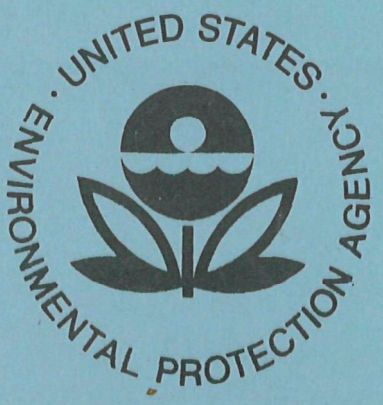
EPA 560/5-75-004

April 1975

LABORATORY TEST METHODS TO ASSESS THE EFFECTS OF CHEMICALS ON TERRESTRIAL ANIMAL SPECIES



FINAL REPORT

Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460

April 1975

LABORATORY TEST METHODS TO ASSESS THE EFFECTS OF CHEMICALS ON TERRESTRIAL ANIMAL SPECIES

Prepared by
Ryckman/Edgerley/Tomlinson & Assoc. Inc.
12161 Lackland Rd.
St. Louis, Missouri 63141

Prepared for
Office of Toxic Substances
U.S. Environmental Protection Agency
Washington, D.C. 20460
Contract/Order No. 68-01-1896
Project Director
Mr. Frank Kover

This report has been reviewed by the Office of Toxic Substances, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

TABLE OF CONTENTS

		Page No.
Letter of Transmittal		i
Title Page		ii
Table of Contents		iii
List of Tables		vii
List of Figures		ix
Abstract		xii
Acknowledgments		xiii
SECTION		
I	CONCLUSIONS AND RECOMMENDATIONS	1
II	INTRODUCTION	2
	Toxic Substances and the Environment	2
	Toxicity Testing: An Historical Perspective	2
	Pertinent Legislation	3
	Methodology Used in this Study	4
III	SPECIAL PROBLEMS	5
	Problems in Toxicity Testing	5
	Variations in Experimental Design	5
	Homeothermic vs. Poikilothermic	5
	Sex, Weight and Age	5
	Random Sampling Variation	6
	Variations in Breeding and Holding Environs	7
	Genetic Variations	8
	Non-Genetic Variations	8
	Genetic Drift	9
	Resistance	9
	Mechanisms	9
	DDT Resistance - A Case Study	11
	Natural Selection	11
	Testing of Dynamic Ecological Associations	12
	Experimental Design of Test vs. Cost	13
	Wild Native Species vs. Domestic Laboratory Species	14
	Chemical Problems in Testing: Degradation	15

TABLE OF CONTENTS
(continued)

		Page No.
	Nonbiological Oxidation, Reduction and Hydrolysis	15
	Nonbiological Miscellaneous Reactions	16
	Biological Oxidation, Reduction and Hydrolysis	16
	Dosages	17
	Biostatistics in Toxicological Testing	17
	Introduction	17
	Randomization	17
	Sample Size	18
	The Normal Distribution	18
	Tests of Hypotheses	19
	Linear Regression and Correlation	19
IV	ANIMAL SUITABILITY	21
	Introduction	21
	Establishment of Animal Groupings	21
	Animal Suitability Tables	22
	Invertebrates	26
	Helminths	26
	Mollusca	29
	Arachnida	31
	Orthoptera	35
	Hemiptera-Homoptera	38
	Coleoptera	40
	Lepidoptera	42
	Diptera	45
	Hymenoptera	50
	Miscellaneous Invertebrates	52
	Vertebrates	53
	Amphibians and Reptiles	53
	Anurana	55
	Chelonia	62
	Squamata	64
	Birds	69
	Pelecanidae and Phalacrocoracidae	73
	Gaviiformes and Podicipediformes	75
	Anseriformes	76
	Procellariiformes	86
	Ardeidae and Threskiornithidae	88
	Gruidae and Rallidae	90
	Scolopacidae	91
	Lari	93
	Picidae	95

TABLE OF CONTENTS
(continued)

		Page No.
	Falconiformes and Strigiformes	96
	Columbiformes	100
	Colinus virginianus	104
	Phasianus colchicus	107
	Miscellaneous Galliformes	109
	Passeriformes	111
	Domestic Galliformes	121
	Galliformes: *Coturnix coturnix japonica*	124
	Exotics	129
Mammals		132
	Insectivora	135
	Chiroptera	140
	Lagomorpha	143
	Rodentia	148
	Carnivora	176
	Ungulates	187
	Primata	205
	Marsupiala	210
	Edentata	213
	Exotic Species	216
Suggested Species		219
	Final List	219
	Selection of Animal Species for Testing	221
V. METHODOLOGIES		225
Introduction		225
	General Scientific Procedure	225
	Selection of Categories	226
	Physical/Chemical Tables	226
Physical/Chemical Methods		239
	Ciliary Transport	239
	Stress	239
	Respiration	240
	Enzymes	241
	Anesthesia	242
	Telemetry	242
	Observe/Monitor	242
	Population Dynamics	243
	Chromatography	244
	Residues	245
	Bioassay	245
	Metabolism	246

TABLE OF CONTENTS
(continued)

	Page No.
Genetics	246
Anticholinesterases	247
Hepatic Studies	247
Endocrinology	248
Anatomy	248
Physiology	249
Histology	249
Pathology	250
Pharmacology	251
Hematology	251
Oology	252
Embryology	252
Other	253
Behavioral Methods	254
Introduction	254
Repellents	254
Discrimination	262
Stress	266
Pheromones	267
Neurophysiology	267
Other	268
Instinct	270
Learning	276
Suggested Methodologies	279
Introduction	279
Suggested Approaches to Toxicity Testing	279
General Studies	283
The Selection of Methods for Toxicity Testing	283
State-of-the-Art	284
The Future	285
VI. APPENDICES	
A. Contacts and Consultants	
B. List of Suppliers	
C. Suggested Species Synopsis Sheets	
VII. BIBLIOGRAPHY	

LIST OF TABLES

Table No.		Page No.
1	Suitability of Invertebrates for Toxicological Testing	25
2	Composition of an Artificial Medium Used for Rearing Noctuid Species	43
3	Suitability - Herpetofauna	54
4	Food Recommended for Amphibian Larvae Young Animals and Adults	57
5	Natural Breeding Habits of Common North American and European Anura	58
6	Suitability of Birds for Toxicological Testing	70
7	Distribution of Indigenous Breeding Waterfowl Species by Zoogeographic Regions	77
8	Incubation Periods of Various Waterfowl	81
9	Fledging Periods of Various Waterfowl	82
10	Some Longevity Records for Waterfowl	84
11	Suitability of Mammals for Toxicology Testing	133
12	Composition of an Experimental Creep-Feed	145
13	Breeding and Rearing Characteristics of the Wild Rats	153
14	Components of the Standard Laboratory Feed Used for Maintaining Microtus ochrogaster in the Laboratory	164
15	Reproduction of M. ochrogaster in the Laboratory at Different Temperatures	165

LIST OF TABLES
(continued)

Table No.		Page No.
16	Reproduction of *M. ochrogaster* in the Laboratory on Different Light Schedules	165
17	Reproduction in Mustelidae Under Natural Conditions	181
18	Growth Ration	193
19	Gestation Ration	194
20	Maintenance Ration	195
21	Artificial Diets for Baby Pigs	196
22	Final List of Suggested Species	219
23	List of Suggested Species from Selected Terrestrial Animal Groups	220
24	Ecological Roles of Suggested Species	223
25	Methodology: Physiological	228
26	Methodology: Behavioral	255
27	Selected Approaches to Toxicity Testing	280

LIST OF FIGURES

Figure No.		Page No.
1	Web Building Enclosure	32
2	Mallard Test Chamber	78
3	Raptor Cages Converted into Mallard Cages	78
4	Mallard Cages (Base 4' x 4')	79
5	Barn Owl Cages of Chicken Wire and Wood, 50' x 6' x 10', with Nest Boxes as Shown	97
6	Nest Box for Barn Owl Cages	97
7	Mourning Dove Cage	101
8	Food and Water Distribution System for Mourning Dove Cages	101
9	Bobwhite Quail Outdoor Holding Pens (Base approximately 4' x 8')	105
10	Starling and Wild Bird Cages	117
11	Wild Bird Traps - Birds Trapped Include Starlings, Sparrows, Robins and Crows	117
12	Coturnix Laying Cages (12" x 10" x 16")	125
13	Coturnix Incubation Chamber	125
14	Coturnix and Mourning Dove Testing Cages (10" x 10" x 12")	126
15	Miniature Swine Transport Apparatus	190
16	Miniature Swine Observation Enclosures (20" x 30" Floor Area)	190
17	Miniature Swine in Holding Pen (8' x 12' Floor Area)	191
18	Deer Holding Pen	202
19	Primate Cages - Squirrel Monkeys - Metal - Commercially Available	206

LIST OF FIGURES
(continued)

Figure No.		Page No.
20	Plexiglas and Metal - Fabricated at Iowa State	206
21	Plexiglas Cage, Fabricated at Iowa State University	207
22	Trophic Triangle	222
23	The Interrelationships of Testing Methodologies	238
24	Diagram of Visual Acuity Chamber	263
25	Cutaway of a Visual Cliff Used as a Test Apparatus	264
26	Discriminatory Device Used in Primate Testing	265
27	Inside of Visual Discrimination Chamber for Primate Testing	265
28	Wiring Diagram of Traffic Counter. Mountain Beaver Burrow with Pictorial Drawing of Burrow Probe in Place	269
29(a)	Runways with Frightening Device at End	271
29(b)	Runway Mechanism for Operating Frightening Device	271
29(c)	Runways, Frightening Device, and Camera Used to Record Chick's Response	271
30(a)	Enclosure for 10 Runways	272
30(b)	Runway with Speaker at Far End	272
30(c)	Holding Area - Point of Release	273
31(a)	Examination Table Prior to Photographing Web	274
31(b)	Examination Table Prior to Photographing Web	274

LIST OF FIGURES
(continued)

Figure No.		Page No.
32	Apparatus for Photographing Web	275
33	An Automated Method for Studying Aggression in Primates	277
34	Succession of Toxic Response	282

ABSTRACT

This report presents a review of test specics and methodologies utilized in toxicological research on terrestrial animals. It involved an extensive survey of available literature, interviews with toxicologists, physiologists and ecologists, and visits to research facilities around the country. The report contains reviews of each major animal group and individual species where specific information was available, that have been used as test subjects in past or present toxicological research programs. Suitability for use in such testing, both actual and potential, and general information relative to laboratory maintenance and possible alternative species is presented for each group. Brief synopses of general characteristics for each group and/or species are also included. Existing methodologies used in toxicological research are discussed and evaluated for their particular applicability to testing programs and general recommendations for approaches to toxicity testing, test animals, and methods are made.

This report was submitted in fulfillment of Project Number , Contract Number 68-01-1896, by Ryckman/Edgerley/Tomlinson and Associates, Inc. under the sponsorship of the Environmental Protection Agency. Work was completed as of April 1975.

ACKNOWLEDGMENTS

The investigation, data handling and report preparation were performed by RETA and consultants. Primary contributors were R. Matter, Project Scientist, W. Zegel, Project Manager, E. Edgerley, Project Principal, L. Hathaway, T. Anderson, K. Cherryholmes, R. Bessent, K. Schaffer, T. Weyenberg, M. Kane, C. Teig and B. Skwiot. C. Hamlin, P. Braden, E. Ratz and M. McReynolds are given special acknowledgment.

A special debt of gratitude is owed to those scientists and technicians from numerous university research teams and private industries who gave of their time, ideas and information.

Keith Long, Director of the Institute of Agricultural Medicine, University of Iowa, who reviewed the draft document and whose comments and interests were very beneficial to the RETA staff is acknowledged with sincere thanks.

RETA extends thanks to the EPA, Washington, D. C. personnel including M. Prival and F. Kover for their interest and schedule considerations.

SECTION I

CONCLUSIONS AND RECOMMENDATIONS

Selection of test organisms should depend primarily on the following criteria:

1. The trophic level at which the test substance is known to effect a reaction, either directly or indirectly.

2. The general habitat in which the test substance is used.

3. The availability of the organism and level of background information on the prospective test species.

4. The degree to which the test species represents a trophic level and accommodates the testing methodologies of the program.

Optimally, multispecies test programs in which a number of trophic levels are represented should produce results of greater ecological significance. Such programs, though more difficult to design, fund and manage than traditional single species tests, will hopefully illustrate composite effects of test substances on ecosystems instead of individual species.

Toxic substances can only be termed detrimental if they adversely affect the reproductivity of a species. Therefore, methodologies used in toxicity testing should be selected for their ability to show short or long-term effects on the survival of the test species (e.g., generation studies at subacute dosages). If this approach is followed, morbidity and functional studies, biochemical analyses and residue determinations are significant only to the extent that they aid in projecting or precisely determining the eventual effects of the test substance on the species' survival (short and/or long-term). Ultimately, toxicity testing results should provide an indication of the relative health of the ecosystem.

SECTION II

INTRODUCTION

TOXIC SUBSTANCES AND THE ENVIRONMENT

This report reviews terrestrial animal species and existing testing methodologies to assess their suitability for use in toxicological testing. A toxic substance has been defined as a man-made chemical or man-caused concentration of chemicals which may come in contact with living organisms other than man, either accidentally or purposely, on a small or large scale. This definition has been used to avoid the problematic aspects of the concept of toxicity. For example, many naturally occurring compounds (certain plant fluids and animal secretions) are toxic if ingested or touched.

Toxicity is incremental, depending on such factors as the concentration of the substance in question, the contact organism and its immune systems, sensitivities, and tolerance levels, and on routes of exposure. Some compounds, which are essential to living systems, are beneficial at low concentrations and lethal at high concentrations (e.g., salts and trace elements). The effects of toxic chemicals can also be very slight and only discernible after long periods of time, even several generations. In wild animals the level could be so low as to be unmeasurable, but still affect the ability of the creature to survive in a natural system.

A more thorough and sophisticated testing system is required to overcome these difficulties in assessing toxic substances. This report provides the basic data on selection of test species and methods necessary to develop this system. It presents the judgments of the scientific community on the suitability of certain animals and methods for use in toxicological testing and, in that light, attempts to determine the adequacy of present testing procedures.

TOXICITY TESTING: AN HISTORICAL PERSPECTIVE

Modern toxicology is considered to have begun with the work of M. J. B. Orfila (1787-1853) who investigated toxic chemicals and their effect on physiology. His two-volume treatise served as a basic reference for many years. Since

Orfila's time, the science of toxicology has grown steadily and incorporated discoveries and technology from chemical and biological disciplines.

A rapid evolution in the science of toxicology has occurred in the past few decades. In addition to the older LD_{50} and percutaneous tests, teratogenic, carcinogenic, biochemical, and behavioral methods have been introduced as more efficient and effective ways of quantifying and defining toxicity. This evolution has been provoked primarily by the increasing amount of chemicals introduced by man into the environment.

PERTINENT LEGISLATION

Toxic substance control legislation deals primarily with pesticide useage. The Federal Food, Drug and Cosmetic Act of 1938 is the cornerstone of all present pesticide legislation in the U.S. This act deals primarily with the controls placed on useage of economic poisons in the natural environment. In 1955, an amendment to the Act defined an orderly procedure for establishing pesticide tolerance levels and provided penalties for violations (1).

The welfare of animals used in biological research is controlled by Public Law 89-544, enacted in 1966 (2), and its amendment, Public Law 91-579 of 1970 (3). The purpose of the original Act was to establish the Secretary of Agriculture's authority to license and regulate suppliers of laboratory animals. The Act as amended in 1970 states:

> The Secretary shall promulgate standards to govern the humane handling, care, treatment, and transportation of animals by dealers, research facilities, and exhibitors. Such standards shall include minimum requirements with respect to handling, housing, feeding, watering, sanitation, ventilation, shelter from extremes of weather and temperatures, adequate veterinary care, including the appropriate use of anesthetic, analgesic or tranquilizing drugs, when such use would be proper in the opinion of the attending veterinarian of such research facilities, and separation by species when the Secretary finds such separation necessary for the humane handling, care, or treatment of animals.

As of 1971, legislation concerning toxic substances (cadmium, mercury, miscellaneous heavy metals, PCB's, etc.,) had been promulgated by a number of different federal agencies. Section 12 of the Federal Water Pollution Control Act passed

in 1970 is concerned with toxic materials discharged into navigable waters. The Clean Air Amendments of 1970 contain a section directly related to hazardous substances. The Department of Transportation regulates transport of hazardous materials with legislation such as the Transport of Explosives Act and Hazardous Cargo Act (4). Current legislation applicable to pesticides includes the Federal Environmental Pesticide Control Act of 1972. Major concerns of this act are registration of pesticides under EPA guidelines, statements of use, and unlawful acts in relation to pesticide sale, distribution and use.

METHODOLOGY USED IN THIS STUDY

Data presented in this report were gathered from a variety of sources by several means. First, a literature search was conducted in over 120 scientific journals. Further relevant literature sources were identified and obtained from bibliographies and from interviews with scientists conducted by phone. In addition to phone contacts, a number of visits were made to particularly interesting and relevant research facilities where information was gathered using sketches, photographs, further interviews and, in some cases, actual observation of testing procedures. Finally, outside consultants and correspondence with the Environmental Protection Agency offered direction and review throughout the preparation of this document. (A list of contacts and visits can be found in Section VI.)

SECTION III

SPECIAL PROBLEMS

PROBLEMS IN TOXICITY TESTING

Special problems inherent in toxicological testing revolve around the ramifications of experimental manipulations of biological systems. Such deliberate interventions by toxicologists are termed treatments. A direct result produced by a specific treatment is described as an effect. The primary goal in designing and conducting a toxicological experiment is to ensure, insofar as possible, that no factor other than the treatment will contribute to the observed effects. Unfortunately, this ideal situation is almost impossible to attain due to extraneous influences. Therefore, it is imperative to insure that all influences except those of the treatment will act equally on the test (treated) animals and the control animals (those exposed to identical conditions excluding the treatment).

VARIATIONS IN EXPERIMENTAL DESIGN

Homeothermic vs. Poikilothermic

The predominance of homeothermic animals in toxicity testing is immediately apparent in a review of the literature. There seems to be greater scientific knowledge pertaining to the physiology, metabolism, biochemistry and behaviorisms of homeotherms than there is for these same parameters in poikilotherms. Poikilotherms, however, pose a special problem in toxicity testing. They must be maintained in a carefully controlled environment since their metabolic rate is a function of environmental temperatures. Environmental control is important to all toxicity testing since inadmissable experimental error would be produced if the metabolic rate of the test animal fluctuated from one exposure of the toxin to the next.

Sex, Weight and Age

Sex, weight and age variations influence many toxicity tests. Researchers using mammals and primates tend to use males because of the problems encountered with the estrous and menstrual cycle of the females. Not only do these cycles arouse the male animals being kept in the

same laboratory, but they also greatly affect the physiology of the two groups. However, it is not scientifically sound to extrapolate male toxicity data to females (5). The fluctuations in hormone levels, coupled with the nervous stress created by the estrous and menstrual cycles, may greatly influence the results of a specific toxicity test.

Unless the toxicity test is aimed at postnatal toxicology, age variation is often introduced since test animals are generally selected from a stock of sexually mature animals (6). The very old animal is almost always excluded from toxicity testing. Closely related to age is the weight of the test animal. When the test animals are sexually mature, their weights are comparable within a certain deviation. Test animals that are procured through animal supply houses are supplied with a controlled, well-balanced diet, and are relatively uniform in weight. Undernourished or extremely obese animals are normally excluded from laboratory supply house stocks.

Age, weight, and sex must be considered when interpreting toxicity test results. The very young animal does not respond (detoxify) to toxic materials nearly as fast as the sexually mature, while the very old animal has even less ability to detoxify poisonous materials. Weight can be the key factor in determining a test animal's tolerance of a certain poison. The undernourished as well as the extremely obese animal is much more prone to a high mortality rate with a given toxin than a well nourished animal of the same species (5).

Random Sampling Variation

Random sampling can be defined as selecting individuals in such a manner that every animal in a population has an equal and independent chance of being chosen. Unfortunately, human errors and natural phenomena frequently bias a "random" sample. When researchers are selecting animals from within a species group, it is easy to introduce variation into the sample by choosing potential test animals that are less alert or slower to escape the collector. The collector is also frequently prejudiced by a potential test animal's well being (e.g., healthy looking fur, skin, teeth, etc.). Thirdly, docile animals within a species group are normally selected over pugnacious fellow-members. These selection tendencies could all greatly bias a toxicity test.

In addition to the problem of random selection within a species group, there is a lack of true random selection

between various animal groups. As stated previously, homeothermic animals are generally selected for research much more frequently than poikilothermic animals. Animals that adapt well to laboratory environments are chosen over those animal groups that do not. Size is another factor which will bias the sampling of various animal groups; small sized (rodents) to moderately sized (sheep) animals are preferred over large animals (moose) due to the limitations of laboratory space. Polyestrous animals are more desirable than monestrous animals such as toxicity test animals, especially in chronic studies dealing with a toxin's effect on fecundity and transplacental migration. These kinds of biases affect the true random selection of toxicity test animals. One must also consider the possibility that the effects demonstrated in the laboratory by a wild animal are not necessarily those which a toxin would produce in the animal's natural environment.

Variations in Breeding and Holding Environs

Variations are introduced into toxicological testing by the ease of procurement of certain animals which are potentially good test animals. Researchers have two main avenues to obtain toxicological test animals: biological supply houses, and actual field capture of test animals. "Animals for Research" (7), a publication of the Institute of Laboratory Animal Resources, National Research Council of the National Academy of Sciences, lists 315 biological supply houses throughout the United States that specialize in selling various species of test animals. The animals available through these supply houses fall into two categories: (1) domestic laboratory animals and (2) animals collected in the wild -- 19 phyla of animals from all over the world.

The major problems associated with animals collected from the natural environment are: (1) the high cost per animal, (2) the possibility of collecting diseased animals and (3) the rarity of certain species. It is not uncommon for large terrestrial animals, e.g., Artiodactyla, to cost $200 to $1,500 per animal, depending on the species used. Even the smaller animals, carnivores and rodents, are generally very expensive compared to most domestic laboratory animals. Animals collected in the wild and then brought into animal supply houses often suffer from disease or stress. The animal may have been captured because it was diseased and not as alert as its more healthy brothers. In addition, the stress and fear involved in the capturing can render these animals more sensitive to toxicants than other members of their species in the wild. Thus, these

animals may introduce significant bias into toxicity testing. Many species sought as test animals also fall into the rare or endangered species category. The Endangered Species Conservation Act of 1969, Public Law 91-135, sets very stringent regulations on the use of endangered animal species as toxicological test subjects. The best solution for obtaining an ample supply of native North American terrestrial animals for toxicological testing is to collect animals that are easily bred and maintained in captivity. Collection of these potential wild test species should be confined to geographical areas where environmental contamination is minimal to avoid collecting animals which have developed resistances. If the animal does not lend itself to breeding in captivity or if it is from an area of heavy environmental contamination, it should be viewed with skepticism as a potential test subject. Repeated collection of a certain species from the wild should also be avoided since this may seriously deplete species numbers. It must be kept in mind that a breeding colony of "wild" animals in the laboratory is very different from a breeding colony in nature, and is subject to a loss of the genetic diversity which enables wild populations in the natural environment to adapt to change.

Genetic Variations

Genetic variations involve a chemical rearrangement of an organism's DNA which produces a mutant form. This variation or change may be very subtle (alteration in a single enzyme's activity) or the change may be very drastic such as the critical shortening of the wings on a bird. Mutation is the original source of variation within a biological system, but it is only through many sexual recombinations that a mutation establishes itself in the species. Mutations occur constantly in biological organisms. The average spontaneous rate for a given gene locus is 1-2 mutations per 100,000 genes per generation (8). Of these mutations, the vast majority are of a detrimental rather than of a useful nature. However, to remain viable under continuous environmental changes, it is extremely important for the population to carry this potential for change.

Non-Genetic Variations

There are instances where non-inherited changes will occur in an animal, altering body structures, functions and behavior, without being transmitted to the next gene-

ration. For example, if certain muscles are used very intensively in an animal they become much thicker and stronger; if one organ is removed such as the kidney, the other kidney will increase in size and degree of function. Skin in areas that receive a lot of rubbing or pressure become very thick and impervious. Animals that are exposed intermittantly for short periods to extremely cold weather or perhaps low oxygen concentrations can learn to acclimate, whereas they would have died had they been suddenly introduced to these environmental conditions.

Genetic Drift

Biological variation in a population is greatly reduced if the gene pool is small; some of the more uncommon variations of the population-at-large will not be represented or will be lost. This phenomenon is commonly referred to as genetic drift, which is simply the random loss or gain of genes rather than loss or gain by natural selection in large gene pools.

RESISTANCE

Resistance is a phenomenon resulting from a combination of biological variations and genetic adaptation. In toxicity testing, resistance is the developed ability of a species to tolerate dosages of toxicants which would once have been lethal to 50 percent (LD_{50}) of the individuals within a population.

Mechanisms

The vast majority of data collected on resistance deals primarily with the various economic poisons. An animal's resistance to an economic poison is observed as the progressive inability of a given compound applied at a fixed rate to achieve control on its target organisms. The mechanisms involved in developing resistance to economic poisons are very similar to those used to avert mortality and morbidity from other toxicants encountered by animals in the natural environment.

It has been discovered that resistance can be induced in laboratory test species by exposing the test animals to toxic levels which kill 90 percent of the population, and then breeding the survivors. Ozburn and Morrison (9) have bred white laboratory mice (Mus musculus) in order to develop a strain that after nine generations is 1.7 times more tolerant to DDT than the controls. Dosages of DDT to which these mice are exposed kill the control animals.

Recent toxicological testing confirms that there are four mechanisms working simultaneously or independently to develop resistance: (1) physiological mechanisms that reduce the penetration and transportation of a toxin to the site of action(10); (2) unusual capacities for storing toxins at inert sites coupled with rapid excretion (10); (3) microsomal detoxification (10); and (4) genetic resistance resulting from selective breeding within a population (9). It has been found that reduced penetration and transportation, rapid excretion and microsomal detoxification are manifested in populations of animals that have never been exposed to toxic materials.

Reduced penetration is significant because even a small reduction in the rate with which a toxin enters an animal may enhance the activity of detoxification centers.

Certain animals have capacities for storing toxins in inert areas of their body which prevent toxins from entering metabolic pathways. Chlorinated hydrocarbon pesticides can be assimilated in the adipose tissue of animals having ample adipose reserve. Once isolated in the adipose tissue, chlorinated hydrocarbons exert little if any effect on the animal until the animal is forced to catabolize the tissue. This delayed reaction can be a significant effect of a toxin in a time of stress. The increased excretion of toxins has not been verified. It is believed that certain enzymes convert foreign substances into less toxic materials by making them more water soluble. These enzymatic reactions are accompanied by reduced tubular reabsorption in the kidney which increases urine output.

Microsomal enzyme systems play a primary role in the metabolism of foreign chemicals. Anything which tends to change the effectiveness of this system in metabolizing toxins alters the resistance of the animal. Microsomal enzyme induction is another phenomenon related to resistance. Chlorinated hydrocarbons are very good microsomal enzyme inducers. Chlordane increases the metabolism of hexabarbital aminopyrene and chloropromazine (11).

Research by Webb et al. (6) offers preliminary support for the thesis that genetics play a role in specific resistance processes. Pine mice (Pitymys pinetorum) were exposed to endrin in apple orchards in Maryland and Virginia and then live-trapped and brought into the laboratory and bred. Offsprings of these field-trapped pine mice were held between 60-90 days after birth and then LD_{50} values were established using endrin. It was

the consensus of Webb et al. that the results of their toxicity studies on first generation offspring raised under a pesticide-free environment offered preliminary support for a genetic basis for the demonstrated endrin resistance. Other genetic work by Cory et al. (12) supports the thesis that exposure to environmental levels of DDT induced chromosomal changes in Drosophila pseudoobscura.

DDT Resistance - A Case Study

The most celebrated case of resistance recorded in the scientific literature is that which houseflies (Musca domestica) have developed to DDT. The work of Sternburg and Kearns (13) demonstrated that resistant houseflies have a high titer of DDT - dehydrochlorinase which allows them to dehydrochlorinate DDT to DDE. There is good information to substantiate a correlation between dehydrochlororinase activity and DDT resistance.

