 nights after dosing (approx. nights), which is very desirable feature for a control program. However, DRC-3776 has a narrow safety margin for cattle as evidenced by the marked rise in prothrombin clotting time at only 3.0 mg/kg. This, together with the retention of DRC-3776 in the liver up to 90 days after dosing (see Table 7), indicates that it should not be used as a livestock systemic for vampire bat control.

DRC-5652

This compound was selected for testing after the disadvantages of DRC-3776 as a livestock systemic were detected. The testing procedure was generally the same as for DRC-3776. The structural formula of DRC-5652 is identical to DRC-3776 except that it contains no chlorine. Test data obtained thus far indicate that DRC-5652 has excellent potential as a livestock systemic for vampire bat control. The evidence for this viewpoint is based on the following factors:

1. It is very toxic to vampire bats (LD_{50} = 0.91 mg/kg as compared to 3.75 mg/kg for DRC-3776), but has a wide safety margin for cattle. A single oral dose up to 5.0 mg/kg produces only a transcient and harmless effect on the clotting ability of host blood (see prothrombin and whole blood clotting time in Table 8).

TABLE 8. Prothrombin and whole blood clotting time response of cattle to DRC-5652 (mean±SE).

Hours post-dosing	Oral dose (mg/kg)					
	0.5 n=4		2.75 n=4		5.0 n=4	
	P.T.*	WBCT**	P.T.	WBCT	P.T.	WBCT
0	18.9±1.4	5.6±0.4	20.8±0.9	6.7±1.0	19.6±1.0	6.6±0.9
12	19.1±1.3	5.5±0.3	22.4±1.1	6.6±0.8	24.3±2.6	6.7±0.9
24	26.4±3.6	5.6±0.3	27.0±1.6	6.3±0.8	30.1±4.0	7.0±1.2
48	45.6±9.9	6.7±0.0	58.1±4.7	7.1±0.4	63.3±9.1	7.5±1.3
72	44.8±11.8	6.7±1.0	95.0±4.8	7.5±0.6	124.2±33.1	9.2±1.5
96	28.8±5.2	6.7±0.6	121.9±24.0	8.5±0.2	183.4±47.2	10.9±2.0
120	22.8±2.6	6.2±0.4	39.6±6.3	9.0±1.4	78.2±8.4	9.1±1.7
144	19.8±1.4	5.5±0.5	24.5±1.7	8.2±0.6	35.4±3.7	9.6±1.3

*Prothrombin time in seconds.
**Whole blood clotting time in minutes.

2. Like DRC-3776, a single dose of DRC-5652 is lethal to bats for an extended period of time. For example, enclosure studies show that one blood feeding from cattle dosed orally at 0.5 mg/kg is lethal to 33% of bats feeding on blood of the treated host for 48 hours after dosing (approximately two feeding nights). Two consecutive feedings produced 100% bat mortality (see Table 9). Data in Table 10 show that one feeding of blood from cattle dosed at 1.0 mg/kg (intraruminal) produces 100% mortality in vampire bats.

TABLE 9. Mortality of vampire bats feeding on beef calves treated orally with DRC-5652 (48 hours postdosing, 0.5 mg/kg).

	Bat mortality	
Animal no.	One feeding	Two feedings
1	1/2	2/2
2	0.2	2/2
3	1/2	2/2
Average (%)	33	100

TABLE 10. Mortality of vampire bats feeding on blood of beef calves with DRC-5652 (1.0 mg/kg, intraruminal).

Hours postdosing	No. calves	Bat mortality from consuming 20 g blood, single feeding
24	3	3/3
48	3	3/3
72	3	3/3
96	3	1/3

3. DRC-5652 appears not to be retained in the liver of treated cattle for a prolonged period of time after dosing (see Table 11 for data on bioassay of cattle liver).

TABLE 11. Bioassay of cattle liver with rats for DRC-5652.

Description	No. rats	No. feeding days	Liver consumed mean (g/kg B.W.)	Mortality
Uncooked control liver	5	7	798.6	0/5
Uncooked liver, 30 days postdosing (2.75 mg/kg)	10	7	693.4	0/10
Cooked liver, 30 days postdosing (2.75 mg/kg)	10	7	778.3	0/10

4. Preliminary data indicate there is no hazard to newborn calves feeding exclusively on milk of cows treated with DRC-5652 up to 2.75 mg/kg. A cow dosed at 0.5 mg/kg contained approximately 0.02 ppm of DRC-5652 at 120 hours after dosing which, if repeatable, is a negligible level from the standpoint of secondary hazard.

8. LIVESTOCK SYSTEMIC TECHNIQUE FOR CONTROL OF VAMPIRE BATS--PRELIMINARY FIELD PHASE

Introduction

Inasmuch as DRC-5652 appears to have excellent potential as a livestock systemic for vampire bat control, a preliminary experiment was conducted to test its effectiveness under field conditions. The experiment was conducted at cattle ranches in the State of San Luis Potosi, Mexico, in October 1971.

Results and Discussion

Cattle suffering from vampire bat predation were treated with a single oral dose of DRC-5652 at 1.0 mg/kg. The results are summarized in Table 12.

TABLE 12. Effectiveness of DRC-5652 as a livestock systemic in vampire bat control.

Parameters	Ranch 1	Ranch 2
No. animals treated (cows, bulls, steers, and young calves)	36	82
No. fresh vampire bat bites, pretreatment	54	73
No. vampire bats netted and released, pretreatment	76	52
No. fresh vampire bat bites, 10 days posttreatment	4	*
No. vampire bats netted, 10 days posttreatment	9	0
% reduction in vampire bat bites	92.6	*
% reduction in bat population	89.5	100

*Unable to determine count.

The control treatment was very effective in reducing the number of fresh bites and the number of vampire bats at ranch 1. However, it was not possible to determine the posttreatment bite count at ranch 2 because of an unexpected influx of cattle egrets that were observed feeding on ticks from the cattle during the posttreatment evaluation period. The experiment will be replicated in February 1972, with certain modifications, on a larger scale to get a clearer indication of the effectiveness of this technique for vampire bat control.

REPRODUCTION - J.J. Kennelly, M.V. Garrison, B.E. Johns, and J.D. Roberts

I. RODENT STUDIES

A. Bait Formulation with DRC-4801

Introduction

Results from previous studies indicated there was an acceptance problem with DRC-4801 treated bait. Whether this problem was due to the concentration of the chemosterilant or the combination of the chemosterilant and the carrier used was not known. Therefore, this study was undertaken to determine: (1) an acceptable carrier and (2) the optimum concentration of chemical to use in the study described in Section I-B.

Results and Discussion

To determine the acceptable carrier, a 5 x 5 Latin square designed study was conducted with Polynesian rats using the following feeds: (1) oat groats, (2) lab chow with 20% dried molasses, (3) oatmeal, (4) wheat, and (5) cracked corn. Results indicated that lab chow (with molasses) was the preferred food, followed by oatmeal, oat groats, wheat and cracked corn in that order.

The optimum concentration was determined by feeding the preferred food from above, formulated at five different concentrations (0.005%, 0.0075%, 0.01%, 0.0125% and 0.0150%). Five adult female Polynesian rats were assigned to each treatment level and were fed untreated carrier for 3 days and treated bait for 4 days. Results are shown in Table 1.

TABLE 1. Bait acceptance with DRC-4801 treated carrier.

No. animals	Mean body wt. (g)	Treatment (%)	Mean bait consumed (g)	Mean DRC-4801 consumed (mg/kg b. wt.)
5	67.8	0.005	0.79	0.67
5	66.6	0.0075	0.46	0.53
5	58.6	0.010	0.24	0.36
5	67.4	0.0125	1.48	2.91[a]
5	59.2	0.0150	0.62	1.48[a]

[a] The high mean value is due to one female that consumed a disproportionate quantity of bait.

TABLE 2. The inhibition of pregnancy in Norway and Polynesian rats given a single oral dose of DRC-4801.

