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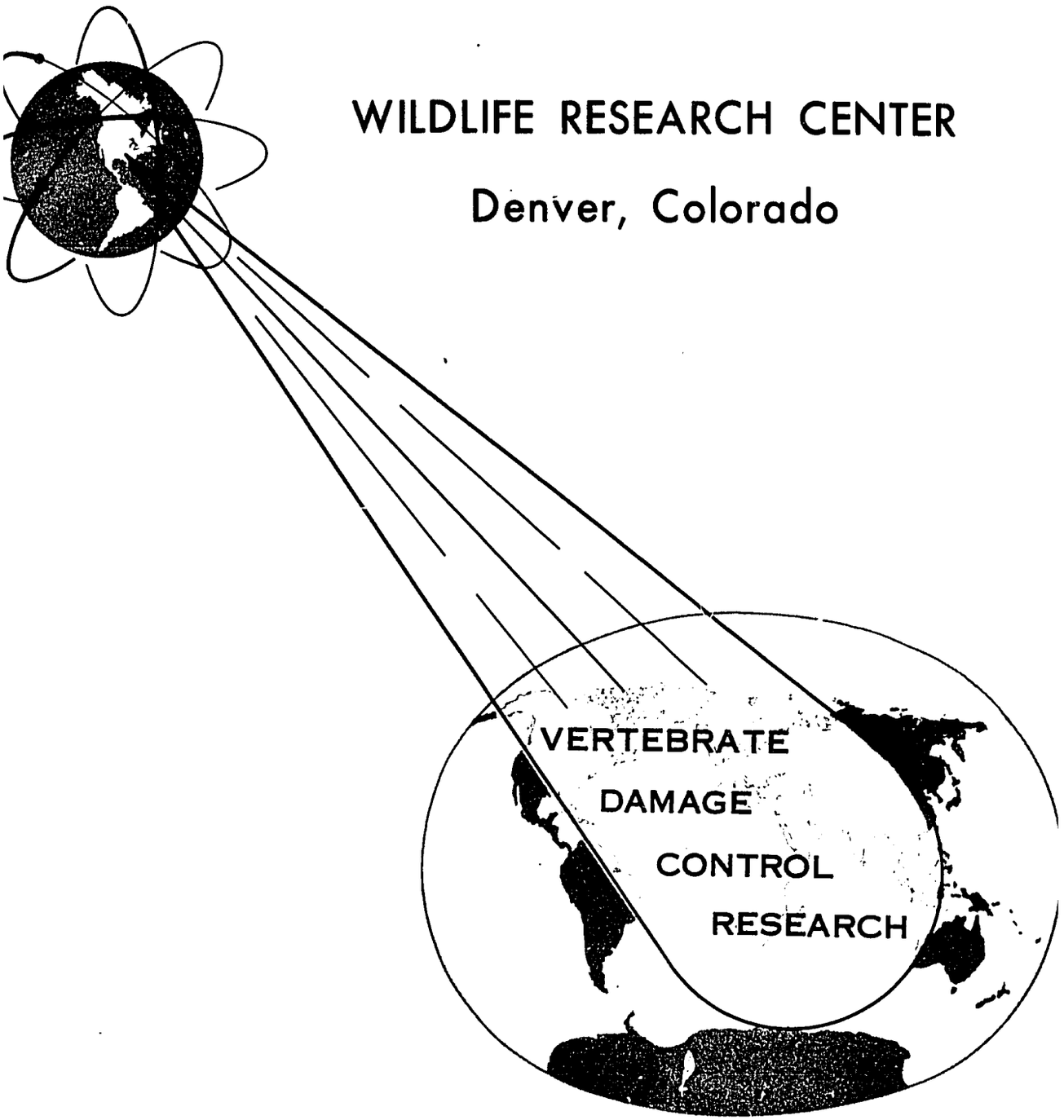

1970 ANNUAL REPORT

A Cooperative Program
of

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

ANNUAL PROGRESS REPORT
Calendar Year 1970

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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

Two hundred eighty-six compounds were evaluated for biological activity as candidate animal damage control agents. Twenty-two candidate toxicants passed the screening program as active compounds; these were referred to the Section of Mammals for their review and selection in the development programs.

A new program is being initiated to evaluate carefully selected candidate control agents based on present-day requirements for registration, structure-activity correlations, and thorough review of existing and new literature. The past empirical screening procedures will be de-emphasized.

Pharmacological studies related to mode of action will be undertaken to develop new leads in more selective means of control.

Additional laboratory equipment, bioassay media, and candidate control compounds were acquired this year through the continued support of the AID program which is concerned with prevention of animal damage, especially that caused by rats and bats in the domestic and foreign problem areas.

A toxicological evaluation of chlorophacinone was conducted with vampire bats and is reported separately by another investigator.

The Repellency Index based on functional groups was completed for publication and will be released in 1971 pending chemical identify release.

The initial studies for converting the data storage and retrieval system to the Wiswesser Chemical Line-Formula Notation are completed.

Four candidate toxicants were tested to determine their efficacy as vampire bat (Desmodus rotundus) control agents. DRC-3776 (chlorophacinone), a slow-acting anticoagulant, was found to have an LD_{50} of 3.75 mg/kg and was selected for further evaluation.

Twenty captive adult vampire bats were placed in an artificial roost measuring 36 x 25 x 26 inches. All bats began utilizing the 13-inch diameter by 5-inch-deep "niche" located in the roof of the roost within 2 days. Earlier studies reporting intense grooming activity by this species

were confirmed by closed-circuit TV observations. Five weeks after the colony was established a single bat was removed and 1.5 cc of Vaseline containing 50 mg of DRC-3776 was smeared on the dorsal surface from the scapular region posteriorly to the uropatagium. The carrier bat was then reintroduced into the colony and was found dead the following day. A total of 18 of the remaining 19 bats subsequently died. The first deaths occurred 5 days after introduction of the carrier bat. Over 50% of the deaths took place by the 7th day and 80% by the 9th day. The last two deaths occurred on day 14.

PHYSIOLOGY AND BEHAVIOR

Physiological, behavioral, and analytical chemistry experiments were conducted pertaining to development of techniques and methodology for controlling damage caused by rodents, vampire bats, birds, and deer. The subject areas are listed along with a brief statement of accomplishmei

1. Taste Preference Comparison of Laboratory and Wild Norway Rats to Basic Taste Stimuli. A comparative taste preference study was carried out with groups of laboratory and wild Norway rats. With the four kinds of taste stimuli employed, each tested at four different concentrations, neither qualitative nor quantitative differences were exhibited by the two strains. The results are presented in a paper entitled "Taste Preference Behavior of Laboratory vs. Wild Norway Rats". The manuscript will be submitted to a technical journal for publication in 1971.

2. Response of Vampire Bats to Basic Taste Stimuli. The taste preference profile of 24 vampire bats (six per taste stimulus) was determined by the foods-together technique. Sucrose, citric acid, sodium chloride (Nacl) and quinine hydrochloride (QHcl) were used to represent the four basic taste qualities of sweet, sour, salty, and bitter, respectively. Test animals were given a choice between a blood standard and blood treated with each taste stimulus at four concentrations. Bats exhibited stereotyped preference behavior but clearly demonstrated taste discrimination ability by significant aversion ($P<0.01$) to the sour and bitter stimulus. Consumption preference of blood containing the sweet and salty stimulus was not statistically different from the standard.

3. Temperature-Preference Response of the Vampire Bat. The temperature-food preference relationship of the vampire bat was investigated via the brief-exposure foods-together principle. Eight bats (four males and four females) were tested in an apparatus designed for individual or simultaneous presentation of two water jacketed drinking tubes containing the test liquids. Four plasma temperatures (57°, 87°, 102°, and 116°F) were tested against 12°F. The plasma temperature was regulated by circulating water through the water jacket from a temperature-controlled water bath. Preference was measured by both choices and consumption. Physicochemical changes occurred at 116° and obviously were factors in acceptance. There were no differences in preference at the other temperatures, indicating that it does not significantly influence preference behavior in the laboratory vampire bat. The results have been presented in manuscript form which will be submitted to a technical journal in 1971.

4. Preference of the Vampire Bat for Freeze-Dried and/or Whole Blood. Eighteen vampire bats were used in a 15-day switchback trial to determine the preference for fresh or freeze-dried blood. A protein taste enhancer was also incorporated in the trial. The vampires consumed reconstituted freeze-dried blood as readily as fresh blood, 18 ml per bat per day in both cases. There was a significant reduction in the consumption of reconstituted freeze-dried blood reinforced with a 5-ft nucleotide.

5. Pheromone Research in the Norway Rat. In order to discover which odors may attract, repel, or socially disorganize the wild rat, our research efforts have been directed toward both a method by which attractancy or repellency values may be reliably assessed in wild rats and a method for observing the behavior of wild rats under simulated field conditions in the laboratory. The goal of developing an assessment method has been largely achieved and the results are presented in a paper in rough draft form entitled "A Four-Choice Open-Field Test for Measuring the Behavioral Effects of Various Odors." The report will be submitted to a technical journal for publication in 1971. Our second objective is primarily directed towards a means of detecting which naturally secreted or excreted odors have important effects on rat behavior. An observation area for wild rats has been constructed and several male-female pairs have been observed under controlled conditions. Present indications point toward a potentially high degree of importance of certain rat body odors prior to sexual contact.

6. Olfactory Reception in the Vampire Bat (Desmodus rotundus). Olfactory perception of 16 vampire bats was tested by measuring heart and respiration rate responses to five 10-second stimulations of compressed air (control) and to the odor of fresh (attractant) and putrified blood (repelling). Preliminary analysis of the data shows that the vampire bat is very sensitive to either the odor of fresh or putrified blood. Data are being tabulated for statistical analyses, detailed interpretation, and publication in a technical journal.

7. Feeding Behavior of Vampire Bats When Exposed to Living and Nonliving Sources of Blood. A bait station for vampire bats is being tested to determine whether the vampire will drink from an open blood supply (bait station) in the presence of a living (host) animal. We will also determine whether caged bats (decoys) will increase this tendency.

8. Hematological Values of the Normal Vampire Bat. The blood of the vampire bat is strikingly different from that of many other mammals. It is high in erythrocytes (9,116,000/mm^3), hematocrit (51%), and hemoglobin (16.4 g/100 ml), indicating either a great need for oxygen (unlikely with the low metabolic rate during rest) or a hemoconcentration due to dehydration. The high blood cation content (172.3 mEq/l) of the vampire together with the high levels of blood urea nitrogen (99.8 mg/100 ml) and glucose (105.4 mg/100 ml) further suggests unusual dehydration and hemoconcentration. Since the vampire does not drink water to relieve this situation, it is probably a normal condition. Quite possibly, the apparent high blood osmolality is utilized by the vampire to rapidly remove and excrete water from the blood it consumes.

9. Isolation and Identification of Deer Repellent Constituents From Putrified Fish. Continuation of the isolation and identification of repellent compounds from putrified fish has involved work on six preparative gas chromatography fractions. Each fraction has been analyzed by combined gas chromatography-mass spectrometry. There were 52 of 124 interpretable spectra resulting in tentative identification of 45 compounds. Specific structure was not obtainable on some of these. Considerable information, however, has been gained about the chemical class constituency of the fractions and synergism thereof.

10. Effectiveness of Chlorophacinone (DRC-3776) as a Livestock Systemic for Control of Vampire Bats (*Desmodus rodundus*). Preliminary research was conducted to explore the potential of using livestock systemics for the control of vampire bats (*Desmodus rotundus*). In the experiment a total of 90 mg/kg of DRC-3776 (anticoagulant) was orally administered to a ruminant animal (goat) in 5- to 15-mg/kg increments over a 2-week period. Blood was collected to determine clotting time and fed to four vampire bats. A fifth bat was allowed to feed directly on the blood of the DRC-3776-treated animal. All five bats died from either single or multiple feeding of host blood.

    Research is underway to develop an analytical procedure to measure DRC-3776 in blood plasma in order to facilitate adjusting the oral dosage so that the circulating blood anticoagulant content will be toxic to vampire bats but safe for ruminants.

11. Cardiac and Operant Behavior Response of Starlings (*Sturnus vulgaris*) to Distress and Alarm Sounds. An experiment was conducted in which the repelling strength of four prerecorded sound stimuli (two alarm and two distress sounds) were compared in terms of heart rate response and key-pecking suppression ability. The sounds were compared using 24 wild starlings in a switchback experiment. Each treatment was replicated twice. Data are being tabulated for statistical analyses and interpretation.

12. Toxicity of Diuretics and Carbohydrates to the Vampire Bat. The Thompson-Weil method of obtaining LD_{50}'s was used to determine the toxicity of diuretics and sugars to vampire bats. Of the diuretics, three, ethacrynic acid, hydrochlorothiazide, and a mixture of hydrochlorothiazide and spirolactone had LD_{50} in excess of 2000 mg/kg body weight. Chlormerodrin had an LD_{50} of 11.4 mg/kg body weight.

    Both carbohydrates used were relatively nontoxic to vampire bats. Sucrose had an LD_{50} of 37,980 mg/kg body weight while that of dextrose was 20,670 mg/kg body weight.