It was first believed that only the cuticle of resistant flies was the site of the enzymatic reduction (14); however, later work has demonstrated that most tissues of the resistant flies are active (16). Refined genetic procedures conducted on the housefly by Lovell and Kearns (17) reflected the fact that dechlorination and resistance were genetically linked. It is also the belief of pesticide toxicologists that the development of DDT dechlorinating ability in the housefly is preadaptive, that is to say, it already existed prior to DDT application.

Resistance is important in toxicity testing because the possibility always exists that researchers will select test animals that possess one or all of the resistance mechanisms for a particular toxin. These biased test animals can produce erroneous dose/effect responses. This is at best misleading and could be devastating if, for example, the results are used to set threshold exposure limits.

NATURAL SELECTION

Natural selection involves a combination of environmental and interspecies pressures. Most species produce more offspring than can possibly survive, necessitating a competition for existence. Structural, functional, and behavioral variations in some individuals provide them with advantages over other individuals who lack these distinctions. These variations must be phenotypic before natural selection can occur. If they are, they will make a much greater contribution to the gene pool of

the next generation. Accumulation of a desirable variation within a population generally facilitates the species adaption to its environment, enhancing its chances for survival.

When the ratio of one characteristic to another changes from 1:00 : 1:00 to 1:01 : 1:00 in each generation, it is known that more individuals with this characteristic are surviving than are individuals without it (8). With a selective advantage of 1 in 100, it has been demonstrated that a dominant characteristic will become established in 99 percent of a population in about 1200 generations (18), a rapid evolution rate. When selection has decreased to 1 in 100,000 or more, evolution is relatively slow. The phenomenon of natural selection and its effect on speciation can introduce a great amount of distorted data in toxicological testing. Species collected in geographical areas where natural selection and resistance have influenced the population will be entirely different from the same species collected in areas where the parameters of selection and resistance are dissimilar. Hence, the dynamic processes of resistance and natural selection must be taken into careful consideration when selecting species of toxicological test animals.

TESTING OF DYNAMIC ECOLOGICAL ASSOCIATIONS

An ecosystem may be generally thought of as the biotic community and abiotic environment functioning together as a system. From a structural standpoint there are four constituents in the ecosystem: (1) abiotic substances which are basic elements and compounds of the environment; (2) producers, the autotrophic organisms that are comprised largely of green plants; (3) consumers, the heterotrophic animals that ingest other organisms or particulate organic matter; and (4) the decomposers, the heterotrophic organisms, chiefly bacteria, that break down complex materials from dead biota.

The use of model ecosystems has recently been recognized as being a valuable tool in toxicity testing. The majority of the environmental toxicological testing to date has involved the use of aquatic ecosystem models. Kapoor et al. have constructed several aquatic model ecosystems to assess the biodegradability of pesticides (19).

The use of terrestrial ecosystems in toxicity testing is still in the developmental stages. Terrestrial model ecosystems containing a predator require sizeable enclosures and refinement (20).

A food web is the system of dynamic transfer of food energy from primary producers (plants) through various trophic levels. It has been estimated by ecologists that food webs allow approximately 10 percent of the energy entering one trophic level to pass on to the next higher level. The least complex version of this type of system takes the form of a pyramid, with each successive population receiving approximately a tenth of the energy received at the level below it.

Unfortunately, nature seldom builds communities with such a simple structure and the use of food webs as tools to measure the biological magnification of environmental toxins is not without problems. Almost invariably, energy is not passed along neatly ordered chains, but instead is transferred to a great many organisms through a highly complex sprawling web of pathways. It is this inherent complexity that becomes one of the principal factors to be considered in investigating how toxic materials are disseminated and concentrated within food webs.

Another factor that becomes a problem in using food webs as toxicity test devices is the nature of the various metabolic processes of the members of a food web. Normally less than 50 percent of the products of metabolism go into the construction of new tissue, the remainder is spent as respiration. The crux of the problem is that some substances within a food web are not involved in respiration, and consequently not excreted efficiently. Therefore, these materials may be concentrated in the tissues twofold or more when passed from one population to another (21).

EXPERIMENTAL DESIGN OF TEST vs. COST

The experimental design of a toxicity test dictates the cost of the research project. In toxicity testing, two major considerations are reflected in costs: (1) retrospective versus prospective studies and (2) field investigations. A retrospective study examines the distribution of concentrations of toxins in the physical aspects of an ecosystem and the biological aspects. From this residue data, an attempt is made to assess the impact of the various toxins on the animals collected. Retrospective tests rely very heavily on data that has already been collected and are usually of a shorter duration and are much less costly than prospective studies.

Prospective studies are those that start with a thesis which is to be experimentally tested, e.g., to ascertain

if exposure to 200 ppb of polychlorinated biphenyls (PCB's) in crayfish will have any chronic effect on the fecundity of raccoons fed on these crayfish. In this example, mature raccoons must be used that are known not to have been exposed to PCB's (laboratory reared subjects, perhaps) and they must be fed for one year or more on dosed crayfish containing 200 ppb of PCB's. During this study period the levels of PCB's in the crayfish and raccoon must be closely monitored. Observations must be made on the reproduction capabilities of these test animals. Even if reproduction is accomplished in these animals, it may be necessary as well to observe their offspring for possible effects. It is clear from this example that prospective testing can become highly complex and in general is more costly than retrospective testing.

Field investigations are usually concerned with monitoring toxic residues in terrestrial animals and comparing these levels to those found in the air, water and soil of the animal's ecosystem. Generally, the volume of biochemical analyses, tissue residue level determinations and histopathology examinations required by field studies are fewer than in laboratory studies. Field investigation studies will frequently require the services of a commercial laboratory to conduct the biochemical, histopathological and residue analyses. Although these two characteristics sometimes make field studies less costly than laboratory studies, it is generally the latter type of study that is more expensive. There are unique features which make laboratory studies more costly than field studies: (1) animal maintenance, including veterinary services; (2) original cost of the test animals; (3) sophisticated laboratory instrumentation; (4) large numbers of technically trained personnel; and (5) the fact that they are generally prospective studies (whereas field studies are generally retrospective).

WILD NATIVE SPECIES vs. DOMESTIC LABORATORY SPECIES

A unique group of domestic animals has been bred for use in biological research throughout the world. This group includes white mice and rats of various strains, crickets, chickens, ducks, guinea pigs, hamsters, gerbils, dogs, cats, etc. The majority of these animals reproduce at relatively fast rates, are easily maintained in a laboratory environment, and have been inbred (especially the white mice and rats) to produce a very homogenous, well-defined animal. Inbreeding results in a loss of the genetic diversity and plasticity which a natural population of animals relies on to adapt to changing environmental

conditions, and, in this sense, laboratory animals are no longer representative of wild populations.

Laboratory rats and mice are excellent test animals where the experimental design calls for consistent, genetically similar animals in large quantities. However, using these domestic animals in environmental toxicological testing may bias the results and not really reflect the reactions that a natural population might exhibit. For this reason, new animals, either naturally or captively bred, should be added to each captive colony, periodically introducing new wild-type genes. The results of toxicity testing on animals from populations whose ancestors have been subjected to natural selection would seem to be less biased and much more indicative of the real impact of a toxin on the natural environment.

Wild animal species recommended for toxicity testing are reviewed in detail in the Animal Suitability section of this report.

CHEMICAL PROBLEMS IN TESTING: DEGRADATION

Once released into the environment, chemical toxicants undergo a continual dynamic change with the rate of change being governed by the chemical stability of the toxin. Environmental toxicants, in general, go through two degradation processes: (1) nonbiological transformations and (2) biological transformations.

Nonbiological Oxidation, Reduction and Hydrolysis

Except for a few locations (i.e., certain soil types), oxygen is a ubiquitous force for environmental transformation of toxicants, especially pesticides. The usual reactions of oxygen focus around the free-radical character of its usual triplet electronic state. However, recent research has revealed the significance of the more reactive double-boned singlet oxygen produced by both light and chemical means (22). Photooxidation at aliphatic carbons is a common reaction in some pesticides. However, DDT is normally very resistant to oxidation - either photochemical or otherwise - given normal temperatures and light.

The removal of hydrogen atoms from organic solvents by free radicals is well understood. Photochemical study of pesticides has revealed that energetic irradiation in methanol will lead to the replacement of aromatic ring halides with hydrogens (22). Strictly chemical reductions

by transitional metal ions are probably very widespread throughout the environment, but have not been reported in the literature. A good example of this type of reaction is the reduction of DDT to DDD by iron porphyrins.

Water, like air, is present to some degree almost everywhere in the natural environment. Water has unique chemical properties: it has high heat capacity, polarity, is a good solvent, and is transparent to uv light; thus it is an extremely important medium for chemical reactions. Pesticide hydrolysis occurs very readily and many researchers are surprised that the extent of these environmental transformations has received so little attention (22).

Nonbiological Miscellaneous Reactions

A number of degradation reactions have been reported under various other environmental conditions. It has been demonstrated that dieldrin undergoes an internal cyclization in the presence of light to form photodieldrin (22). Many organophosphorus esters such as the phosphorothionates will isomerize under the influence of heat and light.

Biological Oxidation, Reduction and Hydrolysis

Generally all biochemical oxygenations of pesticides and other toxins are carried out by low-specificity, mixed-function oxidases often localized in microsomes or similar structures.

Reduction of toxins through metabolic pathways is much less obvious than oxidative processes, perhaps because life exists in and on an oxidizing system. The biological reduction of C-C double bonds has been suggested to explain the intermediates observed in the metabolism of DDT and its chlorinated hydrocarbon relatives; however, simple olefins to not display these reduction products.

It has been found that pesticides react with water within living organisms. The hydrolysis of the epoxide ring of dieldrin has provided one of the most important routes for the metabolic degradation of dieldrin. Hydrolysis, however, like many other types of environmental transformations, is not always straightforward. The primary route for the detoxification of DDT in animals is by the hydrolysis of the trichloromethyl group to a carboxyl group.

DOSAGES

Dosages used on test animals in toxicity testing can be critically important in obtaining reliable test results. As discussed under Biological Variation, individuals from a homologous population display variations. The real nature of these variations is seldom obvious except when the organisms are challenged, such as by exposure to a toxic material. For example: if the toxic agent is capable of producing an observable effect (such as death), then the chemical dose can be selected to produce that effect on each test animal in a series of tests using the same species group. However, it has been demonstrated that, if the effect could be measured by means other than death, then the test would show that not all members of the group responded in the same manner.

Another problem associated with dosages is the method used to administer the toxin. Methods include filling gelatin capsules with a prescribed dose, injecting marshmallows with the toxin by using a microsyringe, and putting the toxin dosage into the animals' normal food or water. It is questionable whether the test animal really receives the intended dosage since some of the dosed food may spill or the loaded marshmallows may be unpalatable.

There are four major routes to administer toxicants: (a) percutaneous, (b) inhalation, (c) oral, and (d) parenteral. Within a species and from species to species, these four routes of administration greatly influence the toxicity exhibited. If not taken into careful consideration, they can introduce variation into the testing.

BIOSTATISTICS IN TOXICOLOGICAL TESTING

Introduction

Toxicological studies should be well-designed to assure the collection of unbiased and precise data which are technically defensible and amenable to biostatistical evaluation. Some of the basic concepts and techniques of sampling design and data evaluation by biostatistical methods are discussed in the following paragraphs.

Randomization

In toxicity testing, the experimental animals must be selected with known probability. As mentioned previously in "Variations in Experimental Design," the manner in which a random sample is obtained can introduce a great

deal of variation into the experiment. The careful use of random selection is the only feasible technique by which to satisfy the "known probability" criterion. For example, knowing the probability of selecting an experimental animal makes it possible to extrapolate from the sample to the population in an objective way.

It becomes obvious that there is a fundamental distinction between a "haphazardly-selected" experimental animal and a "randomly-selected" animal. The distinction can be made that a "haphazardly-selected" animal is one chosen with no conscious bias, whereas a "randomly selected" animal is one chosen when there is consciously no bias (the rationale being that there is consciously no bias since the randomization is planned, and therefore bias is planned out of the selection).

Sample Size

In any toxicological study, the number of animals to be exposed and the number of animals to be employed as controls should be decided early. Sample size is important since frequently too many animals are used which is wasteful, or not enough experimental animals are used which results in invalid data. There are two biostatistical techniques which can be employed to ensure adequate random sample size: estimation of a binominal proportion and estimation of a population mean for measurement data.

The Normal Distribution

Due to unavoidable error in the measurement process, all measurements in toxicity testing are uncertain to some extent. There is, of course, only one correct measurement, which researchers can only attempt to estimate by one mere measurement or by the mean of a number of measurements. If measurements of some treatments are repeatedly redone in a specific test, it is found that most outcomes tend to cluster about a central value while a few deviate. A frequency distribution of the results forms a somewhat irregular histogram with the density of measurements being greatest in the center and diminishing to the right and left. It has been observed that the accumulation of more data tends to make the histogram appear less irregular. This type of measurement distribution is approaching a mathematically defined bell-shaped curve known as the Gaussian curve of error, or simply the normal distribution.

The mean is a biostatistical technique that locates the center of distribution of the x-axis of measurement.

In other words, it is the long-run arithmetical average of all x-values.

The statistical variance is useful in describing the amount of data variability or data dispersion which, in turn, determines the distribution curve's bredth. Variance is the expected value of the squared deviation of a measurement from the mean, or simply the long-run arithmetical average of all such squared deviations.

The square root of the variance is known as the standard deviation. The standard deviation is the distance measured along the x-axis, from the center (mean) to the point of inflection of the curve which is the steepest point.

Tests of Hypotheses

In toxicity testing, frequently some aspect of the study is directed to answering a hypothetical question regarding a population. If the hypothesis is quantifiable, then the hypothesis can be tested statistically by either the T-test, Chi-Square Test, or the F-test.

The T-test can be used to compare a sample statistic (i.e., the mean) from a toxicity experiment with some value for the purpose of making a judgment regarding the population as indicated by the sample data.

If there are more than two alternative categories of response known or assumed, the sample methods developed for binomial populations no longer serve. A useful tool that can be applied to various problems involving enumeration data, without regard to sample size, is the Chi-Square Test.

In biostatistics the F-test is used for testing the equality of variance. The computation of the F-values involves the ratio of two variances, each with associated degrees of random.

Linear Regression and Correlation

In various toxicity testing it is often desirable to investigate relationships between different variables, i.e., rate of weight loss in a test animal and the concentration of toxin in blood serum, or mortality of test animals per unit of time and the concentration of polychlorinated biphenyls in the test animals brain, etc. Toxicologists appreciate the complexity of actual world

relationships between variables and those mentioned above; however, toxicologists may also wish to investigate further approximating these relationships with a straight line.

Such an approximation may prove invaluable if used judiciously in toxicity testing within the limits of the conditions where the relation holds. It is important to recognize that regardless of how well the straight line describes the data, a casual relationship between the variables is never implied. Causality is much more difficult to establish than mere description by a statistical relation.

When studying the relationship between two variables, the data may be taken in one of two ways. One way is to measure two variables, e.g., measure body weight and associated blood serum measurements. Where two variables are measured, the data are termed bivariate. The alternate way is to choose the level of one variable and measure the associated magnitude of the other variable.

Straight line equations may be obtained for each of these situations by the technique of linear regression analysis, and if the object is to predict one variable from the other, it is desirable to obtain such a relation. When the degree of (linear) association is to be examined, no straight line need be derived - only a measure of the strength of the relationship. This measure is the correlation coefficient, and the analysis is termed correlation analysis.

Thus, linear regression analysis and linear correlation analysis are two ways in which linear relationships between two variables may be examined.

By judiciously employing the biostatistical techniques described above, the conclusions drawn from toxicity testing can be made much more valid. It must be remembered that all biostatistical methods are based on an abstraction (math model) which is an approximation of some actual world phenomenon. If the approximation is reliable, researchers have a useful tool; if the approximation is unreliable, researchers can easily draw erroneous conclusions.

SECTION IV

ANIMAL SUITABILITY

INTRODUCTION

After determining the suitability of animals or animal groups for use in toxicological testing, species have been recommended in this section as good potential test subjects. The basic criteria for these recommendations are: (a) the availability of the species; (b) the ability of the species to breed under captive conditions; (c) the ease with which it can be maintained; (d) the previous use of the animal in toxicological testing; and (e) the degree to which a species is representative of the group. Most of the species considered are wild native North American forms. Exotic or domestic animals were substituted only if suitable organisms for a specific group could not be found (see Section III - Wild Species Versus Domestic/Lab Species for further discussion).

Establishment of Animal Groupings

The animal groupings reflect natural, taxonomic associations. Certain mammalian taxa were divided or aggregated because of different or similar food habits, or because of size and/or habitat preference. Invertebrates have been aggregated on the basis of phylogeny due to the paucity of research information.

Sample species for each group were used wherever possible and are meant as general guides to the group. In all cases, the text refers to North American members of the animal groups unless otherwise specified and it should be noted that the stated requirements may differ widely from those of non-temperate species. Indeed, animals of the same species from different latitudes can require different maintenance methods.

A format consisting of 12 major categories has been established for the discussion of the animal groups. Where no information was available in a category for a specific group, that section was omitted from the presentation. Previous research is briefly presented under the category of Toxicity Testing. The large amount of information accumulated makes it difficult to describe each study in detail, but they may be located for further information via the list of references.

An effort was made to identify the specific qualities of a species that make it particularly suited for a certain type of test or procedure. The inclusion of information on habitat preference and ecological roles enables the reader to further evaluate the suitability of an animal for a given test or methodology.

Animal Suitability Tables

The suitability of each animal subgroup for use in toxicological testing is described in a table found in each major animal group section. These tables synopsize the characteristics of the animal group discussed in the text, with central consideration afforded the recommended test species in each group. The final section of this chapter summarizes pertinent information on each suggested form and contains a final list of the most suitable species for testing from each group (general use of the suggested species is further discussed in Section V, Suggested Methods).

INVERTEBRATES

Invertebrates, a very large group, include all animals that do not possess a backbone, from the tiniest single-celled creature to highly developed forms like squids, snails, insects, and crustaceans. Despite their enormous variety and number, invertebrates have been scientifically studied far less than vertebrates, partly because they are not as conspicuous as vertebrates or as directly important to man, and because they are considered by many to be "lower" forms of life. Information is available for relatively few species and these are largely pests and otherwise economically important species.

By far the largest group of invertebrates both in numbers of individuals and species is the insects. Insects belong to the phylum Arthropoda and possess jointed appendages and an exoskeleton. They are one of the three animal groups that have attained true flight, a characteristic which separates them from other Arthropods. Three pair of walking legs, compound eyes, tracheal respiration and metamorphosis also characterize insects.

The information on insects is very heavily skewed in favor of pest species and honeybees, because both groups are of major importance in agriculture. The seemingly unimportant species have largely been neglected, but research on these will increase as more importance is attached to evaluating and assessing the effects of chemicals on whole ecosystems.

Invertebrates can be rewarding as well as difficult test organisms. Their life cycles are complex, often involving many distinctive, morphologically different stages which can cause the researcher some difficult maintenance and rearing problems. The limited knowledge of this group hinders research, and the generally small size of its members restricts testing methods. On the other hand, they are important members of the world ecosystem as basic sources of food for many animal species. Furthermore, most are very instinctual and therefore routine and stereotyped in their behavior so that differences or alterations in behavioral norms can be easily measured. In statistical studies, their characteristics make them potentially good test organisms. Finally, the group offers the researcher a vast array of ecological roles. The greatest amount of research to-date is concentrated in genetics (Drosophila) and bioassays, (Musca).

General criteria for selection of a test species include the position of the species in the food chain, its availability, breedability in captivity, ease of maintenance and previous use in toxicity testing. These criteria are summarized for each invertebrate species investigated in Table 1.

TABLE I. SUITABILITY OF INVERTEBRATES FOR TOXICOLOGICAL TESTING

INVERTEBRATES	Availability	Breedability in Captivity	Ease of Maintenance	Previous Use in Testing	Restrictions	General Suitability	Ecological Role	Habitat Preference	Suggested Species
Helminths	G	G	E	F		G	E	Fo Gr	*Lumbricus terrestris*
Molluscs	G	G	G	P		G	D_2	Fo Gr	*Helix aspersa* *Milax* sp.
Arthropods Arachnids	G	P	G	P	No previous breeding work	F	B_2	Fo Gr	*Araneus diadematus*
Insecta Orthoptera	G	G	G	G		G	B_2,D_2	Fo Gr	*Gryllus pennsylvanicus* praying mantids
Hemiptera Homoptera	G	G	E	F		G	D_2	Fo Gr	*Blissus leucopterus* leafhoppers
Coleoptera	E	E	E	G		E	B_2,D_2	Fo Gr	*Tribolium* sp. *Tenebrio* sp.
Lepidoptera	G	G	E	G		G	D_2	Fo Gr	*Carpocapsa pomonella* *Agrotis ypsilon*
Diptera	E	E	E	E		E	D_2	Fo Gr Do	*Musca domestica* *Drosophila melanogaster*
Hymenoptera	E	E	E	G		E	D_2	Fo Gr Do	*Apis mellifera*
Misc. Arthropods	G	F	G	F		G	E, B_2	Fo Gr	Isopods Centipedes

Legend:

General Ecological Role

A - Large carnivore
B - Small carnivore
  1. General
  2. Insectivore
C - Omnivore
D - Herbivore
  1. Seed, fruit eater
  2. Grass, foliage eater
E - Decomposers

Habitat Preference

Fo Forest
Gr Grassland
De Desert
Aq Aquatic
Do Domestic

E = Excellent
G = Good
F = Fair
P = Poor

Sources: - literature cited in text
- interviews of Scientists

Helminths

Introduction -

This invertebrate group is comprised of several phyla, the number depending upon the classification scheme used. Generally, the flatworms (Platyhelminthes), round worms (Aschelminthes), segmented worms (Annelida), and a few other smaller groups (e.g., Onychophora), are recognized as the major divisions (24).

Worms are distributed throughout the world and occupy many types of ecological niches. Earthworms (class Oligochaeta) are fossorial, and ingest organic materials found in the soil. There are also a great number of parasitic worms ranging from the externally attaching, blood sucking leeches (class Hirudinea) to the basically internal, often highly specialized groups (classes Trematoda, Cestoda, and Nematoda). Whether this last group can be classed as terrestrial is questionable. However, they do infect terrestrial organisms to a great extent. Because of the small amount of data available on worms in toxicological testing and the large extent of the group, most will be treated in this section in a general manner.

The only worms used to any extent in toxicity testing are members of the Oligochaeta because of their close association with soils. Most of the following comments pertain to earthworms.

Caging/Lab Conditions -

The common earthworm species, Lumbricus terrestris (family Lumbricidae), is widely used in zoology courses and in research. They are readily available in large quantities from biological supply houses or from bait dealers. However, if greater quality control is desired, earthworms can be easily reared. Three general methods are used: small box cultures that are handled easily; large containers; and open fields. Any size container will do, but for frequent inspection or sorting, a depth of more than six inches is not desirable. Containers should have drainage holes covered with screen. The bottom should be layered with one-half inch of gravel and, if kept outdoors, covers should be provided (25,26). Temperatures of 50°F to 75°F (depending on the species) should be maintained (25).

Nutrition -

Culturing media should be a combination of organic matter and soil. Any combination of peat moss, manure, leaf mold, sod, decaying sawdust, straw, hay and leaves and one-third soil of high organic content will be a sufficient substrate for most earthworms. Other foods, like table scraps, chick starter, or cornmeal should be added periodically. About 40 g of dried food for 300 worms is a sufficient food addition at any one time (26). The substrate must always be moist, but not wet (25) with an approximate pH of 7 (26).

Breeding/Rearing -

If provisions are made for adequate space, temperature (about 50°F for L. terrestris, higher for some other species), and moisture, earthworms will reproduce successfully in a loose substrate of one-part dung, three parts soil, and five parts peat moss (26). Though earthworms are hermaphroditic, an exchange of sperm takes place during conjugation. The fertilized eggs are deposited in the soil in small pea-sized cocoons.

Special Needs -

Moisture and moderate temperatures are necessary. In nature, worms will burrow until the desired conditions are found. Since this may be impossible in small containers, care should be taken to keep their environment satisfactory.

Ecological Role -

Earthworms occur in upper soil levels and feed on decaying organic matter. They are particularly important as soil mixers, aerators and drainers and serve as food for many insectivores (robins, woodcock, mice, shrews).

Problems with Mass Culture -

A bin 4 x 8 x 2 feet will support 50,000 worms, so crowding is seldom a problem when sufficient moisture and food are available (25).

Toxicity Testing -

Tissue residues in earthworms have been monitored extensively in areas where toxic substances have entered the soil (27, 28). The effect of chemicals on earthworm population levels is of great concern and has been investigated under

various conditions (29, 30, 31, 32), including in the open field (33, 34, 35, 36, 37, 38, 39). The U. S. Fish and Wildlife Service has published a thorough review of the effects of chemicals on earthworms (see Reference 40).

General Suitability -

Worms occupy a variety of ecological niches and occur throughout North America. Their diversity, wide distribution, and importance as a food source for many vertebrate species make them desirable test species. Earthworms are particularly valuable because of their role in soil ecosystems, their past use and ease of maintenance.

Ecological Alternatives -

Isopods, snails.

Molluscsa

Introduction -

Molluscs are mainly shelled invertebrates and most are aquatic; however, the pulmonate snails (class Gastropoda) are common terrestrial forms with a modified mantle for air breathing. Certain slugs are also terrestrial and cause crop damage in some sections of the U. S.

Cepaea nemoralis, a snail, Testacella sp. (carnivorous) and Milax sp., both slugs, have been successfully used in lab research (41). Helix aspersa, a nocturnal pulmonate snail found throughout temperate areas of the world, has been successfully bred and reared in captivity since the life cycles of snails and slugs are similar, the comments in this section, which are primarily concerned with Helix aspersa, can be applied to many other species.

Caging/Lab Conditions -

Colonies of Helix aspersa have been kept in 55 x 40 x 28 cm wooden boxes with a 5 cm layer of damp soil. The boxes were covered with aluminum screen. Room temperature (avg. 23°C) is suggested (42). C. nemoralis has been bred in 20 cm flower pots two-thirds filled with soil. Testacella sp. has been bred in corked glass tubes and fed on earthworms. Milax sp. seems to reproduce better under conditions of varying temperature (41).

Nutrition -

Snails in captivity thrive on lettuce and other fresh vegetables (43). Slugs will very probably do equally well on similar foods. Carnivorous slugs do well on a diet of earthworms (41).

Breeding/Rearing -

Helix aspersa has been successfully bred and reared in quart fruit jars containing 5 cm of damp soil (43). The best results occur when only two individuals are housed in each jar (43). Copulation is extended (the average per pair of 62 pairs was recorded as 6.25 hours) compared with that of other animals. The eggs, which are white and about 3mm in diameter, are oviposited in nests prepared in the soil. In one study, the average number of eggs deposited was 53, of which, on the average, 10 hatched. Sexual maturity occurred within four months after birth (43). The photoperiod affects egg-laying (45).

Special Needs -

Constant moisture and suitable temperatures are required for snails to remain active (43). If sufficient moisture is not present, snails will withdraw into their shells and excrete a seal over the opening.

Ecological Role -

Terrestrial snails and slugs are primary consumers and eat a varied diet of plant materials. They are a food source for larger insectivores.

Longevity -

The average lifespan of snails and slugs is unknown.

Toxicity Testing -

Helix sp. have been subjects in drug testing (46, 47, 48, 49) and in open field pesticide studies where general population fluctuations were measured (50). Learning behavior in Helix is described in reference 42.

General Suitability -

Helix sp. is a very widely distributed snail and abundant in certain moist habitats. In terms of rearing, abundance, and present use, it may be the only suitable terrestrial mollusc for use in toxicity testing. However, other pulmonate snails and slugs, with the exception of the carnivores, may prove to be equally suitable after preliminary maintenance procedures are worked out.

Ecological Alternatives -

Earthworms, some herbivorous insects.

Arachnida

Introduction -

Spiders, mites and ticks belong to the class Arachnida in the phylum Arthropoda. Members of this class are primarily terrestrial and possess two main body segments. The spiders, mites and ticks have four pairs of legs on the cephalothorax and no abdominal appendages. They have compound eyes but lack antennae. Many Arachnids have poison glands and some spiders and ticks are able to produce silk from special glands in the abdomen. Other members of this class are scorpions and harvestmen.

Caging/Lab Conditions -

These invertebrates can be very easily maintained in almost any container from individual glass tubes to covered terraria and fruit jars. Mites and ticks are parasitic and can be kept with their host in its enclosure. However, the life cycles of ticks vary, some requiring only one host and some up to three different host species, with maintenance conditions changing accordingly. Boophilus sp. and Dermacentor sp. are examples of some species that have been used in the laboratory (51).