Treatment	No. of females	Mean body weight (g ± S.D.)	Mean quality eaten (mg/kg ± S.D.)	Mean no. days (% S.D.) To parturition	Mean no. days (% S.D.) Delays in days
			Norway rat		
0.0%	9	273.8 ± 39.7	---	26.0 ± 4.5	-- [e]
0.0012%	9	255.7 ± 35.3	0.21 ± 0.18 [b]	30.9 ± 4.6	4.9
0.012%	7	255.8 ± 54.1	2.00 ± 1.05 [b]	37.3 ± 6.8	11.3
0.5 [a]	7	269.8 ± 60.5	0.5 ± 0.0	37.6 ± 3.6	11.6 [d]
			Polynesian rat		
0.0%	7	70.7 ± 8.2	---	27.0 ± 3.0	-- [e]
0.0012%	3	70.0 ± 6.0	0.46 ± 0.19	32.0 ± 6.1	5.0
0.012%	7	65.8 ± 7.3	2.16 ± 1.68	45.1 ± 7.6	18.1
0.5 [a]	6	65.7 ± 10.8	0.50 ± 0.0	38.7 ± 7.3	11.7 [d]

[a] Each female dosed via gavage to provide 0.5 mg/kg body weight.
[b] Chemosterilant treated bait presented for 1 night (16 hours) and actual dose was based on weight of eaten bait.
[c] Based on respective control (0.0%) values.
[d] Significantly ($P<0.05$) different from control.
[e] Any means not connected by the same line are significantly different ($P<0.05$).

B. Evaluation of an Antifertility Compound, DRC-4801

Introduction

The initial work with DRC-4801, a synthetic estrogen, was reported in the 1970 Annual Report. This report deals with the effect of DRC-4801 in regards to: (1) delaying the onset of pregnancy, (2) the induction of sterility in offspring nursing treated mothers, and (3) controlling the ultimate size of closed colony populations.

Results and Discussion

The effectiveness of DRC-4801 in delaying the onset of pregnancy was studied with the Norway and Polynesian rats. Results are shown in Table 2.

As can be seen in the table, the chemosterilant was effective in increasing the interval between pairing and parturition in both the Norway and Polynesian rats. A formulation of 0.012% DRC-4801 was adequate to obtain significant delays in fertility in both species of rodents.

The results of the study to determine the effect of DRC-4801 on fertility of offspring nursing treated mothers are shown in Table 3.

TABLE 3. The effect of DRC-4801 on litters nursing from treated Norway females.

		Females		Males		
Treatment	Dose level[a] (mg/kg b. wt)	Number	Percent affected	Number	Percent affected	Average testis wt (mg)[b]
I	0.0	31	0.0 [c]	18	0.0 [c]	0.5558 [d]
II	0.01	34	76.5	12	0.0	0.5633
III	1.0	17	100.0	20	20.0	0.4788
IV	10.0	18	100.0	14	78.6	0.4042

[a] Chemosterilant delivered by gavage.
[b] Testis weight per gram of body weight X100.
[c] Any means not connected by the same line are significantly different ($P < 0.01$).
[d] Any means not connected by the same line are significantly different ($P < 0.05$).

Our results clearly show a dose-dependent effect. A highly significant ($P<0.01$) effect of the chemosterilant with female pups (distended uteri and inactive ovaries) was found when the treated groups were compared to the control group. A less significant ($P<0.05$) effect (lesions on caput epididymidis, necrotic tubules, corpus epididymidis being void of sperm, and atropied testes) was found in males from the high treatment level (10 mg/kg b. wt). A dose-related effect on testes weight was also observed.

The chemosterilant was offered to the animals via treated food (ground lab chow with 20% dried molasses). The results show a decreased consumption of DRC-4801 during the second and third feeding periods. During the fourth feeding period the carrier bait was changed to oatmeal (with brown and maple sugars) and the concentration increased from 0.005% to 0.0125%. The consumption of DRC-4801 increased over previous feeding, but the subsequent feeding dropped to an unacceptable level. The difference found among pregnancies and implantations when treated groups are compared to controls indicate the estrogen is capable of significant antifertility effects.

TABLE 4. The effect of DRC-4801 on the fecundity of small colonies of Polynesian rats.

					Avg. amt. DRC-4801 received				
					Feeding periods mg/kg b. wt				
Treatment	No. orig. females	No. females removed	No. preg.	No. implants	1	2	3	4	5
Control	20	30	13	37	0.0	0.0	0.0	0.0	0.0
Treated	20	17	4[a]	10[a]	0.80	0.58	0.18	1.02	0.20

[a] Significant at the 5% level.

Although there presently exists a problem of continuous acceptance on repeated baiting of DRC-4801, the above results clearly indicate the chemosterilant shows promise as an agent in rodent control and underscores the need once again to develop more acceptable baits.

C. Evaluation of a Taste Enhancer, DRC-1813

Introduction

A taste enhancer, DRC-1813, was obtained from a collaborator to evaluate its potential as a masking agent for DRC-4801 treated baits.

Results and Discussion

The effectiveness of DRC-1813 to increase the consumption of DRC-4801 treated bait is shown in Table 5.

The results show the enhancer, DRC-1813, had a nonsignificant effect on the consumption of DRC-4801 treated bait. Although no significant differences were found, the data, as shown in Table 5, indicate the consumption of bait with no enhancer (0.0%) was greater than any other bait with enhancer.

II. AVIAN REPRODUCTIVE STUDIES

A. Sterilization of Male Red-winged Blackbirds

Introduction

It is questionable whether sterilization of male red-winged blackbirds would effectively reduce the number of young born to females on the territory of the sterilized males. If true, it suggests that development of a chemical sterilization technique would be a worthwhile undertaking; if not, it would be pointless to divert time and funds into a research and development program with that as an objective. One way to resolve the question without first spending undo time on the search for and development of an effective inhibitor of reproduction for male redwings is to surgically sterilize some breeding males. Such a study was undertaken as a cooperative effort with the Section of Birds. The primary objective was, as stated above, to determine if sterilization of territorial males would significantly reduce the number of young produced by the associated females. The results of the study are presented in the 1971 Annual Progress Report for the Section of Birds.

TABLE 5. Consumption of DRC-4801 treated bait containing DRC-1813, a bait enhancer (four rats per treatment).

	Percent DRC-1813 in bait[a]											
	0.0		2.5		5.0		7.5		10.0		12.5	
Days	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.
	(Mean grams consumed)											
1	3.6	0.9	1.9	0.9	1.7	1.0	2.5	1.1	4.0	0.6	2.8	0.4
2	1.7	0.2	2.9	0.1	0.5	0.0	1.4	0.2	1.2	0.0	2.1	0.1
3	0.3	0.7	2.1	0.0	0.5	0.0	0.6	0.2	1.4	0.0	1.0	0.1
4	1.0	0.6	4.3	0.0	1.4	0.0	1.9	0.0	2.2	0.2	1.9	0.0
Grand mean	1.6	0.6	2.8	0.2	1.0	0.2	1.6	0.4	2.2	0.2	2.0	0.2

[a] Cont. = lab chow only; treat. = lab chow + 0.005% DRC-4801.

This Unit's involvement was primarily oriented towards (1) the development of the surgical technique and (2) initiating studies on some of the pertinent basic reproductive mechanisms of red-winged blackbirds. The latter consisted of (1) estimating how long after a female redwing is capable of producing fertile eggs and (2) estimating the duration of spermatogenesis in the testes of adult males. The results of these studies are presented below:

Results

The seminal sacs, the sperm storage organ for most avian species located on each side of the cloaca, becomes very prominent in red-winged males during the breeding season. Each is quite convoluted and, although but a single continuous duct, it gives the appearance of being made up of many tubules. Sterilization is accomplished by both ligating and cutting this duct at its most distal point, the point where it enters the cloaca. The procedure that proved most successful follows:

Each live-trapped male is first anesthetized with metaphane, an inhalation type of anesthetic administered much like ether. One person should monitor the anesthesia to avoid overdosing the bird. The bird is secured to a board (ventral side up) by taping the legs and the feathers around the cloaca are plucked until the seminal sacs are clearly visible underneath the skin. The area then is thoroughly disinfected with Zephrin. An incision approximately 5 mm long is made directly over the seminal sacs; the direction of the incision is entirely at the discretion of the operator. It may be helpful at this point to bathe the incision with a few drops of epinephrine (1:1,000 dilution) to retard bleeding in the area. Using blunt dissection the incision is expanded and the seminal sacs teased out until the point where the seminal duct enters the cloaca is located. Two ligatures (5-0 chromic cat gut) are placed around the duct after which the duct is cut between the two ligatures. The seminal sac is replaced and the skin is sutured (three sutures are usually adequate). The procedure is repeated for the opposite side. After the males were fully recovered (about 20 minutes after surgery is completed) the birds were released.