REPRODUCTION

Ovarian samples were obtained at 12 and 24 weeks of age from four female beagles that had been injected with tritiated thymidine (thy-H^3) at either 0 or 14 days of age. The proportion of radioactive ova ranged between 2.6 and 7.6 percent. The low values were due to either the time of isotope injection or many of the ova formed at the time of injection degenerated

before 12 weeks. Adult beagles similarly injected during either proestrus or estrus failed to show radioactive ova in ovaries removed 10 days later. The adult beagle results suggest that no new ova are formed during those two phases of the estrous cycle.

The ED_{50} for a canine chemosterilant, DRC-4802, was estimated to be between .1 mg/kg and 1.0 mg/kg for adult female beagles.

Preliminary results of a comprehensive study of the coyote endocrine system involved with the initiation of the breeding season and the maintenance of pregnancy were discussed. The five females in the study had an average estrus period of 9.4 days beginning the first week in March. The blood estrogen and progesterone values have not been analyzed yet.

The duration of spermatogenesis in coyotes was estimated to be 54.4 days and epididymal sperm transport averaged 14.6 days. The comparable figures for the Polynesian rat are 56.1 and 18.0 days respectively. A manuscript of a study of coyote spermatogenesis was completed, edited, and is scheduled for transmission to Washington, D. C., for approval.

Two chemosterilants, DRC-4801 and DRC-4802, were tested for possible use in Polynesian and Norway rat control. Female Norway rats showed a 21.3 and 20.1 day delay in the onset of pregnancy when orally dosed with .5 mg/kg with DRC-4801 and DRC-4802 respectively. The former compound delayed pregnancy in Polynesian rats for only 7.6 days. The results with DRC-4802 are incomplete for the Polynesian rat.

Several male chemosterilants were tested in rodents. U-5897, a chemical that induces permanent sterility following a single oral dose, was effective only in Norway rats; Polynesian, ricefield, and black rats showed a poor, if any, antifertility response to the chemical. The chemical also was ineffective as a chemosterilant in prairie dogs, pocket gophers, and jackrabbits. An analog of U-5897 was tested in Norway and Polynesian rats and found to be ineffective as a chemosterilant.

Two colonies of Norway rats were established to determine if a male chemosterilant could effectively limit population size. To simulate a chemosterilant's effect, 85 percent of the males in one colony were bilaterally vasectomized prior to the start of the study. No marked differences were noted between the two colonies.

A description of the procedures developed to establish and maintain breeding colonies of Norway and Polynesian rats is discussed.

ELECTRONICS

A questionnaire was distributed to wildlife research personnel throughout the United States requesting an identification of the major areas where biological information is most needed to advance research programs. These priorities for research shall now be used to focus on specific electronics development rather than dissipate efforts on novel applications or minor problems. The results of this questionnaire are summarized in a manuscript entitled, "Priority Listing for the Development of Wildlife Research Methodology" which has been submitted for publication in 1971.

Detailed laboratory tests have been conducted to evaluate the possible use of solar energy converters as rechargeable power sources for low power biotelemetric instrumentation. The solar energy conversion efficiencies measured in our experiments are sufficiently high to merit further investigation in the field environment. A small illumination recorder (3 g) has been designed which will measure the total amount of time a given species spends in sunlight illumination above some defined threshold. The results of our laboratory experiments have been prepared in a manuscript entitled, "Solar Energy Sources for Biotelemetry Transmitters" and submitted for publication in 1971.

A limited amount of work was conducted in the audio and ultrasonic spectrum, this being mainly in a supporting role to the Unit of Physiology. Selected audio recordings were supplied for research studies evaluating sounds to frighten starlings. Research programs involving the vampire bat have requested ultrasonic recordings of the bats' cries for which the instrumentation is being perfected.

The fabrication and development of miniature (2 g or less), 30 megahertz (MHz), radio-frequency (rf), beacon transmitters for pest species continue to require a great deal of time for electronics personnel. Improved circuit component selection, faster construction techniques, and greater product reliability were all achieved this year. In excess of 360 rf beacons were constructed, quality inspected, and provided for the field biologists. In addition, a smaller (0.7 g), short range (90-200 ft), rf beacon was developed for use on 15- to 25-g mammals. The description of this newest animal locator is presented in a manuscript entitled, "Radio-Frequency Locator for Small 15- to 25-Gram Mammals" which has been submitted for publication in 1971. A second manuscript entitled, "A Miniature 30 MHz Collar Transmitter" is being edited and will be submitted for publication.

A concerted effort was directed this year to the updating of the instrumentation available at the electronics laboratory and to provide the field biologists with equipment suitable to facilitate their work. This newly acquired laboratory equipment can be utilized in all aspects of bioelectronics research with an immediate goal of evaluating many of the existing commercially available biotelemetric devices.. The ultimate objective is to establish specifications for various types of electronic instrumentation to aid biologists in the proper selection of equipment to perform a specific research requirement.

BIRD DAMAGE CONTROL RESEARCH

LATIN AMERICA

In 1970, two specific problems were investigated in field studies--dickcissel (Spiza americana) damage to grain sorghum in Venezuela and Mexico. Serious damage to sorghum by fringillids, blackbirds, parakeets, and doves has been reported in various Latin American countries. Birds appear to attack this crop more readily than even corn and rice.

Tests in the United States indicate that the fright-producing chemical, 4-aminopyridine (DRC-1327), when applied to grain baits is an effective tool for reducing blackbird damage to corn. In an initial field test in a Venezuelan sorghum field, it appears that dickcissels can be successfully frightened from DRC-1327-treated areas. Similar success was obtained in baiting a sorghum field in Mexico; a 3-hectare planting was repeatedly cleared of dickcissels with extremely high dilution ratios of DRC-1327-treated baits.

The safety and field efficacy of several compounds of proven utility in bird damage control were studied in depth to provide information necessary for up-coming registrations. Laboratory data on the acute and chronic toxicity and reproductive effects of the avian carbamate repellent and stupefacient, DRC-736, and the stupefacient, DRC-1327, were collected. Other agents were also evaluated in the laboratory.

Plant translocation of DRC-1327 in corn tissue and soil degradation studies were continued in anticipation of future registration of the compound.

BASIC RESEARCH IN MAMMAL AND BIRD DAMAGE CONTROL

CHEMICALS - P. J. Savarie (July-December), W. A. Bowles, G. J. Dasch, and R. W. Dutton

I. Chemical Screening Activities

A. Introduction

The major problems, responsibilities, and work load of this Unit are concerned with screening contracted, solicited, and purchased chemicals to determine their efficacy as toxicants or repellents to control animal damage. Candidate compounds are evaluated with standard bioassay procedures which include: (1) coating them onto wheat seeds and measuring food acceptance when offered to deer mice (*Peromyscus maniculatus*), and (2) determining their approximate lethal dose by gavaging deer mice.

Data on active candidate compounds are supplied to research units responsible for establishing specificity of biological action with advanced studies as described in the Annual Progress Reports for Work Unit F-16.1, 1963-67. Computorized printouts of test results for each supplier's compounds are distributed to them quarterly, or as data are made available by field stations following completion of their pen or field studies. All data pertaining to results obtained in the screening program are on file at the Denver Wildlife Research Center.

The continuing service status of the Unit was retained this year with no changes in the routine laboratory procedures outlined in the 1965 Annual Report and revised in the 1966 Annual Report.

B. Results and Discussion

The Unit processed 286 new compounds this year which were submitted by 11 manufacturers. These totals represent decreases over 1969 in both numbers of compounds evaluated and manufacturers submitting compounds to be tested. Initial bioassay tests were run on 207 of these compounds, 22 of which exhibited sufficient activity to require establishing the approximate lethal dose.

A portion of the AID allotment was used to purchase unique chemicals that are analogs or homologs of known biologically active chemicals containing reactive nuclei, or having peculiar spatial configurations, or any molecules with simple or complex combinations of functional groups. These compounds procured in this manner are those not normally offered by contractural or solicited sources, and they are needed to advance knowledge at the DWRC concerning effective toxicants whose identities are known. The remaining allotment was used to purchase basic equipment and supplies including bioassay media necessary to support and maintain this service activity.

Progress was made this year in obtaining the identity of compounds submitted by manufacturers. All of the companies now identify the functional groups or class of compound which is submitted, and in many instances, the structural formula and chemical name are revealed. This is a big improvement when one considers that in past years over 3,000 compounds were evaluated but we have neither the chemical name nor structural formula. Identity of compounds submitted is needed to prevent repetitious screening, alert investigators to the potential hazards of the chemicals, and to avoid classes of chemicals which have been banned for use on Federally controlled land.

Preliminary investigation has revealed that a class of compounds, the pyrrolidinones, might have application for the control of rodents or bats. One compound in this series, oxotremorine (DRC-5826), was particularly interesting. The LD_{50} of this compound by oral administration to adult vampire bats (Desmodus rotundus) was found to be 0.15 mg/kg (95% confidence limits = 0.109-0.256). The mode of action of compounds in this series is thought to be through the mimicry and/or release of acetylcholine at the cholinergic synapses. Further studies into this class of compounds are being initiated.

C. Conclusions

During the past 12 months, this Unit has been in a transition stage of converting from a purely empirical approach to one of selectivity as to the modes of action of potential control agents. This transition has necessitated the need for an extensive literature review on the modes of action of compounds currently being used in the control programs. Also, the stringent registration requirements for pesticides has made it ever more important that as much information as possible be obtained from the existing literature sources before extensive laboratory studies

D. Literature Reviewed

Haslett, W. L., and D. J. Jenden. 1961. Pharmacological properties of oxotremorine, an active metabolite of tremorine. Pharmacologist 3:2.

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

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

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, Michigan, 222-226.

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

Completion of the correlation publication is pending the decision of a manufacturer to release the chemical identities of a group of tin compounds that were the most effective of all groups evaluated by the barrier test. Although the manufacturer has insisted that the names of all of his active compounds be withheld from this publication (at the advice of his patent attorney until they can obtain a more favorable patent position), another appeal has been made asking for a review of the present status of these compounds and their authorization for us to list them in the barrier study section.

The table listing results of compounds appraised by the barrier test method will require revision if permission to include the tin complexes is granted, as will the text portion; if consent is refused, then they will stand as written. However, that portion of the publication relating to the results obtained by the Patuxent food acceptance test method has been reviewed by Dr. John J. Pratt, Jr., sponsor from the U. S. Army Natick Laboratories. The unreviewed material, either revised or as is, will be forwarded to him as soon as the final word is received from the manufacturer.

A list of compounds screened by the concentration-effect method for rodent repellency as developed by the Denver Center is in preparation. The compounds are listed sequentially by DRC number, chemical name, 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 preliminary study and initial steps taken to accomplish this objective 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

Retrieving information stored in our present data system is no longer rapid nor practical as the mechanics involved in sorting and handling a deck of more than 5,000 cards restricts its utility adding to or updating of the more than 200,000 bits of information presently encoded in the Keysort deck is limited by the physical size of the card format and the encoding scheme is not so useful as in the original application. Indexing chemical compounds by our functional group classification notation is not compatible with the precise and compact machine language required by ADP procedures.

These deficiencies were anticipated in the preliminary planning of the present data system and it was conceded then that the Keysort system eventually would be replaced. Subsequent investigation of other information handling procedures revealed that recently one method has been widely acclaimed and adopted by many who are engaged in coding chemical structures with notation schemes.

This system, the Wiswesser Line-Formula Notation or WLN, describes a procedure for expressing the structural configuration of a molecule as a linear string of symbols.

The resulting notation is compact, unique and unambiguous and particularly suitable for indexing and computor applications. It requires only the upper case letters, the digits, and four punctuation marks, plus the space to encode a chemical structure. The basic rules include using letter symbols to denote elements or functional groups and numbers to express alkyl chain lengths or ring sizes. The punctuation signs imply special meanings peculiar to indexing and notation procedures in representing two-dimensional graphic formulas by alphanumeric ciphers. The rules governing each WLN operation in codifying chemical compounds are too numerous for this report but they may be found in several of the references listed.

Currently there are more than 20 industrial, academic, and government organizations using the WLN for internal data stores. Contact was made with several of them and two expressed a willingness to assist us in converting to the WLN and its ADP processes. Mr. E. H. Schwanke, Chief, Industry Liaison Office, Research Laboratories, U. S. Army Edgewood Arsenal, visited the Center this year to discuss initial steps of the conversion program. At that time, arrangements were made whereby he would send us bioassay data on 10,000 compounds screened at their Toxicological Center; he also agreed to provide a copy of their format used to encode test results onto the 80-column standard IBM card. At a later date, one of his staff members encoded and returned 100 compounds selected from our files to serve as illustrative examples.

After discussing our problem with Mr. W. J. Wiswesser, the originator of the WLN and presently located at the U. S. Army Ft. Detrick Laboratories, he expressed a keen interest in helping as he thought that our operation was ideally suited for his system. He has since supplied several reprints and is now assisting by coding unrestricted compounds until we become operational with the WLN system.

C. Conclusions

Those shortcomings cited above point out that the present data storage and retrieval system is no longer functional as a serviceable information repository and changes are indicated. The major change involves a conversion to the WLN as it appears that this system is the one of choice over all other notation schemes. This is the optimum time to effect a changeover since previous commitments relative to the publications are fulfilled, and a full-time effort on this activity is now possible.