Spiders are also relatively easy to keep in the laboratory. Dr. Peter N. Witt and his colleagues from the North Carolina Department of Mental Health have very successfully used web patterns in orb-weaver spiders to test the effects of drugs. Araneus diadematus is their best subject. (see Toxicity Testing for details). Special 50 x 50 10 cm aluminum enclosures (See Figure 1) with removable glass windows and provision for air circulation were constructed for web-spinning. To encourage nightly spinning, a photoperiod of 13L:11D has been used. A temperature change (colder at night) is also important (52).

Nutrition

The feeding of mites and ticks often requires a host. All their nutritional requirements are met by the blood meals they extract (51). Spiders are fed flies and mealworms. They require water, which can be provided by spraying a ball of cotton placed in the cage or, in lab reared individuals, administered by a syringe placed up to the animal's mouth (53).

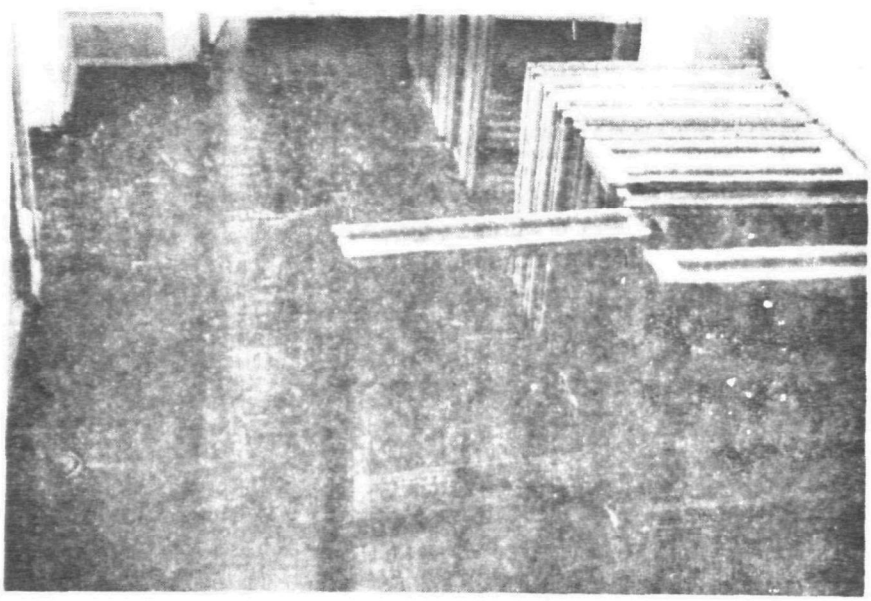


Figure 1. Web building enclosure.
Raleigh, NC

Source: (52,53)

Breeding/Rearing -

Breeding mites and ticks is simple when the appropriate host is provided. The instar stages vary in number and length, but if their natural host is available, a continuous supply of organisms can be produced. Female ticks usually fall from the host after engorgement and mating to lay their eggs, necessitating some method of collection and housing (such as glass vials) (51). Success at breeding spiders in captivity has been minimal, probably due to the lack of effort. Most supplies of spiders are acquired by gathering cocoons in the fall and hatching the young in the lab (53).

Special Needs -

The special needs of parasitic mites and ticks have been described above under Caging/Lab Conditions. Spiders need a cold-dark, warm-light rhythm in order to build webs nightly. Water should also be given frequently (53).

Ecological Role -

Mites and ticks are parasitic on plants and animals, deriving their sustenance directly from the fluids of their hosts. Spiders are carnivorous invertebrates whose food consists entirely of small animals, primarily insects. All Arachnids are potential food sources for insectivores.

Longevity -

The lifespan of mites and ticks are relatively unknown. Spiders of the genus Araneus can live up to 18 months (53) and Tarantula and wolf spider life spans are measurable in terms of years.

Problems with Mass Culture -

Spiders need more research on breeding before they can be mass-bred under controlled conditions.

Toxicity Testing -

Because certain mites are serious crop pests, a significant amount of toxicological research has been conducted using these mites as test species (54, 55, 56). Spiders have been used in drug testing to some extent. Since some groups build very uniform webs, the researcher has a very

discriminating parameter for measuring drug effects. The application of this technique to the testing of toxic substances seems rather simple (53).

General Suitability -

Mites and ticks are easily maintained under controlled conditions and because many derive their substenance from the fluids of their hosts, they might be used in a variety of ways to monitor the host.

Spiders are excellent test subjects because they are predators on many insects, they are relatively easy to maintain, if not to breed, and their web-building provides a very useful experiment tool. Araneus diadematus has been a most successful web-weaver and quite common in the Eastern U.S. Mr. Leonard Pankhurst, 204 Stroud Street, Canastota, New York, 13032, supplies this species in limited quantities (52).

Ecological Alternatives -

For spiders, centipedes.

Orthoptera

Introduction -

This order includes many large and well-known insects: crickets, grasshoppers, roaches, locusts, and praying mantids. Most are plant feeders and can be very destructive to man's crops. A few species are predacious (57). The Orthopterans usually have wings (two pair) and their mouthparts are of the chewing type. Auditory stimuli are important for intraspecific communication and oval eardrums, or tympana, are the receptor organs. Stridulations (the songs) are the results of rubbing certain body-parts together (e.g., hind femora and wing).

Most past toxicological research has been done on wide-ranging and easily obtainable species, the common cockroach (Periplaneta americana), the field cricket (Gryllius pennsylvanicus), the locusts (Schistocera gregaria and Locusta migratoria), and the Mantids (Mantis sp., Stagmomantis sp., Tenodera sp.).

Caging/Lab Conditions -

Crickets, G. pennsylvanicus, have been housed in a great variety of containers. Successful breeding has occurred in cages with a moist sand substrate to facilitate oviposition. Stock cages 42 x 18 x 18 inches made of aluminum screen will house 250 adults. Five hundred nymphs can be reared together in an 11 x 7 x 8 inch glass aquarium. A 16-hour photoperiod at a temperature of about 24°C and a relative humidity of 60 to 65 percent are appropriate conditions for rearing crickets (58).

Roach colonies have been kept in larvae cages 40 cm high by 20 cm in diameter with plastic sides, metal bases and perforated lids. The ten hardboard platforms provided added surface area and relative seclusion for the roaches. A 70 percent relative humidity and a temperature of 27.5°C were maintained for the colonies. Any enclosure with layered surfaces to increase the area should be adequate for roach maintenance (59).

Locusts and grasshoppers have been housed in many types of enclosures. Electric lights of 25 watts are suggested as a heat source (60).

Praying mantids can be kept at 75° to 88°F and at a relative humidity of 50 to 70 percent, but after the fifth molt they become cannabalistic and should be separated (61).

Nutrition -

The main diet of crickets in one lab consists of a mixture of biscuit meal, dried milk, Bemax and dried yeast in a ratio of 50:5:40:5. Fresh greens are provided about once a week. A constant supply of water is required (62).

Cockroach diets can have many components. Table scraps, dog food, and rat food are suggested. Water should be constantly available (59).

Fresh grass and dry wheat bran are the components of one diet for locusts. Water is necessary at all times (60).

Praying mantids will eat almost any living insect of an appropriate size (61).

Breeding/Rearing -

Some crickets breed all year long, while others have but one generation per year (58). For all types, moist sand should be provided for oviposition and the eggs removed to petri dishes for hatching. Incubation takes 15 days at 24°C but hatching can only take place after a three month diapause period at 4°C (62).

Cockroaches oviposit their eggs in dried food mixtures, from which they can be removed with a sieve and incubated at a relative humidity of 70 per cent (59).

In the lab, locusts lay eggs in moist well-packed sand. The pods can be removed and incubated at 32°C and will hatch in 11 to 25 days, depending on the species (60).

All Orthopterans have simple metamorphosis: the nymphs hatch from the egg as miniature adults except for the wings and achieve adulthood by periodic molts.

Special Needs -

Most insects need high humidity and a constant supply of water.

Ecological Role -

Crickets and roaches are omnivorous insects and will feed on many kinds of organic matter. Locusts and grasshoppers are vegetarians and can occur in very large numbers, sometimes defoliating the countryside. Praying mantids are predators and feed primarily on insects. All of these species are possible food items for insectivorous invertebrates and vertebrates.

Longevity -

Few representatives of this order live longer than one season as adults.

Problems with Mass Culture -

Adult praying mantids cannot be housed together for they tend strongly toward cannabalism.

Toxicity Testing -

Cockroaches are extensively used in toxicity testing in a great number of ways, e.g., effects of pesticides on enzymes (63, 64, 65) and the nervous system (66). Orthopterans have been used in studies of metabolic reactions (67, 68, 69, 70) and in open field studies (71).

General Suitability -

The insects in this order are easily maintained and very abundant. Roaches, grasshoppers, crickets and praying mantids are available commercially from suppliers. Praying mantids, being strictly carnivorous and relying heavily on insects for food, might accumulate certain chemicals or be more heavily exposed to target animals. This ecological role might make them especially interesting test subjects.

Ecological Alternatives -

For crickets, leaf hoppers. For praying mantids, carnivorous beetles.

Hemiptera-Homoptera

Introduction -

Hemipterans are true bugs. Their upper half-wings, or hemelytra, are proximally tough and leathery, and distally membranous. Their mouth parts are the sucking-piercing type, enabling them to extract vital juices from plants and animals (57). The Homoptera are closely associated with the bugs and share many characteristics with them. A distinct difference is implied in their name: both pairs of wings are similar and entirely membranous. However, some members of this group are wingless. All Homopterans are plant feeders (57). Both orders undergo simple metamorphis.

Caging/Lab Conditions -

There are no special requirements for rearing most of the common species of Hemiptera and Homoptera, though the life cycles of some groups may present difficulties (e.g., cicadas). Milkweed bugs (Oncopeltus fasciatus) can be reared in small glass tubes.

Nutrition -

In natural situations all of these insects utilize the fluids of many kinds of plants and animals.

Ecological Role -

Hemipterans and Homopterans are feeders on organic fluids, primarily plant juices. They can be destructive agricultural pests. These insect groups are food for insectivorous invertebrates and vertebrates.

Longevity -

It is doubtful that most adult Hemipterans and Homopterans will live longer than one year. Cicada larvae however, care long-lived and could provide a unique means for toxicity in soil ecosystems.

Toxicity Testing -

Chinch bugs (Blissus) are serious pests of cereal crops. The effects of chemical control measures on their numbers have been studied (72). Assassin bugs (Triatoma) are blood-sucking insects which transmit a Trypanosome known as Chagas disease in South America. The effects of chemicals on this species have also been studied (73, 74).

Aphids or plantlice are a large group of Homoperans and frequent pests on vegetation. Toxicity testing with aphids have been concerned with their control (75, 76).

General Suitability -

The herbivorous aphid species are good selections for use in studies of environmental contaminants that may accumulate or deposit on vegetation.

Ecological Alternatives -

For aphids, herbivorous invertebrates.

Coleoptera

Introduction -

About 40 percent of all known insects are beetles. They comprise the largest order of animals, with well over 25,000 species in the U.S. alone. The sizes, habits, and ecological niches of beetles vary greatly. Some are phytophagus, some predacious, some scavengers, some saprophagus, and others parasitic on plants and animals, Beetles are found almost everywhere in freshwaters and on land (57). The Coleopterous (sheath-wing) insects have four wings, the top pair a thick, hard set (elytra) to protect the membranous under set. Mouthparts are basically of the chewing type though some are modified for sucking. Metamorphosis is complete. The life cycles vary considerably within the group (57).

Beetles that have been used in research are frequently pest species, though not exclusively. Included are ground beetles, [Harpalus, Agonum (77), Feronia (39)], lady beetles, [Hippodamia, Coleomegilla (78, 79)] and flour beetles, [Tribolium (80)]. Mealworms, Tenebrio sp. are commonly reared as food for insectivorous animals (81).

Caging/Lab Conditions -

Depending on their size and habits, beetles are relatively easy to maintain under controlled conditions. The proper temperature, 25° to 30°C, and humidity, 50 to 65 percent, are essential to all insects. Insect breeding may depend on the photoperiod. A list of species bred in Great Britain, their nutrition and temperature requirements is found in reference 81.

Nutrition -

The food habits of beetles are so varied that a general statement about them would be useless. A close matching of natural foods has been the general system in animal maintenance and sould be acceptable for beetles. For a large number of pest species, artificial diets have been determined and a keyed list of them can be found in reference 81.

Breeding/Rearing -

Many species are easily bred in captivity, given the proper medium e.g., grain weevils (Sitophilus) on cereal grains,

or mustard beetles (Phaedon) on potted cabbage, turnips or radish plants. Tenebrio sp. can be bred year round with great ease (81).

Ecological Role -

Some beetles are pests on agricultural crops and other are predacious ground dwelling species (e.g., Harpalus) (82). Others feed on fungi and carrion. All beetles are potential food for insectivorous invertebrates and vertebrates.

Longevity -

Like most other insects, the generation time of beetles is seldom longer than a year and it is unusual when adults live for more than one year.

Toxicity Testing -

Insects are the primary pests which afflict man and his domestic stock and crops, and millions of dollars are spent yearly for insect control. By far, the majority of toxicity testing using beetles has been concerned with pest species. Pesticide residues in many species have been studies including Popillia, (83, 84, 85); Tribolium (80); ground beetles (36, 39, 77, 82); and lady beetles (78, 79). Large numbers of toxic chemicals have been tested on beetles (78).

General Suitability -

Beetles are an ecologically diverse group which generally appears to be attractive for use in toxicity testing.

Ecological Alternatives -

For ground beetles, centipedes. For scarab beetles, millipedes. For plant eaters, Hemiptera and slugs.

Lepidoptera

Introduction -

Butterflies and moths are conspicuous and well-known insects, among which are some of the most beautiful animals we know. However, the adults that please the eye are often metamorphosed from destructive phytophagus larvae that are major insect pests of cultivated plants. The adult mouth parts are the sucking type and metamorphosis is complete in the species of this order. The larval forms can produce silk for cocoons and shelter (57).

Many Lepidopterans have been reared under lab conditions. Three general methods - two for pest species and one for nocturnal moths (the largest family of Lepidoptera) will be discussed.

Nutrition -

Some Lepidopteran larvae are general feeders and others are very specific, feeding on one plant species exclusively. Lab diets must reflect these natural habits and requirements.

Breeding/Rearing -

The black cutworm (Agrotis ypsilon) has been difficult to rear due to cannabalism, but because of its economic importance, a method has been found by some Canadian researchers. Pupae are obtained from nature and after emergence the adults are held in 12 x 12 x 16 inch cages with sliding glass panels to permit air circulation. Twenty moths per cage is the normal capacity and ambient temperature is 80 $\pm$ 2°F. Gallon jars with moist paper are used to supply an egg laying environment and a light-dark photoperiod is maintained. Pyrex trays 12 x 8 x 2 inch with 1 inch moistened sand are provided for larvae. At the initiation of the fifth instar, each larva is separately placed in a small flint jar with a 1.5 inch layer of a 1:1:1 sand-sandy loam-vermiculate mixture with about 6 percent moisture added (86).

The codling moth (Carpocapsa pomonella) is reared in 53.3 x 30.5 x 7.6 cm stainless steel trays with a 10 to 13 mm layer of burrowing medium on the bottom. To prevent contamination and dehydration, a thin layer of paraffin is sprayed over the surface with a specially designed apparatus (87). Larvae of Noctuid moths have been successfully reared in the lab from eggs in 6 ounce waxed paper cups with

standard cardboard lids. A diet, shown in Table 2, is placed in each cup. The number of larvae per cup depends on the species (88).

Adult black cutworm larvae are fed by means of dental cotton plugs soaked in a 10 percent dextrose solution suspended in the cages on strings. Larvae are fed red clover or tobacco (86).

Artificial diets for codling moths are based on ingredients such as wheat germ, soybean meal, rice flour, pinto beans, alfalfa meal, cottonweed meal, corn meal and carrot powder (87).

Noctuid larvae have been successfully reared on the medium described in Table 2 below.

Table 2. COMPOSITION OF AN ARTIFICIAL MEDIUM USED FOR REARING NOCTUID SPECIES

Ingredient	Grams for 120-150 Cups Present Method
Soaked lima beans	
Soaked pinto beans	2133
Dried brewers' yeast	320
Ascorbic acid	32
Methyl p-hydroxybenzoate	20
Sorbic acid	10
Formaldehyde (40%)	20
Agar	128
Water	6400

Source: 88

Special Needs -

Excessive humidity can cause fungi and bacteria growth on insect growth media and infections of the animals themselves. On the other hand, a lack of moisture and the resulting dehydration can also cause mortality in lab colonies. A careful balance of temperature and humidity is mandatory (87,88).

Special diets for the various life stages of Lepidopterans may be expensive and time-consuming to prepare.

Ecological Role -

The larvae of butterflies and moths, often severe agricultural pests, are economically much more important than the adults, some of which never feed. They frequently supply food for insectivorous predators.

Longevity -

Most Lepidopterans have an annual cycle. Eggs or pupae overwinter. The larvae feed voraciously and pupate. Adults often have the sole task and ability to mate and lay eggs.

Problems with Mass Culture -

Many larval forms are cannibalistic, especially in crowded quarters. Isolation may be necessary during certain instar stages (86,88).

Toxicity Testing -

Much of the research with butterflies and moths has been with pheromones (89,90,91,92,93) often with the goal of achieving population control. However, a few toxicity studies are available (94,95,96).

Researchers have found a way of closely scrutinizing the flight of insects and the comparison of certain measurements between controls and test organisms gives a reliable, behavioral test (97).

General Suitability -

Some Lepidopterous insects can be routinely bred and reared in captivity and can make excellent test animals.

Ecological Alternatives -

Other phytophagous insects.

Diptera

Introduction -

The "flies" are a well-known group of insects and one of the larger orders. They possess only one pair of wings - the front pair. The hind set are reduced to balancing, knob-like organs. Mouth parts are sucking and metamorphosis is complete.

Most flies are small soft-bodied insects, many of which are economically important. Mosquitoes, stable flies, house flies, and blow flies are pests of man and other animals. Many diseases can be transmitted by Dipterans. Many other flies are predacious on harmful insect species, some are important as pollinators, and still others are useful scavengers (57).

Most of the available maintenance procedures are concerned with pest species and these will be reviewed. A group of smaller insects (family Chironomidae) has also been successfully reared in the lab and will also be discussed below with mosquito (family Culicidae) rearing techniques.

Caging/Lab Conditions -

Larger flies (i.e., Muscidae, Calliphoridae) can be kept in any escape-proof container at a temperature of 27.5 ± 1°C and relative humidity of 50 to 55 percent. Windows are not necessary but proper air circulation is important. Wooden cages (45 x 45 x 45 cm) with screen sides that can be dismantled for cleaning are used to keep adult house flies (Musca domestica), and stable flies (Stomoxys calcitrans) (98, 99). A minimum of 50 cm^3 for each adult is a good guide for space requirements (98).

Mosquito adults (Culex, Aedes, Anopheles) can be easily housed in 18 x 18 x 18 inch plywood cages screened with 18 mesh screen. In most fly cages, a muslin sleeve is attached (24 inches long, 9 inches circular opening) to provide access while preventing the escape of the animals. Temperatures between 24° and 27°C and relative humidity around 80 percent are satisfactory ambient conditions. A 12L:12D photoperiod enables Anopheles quadrimaculatus to carry out its normal life cycle (100).

Chironomids can be housed in cages similar to those of mosquitoes, but because they may need ample space to swarm in order to reproduce, larger enclosures (5.25 x 4.5 x 7 feet)

must be provided with a 75 gallon aquarium filled with mud for the floor (see Breeding/Rearing for further discussion). Chironomus plumosus will breed under such conditions with an ambient temperature of about 25°C and a relative humidity of 70 percent (101).

Fruitflies (Drosophila), can be easily raised in the lab in many sizes of cages and are widely used in many types of research (102).

Nutrition -

Adult houseflies and blowflies are provided with a shallow dish of sugar for feeding. Stable flies are fed blood offered in soaked cotton, the live host, or in dishes on the floor of the cage. Water soaked cotton pads should be available for drinking in all fly enclosures (98, 99, 103).

Larval houseflies and blowflies are reared on many media, the general ingredients consisting of various combinations of dog biscuits, fish, wheat bran, alfalfa meal, baker's yeast, malt extract, manure, sugar, and water (98, 103). Larval stable flies are more difficult to rear. Stomoxys cultures should be fed the diet found in reference 99.

Only small amounts should be given at one time until larval consumption increases. Shallow flat dishes are suitable holding containers for larvae and medium. A pH of above 7 is desirable for the medium, and using only 24-hour old medium assures that newly hatched larvae will not encounter acid conditions (99).

Adult mosquitoes can be maintained on soaked seedless raisins or a 10 percent sugar solution in saturated gauze pads. Females get a blood meal via a shaved guinea pig three times a week (100). Adult female mosquitoes can also be fed on shaved rabbits. Larval mosquitoes are fed a diet of wheat flour, dry brewer's yeast, dried beef blood, and non-fat dry milk. The finely ground mixture is sprinkled sparingly on the surface of the water (100).

Breeding/Rearing -

The procedure for breeding houseflies is to transfer adults to a cage made of gauze with a plywood bottom (see Caging) (104). Cotton-wool pads soaked with a milk-water solution (1:1) provide an oviposition site. The pads with

eggs are then transferred to jars with rearing medium and muslin caps. About eight days after the eggs are laid, larvae will migrate to upper layer of medium and pupate. This layer is removed to an adult cage where emergence takes place (98).

Ground horse meat is the medium most often used for oviposition by blowflies. Eggs remain in the meat and are transferred to larvae medium in glass jars. They hatch in 24 hours. In eight days they pupate and emergence occurs six days later. Sawdust is used as a sponge to soak up excess moisture from the meat in the culture jars (103).

Oviposition cages for stable flies are of the same construction as adult holding cages, but smaller in size (25 x 25 x 25 cm). Oviposition occurs more readily in confined spaces. About 6.5 days after emergence, well-fed flies will produce the first eggs, but full production doesn't occur until the 10th day, when the transfer of gravid adults to oviposition cages is suggested. Eggs are laid through perforations in a zinc floor and caught on the black cloth surrounding the cage. Flurorescent lighting and adult food should be present. About 60 to 70 percent of the eggs will hatch and placed on a culture medium, will pupate in nine to ten days. Pupae are placed in adult cages for emergence (99).

Mosquitoes (Anopheles quadrimaculatus) oviposit in 8.5 x 4.5 x 2 inch enamel dishes of aerated tap water two to three days after a blood meal. Eggs are transferred to rearing pans (9 x 15 x 2.5 inch). Dechlorinated water should be used. After hatching, larvae are separated to 500 per pan and fed the larval diet (see Nutrition). Pupae are transferred to the holding cages for emergence. It should be noted that different species of mosquitoes have different specific requirements, though the rearing pattern for A. quadrimaculatus is, in general, characteristic for other species (100).

Midges (Chironomus plumosus) have been bred and reared in the laboratory. The Chironomid group swarm under specific conditions, and successful breeding depends on suitable ambient temperature and light. Cages are lit by fluorescent and incandescent lamps and natural conditions are simulated by a gradual increasing and diminishing of intensities. Swarming takes place in the "evening". Some successful breeding has taken place in small cages (3.0 x 1.9 x 1.5 cm) (105). Larvae will develop to the fourth instar in the mud substrate at the cage bottom. Trainer's Dog rewards are added as food after they are given thorough soaking. Pupation occurs in the substrate and adults emerge after nine to 13 days (101).

Special Needs -

Diets and maintenance requirements are different for each species. There are many entomology laboratories throughout the United States which can supply procedural data and special needs for many Dipteran species.

Behavioral Restrictions -

Life cycles can be very complex and difficult. Species-specific behavioral idiosyncrosies abound in this animal group. These facts can make lab breeding/rearing difficult or at least, demand special provisions (e.g., swarming cages for Chironomids).

Ecological Roles -

Many adult Dipterans are vectors of disease and nuisance pests of other animals. However, they also can represent staple foods for insectivorous predators (i.e., bats, swallows, frogs). Aquatic larvae are frequently major food sources for fish in quiet waters.

Longevity -

It is doubtful that adult Dipterans live for more than one year; they probably live for a much shorter period of time.

Toxicity Testing -

Dipterans have been the subjects of conditioning experiments, with mixed success (106, 107). They have been used in a great number of experiments with toxic substances to determine mode of entry (108, 109) and general effects (80, 110, 111). Sublethal effects on reproduction, behavior under stress conditions (112) and physiology have also been studied (113, 114, 115). Insect resistance to toxic substances (116, 117, 118) and even the effects of toxic substances on Dipteran eggs have been studied (119).

General Suitability -

Many flies have been used in research and for a good number of species the maintenance procedures are available. This makes them worth consideration as test species. The double association of some forms (e.g., mosquitoes) to aquatic and terrestrial systems at different times during their life cycles may make them particularly suitable subjects in experiments in which land-water transfer of a substance is to be studied.

Ecological Alternatives -

Blood sucking Hemipterans.

Hymenoptera

Introduction -

The Hymenoptera (membrane-wing) contain what man regards as the most beneficial group in the insect kingdom, the honeybees. There are also a good number of wasps that parasitize other insects, many important pollinators and several interesting social groups. Ants, sawflies, Ichneumons, Chalcids, wasps and bees all belong to this order. They have chewing and chewing-sucking mouthparts and undergo complete metamorphosis. Bees are the only members that have been maintained to any extent.

Caging/Lab Conditions -

Housing of honeybee (Apis) colonies is an old and well-known practice and there are very extensive texts available on the subject. Bumble bees (Bombus) are also important as pollinators of legume crops and have been bred and reared in the lab. Wooden nest boxes contain two compartments: a feeding and a brood chamber. Nesting material such as dried grass or cotton is supplied, and, for feeding, a honey-and-pollen mixture. The queens are introduced to the nest and their natural nesting instinct usually takes over. Some reports attribute success in colony establishment to the addition of some workers to the nest with the queen. Queens are caught in nature and fed on pollen and honey. After the establishment of colonies in the lab, they have been set outdoors to do their pollination job. Successes have been reported for about 30 species. Some success at overwintering queens has also been reported (120).

The Agricultural Research Services, USDA, Tucson, Arizona, has utilized honey bees (Apis mellifera) in toxicity testing under lab and field conditions. Their lab cages are 6 x 6 x 2 inches with 100 bees per cage (121).

Nutrition -

Bumblebees have been kept in the lab on honey and pollen (120). Honeybees have been maintained on 60 percent sucrose and pollen (121). The larvae require no special diet because of the care given them by the adults.

Breeding/Rearing -

Once a bee colony is established it will be self-perpetuating for at least that season. Honeybee colonies remain intact over winter and may last for many years, but whether colonies which have been kept in the lab year-round are still able to reproduce is uncertain, because most have been initiated for research outdoors.

Special Needs -

Nest boxes are necessary for the colony.

Ecological Role -

Many Hymenoptera are important as pollinators and as parasites on other insects. They feed on pollen, plant juices, and many other liquid foods.

Longevity -

The lifetime of most adult Hymenopterans can be measured in months.

Toxicity Testing -

There have been numerous studies of the resistance of Hymenopterans to the adverse effects of toxic substance (122, 123, 124, 125, 126, 127, 128). General studies both in the lab and field have also been completed (129).

General Suitability -

The social species of insects, e.g., ants and bees, appear to be very interesting potential subjects because there already exists a considerable body of knowledge on their social organization and population dynamics and because some species are relatively easy to keep and simple to work with (121). Studies at the population level and of sub-lethal effects on behavior could easily be performed and the results reviewed in the light of known patterns.

Miscellaneous Invertebrates

This section discusses a group of insects which have not been extensively studied in the laboratory, but which may prove to be useful subjects for toxicity testing. Included here are the soil arthropods and those that are closely associated with ground and other substrates, as well as other miscellaneous groups. Centipedes (Chilopoda) and millipedes (Diplopoda) are interesting animals and play important roles in the micro-systems in which they exist: centipedes are predators, sucking the fluids from their prey through their mandibles; millipedes feed mainly on dead organic matter. Both have been used as test animals (130).

Springtails (Collembola) are apterygote insects that locomote by means of a forked structure which, extended, causes the animal to jump. Springtails are inhabitants of leaf litter, decaying logs and fungi. They are basically vegetarians.

Thrips (Thysanoptera) are also insects and plant feeders. They occur on all types of vegetation and can be serious pests of cultivated plants.