To obtain some information on the duration of the postcopulatory fertile period in redwings, females were obtained at various times during nesting and were either killed immediately (Group I) or isolated from males for 10 days and then killed (Group II). At necropsy, each tract was checked for the presence of sperm by flushing it with saline; a second check was made by a histological examination of the uterovaginal sperm host glands.

Sperm were recovered in the saline flushings from one Group I bird shot during nest building. The flushings from the other birds were negative, but histological checks showed that most of the birds in both groups had sperm stored in the uterovaginal glands. The number of sperm varied according to when the bird was captured, with the highest numbers found in birds caught during nest building or egg laying. At the start of incubation, sperm numbers appeared to decrease, until at hatching or shortly thereafter there were vey few. It seems unlikely that the fertility persists for the same period, although the mere presence of sperm indicates it is a possibility. Quite likely, fertility follows a pattern similar to sperm numbers and begins to decrease as the bird starts to incubate the clutch.

One other study concerned with the basic reproductive mechanisms of red-winged males was undertaken. The objective was to estimate the approximate number of days required for the development of mature spermatozoa from spermatogonia to assist in designing a study that would provide a more precise estimate of the total time required as well as enable one to illucidate the more pertinent events occurring during spermatogenesis. Tritiated thymidine was injected (i.p.) into 10 adult male birds and testicular samples removed at 3-day intervals up to 21 days. Labeled germ cells were observed at 12 days but few to none were observed at 15 days or more. If spermatogenesis in redwing males is at all similar to the process as it is known to exist in most mammals, the development of spermatozoa from spermatogonia requires 16-20 days.

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

I. ACTIVITY-MORTALITY SWITCH FOR BEACON TRANSMITTERS

A. Introduction

A questionnaire was distributed to wildlife research personnel in 1970 to ascertain those problem areas where biological information is most needed. The objective of this inquiry was to establish a priority index which could be used as a guide in setting developmental work for the Electronics Unit. The analysis of this questionnaire indicated that a method for indicating mortality in an animal population was the major concern of those scientists responding to the inquiry. With this basic need established for a mortality indicator, the Electronics Unit initiated a study plan to develop electronic circuitry capable of indicating activity patterns and mortality.

B. Results and Discussion

The selection of a proper sensing technique to determine mortality becomes very important if numbers of animals are to be instrumented. Ideally, one would prefer to sense a major physiological function which would clearly announce the moment of death. Unfortunately, no reliable technique could be conceived which did not require the implant of a sensor. Implanted electronic packages in wild animals have been ruled out as too expensive, time consuming, and of questionable reliability.

The electronic circuitry developed for the activity-mortality indicator has been tested on a short-term basis in the laboratory. It still remains to test the circuit on a live animal over an extended period.

The operation of the circuit is based upon an electronic timing mechanism. Very crudely, the circuit can be considered an unbalanced wheatstone bridge with a quiescent current designed to flow through the common bridge component. The motion-activated switch reverses the bridge balance and causes the opposite polarity of current flow through the common bridge component. The voltage across the common element is sensed with a switching transistor which can provide a "turn on" signal to a radio-frequency (rf) beacon.

A normal animal beacon transmits a sequence of radio frequency pulses. The addition of the activity-mortality indicator causes the transmitter to emit extra pulses whenever there is a closure of the motion-activated switch. Should the mortality indicator not receive a motion switch closure in a prescribed time, the mortality indicator overrides the pulsing circuit in the radio frequency beacon and causes the transmission of a continuous rf signal. The activity-mortality circuit thereby provides to a research biologist a relative index of activity as well as an indication of death.

II. ANIMAL RADIO-FREQUENCY BEACON TRANSMITTERS

A. Introduction

Biological data obtained through animal location and movement studies utilizing miniature rf transmitters have greatly enhanced man's understanding of animal and bird populations. The continued demand for transmitters of proven designs plus the development of improved circuitry annually require much time. Developmental work was initiated early in 1971 on a new bird and bat transmitter operating at 164 MHz. These transmitters were used successfully in the field in November with excellent results.

B. Results and Discussion

The Electronics Unit supplied miniature animal transmitters of both collar and linear antenna designs to its field biologists. At 30 MHz, the uniformity of the constructed transmitters is very encouraging, indicating a proper selection of critical components. Each transmitter is sent to the field with data listing the duty cycle, average current, pulse current, and relative radiated output power. These parameters enable the biologist to properly select and fit the transmitter to the animal.

The recently designed bird and bat transmitter at 164 MHz is the most sophisticated circuitry which the Electronics Unit has supplied to the field. It is a two-stage transmitter (i.e., two transistors) which utilizes a miniature frequency crystal. The miniature quartz crystal is a major breakthrough for transmitter operating at this frequency. This new component allows the transmitter to be constructed with a total weight of 1.4 g exclusive of the battery.

This latest transmitter design also incorporates a different type of transistor than previously used and specially wound, high Q, coils which are not commercially available. Although we have delivered these transmitters to field biologists, studies relating to component parameter selection must be conducted to aid in the uniformity of transmitter construction.

The uniformity of the animal transmitters produced by the electronics laboratory has been a major concern. The laboratory has now established means for measuring the relative radiated output power of these transmitters at both 30 and 164 MHz. Electrical current measurement techniques have been standardized which provide an absolute measurement of the average current drain for any particular transmitter. This current measurement allows the biologist to predict the life of a transmitter with an accuracy dependent upon the battery specifications.

An effort was extended this year to develop an integrated circuit for use as an animal transmitter. Design specifications were sent to six semiconductor facilities in an attempt to solicit their interest in the possibility of supplying animal transmitters. Only one of these firms submitted a bid to develop the technology for transmitter construction. To date, no conclusive decisions have been made concerning the allocation of funds to pursue this production technique.

III. RECEIVING EQUIPMENT DEVELOPMENT

A. Introduction

The height of a receiving antenna is a significant factor in the reception of rf signals having amplitudes of the same order of magnitude as the thermal noise background. Quite often, only a slight elevation in the receiving antenna means the difference in the acquisition of a weak signal. The collapsible mast developed for mobile use provides animal tracking capabilities which would otherwise be very difficult.

B. Results and Discussion

The collapsible antenna mast was developed about the scissor jack principle. The drive mechanism is a 12-v starter motor with reversing capabilities. The collapsed mast is about 4 ft high and extended it is 18 ft. The total height that an antenna system can be elevated above the ground is about 25 ft with the added height of a vehicle considered. The time required to raise the mast to its full height is approximately 20 sec.

The major problem encountered with this mast has been the collection of dirt on the drive mechanism. Modifications are being considered to cover part of the mechanism with bellows for protection from road film. The required starting torque for the drive motor has been greatly reduced by the addition of counter-balancing springs attached at the pin joints of the scissor arrangement.

Some antenna development work was conducted this year to provide a highly directional receiving antenna at 164 MHz. The adcock antenna was found to possess many of the desirable characteristics. This antenna is basically two half wavelength dipoles cross-wired and spaced at one-quarter wavelength. Through t
an impedance matching network was developed
a receiver. Biologists shall be asked to e
antennas in the near future.

IV. AUTOMATIC TRACKING STATION

A. Introduction

The development of a system capable of automatically recording an animal's position as a function of time and simultaneously formating the data for a direct input into a digital computer would be a tremendous asset for wildlife research. Such is the goal for the

equipment ordered by the DWRC from Southwest Research Institute. The overall objectives for this equipment are to be able to sequentially collect location information from 12 instrumented animals and record this data on a paper punched tape as well as a teletypewriter printout.