The first step involves learning rules, regulations and requirements relative to encoding and decoding chemical structures by the WLN system. There are no formal courses offered at the institutional level for training in this discipline. However, an occassional Tutorial Session is held during American Chemical Society national meeting that would be of unquestionable value to the project, in time and money, if permission to attend is obtained.

Once the methodology is mastered, it then becomes essential that all chemical compounds tested as control agents be ciphered for ADP applications. There are some 8,500 identified compounds tested at Denver and Patuxent Research Centers as repellents, approximately 1,000 structured compounds appraised at Denver as bird toxicants, repellents or behavioral altering drugs, and about 800 more named compounds evaluated as general control agents. There are more than 3,000 DRC-numbered compounds for which we have at least the reduction in food acceptance and the ALD values, but the data are useless as the chemical identities were withheld by the supplier.

After establishing our reference file on 10,000 WLN-encoded compounds complete with bioassay data, we can seek cooperation on an information exchange basis with other agencies using this notation scheme. Some of those are Food and Drug Administration, National Bureau of Standards, National Library of Medicine, and the U. S. Army Edgewood Arsenal and Ft. Detrick installations.

Another approach in obtaining background and updated test data is by addressing an inquiry to industrial organizations, who are also WLN users, asking if they are willing to exchange bioassay data on a mutually cooperative arrangement. Some of the pharmaceutical and manufacturing companies generating LD_{50} data for many compounds that are novel or untested by us are Dow Chemical Company, GAF Corporation, Goodyear Tire and Rubber Company, Hoffman-LaRoche, Inc., Eli Lilly and Company, G. D. Searle and Company, and others.

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IV. Laboratory Evaluation of Bat Control Agents

A. Introduction

Vampire bat control aimed at reducing the incidence of rabies transmitted to livestock is a problem due to the difficulty of locating the roosting sites of these animals. A promising new approach involves mist netting a few vampires in the vicinity of corralled or tethered cattle, applying a control agent to captured individuals, and releasing them to return to their roost(s) where other members of the colony would be contaminated. The rationale leading to this concept is as follows:

1. It is normally easy to mist net a few vampires around corralled or tethered cattle subject to predation.

2. Vampires utilize the same location within a roost over extended periods of time (months or years). These sites generally consist of a crevice, crack, or concavity in the roof of the cave, mine, etc., and normally do not exceed 1 to 3 ft in diameter. Frequently only one such niche is found within a roost and seldom are there more than two or three.

3. The number of vampires utilizing each niche is usually not large (5-150), and in the case of tree roosts, probably averages only 5-50.

4. Vampire bats commonly share the same cave with other beneficial species of bats; however, they always utilize a separate niche or roosting site within the cave. Contact between species seldom, if ever, occurs excepting, perhaps, in the case of an individual bat infected with rabies.

5. Vampires roost in extremely close proximity with each other and body contact between individuals is the norm.

6. Greenhall (1965) reported on certain aspects of the grooming behavior of the common vampire indicating that grooming is thorough and that considerable time is spent at this activity. Crespo, Linhart, and Burns (submitted for publication) found that foot-mouth contact is frequent and that captive vampires spent an estimated 2 hours per day grooming.

The initial phase of this study seeked to:

1. Find a suitable toxicant.

2. Find a suitable carrier or vehicle for the control agent.

3. Confirm grooming activity within a captive vampire colony.

4. Determine the efficacy of this approach to control in the laboratory by introducing a treated bat into a captive colony of vampires.

An artificial "roost" measuring 36 x 26 x 25 inches was constructed of 1/2 inch plywood and lined with plastic sheeting. A plexiglass door was attached to the front of the roost to permit direct observation of the bats. Plastic bird feeders containing defibrinated bovine blood (provided daily) were inserted into the side of the roost and bats were permitted to feed at will. A 13-inch-diameter hole was cut into the top of the roost and a metal basin 13 inches in diameter and 5 inches deep was inverted over the hole to provide a "niche" within which the bats could roost. Metal window screen was attached to the "roof" of the basin so as to provide a surface from which the bats could hang. A 1-inch strip of wood covered with screening was suspended from the niche to the floor of the roost as most of the bats utilized had lost the ability to fly due to extended captivity. This "ladder" permitted the bats to descend from the niche to the floor of the roost to feed and otherwise move about.

Four candidate toxicants were chosen on the basis of recommendations solicited from DWRC personnel. These were DRC-714 (Gophacide), DRC-3492 (6-aminonicotinamide), DRC-5826 (oxotremorine), and DRC-3776 (chlorophacinone). Suspensions of DRC-714, -3492, and -3776 (in gum tragacanth), and DRC-5826 (in saline) were gavaged to individually caged adult vampires and LD_{50}'s were subsequently determined by the Thompson (1947) method.

Candidate vehicles or carriers were tested by adding either 50 mg of Tracerite, an inert metallic fluorescent powder, or 50 mg of an acrylic polyester fluorescent paint pigment, and applying the mixture to the dorsal surface of individually caged vampires. Each bat was then checked several times daily with a 125-watt, UV light (3600 A. U. to 3700 A. U.) to determine the persistence and translocation of carrier and fluorescent material to elsewhere on the body, as well as the degree to which it was removed from the hair by grooming. Ten different candidate carriers were tested, of which three appeared worthy of additional study. The three candidates that appeared most promising were (1) a saturated solution of acetone and an acrylic polyester fluorescent paint pigment, (2) Vaseline, and (3) a sticky material consisting of Abalyn (a rosin), mineral oil, and polyethylene (Epoline-10). Fifty milligrams of fluorescent paint pigment were added to the Vaseline and to the formulation of sticky material to permit use of a UV light for the purpose of determining results. A single bat was removed from the 20-bat colony described elsewhere and one of the three carriers was applied to its dorsal surface. The carrier bat was then reintroduced into the colony and the colony checked several times daily with UF light for 2-3 days to determine the degree of translocation to other colony members.

The intense grooming activity of captive vampire bats has been described by Greenhall (1965) and Crespo, et al. (submitted for publication). A single night was spent observing the 20-bat colony by means of closed-circuit TV. A 25-watt, red light was placed inside the roost 3 days before observations began in order to condition the bats to the light required for TV transmission. The TV monitor was located in an adjacent room and observations were made intermittently for half-hour intervals from 7:30 PM to 12:30 AM for 1 night only. Data were recorded as to movement and grooming activity of individual bats within the colony as well as contact between colony members.

One candidate toxicant (DRC-3776) and one of the three most promising carriers (Vaseline) were tested in the bat colony. Fifty milligrams of DRC-3776 were dissolved in 1.5 ml of melted Vaseline and the mixture was permitted to cool to room temperature. One of the 20 bats in the colony was removed and the mixture was applied to the dorsal surface of the bat from the scapular region posteriorly to the uropatagium. The plexiglass door was covered with opaque paper to reduce external stimuli and the treated bat was reintroduced onto the floor of the roost at the end of the workday (4:00 PM) to further minimize disturbance by personnel normally working in the room. The roost was subsequently checked once daily at 4:00 PM for 31 days and all dead bats were removed from the colony. Date of death, band number, and other pertinent notes were recorded.

B. Results and Discussion

1. Candidate toxicants - Of the four compounds given by gavage to vampire bats to determine LD_{50} levels, DRC-3776 (chlorophacinone) appeared the most promising. The LD_{50} of this slow-acting anticoagulant was 3.75 mg/kg (95% confidence limits = 1.66 mg-8.43 mg). The highest dose administered (140 mg/kg) required 1.5 days for death to occur. Intermediate doses of 8 and 5.5 mg/kg required from 5-13 days, whereas at the lowest levels, 3.7 and 2.5 mg/kg, deaths occurred from 9-16 days later. Several animals gavaged at the latter dose rates survived.

2. Carriers - Two of the 10 different candidate carriers or vehicles tried appeared superior. Both Vaseline and the acetone-fluorescent paint pigment upon introduction into the 20-bat colony resulted in contamination of all or nearly all bats. The day after introduction of the bat treated with Vaseline all members of the colony were contaminated to varying degrees. Fluorescence was most noticeable on the forearms, legs, and feet. Contamination of the colony with the acetone-paint pigment was a result of gradual flaking or powdering off of the dried mixture from the back of the carrier bat that resulted in more uniform contamination than in the case of Vaseline. However, unlike Vaseline, which clings to the fur, a considerable quantity of the acetone-paint pigment fell to the floor of the roost.

3. Grooming behavior within the colony - Observations via closed-circuit TV were admittedly limited but did serve to confirm previous observations of grooming behavior by Greenhall (1965) and Crespo, et al. (submitted for publication). Observations revealed that from one-third to one-half of the bats were grooming at any one time. Several instances were noted of one bat grooming another (with the tongue) and of one bat "embracing" another by folding its wings about the body of the latter. Considerable contact between bats was observed and grooming commonly took place when bats were in close proximity (i.e., touching or nearly so), despite considerable space within the "niche" not being utilized by other members of the colony. Movement of individual bats from one position to another within the niche was commonly observed. The above observations indicate that contamination of the colony by introduction of a "carrier" bat would likely take place.

4. Mortality within colony following contamination by DRC-3776 - Excellent results were obtained following introduction of bat-carried DRC-3776 into the roost that contained 20 vampires (8 females, 12 males). The carrier bat was found dead the morning following reintroduction into the roost and 18 of the remaining 19 bats subsequently died. The number of deaths, by day, are shown in Table 1. The first deaths occurred 5 days after introduction of the carrier bat. Over 50% of the deaths took place by the 7th day and 80% by the 9th day. The last two deaths occurred on day 14.

TABLE 1. Number of deaths by day following contamination by DRC-3776

Day	Number deaths	Day	Number deaths
1	1 (carrier)	8	1
2	0	9	4
3	0	10	0
4	0	11	1
5	4	12	0
6	1	13	0
7	5	14	2

Blood intake was reduced the first 2 days following introduction of the treated bat and subsequently returned to normal. Numerous bats were observed hemorrhaging from various parts of the body prior to death and subcutaneous hemorrhages or purpuras were seen on some of the extremities and on the lateral portions of the body at the junction of the patagium and body trunk. Dead animals were not autopsied.

Based on the above results, it is felt that field trials should be undertaken in order to determine the efficacy of this technique for controlling vampire bats.

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

I. Taste Preference Comparison of Laboratory and Wild Norway Rats to Basic Taste Stimuli

A. Introduction

From the standpoint of experimental efficiency, there are advantages to using domesticated laboratory rats in screening for taste and flavor enhancers even though the results may eventually receive application in the field on wild Norway rats and other species of problem rodents. A group of 24 Long-Evans hooded rats was compared to a group of 24 wild Norway rats with respect to the four taste qualities: sweet, sour, salty, and bitter. Each rat was tested on one of these taste stimuli at four different concentrations.

B. Results and Discussion

For each taste stimulus, there were statistically significant concentration effects for both rat strains. However, in no case was there a rat strain effect implying that the effects of domestication in laboratory rats have not produced a substantial change in taste preference. Variability of taste preference response for both strains of rats appeared to be similar in value and both strains showed systematic changes in variability as concentrations were varied for sweet and bitter stimuli. Means and standard errors for the groups of rats are presented in Table 1.

Table 1. Mean Percent Preference Responses and Standard Errors for Four Taste Stimuli, Four Concentrations, and Two Strains of Norway Rats

	Conc. 1		Conc. 2		Conc. 3		Conc. 4	
	Hood	Wild	Hood	Wild	Hood	Wild	Hood	Wild
				Sucrose (Sweet)				
Mean	47.39	52.30	57.87	64.74	78.87	79.15	88.71	91.88
S.E.	19.68	19.67	23.84	26.08	28.31	29.31	30.63	28.60
				Sodium Chloride (Salty)				
Mean	59.25	60.30	52.98	65.41	49.39	53.84	31.91	29.76
S.E.	20.08	23.26	17.32	28.11	18.98	28.44	17.96	18.45
				Quinine Hydrochloride (Bitter)				
Mean	53.43	47.84	48.93	46.85	27.04	19.20	1.92	4.01
S.E.	20.64	15.72	17.58	15.05	17.60	19.03	4.26	6.45
				Citric Acid (Sour)				
Mean	50.41	54.03	53.57	52.14	40.95	34.64	13.81	15.79
S.E.	15.87	18.38	18.39	17.55	15.38	19.48	14.64	17.01

II. Response of Vampire Bats to Basic Taste Stimuli

A. Introduction

There is a need to explore the possibility of enticing vampire bats (*Desmodus rotundus*) to eat from blood bait stations as a means of applying chemical control agents. In order to adequately explore the potential of this concept there is a need to know the preference profile of vampires for the basic taste qualities.

B. Results and Discussion

Sucrose, citric acid, sodium chloride (Nacl) and quinine hydrochloride (QHcl) were used to represent the four basic taste qualities of sweet, sour, salty, and bitter, respectively. Each taste stimulus was tested with six bats against untreated blood (standard) at four concentrations with an automated two-choice preference tester. Different bats were used to test each taste stimulus (total of 24 bats) and each preference observation was replicated twice and averaged. Test concentrations and preference response is shown in Tables 2 and 3.