Another Arthropod group are the Isopods, or pill bugs. They are frequently found around decaying matter and play an important role in the decomposition of organic solids. Isopods are abundant throughout North America and because of their saprophagous feeding habits and abundance, may prove to be very good test animals. They have been used previously (131).

Scorpions (Arachnoidea) are rapacious arthropods that occur primarily in subtropical and desert regions of the United States. They nocturnally feed on insects and spiders, first killing or immobilizing them with the sting apparatus on the distal tip of the abdomen. Scorpions are relatively easy to keep. Palamnaeus gravimanus have been kept in wood cages 3 x 2.5 x 1 foot with a soft earth substrate for burrowing. Wads of wet cotton kept them moist and cockroaches were the staple food. Some evidence indicates they may be quite suitable for use in toxicity testing (132).

VERTEBRATES

Amphibians and Reptiles

Amphibians and reptiles, the herpetofauna, are poikilothermic vertebrates that inhabit both terrestrial and aquatic environments of all types. The herps are basically egg laying animals. Amphibians usually require water for successful reproduction. Reptiles on the other hand, through evolution,have developed a land egg which can be deposited in many types of terrestrial nests. Some reptilian species are live-bearers and do not lay eggs.

Most reptiles and amphibians are carnivorous and feed on insects, other invertebrates, young birds, eggs, and small mammals. However, a few lizards and turtles are herbivorous.

Amphibians and reptiles have been used in laboratory research in a few ways that may prove applicable to toxicity testing. For example, amphibian eggs are excellent for embryological studies and are often used in college courses. In toxicological research, however, there has been very little attention paid to the poikilothermic vertebrates probably because of their lack of economic importance.

The following text discusses species which are judged to be potentially suitable for toxicity testing based on their ecological importance, availability, ease of laboratory maintenance, and reproductive success in captivity. Because of the paucity of information available in the literature on herpetofauna that might aid in such a selection, a number of herpetologists were consulted for suggestions. A summary table indicating the suitability of herpetofauna for toxicology testing is provided in Table 3.

TABLE 3. SUITABILITY - HERPETOFAUNA

HERPS	Availability	Breeding in Captivity	Ease of Maintenance	Previous Use in Testing	Restrictions	General Suitability	Ecological Role	Habitat Preference	Suggested Species
Amphibians	E	G	F	F	Pure water Housing methods	G	B_2	All	*Rana pipiens* *Ambystoma mexicanum*
Turtles	G	F	G	P	Captive breeding	F	B_2 D^2	Aq Fo	*Terrapene* sp. *Chelydra serpentina*
Lizards	G	G	G	P		G	B_2	Fo,Gr De	*Anolis carolinensis* *Phrynosoma cornutum*
Snakes	G	F-G	E	P		G	B_1	All	*Thamnophis sirtalis* *Elaphe* sp.

Legend:

General Ecological Role

A - Large carnivore
B - Small carnivore
  1. General
  2. Insectivore
C - Omnivore
D - Herbivore
  1. Seed, fruit eater
  2. Grass foliage eater

Habitat Preferences

Fo Forest
Gr Grassland
De Desert
Aq Aquatic
Do Domestic

E = Excellent
G = Good
F = Fair
P = Poor

Sources: - literature cited in text
- interviews of Scientists

Anurana

Introduction -

Amphibians are so named because they metamorphose from aquatic forms into air-breathing and often terrestrial tetrapods. The aquatic forms of frogs and toads, the tadpoles, have provided scientists with unique opportunities to study developmental biology.

There are six families of Anurans in North America: the spadefoot toads, Pelobatidae, the narrow-mouthed toads, Microhylidae, the other toads, Bufonidae, the leptodactylid frogs, Leptodactylidae, tree frogs and their allies, Hylidae, and the true frogs, Ranidae. Of the true frogs, the leopard frog (Rana pipiens) has been utilized by researchers for many types of investigations. It is a very abundant and widespread species that occurs in many kinds of waterbodies throughout the U. S., Mexico, and Southern Canada (133). Many other species of toads (Bufo) and true frogs (Rana) are locally abundant and also bred, reared and maintained in labs throughout the U. S. However, since the leopard frog is the major subject in amphibian research, the following text is focused primarily on this specie.

Caging/Lab Conditions -

For adult frogs, just about any waterproof enclosure with available water is sufficient to house large numbers (134). Plastic tanks, large sinks (22 x 14 x 14 inches) for about 25 adults (135), and plastic rat cages (22-3/4 x 11 x 6 inches) with perforated stainless steel lids (136) have been suggested cages. Adults can also be maintained for weeks under refrigeration (4°C) in water. No feeding is necessary during this time. The water, however, should be changed three times weekly (134). Further enclosure specifications and handling techniques can be found under Breeding/Rearing.

Water for use in amphibian cages should be unchlorinated and unfluoridated, especially water in which larval forms are kept (134, 137, 138, 139, 140).

Proper temperature regimes are quite variable for adults, and should depend on the state of mobility desired and the techniques to be performed. Certain anesthesia doses are differentially effective at different temperatures (141). For active adult animals, 18° to 20° C is suggested (134).

Larval anurans are affected in their sexual development by ambient water temperatures. For normal results, a 15° to 25°C range is sufficient (134).

The Mexican axolotl, Ambystoma mexicanum, have been maintained in the laboratory in containers made of asbestos cement at the time they reach 5 mm, for caging specifications see reference 142. Water in the salamander containers should be unchlorinated and circulated constantly (142).

Work in the Amphibian Facility at the University of Michigan, Ann Arbor, is proving the feasibility of mass producing Rana pipiens as well as other amphibian species (137).

Nutrition -

Cooked lettuce and various other greens have been most often used as food for tadpoles (137, 138), but one nutritional study showed a 300 percent weight increase at seven weeks in larvae fed Purina No. 1 trout chow over those fed just lettuce (143). Natural pond plankton and bits of liver have also been successfully used to rear tadpoles (148). Spinach, however, has been shown to cause kidney stones in some amphibians (144) and is not recommended (137).

For recently metamorphosed frogs, small mosquitoes are excellent food, and can be replaced by flies (137), crickets and liver bits (134) as they mature. Adults have survived in excellent condition by being fed twice weekly, crickets (two to three) alternated with worms (134).

Suggested diets for the appropriate life stage for amphibians are presented in Table 4. Adult axolotls thrive on beef heart with vitamins A, D, B complex and powdered $CaCO_2$ added (142).

Breeding/Rearing -

The breeding and rearing of most amphibious species are more complicated than those of birds or mammals because amphibians show no parental care for the young. In frogs and toads, the eggs are laid in water and fertilized externally by the males during amplexes. (Salamanders have internal fertilization by means of spermatophores deposited by the male and taken up by the females into her cloaca.) The eggs are left to develop, hatch and mature on their own. Therefore, rearing techniques must include care for the organisms from the zygote stage to the adult stage. Table 5 presents the natural breeding habits and numbers of eggs for some North American and European Anurans.

Table 4. FOOD RECOMMENDED FOR AMPHIBIAN LARVAE YOUNG ANIMALS AND ADULTS

Stage In Life History	Anura
Newly hatched larvae	Boiled spinach or lettuce, green part of Romaine lettuce; for Xenopus: dried nettle or alfalfa powder, dried yeast, egg or liver powder
Older larvae	As above
Newly metamorphosed animals (terrestrial)	Drosophila
Juveniles (terrestrial)	Larger Drosophila species, mealworms, small earthworms, crickets
Metamorphosed animals (more or less aquatic) and large, neotenic larvae (e.g., axolotl)	For Xenopus: Tubifex, Enchytraeus, small pieces of liver; later: earthworms and beef heart cut into small blocks
Adults (aquatic)	For Xenopus: earthworms, beef heart cut into small blocks
Adults (terrestrial)	Larger species of Drosophila, house flies, mealworms, crickets, grasshoppers and other insects.

Source (142)

Table 5. NATURAL BREEDING HABITS OF COMMON NORTH AMERICAN AND EUROPEAN ANURA

SPECIES		Breeding Season (in northern latitudes)	Approximate Number of Eggs Laid by One Female, and Mode of Deposition
Scientific Name	Common Name		
Rana catesbiana	Bullfrog	June-July	10,000 or more, in large film on surface, 60 cm across
Rana clamitans	Green frog	June-August	1000-4000, in film on surface, less than 30 cm across
Rana palustris	Pickerel frog	April-May	2000-3000, in firm globular masses attached to twigs
Rana pipiens	Leopard frog	April-May	3000-5000, in flattened sphere
Rana sylvatica	Woodfrog	March-April	2000-3000, in globular mass
Hyla crucifer	Spring peeper	April-June	800-1000, laid singly among plants
Hyla versicolor	Treefrog	May-July	1000-2000 in small packets of 30-40 eggs
Bufo americanus	American toad	May-June	4000-8000, in two long strings
Bufo Fowleri	Fowler's toad	May-June	Up to 8000, in two long strings
Rana esculenta	Edible frog	May	1600-1900, in clumps
Rana temporaria	Common frog	February-April	1000-4000, in large clumps
Bufo bufo	Common toad	March-April	3000-4000, in two long strings

SOURCE: (138)

George W. Nace, et al., began an amphibian maintenance program in the mid-1960's at Ann Arbor, Michigan and has very successfully bred and reared a variety of frogs, and salamanders, with the purpose of developing genetically defined strains. A general review of their techniques and inserts from various other maintenance programs follows (for specific detail, see 137).

It has been discovered that breeding can be induced in Anurans by injecting the animals with pituitary tissues from other frogs, and progesterone (145). Females can be injected and stripped of their eggs which are then inseminated by sperm from excised testes, or males can also be injected and natural breeding behavior allowed to occur. The artificial method allows for unique egg-sperm distributions or for other specific handling techniques. (The specific procedure for inducing ovulation can be found in reference 138). Generation time (at Ann Arbor, Michigan) is 13 to 15 months.

Embryos are held in enamel pans and water (unchlorinated) is changed every third day. When the tadpoles begin to swim they are transferred into specially designed bottles which hold 25 to 75 larvae, depending on the species and size of individuals. These are one-gallon bottles with their bottoms removed. A one-hole cork is fitted in the neck, they are positioned upside-down and a leveling-drain tube extends from it to the desired water level. A wire screen keeps the tadpoles from the narrow neck and from debris that collects at the bottom of this aquarium. Water is slowly added to the system and circulated through it. There is good evidence that the average growth rate of tadpoles decreases as the density rises (138).

Metamorphosed animals are transferred to larger plastic containers with appropriate water circulation. These are tipped up on one side leaving a pool at the lower end and a terrestrial area at the other.

Adults are housed in larger containers that can be easily cleaned and that provide both aquatic and terrestrial areas.

Salamanders breed from January to June. After two to three months some species can be bred again. Breeding is conveniently induced in the Mexican axolotl by a sudden decline in temperature (22°C to 12°C for two days). Males and females are kept separately except to breed and put together into the colder water. The breeding chamber should be sheltered from light and no food provided until after oviposition on plastic tubes (13mm diameter) fitted to aquarium sides below the water line. Eggs are reared in 14° to 18°C water in shallow trays. During culture, crowding should be

avoided. Larvae should be fed Artemia or small Daphnia obtainable from supply houses. Mosquitoe larvae also can be used. Containers should be cleaned of detritus daily. Sexual maturity is achieved in males at about seven months and in females in nine or ten months (142).

Special Needs -

Larval forms need fresh, unchlorinated water, which must be changed frequently. Tap water can be treated in various ways to remove the chlorine (134).

Fresh live insects must be continuously available for adults (135, 137).

Embryos and tadpoles need special handling (137).

Ecological Role -

Anurans are carnivorous animals that feed on a great variety of invertebrate species, particularly insects. Many larger predators utilize them as a food source. Birds, snakes, turtles and mammals feed on the adults, and the tadpoles provide food for predators associated with aquatic habitats (133).

Longevity -

Rana pipiens has achieved life spans of three years in the laboratory (134).

Problems with Mass Culture -

Mass culture of anurans requires a large supply of unchlorinated water (134,137) and sufficient lab personnel to feed and maintain the various life stages.

The susceptibility of frogs and toads to parasites is well known and health maintenance programs including penicilin inoculations, etc., may be required (137,146).

Toxicity Testing -

Larval and adult frogs and toads have been used in studies of the effects of pesticides on survival and reproductive behavior in outdoor enclosures (147,148,149,150,151) and under lab conditions (152,153,154,155,156). Heavy metal (157) and irritant vapor (158,159) studies have also been performed. Their use in cancer research is very extensive (160,161,162,163,164).

General Suitability -

In toxicity tests, the accessibility of the early life stages of anurans and the sensitivity of frog skin makes them very suitable subjects. An increase in breeding amphibians in recent years has made them more available than in the past.

Rana pipiens is the most likely subject due to its wide use in many research areas, its availability and the large body of basic information that has been gathered. Ambystoma sp. are salamanders that have received much previous attention and are being bred at the University of Michigan Amphibian Facility (137).

Ecological Alternatives -

Turtles, Lizards.

Chelonia

Introduction -

Turtles are reptiles with a very long history on earth. They are shelled organisms that are mostly associated with aquatic environments, but wholly terrestrial species are not uncommon. The feeding habits of turtles are quite variable and most species seem to be omnivorous, feeding on a great variety of plants and animal materials. They are found in many natural habitats, particularly around ponds, streams and rivers.

Caging/Lab Conditions -

Aquatic turtles have been adequately housed in concrete tanks (130 x 72 cm) lined with paraffin through which unchlorinated water was constantly circulated and maintained at a depth of 25 cm. Wooden platforms were provided onto which the turtles could crawl. Terrestrial species were kept in dry tanks and provided with water for drinking (165).

Terrariums are also excellent enclosures for keeping turtles, and are easily transported (133).

Nutrition -

Ground horsemeat and lettuce is a suitable diet for many species of turtles (165) and raw hamburger and table scraps are also recommended (133). In the wild they consume insects, crayfish and other invertebrates.

Breeding/Rearing -

No information is available on breeding turtles in captivity. Since they are egg layers, the collecting of eggs from natural nest sites is a possibility. Most species produce large amounts of eggs yearly.

Ecological Role -

Turtles are basically omnivorous reptiles and are important elements in the aquatic systems. They are predators on all types of invertebrates and some species are avid consumers of aquatic vegetation.

Problems with Mass Culture -

Turtles are susceptible to a great variety of parasites and diseases including tuberculosis and many types of Salmonella (166).

Toxicity Testing -

Turtles have been used in terrestrial DDT studies (167) and parathion distribution studies in aquatic ecosystems (152).

General Suitability -

Turtles might prove very suitable for testing. Their courtship rituals are unique and may supply a means by which behavioral modifications, etc., might be studied. However, their external dermal skeleton makes minor surgical techniques difficult.

Their morphology and taxonomy are known and sexes are easily distinguished (165). Terrapene sp. has a number of terrestrial species and is recommended for use in the toxicity testing of terrestrial forms. Young snapping turtles (Chelydra serpentina) are frequently used in laboratory experiments, and though primarily an aquatic species, they are a likely second choice.

Squamata - Lizards

Introduction -

The lizards are represented in North America by five families: the iguanids (Iguanidae), the skinks (Scincidae), the glass lizards and alligator lizards (Anguidae), the whiptails (Teiidae), and the geckos (Gekkonidae).

Caging/Lab Conditions -

Lizards can be housed in terrariums (133), wooden cages with glass doors (168), or nearly any escape proof enclosure. Many lizard species are desert or tropical forms and, being poikilothermic, require rather warm ambient conditions. Some recommended temperatures are 85° to 90°F for iguanas (169) (this can be achieved locally by infrared lamps) and 75° to 85°F for gila monsters (168). Arboreal species should have appropriate props (170).

Photoperiod is important for the regulation of the endocrine system in some lizards and should be regulated if animals are kept indoors (171). Light might also influence feeding in certain lizard species and it has been reported that reluctant feeders can often be induced to eat by putting them under a wide spectrum artificial light (170).

Nutrition -

Most lizards are carnivorous and can be maintained on a large variety of foods. However, there are some native herbivorous species, such as chuckwalla, in the southwest. Iguana iguana, a common South American lizard seen frequently in pet stores is also herbivorous and can be satisfactorily maintained on fresh green vegetables (169).

Carnivorous species like Sceloporus, a native iguanid, have been fed larval wax moths (Galleria mellonella) (172) and other easily obtainable insects. Some desert lizards will not eat if their body temperature is below 35 to 40°C.

Gila monsters, Heloderma, have been maintained for years on a diet of hens eggs (one every two weeks). Ground whole rat was substituted for a time with no significant effect on weight (168).

Water should be provided ad libitum, though certain desert species will make little use of it. Some Anolis may not

drink at pools, but will only take water in the form of dew off of leaves. Spraying the cage may be the only means of watering this lizard group (173).

Breeding/Rearing -

There is no information concerning captive breeding in lizards. They are egg layers and usually breed in the spring in nature.

Special Needs -

Infrared lamps or some other local heat source may be required for lizard cages.

Live food may be necessary because lizards respond to movement and under natural conditions attack only moving prey.

Behavioral Restrictions -

Many desert lizards are highly territorial (e.g. Crotaphytus) and there may be some difficulty in caging individuals of this species together in any numbers. However, Anolis can be housed in rather large quantities in cages with sufficient vertical twig surface for all individuals (173).

Ecological Role -

Lizards are both primary consumers, insectivorous, and carnivorous (Gila monsters eat eggs, young birds and mammals). They all, in turn, are consumed by predatory snakes, birds and mammals.

Problems with Mass Culture -

Lizards, like most reptiles, are relatively unprolific. Their breedability in captivity has not been thoroughly investigated and their potential as laboratory colonial animals does not appear to be very good.

Toxicity Testing -

Lizards have not been extensively used as subjects for toxicity testing. However, field investigations of DDT effects on faunal elements have included lizards (174).

General Suitability -

Their poor availability and the lack of knowledge of their

reproductive behavior in laboratory situations makes lizards rather poor candidates for wide use as toxicity experiments. Anolis carolinensis has been successfully bred in captivity and would be the most likely candidate (175).

Ecological Alternatives -

Toads, snakes.

Squamata-Snakes

Introduction -

Snakes are more closely related to lizards than to other reptiles. They are legless poikilothermic vertebrates that are significant predators on smaller animals. North America has three families: the constrictors, Colubridae, the rattlesnakes, Viperidae, and the coral snake, Elapidae. Snakes of the last two groups are venomous (133).

Reptiles differ from amphibians in their ability to breed without returning to an aquatic environment and in possessing internal fertilization. Their eggs are laid in a terrestrial nest. There is no metamorphosis.

Snakes inhabit many types of habitat and are carnivorous in their natural feeding habits.

Caging/Lab Conditions -

Snakes are easily housed in terrariums with tightly sealed lids (133), but can be kept in various types of cages. Pea gravel or artificial grass are recommended substrates. Fibrous substrates have been found unsuitable. Rocks and/or branches should be available to all snakes as aids in ecdysis (171,173).

Preferred ambient temperatures range from 26° to 36°C depending on the species. Local heat (infrared lamps) if supplied, will allow the snakes to position themselves in order to achieve a desired body temperature.

Humidity can be critical for some snakes and a range from 33 to 60 percent is recommended. At very high humidities snakes succumb easily to bacterial infections and at low humidity levels the danger of dehydration is always present (171).

Proper lighting is important for feeding responses in some reptiles and sufficient light is most important (171).

Anesthesias for snakes are variable (141,176,177,178,179).

Nutrition -

Carnivory is the rule with snakes. Some species may need a visual stimulus to attack and eat (173).

Water should be supplied in open bowls or other containers ad libitum.

Breeding/Rearing -

Very little information is available on the breeding and rearing of snakes in captivity.

Special Needs -

A source of heat is necessary for snakes. Live food may be an additional requirement for some species.

Behavioral Restrictions -

Some species are prone to biting each other and their keepers.

Ecological Role -

Snakes are predators on various small mammals and other animals. In turn, they are a food source for larger predators, especially birds and mammals.

Problems with Mass Culture -

Reproductive potential seems to be rather low for snakes.

Toxicity Testing -

Snakes (Opheodrys) have been analyzed for DDT content in open areas where spraying took place (174). In toxicity testing their use has been very limited, but the effects of radiation on some species is known (180).

General Suitability -

It would be difficult to obtain a large number of snakes for testing. Thamnophis sirtalis has been successfully bred in captivity and is suggested for that reason. Elaphe obsoleta is a common snake and a likely second choice.

Ecological Alternatives -

Lizards, turtles.

Birds

Birds are a fairly large vertebrate group (about 1,780 species in North America) that is unique because all of its members are feathered. With the exception of such birds as emus, ostriches, penguins and a few South American waterfowl, all birds are capable of flight. They are as diverse in their ecological roles as they are in physical size. Despite these facts, far fewer scientific endeavors use birds as subjects than mammals for a number of reasons. First, most birds are protected by federal and state laws. Second, birds have a high metabolic rate which means they need a constant supply of food, and migration is an innate urge which makes the maintenance of many species very difficult. Lastly, even when the birds can be maintained, they still may not breed in captivity (this is true of other animal groups as well). Because of relaxed statutes, easier maintenance and breedability, exotic species have received the most attention in labs. Wild North American species have been used only infrequently, so many gaps occur in the data presented here.

The life cycles of birds are complicated and controlled by their endocrine glands which are stimulated by the photoperiod of their surrounding environment. Establishment and maintenance of appropriate photoperiods is a major problem in breeding birds.

In spite of these difficulties in using birds in the laboratory, they are potentially very valuable subjects. First, birds are very important in the world ecosystem. They are primary and secondary consumers, feeding on plants, invertebrates and vertebrates alike. They in turn are food for mammalian predators (including man), a few amphibians and reptiles, and a few species of birds. Second, most birds are highly instinctual and therefore are routine and stereotypical in certain aspects of their behavior. This means that differences or alterations in some behavioral norms can easily be measured. Last, a vast array of ecological roles can be seen in this group of animals.

While limited background information hinders in-depth testing of most birds, it is evident, nevertheless, that many avian species are good indicators of environmental quality and good potential test subjects. A summary table indicating the suitability of birds for toxicology testing is provided in Table 6.

TABLE 6. SUITABILITY OF BIRDS FOR TOXICOLOGICAL TESTING

	Availability	Breeding in Captivity	Ease of Maintenance	Previous Use in Testing	Restrictions	General Suitability	Ecological Role	Habitat Preference	Suggested Species
Pelicans and Cormorants	F	P	F	P	*Large size, slow reproducers, expensive to house	F	D_2	Aq	Phalacrocorax curitus
Loons and Grebes	F	P	F	P	*Don't live well in captivity	P	D_1,D_2	Aq	Podilymbus podiceps
Waterfowl	E	E-G	E-G	G-F	*	E	A_4,B, A_3	Aq	Anas platyrhynchos Branta canadensis
Shearwaters and Petrels	P	P	P	P	*Don't live well in captivity	P	D_1,D_2	Aq	Puffins griseus
Herons and Ibises	G	P	F	P	*Don't live well in captivity	P	D_1,D_2	Aq	Puffinus griseus
Cranes, Rails and Coots	E	P	F	P	*Hard to house, don't breed in captivity	F	D_1	Aq-Ed	Fulica americana
Woodcock	E	P	P	P	*Very high strung, hard to feed	P	D_1	Fo-Ed	Phalohela minor
Gulls and Terns	E	P	P	F	*Don't breed in captivity	F-P	D_1,D_2	Aq	Larus argentatus
Woodpeckers	E	P	F	P	*Don't breed in captivity	F-P	A_3,C_1	Fo	Melanerpes erythrocephalus
Raptors	F	P	F	F	**Require large cages	F	C,E	Fo-Gr-Do	Falco sparverius Tyto alba
Doves and Pigeons	E	E	E	E	**	E	A_3,A_4	Gr-Do	Columba livia
Bobwhite Quail	E	E	E	E	*Cannibalistic if not "de-beaked"	E	A_3,A_4	Fo-Gr	Colinus virginianus
Pheasant	E	E	E	E	*Cannibalistic if not "de-beaked"	E	A_3,A_4	Go-Gr	Phasianus colchicus

TABLE 6 (CONT.). SUITABILITY OF BIRDS FOR TOXICOLOGICAL TESTING

	Availability	Breeding in Captivity	Ease of Maintenance	Previous Use in Testing	Restrictions	General Suitability	Ecological Role	Habitat Preference	Suggested Species
Miscellaneous Galliforms	E	E	E	F	*Cannibalistic if not "de-beaked"	E	A_3,A_4	Fo-De	Dendragupus obscurus
Perching Birds - Granivores	E	P	G	P	*Territoriality	F	A_3,A_4	Fo-Gr	Melospiza melodia Richmondena cardinalis
Perching Birds - Insectivores	E	P	F	F	*Territoriality	F	A_2,C	Fo-Gr	Turdus migratorius
Perching Birds - Omnivores	E	F-P	E-G	G	**u-starlings, weaver finches, territoriality	G	C,A_3, A_4	Fo-Gr-Do	Sturnus vulgaris Passer domesticus
Domestic Galliforms	E	E	E	E	Cannibalistic if not "de-beaked"	E	A_3,A_4	Do	Gallus gallus
Japanese Quail	E	E	E	E	Cannibalistic if not "de-beaked"	E	A_3,A_4	Do	Coturnix coturnix
Exotics	E	F	G	P	**Many species are poor breeders in captivity and unavailable in large numbers	G	A,C_1, C_2,	Do	Melopsittacus undulatus

TABLE 6 (CONT.). SUITABILITY OF BIRDS FOR TOXICOLOGICAL TESTING

LEGEND

ECOLOGICAL ROLE

A. Herbivorous - Terrestrial
   1. Nector Feeding
   2. Fruit Eating
   3. Seed Eating
   4. Granivores

B. Herbivorous - Aquatic

C. Carnivorous - Terrestrial
   1. Insectivorous
   2. Other Invertebrates
   3. Vertebrates

D. Carnivorous - Aquatic
   1. Invertebrates
   2. Vertebrates

E. Carrion Foragers

C_o = Nestling Diet

HABITAT PREFERENCE

Fo	Forest
Gr	Grassland
De	Desert
Aq	Aquatic
Ed	Edge
Do	Domestic

E = Excellent
G = Good
F = Fair
P = Poor

RESTRICTIONS

*All species protected by legislation

**Some species protected by legislation

^{u}Unprotected

Sources: Literature cited in text, interviews of scientists

Pelecanidae and Phalacrocoracidae

Introduction -

These two families are represented by the pelicans and cormorants. Pelicans are large, bulky water-birds with long flat bills and tremendous throatpouches. Cormorants are dark water-birds the size of loons and geese. Both are primarily coastal birds, are active during the day, and feed on aquatic animal life. They are colonial nesters and migrate to their northern breeding grounds each year where most nest on the ground along the rocky shores. They make their nests from pebbles and other debris they find on the nesting site. Like most other birds, they are protected by legislation.

Caging Conditions -

A cage 6 x 6 feet made of wood, cement and chicken wire, painted, and fitted with indoor-outdoor carpeting will house three pelicans or six cormorants. This cage was equipped with tree trunks and large tanks for bathing and drinking (181).

Nutrition -

In captivity, pelicans and cormorants can be maintained on a diet of fish (182). Food and water should be given ad libitum.

Breeding/Rearing -

Pelicans and cormorants usually lay two eggs per clutch and one clutch per year (183). Their young are altricial and born with their eyes closed. They are also nidicolous (remain in the nest until fledged) (183). These birds are colonial nesters (182).

Behavioral Restrictions -

These birds are large and have voracious appetites (182).

Ecological Role -

Pelicans and cormorants are at the top of their food chain. They are carnivores and feed mainly on marine vertebrates.

Problems with Mass Culture -

Pelicans and cormorants are difficult to maintain in captivit for long periods of time and are even harder to breed in captivity (184). They do not mature rapidly as smaller birds do and they lay small clutches (183). They require a good deal of space and are expensive to feed (182).

Toxicity Testing -

Because of maintenance problems most of the work on these birds has been done in the field. DDT and toxaphene levels have been checked in birds found dead in the field (185). DDT and its metabolities were studied in penned cormorants (186). The largest body of knowledge comes from oological studies done in the field (184, 187, 188, 189, 190).

General Suitability -

Pelicans and cormorants make good subjects for short-term and field studies because they are large and easily marked for identification. Their eggs are also easily obtainable. They feed on a wide variety of marine organisms thus making them good indices for environmental contamination levels. The difficulty encountered in maintaining and breeding them makes them poor candidates for chronic toxicity testing. The cormorant was chosen as a selected species because it has been used more frequently in laboratory tests.