B. Results and Discussion

The automatic tracking station operates in the following manner: Three antenna sites are located in the field; each site consists of two ground plane antennas separated by less than one wavelength. These pairs of antennas utilize the interferometer principle and resolve a received electromagnetic wave into two phase related electrical signals which can be correlated directly with the azimuthal bearing of the received signal. The phase locked electrical signals are utilized as gating controls for a digital phase detector which converts the analog information into a digital format. This digital information is printed by the teletypewriter and also punched on a paper tape. The teletypewriter sequentially prints the azimuth bearing information from each of the three antenna sites, at which time the receiver is switched to another channel and the process repeated. A total of 12 frequencies, and therefore 12 animals, can be monitored by the automatic tracking system.

Several modifications to the prototype equipment delivered by Southwest Research Institute would be desirable for ease of field maintenance. All 24-v power supply requirements shall be eliminated through a change in the system's component selection. Tests will be conducted in the rearrangement of the system's components which could possibly eliminate much of the interconnecting cabling and some component parts. Circuit design changes shall be incorporated in an effort to improve the overall sensitivity of the receiver package. Finally, modifications of the analog-to-digital computer shall be utilized to shorten the checkout procedures when trouble shooting the digital logic circuits.

V. 164 MHz CONVERTER FOR THE JOHNSON 350 DF RECEIVER

A. Introduction

The DWRC and its associated field stations have purchased about 20 Johnson 350 DF, 30 MHz, crystal-controlled receivers. This represents a substantial capital investment. Since the DWRC also has a frequency assignment at 164 MHz, it is desirable to have the potential of frequency converting these 30-MHz receivers to the higher frequency assignment. Thus, a possible frequency conversion technique has been developed which would cost about $100 per receiver and result in a loss of sensitivity of 2 to 3 dbm (i.e., decibels referenced to one milliwatt).

B. Results and Discussion

The frequency conversion of the Johnson 350 DF receivers was initiated through the selection of a commercially available broadband 164 MHz to 30 MHz frequency converter. This converter was selected on the basis of sensitivity, operating voltage, and physical size. Modifications were then made to the Johnson 350 DF to change its channel separation to 25 kilohertz (KHz) from its design 10 KHz. Finally, changes had to be made in the fine tuning adjustment of the Johnson receiver to account for the broader channel separation.

One Johnson 350 DF receiver has been frequency-converted to operate at 164 MHz. There has been a loss of receiver sensitivity of about 2 to 3 dbm. Further work is projected for this receiver conversion in an attempt to increase the sensitivity and improve the fine tuning adjustment.

BIRD DAMAGE CONTROL RESEARCH

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

INTRODUCTION

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

Although there has been quite a bit of information published on bird repellents, few repellents, if any, have proven to be effective when used in damage situations covering large areas. However, recent breakthroughs in field test design and the continued success with DRC-736 has allowed an expanded program in this area.

The use of reproductive suppressants is becoming widely accepted as a potential technique for managing mammalian or avian populations. Reproductive inhibitors have been extensively evaluated for insects and mammals and a number of compounds have also been examined for domestic

fowl. However, very little work has been done on wild birds. In past programs conducted by other researchers, major emphasis has been put in finding compounds that would inhibit some phase of the reproductive cycle in females. It seems that a more logical approach in migratory species is to direct efforts at discovering a compound that would be effective on males.

The use of stupefacients is a recent approach to controlling damage by birds. These compounds either produce unique behavioral actions in birds, which elicit a fright response from other members of their flock and, as a result, treated areas are evacuated; or immobilize birds, allowing their removal from damage or nuisance situations. Our most promising compound (DRC-1327) is now in the final stages of registration for use on ripening corn.

The toxicant research has been oriented towards finding suitable contact toxicants for use in urban and rural starling and blackbird roosts. This research has been de-emphasized.

A four-step development program has been initiated to discover and register compounds for reducing damage by birds: (1) initial testing on red-winged blackbirds (reproductive suppressant studies will use Coturnix quail initially); (2) advanced testing on target and related species; (3) preliminary field studies with candidate materials; and (4) plant translocation and degradation, and leaching-degradation studies in soils of compounds of proven utility that are pending registration. Due to the increasing concern of the Bureau and the new public awareness regarding chemical contamination of the environment, the fourth step in our development program is expanding to provide answers on the safe use of potential bird damage control compounds on our lands and waters. Procedures used in conducting these four steps are reported in Annual Progress Reports F-9.1, F-9.2, F-9.3 from 1961 through 1967, and in DF-102.2 from 1968 to present.

I. INITIAL TESTING

A. Repellents

Of 21 compounds evaluated as red-winged blackbird repellents, none were repellent below 0.100%.

B. Reproductive Suppressants

Three of the four phases of this study which are detailed in Study Plan Outline DF-102.2-27 (Evaluation of the male Coturnix quail as a test species for use in a chemosterilant development program) have been completed. A brief summary of the results follows:

Six chemosterilants (azacosterol, busulfan, α-chlorhydrin, isopropyl methane sulfonate, mestranol, and triethylene melamine) were tested on: (1) adult breeding male Coturnix quail, (2) adult male Coturnix with undeveloped testes since hatching, and (3) 12-day-old male Coturnix chicks. Of the six compounds, azacosterol (100 mg/kg po), and α-chlorhydrin (100-316 mg/kg po) had no effect on any of the three groups tested. Mestranol (100-316 mg/kg po) caused delayed sexual development in 12-day-old chicks but did not affect the other two groups. Triethylene melamine (10-31.6 mg/kg po) was ineffective when given to 12-day-old chicks, but in adult males with undeveloped testes resulted in infertility of 85% of the test birds. All adult male Coturnix in breeding condition were permanently sterilized by 31.6 mg/kg po doses, although libido was unaffected. Busulfan (10-100 mg/kg po) had no effect on 12-day-old quail. When given to adult male birds in breeding condition, 100 mg/kg resulted in permanent sterility but mortality was excessively high (85%), while 31.6 mg/kg po doses resulted in transient infertility for a period of 10 days. The latter dosage, given to adult males with undeveloped testes, delayed sexual maturity slightly, but had no other effects.

Isopropyl methane sulfonate (100-316 mg/kg po) was perhaps the most effective chemical. Treatment of 12-day-old chicks with 316 mg/kg po resulted in delayed sexual maturity in all birds and complete infertility in 40% of the males. The same dosage when given to adult males with undeveloped testes again resulted in delayed sexual maturity with 60% of the treated birds being infertile. Treatment of adult males in breeding condition resulted in 100% permanent sterility beginning 16 days after treatment; libido was not affected.

C. Stupefacients and Toxicants

Nine of 17 compounds examined for toxic activity in red-winged blackbirds indicated activity (Table 1). No compounds were specifically examined as stupefacients during the reporting period.

TABLE 1. Compounds toxic to red-winged blackbirds at 100 mg/kg or less.

DRC No.	LD_{50} mg/kg	95% CL
1806	5.62	3.16-10.0
3324	75.0	42.1-178
3970	1.00	0.56-1.78
4800	24.1	NC
5533	56.2	31.6-100
5534	100	56.2-178
5539	31.6	17.8-56.2
5540	75.0	NC
5977	75.0	NC

II. ADVANCED LABORATORY TESTING

A. Repellents

1. DRC-736

Laboratory studies continue to indicate that DRC-736 is highly repellent to most avian species tested. Of seven species tested, ring-necked pheasants, brown-headed cowbirds, and bobwhite quail are the least sensitive, while tricolored and red-winged blackbirds, house sparrows, and common grackles are the most.

(a) Acute oral toxicity of DRC-736 to robins was assessed and an LD_{50} of 7.50 mg/kg resulted, which is similar to the values obtained on most avian species. The repellency (R_{50}) to bobwhite quail was determined to be >0.100%. R_{50} values for the brown-headed cowbird, ring-necked pheasant, and California quail are all <0.562% but >0.100%.

(b) The repellency of DRC-736 to redwings and common grackles was determined in a series of cage tests. Sorghum plots, 6-ft square, were treated at 1, 3.2, and 10 lb/acre and damage caused by redwings was reduced from 53% to >85% depending upon the treatment level and the evaluation technique used. Statistical evaluation of yield data did not give significant differences in treatments because of wide variations in predicted yields; however, all three treatments had significantly ($P<0.05$) less damage than the control using visual estimates of damage. No significant difference could be determined in damage reduction between the three treatments although damage increased as the treatment level decreased.