TABLE 2. Taste stimulus test concentrations (C) (gms/100 ml of blood)

Stimulus	C_1	C_2	C_3	C_4
Sucrose	0.034	5.682	11.364	17.114
Sodium Chloride	0.006	0.971	1.942	2.925
Citric Acid	0.002	0.021	0.210	1.150
Quinine Hcl	0.004	0.040	0.400	2.188

TABLE 3. Preference consumption response (%) of vampire bats to taste stimulus test concentrations

Stimulus	C_1	C_2	C_3	C_4
Sucrose	58.1 ± 5.1	49.2 ± 4.4	44.9 ± 3.4	45.6 ± 3.6
Sodium Chloride	51.6 ± 4.0	49.6 ± 2.7	40.7 ± 5.8	43.4 ± 6.6
Citric Acid	51.1 ± 5.5	49.4 ± 4.1	46.6 ± 1.4	18.5 ± 1.8
Quinine Hcl	44.4 ± 6.3	43.6 ± 2.6	32.5 ± 2.6	23.5 ± 2.4

Bats exhibited a sterotyped behavior when confronted with the 2-choice situation in the experiment. Such behavior is to be expected since Desmodus depends solely on the blood of living hosts for energy and, unlike rodents, has no need for highly developed food preference behavior. In spite of the vampires' poorly developed preference behavior, they showed some aversion to the C_3 and C_4 levels of sucrose and sodium chloride. However, on an overall basis, consumption preference of sucrose and sodium chloride-treated blood was not statistically different from the standard. On the other hand, bats displayed significant aversion ($P<0.01$) to citric acid and quinine hydrochloride. The aversion was especially pronounced at the C_4 test concentration of citric acid and the C_3 and C_4 levels of quinine hydrochloride.

While Desmodus exhibits a stereotype preference behavior, the results clearly show that they have taste discrimination ability. In terms of vampire bat control, the results indicate that Desmodus would show no taste aversion to blood treated with low to moderately high concentration of sweet and salty tasting toxicants, but bitter and sour tasting toxicants may produce bait shyness at even a low concentration.

III. Temperature-Preference Response of the Vampire Bat

A. Introduction

The difficulties of applying a chemical agent at either the home caves or feeding sites (unconfined range cattle) has prompted our investigation of the bait station concept. Ideally, bats would be attracted to a bait station (either live or artificial), whereupon, chemical control methods are applicable. One consideration in the artificial bait station concept has been the role of temperature in the vampire's food preference. This experiment is an effort to answer that question.

B. Results and Discussion

We used a preference-testing technique employing the brief-exposure foods-together principle. The physicochemical characteristics of whole blood made it necessary to use blood plasma in the experiment. Since the laboratory bat accepts plasma as readily as whole blood, this was only a minor adjustment. Plasma temperatures of 57°, 87°, 102° (body temperature of cattle) and 116°F were tested against ambient temperature (72°F).

The preference testing apparatus and procedure was a modification of that used by Young and Kappauf (Am. J. Psychol. 75:482-484). The test chamber was a 6 x 10 x 6.5-inch plexiglass box with a 1/2-inch hardware cloth floor. Two drinking tubes (1-1/4 inch apart laterally) were attached to a sliding mechanism on the front of the chamber. The operator could either present the tubes in pairs or individually. The inside front of the chamber was modified with a "bench" 1-1/4 inch high that enclosed the drinking founts. Two holes in the top allowed feeding by the bat but prevented contact of any part of the body other than the tongue.

The drinking tubes were prepared by adapting a 50-ml graduated buret (for measuring consumption) with a water jacket and fount for drinking. Plasma temperature was maintained by circulating water from a temperature-controlled water bath through the water jackets. Each tube had a 28-gauge platinum wire embedded in the boot of the fount and protruding 2 mm into the liquid. A drink-ρ-meter mechanism attached to this and to the wire floor permitted sensing the tongue contacts with the plasma. Elapsed time meters and impulse counters in the circuit permitted the determination of drinking time and tongue contacts respectively.

Eight animals (four males and four females) were trained to drink from either tube. Twenty choices in two sets of 10 (test positions reversed) were offered each day. The test animal sampled each tube for 1 second before the two tubes were presented together. The choice criterion was 4 seconds of continuous drinking from one of the tubes. Consumption was recorded from the burets at the end of 10 choices.

Choice preference was determined by chi-square analysis where the observed values were the number of choices made on 23°C plasma, and the expected was 1/2 the total number of choices made by an individual. Thus, our hypothesis was that the bat made the same number of choices on ambient temperature plasma as it did at the treatment temperature. The probability of no difference was greater than 0.5 in all cases except one (47°C) where $P = 0.35$. Paired "t" tests were used in comparing preference based on consumption. There were no differences between the treatment and control at the 0.05 level of significance for any treatment except 47°C.

Consumption is probably a more reliable measurement of preference although the two results were comparable. It can safely be concluded that plasma temperatures other than ambient will not improve the preference selection of the laboratory vampire bat.

IV. Preference of the Vampire Bat for Freeze-Dried and/or Whole Blood

A. Introduction

The natural, and only, food of the vampire bat is whole fresh blood. The laboratory confinement of these animals, therefore, requires the continual procurement of fresh blood (fresh blood deteriorates quite rapidly and must be acquired fresh every 3 or 4 days in order to insure acceptable comsumption by the vampire). The feasibility of an artificial bait station also depends on a stable source of blood. Freeze-dried blood would provide an excellent source of food for the vampire that would be stable for long periods of time even without refrigeration.

This study was conducted to obtain information on the preference, as measured by consumption, of vampire bats for fresh or freeze-dried blood.

B. Results and Discussion

Eighteen vampire bats were used in a sensitive switchback trial for more than two treatments (Lucas, H. L. 1956. Switchback trials for more than two treatments. J. Dairy Sci. 39:146-154). Consumption data was obtained on fresh blood, reconstituted freeze-dried blood, and reconstituted freeze-dried blood with a protein taste enhancer (5-ft nucleotide) added.

The vampire bats consumed the reconstituted freeze-dried as readily as fresh blood, 18 ml per bat per day, indicating that freeze-drying had not decreased the palatability of the blood. Consumption of reconstituted freeze-dried blood reinforced with the protein taste enhancer, however, was significantly reduced from that of fresh blood. Emphasizing, perhaps, that new or novel taste stimuli are not always the best answers to bait acceptance.

Freeze-dried blood provides an ideal food for the maintenance of laboratory vampire bats. One that has long-keeping qualities, does not need refrigeration, is easily reconstituted, and is accepted by the vampire bat as readily as fresh blood.

Pheromone Research in the Norway Rat

(I. A Four-Choice Open-Field Test for Measuring the Behavioral Effects of Various Odors)

A. Introduction

The sensitivity and reliability of an automated four-choice, open-field test for the effects of various odors on rat behavior has been shown to be adequate for screening candidate attractant or repellent odors. Initial tests with this apparatus have included uniformity data for food odors, attractancy measurement for food and urine odors, and repellency measures of two repellents, tributyltin chloride (TBTC) and Rotran (R-55).

B. Results and Discussion

The uniformity trial data initially collected with the open-field test indicated that there were no sources of physical bias leading to preference for one particular odor station over any other odor station. These data were collected on 20 albino rats with each animal receiving five trials and initial choice of odor station as the dependent measure. However, it was noted that any confinement of the animals at a particular odor station and between-trial handling led to progressively longer response latencies as tests were conducted. In order to increase efficiency of testing, each animal was retested in a free-choice condition for 30 minutes and activity along with elapsed time measures were recorded for each rat and each odor station. This second uniformity test indicated no significant differences between odor stations for activity (photocell breaks) but significant differences for the elapsed time measure. The delivery system for the odorant sources has been modified and will be retested for uniformity of the elapsed time measure.

For attractancy measurement of food odor and female urine odor, a group of 20 male albino rats was tested with the activity measure. One of the odor stations was baited with either urine or food and the other three contained distilled water. For repellency measures on TBTC and R-55, the repellents were mixed with ground rat food and one odor station contained the repellent odor while the other three odor stations contained untreated food. For both repellents, an initial odor test was first conducted and a second odor test was conducted after each animal had been allowed to orally sample the repellent. The results of all odor tests are presented in Table 4.

TABLE 4. Photocell Activity Measure of Odor Attractancy or Repellency

Odor Type	$\bar{x}$ Control ± S.E.	$\bar{x}$ Odor ± S.E.	Significance Level
Food	12.00 ± 6.39	85.58 ± 31.72	.001
Urine	15.79 ± 8.85	43.95 ± 28.50	.001
$TBTC_1$	24.60 ± 8.93	21.40 ± 13.47	n.s.
$TBTC_2$	32.60 ± 12.95	11.20 ± 7.91	.001
$R55_1$	20.00 ± 8.81	20.30 ± 9.63	n.s.
$R55_2$	23.50 ± 13.43	17.60 ± 10.60	n.s.

(II. Observations on the Behavioral Effects of Odors Excreted or Secreted by the Wild Norway Rat)

A. Introduction

By observing the behavior of wild rats with natural conditions simulated to the highest degree possible in the laboratory it may be possible to detect the important sources of odor eminating from glands or external excretions. Presently, investigations are being carried out to closely observe the behavior of pairs of male and female wild Norway rats while the females are in estrus and diestrus conditions.

B. Results and Discussion

Preliminary observations suggest that as the female rat approaches maximal sexual receptivity, the male rat increases the frequency of licking and sniffing the female genitalia. However, even when the female is in the diestrus condition there is a moderately high frequency of olfactory-oral examination of the female by the male. It is difficult to characterize this olfactory investigative behavior since in some pairs the female will show more attention to the male than the male to the female. Part of this problem may stem from the unknown ages of the wild caught rats. These same conditions will be observed with wild ricefield rats and the age factors may be controlled by laboratory breeding of these wild strains.

VI. Olfactory Reception in the Vampire Bat (*Desmodus rotundus*)

A. Introduction

There is anatomical evidence to show that the vampire bat has a well-developed olfactory system. Such evidence suggests that olfaction may be of primary importance to the blood-eating vampire in locating prey. However, there is no experimental data pertaining to odor perception in *Desmodus*. In order to gain insight into the functional significance of their olfactory system, we measured cardiac and respiratory rate responses of 16 vampire bats to the odor of fresh (attractant) and putrified blood (repellent). The test apparatus consisted of a small animal chamber coupled to a olfactometer to deliver odor to each test bat. HR and RR were recorded from implanted electrodes. Bats were allowed to adjust to the chamber environment before testing. On test day, 1 minute of prestimulus baseline HR and RR were recorded, after which each bat was subjected to five 10-second odor stimulations at 5-minute intervals. Immediately prior to the first odor stimulation, 10 seconds of compressed air was injected and response recorded as a check for odor or auditory cues in the test system.

B. Results and Discussion

The experiment has been completed. Preliminary analyses of the data show that the vampire is very responsive to the odor of fresh and putrified blood. Data are presently being tabulated for detailed statistical analysis and interpretation. We plan to publish the results in a technical journal in 1971.

VII. Feeding Behavior of Vampire Bats When Exposed to Living and Nonliving Sources of Blood

A. Introduction

The common vampire bat feeds exclusively on fresh blood which must be supplied either by a living host or in an open container. It would be a simple matter to bait and control the vampire if it were an opportunist and would take blood from an open container rather than from a living animal, which is more difficult and dangerous to the bat. We have designed this experiment to determine whether the wild vampire would prefer the easy approach and take blood from an open container in the presence of a living animal.

B. Results and Discussion

The experiment is being conducted in a flyway at the Denver Research Center using a sensitive changeover design where each bat will be used as its control.

The design is as follows:

	B_1	B_2	B_3	B_n
Day 1	D	ND	D	ND
Day 2	ND	D	ND	D
Day 3	D	ND	D	ND

B_1---B_n = bats; D = decoys; ND = no decoys

The criteria of success, of course, will be the drinking of blood from an open container in the presence of a living animal (rabbit). Measurements taken will include (1) tongue contacts with the blood in the open container, and (2) consumption of blood from the open container. Three vampire bats have been tested to date. This is an insufficient number from which to draw any conclusions and 12 more bats will be tested as soon as they can be obtained from Mexico.

VIII. Hematological Values of the Normal Vampire Bat

A. Introduction

Hematological parameters are of great value in a better understanding of the vampire bat and in the subsequent development of control techniques. They accurately reflect both normal and abnormal physiological functions as well as the effects of various pharmacological agents.

B. Results and Discussion

The post-absorptive (24 hours after feeding) blood of normal vampire bats contains interesting levels of several of the 20 parameters evaluated. Erythrocyte counts, hematocrits, and hemoglobin levels are elevated when compared to those found in most other mammals. The high levels of these parameters and the subsequent hemoconcentration,

in the absence of the need for additional oxygen, can be attributed to the copious diuresis of the vampire during and immediately after feeding. Its refusal to drink water at any time is also a causative factor. Vampire blood is high in sodium (Table 5) which is the major cation contributing to the very high plasma cation levels of 172.3 mEq/l which also indicates a severe dehydrated condition or perhaps a hemoconcentration.