Ecological Alternatives -

Gulls, terns, herons, shearwaters.

Gaviiformes and Podicipediformes

Introduction -

These two orders are represented by loons and grebes. Loons are swimming birds that are larger than ducks but have shorter necks than geese. Grebes are duck-size. Both loons and grebes are expert divers. They have elaborate courtship displays, like those of ducks and geese. Both groups are ground nesters and migrate to their breeding grounds each year. Loons are basically northern birds ranging from the north edge of the United States to Labrador and Newfoundland. Some populations winter in the Gulf of Mexico. Grebes are more widespread in their distribution and are found throughout the United States. Grebes, like most birds, are diurnal, while loons are crepuscular. Both groups are protected by legislation.

Breeding/Rearing -

Loons and grebes usually lay two eggs per clutch and one clutch per year. Their young are precocial and hatch with their eyes open. Like most precocial young, they are nidifugous (183).

Ecological Role -

Loons and grebes are aquatic carnivores and feed on a variety of vertebrate and invertebrate species.

Problems with Mass Culture -

These birds are poor breeders in captivity (182).

Toxicity Testing -

DDT and toxaphene have been applied to the habitat of loons and grebes. They have also been fed in capsule form to these birds (185).

General Suitability -

Loons and grebes are fairly available, but they are poor breeders in captivity. The pied-billed grebe was chosen as a selected species on the basis of its availability.

Ecological Alternatives -

Diving Ducks.

Anseriformes

Introduction -

There are 43 species of waterfowl in the U.S. (see Table 7), nine species of swans and geese, and 34 species of ducks. All species of waterfowl have webbed or at least partially webbed feet to increase swimming efficiency. They have down-covered precocial young that are able to swim soon after hatching. The individual species exhibit a remarkable diversity in size, form, behavior, and environmental requirements. They range in size from the pygmy geese (Nettapus) to the trumpeter swan (Cygnus cygnus) and have a variety of bills which are modified to suit the individual species' feeding habits.

Some species such as the mallard (Anas platyrhynchos) are practically omnivorous in their diet. Others, such as the common shoveler (Anas clypeata) are purely strainers of aquatic life. A flock of geese can cause significant damage to an agricultural grain crop.

Waterfowl are migratory and the largest concentration of breeding is in Manitoba and the potholes of the Dakotas. A less impressive number of them breed along the coasts of North America. A few refuges have established flocks of year 'round residents, especially in the case of the Canada goose (Branta canadensis) (191).

Caging/Lab Conditions -

A recommended pen for ducks is 30 x 15 x 6 feet. This will house five adult birds. The birds must be provided with access to water 10 inches or more deep (192). Pens of this size with nest boxes, 250 gallon water troughs and feeders, were used to breed mallards (193). A 14L:10D photoperiod is recommended (194). See Figure 2 for the mallard test chamber used at the Denver Wildlife Research Center and Figure 3 for the cages used. See Figure 4 for the outdoor mallard cages used at Patuxent Wildlife Research Center.

A more elaborate structure is a 12 x 7 foot room with one-way glass windows so that the birds can be observed without being disturbed (194).

Nutrition -

Commercial game bird breeder ration is available, but many labs supplement this with millet, cracked corn, wheat, milo, and barley, all fed ad libitum (195). An alternate diet

Table 7. DISTRIBUTION OF INDIGENOUS BREEDING WATERFOWL SPECIES BY ZOOGEOGRAPHIC REGIONS

	Palearctic[1]	Nearctic[2]	Neotropicals[3]	Australian[4]	Ethiopian[5]	Oriental[6]
Magpie Goose (1 sp.)				1		
Whistling Ducks (9spp.)		2	4	2	3	3
Swans and Geese (21 spp.)	15	9	2	2		
Freckled Duck (1 sp.)				1		
Sheldgeese and Shelducks (15 spp.)	4		6	2	3	
Steamer Ducks (3 spp.)			3			
Perching Ducks (13 spp.)	2	1	4	3	4	3
Dabbling Ducks (39 spp.)	11	9	10	8	8	3
Pochards (16 spp.)	6	5	2	2	2	1
Sea Ducks (20 spp.)	14	16	1	1		
Stiff-tailed Ducks (8 spp.)	1	1	3	2	1	

[1]Europe, Asia north of the Himalayas, and northern Africa

[2]North America, Greenland, northern Mexico, and Hawaiian Islands

[3]South and Central America (except Mexican uplands), and West Indies

[4]Australia, New Zealand, New Guinea, Melanesia, and Polynesia

[5]Africa (except northern part), southern Arabia, and Madagascar

[6]India, Southeast Asia, Sumatra, Java, Borneo, and Philippine Islands

Source: (196)

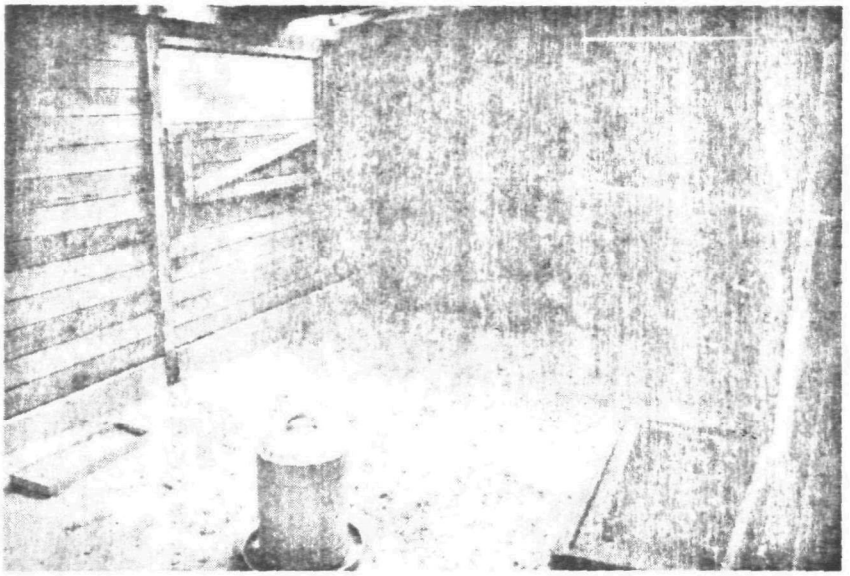


Figure 2. Mallard test chamber. Denver Wildlife Research Center, Denver, Colorado

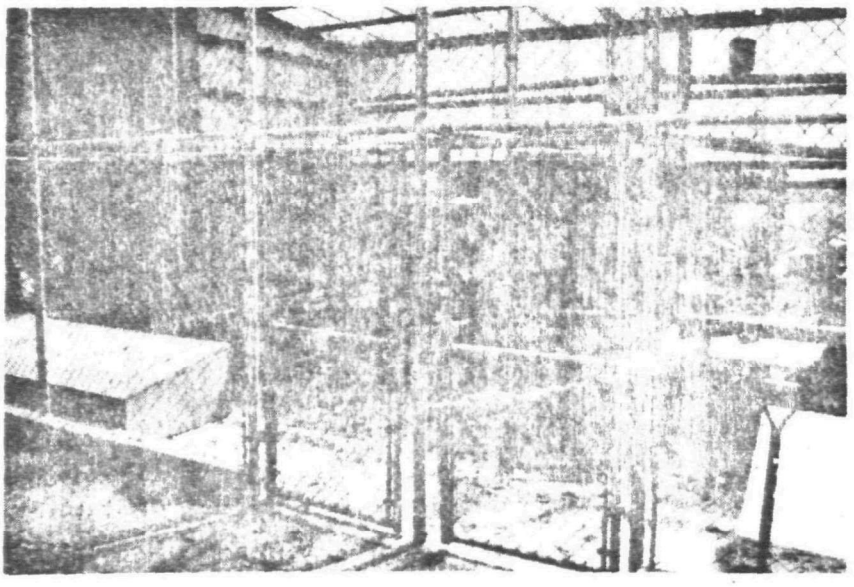


Figure 3. Raptor cages converted into mallard cages.

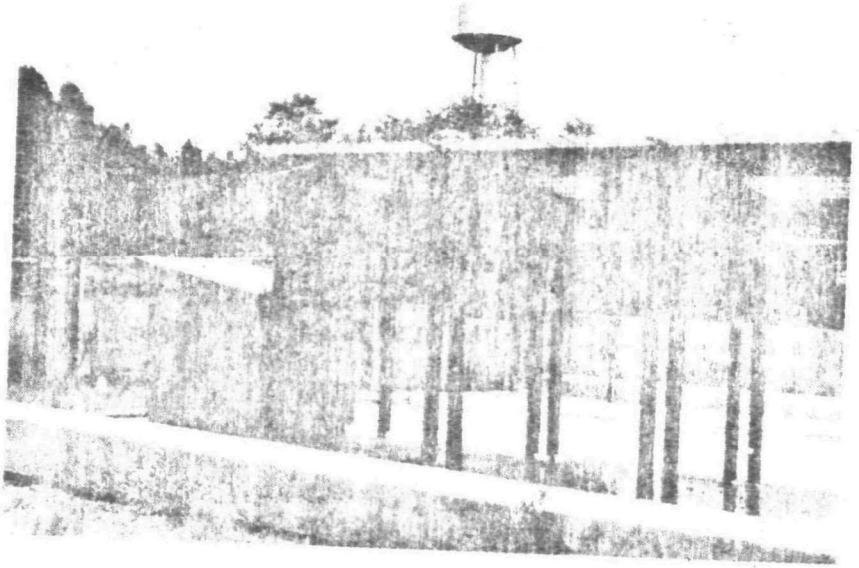


Figure 4. Mallard cages (base 4' x 4').
Patuxent Wildlife Research Center

consists of cracked corn with quartz and oyster shell grit fed ad libitum (192). In the wild, grazers, such as B. canadensis feed on all sorts of grains, while aquatic strainers such as A. clypeata feed primarily on spaeriids, gastropods, mayflies, and caddisflies. Waterfowl consume an average of 230 gm of food per day (191). Water should be available at all times.

Breeding/Rearing -

Waterfowl are territorial during the breeding season. Some, such as the gadwall (Anas strepera), Canada goose (B. canadensis), and greater scaup (Aythya marila) are colonial nesters. Waterfowl seek nesting sites that suit their individual ecological niches. Hence, they are found nesting on land, on improvised platforms, in tree holes, on limbs, and over water in marshy areas.

Hole nesters such as the wood duck (Aix sponsa) have larger clutches and longer incubation periods than ground nesters (see Table 8). Clutch size in all waterfowl varies with environmental and physiological stimuli and with the breeding abilities of the adults. In general, a large clutch size means small eggs and vice versa (191). Some ducks such as the ruddy duck (Oxyura jamaicensis) lay their own weight in eggs (191).

There is a great variety in average clutch size between species (5.1 to 9.3 eggs per clutch) and also in the time it takes to lay the clutch (191).

Renesting, dump nests, parasitic layers, and indeterminate layers are some phenomena which are characteristic of the duck nesting period. The best known parasite layer is the redhead (Aythya americana). Mallard (Anas platyrhynchos) and pintail (Anas acuta) are the most persistent renesters. Gadwall (Anas strepera) are indeterminant layers (191).

Geese and swans become mature in their second or third year. Ducks mature in their first, but are usually not successful breeders until their second year. Waterfowl young are all precocial. For an idea of fledging periods see Table 9.

Imprinting is a behavioral phenomena found only in precocial young. During a relatively brief period shortly after hatching, the young will develop a permanent attachment for any moving object presented to it whether it be a human, a dog or a matchbox on a string (191).

Table 8. INCUBATION PERIODS OF VARIOUS WATERFOWL

Species or Group	Days
Magpie Goose	28-30
Whistling Ducks	26-30 (White-backed Duck 31)
Swans	34-40
True Geese	24-30 (Ross' Goose 21-23)
Sheldgeese and Shelducks	28-30
Perching Ducks	28-32 (Brazilian Teal 25; Muscovy Duck 35)
Dabbling Ducks	21-28 (Hottentot Teal 18-20; Crested Duck 30)
Pochards	23-29
Sea Ducks	25-30 (Bufflehead 22; Goosander and Harlequin Duck 30-32)
Stiff-tailed Ducks	21-27 (Musk Duck and Black-headed Duck unknown)

Source: (196).

Table 9. FLEDGING PERIODS OF VARIOUS WATERFOWL

Species	Days
Magpie Goose	82
Fulvous Whistling Duck	55-63
Mute and Trumpeter Swans	100-120
Black and Bewick's Swans	105
Pink-footed Goose	58
Graylag Goose	53-57
Snow Goose	38-49
Hawaiian Goose	70-84
Canada Goose (large races)	63-86
Cackling Canada Goose	42
Ross' Goose	42
Freckled Duck	58-80
Shelducks	49-56
Egyptian Goose	88
Gadwall	49-63
Mallard	49-60
Northern Shoveler	39-60
Pintail	38-52
Blue-winged Teal	35-44
Redhead	56-73
Canvasback	54-68
European Pochard	49-56
Ring-necked Duck	49-56
White-winged Scoter	63-77
Common Goldeneye	56-62
Ruddy Duck	49-66

Source: (196).

Behavioral Restrictions -

Most waterfowl are very territorial during the breeding season. It is necessary to minimize crowding during this time if reproductive studies are being carried on.

Ecological Role -

Waterfowl are primarily herbivores, utilizing many plant foods but preferring grains and aquatic vegetation. In late spring and summer they become insectivorous, scouring the lakes and rivers for all forms of insect life. Ducklings, goslings and cygnets thrive on this high protein diet. During the nesting period waterfowl prefer secluded sites near small bodies of water. Once the young are off the nest the families head for open bodies of water where the young are preyed upon by turtles, hawks and larger carnivores (191).

Longevity -

Waterfowl are rather long-lived, but between species there is a wide range of life spans. For a more specific list see Table 10.

Problems with Mass Culture -

Waterfowl are protected by legislation and so might be hard to procure.

Ducks, geese and swans can be heavily parasitized but the effects of this are not known. Juveniles are more prone to parasitism, because of their feeding habits, than adults are. Bacteria pose the greatest threat to waterfowl although fowl cholera, avian tuberculosis and malaria also occur. Infestations with trematodes, mites and leeches are common (191).

Toxicity Testing -

Because of their large size, ecological role, and importance in hunting, much testing has been done with waterfowl, although work with captive birds has been limited because of the stringent migratory bird code. Most of the penned studies have used the oral route of exposure for pesticide testing (193,195,197,198,199,200,201). Disease studies have been another area where oral administration was used (202). Heavy metals and pesticides are also introduced into wild populations by the oral route (203,204,205,206,207). This has been the major route of exposure for heavy metals too (192,208). Spraying enclosed fields and ponds has been another field study method (209,210,211).

Table 10. SOME LONGEVITY RECORDS FOR WATERFOWL

Species	Maximum Age in Years
Magpie Goose	26 (captivity)
Wandering Whistling Duck	15 1/4 (captivity)
Trumpeter Swan	32 1/2 (captivity)
Whistling Swan	19 (captivity)
Graylag Goose	26 (captivity)
Canada Goose	33 (captivity); 23 (wild)
Egyptian Goose	25 (captivity)
Northern Green-winged Teal	20 (wild)
Common Mallard	20 (captivity); 16 (wild)
Canvasback	19 (captivity)
European Pochard	20 (captivity; fertile entire period)
Redhead	16 1/2 (captivity)
Common Goldeneye	17 (wild)

Source: (196).

Post mortem metabolism studies are another area of study (113). Yolk injection is a common method of pesticide and drug study. The stomach tube has been used in toxicological testing (212). Tests for the presence of certain metals have also been developed (213). Reference 214 has extensive tests of pesticides on waterfowl.

General Suitability -

Waterfowl are large enough to be easily handled. Many of them will breed readily in captivity, especially the mallard (Anas platyrhynchos) and black duck (Anas rubripes). Some waterfowl species are indeterminate layers (e.g., A. strepera) and will continue to renest if their eggs are destroyed or removed. This makes them good candidates for oological studies as they offer an almost unlimited supply of eggs during the breeding season. Because of their sensitivity, they are good models for ecological indices. Finally, most species adapt readily to laboratory conditions. A major drawback to their use in toxicological research is the migratory waterfowl code. The Canada goose and mallard duck were chosen as selected species because of their abundance and adaptability to laboratory conditions.

Ecological Alternatives -

Other waterfowl of the world.

Procellariiformes

Introduction -

This order is commonly called the tubenosed swimmers because of the peculiarly shaped openings of the birds' nasal passages. These sea birds have the ability to convert sea water to usable drinking water by means of their nasal salt glands. Shearwaters and petrels are usually found far out at sea. They are gull-like birds but their flight is distinctive. They are migratory and have diurnal habits. Their populations are small compared to most other birds and they are protected by legislation.

Caging Conditions -

Sea birds do not keep well in the lab, and to date, no successful arrangement has been made for long term laboratory studies of them.

Nutrition -

In nature, sea birds eat fish, crustacea, cephalopods, and macroplankton (215,216). It is not necessary to give them freshwater as they can convert sea water with their nasal salt glands. However, food and water should be supplied ad libitum.

Breeding/Rearing -

Shearwaters and petrels are colonial nesters (215). They have altricial young which are nidicolous. Most petrels are born with their eyes closed, while other sea birds are born with their eyes opened (183). The Bermuda petrel is wholly pelagic and visits land only to breed (216). Most lay one egg per year (183,215). If their nest is destroyed, they will not lay again that year (215). Incubation varies from six weeks to 11 weeks, depending on the species. Young petrels fledge at 9 1/2 weeks while shearwaters take 13 to 14 weeks (215). Young sea birds never breed their first year (215). The incubation period broadly correlates with the fledging period but apparently is not affected by egg size. Fledging period does not correlate with body size. The young have fat deposits (subcutaneous and visceral) which probably act as reserves when food is scarce (215).

Ecological Role -

Sea birds are aquatic carnivores feeding on both vertebrates and invertebrates.

Problems with Mass Culture -

Sea birds, although not hard to obtain, are not easily maintained in captivity and are hard to breed (215).

Toxicity Testing -

Because of their limited number and poor adaptation to laboratory conditions, little testing has been done on sea birds. What little has been done, has been conducted in the field. The effects of DDT and organochlorines on population dynamics have been studied (216,217,218,219). Gas liquid chromatography has been used on PCB concentrations (220).

General Suitability -

Sea birds range widely so they are not suitable for localized studies. They are slow reproducers (183). Their existing populations are too small for extensive testing to be done (216). Sea birds have not yet been successfully maintained for long periods under laboratory conditions. The sooty shearwater was chosen as a selected species on the basis of its relative abundance.

Ecological Alternatives -

Gulls and terns.

Ardeidae and Threskiornithidae

Introduction -

These two families are commonly known as herons and egrets (Ardeidae) and ibises (Threskiornithidae). They are all stork-like wading birds with long necks, long legs and pointed, herons and egrets, or decurved, ibises, bills. They are semi-aquatic birds associated with coasts, marshes and rivers. They are large birds with body lengths ranging from 11 inches, least bittern, to 52 inches, Great blue heron (221). They are primarily colonial tree nesters and their colonies are called rookeries. Herons, egrets and ibises are diurnal and migratory. There were never large numbers of them, but man's encroachment has made many of them endangered species. For this reason, they are all protected by legislation.

Caging Conditions -

An outdoor cage, made of chickenwire with a wooden frame and measuring 50 x 10 x 6 feet has been successfully used (20).

Breeding/Rearing -

Herons and their allies usually lay a clutch of three eggs once a year (222). Their young are altricial but have their eyes open at hatching. As with other altricial young, they are nidicolous (183). Young ibis fledge at about five weeks of age (222).

Ecological Role -

Herons, ibises and egrets are aquatic carnivore. They feed on both vertebrates and invertebrates.

Problems with Mass Culture -

Herons and their allies are slow breeders. They require a good deal of space because of their size and habits (182).

Toxicity Testing -

These birds have been used very little in toxicological studies. However, DDT and toxaphene have been fed to them in capsule form as well as sprayed in their natural environment (185). General pesticide studies have been done on population dynamics in the field (222).

General Suitability -

Size is a somewhat limiting factor in using some herons. As with most other birds, Ciconiids are protected by law. They are fairly easy to maintain in captivity; however, they are not easy to breed. The American egret was chosen as a selected species because it typifies this group of birds.

Ecological Alternatives -

Cranes, rails, coots.

Gruidae and Rallidae

Introduction -

The first family is represented by the cranes, the second family by the rails and coots. Cranes are found on the prairie and, like rails and coots are semi-aquatic. Cranes are large stork-like birds while rails and coots are plump somewhat chicken-like birds. Both families have secretive habitats, are shy and much more often heard than seen. Cranes, rails and coots have diurnal habits, are migratory and are protected by legislation.

Breeding/Rearing -

Gruiiforms have precocial young that are born with open eyes. Like most precocial birds, they are nidifugous (183).

Ecological Role -

Cranes, rails and coots are primarily aquatic carnivores and feed on invertebrate populations.

Problems with Mass Culture -

Cranes are slow reproducers (182).

Toxicity Testing -

Not much testing has been done on this group, however, tissue residual studies have been done using dieldrin and organochlorides (223,224). Hatchability studies have also been done on a limited scale (223).

General Suitability -

Rails and coots are readily available for study, but cranes are not nearly as numerous. The American coot was chosen to represent this group because of its abundance.

Ecological Alternatives -

Herons, ibises, ducks.

Scolopacidae

Introduction -

Shorebirds have not been used very extensively in laboratory research. The most widely used member of this group is the woodcock (Philohela minor) and for this reason it will be discussed here. The woodcock is a little larger than the bobwhite quail and has an extremely long bill. It inhabits woodland swamps and leafy thickets throughout the United States. At dusk, during the breeding season, it makes aerial displays, calling loudly, and beating its wings. The woodcock is migratory, territorial and protected by legislation.

Caging Conditions -

Various types of cages have been employed to house woodcock, ranging from a 20 x 40 x 8 feet field enclosure and a 4 x 4 x 5 feet cage resting on the ground, to a 22 x 29 x 30 inch stainless steel cage in a laboratory. All cages had water and food pans (225). Hemmed burlap was placed over the top and front of the cages to prevent the birds from flushing and injuring themselves at the least disturbance. The burlap was hemmed to keep the woodcocks from becoming ensnared in its ends and injuring themselves. Injuries of birds attempting to flush were also reduced by clipping feathers from one wing, making the cage walls opaque and using high cages or false ceilings of fabric (225). The smaller cages were found to be more satisfactory because they allowed the birds to find their food more readily. Smaller steel cages also helped eliminate the sanitation problem common in the maintenance of woodcock.

Nutrition -

Woodcocks were fed a diet of earthworms, mealworms and fly larvae placed in peat moss in food pans (225). Because the bird has a high metabolism rate, food and water should be supplied ad libitum (225).

Breeding/Rearing -

Woodcocks have precocial young that are born with their eyes open (183). They are nidifugous (183).

Special Needs -

Woodcock require a constant supply of fresh, live earthworms (38,225).

Behavioral Restrictions -

Woodcock are high strung and therefore prone to injuries in caged conditions. Their cages are impossible to keep clean and being nonperching birds they live on the contaminated floors, causing themselves to become soiled (225).

Ecological Role -

Woodcocks are carnivorous, feeding on invertebrates they find near the shores of ponds, lakes and streams.

Problems with Mass Culture -

The major problem with maintaining woodcocks is keeping an adequate supply of fresh, live earthworms (38,225). This is particularly hard to do in winter. Another important problem arises from the unsanitary conditions under which the birds are caged; foot infections are a particular problem (225). Cages must be built to maximize cleaning efficiency and minimize self-inflicted injuries of the birds.

Toxicity Testing -

Because of the effort it requires to keep woodcocks, not much work has been done with them. DDT studies (226,227, 228) and Heptachlor studies (228,229) have been conducted however, in addition to general pesticide studies (27,230).

General Suitability -

Woodcocks are fairly available and socially tolerant of one another. They are relatively hardy in captivity, withstanding heat and cold well and fighting off a wide range of minor injuries and infections. However, they are costly and troublesome to feed and create a difficult cage sanitation problem. Cages must be built to minimize injuries as woodcocks are far more prone than other birds to injure themselves (225).

Ecological Alternatives -

Snipe.

Lari

Introduction -

The suborder Lari is composed of two groups of birds: gulls and terns. They are all colonial nesters. Gulls and terns are primarily coastal birds, but some species are also found along river banks. In any case, they are associated with the aquatic environment. Gulls range in size from the great black-backed gull (Larus marinus) (28-31 inches long) to the Bonaparte's gull (Larus philadelphia) (12-14 inches long) (221). The most widely known gull is the herring or "sea" gull. Terns are the size of smaller gulls and are more streamlined than a gull. Members of the suborder Lari are diurnal, migratory and protected by legislation.

Nutrition -

Gulls and terns eat a variety of aquatic life, both vertebrate and invertebrate. In captivity, food and water should be supplied ad libitum.

Breeding/Rearing -

Gulls and terns usually lay four eggs to a clutch and one clutch per year (183). They are colonial nesters and will renest if their nest is destroyed early enough in the season (184). The young are precocial and have their eyes open when they hatch, but they are nidicolous (183); the average incubation time is 27 days, and the young are able to fly at 43 days (231).

Ecological Role -

Gulls and terns are carnivorous in the wild, feeding on vertebrates and invertebrates alike. They have become scavengers of refuse dumps.

Problems with Mass Culture -

They do not live well in captivity (185). Like other birds they are hosts for fleas, mites, ticks, and lice(182).

Toxicity Testing -

Most testing has been done in the field because long term studies are not possible at present in the laboratory. The primary route of exposure has been oral and the birds have been tested for DDT (185, 231), PCB's (220,232,233, 234, 235), organochlorines (210), chlorinated hydrocarbons (236,237), general pesticide (231) and pollution effects (184) as well as eggshell changes (188).

General Suitability -

Gulls and terns are hard to maintain in captivity (185). For short term studies and ecological studies they make good subjects because they are colonial nesters and it is relatively easy to capture large quantities of birds in a short time. Their eggs are also easily obtainable. Herring gulls were chosen as a selected species because of their abundance and previous use in the laboratory.

Ecological Alternatives -

Pelicans, cormorants.

Picidae

Introduction -

This is the family of chisel-billed tree-climbers known as woodpeckers. These birds have stiff, spiny tails which act as props as they hitch their way up the trees. The males of most species have some amount of red on the head. They are represented throughout the U. S. in woodlands. They are hole nesters, usually excavating their homes in tree trunks. Woodpeckers are diurnal and migratory. Like most birds, they are protected by legislation. Very little is known concerning their adaptability to laboratory conditions.

Breeding/Rearing -

Woodpeckers lay two to six eggs per clutch. There is a one-day laying interval between eggs and a 12-day incubation period (215). The young are altricial and are born with their eyes closed. They are nidicolous (183) and usually fledge in about 24 days (215). Sexes are readily distinguishable in breeding adults (182).

Ecological Role -

Woodpeckers primarily eat insect larvae which inhabit the interior of tree trunks and limbs but also eat seeds. They are a major check on insects that attack trees.

Problems with Mass Culture -

All birds are hosts for fleas, mites, lice and ticks and can become heavily infested with them (182).

Toxicity Testing -

Very little work has been done with woodpeckers in this area. Field spraying has been done, however, and the population dynamics that result have been studied (238).

General Suitability -

The red-headed woodpecker has been chosen as a representative of this group because it is abundant. However, no member of this group of birds is judged to be very suitable for testing.

Ecological Alternatives -

Brown creeper, nuthatch.

Falconiformes and Strigiformes

Introduction -

The Falconiformes are eagles, hawks, and falcons. Strigiformes are owls. Most of these birds are impressive in size and perform spectacular aerial displays. Of all the birds, they are probably the best known in American folk stories. Their feathers are especially adapted for soaring, sudden aerial dives and silent flight. Their beaks and claws are modified into sharp cutting and tearing implements with which they can grasp, kill and eat their prey. Members of the order Falconiformes are diurnal, while Strigiformes are nocturnal. Like most other North American species of birds, raptors are migratory. Some of them, such as the bald eagle (Halioeetus leucocephalus) are on the Federal endangered species list. Although some species are very restricted in their distribution, most are represented at least by a subspecies throughout the United States. With the exception of eagles and fish hawks, which prey on fish, all raptors are associated with terrestrial environments. Most raptors build their nest platforms in high tree tops or fairly inaccessible nooks and crags. The sparrow hawk (Falco sparverius) is an exception as it is commensal with humans. It can be found nesting in attics, and other nooks and crannys of barns, factories, school buildings, churches, etc. (182).