In a large cage containing three replications of four treatments (0.32, 1.0, and 3.2 and 10 lb/acre) plus controls, the three highest levels had less damage than controls and the 0.32 lb/acre showed no difference. Differences in damage, however, due to an uneven yield in the plots, could not be detected statistically. When individual 6-ft square plots of sorghum treated with 1 lb/acre DRC-736 and controls were offered to common grackles, visual estimates indicated significantly less ($P<0.01$) damage in the treatment.

Results of this test are contained in more detail in "Determining concentration effect levels of two repellents on mature grain sorghum"--Report of Study No. 24, Work Unit DF-102.2, by Schafer et al., Dec. 16, 1971.

(c) The repellency of DRC-736 as a foliar spray on lettuce and sugar beet seedlings was investigated using three species; red-winged blackbirds, house sparrows, and white-crowned sparrows. Treatments were at effective rates of 3-10 lb/acre on 9-inch square flats planted to contain 25-100 seedlings.

Redwings showed their distaste for sugar beet seedlings by clipping the stem off just below the leaves. No treatment effect could be determined since none of the seedlings were consumed. Many treated seedlings were thrown out of the flats whereas untreated remained where they fell in the flat. A test with lettuce indicated that seedlings sprayed with 3 lb/acre DRC-736 sustained only 1/3 as much damage as the controls.

White-crowned and house sparrows were repelled from lettuce seedlings treated at 3 to 10 lb/acre with the ratio of damage on control flats to treated flats ranging from 2.8:1 to 31.8:1 in number of seedlings destroyed. Sparrows learned to avoid the treatment within a short time and once they were exposed to DRC-736, repeated exposure resulted in quicker avoidance.

More detailed data on this study is available in "The Bird Repellency of DRC-736 Foliar Sprays on Lettuce Seedlings"--Technical Report No. 32, Work Unit DF-102.2, by Schafer et al., October 1971.

(d) DRC-736 was determined to have no phytotoxic effects on lettuce or sugar beet seedlings when applied at 3 lb/acre.

More detail on this study can be found in "The Phytotoxicity of DRC-736 on Lettuce and Sugar Beet Seedlings"--Technical Report No. 23, Work Unit DF-102.2, by Schafer and Cunningham, September 1971.

2. DRC-3512

This compound has shown what was considered to be good repellency in tests conducted by the manufacturer and is less toxic than DRC-736. To verify those results, a number of comparative tests were conducted and the results are given in Table 2. It was decided to continue studies with DRC-736 and curtail studies with DRC-3512.

TABLE 2. Comparative repellency and toxicity of DRC-736 and DRC-3512 to several avian species.

	DRC-3512		DRC-736	
	R_{50} (%)	LD_{50} (mg/kg)	R_{50} (%)	LD_{50} (mg/kg)
Red-winged blackbird	>1.000	>100	0.089	4.7
Common grackle	>1.000	>100	0.056	10
Brown-headed cowbird	0.178	>100	0.318	7.5
Bobwhite quail	>1.000	100	>1.000	25
Ring-necked pheasant	0.316	>100	0.421	56-1000
House sparrow	0.821	>100	0.042	18
Robin	-	100	-	7.5
Common pigeon	-	>100	-	13

B. Reproductive Suppressants

1. Selection of Male Chemosterilants

A considerable effort has been expended in reviewing the literature on chemosterilants in avian, mammalian, and insect species. Results of this review are contained in "A Summary of the Effects of Chemical Agents on Reproductive Potential in the Mammalian and Avian Male, with Comments on the Feasibility of Developing an Avian Male Chemosterilant Program"--Technical Report No. 27, Work Unit DF-102.2, by Schafer, May 1971; and in "An Evaluation of the Male Coturnix Quail as a Test Species for Use in an Avian Chemosterilant Program"--Study Plan Outline DF-102.2-27, by Schafer *et al.*, March 1971.

2. Acute Toxicity and Acceptance

Acute toxicity determinations and initial acceptance studies of six potential chemosterilants; busulfan, α-chlorhydrin, mestranol, azacosterol, Isopropyl methane sulfonate, and triethylene melamine (TEM) were conducted on red-winged blackbirds and Coturnix quail. Results indicated that there is a considerable difference in the toxicity of the chemicals between these species. The first three compounds were more than 10 times as toxic to male redwings than male quail, while the remaining three showed little variation. Triethylene melamine and azacosterol were also shown to cause some aversion in acceptance studies. Results are shown in Table 3 and are reported in more detail in "Acute Oral Toxicity and Acceptance of Six Selected Chemosterilants to Coturnix Quail and Red-Winged Blackbirds"--Technical Report No. 30, Work Unit DF-102.2, by Schafer *et al.*, June 1971.

TABLE 3. The acute oral toxicity and acceptance of six chemosterilants to Coturnix quail and red-winged blackbirds.

	Acute LD_{50} Coturnix (mg/kg)			Acute LD_{50} redwings (mg/kg)	Acceptance 1% treated food-redwings (%)
Compound	♂	♀	Combined	♂	♂
Azacosterol	421	562	487	421	89.6
Busulfan	750	316	421	56.2	99.2
α-Chlorhydrin	316	421	365	23.7	100
IPMS	>1000	562	1000	562	100
Mestranol	>1000	>1000	>1000	>1000	100
TEM	100	133	116	5.62	82.4

C. Stupefacients and Toxicants

1. DRC-735

Dermal application on house sparrows resulted in no activity up to 100 mg/kg.

2. DRC-736

The immobilization and lethal levels of this compound were determined for robins by oral intubation. The margin between immobilization and death is 2.4. Dermal application on house sparrows resulted in no activity at 100 mg/kg.

3. DRC-632

The acute dermal toxicity of this organophosphate was determined to be 2.42 mg/kg for house sparrows. This is comparable to data gathered on other species.

4. DRC-5526, 5527, 5528, and 5529

These four closely related organophosphates have been extensively investigated for acute oral toxicity to a variety of species (Table 4). Of the four compounds, DRC-5528 has the most potential for sparrow and pigeon use due to its low toxicity to quail and pheasants.

5. DRC-5534 and 3776

These two anticoagulants were evaluated as potential avian toxicants for use in situations where long-term, repeated feeding would be possible. Of the two compounds, DRC-5534 at 0.05% was superior, producing 60% mortality in redwings over a 2-week period. Neither compound was extremely aversive, but DRC-5534 was consumed at a lower rate than DRC-3776. Details of these tests can be found in "The Acute Oral Toxicity, Repellency, and Chronic Toxicity of Two Anti-Coagulants to Red-winged Blackbirds"--Technical Report No. 31, Work Unit DF-102.2, by Schafer, August 1971.

TABLE 4. Acute oral toxicity of four organophosphates to a variety of avian species.

Species	Acute oral LD_{50} (mg/kg)			
	5526	5527	5528	5529
Red-winged blackbird	18	2.4	1.3	2.4
Starling	13	13	2.4	13
Brown-headed cowbird	--	2.4	2.4	10
Common grackle	--	5.6	2.4	7.5
Common pigeon	--	10	7.5	7.5
Bobwhite quail	--	5.6	13	1.3
Ring-necked pheasant	--	10	13	1.0
House sparrow*	--	5.6	1.8	3.2
Rat	750	375	175	>1000

*Acute dermal LD_{50}'s are: 5527 - 7.5 mg/kg; 5528 - 7.5 mg/kg.

III. FIELD STUDIES

A. Repellents

1. DRC-736

(a) Ripening grain treatments

Introduction

Blackbirds cause economic losses throughout the United States by damaging ripening grains. Rice and sorghum are the two crops that bear the brunt of this damage. Of major importance is the damage caused to rice because it is a high-yielding, high-priced crop. One promising method for reducing damage appears to be chemical repellents applied to the ripening grain heads prior to expected damage. Tests conducted in California and Oklahoma in 1970 with DRC-736 showed the potential of this compound for reducing damage to maturing rice and sorghum, respectively, when used as a head spray. More tests were needed in 1971 in these same areas to gather additional information on both DRC-736 and on application techniques.