TABLE 5. Mean, standard deviation, and coefficient of variation of 20 parameters of vampire bat blood

Parameter	Unit	No.	Mean ± S.D.	C.V. %
Erythrocytes ($X10^6$)	mm^3	19	9.116 ± 1.796	19.7
Leucocytes	mm^3	19	4.358 ± 2.789	64.0
Differential Leucocyte Count				
Lymphocytes	mm^3	19	24.1 ± 14.7	61.1
Monocytes	mm^3	19	4.4 ± 3.3	74.8
Neutrophils	mm^3	19	69.3 ± 15.0	21.7
Eosinophils	mm^3	19	0.2 ± 0.4	237.3
Basophils	mm^3	19	1.3 ± 1.7	129.3
Hematocrit	%	19	51.0 ± 9.4	18.3
Electrolytes				
Sodium	mEq/l	25	161.8 ± 7.6	4.6
Potassium	mEq/l	21	2.9 ± 0.7	23.3
Calcium	mEq/l	31	4.8 ± 0.6	10.7
Magnesium	mEq/l	26	2.8 ± 0.6	22.5
Chloride	mEq/l	22	124.2 ± 6.2	4.9
Biochemical Values				
Hemoglobin	g/100 ml	31	17.4 ± 3.2	18.6
Cholinesterase	M/min/gHb	31	10.8 ± 4.0	37.0
Glucose	mg/100 ml	30	105.4 ± 57.2	53.4
Blood Urea Nitrogen	mg/100 ml	21	99.8 ± 11.9	11.6
Total protein	g/100 ml	26	6.7 ± 0.8	11.7
Uric acid	mg/100 ml	22	1.1 ± 0.6	59.8

Blood urea nitrogen (BUN) is another parameter which is found in high levels in the blood of vampire bats. BUN levels such as found in this study would indicate severe kidney damage in man. However, in the vampire bat these levels appear to be the normal result of high dietary protein levels and hemoconcentration.

In retrospect the post-adsorptive blood picture of the vampire bat indicates a hemoconcentration and high osmolality. This high concentration of the circulating blood would appear to be the means by which the vampire so rapidly removes water from the dietary blood.

IX. Isolation and Identification of Deer Repellent Constituents From Putrified Fish

A. Introduction

Considerable progress has been made in solving the animal damage problem in reforestation areas on several species. The browse damage by deer and elk, however, is still with us and probably is the most serious problem today.

Chemical deer repellents are still considered the best solution to the problem. Several years have now been invested in attempting to isolate and identify the deer repellent constituents from putrified fish. It is a complex and difficult task but progress is being made.

B. Results and Discussion

In the 1969 Annual Progress Report we reported techniques that were used to separate Fraction T into eight subfractions; six preparative gas chromatography fractions and two high boiling sweep-co-distillation fractions.

The six preparative gas chromatography fractions have been analyzed by combined gas chromatography-mass spectrometry analysis. Compounds separated on a 50-ft surface-coated open tubular (OV-17) column were analyzed on an A.E.I. M.S. 12 mass spectrometer. Regardless of the extensive fractionation preceding this analysis, the mixtures in each fraction were so complex as to make this analysis extremely difficult. Only 52 of 124 spectra were interpretable resulting in the tentative identification of 45 compounds many of which are only identified as to class and molecular weight.

Although we cannot hope for an extensive identification of compounds present in these fractions with the present program, a wealth of information has been gained from the G.C.-M.S. analysis. Several classes of compounds of a specific molecular weight range have been pinpointed. We have gained enough experience to know that the deer repellent characteristics of this material are probably the result of a complex synergism. Possibly by the correct combination of compound classes we can make a good deer repellent.

We have obtained many of the tentatively identified compounds or their close homologs. Attempts are being made to confirm the identity by mass spectra and gas chromatography retention times. Testing of classes and class combinations will be accomplished by the deer preference testing procedure soon to be implemented at the Olympia, Washington, field station.

X. Effectiveness of Chlorophacinone (DRC-3776) as a Livestock Systemic for Control of Vampire Bats (*Desmodus rotundus*)

A. 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. Calculations based on vampire blood consumption, toxicity, and blood dilution, show that it is mathematically feasible to administer a systemic to host animals for vampire control. However, research is needed to determine the actual potential of using a systemic for controlling *Desmodus*, and if feasible, develop methodology to safely administer it to livestock in Latin America so that the host blood will be lethal to the bat.

B. Results and Discussion

A preliminary experiment was conducted to determine whether the principle of oral administration of a systemic would produce host blood toxic to vampire bats. DRC-3776 (chlorophacinone, an anticoagulant) was selected as the first candidate systemic because it is highly toxic to vampire bats (LD_{50} 3.75 mg/kg) and vitamin K counteracts its prothrombinopenic effects.

In the experiment a total of 90 mg/kg of DRC-3776 was administered to a goat in 5- to 15-mg/kg increments over a 2-week period. During the dosing period blood was collected for the determination of clotting time and to be fed to four vampire bats. Two of the bats were each fed 10 ml

goat blood once and two were each fed 10 ml of blood twice on consecutive days. Also, a fifth bat was allowed to feed on the goat for 3 days. All five bats died within 3 to 10 days after ingesting the goat blood. We are encouraged by the initial results. At the present time an analytical procedure is being developed to measure DRC-3776 blood plasma. Once the procedure is perfected an attempt will be made to adjust the dosage so that the circulating blood level will be toxic to vampire bats but safe for ruminants.

XI. Cardiac and Operant Behavior Response of Starlings (Sturnus vulgaris) to Distress and Alarm Sounds

A. Introduction

A variety of auditory stimuli have been used experimentally and in practice for many years to repel nuisance birds from problem areas. Such an approach to alleviating bird problems is appealing in that wild birds are sensitive to sound stimuli of biological origin and such stimuli are harmless to both target and nontarget species. Unfortunately, the success of auditory repellents in bird control programs has been variable. In many cases, this stems from the use of nonspecific sounds that produce only a weak physiological and behavioral response, hence, rapid habituation and loss in repellent strength.

In an effort to identify strong auditory repellents for control programs, an experiment was conducted to rank the repellent strength to starlings of four prerecorded alarm and distress sounds in terms of physiological and behavioral response.

B. Results and Discussion

The four stimuli (two alarm and two distress sounds) were compared in terms of telemetered heart rate and key-pecking suppression with 24 starlings in a switchback experiment. Each treatment was replicated twice. The experiment has been completed. Data are presently being tabulated for statistical analysis and interpretation. We plan to publish the results in a technical journal in 1971.

XII. Toxicity of Diuretics and Carbohydrates to the Vampire Bat

A. Introduction

The vampire bat has a unique renal system; it urinates copiously upon feeding (presumably to lighten its weight for takeoff and to insure the ingestion of sufficient solids to satisfy its nutritional requirements),

with the result that it has very little water left for the digestion of its food and the elimination of wastes. It is plausable, therefore, to determine the effects of several diuretics with a view to controlling the vampire through excessive diuresis.

The extremely poor digestibility of carbohydrates by the vampire bat has led to the investigation of these compounds to determine their toxicity to the vampire bat.

B. Results and Discussion

A modification of the Thompson-Weil method for obtaining LD_{50}'s was employed to determine the toxic effects of four diuretics: (1) ethacrynic acid, a powerful, dose-dependent diuretic; (2) hydrochlorothiazide; (3) a 50-50 mixture of hydrochlorothiazide and spirolactone; and (4) chlormerodrin, which contains the equivalent of 10 mg of nonionic mercury for each 18.3 mg. Ethacrynic acid, which is one of the most successful diuretics for human use, required 2051 mg/kg of body weight for an LD_{50}. Both the hydrochlorothiazide and the mixture of hydrochlorothiazide and spirolactone had LD_{50}'s in excess of 2000 mg/kg of body weight. The mercury containing chlormerodrin was the most effective of the diuretics with an LD_{50} of 11.4 mg/kg of body weight. The toxic effect was produced, however, not by an excessive diuresis, but probably by mercury damage. In fact, this compound had the effect of preventing urination. From the above results it appears that it is extremely difficult to alter the water balance of the vampire bat by the use of a diuretic.

In previous work we have found that the vampire bat's utilization of carbohydrates is extremely poor and that some sugars might be detrimental in moderate quantities. In order to assess the toxic properties of carbohydrates, LD_{50}'s were obtained on sucrose and dextrose. Sucrose had an LD_{50} of 37,980 mg/kg while dextrose was only slightly lower at 20,670 mg/kg body weight. It is apparent, therefore, that while the vampire does not utilize carbohydrates to any great extent, it nevertheless can tolerate relatively large quantities of these compounds.

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

I. Rodent Studies

Spermatogenic Cycle of Polynesian Rats

A. Introduction

A study of the spermatogenic cycle and duration of epididymal sperm transport was undertaken as part of a major study of the reproductive patterns of Polynesian rats. The general objective was to establish criteria whereby the selection and evaluation of male antifertility compounds could be accomplished. The specific objectives of this phase of the work were (1) to define the duration of the spermatogenic cycle and its major subdivisions, (2) to define and estimate the duration of the stages of the spermatogenic cycle, and (3) to establish the time required for epididymal sperm transport. The original design failed to provide information at two critical points during the study and evaluation had to be withheld until the necessary data was obtained.

B. Results and Discussion

The duration of the spermatogenic cycle was found to be 56.1 days. However, the delineation of each of the spermatogenic stages and an estimate of their duration has not been completed due to the need for additional data. Epididymal sperm transport time, the third objective, has been estimated and the results are presented in Table 1.

TABLE 1. Estimates of the duration required for sperm transport through the epididymis

Epididymal section or organ checked	Days after isotope injection: First appearance labeled sperm	Maximum percent labeled sperm	Maximum number of total grains	Mean days elapsed from the previous point checked
Caput epid.	40	43	44	
Corpus epid.	45	49	49	5.3
Cauda epid.	53			8.0
Vas def.	58			5.0

The data indicate epididymal sperm transport requires about 18 days (58-40) in the sexual rested Polynesian rat. This duration added to the 56.1 days needed for sperm development gives a total duration of 76.6 days from the start of sperm development until their availability for fertilization. These values, when combined with those to be obtained on the duration of each spermatogenic stage, will enable one to (1) evaluate where and when a particular chemosterilant exerts its action and (2) predict when a rat will become infertile following a chemosterilant. Further, the information will be useful if and when data is required for registration of a male chemosterilant for this species.

Evaluation of Antifertility Compounds

1. DRC-4801 and DRC-4802

A. Introduction

Two new synthetic estrogens (hereafter designated DRC-4801 and DRC-4802) were offered to this unit as potential rodent chemosterilants. The compounds, provided by two cooperators, were both reported to be more effective in reducing the fertility of white rats than mestranol, a highly potent synthetic estrogen. Both chemicals were reported to have a dual effect; the initiation of pregnancy was delayed 2-3 weeks in females given a single oral dose, and young female rats nursing treated mothers were permanently sterilized. Since the effectiveness of both compounds on the inhibition of reproduction in Norway and Polynesian rats is unknown, tests were initiated to determine whether DRC-4801 or DRC-4802 showed any potential as a rodent chemosterilant.

B. Results and Discussion

The study was divided into two parts corresponding to the two main effects of the chemicals. Part I is now completed and the results are summarized in Table 2.

TABLE 2. The inhibition of pregnancy in Norway and Polynesian rats given a single oral dose of one of two synthetic estrogens, DRC-4801 and DRC-4802

		DRC-4801				DRC-4802			
				Mean no. days				Mean no. days	
Treat	Dose (total mg given)	No. of females	Mean quantity eaten (mg/kg)	To whelp	Delay[c]	No. of females	Mean quantity eaten (mg/kg)	To whelp	Delay[c]
				Norway rats					
I	0	9	-	26.0	-	9	-	29.7	-
II	0.127[a]	10	0.5	47.3	21.3	8	0.5	49.8	20.1
III	0.175	10	0.20[b]	34.8	8.8	8	0.23	41.2	11.5
IV	1.750	8	1.85[b]	41.4	15.4	10	2.62	43.2	13.5
				Polynesian rats					
I	0.0	10	-	43.7	-				
II	0.033[a]	9	0.5	51.3	7.6				
III	0.058	10	0.45[b]	58.6	14.9				
IV	0.583	10	3.9[b]	54.5	10.8				

[a] Each female dosed via gavage to provide .5 mg chemosterilant/kg body weight; average dose was as shown.
[b] Chemosterilant-food presented for 1 night (16 hours) and actual dose was based on weight of uneaten food.
[c] Based on control values.

Part II will be completed in mid 1971 and will be reported next year. DRC-4801 was more effective in the Norway rat than DRC-4802. In the former, pregnancy was significantly ($P < .05$) delayed in all three treated groups over that observed in the controls. DRC-4802 significantly delayed parturition only in Treatment II (gavage), but not in the other two treatments. However, DRC-4801 did not significantly alter reproduction in the Polynesian rats. The results for Polynesian rats dosed with DRC-4802 are not yet available.