Caging Conditions -

A recommended outdoor pen for a pair of sparrow hawks is 8 x 12 x 7 feet with a roof to keep predators out. The cage consists of a wooden framework with 1 x 1-1/2 inch mesh partially sunk in the ground. Each cage has a nest box 10 x 10 x 15 inches with a 3 inch diameter opening and a slab perch below the opening. Larger scaled (50 x 20 x 6 feet) versions of this cage were used to house multiple pairs of hawks (239). An alternate cage for a pair of sparrow hawks is 6 x 6 x 8 feet solid wire (240). See Figure 5 (Cage) and Figure 6 (Nest Box) for the outdoor housing used for barn owls at the Patuxent Wildlife Research Center.

Nutrition -

Sparrow hawks have been maintained on day-old dead cockerels (240), but a more rounded diet consists of ground beef (mostly muscle, liver, tongue, jowls and heart), vitamins, minerals and turkey breeder crumbs (239).

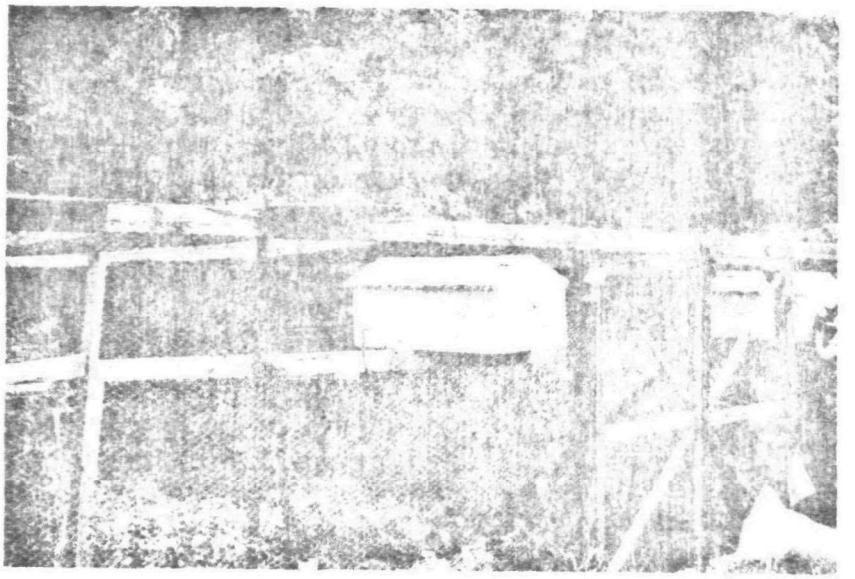


Figure 5. Barn owl cages of chicken wire and wood, 50' x 6' x 10', with nest boxes as shown. Patuxent Wildlife Research Center (Dr. I. Klaus)

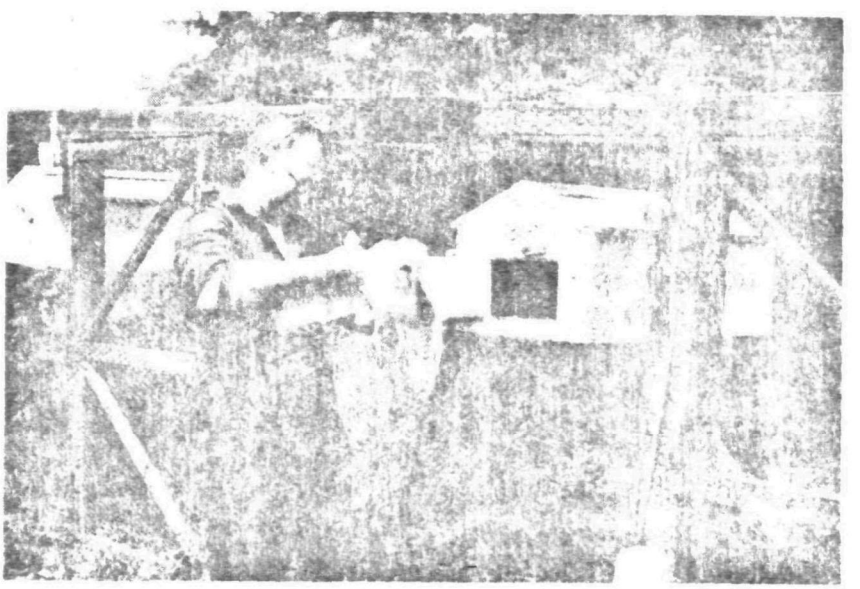


Figure 6. Nest box for barn owl cages. Patuxent Wildlife Research Center (Dr. I. Klaus)

A combination of whole white rats, white mice or hamsters, chicken heads and skinned chicken necks can be substituted for the ground beef (239). This diet will sustain most other hawks and owls also, but eagles and fish hawks must b given fish (182). Food and water should be available ad libitum.

Breeding/Rearing -

Sparrow hawks lay three to seven eggs per clutch and will breed their first year. Pairs need not be housed separat (239,241). Peregrine falcons also lay three to seven eggs per clutch, and lay one clutch per year (239). Prairie falcons lay two to five eggs per clutch per year (242). Peregrine and prairie falcons cannot be housed in multiple pairs. There is usually a two to three day laying interval between eggs in raptors and incubation generally takes 30 days. The usual fledging period is also 30 days (215). Raptors have altricial young which are born with closed eye They are nidicolous (183). Most raptors do not breed until they are two to three years old (182).

Special Needs -

Raptors need some types of roughage in their diet to help them form fecal pellets. This can be in the form of feathers, fur or crumbs (239). They need shelters to escap the wind if they are kept outdoors (239). They also need smaller mesh around the cage by the nest box to keep mammal ian predators away from the eggs and young (239).

Behavioral Restrictions -

Wild adult raptors never tame and even hand-reared young must constantly be worked with to keep them gentle. Even the smaller hawks are capable of inflicting deep lacerations.

Ecological Role -

With the exception of eagles and fish hawks, raptors are carnivorous mammalian predators and constitute a major chec on small mammal populations. Eagles and fish hawks are pre dators on fish populations.

Longevity -

Next to members of the parrot family (Psittacidae), raptors are probably the most long lived birds on earth.

Problems with Mass Culture -

Raptors are relatively slow reproducers and require large pens for breeding and rearing (182). These birds are hosts for external parasites also, but not to the extent that most birds are (182).

Toxicity Testing -

Most testing has been done on wild trapped birds. The birds were usually fed the toxic substances in foods. Whole bird analysis of DDT has been done on sparrow hawks (243,244), PCB analysis with most raptors (240,242,245,246), and Methyl mercury has been studied in the red-tailed hawk (247). Finally, egg shell thinning as the result of organchlorines has been studied in nearly all raptors (138,184,188,210,237, 248), while DDT residue studies have been done with bald eagles (249).

General Suitability -

Raptors are fairly available and fairly easy to maintain but with the exception of sparrow hawks they are hard to breed in captivity and they are slow reproducers. The larger species are somewhat hard to handle. However, they can be easily marked for identification and are good indicators of pesticide levels in their environment. The barn owl and sparrow hawk were chosen from this group because of their abundance and previous use in testing.

Ecological Alternatives -

Kites, shrikes.

Columbiformes

Introduction -

This group is unique in North America because it feeds its young crop (or pigeon) "milk", a pasty substance produced in the bird's crop. Both male and female birds are capable of producing this substance. The domestic pigeon is the most common and widespread member of this group. Most birds of this group are diurnal, migratory, and territorial. The domestic piegon is an exception to the last two of these classifications. Except for the domestic piegon, these birds are protected by legislation.

Caging Conditions -

Cage specifications differ as the number of birds to be caged varies. For a cage designed to house one bird, a 20 x 29 x 19 cm steel rat cage (250), and a 40 x 40 x 64 cm cage made of adequate materials (251) have been suggested. Pairs should be kept in 7.3 x 2.5 cm^2, welded wire cages, having an area of 45.7 cm^2. A perch, food can, automatic watering device and a nest box (15.2 cm^2, 5.1 cm deep) should be provided (252). A cage having the dimensions 115 x 13[illegible] x 229 cm has also been suggested for a pair of birds (253). A 14L:10D photoperiod is recommended for breeding pairs (251, 252, 253). Cages should be kept in an environment having a temperature of 20°C $\pm$ 1°C (253). See Figure 7 for the caging system used at the Denver Wildlife Research Center.

Nutrition -

Various diets have been suggested for this group of birds. One includes a strictly commercial pellet diet while the other incorporates a mixture of wheat, maize and tick beans (251). Grit should be available to the birds at all times as a digestive aid and mineral supplement (182). See Figure 8 for the feeding and watering system used at the Denver Wildlife Research Center.

Breeding/Rearing -

In the wild, this group of birds breeds all year. A reduced rate of breeding is generally noticed between October and December (254). Domestic pigeons breed readily in captivity and are available from commercial breeders (255). Under tropical conditions, two eggs make up a clutch. The usual laying interval between eggs is 1.5 to two days, the

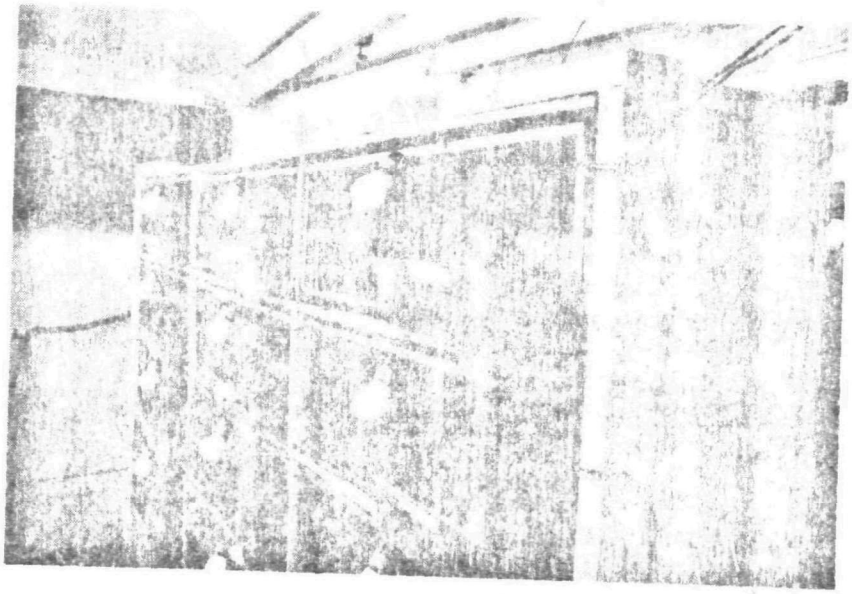


Figure 7. Mourning dove cage. Denver Wildlife Research Center, Federal Center, Denver, Colorado

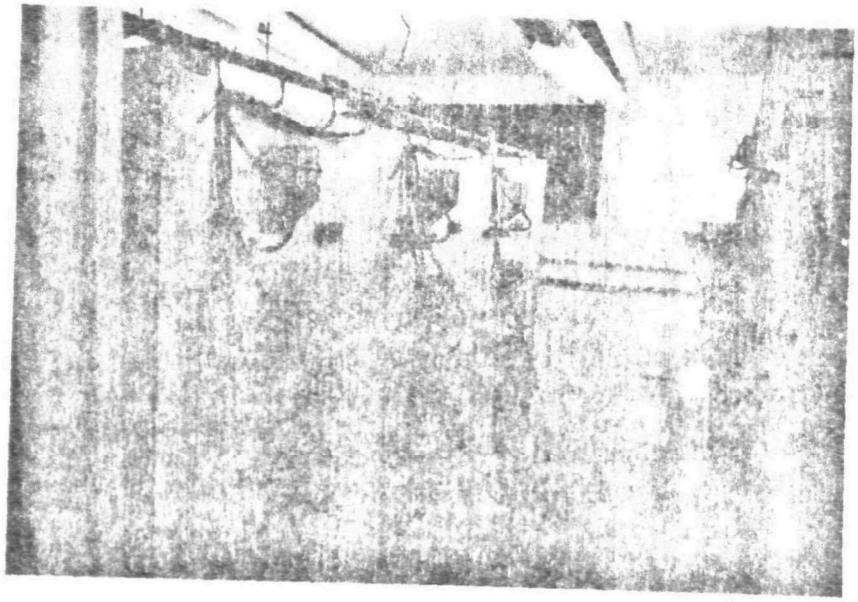


Figure 8. Food and water distribution system for mourning dove cages. Denver Wildlife Research Center, Federal Center, Denver, Colorado

incubation period is 15 days, and the fledging period is 17 days (215). Adult birds stay sexually active for years (256). Fertility in these birds ranges from 83.3 percent to 92.9 percent, while hatching occurs in 81.9 to 90.5 percent of eggs produced (252). In general, experienced pairs prove to be more successful propagators than inexperienced pairs (252).

At birth, these birds have their eyes closed and they are both altricial and nidicolous (183).

Ecological Role -

Pigeons and their allies eat seeds and grains. They are preyed upon by domestic cats, other carnivorous mammals, and birds of prey.

Toxicity Testing -

Much toxicity testing has been done using pigeons, ring doves and mourning doves as test specimens in the laborato PCB's have been administered to these birds to examine effects on eggshells (257) and to measure residue (253). Methods of PCB administration include oral injection (253, 257), and intraperitoneal injections (257). Lead residues have been analyzed at laboratories in birds wounded by hunter shotgun pellets (258). The synergistic effects of dieldrin and DDE on the thyroid gland (251) have been studi along with steroid breakdown due to dieldrin and DDT administered through the diet. The reproductive effects of DDE (250), the effect of DDT on the thyroid gland, and metaboli (256,259) have been observed after oral administration. Res dues have been analyzed in eggs and tissues after aldrine and dieldrin have been orally administered through a bird's diet. Acute toxicity has also been measured (260). Residu studies have been performed using dieldrin which was administered orally (255). Breeding and reproductive effects o mestranol when added to synthetic grit have been studied (252,261). Parathion metabolism and liver activation have been investigated (152). Acute toxicity of a wide variety of pesticides has been investigated (199).

Anesthetic effects of drugs and other materials on this group of birds have been evaluated. Pentobarbital and ketamine, injected into the pectoral muscle, have been used to evaluate these effects. Anesthesia chamber experiments have also been documented (262).

Various behavior studies have been performed on this bird group. The behavioral effects of pentobarbital (263), chloropromazine (264), and promazine (264), orally administered, have been evaluated. The reversal of discriminatory performance due to orally administered scopolamine has been observed (265). SC-12937 has been placed on food to evaluate a bird's taste discrimination (266). General drug tests and their relation to behavior have been documented (267), and numerous Skinner-type behavior experiments have been performed (268,269,270,271,272).

Field studies on these birds include an evaluation of breeding effects after exposure to SC-12937 via wheat, and of lead residue in tissues of birds to determine air pollution levels (273).

General Suitability -

This group of birds has been found to be fairly suitable for testing because they are large enough for good samples, yet they are easily housed and marked. Pigeons, ring doves, and mourning doves also have good temperaments (182). Easy conditioning and training are other advantages of this bird group (267,272). The rock dove (domestic pigeon) has been chosen to represent this group on the basis of availability and previous use in testing.

Ecological Alternatives -

Seed-eating passerines.

Colinus virginianus

Introduction -

This is the bobwhite quail, one of the smallest members of the Galliformes. It is about the size of a meadowlark (8-1/2 to 10-1/2 inches long) and is chiefly an eastern bird, found in farming country from the Gulf of Mexico north to Ontario. Local populations of it are found in Texas and Florida. The bobwhite quail is a denizen of open country and is usually found in family groups. Quail are diurnal creatures, foraging during the day and roosting on the ground in their characteristic wagonwheel coveys at night. Quail are migratory upland game birds and are protected by legislation.

Caging Conditions -

A recommended cage is constructed of one inch wire mesh in dimensions of 6 x 10 x 8 inches. Twenty-four of these cages constitute a rack in the laboratory (274). An alternate pen, designed to hold a larger number of birds, is 4 x 6 x 1.5 feet (275). Quail have also been housed outdoors in 3 x 6 foot pens with wire netting roofs to guard against predators (276). The outdoor holding pens used at the Patuxent Wildlife Research Center are shown in Figure

Nutrition -

A variety of commercial products have been used, ranging from Purina F&M Game Bird Chow (274) and Purina Turkey Starter Mash (277) to Purina Pigeon Chow (278). All foods and water were given ad libitum.

Breeding/Rearing -

Bobwhite quail eggs have a 25-day incubation period (276). The young are precocial and hatch with their eyes already open (183). As with other precocial birds, quail young are nidifugous (183). Sexes are visually distinguishable in adults (182).

Behavioral Restrictions -

Young quail should be "debeaked" to prevent cannibalism (27

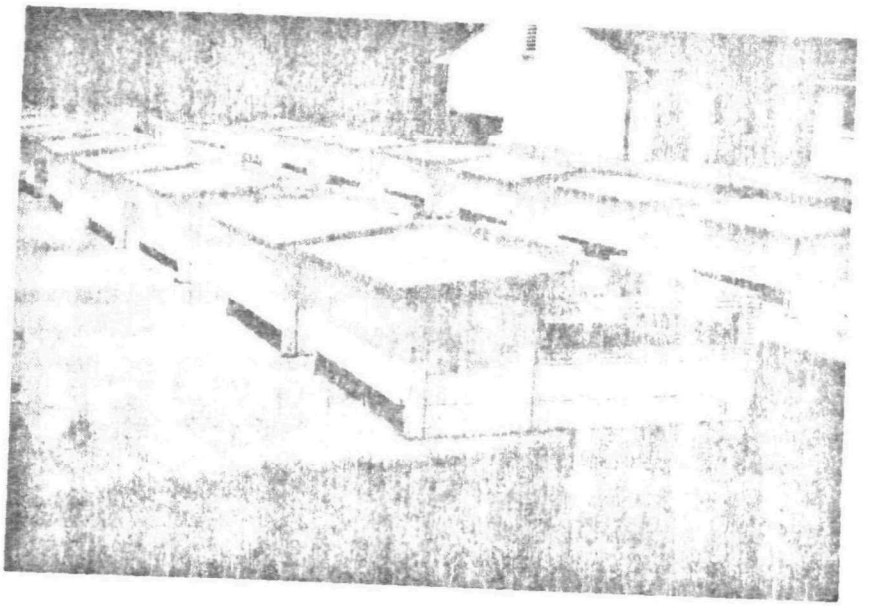


Figure 9. Bobwhite quail outdoor holding pens (base approx. 4' x 8'). Patuxent Wildlife Research Center

Ecological Role -

Quail eat seeds and grains. They are prey for larger mammalian carnivores. Their young are preyed upon by raptors as well as mammalian carnivores.

Problems with Mass Culture -

Birds can become heavily infested with a number of fleas, mites, ticks and lice (182).

Toxicity Testing -

In all penned studies the toxic substances were fed in dietary form. Blood parameters (274), behavioral (280) and residue studies (278) have been done with DDT. Population dynamics were studied using chlorinated hydrocarbons (281, 282) and PCB's (283). Assays were done with aroclor (277) and chlorinated hydrocarbons (284). General observation studies were done with fenuron (279). Residue studies were done with mosquitoe larvicides (275) and mirex (276). Thyroid studies were done using calcium deficient diets (280). Studies on bait acceptability were done with chemical repellants (285). Population dynamics were also studied in fields sprayed with heptachlor (286).

General Suitability -

The bobwhite is a prolific breeder and does not require much housing space. It is readily available and easy to maintain. For these reasons it is a recommended species.

Ecological Alternatives -

Coturnix, pheasant, grouse.

Phasianus colchicus

Introduction -

This is the ring-necked pheasant, an introduced bird in the Americas. It has become established in farming country, mainly north of the Mason and Dixon Line. A long pointed tail readily distinguishes it from other upland game birds. The pheasant is the largest of the wild gallinaceous birds and, like all members of this, it is a diurnal creature. The pheasant is a denizen of open country and an excellent bird for sportsmen. It is protected by legislation. Although the pheasant is not as mobile as passeriforms, it is still considered to be migratory.

Caging Conditions -

A pen complex 22 x 6.1 x 1.7 m was portioned into 27 individual cages. Of these, nine pens were 2.4 x 2.4 x 1.7 m with dirt runways and 1.2 x 1.2 x 1.7 m plywood shelters. The other 18 pens were only half as wide but otherwise identical (287).

Nutrition -

As with all birds, food and water should be fed ad libitum (282,287). A basal diet was that recommended for nine to eighteen week old pheasants in the Poultry Formula Guide (288).

Breeding/Rearing -

Like other gallinaceous birds, pheasant young are precocial and hatch with their eyes open (183). They are nidifugous (183). Sexes are distinguishable in adults (182).

Behavioral Restrictions -

Pheasants are cannibalistic in close quarters. To prevent this, young chicks should be "debeaked" (289).

Ecological Role -

Pheasants are primary consumers, feeding on seeds and grain. Young pheasants, however, are primarily insectivorous for the first weeks of their life.

Problems with Mass Culture -

Birds can become heavily infested with ticks, lice, mites and fleas (182).

Toxicity Testing -

Numerous studies have been done on the pheasant. The two major routes of exposure to toxic substances are mixing the substance with chow and force feeding gelatin capsules. The former method was used to study population dynamics with dieldrin (290,291) chlorinated insecticides (282), chlorinated hydrocarbons (281), DDT and toxaphene (291) and ethyl mercury p-toluene sulfonanilide (292). It was also used in monitoring studies with pesticides (293), metabolism studies with PCB (294), residue studies with heptachlor and ^{14}C-lindane (295), behavior studies with PCB's and general physiology studies with DDT (296). The latter method was used to study dieldrin residues (29 PCB residues (298), eggshell thinning caused by dieldrin (299), pharmacodynamics of dieldrin (300) population dynamics caused by dieldrin (301)and stress due to PCB's (302). Behavior due to dieldrin (303), LD_{50}s of pesticides (199), monitoring of aldrin (304), residue studies on organochlorine insecticides (248, 305), aldrin (306), and organochlorines in general (210) were also done in the field.

General Suitability -

Pheasants breed readily in captivity. They are easily maintained although they require larger cages than most other gallinaceous birds.

Ecological Alternatives -

Grouse, turkey.

Miscellaneous Galliformes

Introduction -

This group includes several species of grouse, prairie chicken and partridge. The birds on the whole are chicken-size or slightly smaller. Of the galliforms, this group has the most spectacular courtship displays. The booming grounds of the prairie chicken and drumming logs of the grouse attract many amateur and professional ornithologists each year. These displays are used to establish breeding territories and also to attract mates. Grouse designate certain trees in their territories as roosts and use these trees habitually. Galliforms are classed as upland game birds because of their topographical distribution and the sport they provide for hunters. Like other gallinaceous birds, they are diurnal creatures. They are migratory and are protected by legislation.

Caging Conditions -

A number of different cages have been used for housing gallinaceous birds. A 24-1/2 x 2-1/2 x 2-1/4 inch concrete tank with wire mesh top is recommended (307). An alternate type of housing is 20 x 10 x 6 feet pens constructed of fish netting on steel posts (307). A third method of housing consists of pens 8.1 x 16.2 feet with turf floors and mesh roofs (308).

Nutrition -

Commercial game bird chow in pellet form has been fed these birds (308). Food and water were given ad libitum.

Breeding/Rearing -

Gallinaceous birds have precocial young that hatch with their eyes open. They are nidifugous (183). Grouse reach sexual maturity in approximately 112 days (309).

Behavioral Restrictions -

Gallinaceous birds are cannibalistic and need to be "de-beaked" (289).

Ecological Role -

Galliforms are primarily seed and grain eaters. Their young, however, eat insects as well for the first weeks of their life. All these birds are preyed upon by mammalian carnivores.

Problems with Mass Culture -

Birds are hosts for a number of ticks, mites, fleas and worms (310).

Toxicity Testing -

Zectran was fed to birds in capsule form followed by gross observation and monitoring (307). Dieldrin and malathion were mixed with game chow and general behavior disruptions in the peck order were studied (311). Chlorinated hydrocarbons and organophosphate were mixed with chow and fed to the birds. Various parameters of population dynamics were then studied (308). Field studies include work with phosphamidon (312), DDT (313), and zinc phosphide baits (314). Telemetric studies were also done with dosed birds (307, 31 316).

General Suitability -

Gallinaceous birds are easy to maintain and breed in captivity. They are particularly useful in field and laboratory telemetric studies because of their physical size and high fidelity to their territories (307). The blue grouse has been chosen to represent this group because of its previous use in testing.

Ecological Alternatives -

Coturnix , bobwhite, pheasant.

Passeriformes-Granivores

Introduction -

This group includes the cardinal, song sparrow and other finches. These are mostly small, sparrow-size birds and are distributed throughout North America. They are inhibitants of wooded areas and fields and are associated therefore with a terrestrial environment. Members of this group are diurnal, migratory, highly territorial and protected by legislation.

Caging Conditions -

Different investigators have used different sized cages to house this bird group. One cage used measures 120 x 60 x 60 cm; the floor, roof, and back of this cage are wood, while the front and sides consist of 12 mm mesh (317). Food and water must be available at all times. A nest box should be provided in the cage, or materials for building nests may be put at the birds' disposal (318). Another investigator specifies a cage 61 x 41 x 38 cm for a single bird (319). Large communal aviaries are recommended for long term experiments (320).

Nutrition -

In the lab, these birds are usually fed either pigeon chow (275), canary or millet seed (319), or a mixture of biscuit meal, dried milk, soya bean meal, and maw seed. The mixture diet is considered good for breeding (319). Cuttle bone should be available and grit should be provided as a digestive aid and mineral supplement (182,319).

Breeding/Rearing -

This bird group commonly has four nestings a season with two to five eggs per nesting. The female usually incubates the eggs, and incubation lasts 13 to 14 days. Hatchlings usually leave the nest after nine to 11 days, and are considered fleged at 45 days. A complete molt occurs, in both young and adults, during August or late summer (288).

Behavioral Restrictions -

These birds are highly territorial and so require separate cages for each breeding pair (182).

Ecological Role -

Cardinals, sparrows and finches serve as granivores in the food chain. Small mammals commonly eat their eggs, and cowbirds parasitize them (321).

Longevity -

A song sparrow's life span in the wild is approximately two years (322). A cardinal lives between three to six years in a natural environment (321).

Problems with Mass Culture -

The main problem encountered with mass culture is parasitism. Common parasites on this bird group are lice, flies, mites and ticks (321).

Toxicity Testing -

Limited toxicity testing has been performed on this bird group. Acute toxicity of mosquitoe larvicides has been evaluated after ingestion (275). Reproductive effects of orally administered DDT (319) and residue analysis (323) have been documented.

Various non-toxicological investigations have been done including prehatching and hatching behavior (324), flocking behavior (317), hostile, sexual, and social behavior, and adaptive behavior (325).

General Suitability -

This group, although readily available in the wild, does not do well under laboratory conditions. They are relatively difficult to breed in captivity, although there has been some success with house finches (320). Cardinals, song-sparrows and house finches were recommended as test species because of their numbers and somewhat successful breeding in captivity; the house finch was also chosen because it is representative of western birds.

Ecological Alternatives -

Blackbirds.

Passeriformes-Insectivores

Introduction -

This category is represented in laboratory testing by the families Turdidae (thrushes), Troglodytidae (wrens), Alaudidae (larks), and Hirudinidae (swallows). Most species are highly territorial with monogamous mating systems the rule. This presents inherent problems for attempting to rear them under controlled laboratory conditions.

Most passeriformes are diurnal. The swallows are crepuscular. All are migratory and most winter in Central America.

Although they are associated with streams and other bodies of water, insectivorous passerines are purely terrestrial denizens. With the exception of the horned lark which nests on the ground, and the swallows, which nest where their common names suggest (i.e., banks, cliffs, barns, chimneys), these birds are tree and shrub nesters.

Virtually all species of passerines are protected by legislation which makes them generally unavailable from the wild.

Caging Conditions -

A recommended communal cage is 9 x 12 x 7 feet. This will house 20 to 70 individuals (326). Ten birds can be housed in a cage 20 x 15 x 22 inches (327). Individual metabolism cages for these birds are 9-3/4 x 7 x 8 inches (327). To stimulate spermatogenesis, birds were maintained on a 15L:9D photoperiod (328) at 70° to 80°F (329).

Nutrition -

These birds feed on a variety of insect life including beetles, true bugs, spiders, caterpillars, ants and other small Hymenoptera. Grasshoppers, crickets, craneflies, moths, millipedes and snails are minor food items while dragonflies, mites, sowbugs, daddy-longlegs and pseudoscorpions are merely tasted (330).