Results, Discussion, and Conclusions

Rice

In California, field tests were conducted at two sites in the Sacramento Valley. At the first test site, 2.0 lb/acre methiocarb, when applied with a boom sprayer, significantly ($P<0.05$) reduced the amount of blackbird damage. At the second test site, when a 3.0 lb/acre application of methiocarb was applied by aircraft, no conclusions could be reached because of differential as well as insufficient bird pressure on the test plots. Some slight repellency was also suggested here, however.

Results of this test are contained in more detail in Report of Study No. 32, Work Unit DF-102.2, "Evaluation of Low-Level Applications of Methiocarb for Preventing Birds from Damaging Ripening Rice" by DeHaven *et al.*, November 1971.

Grain Sorghum

Results from spraying 3.0 lb/acre DRC-736 on three 1-acre plots at Tishomingo National Wildlife Refuge, Oklahoma, are incomplete and difficult to evaluate because a reliable damage appraisal technique for detecting differences in damage among treatments is lacking.

However, visual estimates of damage in the test plots indicated that DRC-736 was effective for reducing damage caused by red-winged blackbirds.

About 1,500 sorghum heads were collected from the test plots and are being stored pending the development of a damage appraisal technique (see Work Unit DF-102.3).

Safflower and Sunflower

In California, tests were also conducted in ripening safflower and sunflower. In three different tests, DRC-736, applied at a rate of 2.0 to 3.0 lb/acre, apparently provided little, if any, protection from bird damage. It is believed that the poor results may have been due to problems in application of methiocarb to these crops rather than lack of repellency. Laboratory studies are needed to devise effective formulations and application techniques before further tests are conducted.

(b) Ripening fruit treatments

Introduction

Many species of birds create serious economic losses throughout the United States by damaging ripening fruits. Cherries and grapes are two of the major orchard crops that are damaged. The species involved are often most desirable, such as robins and orioles, so it is essential to find nonlethal methods for reducing the damage. One promising technique is to treat the ripening fruit with chemical repellents.

Both laboratory tests and field studies have shown that DRC-736 is an effective repellent for preventing bird damage to sprouting corn and ripening rice and sorghum. Therefore, initial tests were undertaken in cooperation with the Division of Wildlife Services in Michigan and New Hampshire and other tests were conducted in Washington to determine its effectiveness for preventing bird damage to ripening fruits.

Results, Discussion, and Conclusions

In Michigan, several trees of two varieties of cherries, *Prunus mahalob* (sour) and *P. aviurm* (sweet), were sprayed until dripping with a 1 lb/100 gal water formulation of DRC-736. Damage was caused primarily by robins and common grackles in the sweet cherry orchard and by starlings in the sour cherry orchard.

Preliminary results from both orchards showed highly significant ($P<0.001$) differences in damage between treatments and controls. Random samples in the sweet cherry orchard showed that the controls received about five times as much damage as the treated (36% vs 7%). In the sour cherry orchard, over 50% damage occurred in the controls and under 20% in the treated.

In Washington, tests were established using two treatment levels on cherry trees (1/3 and 1 lb DRC-736/100 gal water). However, no usable information was obtained due to light and uneven bird pressure.

In New Hampshire, grapes were sprayed until dripping wet with the same formulation of DRC-736 (1 lb DRC-736/100 gal water). Damage in this vineyard was caused mostly by robins, but starlings, catbirds, and scarlet tanagers also fed heavily. Protection was not pronounced during the first week of the test because of light bird pressure, but was dramatic when bird pressure increased. Random samples (clusters) of the two most vulnerable varieties weighed six times more from treated than from untreated vines.

Results from the grape tests are contained in more detail in Report of Study No. 33, Work Unit DF-102.2, "The Effectiveness of Methiocarb for Protecting Ripening Grapes from Bird Damage in New Hampshire" by Bollengier et al.

B. Reproductive Suppressants

Introduction

One approach to managing depredating populations of birds is controlling their birth rate. Therefore, studies were undertaken in 1971 in cooperation with the Ecology Unit in the Section of Birds and the Section of Basic Studies to determine whether sterilization of wild adult territorial male red-winged blackbirds will significantly reduce the number of young produced by their associated females. This was accomplished by mechanically sterilizing (via vasectomy) a number of redwing males in cattail marshes in the Denver area.

Results, Discussion, and Conclusions

Nesting information was obtained from territories of seven male red-winged blackbirds that were successfully sterilized via bilateral vasectomy. During the period when infertile clutches could have appeared, 40 clutches were laid on their territories. Twenty-seven (68%) of the clutches were fertile, as compared to 96% of 45 control clutches. The percent of fertile clutches on each territory ranged

from 0 to 86. Comparisons of distance and fertility indicated that the more isolated males tended to have a lower percentage of fertile clutches. The percentage of infertile clutches in the 30-39 day period after sterilization, as compared to the 10-19 and 20-29 day periods, indicates that length of time after sterilization did not affect the number of infertile clutches during these time periods.

More detailed information on this study is contained in Report of Study No. 30, Work Unit DF-102.2, "Effecting Male Sterility on a Breeding Population of Red-winged Blackbirds" by Bray, Kennelly, and Guarino.

C. Stupefacients

1. DRC-1327

Broadcast-baiting studies with DRC-1327-treated cracked corn baits in South Dakota have shown continued success in protecting ripening corn from red-winged blackbirds. Studies in Oklahoma conducted on windrowed peanuts and standing sorghum yielded no information because of a lack of bird pressure in treated fields. In Africa, red-billed queleas responded well to DRC-1327 dosing but results from field exposure were inconclusive due to lack of bait acceptance.

Results from these DRC-1327 field studies are more fully reported in Work Unit DF-102.3.

D. Toxicants

1. DRC-1347

Introduction

Starlings and blackbirds create problems in many areas of the United States and methods for reducing losses caused by these species are needed. One approach to solving the problem is direct population reduction of these species by spraying their winter roosts with contact toxicants. Presently, there are no safe, selective, effective, and economical toxicants that can be used. However, an experimental toxicant, DRC-1347, is highly toxic to starlings and blackbirds and has a rapid deterioration rate. DRC-1347 has proven to be an effective contact toxicant in reducing starling populations in urban structures by treating roosting ledges, but inconclusive results were obtained in initial vegetative roost spraying trials in earlier years with this compound in Texas and Idaho. More trials are needed to determine whether DRC-1347 has potential in the field as a roost spray.

In eastern Texas, near Hillsboro, starlings and blackbirds roost in small patches of "Georgia" cane (Arundinaria gigantea) during winter. These roosts are ideal test sites because of their small size and the large number of birds that use them; they are isolated, and few are on impounded watersheds.

Results, Discussion, and Conclusions

Aerial application of 7 lb of DRC-1347/acre resulted in a negligible blackbird and starling kill. Only four birds were found dead in three cane roosts (each <0.2 acres) each of which contained about 3,500 redwings, starlings, and cowbirds. Studies with DRC-1347 have since been discontinued.

IV. SOIL AND PLANT STUDIES: DRC-1327 (4-Aminopyridine) REGISTRATION

Introduction

Previous soil work with 4-aminopyridine (1969 Annual Progress Report DF-102.2) has shown the compound to be adsorbed quite strongly to alkaline soils and therefore resistant to leaching; these residues, in instances of repeated soil application or misuse treatment levels, could accumulate in certain soils and become absorbed by future crops planted in the treated areas. Thus, the degradation of 4-aminopyridine in soils is a key factor as to whether the compound and/or metabolites would be present in sufficient quantities to be absorbed and translocated by plants.

Preliminary soil degradation studies (1970 Annual Progress Report DF-102.2) were designed to broadly evaluate the breakdown of the chemical in three alkaline soils (typical of those in the western United States) under both anaerobic and aerobic conditions. Although 42%-60% of the soil-applied ^{14}C-4-aminopyridine was aerobically degraded to $^{14}CO_2$ over a 2-month period, the environmental conditions existing during this experiment were ideal for breakdown. Thus, additional soil leaching-degradation data were obtained during this reporting period to provide a more satisfactory prediction of the persistence of the compound in both alkaline and acidic soils, representative of those found in the western and eastern sections of the United States, respectively.

Anticipating future submission of a registration petition for 4-aminopyridine in sorghum, studies concerning the absorption, translocation, and degradation of the chemical in this crop were initiated during 1971.