2. DRC-4800 (U-5897)

A. Introduction

U-5897 is an alpha-chlorohydrin that, depending on dosage, has both a reversible and irreversible antifertility effect on male Norway rats. Since the irreversible effect can be induced with a single oral dose, the chemical attracted attention for potential use in other problem species. Two different studies were undertaken. In the first study, five dose levels of U-5897 were tested on Polynesian (Rattus exulans), black rats (R. rattus), ricefield rats (R. argentiventer), and for comparative purposes, Norway rats (R. norvegicus). The second study was a cooperative effort with the Section of Mammals in which the effectiveness of the chemical was tested in prairie dogs (Cynomys ludovicianus), pocket gophers (Thomomys talpoides), and jackrabbits (Lepus californicus).

B. Results and Discussion

The results of the first study have been published (Kennelly, J. J., M. V. Garrison, and B. E. Johns, Laboratory Studies of the Effect of U-5897 on the Reproduction of Wild Male Rats, J. Wildl. Mgmt., 34(3): 508-513, 1970). In view of the specificity of U-5897 as a rat chemosterilant, two preliminary feeding trials were conducted. The objective of one trial was to determine whether the effective dose level changed when U-5897 was offered ad libitum in the food of Norway rats.

The second trial was to determine if epididymal lesions could be induced in Polynesian rats when U-5897 was added to the food. Our earlier studies (publication cited above) showed that no lesions developed when Polynesian rats were dosed by gavage at the same levels producing lesions in Norway rats. It was thought that the trauma associated with handling the rats at dosing could have significantly altered the rat's metabolism of U-5897. The results of both feeding trials are presented in Table 3.

TABLE 3. Effect on the epididymides of male norway and Polynesian rats fed U-5897

Treatments					
No.	Percent U-5897 in bait[a]	Bait material	No. males	Mean dose U-5897 mg/kg (± S.D.)	No. males with bilateral epididymal lesions
		(Norway rats)			
I	0	Lab chow[b]	5	0	0
II	1.0	Lab chow	5	33.1 ± 8.8	0
III	2.0	Lab chow	5	51.4 ± 13.4	1
IV	3.0	Lab chow	5	94.6 ± 27.6	4
V	1.0	Dry dog food[c]	3	214.3 ± 157.8	2
VI	2.0	Dry dog food	2	153.2 ± 24.0	1
		(Polynesian rats)			
I	0	Lab chow	5	0	0
II	1.5	Lab chow	3	136.7 ± 15.9	0[d]
III	1.0	Dry dog food	3	175.8 ± 67.0	0
IV	2.0	Dry dog food	5	275.7 ± 138.8	0

[a] Exposed to bait for one night (16 hours).
[b] Purina Laboratory Rat Ration.
[c] Gaines Kennel-ration.
[d] One male had a unilateral lesion.

Apparently the route of administration affects the Norway rats' response to U-5897. In the earlier studies all males dosed at 100 mg/kg or greater developed epididymal lesions but in this trial several rats received even greater doses and still did not develop lesions. Also, no deaths occurred in this study, yet some males received total doses exceeding the lethal dose observed in the earlier study. A total of 11 Polynesian rats received some U-5897 and, except for a unilateral lesion in one male, no antifertility effect was elicited. Further, none of the treated Polynesian males died from the U-5897 although, on the basis of the earlier tests, some rats ingested lethal levels. The most significant development apparent in these trials was the differential response obtained between the two methods of dosing; gavage vs. chronic

ingestion via the food. The results strongly suggest further studies i this area should be initiated especially for those chemicals that show sufficient potential to be considered for field tests.

The results of the cooperative study with the Section of Mammals are shown in Table 4. None of the species tested developed epididymal lesions further demonstrating the specificity of U-5897, especially for Norway rats. The chemical was toxic at the high levels killing every male dosed with 300 mg/kg. In view of these results, no further testin of U-5897 on other mammalian species is planned.

TABLE 4. Effect of a single oral dose (gavage) of U-5897 on prairie dogs, pocket gophers, and jackrabbits

Species[a]	Treatment (mg U-5897/kg)	Number of animals	Number of animals With lesions	Number of animals Died
Prairie dog	Control	6	0	0
	100	6	0	0
	200	6	0	3
	300	6	0	6
Pocket gopher	Control	5	0	0
	100	5	0	1
	200	5	0	3
	300	5	0	5
Jackrabbits	Control	6	0	0
	100	6	0	0
	200	6	0	3
	300	6	0	6

[a] All males were sexually active when treated.

3. DRC-1811

A. Introduction

Discussions with Dr. A. B. Borkovec in charge of chemosterilant investigations for the USDA's Entomology Research Division, revealed that they had a class of insect chemosterilants that were quite similar to DRC-4800 (U-5897), a male chemosterilant under study at the DWRC. One of the compounds, ENT-26304 (hereafter designated DRC-1811), was found to be a much more effective insect chemosterilant than DRC-4800. If any sterilizing effect could be found in rats treated with DRC-1811, Dr. Borkovec had several more analogs of the compound that he felt would merit further tests in rats. Therefore, a brief trial with DRC-1811 was undertaken using both Norway and Polynesian rats.

B. Results and Discussion

The results of the tests are summarized in Table 5. It is evident from the table the compound had no demonstrable effect on either species. No further testing of this group of compounds is planned. However, Dr. Borkovec has volunteered to inform us if and when any compounds showing potential as chemosterilants appears in the course of their search for insect chemosterilants.

TABLE 5. Effect of DRC-1811, a halohydrin, on Norway and Polynesian rats (dosed via gavage)

Species	No. males tested	Dose level (mg/kg)	Results: No. males died	Results: No. males with epididymal lesions
Norway	3	100	0	0
	3	200	0	0
Polynesian	5	100	0	0
	5	200	0	0

4. DRC-1810

A. Introduction

This is a male antifertility compound. Preliminary reports from the manufacturer indicated it may be useful as a rodent control agent. Accordingly, laboratory tests and breeding trials were undertaken to decide whether field tests were warranted.

B. Results and Discussion

The laboratory and breeding results were reported in the 1968 Annual Report and a manuscript was prepared in 1969. However, the cooperator, Smith, Kline, and French Company, requested we hold the manuscript from publication until they cleared up several matters concerning the compounds' registration. Clearance to publish was not granted until January 1971. The manuscript will now be submitted to the Center's editor and hopefully will be considered suitable for publication.

Control of Wild Rodent Populations Via
Reproductive Inhibitors

1. Fecundity of a Norway rat colony with 85 percent of the males sterile.

A. Introduction

The recent discovery of a chemical that permanently sterilizes male Norway rats focuses attention on the question: how effective will a male chemosterilant prove to be in a free-breeding colony of Norway rats? Clearly, sterilization of a high proportion of the breeding males in a population will have no impact whatsoever if the remaining fertile males impregnate the females. To obtain an answer to this question, 85 percent of the males in one of two otherwise similar populations of Norway rats were sterilized by bilateral vasectomy and the populations compared.

B. Results and Discussion

Initially each colony consisted of 20 males and 20 females. Seventeen of the males in one colony (treated) were bilaterally vasectomized 12-14 days prior to the start of the study. Data summarized in Table 6 shows this interval between vasectomy and initiation of the colonies was sufficient to render the treated males sterile. Note that males bred as early as 5 days after vasectomy failed to impregnate females.

TABLE 6. Fertility of laboratory rats 5, 10, 15 and 20 days after vasectomy

Days after vasectomy: Male mated with female	Necropsy of males	No. of animals: Males[a]	No. of animals: Females tested[b]	No. of vaginal plugs: With sperm	No. of vaginal plugs: Without sperm	No. females pregnant
5	7	5	20	0	15	0
10	12	5	20	0	21	0
15	17	5	20	2	18	0
20	22	4	16	0	13	0
Control	22	7	28	35	2	9

a/ All males were proven fertile prior to the study.
b/ Two estrus females on each of 2 consecutive days were placed with each male on the day post-vasectomy indicated. Control males were paired on day 20 and 21 after sham operation and necropsied on day 22.

The study was terminated after 105 days and every animal in each colony was caught and autopsied. A summary of the number of animals recovered is shown in Table 7.

TABLE 7. Summary of animals recovered ($\geq$25 g body weight)

	Original stock: Males	Original stock: Females[a]	Juveniles: Males	Juveniles: Females	Juveniles: Total
Control	19	19	69	61	130
Treated	20	19	63	47	110

a/ One female in each colony was found dead during the first week of the study.

Except for one control male, each original male was recovered. The total number of new individuals was 130 and 110 for the control and treated colonies respectively. Comparisons between the two colonies with regard to the number of pregnancies and litter size of the original stock of females in shown in Table 8.

TABLE 8. Pregnancy and litter size comparisons between the original control and treated colonies

Colony	No. of females completing indicated no. pregnancies				No. pregnant females (autopsy)	Total no. pregnancies	Estimated mean litter size[a] ± S.D. First litter		Second litter	
	0	1	2	3			No. females	Mean no. young	No. females	Mean no. young
Control	0	5	13	1	4	38	18	10.8 ± 3.4	13	10.0 ± 3.2
Treated	2	3	13	1	7	39	16	8.6 ± 2.7	13	10.3 ± 1.6

[a] Based on placental scar counts.

The total number of pregnancies was 38 in the control and 39 in the treated colony. Even more significant was the fact each colony showed the same number of multiple pregnancies (Table 8). If the sterilized males had a pronounced effect, a greater number of pseudopregnancies would have occurred resulting in a decreased number of multiple pregnancies in the treated colony. The most likely explanation is that the females mated with many different males during estrus with a reasonable probability that a fertile male was encountered.

We concluded from this study that a single application of a chemosterilant, affecting 85 percent of the males, would be ineffective in controlling a Norway rat population. All the data that contribute towards this conclusion have not been presented here; a manuscript that contains these data and pertinent arguments has been submitted for publication.

Establishment of Breeding Colonies of Polynesian and Norway Rats

To efficiently and (hopefully) successfully conduct both basic studies of rodent reproduction and to evaluate rodent chemosterilants, it is necessary to establish breeding colonies of the target species. Once a breeding

colony is established, the researcher is able to (1) provide a standard and routine breeding program, (2) provide controlled test conditions, (3) manipulate the animals as called for in the design, and (4) provide a standard stock of known-age animals. At least one and sometimes all four of the above items are necessary for carrying out reproductive studies. The objective of this study was to establish (1) Polynesian rat, and (2) Norway rat breeding colonies.

B. Results and Discussion

Norway Rats

During 1969 and 1970, approximately 500 rats were trapped and either tested or used in the development of a laboratory colony. After several preliminary studies it was found that the following physical conditions and handling techniques were necessary prerequisites for the successful establishment of a Norway rat breeding colony:

a. Trapped rats held in individual cages containing water and a commercial rat food a minimum of 1 week before pairing.

b. The male should be placed in a rat cage (14" x 9-1/2" x 7") a minimum of 2 days before the female is introduced.

c. Each cage should contain a shelter (e.g. 1-gallon can).

d. 18-20 days after pairing the male should be removed.

e. Nesting material should be provided (small 5-6" burlap squares work well).

f. Wean the young at 21 days of age.

The results of two Norway rat breeding trials are summarized in Table 9.

TABLE 9. Summary of the results of two Norway rat breeding trials

	No. of pairs	Litters		No. of young weaned	Average weaned litter size
		Born	Weaned		
Trial 1	66	22(33%)	16(24%)	119	7.4
Trial 2	40	34(85%)	27(68%)	224	8.3

The majority of the rats in trial 1 were paired during the fall months in a room with the temperature (72-74°) but not the light artificially regulated. In trial 2 the rats were paired in November under the similar conditions except the light was controlled to provide a light-dark cycle of 14-10 hours daily. The results indicate the importance of controlled lighting during a season of the year when breeding is normally low.

Polynesian Rats

Approximately 700 Polynesian rats trapped in Hawaii and shipped to Denver over a 3-year period (1967-1970) were tested or used in the development of a laboratory colony. The conditions and techniques used to breed and raise the Polynesian rats were similar to those followed with the Norway rats except:

a. The floor of the rat cage had a smaller mesh wire and a 7" x 4" electric conduit tube was placed in the cage instead of a 1-gallon can.

b. The male and female were introduced into the cage at the same time (the female should never be larger than the male).

A summary of the Polynesian rat breeding trial is shown in Table 10.

TABLE 10. Summary of the results of Polynesian rat breeding trials

	Event		
Months	No. females mated	No. litters	Success (%)
Jan.	6	3	50
Feb.	11	5	45
Mar.	7	3	43
April	5	4	80
May	2	2	100
June	19	13	68
July	17	7	41
Aug.	17	4	24
Sept.	15	8	53
Oct.	8	3	38
Nov.	7	1	14
Dec.	6	4	67
Total	120	57	47.5

It is quite apparent that breeding success varied from month to month but the reasons for the variation, if indeed they are real, remain obscure. Since the environmental conditions (temperature, light, and food) were standard throughout the year, it suggests that either (1) other environmental conditions are at fault (e.g. humidity), or (2) the Polynesian rat differed physiologically from the Norway rat. Examples of the latter are age differences and prior breeding histories. Attempts will be made to improve the breeding success of Polynesian rats.