Breeding/Rearing -

Passerines usually lay five to six eggs per clutch with a one-day laying interval between eggs (330). The incubation

period is usually 13 to 16 days (215, 330). The young are altricial and are born with eyes closed (183). Temperature is an important factor in determining the length of incubation (330). They are nidicolous (183) and fledging on the average takes 13 days (215). Sometimes 3 broods are reared per season (330). Both parents usually care for the young after hatching (330). Swallow eggs have a 20 day incubation period and young are fledged 40 days after hatching (215). Adult passerines undergo a complete postnuptial molt in August and young birds undergo a partial juvenal molt at this time also (330).

Behavioral Restrictions -

Passerines hare highly territorial birds and so require individual cages for each breeding pair (182).

Ecological Role -

Wrens, swallows, thrushes and horned larks are all insectivores. Wrens are forest foragers, swallows and horned larks are denizens of open country, and thrushes occupy both habitats. All are preyed upon by carnivorous mammals and predatory birds.

Longevity -

The average life span for wild birds is one to three years, but passerines have been known to live 12 years (331).

Problems with Mass Culture -

Passerines are territorial so each pair must be kept in separate cages if breeding studies are undertaken (182). Passerines can become heavily infected with mites and ticks (330). Swallows are poor breeders in captivity and cannot be easily maintained for long periods in the lab (182).

Toxicity Testing -

Birds were dosed by mixing the toxic substances with their food or by loading the food with the substance. DDT was fed and residual studies were done (326, 332). DDT was also used in field studies of populations (333, 334) and residue (37, 335, 336, 337). Loaded food was used to study the residue and pharmacodynamics of dieldrin (38). Methoxychlor was fed in field and lab residual studies.

General Suitability :-

Because they require a constant and abundant supply of insects, they are poor candidates for testing subjects. The robin is the best candidate among them because of its distribution and abundance.

Ecological Alternatives -

Vireos, warblers, goatsuckers.

Passeriformes-Omnivores

Introduction -

This group includes four North American families: Sturnidae (starling), Corvidae (jays and crows), Icteridae (blackbirds) and Ploceidae (weaver birds). Ecologically these families range from prairie and open field denizens (meadowlarks) to early successional and edge species (grackles, cowbirds, redwings) to late successional woodland (jays and crows). With the exception of the house sparrow (Passer domesticus) and the European tree sparrow (P. montanus), which are both permanent residents, these birds are all migratory. Most species are highly territorial and monogamous, breeding in loose but discrete colonies and foraging and roosting in flocks at other times of the year. Many of these birds are not protected by the Migratory Bird Treaty Act and so they are readily available from the wild.

Grackles and redwings are usually found around water, while the other members of this group are usually associated with purely terrestrial environments. Blackbirds generally nest in swampy or marshy areas or wet meadows. Jays and crows usually nest in tree tops and weaver birds and starlings, who are commensal with man, nest in almost any available nook or cranny.

Caging/Lab Conditions -

Because these passerines are so diverse in their physical sizes, a number of different cage dimensions are in use. For the sparrow-sized members of the group, a cage 7 x 7 x 7 feet will hold 50 to 100 birds indoors for a four to six week period (285). A cage 2 x 2 x 2 feet will hold seven to ten of these birds for three days (285). Individual test cages are 6 x 9 x 6 inches. One inch mesh poultry netting is used in all cases. A community cage for grackles, red-wing blackbirds and other blackbirds is 9 x 12 x 7 feet; this will house 20 to 70 individuals (326). Ten birds of this size can be housed in a cage 20 x 15 x 22 inches (327). Individual metabolism cages for these birds are six inches cubed (339, 340) or 9-3/4 x 7 x 8 inches (327). Caging requirements for crow-sized passerines were not available. See Figure 10 and Figure 11 for cages and traps used at the Denver Wildlife Research Center.

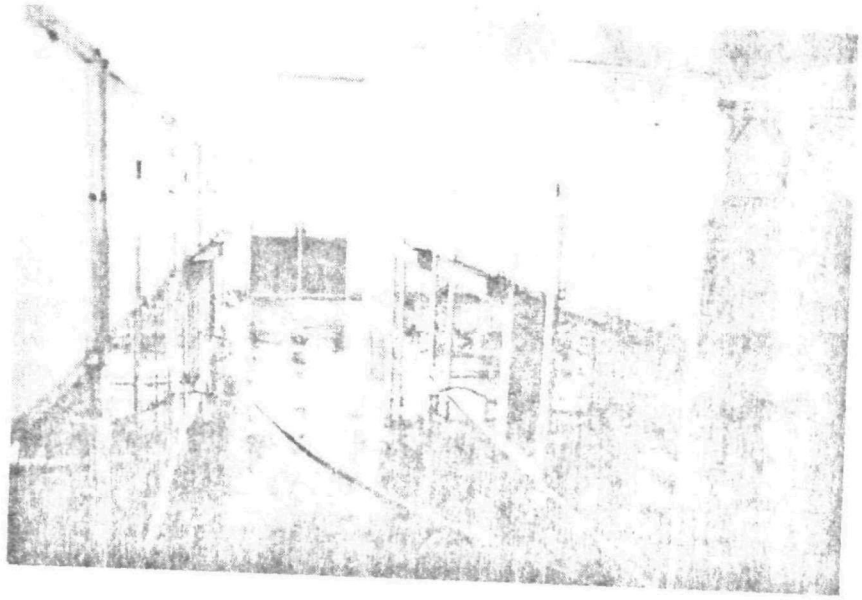


Figure 10. Starling and wild bird cages. Denver Wildlife Research Center, Denver, Colorado

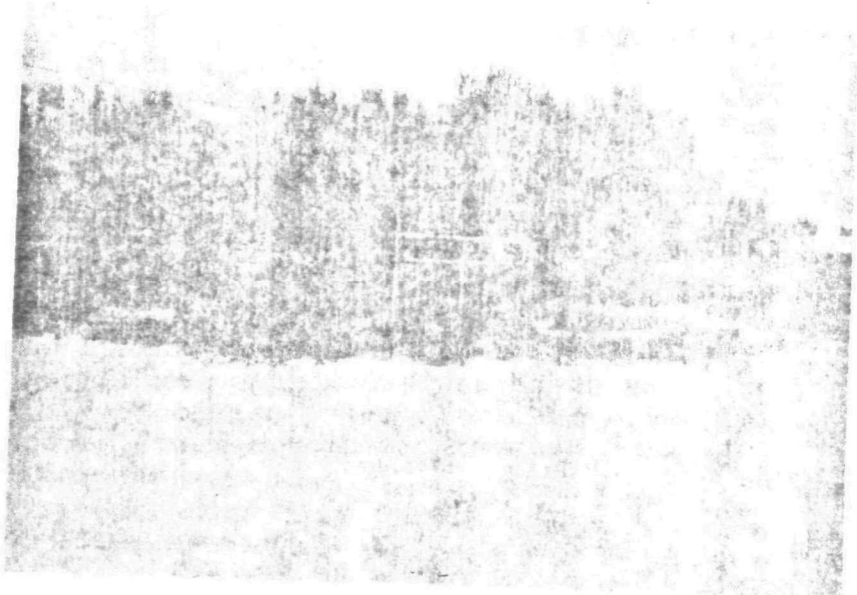


Figure 11. Wild bird traps - birds trapped include starlings, sparrows, robins and crows. Denver Wildlife Research Center, Denver, Colorado

To stimulate spermatogenesis, birds were maintained on a 15 L: 9D cycle (328) at 70° to 80°F (329).

Nutrition -

Pigeon chow checkers (275) and chick starter mash (335) were used to feed house sparrows. Hulled rice (285, 327) turkey starter crumbs (326, 340, 341), chicken mash (342) and a cracked corn and oats mixture (339) were used to feed the smaller blackbirds. Crows, bluejays and ravens were fed pigeon chow checkers (275). A suggested diet for breeding smaller omnivores is a seed mixture (75:25:1 - canary - rape - thistle) supplemented with petamine, collard greens, lettuce and mealworms (343). Water and food should be given ad libitum.

Nesting food consists of the following: five pounds poul cage bird nesting food, two pounds yellow cornmeal, 10 ounces Kretschmer wheatgerm with sugar and honey, two po ten ounces quick Quaker oats and one teaspoon iodized sal These ingredients are mixed thoroughly and refrigerated until used. To this is added dried crushed wholewheat bread and hard boiled eggs as follows. Equal volumes of the first mixture and wheat bread are mixed, then to ever 1 1/2 cup of this mixture, one finely ground hard boiled egg and shell is added. All this is mixed thoroughly and enough water is added to make the mixture crumbly (343).

Breeding and Rearing -

Like other passerines, blackbirds and their allies have altricial young that hatch with their eyes closed (183, 344). They are nidicolous (183). In general, passerines lay two to six eggs per clutch, have a one day laying interval between eggs, a 13 day incubation period and a 13 day fledging period (215). The incubation and fledgin period is longer for jays and crows. Passerines usually rear two clutches per breeding season, and the first clut is generally larger. Both male and female partners incu- bate the eggs and feed the young. Starlings (Sturnus vulgaris) have a clutch size of four to six eggs, incubat takes 12 days and there is a 21 day fledging period after hatching (344). House sparrows (Passer domesticus) have clutch size of three to six eggs, an 11 day incubation period and a 14 day fledging period (345). They rear two to four clutches per breeding season (March to September) (345, 346).

Redwings (Agelaius phoeniceus) usually have four eggs per clutch and fledge 11 days after hatching. Two clutches per season is standard (310). Cowbirds (Molothrus ater ater) are completely parasitic, but an egg laying potential of four to five eggs has been suggested. Incubation is believed to take 12 days (310). Grackles (Quiscalus quiscula) also lay four to five eggs. The incubation period is 14 days and the young are fledged 18 days after hatching (310).

All junenile passerines have a partial postjuvenal molt in early August and adults have a complete postnuptial molt a few weeks later (310). Omnivorous passerines as a whole are sexually mature and breed at one year of age (310).

Behavioral Restrictions -

Most of these species are highly territorial and so require separate cages for each breeding pair (182).

Ecological Role -

Blackbirds and their allies are omnivores, feeding primarily on grains, seeds and insects. All these birds are considered to be agricultural pests, but cowbirds are really more beneficial than harmful. Passerines are food for mink, weasel, owls, hawks, and falcons. In addition their young are often attacked by other species of omnivorous passerines.

Longevity -

Life expectancy in nature for smaller members of this group of passerines is three years, but they have been known to live up to 12 years (331). The larger members, such as the crow (Corvus brachyrhynchos) can live past 20 years of age (182).

Problems with Mass Culture -

The house sparrow (Passer domesticus) is a carrier of St. Louis encephalitis (346). All birds are parasitized by lice, flies, mites and ticks (310).

Toxicity Testing -

Pesticide studies were undertaken using a stomach tube and LD_{50}'s were done (347). Field applications of pesticides were used for tissue analysis (335), eggshell thinning

studies (188), and residue (210). DDT was given in loaded food and necropsy was done (342). LD_{50}'s were done on birds fed mosquito larvicides (275) and DRC-1339 bait (34 LD95's were conducted on birds fed strichnine bait (339). SC-12937 was fed and physiological and histological studie were undertaken (328). A field study on the repellant power of 4-Aminopyridine was also undertaken (349).

General Suitability -

Bluejays are particularly sensitive to toxic substances (275). This group of passerines is fairly easily raised in captivity (182). The starling and house sparrow were chosen to represent this group because they are numerous and not protected by legislation.

Ecological Alternatives -

Most non-grain eating seed-eaters.

Domestic Galliformes

Introduction -

This group is comprised of two species: the chicken (*Gallus*) and the turkey (*Meleagris*). Both of them have down-covered, precocial young. Many different strains of these domesticated birds have been developed for commercial purposes but the leghorn is probably the best known. For this reason, the text that follows will concern itself only with the leghorn unless otherwise stated. Chickens, like all gallinaceous birds, are grain and seed eaters and are strictly terrestrial in their habits. They are ground nesters, but they roost in trees.

Caging Conditions -

A recommended cage for domestic fowl is 30 x 20 x 8 inches will house ten to 50 chicks. For animals used in experimentation, a pen 15 x 10 x 8 inches will house ten to 50 chicks. Individual cage units and brooder facilities are available commercially. Shavings, peat moss or commercial litter should be used on the floor of the cage (289). A 14L:10D photoperiod should be observed. Relative humidity for day-old chicks should be 78 percent, for five-week old chicks 65 percent, and for adults 45 to 55 percent (289, 350). Adult domestic fowl should be kept at a temperature of 23.9°C (350). A restrainer for blood collection has been described in 351.

A commercial brand of chow was fed, food and water were available *ad libitum* (289).

Breeding/Rearing -

The chicken has been domesticated for so long that it no longer has a clutch size; a hen lays for a few months continually and then ceases for a few weeks (352). Chickens have an incubation period of 21 to 23 days. They mature sexually in 20 to 22 weeks. At four weeks, secondary sex characteristics appear and sexing becomes a relatively unskilled job. Before this time vent sexing and feather sexing are used. There are two types of feather sexing - growth rate of primaries and color of feathers. Both of these traits are sex linked (352). One cock can service several hens (352).

Special Needs -

A supply of calcium must be available for good egg laying results (353). Coarse textured food is preferred over a food with fine texture (354). Drafts should be avoided and noise and excitement should be kept to a minimum. The pens should be kept dry and sudden changes in temperature should also be avoided (289). For the first four to six weeks, temperature control is most important. Chicks should be started at 90 to 95°F and gradually decreased (approximately 5° per week) to 60 to 65°F (352).

Behavioral Restrictions -

Fowl have cannibalistic tendencies. To help eliminate these tendencies they can be "debeaked" as young chicks (289, 352) and the light intensity can be diminished (352).

Ecological Role -

Because they are domesticated and have been for centuries, it is doubtful that these birds can be considered to have an ecological role in the wild.

Longevity -

Domestic fowl, like other larger birds, are fairly long-lived. Age limits have not been definitely established, however, as the adults are usually sacrificed after a few years (352).

Problems with Mass Culture -

Fowl are prone to many parasites and diseases. Some, such as Newcastle's disease, bronchitis, fowl pox and coccidiosis can occur in epidemic proportions unless controlled by vaccines (352). Other diseases and parasites are common but can usually be eliminated without much effort or loss to the flock (352).

Toxicity Testing -

Because they can be readily supplied and their biology and anatomy are well-known, chickens are frequently used in toxicity testing. Almost every conceivable route of exposure has been used on adults and juveniles. Injecting chemicals into yolk sacs (355, 356) and studying the

development of embryos (357) are some of the experiments done with the eggs themselves. Ciliary transport studies (358, 359) and teratological studies have been done (360, 361, 362, 363) along with disease studies (350), enzyme studies (364) drug studies (363, 365, 366, 367), population dynamics studies (368, 369, 370, 371), histological studies (372), x-ray morphology studies (373) and stress studies (374).

Methods of tissue culture (375, 376) and devices for collection of excretions (377) have been developed. The piezoelectric transducer has also been used in domestic fowl study (378).

General Suitability -

Chickens are large yet easily handled. They can readily be marked for later identification (289). They are obtainable in large numbers from commercial breeders on short notice. They are continuous nesters, so ecological studies could be carried out year-round. Chicks can be housed in limited space fairly cheaply.

Ecological Alternatives -

Jungle fowl, wild turkey.

Galliformes: Coturnix coturnix japonica

Introduction -

The Japanese quail was introduced into this country by the Missouri Department of Conservation in an effort to supplement existing game bird species. Coturnix are now found in several states. It is slightly smaller than the bobwhite quail in size and far more amenable to laboratory conditions. Because it is an upland game bird, it is associated with a terrestrial environment. Like other gallinaceous birds, it is diurnal in habits and migratory.

Caging Conditions -

Several cages have been used in the laboratory testing of Coturnix. A frame of 3 x 6 x 1.5 feet covered with 1/2 inch mesh hardware cloth is suitable to house 20 adult birds (379). Cages to house 25 brooding birds have a floor surface area of 69 x 100 cm and a height of 24 cm; three sides are mesh and a fourth side is sheet metal. This cage also serves as a test chamber in behavior studies of Coturnix (380). Breeding cages for Coturnix having variable dimensions may be purchased from GOF Mfg. Co., P.O. Box 8152, Savannah, GA 31402 (381). Laying, incubation, and testing cages used at the Denver Wildlife Research Center are shown in Figures 12, 13, and 14 respectively.

Diurnal light schedules differ. Some workers recommend a 14L:10D photoperiod (379, 382, 383, 384); others recommend an 18L:6D photoperiod (385). Cages should be kept in an environment of 60 to 80 percent humidity (379). Temperature in brooding units should range between 29.3°C to 37.8°C (380).

Nutrition -

It is common practice to feed Coturnix commercially prepared game bird feed in experimental laboratories. In some cases, the birds are given turkey starter ration which is approximately 22 percent protein (380, 386, 387). Some labs place Coturnix on feeding regimes. An example is Game Bird Startena (Purina) for two weeks; then Game Bird Growena is increasingly mixed with Game Bird Startena until Growena becomes the exclusive diet of the bird. After eight weeks the young bird's diet consists exclusively of Game Bird Layena (379, 381). An oyster shell should be available to the birds at all times to provide calcium for

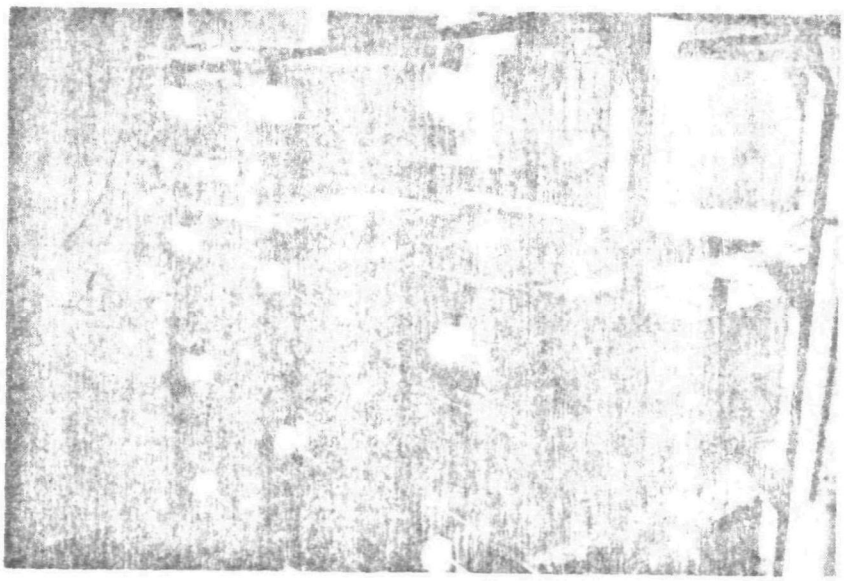


Figure 12. Coturnix laying cages (12" x 10" x 16"). Denver Wildlife Research Center, Federal Center, Denver, Colorado

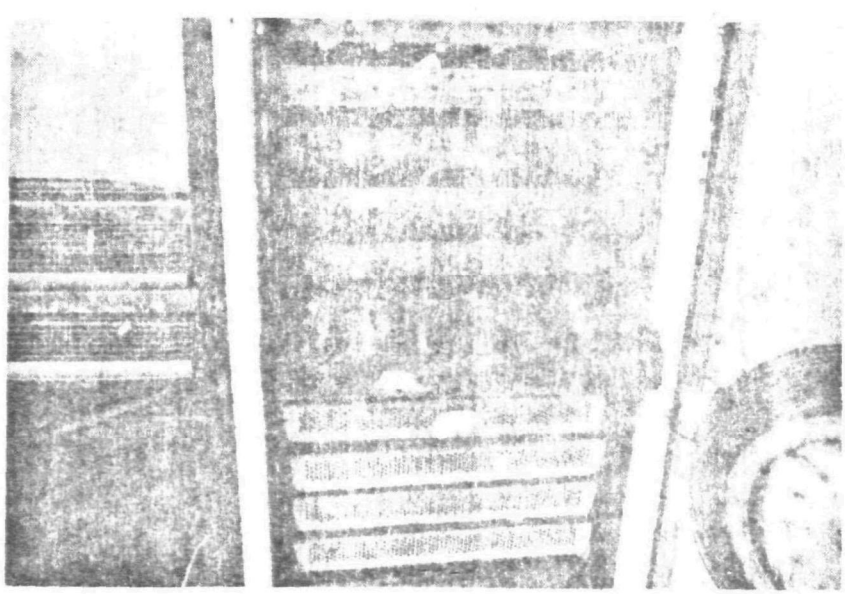


Figure 13. Coturnix incubation chamber. Denver Wildlife Research Center, Denver, Colorado

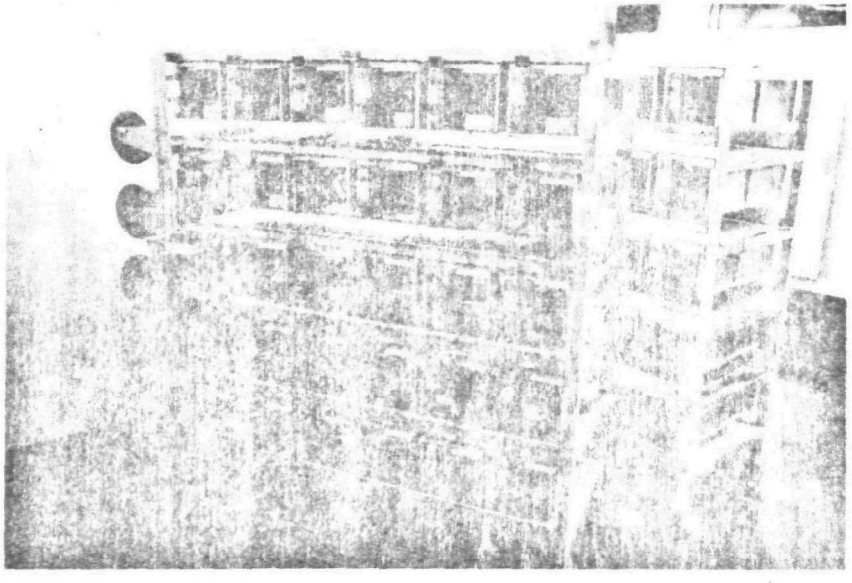


Figure 14. Coturnix and mourning dove testing cages (10" x 10" x 12"). Denver Wildlife Research Center, Denver, Colorado

general good health and for adequate egg development in females (382, 386).

Food and water should be available at all times because of the high rate of metabolism of Coturnix (379, 380).

Breeding/Rearing -

The natural breeding season for Coturnix is April to September. A year-round breeding season can be induced in the lab by providing the birds with the correct lighting and photoperiod. Eggs laid by Coturnix required an incubation period of 16 days ± eight hours at a temperature of 100°F. It has been found that temperatures above 102°F are harmful to developing embryos. For purposes of embryological study, eggs may be held at 25°C for as long as two weeks before incubation without damage (388).

Coturnix are born with their eyes open, they are precocial and nidifugous (183).

Behavioral Restrictions -

Migratory restlessness occurs from April to September and birds may damage themselves when they fly against their cage (379).

Ecological Role -

Japanese quail are seed and granivores primarily although newly hatched young feed on insects. They are prey for predatory birds and carnivorous mammals.

Toxicity Testing -

A wide variety of toxicity testing has been performed on Coturnix with numerous test substances. Enzyme assays (386, 389, 390, 391), egg production and shell characteristic studies (382, 392), physiological effect studies (326, 393), acute toxicity studies (249), stress studies (394) and residue studies (392, 395) have been conducted after the oral administration of DDT and DDE to these birds. The effects of oral administration of dieldrin and PCB's have been evaluated through egg studies (396), acute studies (249), and tissue residue studies (255, 396, 397). Sleep studies have been documented after the oral administration of PCB's and terphenyls (398). Reproductive effects have been evaluated upon oral administration of Hexachlorobenzene (399), and physiological and endocrinologic effects

of Kepon have been evaluated after it was administered in the birds dret (385). Enzymatic effects have been examined upon dietary application of Bidrin and Azodrin (400), and parathion and guthion (401). Coturnix sterility has been studied after egg application of Mestranol (402). Pesticides have had their effects evaluated, after oral application, in physiological testing (403), sleep testing (384), synergistic testing (293), behavior testing (380), and residue testing (404). A crop-tube method for administering pesticides for acute toxicity tests has been documented (199). Solitary PCB testing has been performed to evaluate liver residue, biological effects, and behavior after oral administration (380, 405). The development of different pesticides has dictated acute, subacute, and chronic testing after oral administration of a particular material (406).

General Suitability -

The Japanese quail is considered a good laboratory animal because it is hardy, grows rapidly, reaches sexual maturity early, and is a prolific egg producer under lab conditions (379, 383, 400, 406, 407). It also has simple housing requirements (379, 383, 406, 407), and is considered representative of upland game birds (199, 406). Research with these quail has become sophisticated to the point that strain numbers have been assigned.

Ecological Alternatives -

Bobwhite quail, ring-necked pheasant.

Exotics

Introduction -

This group encompasses a wide variety of foreign species including members of the parrot family, mynahs, and finches. The most popular and well-known member of the group is the parakeet or budgerigar. Along with canaries, mynahs and cockatiels, it is sold by the thousands as a household pet. The physical size of these exotic birds is as varied as their distribution and ecological niches, ranging from the large, brilliant colored macaws to the almost hummingbird-size finches. The bills and claws of these birds reflect the peculiar habits each has acquired from evolution. Many members of this group are considered to be the most intelligent birds in the world. Parrots, parakeets and mynahs can be taught to talk, whistle, do tricks and solve problems. Most of the exotic birds are colonial nesters and are tropical inhabitants. Many of them are native to Australia, Africa, Central and South America.

Caging/Lab Conditions -

Standard breeding cages, nest boxes, and next bowls are available commercially for the smaller members of this group such as the finches and parakeets. Lovebirds can use parakeet cages, but they need larger nest boxes with bigger holes (408). Nest material for canaries and finches can be bought commercially (408). Cages for small finches should be 24 x 12 x 12 inches and at least four feet long for larger finches. Perches should be made from twigs or branches rather than doweling (408). A suitable nest box for cockatiels is 9 x 9 x 15 inches with two to three inches of coarse sawdust or woodchips on the floor and a ladder of hardware cloth stapled inside the box from the entrance hole to the bottom of the box (408). Birds accustomed to flying should be housed accordingly (354). An alternate method of housing is the aviary. This should be eight feet high with four foot high doors, a small entrance chamber to prevent birds from escaping, and a shelter of some sort for the birds at one end (354). Planted aviaries are best (408).

Nutrition

Commercial chows are available for all these birds. Sunflower seeds should be used to supplement this diet in seed eating birds. Mealworms, flies, maggots, crickets and cockroaches have been used to feed the insectivorous

members of this group (409). Water should be kept availa ble at all times with these birds (410).

Breeding and Rearing -

Lovebirds are sexually distinguishable only by surgery. They lay three to eight eggs per clutch and have a 23 day incubation period. The young are fledged at seven weeks (408). Parakeets average three to four eggs per clutch and can have as many as six eggs per clutch. They have an 18 to 23 day incubation period and the young are fledged at six to nine weeks of age. Parakeets become sexually mature at six months and at this time the sexes are fairly easy to distinguish (408). Parakeets will breed year-round, starting a new clutch of eggs before the old cluth is fledged. In order to insure quality stock, a pair should only be allowed to raise three overlapping broods before they are given a rest of a month or so (408). Canaries show no sexual dimorphism, except that mature mal sing while females do not. They lay three to five eggs pe clutch and breed year-round (408). Some finches are sexua dimorphic. They have the shortest incubation time of exot birds -- only 12 days, and their young are fledged at two to three weeks (408). Sexes are distinguishable in cocka· tiels but their breeding habits are not well-known. Mynah and parrots show no sexual dimorphism and many species do not breed readily in captivity (408).

Special Needs -

Routine care and definite scheduling are a must for these birds (213). Social birds should not be kept in individual cages if they can still hear and see members of their own species (409). Adequate flying space is a must (354, 409). Ground foragers should be fed on the ground and tree foragers should have their feeders placed higher (409). Grit is essential for all exotic birds along with vitamins (410). Parakeets need iodine in their diet (410). Parrots and parakeets need pieces of tree branch to chew on and keep their bills trimmed (408). Lovebirds need a supply of green twigs for moisture for hatching eggs (408).

Behavioral Restrictions -

Larger birds such as parrots and mynahs can give nasty bites. Lovebirds can only be caged with members of their own species as they are vicious in interspecific encounters (408).