A. Soil Studies with ^{14}C-4-Aminopyridine

1. Movement within alkaline and acidic soils

Seven representative soils were treated in triplicate (contained in modified chromatographic columns) with sufficient ^{14}C-4-aminopyridine (specific activity 0.16 mc/mM) to provide a concentration of 10 ppm. The columns were maintained in a growth chamber at constant temperature and humidity during the test. Sufficient water was added to the soils every 2-3 days to produce a runoff of approximately 10 ml. At each sampling period the radioactivity present in the effluents was analyzed by liquid scintillation spectrometry. After 20 days, the soils were removed from the columns, sectioned into about 1-inch segments, extracted with acetone, and the resulting extracts and residual soils retained for analysis.

Results, Discussion, and Conclusions

There was very little movement of the radiolabeled compound through any of the alkaline soils after application of approximately 7 inches of simulated rainfall, the amounts ranging from 0.02% to 0.18% of the initial application; detectable quantities of radioactivity were received in the runoff from only one of the three acidic soils. The quantity of radiolabeled compound present in the top 1-inch sections of the alkaline soils varied from 95.1% to 98.8% for the loamy sand and loam soils, respectively; essentially all the radioactivity recovered from the acidic soil samples were present in the upper 1-inch layer.

2. Degradation in alkaline and acidic soils

Six soils differing in pH, organic matter, and physical composition, were treated with 10 ppm ^{14}C-4-aminopyridine and incubated at two temperatures and two moisture levels for 3 months. The rates of degradation to $^{14}CO_2$ were monitored at 2-week intervals. Upon completion of the study, the soils were extracted with acetone, and the extracts and soil residues retained for metabolite analysis.

Results, Discussion, and Conclusions

The results of this study demonstrated the importance of soil texture, organic matter, pH, temperature, and moisture on the degradation of this compound. The most extensive breakdown (54.2%) of the radiolabeled material occurred in a well-aerated, slightly alkaline soil (pH 7.8). Also, these data suggest that 4-aminopyridine residues are more tightly bound to acidic than alkaline soils, and therefore are less available for microbial and/or chemical degradation.

B. Plant Studies

1. Phytotoxicity of 4-aminopyridine to grain sorghum

One-month sorghum seedlings were transferred to nutrient cultures containing 10 or 100 ppm 4-aminopyridine and maintained in these solutions for 1 week. After this period, one group of plants was harvested and the fresh and air-dried weights of the tissues recorded. In order to evaluate possible latent toxic effects of the chemical, two additional groups of plants were removed from the treated cultures after 1 week and transferred to untreated solutions for additional periods of either 1 or 2 weeks.

Results, Discussion, and Conclusions

There were no visible toxic effects, e.g. chlorosis, noted at either treatment level. The volume of water absorbed (measure of transpiration) was greater for the treated plants than for the controls, resulting in a 9.7% and 6.2% increase for the 10 and 100 ppm concentrations, respectively. Although both concentrations of 4-aminopyridine appeared to stimulate shoot and root growth, the amount of variation between individual plants was too great for these values to be statistically significant.

2. Absorption and translocation of ^{14}C-4-aminopyridine in 1-month sorghum

Sorghum was cultured in nutrient solutions containing 5 ppm ^{14}C-4-aminopyridine (specific activity 0.14 mc/mM) for periods of 1 hour, 1 day, or 7 days. At each sampling period, the plants were divided into three groups and the translocated radiolabeled material analyzed by either Schöniger Combustion - liquid scintillation spectrometry, autoradiography, or TLC-autoradiography.

Two additional groups of plants, which had been growing in the radiolabeled cultures for 1 week, were transferred to untreated cultures for an additional 7 days; these plants were then harvested and treated in similar manner.

Additional plants grown 2 weeks in the labeled ^{14}C-cultures were extracted with methanol and acetone; after concentration under vacuum and nitrogen, followed by pigment removal, aliquots of the concentrates were analyzed by TLC-autoradiography for metabolites; both cellulose and alumina TLC plates were used for this work, with the cellulose plates giving the best resolution of the extract components. The cellulose adsorbent was removed from the plates and the radioactivity contained therein, determined by liquid scintillation spectrometry.

Results, Discussion, and Conclusions

The residual radioactivity contained in the sorghum tissues demonstrates that this species has a very efficient root system, with 1.76% of the initial application being absorbed/adsorbed by the roots within 1 hour; the quantity of radioactivity detected within the shoots during the same period was 0.74%. After placing the roots of treated plants in untreated cultures for 7 days, a decrease in radioactivity occurred, which strongly suggests that a substantial quantity of the root-contained compound is present in a free form and not incorporated into any plant components; during this same period translocation of the labeled material continued from root to shoot areas.

Autoradiograms prepared from freeze-dried sorghum tissues treated 1 hour to 7 days all contained ^{14}C-activity, which further substantiates the combustion-liquid scintillation work.

Results of the TLC metabolite analysis are presented in Table 5. These data show that some degradation of the translocated ^{14}C-4-aminopyridine occurred within 2 weeks in both the shoot and root tissues. Moreover, it appears that the metabolites extracted are similar in both types of tissues.

TABLE 5. Degradation products detected in shoot and root extracts after 2 weeks in ^{14}C-4-aminopyridine.

		% of total radioactivity recovered from cellulose TLC plates	
Spot no.	Rf	Shoots	Roots
1	0.85 (Parent ^{14}C-4-aminopyridine)	93.3	90.0
2	0.68	3.6	4.4
3	0.58	0.7	1.1
4	0.26	0.7	0.5
-	Miscellaneous minor components occurring between spots 1 and 2 and 3 and 4	1.7	4.0

3. Absorption and translocation of ^{14}C-4-aminopyridine in 3-month sorghum

Sorghum was cultured under growth chamber and greenhouse conditions for approximately 3 months, at which time the heads were in various stages of development.

These plants were then transferred to cultures containing 5 ppm of ^{14}C-4-aminopyridine and maintained in these solutions for 1 week. The translocated compound was then analyzed by a combination of Schöniger Combustion - liquid scintillation counting techniques.

Results, Discussion, and Conclusions

Analysis of the intact heads from six plants (Table 6) revealed a wide range in detectable radioactivity, with the more mature heads generally containing a greater concentration of the radiolabeled compound.

TABLE 6. Radioactivity translocated into intact sorghum heads of 3-month plants growing in 5 ppm ^{14}C-4-aminopyridine.

Fresh weight of intact head (g)	Radioactivity recovered	
	dpm/head ($\times 10^3$)	ppm*
7.1	Not detected	-
8.6	2.9	0.09
49.5	7.2	0.04
55.5	13.6	0.06
64.1	1.1	0.01
66.9	18.4	0.08

*Calculation based on fresh weight and assumed all radioactivity present as parent compound.

The vegetative tissues from these plants, together with seed from treated sorghum plants, are presently being analyzed for residual radioactivity.

V. MISCELLANEOUS STUDIES

In addition to the four-step development program outlined in the introduction to this report, miscellaneous studies have been conducted on candidate chemicals and formulations to achieve greater effectiveness and safety; to eliminate erratic results obtained in the field; to learn more about the basic properties of compounds; and to gather additional data needed for registration of candidates.

(a) Germination studies were conducted with DRC-736 on several plant species.

Personnel of the Davis field station submitted two varieties of rice seed (previously treated with 3.2 and 10 lb/acre DRC-736) to determine if application of the compound to the ripening grain would adversely affect the seed viability. Within 7 days, 97% of the controls had germinated, with 96% and 100% germination recorded for the 3.2 and 10 lb/acre-treatments, respectively, indicating no apparent phytotoxic effects for the DRC-736 treatments. More detail on this study is contained in "Germination of Rice Seed Treated at Ripening Stage with DRC-736"--Technical Report No. 28, Work Unit DF-102.2, by Cunningham and Starr.

Loblolly pine seeds were treated with 1% DRC-736 and the germination of these seeds compared with a standard pine seed repellent containing 6% Arasan and 2% endrin. Although germination of the DRC-736-treated seeds was somewhat less than the controls (71% vs 79%), it was greater than that obtained in the standard Arasan-endrin treatment (66%). More detail on this study is contained in "Germination of Loblolly Pine Seed Treated with DRC-736"--Technical Report No. 21, Work Unit DF-102.2, by Cunningham and Starr.