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

I. Priority Index for Bioelectronics Research

A. Introduction

Improved techniques and methodology for wildlife research are continually being sought. Electronic equipment, with its inherent remote sensing capabilities, becomes a prime candidate for providing a solution to many biological questions. In fact, the demand is so extensive that an individual group can soon become entangled on just where to emphasize their work.

Seeking to establish meaningful long range goals, the Electronics Unit queried a broad spectrum of the wildlife research community concerning their fundamental needs. We did not ask them to limit their answers directly to problems they felt had electronic solutions. The response indicated from our questionnaire showed distinct research priority trends which should greatly enhance the overall value of our future research program.

B. Results and Discussion

The questionnaire circulated by the Electronics Unit was prepared in two stages. First, a letter of inquiry was sent to 4 wildlife research laboratories and 18 Cooperative Wildlife Research Units within the Bureau of Sport Fisheries and Wildlife, requesting a priority listing of their major needs in research methodology. Secondly, all the research problems presented from this first inquiry were summarized into a three-level topical outline which was mailed to about 60 researchers in questionnaire form. The questionnaire instructions directed the researchers to set their levels of priority on those topics in the outline which were of fundamental concern.

Forty-two responses were obtained from our questionnaire. These were tabulated and the research topics weighted and ranked according to the votes received at each priority level. The results of the priority rating for the five major topical categories are shown in the following table:

TABLE 1. Priority ranking of the five major categories in the outline, based on votes from 40 questionnaires

Major category	No. of votes for priority 1	2	3	4	5	Overall ranking
	Weighting Factor 5	4	3	2	1	
I. Wildlife Field Data	31	2	1	4	2	1
II. Physiological Data	5	4	12	9	8	3
III. Behavioral Data	1	18	10	9	1	2
IV. Environmental Measurements	3	9	7	7	12	4
V. Data Processing and Collection	0	7	9	8	14	5

The top 15 priority topics at the third level of the topical outline are listed below:

1. Mortality Studies
2. Census Techniques
3. Activity
4. Home Range
5. Natality Studies
6. Seasonal Migration
7. Capture Techniques
8. Sex Ratios
9. Longevity
10. Introduction of New Species
11. Habitat Usage
12. Sensory Preferences
13. Contamination Levels
14. Electrophysiological
15. Social Order and Communications

II. Utilization of Solar Energy for Biotelemetric Devices

A. Introduction

The power source for most biotelemetric instrumentation imposes severe weight and volume restrictions. Any long-term studies must be augmented with either an adequate energy source to run the duration of the test or a procedure to regularly replace or rejuvinate the power supply. Solar power converters (solar cells) offer the instrumentation designer an opportunity to intermittently recharge a depleting secondary battery charge without disrupting an animal's behavior. If such a recharging system can be made light-weight, reliable, efficient, and with a resonable attachment to a test animal, biotelemetric instrumentation would be greatly improved as a research tool.

B. Results and Discussion

The manuscript prepared and submitted for publication describes in detail the laboratory tests conducted using solar panels as a recharging current source for nickel cadmium button cells. A general discussion is given to clarify some of the circuit component trade-offs which can be made to meet various specifications and still maintain a good energy conversion efficiency. Rules of thumb for estimating solar panel and battery sizes for any given electrical power requirement are stated.

Three types of operation are possible with a rechargeable battery system: continuous, extended, or intermittent duty. A continuous operation implies that power must be supplied 24 hours a day throughout the component life of the instrumentation package. In the extended mode, the designer admits that solar illumination time is inadequte to keep the battery recharged indefinitely, but that it is sufficient to extend the effective battery capacity a number of times. The intermittent duty implies that the instrumentation is powered only during and immediately after those periods of time that the solar panel has been illuminated.

The defining equation in order to maintain energy balance in a solar cell recharging system can be stated as $I_c T_c = E\ I_1 T_1$, where I_c = average charge current, T_c = average hours of charging time per day, E = charge replacement ratio for a given battery type, I_1 = average load current, and T_1 = average hours of battery load time per day. Our recorded data covering in excess of a year of experimental time shows considerable component latitude with this idealized energy balanced situation. Nickel cadmium cells can sustain short-term over-charging with little degradation, and the advertized charge replacement ratio of 140% appears to be about 10% high.

Two caged starlings were instrumented with solar cell rechargeable, 30 MHz transmitters weighing about 6 g. The transmitters were monitored over a 60-day interval. The abnormal behavior of the birds precludes any conclusions concerning overall system performance, but a great deal of information was obtained concerning attachment techniques and solar panel placement.

A 3-g illumination recorder has now been designed to measure how much charging time can be expected when mounting a solar panel on any given test species. This is a logical approach and will eliminate any preconceived notions which biologists may harbor. Data are currently being gathered from recorders which have been mounted on test species for known periods of time.

III. Sound and Ultrasonic Studies

A. Introduction

No formal studies were planned utilizing sound or ultrasonics as an exciting stimulus. However, owing to previous work, Mr. Pearson was requested by the Unit of Physiology to assist in the preparation of an experiment to evaluate natural and synthetic sounds as fright stimuli for starlings. This preparatory work consisted of evaluating the acoustical test chamber and recording the timed magnetic tapes.

Work in the ultrasonic spectrum involved checking out existing electronic instrumentation to determine if a suitable recording system could be perfected. To date, incompatibilities among the various system's components have precluded obtaining the desirable flat frequency response, but preliminary recordings have been produced.

B. Results and Discussion

The extent of participation in this work segment did not require conclusive data.

IV. Animal Radio-Frequency Locator Transmitters

A. Introduction

The requirements for biological data concerning the movement and location of animals and birds are evidenced by the large number of requests obtained for radio-frequency animal transmitters. The work

load imposed by these large orders for transmitters has caused us to survey the possibility of contracting with a manufacturer. A limited success with a local manufacturer was achieved this year in this endeavor, but the time savings has not been as great as anticipated. It is a continuing effort for the electronics laboratory to update and improve the overall performance of these rf beacons. Our reception range is admittedly limited for studies involving far-ranging animals and birds, but seems to be adequate for rodent control research. The development of a smaller rf beacon during 1970 now allows biologists to evaluate toxicant-induced mortality on animals previously too small to instrument.

B. Results and Discussion

Proper component selection for miniature animal transmitters has required much laboratory time. For example, the mercuric oxide batteries currently used as power sources apparently must be derated for our application. Repeatability in laboratory discharge tests is poor and the field data shows even worse correlation. Investigations are under way for an improved power supply. A second example of component evaluation would be our research for a miniature, variable, high Q inductor optimizing the oscillator to antenna impedance match in the linear antenna (bird) transmitter. The radiated output power from these transmitters can be significantly improved through specially wound toroidal inductors, but the time and effort involved make it impractical for a production item. Alternate methods have been and will continue to be sought.

Arrangements were made this year with a local manufacturer to aid in the construction of animal transmitters. Component selection, quality control, and final evaluation were still conducted by our electronic technicians. This contracting arrangement was reasonably efficient and resulted in about a 30% cost savings to us for the transmitter assemblies.

The two manuscripts submitted for publication detail the rodent rf beacons which can be supplied Bureau personnel, and will not be replicated in this report.

V. Laboratory and Field Equipment

A. Introduction

Electronics personnel are experiencing increased pressure to act as technical advisors to wildlife biologists in formulating research programs. This work often involves comparative evaluations of commercially

available instrumentation. With recently acquired government excess property laboratory test equipment, we can now make comparative radio-frequency receiver sensitivity tests, basic antenna impedance measurements, and comparative radiated power from transmitters at 30 and 164 MHz. It is also anticipated that this additional equipment can be used to aid in basic research being conducted in other Units at the Research Center.

B. Results and Discussion

The field stations involved in animal telemetry studies have been provided with technical assistance whenever functionally possible. Five major field stations were provided with 12-channel, portable, 30 or 164 MHz, signal generators to aid in field checking their receiving systems. A number of collapsible, 30 MHz, portable, loop antennas were sent field personnel for evaluation. These antennas are modified military units obtained from government excess property and have been well accepted. A 6-inch diameter, 50-ohm loop antenna for 164 MHz has been designed upon request and is now under field evaluation. Reference antennas at 30 and 164 MHz have been constructed for comparing radiated output power and conversion efficiencies of animal location transmitters. The laboratory continues to service, calibrate, and update the Johnson 350 DF receivers used by our field personnel. The recently acquired electronics test equipment allows for these design and service functions to be performed with more surety and accuracy than previously possible.

BIRD DAMAGE CONTROL RESEARCH

LATIN AMERICA

Study of Specific Problems: Dickcissel Damage to Grain Sorghum in Venezuela--J.W. De Grazio and K.H. Larsen

A. Introduction

In Venezuela, dickcissels that congregate in wintering flocks feed intensively in maturing grain sorghum fields in December and January. In one affected area near Acarigua, Venezuela, biologists of the Denver Center viewed birds and damage in January 1969 (Besser and De Grazio, 1969a). Dickcissels have also been reported to cause crop losses in several Latin American countries in November and April during fall and spring migrations (Besser, 1968).

Tests in the United States indicate that the fright-producing chemical DRC-1327, is an effective tool for reducing blackbird damage to maturing field corn (De Grazio, 1964; Woronecki, et al., 1967). Durin January 1970, an initial field test was conducted to determine if DRC-1327 has utility in protecting grain sorghum from dickcissel damag and to learn more about the bird and the problem. Data were also acquired on caged birds for potential useful chemical agents on dickcissels, parakeets, and other species noted feeding in sorghum fields.

B. Results and Discussion

A 3-acre sorghum field was the test site for the compound DRC-1327. Approximately 2,000 dickcissels were feeding in the field prior to treatment. Treated sorghum diluted with five parts of untreated sorghum was scattered in three 25-ft swaths in the field prior to the birds' morning arrival. Reactions of DRC-1327-affected dickcissels caused the flock to abandon the field. New flocks replaced frightened flocks but by mid-morning the entire field was clear.

In addition to the field tests, dickcissels, brown-throated parakeets (Aratinga pertinax), ruddy ground doves (Columbigallina talpacoti), an scaled doves (Scardafella squammata) were force-fed sorghum seeds treated with various concentrations of DRC-736 and DRC-1327.

Dickcissels dosed with DRC-1327 sorghum seeds gave the best reaction between 15-25 mg/kg; there was no visible reaction at 8.5 mg/kg and 32-38 mg/kg appeared to be too potent. The carbamate compound DRC-736 appeared to have a fairly wide margin between immobilization and death all of six birds survived a 2.6-4.2 mg/kg dose on sorghum seeds, and three of the six were catchable. The average weight of 20 birds was about 25 g, ranging from 22 to 30 g.

Brown-throated parakeets readily gave distress cries when dosed with DRC-1327 as noted with orange-fronted parakeets (Aratinga canicularis) in Nicaragua (Besser and De Grazio, 1969b). The most effective dose appeared to be about 11-15 mg/kg on sorghum seeds. Some were able to fly between periods of distress and some uttered periodic distress calls for as long as 2 to 9 hours. The average weight of 12 birds was about 75 g, ranging from 67 to 82 g.

Ruddy ground doves survived a dose of 20 mg/kg of DRC-1327 on sorghum seeds and died at 32 mg/kg. Scaled doves survived dosages of 3.7 and 4.3 mg/kg.

C. Conclusions

In the intital test of baiting sorghum fields with DRC-1327-treated baits, it appeared that dickcissels could be successfully frightened from treated areas. Preliminary laboratory information of the effects of DRC-1327 and DRC-736 on some Venezuelan problem species was gathered. Additional data should be obtained to determine exact LD_{50}'s

I. Study of Specific Problems: Dickcissel Damage to Grain Sorghum in Mexico--J.F. Besser and J.W. De Grazio

A. Introduction

Damage to grain sorghum by birds is one of the more common agricultural problems in Latin America. From 1967-69, a particularly severe bird problem had occurred each April in Tabasco, Mexico, where grain sorghum is one of the chief crops grown on newly cleared jungle and drained areas in the basin of the Rio Grijalva. Investigations were made in these areas from April 13 to April 24, 1970.

B. Results and Discussion

The bird species causing most of the problem in grain sorghum in Tabasco was the dickcissel. However, only about 25,000 dickcissels were observed in the area. They did not concentrate in any particular sorghum field, but fed rather uniformily over all fields. Populations were not dense enough to warrant testing control methods during the period of observations. It was noted that dickcissels preferred water grass and panic grasses to grain sorghum. It may be that dickcissels cause severe damage to cultivated crops only in those years when wild grass seeds are relatively scarce.

In Vera Cruz the grain sorghum problem at the Cotaxtla station was unique in that this station's 3-hectare sorghum planting was the only one ripening in this area at this time.

Dickcissels readily took baits broadcast at extremely high diluting ratios (1:50), and those affected by the chemical fright-producing agent, DRC-1327, displayed distress reactions for long periods. Although the field was repeatedly cleared of dickcissels with DRC-1327, these birds or other migrants returned on 2 subsequent days.

Red-eyed cowbirds (Tangavius aeneus) that shared equally in causing damage did not appear to take bait readily from the ground within sorghum plantings, and those that were affected usually died within a few minutes after the onset of cries.