Lettuce seedlings were sprayed with a 3.2% aqueous solution containing 75% WP DRC-736 plus 0.25% latex. There were no phytotoxic effects noted during the 4-day observation period.

(b) The use of Dasanit granules on fairways and greens of golf courses as an insecticide has been reported to result in avian mortality. A test was conducted to determine if Dasanit granules that were dyed with Sudan yellow and Victoria green would be less hazardous than undyed granules when spread on turf surfaces. The combination of these colors simulates the color of turf and it was hoped they would become less hazardous and less attractive to birds.

There were no differences in mortality between groups of redwings offered undyed and dyed Dasanit granules after a 3-day period. More detail on this study is contained in "The Effects of Color on Reducing the Hazards of Dasanit Granules to Red-winged Blackbirds"--Technical Report No. 29, Work Unit DF-102.2, by Guarino, Schafer, Brunton, and Lockyer.

VI. COOPERATIVE STUDIES

The Chemical Development Unit of the Section of Birds has provided numerous services to other units within the Division of Behavioral and Environmental Research during the past year. It has been involved in cooperative research with other Bureau agencies and other Federal and non-Federal agencies including: Patuxent Wildlife Research Center, Northern Prairie Wildlife Research Center, Division of Wildlife Services, Division of Wildlife Refuges, the USDA, county agents in several states, colleges throughout the country, UNDP-FAO, USAID, and private industry.

(a) A cooperative study was initiated with personnel of the North Dakota State University involving DRC-1327 (4-aminopyridine) in sunflowers. These studies, which will involve primarily the translocation and metabolism of DRC-1327 by this crop, were begun in anticipation of future registration of the compound for use as a blackbird damage control agent in sunflowers.

(b) In November 1971, a study was conducted at Ft. Lamy, Chad, under the auspices of USAID to determine the relative acute oral and dermal toxicity of 20 chemicals on the red-billed quelea (*Quelea quelea*). The purpose of this test was to provide information which can be used to compare quelea sensitivity to chemicals with domestic species such as house sparrows or red-winged blackbirds. It was determined that quelea are very sensitive to orally and dermally administered chemicals (Table 7).

Since redwing and house sparrow data are still incomplete, the comparison of these species to queleas will be reported in 1972.

TABLE 7. Results of toxicity tests conducted on *Quelea quelea*, Fort Lamy, Chad.

DRC No.	Acute LD_{50} (mg/kg)				Oral rat LD_{50} mg/kg
	Oral	95% CL	Dermal	95% CL	
142	1.33	NC	1.33	NC	19
632	1.33	NC	1.78	1.00-3.16	230
736	4.21*	NC	100	56.2-178	100
1143	1.33	NC	1.78	1.00-3.16	7
1150	1.78	1.00-3.16	1.78	1.00-3.16	6
1324	3.16	1.78-5.62	7.50	NC	84
1327	5.62	3.16-10.0	>100	-	20
1329	0.24	NC	1.42	NC	6
1339	31.6	17.8-56.2	-	-	1500
1347	-	-	42.1	NC	>1500
2538	1.33	NC	4.21	NC	22
3533	56.2**	NC	-	-	400
4226	2.42	NC	>100	-	1750
4241	0.75	NC	1.33	NC	75
4917	0.42	NC	100	56.2-178	5
5527	2.42	NC	2.42	NC	375
5528	0.75	NC	2.42	NC	175
5529	5.62	1.78-17.8	75.0	NC	1000
5569	1.78	1.00-3.16	10.0	5.62-17.8	30
5781	4.21	NC	5.62	3.16-10.0	4

*ED_{50} - Immobilization with recovery 2.42 mg/kg.
**ED_{50} - Immobilization with recovery 13.3 mg/kg.

FIELD METHODOLOGY - John W. De Grazio

BIRD PROBLEMS IN LATIN AMERICA AND AFRICA

A. Dickcissel Damage to Grain Sorghum in Venezuela

Introduction

In Venezuela, dickcissels congregate in January in wintering flocks, and feed intensively in maturing grain sorghum fields. A field trial in Venezuela in January 1970 indicated that the fright-producing chemical DRC-1327 is effective in protecting grain sorghum fields from dickcissel damage. Further field testing was attempted in January 1971.

Results, Discussion, and Conclusions

Dickcissels did not feed in maturing sorghum in January 1971 because late December rains resulted in lush growths of weed and grass seeds. Although there were probably as many birds as in previous years, flocks fed randomly over a wide area as a result of abundant natural food. A 1-million-bird roost located last year near Hacienda Guache did not form this year, possibly because no sorghum was grown at Guache in 1971. A new roost was found 13 km northeast of Payara in 50 acres of mature sugar cane. Flocks from the 1.5-million-bird roost were noted feeding on natural food sources. Area residents believe that there is a resident population of dickcissels in the Payara area.

About 175 dickcissels were caught in three evenings in mist nets near the sugar cane roost. Of these, 123 males and 50 females were banded and 52 banded birds were color-marked with orange facilon leg streamers.

B. Bird Damage in Colombia

Introduction

Additional information on bird damage problems in Colombia was obtained through discussion with AID field station and ICA personnel, and by traveling to agricultural areas in the Cauca, Sinu, and Magdalena Valleys.

Results, Discussion, and Conclusions

In the Cauca Valley, eared dove damage to emerging soybeans begins in late February-early March and ends in early December, according to Dr. Roy Thompson (Centro Internacional de Agricultura Tropical). Doves nest from December to February in the area, and large roosts form after the nesting season. Work with DRC-736 treatments to emerging soybean plantings will continue.

In the Sinu Valley, about 50 brown-throated parakeets were observed in experimental corn and sorghum plantings. Yellow-hooded blackbirds also feed in corn. Shiny cowbirds, yellow-hooded blackbirds, and seedeaters were implicated in damage to milk and mature rice during August and September. Purple gallinules, which nest in irrigated rice fields, are a problem from May to September.

In the Magdalena Valley, about 100 green-rumped parakeets were observed in a commercial sorghum field, and another small flock was seen in experimental sorghum. Green-rumped parakeets and shiny cowbirds were noted in weedy rice east of Ibaque, and damage was quite heavy adjacent to nearby cover.

Initial phases of study plans were formulated in discussions with AID personnel.

C. Quelea Damage in Africa

Introduction

The red-billed quelea or black-faced diock, a member of the weaver finch family (Ploceidae), has the distinction of being the most numerous, and perhaps the most destructive, avian species in the world. The natural diet of the quelea consists of small grass seeds. When preferred foods become scarce, however, enormous damage to crops can occur. Since 1950, hundreds of millions of queleas have been destroyed by explosives and aerial applications of toxic pesticides such as parathion and fenthion. Neither appreciably reduced populations nor decreased crop damage has resulted from these lethal control methods, except in some local areas. In 1971, under the auspices of USAID and FAO, personnel of the Section of Birds made two visits to Africa to evaluate the potential of damage control techniques developed in the U.S. for quelea control. Evaluations of new chemical control agents are presented in the Section entitled Development of Chemicals.

Results, Discussion, and Conclusions

In a food preference test at Ft. Lamy, Chad, quelea consumed 98% of the millet (Pennisetum) offered, 50% of the Echinochloa, and negligible amounts of red and white milo, durham wheat, and white rice. The average millet consumption was about 4 g per day.

Quelea responded well to the chemical frightening agent, 4-aminopyridine. Vocal distress displays lasted for periods of up to 5 hours. However, preliminary field trials, with sorghum bait in an area in which queleas were feeding largely on Echinochloa, and another trial with Pennisetum bait where queleas were damaging sorghum, were discouraging. Queleas may be difficult to bait by conventional broadcasting, but spraying portions of the crop, particularly sorghum, should be effective.

Encouraging results were obtained in repellent tests with queleas. None of the 20 birds tested took measurable amounts of millet treated with 0.1% methiocarb. Queleas also took nine times more untreated millet than 0.01% methiocarb-treated millet. It appears that queleas will be able to detect levels of methiocarb as low as 30 ppm, only slightly above tolerance levels sought for fruits consumed by humans. At 1000 ppm, methiocarb produced immobilization of queleas for as long as 3 hours.