Baitings with DRC-1327 were far more effective during wind-still periods than when winds were strong (10-20 mph), as affected birds in aerial displays were quickly blown from the fields leaving the potential audiences behind damaging sorghum. Dickcissels particularly were easily drifted from the fields by even moderate winds. At Vera Cruz, winds were often strong in mid-afternoon, but early mornings were almost always calm. Effectiveness of treatments in clearing fields was correlated to wind speed. Also, avian predators that appeared on several occasions apparently aided somewhat in reducing bird feeding.

C. Conclusions

Damage to grain sorghum in April in Tabasco is chiefly caused by migrating dickcissels. Severity of damage is likely correlated to the availability of alternate foods. Damage to commercial plantings in Tabasco in April 1970 was minor.

Dickcissels readily took baits broadcast at extremely low rates within sorghum plantings. Dickcissels affected by taking baits treated with the chemical fright-producing agent, DRC-1327, displayed distress reactions for long periods. Although a sorghum field was cleared of dickcissels repeatedly with DRC-1327, these birds or other migrants returned on 2 subsequent days.

The value of chemical agents will be correlated to length of stay of dickcissel flocks. If the same birds cause damage for only a few days these agents will be of little value; if the birds are there for several weeks, however, they will either become "educated" by chemical frightening agents, or populations will be reduced substantially by its lethal effects.

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II. Laboratory Studies of Damage Control Agents--J.L. Guarino, E.W. Schafer, R.B. Brunton, and N.L. Lockyer

A. Introduction

Bird problems are world-wide and damage occurs to agricultural crops and to military and civilian aircraft. Nuisance problems also occur in many urban areas. Although a variety of methods has been tried to minimize 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. In special situations, toxicants need to be developed further for limited use on certain species of birds.

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 discovery of two highly repellent compounds have allowed an expanded program in this area. Much effort in the past year has been directed at developing one of these compounds for eventual commercial use on seeds.

The use of stupefacients is a recent approach to controlling damage by birds. These compounds either produce unique behavioral reactions 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 is now in the final stages of registration for use on ripening corn.

Because of the development, registration, and successful use of DRC-1339 as a starling toxicant at cattle and poultry feedlots, the toxicant program is now oriented more towards finding suitable contact toxicants for use in urban and rural starling and blackbird roosts.

B. Results and Discussion

1. Initial Testing

Repellents

Of 71 compounds evaluated as red-winged blackbird (*Agelaius phoeniceus*) repellents, 54 were not repellent below 1.0% and 10 were repellent between 0.317% and 1.00%. All these compounds were rejected from further testing. Of 7 compounds repellent at 0.316% or less, 2 were active below 0.100% (Table 1).

TABLE 1. Compounds repellent to red-winged blackbirds at 0.316% w/w or less

DRC #	R_{50} redwings (%)	95% confidence limits
4269	0.237	0.131-0.429
4272	0.147	0.044-0.486
4297	0.316	0.059-1.694
5500	0.042	0.020-0.087
5501	0.282	0.160-0.495
5503	0.056	0.015-0.212
5518	0.272	0.160-0.495

Stupefacients and Toxicants

Only 7 of 23 compounds examined for toxic or stupefacient activity in blackbirds indicated any activity in either area (Table 2).

TABLE 2. Compounds active as toxicants or stupefacients to red-winged blackbirds at 100 mg/kg or less

DRC #	LD_{50} (mg/kg)	95% CL	TI_{50} (mg/kg)	95% CL	SF
4271	3.2	1.0-10	1.3	NC	2.4
4292	24	NC*	---	--	---
5500	100	56-178	---	--	---
5526	18	10-32	7.5	5.6-10	2.4
5527	2.4	NC	1.0	0.56-1.8	2.4
5528	1.3	NC	0.75	0.56-1.0	1.8
5529	2.4	NC	---	--	---

*Not calculable.

2. Advanced Laboratory Testing

Repellents

DRC-736

Laboratory studies continue to indicate that DRC-736 is extremely repellent to most avian species tested. Of seven species involved, ring-necked pheasants (*Phasianus colchicus*), brown-headed cowbirds (*Molothrus ater*), and bobwhite quail (*Colinus virginianus*), are the least sensitive, while tricolored (*Agelaius tricolor*) and red-winged blackbirds, house sparrows (*Passer domesticus*) and common grackles (*Quiscalus quiscula*) are the most.

The following studies were conducted during the past year regarding the registration of this material as a seed repellent for corn and other crops:

a. The acute toxicity of DRC-736 was assessed on Coturnix (Coturnix coturnix) and bobwhite quail, pheasants, budgerigars (Melopsittacus undulatus), tricolored blackbirds and red-winged blackbirds. Of particular interest was the toxicity of DRC-736 to pheasants since several determinations have resulted in a number of different values. This year, adult male and female pheasants in separate groups of six were dosed with 10 to 316 mg/kg using 1/4-log intervals. Mortality gradually increased 10 to 32 mg/kg, peaked at 56 to 100 mg/kg, then fell off gradually at 178 to 316 mg/kg. Peak mortality was only 50%. The lower toxicity at higher levels cannot be explained unless it is associated with regurgitation or poor absorption from the gut. Pheasants dosed at 5, 10, 14, and 26 weeks of age indicated a diminution of toxicity with age.

b. The chronic toxicity of DRC-736 was determined on common grackles, mourning doves (Zenaidura macroura), and Coturnix quail.

   Common grackles fed 100 ppm DRC-736-treated food for 30 days suffered severe weight loss due to a 40% reduction in food consumption. Twenty-four percent of the birds died.

   Mourning doves fed 100 and 316 ppm DRC-736-treated food for 30 days experienced no observable treatment effects other than a reduction in food consumption. However, 83% of the doves fed at 1,000 ppm died, and weight gain and food consumption of the group was severely reduced from controls. Coturnix quail fed 100 ppm DRC-736-treated food for 28 days suffered no observable effects, but there was 17% and 33% mortality in quail fed 316 ppm and 1,000 ppm respectively.

c. The effects on reproduction of subacute and chronic doses of DRC-736 were determined on Coturnix quail. There were no observable effects in birds fed 100 ppm DRC-736 in their diet. Egg production was significantly depressed in quail fed 316 ppm, probably due to a 20% reduction in food consumption. Egg production, hatchability, body weight, and food consumption were severely depressed in quail fed 1,000 ppm in their diet. At this level, all eggs laid after 2 weeks of treatment had thin shells.

   Subacute dosing of male and female Coturnix quail with 70% of an LD_{50} dose had no apparent effects on reproductive performance.

Stupefacients

DRC-735

The immobilization and lethal levels of this immobilizing agent were determined on budgerigars. Although DRC-735 has an extremely rapid onset of action, it continues to show an insufficient safety margin between lethal and immobilizing levels to be of value.

DRC-736

The immobilization and lethal levels of this compound were evaluated on six species: Coturnix and bobwhite quail, ring-necked pheasant, budgerigar, and tricolored and red-winged blackbirds. In redwings, an injection of DRC-736 was found to give the same response as orally ingested material at similar levels. The oral ingestion of DRC-736 produced insufficient safety margins for the compound to be a useful immobilizing agent for the species involved other than the blackbirds.

DRC-1327

Laboratory studies have been directed at gathering data pertinent both to the pending registration of this compound for use on blackbirds in ripening cornfields and to the safety of the compound to the environment. These included the following:

a. Acute toxicity studies that provided data on nine additional species: blue-black grassquit (*Volatinia jacarina*), orange-fronted parakeet, shiny cowbird (*Molothrus bonariensis*), ruddy-breasted seedeater (*Sporophila minuta*), Coturnix quail, budgerigar, tricolored blackbird, ring-necked pheasant, and red-winged blackbird. The studies on the first four species were conducted in conjunction with our AID program to alleviate bird damage in Latin America. Redwing studies involved the use of routes of administration other than oral to determine actual toxicity levels of DRC-1327 in the body.

b. Chronic toxicity studies were run on mourning doves showing that 31.6 and 100 ppm DRC-1327-treated feed had no effect in a 30-day test. However, 316 ppm DRC-1327-treated food resulted in 50% mortality and significantly reduced weight gain and food consumption of the survivors.

Similar studies with Coturnix quail showed that 31.6, 100, and 316 ppm DRC-1327 in their feed produced 8% mortality in the 316 ppm group due to acute poisoning, and mortality in hatched chicks was unusually high in a 2-week posttest period at all levels. All birds fed a 1,000 ppm DRC-1327 diet died within 3 weeks.

c. The effects on reproduction of chronic and subacute doses of DRC-1327 were also determined for Coturnix quail. The chronic feeding of 31.6 ppm DRC-1327 to adult breeding quail caused a significant increase in the number of infertile eggs with a concurrent decrease in hatchability after withdrawal of the chemical. None of the other measured parameters (such as thin-shelled eggs) varied significantly from pretest levels; however, chick mortality during the posttest period seemed to be unusually high. Thin-shelled eggs were measured by actual mechanical breaking of the egg during marking, by cracks, or by a dried-out interior, while infertile eggs were those that did not develop a viable embryo. Thus, it is possible that a slight decrease in shell thickness could affect fertility by dehydrating the albumin and yolk, but not enough to either crack or break or completely dry out.

Chronic exposure to 100 ppm DRC-1327 produced fewer infertile eggs, and significantly more thin-shelled eggs. Hatchability was depressed and although not significant, it was marked, and mortality of hatched chicks was again high during the 2-week posttest period.

The feeding of 316 ppm to quail for 28 days significantly depressed egg production during the 2-week posttest period. The percentage of thin-shelled and infertile eggs also increased, but the increases were not significant. Hatchability decreased, but again, not significantly. Mortality of hatched chicks was also unusually high, and one adult male died of acute poisoning symptoms the ninth day of the test.

Egg production dropped sharply at 1,000 ppm after the second day of exposure to treated food. Food consumption and body weight were significantly depressed immediately after treatment began and all birds died within 3 weeks.

Male and female birds acutely dosed at 70% of their LD_{50} failed to show any effects on reproductive performance.

d. A cage study was conducted in which groups of seven mourning doves were offered DRC-1327-treated baits at field use levels to determine baiting hazards to doves. Using 3% DRC-1327-treated cracked corn diluted 1:99 with untreated corn, five of the seven doves died within 2-4 days after treated material was introduced in the cage. Bait treated with 2% chemical and diluted 1:99 resulted in three dead birds within 3-6 days after treatment began.

DRC-3551

This compound has been shown to be a potentially effective immobilizing agent for capturing doves and pigeons. During the past year we have attempted to refine our evaluations of treated baits in the laboratory. Tests indicate the 0.5% to 1.0% w/w concentration on cracked corn or whole corn should be effective on pigeons. Similar tests conducted with blackbirds indicated that a 4% cracked corn bait diluted with 9 parts untreated was an optimum treatment level.

An evaluation of DRC-3551 on budgerigars indicated an acceptable safety margin for this species.

Toxicants

DRC-1339

Acute oral toxicity studies showed a number of additional species to be susceptible to this oral toxicant including ring-necked pheasants, tricolored blackbirds, budgerigars, and Coturnix quail. Species susceptibility continues to show wide variations with hawks, fringillids, and psittids being extremely resistant; columbids and most waterfowl moderately sensitive, and most other birds extremely sensitive (1-10 mg/kg).

DRC-1347

Toxicity determinations were made on tricolored blackbirds and budgerigars with results similar to those reported for DRC-1339.

DRC-3895

This potential pigeon and sparrow toxicant was found to be only moderately toxic to budgerigars. Development work on this compound has been curtailed because of poor field trial experiences (regurgitation and poor acceptance).

III. Plant Translocation and Soil Degradation Studies--R.I. Starr and D.J. Cunningham

A. Introduction

Results of the DRC-1327 translocation studies in corn (1969 Annual Progress Report DF-102.2) indicate it would be unlikely to detect the chemical in corn grown in soil treated with baits containing this compound, largely because of the tenacity with which the material is adsorbed to soil particles. This was further substantiated by data contained in a report prepared by personnel of the Hazleton Laboratories. However, because of this resistance to leaching, DRC-1327 residues could possibly accumulate in certain soils and become absorbed by future crops planted in the treated areas in instances of repeated soil application or misuse treatment levels. Thus, the rate of loss of 4-aminopyridine from soils (as governed by such factors as leaching and degradation) is of prime importance as to whether the compound would be present in sufficient quantities to be absorbed and translocated by plants.

B. Results and Discussion

The rates at which ^{14}C-4-aminopyridine degraded to $^{14}CO_2$ under aerobic and anaerobic conditions were determined over a 2-month period. These data (0.2%-0.3% anaerobic vs. 42%-60% aerobic) emphasize the importance of soil aeration as related to breakdown of the chemical. Although 42%-60% of the soil-applied ^{14}C-chemical was completely degraded to $^{14}CO_2$, the environmental conditions (oxygen, moisture, temperature) existing during this experiment were ideal for breakdown. Additional degradation studies under other climatic conditions are planned in order to satisfactorily predict the persistence of 4-aminopyridine in soils.

Anticipating future registration of DRC-1327 as a bird damage control agent in sorghum, studies concerning the absorption, translocation, and degradations in this crop are in progress.