Ecological Role -

These birds feed primarily on seeds, grain or fruit. When not kept in check by their natural predators (snakes and carnivorous mammals), they can inflict serious damage on agricultural crops.

Longevity -

Larger birds such as mynahs and parrots live 20 years or longer. Parakeets have been known to live 12 to 15 years and the small finches probably live three or four years in captivity (182).

Problems with Mass Culture -

With the exception of finches, these birds are all very noisy (407). They are extremely messy eaters and sanitation can become a problem (182). The insectivorous species require a constant supply of live insects (408). All are hosts for fleas, ticks, lice and mites (182).

General Suitability -

The smaller birds are easily bred and prolific. All of these birds are relatively easy to maintain. The Australian parakeet, or budgerigar, is recommended as a test species because its initial cost is low and its life history and diseases are best known in this group. It is also the most prolific breeder of the group.

MAMMALS

Mammals are a homeothermic group of vertebrates characterized by the presence of hair and milk producing glands. They are considered by many to be the dominant group of animals on the earth today. Man and most of his domestic animals belong to the class Mammalia and probably for this reason our biological knowledge of this class far exceeds what is known about the rest of the animal world. This is particularly true in the medically-oriented scientific disciplines such as physiology.

While most research has been concerned with the economically important mammals, other species are valuable to their ecosystems and have recently received increased attention.

The following text attempts to consider all mammal groups in light of present knowledge and to select from each, one or two species suited for laboratory testing of the effects of chemicals on terrestrial species. A summary table indicating the suitability of various mammal species for toxicology testing is provided in Table 11.

TABLE 11. SUITABILITY OF MAMMALS FOR TOXICOLOGY TESTING

MAMMALS	Availability	Breeding in Captivity	Ease of Maintenance	Previous Use in Testing	Restrictions	General Suitability	Ecological Role	Habitat Preference	Suggested Species
Shrews	G	P	F	F	Difficult to house	F	B_2	Fo-Gr	*Blarina brevicauda*
Bats									
Vespertilionidae	F	F	G	P	Breedability in captivity poor	F	B_2	Fo	*Eptesicus fuscus*
Phyllostomatid	P_a	F	G	P		F	D_1		
Rabbits and Hares	E	E	E	E		E	D_2	Fo-Gr	*Sylvilagus floridanus*
Desert Rodents	G	F	G	P	Territoriality	F	D_1 (C)	De	*Dipodomys ordi*
Wild rats	G	E	E	G		G	C	Fo-Gr-Aq	*Neotoma lepida* *Oryzomys palustris*
Ground squirrels and allies	G	F	G	G	Not social animals	G	C	Gr	*Spermophilus beecheyi*
Chipmunks and allies	G	F	G	F	Not social animals	F	C	Fo-Cr	*Eutamias minimus*
Voles and allies	E	E	E	E		E	D_2	Fo-Gr	*Microtus pennsylvanicus* *M. ochrogaster*
Deermice and allies	E	E	E	E		E	D_1 (C)	Fo-Gr-De	*P. manículatus*
Lab rats and mice	E	E	E	E		E_b	C	Do	*Mus musculus* *Rattus norvegicus*
Large herbivores	F	G	G	G		G	D_1-2	Gr-Aq	*Myocastor coypus*
Large carnivores	P	G	G	P	Unavailable in large numbers	P	A	Fo-Gr	*Vulpes vulpes*
Small carnivores	G	G	G	G	Some very hard to keep	G	B-C	Fo-Gr-Aq-De	*Mustela vison*
Domestic carnivores	E	E	E	E		E	C	Do	*Canis familiaris*
Horse and allies	E	E	G	G	large size	G	D_2	Do	
Swine and allies	E	E	G	E		E	C	Do-Gr	Miniature swine (*Sus*)
Sheep and goats	E	E	G	E		E	D_2	Do	*Ovis* sp.
Deer and allies	G	G	G	F	large size	G	D_2	Fo-Gr	*Odocoileus virginianus* *O. hemionus*
Primates	F_c	G	G	G	long generation time	G	C	Fo-Gr	*Saimiri sciureus*
Opossum	G	G	G	F	very susceptable to disease	G	C	Fo-Gr	*Didelphis marsupialis*
Armadillo	F	F	F	P	difficult to house	F	C	Fo-Gr	*Dasypus novemcinctus*
Exotic species	E	E	E	E		E	C		

TABLE 11 (CONTINUED). SUITABILITY OF MAMMALS FOR TOXICOLOGY TESTING

Legend:

General Ecological Role

A - Large carnivore
B - Small carnivore
    1. General
    2. Insectivore
C - Omnivore
D - Herbivore
    1. Seed, fruit eater
    2. Grass foliage eater

E = Excellent
G = Good
F = Fair
P = Poor

a - Tropical species availability is good in tropics
b - The suitability of long inbred (homozygous) populations in toxicological testing is suspect.
c - No native non-human species

Habitat Preference

Fo Forest
Gr Grassland
De Desert
Aq Aquatic
Do Domestic

Sources: - literature cited in text
- interviews of Scientists

Insectivora

Introduction -

Two of eight Insectivora families are represented in North America: Soricidae (shrews) and Talpidae (moles). There is little literature on moles and they will not be treated in detail in this report. Crandall (411) gives one account of a captive Condylura cristata, and two accounts from other authors for Scalopus aquaticus. Most of the seven U.S. mole species are restricted to a few states. The eastern mole, (Scalopus aquaticus), however, has a large distribution (34 states); it is found generally east of the 100th meridian and south of 43° latitude (412). Of the 27 U.S. species of shrews, three have wide distributions. Cryptotis parva [the least shrew (or lesser short-tail shrew], and Blarina brevicauda (the short-tail shrew), are generally found east of the 100th meridian. C. parva occurs in 31 states, mostly south of 43° latitude. B. brevicauda occurs in 37 states. Sorex cinereus is a northern species, distributed roughly from 40° latitude to the Arctic Ocean. Identification of shrew species is often difficult and should be verified (412).

B. brevicauda, the principal subject of this section, lives in "well wooded. . . low ground" (413), mature beech-maple forest with abundant leaf litter (414), and sometimes in grasslands (415). Like other shrews, it is fossorial, but not so completely as moles. The short-tail shrew maintains two nests, a breeding nest about 2 x 2-3/4 inches which is lined with grass, whole or broken leaves, and sometimes mouse fur and a resting nest described as about the size of a large apple (413). Hamilton (413) found a dung pile about four inches from a breeding nest and, commonly, snail shells in the runways. Scats are usually piled on one side of the runway near the nest.

Caging/Lab Conditions -

Shrews do well in cages made in a variety of materials and sizes, plastic (14 x 18 inches) and glass have been used, with cages made as small as 20 x 2 inches (314, 411, 416). There is little danger of shrews jumping out of their cage as mice do. For example, Blus (417) kept shrews in 2 x 1 x 1 foot (288 square inches) open-topped aluminum boxes. In designing cages, it should be remembered that Rood (416) observed the animals frequently licking screen sides and tops of cages (without speculating on the cause

of this behavior) and that Blus (417) rejected wire cages because of the inadequacy of the substrate.

Shrews are frequently provided with a tin can (411, 413, 418) for a nest, but have been forced to sleep in the open, living months with no cover and very little soil (416). When cover, such as sod, is provided, the shrews use it (416) Cotton (418), leaves, and grass (411, 416, 417) have been provided as nest material. Shrews treat nest materials in a variety of ways: the softer may be placed inside the rougher; they may be burrowed into (417), or dragged into a corner, and they may be ignored when there is a good layer of soil in the cage where the animal can enlarge one tunnel for a sleeping chamber (416). Two nests are provided a pair of shrews by Blus (417), who also placed a plywood platform supported by dowel legs above the nest chamber to support the weight of a water dish and to provide additional cover. Usually, captive shrews are supplied with soil - from just enough to cover the bottom of their cage, to several inches (411, 416) - or sphagnum (413, 417). Shrews immediately start to dig in either substrate, honeycombing it with tunnels (see 416 for description of digging behavior) (417). Other beddings are wood shavings (418 genus unspecified), pine chips (417), corn cob litter (417), peat (417), grass [(413 genus and condition unspecified)]. Blus (417) identifies milled sphagnum peat moss, three inches deep, as the best substrate.

Rood (416) and Blus (417) kept the animal room at 70°F. Doremus (419) kept the room at an "air-conditioned" 17°C (referring to temperature, not quality or humidity) and controlled lighting to "approximate outside conditions" (probably off-on roughly with sunset-sunrise, ignoring brightness and spectrum). Blus (417) kept a regimen of 14 hours continuous fluorescent light followed by ten hours continuous darkness.

Only Hamilton (413) mentions odor, which he found most pronounced in April, lingering two days after a pair of shrews had been removed from the room. No authors have reported on the method and frequency of cleaning cages. Some shrews dropped scats in the sphagnum and the water, and never in the nest box, and a female with a litter left scats in the corner of the nest box, rarely venturing from the nest (413). Blus' animals usually defecated and urinated in one corner of the next box, either on or below the surface (417).

Nutrition -

Shrews are very sensitive to shortages of water and food but the amounts required by Blarina have probably been confused by experiences with Sorex (418). Blarina consumes less than one-half their body weight in food every 24 hours (416). Blus (417), however, provided 15 to 20g food per day per individual. In nature, soricids consume earthworms, slugs, snails, insects, mice, fungi, berries, seeds, and greens (411, 420). In captivity, they have survived on an array of foodstuffs: ground raw horse meat, bone meal with cod liver oil, chopped hard-boiled egg, earthworms, mealworms, mice, diced fruits, greens, and cottage cheese (416). Additional items are dead shrews, dead birds, chicken, canned dog food, peanuts, crackers, whole wheat bread, cheese (416). There is much individuality in food preferences (416). Pearson (421) fed a mixture of hamburger, ground liver, and dry commercial dog food; Mann (418) substituted commercial dog food for the meat portion. Mann kept shrews up to 16 months solely on canned dog food, starting the animals on it immediately upon their arrival. Doremus (419) kept animals on a basic diet of canned dog food, occasionally supplementing with mealworms and raw beef. A mixture of "ground carcasses of laboratory rodents (61.3 percent), chicken heads (15.4 percent), turkey breeder ration (7.1 percent), Vionate, a vitamin supplement (0.5 percent), and bone meal (0.3 percent)" was fed by Blus (417). Rood (416) found that most shrews readily ate dead shrews. A shortage of food may induce cannabilism in a group (418).

Breeding/Rearing -

The most complete description of breeding captive Blarina is that of Blus (417). The average number of young is five to seven (413, 417) with a range of four to ten (413, 422). The peak of breeding activity in nature is in April (413, 414, Ithaca, New York) when there is maximum testes development and captured females have embryos. In May and early June, females are nursing. Breeding is minimal in late August and early September (413, 415). Dapson (414) concludes that breeding may occur throughout the year. There are probably spring and late summer litters, and occasionally, a vigorous female produces a third litter (413, 415). Crandall (411) reports that E. P. Walker had a captive Cryptotis parva female which produced 66 young in one year. Some individuals breed one or two months after birth (414, 423). As a rule, males with testes greater than 5 by 7 mm have sperm in the cauda epididymis if the accessory reproductive glands are developed (414). The

gestation period is 21 to 28 days (413). Hamilton (422) believes post-partum estrus is normal for wild Blarina, as well as for Sorex fumeus and S. cinereus.

The development of the newborn shrew has been described in detail by Hamilton (413). At birth, the shrews are dark pink and the size of a honeybee. A wild female was observed moving each of her young (about nine days old) by the belly when the nest was disturbed in the wild. By 13 days, shrews weigh about 9 g; mammae are visible on the females; the ears are open; the side glands are obscured; the mid-ventral bare patch is prominent. Litters have been kept together up to one month following weaning (417).

Special Needs -

Shrews are very sensitive to food and water shortages and require continuous supplies of both.

Behavioral Restrictions -

The greatest concerns in maintaining shrews in captivity are their voracious appetites and pugnacious behavior, both over-rated. Although Blair (415) found that Blarina are not territorial, most researchers keep shrews in individual cages except when pair are mated (417). Shrews pair for very long times in the wild, perhaps for life, and parents may stay together until the young are born (413). Rood (416) describes the behavior of captive shrews in various combinations and as strange animals are introduced. Individuals are marked with #1 bird bands on the hind legs (416) or by toe-clipping (415, 424). Playful pushing and snuggling occurs among a few individuals living together with a minimum of quarrel (416). The important factor is the disposition of the individuals involved, with age and sex only secondarily important. Rood (416) doubts that Blarina are "as solitary as is generally believed."

Ecological Role -

Shrews are energetic predators in the ecosystem, consuming quantities of earthworms, snails, and slugs, various insects, and mice. The role of shrews in controlling mice has been disputed and probably depends on the local population as well as individual differences (416).

Longevity -

The longevity record for Blarina brevicauda may well be that established at the National Zoological Park of two years, three months, and one day (411). Life expectancy in nature is 20 months (414). The age tc toothwear relationship is curvilinear (414).

Problems with Mass Culture -

No diseases have been identified for Blarina (413).

Toxicity Testing -

Shrews have not been used much as subjects for toxicity testing except in field studies where lead content was analyzed (425).

General Suitability -

Blarina has had previous maintenance work completed (416, 417), and is of a suitable size to be easily housed in relatively small cages. Its voracious appetite and abundance in nature makes Blarina a good candidate for use in toxicological testing.

Ecological Alternatives -

Other shrews, moles.

Chiroptera

Introduction -

Bats (Chiroptera) are the only mammals capable of true flight. Almost all North American bats (about 30 species) are members of the family Vespertilionidae, and are nocturnal, insectivorous, and weigh no more than a fraction of an ounce. During the day they roost in various types of shelters including caves, attics, trees, and old mine tunnels (412). Some species are migratory, changing their range with the seasons and availability of insects. Other bats hibernate in caves and buildings during the winter. Four Phyllostomidae are found in the southwestern United States and feed on pollen and nectar. Six species of Molossidae occur in the United States and are primarily insectivorous.

Caging/Lab Conditions -

Because of the small size of bats, many different cage enclosures have been successfully used. A 20 x 20 x 25 cm cage was used to house 12 individuals. A wood-wire mesh structure 80 x 92 x 128 cm with a roosting box 80 x 92 x 51 cm was used to house a bat colony of unknown size (426). Bats have been individually housed in pint ice cream containers with wire mesh lids (427), and in battery jars 15 cm wide and 20 cm high (428). All types of materials - wood, metals, etc. can be used for cages.

Sanitation of cage facilities seems to be the major difficulty in the maintenance of bats. Overcrowding leads to sanitation problems and much time is spent cleaning the cages (428, 429, 430). Paper on the bottom of the cages should be changed daily (431).

Because bats are nocturnal, they require dim light for feeding. Other special needs include room to exercise or to fly (431), chitin in their diets (428), and water made available in such a way that bats can readily find it. High relative humidity (71-81 percent) and high ambient temperatures (78-84°F) increase their activity and facilitate the feeding process (427, 428, 432). The use of light bulbs to provide local heat is a common practice (427).

A photoperiod of 13L:11D, a 21-28°C temperature range, and a relative humidity of 55-92 percent, have proven effective in maintaining Phyllostomid colonies (426).

Nutrition -

Bats eat a great variety of foods in captivity. One elaborate diet consists of hard-boiled egg yolk, dry cottage cheese, ripe banana, live mealworms, six drops of liver extract with iron, six drops of wheat germ oil, and three gms of milti-vitamin preparation, all mixed in a blender. If desired, it can be frozen. This preparation is given ad libitum (428). Insects are a necessary component of the diet of insectivores because the chitin aids the formation of fecal pellets (428).

Successful diets for Phyllostomids include young birds, insects, raw beef, banana (433), cereal, wheat germ, milk, powder, protein and mineral supplements, corn oil mixture in a fruit base (426, 429). Mealworms are used widely as a staple food for bats in captivity (434).

Breeding/Rearing -

North American bats do not usually breed in captivity (431). In nature copulation takes place in late autumn and birth in spring. The sperm is stored for several months in the body of the female (429, 433).

Gestation lasts 56-100 days in Corynorhinus (435) and is rather variable in many species. Usually only one or two young are produced each year (429). There have been a number of tropical bats maintained in captivity with mixed breeding success (436).

Behavioral Restrictions -

One drawback to keeping bats, since bats usually forage on the wing, is that they must be conditioned to feed in cages from the food source (see 427 for a thorough description of techniques). North American bats are hibernators and at roost they assume the temperature of their immediate environment. If temperatures are low enough they will enter into a semi-torporous state every day. The seasonal hibernation pattern conflicts with long-term physiological experiments and many researchers have resorted to tropical species which do not hibernate (434).

Ecological Role -

The bats of North America are primarily insectivores feeding entirely on airborne species. Bats are known to locate their prey by means of ultra-sound and navigate with ease in the absence of light. Their ecological role is not fully understood.

The leaf-nose bats, Phyllostomidae, feed in part on pollen and nectar, acting as pollinators of certain angiosperms specifically adapted to their visitations.

Longevity -

For Mexican free-tailed bats, (Tadarida brasiliensis), the life span is greater than 4.5 years in captivity. Pacific pallid bats have lived to eight years, three months and certain Desmodontidae average five to six years (433).

Toxicity Testing -

Bats are rather unusual members of the laboratory world. Myotis lucifugus has been used for taste reception work (437), and the Mexican free-tailed bat (Tadarida brasiliensis) (427) for metabolic versus temperature studies.

The little brown bat (Myotis lucifugus), has been shown to be highly sensitive to DDT (438) and studies have been used also for the effects of ethyl alcohol on subcutaneous microcirculation (439).

General Suitability -

Most species of North American bats are not plentiful enough to withstand large harvests to stock lab colonies. The possibilities for breeding in captivity are relatively unknown. This, added to the seasonal activity changes of temperate-region species, makes them poor subjects for wide use in toxicity testing. Tropical species have a wide range of ecological niches and may be better test subjects (434).

If a North American species is chosen to begin lab breeding/rearing work, Eptesicus fuscus, because of its large size, is recommended (434).

Ecological Alternatives -

Other bats, insectivorous animals, (wrens, shrews).

Lagomorpha

Introduction -

The Lagomorphs are composed of two families, the rabbits and hares (Leporidae) and the pikas (Ochotonidae). Though they resemble the rodents, there is good evidence that they have been separately evolving for a very long time in terms of mammalian history.

Rabbits (Sylvilagus) and hares (Lepus) have long ears and long hind legs modified for saltatorial locomotion. In warmer climates, they are carriers of tularemia. There are about 15 native species that are basically openland-edge animals. The largest hares are about 26 inches long and weigh up to 12 pounds. Leporids are basically herbivorous (412).

Pikas, Ochotona, are inhabitants of talus slopes in the Northern and Central Rockies. There are only two native species of this smaller, rat-sized, tail-less animal. They store food in hay piles and feed almost entirely on grasses and herbs (412)..

In research, the domestic New Zealand white rabbit, Oryctolagus cuniculus, is utilized almost exclusively, and the following comments refer to this species unless otherwise stated. They weigh from nine to 14 pounds and are very gentle lab animals (289).

Caging/Lab Conditions -

Recommended cages for New Zealand white males are 30 x 18 x 18 inches, which are also sufficient for a breeding area. Does with litters need larger enclosures (30 x 36 x 18 inches) and a nest box, 18 x 9 x 7 inches, with fresh straw bedding. Temperatures from 65-75°F are suitable and relative humidity of 50 percent is ideal, but not a necessity. A 14L:10D photoperiod is recommended. Drafts should be avoided (289).

A breeding pair of European hares, (Lepus europaeus) were successfully housed in an 8 x 6 x 8 foot brick, outdoor enclosure with a concrete floor and a wire front which was covered in winter. One-half of the floor was covered with straw (440).

Snowshoe hares have been kept in captivity. One-half inch wire cloth flooring is suggested as a deterrent to the spread of coccidiosis, a perennial problem with this species. This cage alteration increased survival of new born rabbits from 11.6 percent (when they were born on the ground) to 63.3 percent (441).

For handling lab rabbits the restraint device described in reference 442 has proven very effective.

Nutrition -

Commercial rabbit pellets are available, but many labs supplement the diet with greens, raw vegetables, hay, and oats (441). Adults should receive four to six ounces of food daily, pregnant females six to eight, and lactating does eight to 16 ounces. Growing juveniles need from one to two pounds per diem (289). Pikas have been maintained on lettuce, cabbage, raw potatoes, dandelions, grass, oatmeal, and bread (441). Lagomorphs are coprophagus and apparently obtain some nutritional benefit from this habit (443). Water should be supplied ad libitum to all species.

Growth of suckling rabbits has been shown to increase faster in creep-fed individuals than in those reared naturally (444). Creep-feeding is the practice of self-feeding concentrates to young suckling animals in a separate enclosure away from mothers. The composition of one experimental creep-feed diet is shown on Table 12.

Breeding/Rearing -

Domestic rabbits and many wild Lagomorphs are polyestrous. The gestation period for New Zealand whites is 30-32 days, litter size varies from one to 18 with an average of eight, the young are weaned in eight weeks and both males and females can breed approximately six months from birth. Females can be rebred about 35 days after parturition. The breeding life of females and males is one to three years. One buck can service six to ten females in a breeding colony. Pair mating is recommended in the buck's cage (289).

European hares have a 42-day gestation period, snowshoe hares 37 days, and pikas 32 days. There has been some success breeding pikas in captivity (441).

In the wild, cottontails (Sylvilagus floridanus) gestate for 27 to 30 days, and produce three to five litters per annum. The number of young per litter is four to seven (412).

Table 12. COMPOSITION OF AN EXPERIMENTAL CREEP-FEED*

Protein, 22 percent; fat, 5 percent; fiber, 13.4 percent
Ingredients: Dehydrated alfalfa meal, soybean meal, oats, red wheat bran, distillers dried corn solubles, skimmed milk, salt, anise oil powder, vitamin A, balance of trace minerals, vitamin pre-mix, and vegetable fat or oil.

Mixing Formula:	Percent Of Total	Pounds Per Ton
Dehydrated Alfalfa Meal	37.5%	750
Soybean Meal (expeller)	10.0	200
Oats	10.0	200
Red Wheat Bran	15.0	300
Distillers Dried Corn Solubles	13.5	270
Skimmed Milk	11.5	230
Vegetable Fat or Oil	1.25	25
Salt (NaCl)	0.5	10
Vitamin Pre-mix		7.5
Anise Oil Powder		4.5
Trace Minerals		2
Vitamin A		1
	100%	2,000 pounds

*Feed was a crumble in Trial 1 and pelleted at 3/32" diameter in Trial 2. Also, in Trial 2, the formula was modified slightly by Albers Milling Company. Essentially, the modification was to increase the level of protein and reduce the level of fat.

SOURCE: (444).

Behavioral Restrictions -

The restrictions associated with maintaining domestic rabbits are very minor. Wild species may have some difficulty adjusting to caging. Frequently the cage elicits an escape response which sends the animal nose first into the wall of the cage, often with sufficient force to cause self-injury (441).

Pikas are very territorial creatures in their natural environment. This behavior may restrict attempts to cage a number of them.

Ecological Role -

Rabbits and hares are primarily herbivorous, utilizing many plant foods, but preferring grasses and herbaceous materials (412). They are typically openland-edge inhabitants and remain active year round (443). Pikas have similar food habits and are easily detectable on the talus slopes where they live because of the "hay" piles they build in the fall as storage heaps (412).

All Lagomorphs are desirable prey for larger predaceous birds and mammals.

Longevity -

In captivity, eastern cottontails have lived to five years and European hares to 11 years of age (441).

Problems with Mass Culture -

Rabbits and hares are quite susceptible to a variety of ills, particularly coccidiosis which is highly communicable and causes high infant mortality when it occurs (441).

Toxicity Testing -

Domestic rabbits are a rather popular subject in American laboratories because of their gentleness and in some cases, their relatively large size. They have been used in many types of toxicity experiments, particularly those testing dermal reaction where the substance is directly applied to the shaved skin (445, 446, 447, 448, 449, 450, 451). Other routes of exposure are also utilized with a great variety of substances. Work with wild species is limited to a few pesticide studies of free roaming subjects (452).

General Suitability -

Domestic rabbits are readily available and easy to use in toxicity testing. The applicability of such work to natural populations is difficult to judge.

Wild species, particularly the eastern cottontail (*Sylvilagus floridanus*) and a western species (*S. audubonii*) are available and should prove adaptable to laboratory conditions.

Ecological Alternatives -

Woodchucks, nutria.

Rodentia: Heteromyidae

Introduction -

The Heteromyidae, the family of kangaroo rats, and pocket mice, are found in the southwestern United States and in Mexico. They are specially adapted to desert habitats. They have fur-lined cheek pouches. The large forms have modified rear limbs for leaping and very long tails used for balance. All Heteromyids are nocturnal. There are more than 20 species of pocket mice, one kangaroo mouse, and 14 species of kangaroo rat in North America. The rats are the largest, with four to eight inch bodies and weighing from two to five ounces. Pocket mice are similar to laboratory mice in size.

Caging/Lab Conditions -

For the kangaroo rats (Dipodomys) glass front metal cages, 2 x 2 x 3 feet with dried leaves for a floor and a small shelter, have been used. A quantity of sand was supplied and used by the rats for cleaning their pelage (453). Other cages, 10 x 11 x 16 inches (3 x 4 m) are also adequate, but for breeding a larger enclosure, 5 x 7 x 2 feet, is recommended. Shelters such as small boxes or mailing tubes and a sand substrate are also desirable (454).

Pocket mice (Perognathus) were routinely housed in 12 x 12 x 12 cm cages with sand bottoms (455). It was found that the pocket mouse (P. flavus) tolerates physiological stresses very well such as sound, heat, vibration, and G forces (456).

The Heteromyids, though desert forms, are greatly affected by exceedingly high or low ambient temperatures (457) and should be protected from them.

Nutrition -

Kangaroo rats were fed a diet of 26 percent rolled oats, 25 percent rolled barley, 20 percent meat meal, 10 percent powdered milk, 11 percent whole wheat kernel, 2 percent cod liver oil, 2 percent sunflower seeds, and traces of birdseed, salt, powdered calcium carbonate. Lettuce was provided every other day. They seemed to do equally well on just barley, sunflower seeds, and lettuce (454).

Pocket mice have been successfully nourished for seven years on mixed seeds alone (453). A more varied diet included dog food, greens, and vegetables (453). Desert rodents may need green vegetation to induce breeding in the spring.

The water requirement of heteromyids is negligible in the laboratory; even when water is made available, they do not drink it (453).

Breeding/Rearing -

Heteromyids are very territorial and will usually destroy each other when two or more are caged together. Increased cage size may improve the success of co-habitation (455, 458). In one case, individual kangaroo rats were kept separate until estrous and then a pair were placed together in a neutral cage, 30 x 30 x 10 inches. This resulted in pregnancy in six of ten females (459). Successful lab breeding is recorded for D. ordii, D. merriami, and D. deserti in large rooms (453). Some success at group living has been reported for the Fresno kangaroo rat, D. nitratoides, but only in large cages with rather violent initial skirmishes (453).

All heteromyid rodents are seasonally polyestrous. The gestation period in captivity for Dipodomys is 29 to 32 days and varies little from one species to another (453). Young of D. merriami and D. deserti were weaned at 22 to 25 days and were fully mature at 90 days. Sex of the young can be determined after 11 days (454). The number of young per litter for Dipodomys is two to four (459).

Behavioral Restrictions -

Though heteromyids are nocturnal, some specimens have overcome this habit in the lab. Under red light, these rodents have been quite active (453).

Kangaroo rats are very easy to handle and are essentially very gentle creatures (453). However, because of their territorial instinct, communal caging may not be possible, with the exception of a few species.

Ecological Role -

Desert heteromyid rodents are primarily seed eaters and serve as food for many predators. They are particularly important in snake diets.

Longevity -

In captivity, the life span of various pocket mice (Perognathus) differs from four years (P. parvus) to seven years (P. longimembris) to eight years (P. fallax) (453). Their mortality in nature is much higher as would be expected.

Toxicity Testing -

Because of the unique fashion in which these desert rodents metabolize water, their physiology has been rather well studied. Some blood work has been done for kangaroo rats (454).

The effects of psychomimetics like LSD-25 and psilocybin have been studied as has the uptake of I^{131} in the thyroid of D. merriami (454). Kangaroo rats have also been used in a field study on the effect of a rodenticide (Gophacide) (460). They seem very suitable for these experiments.

General Suitability -

Kangaroo rats and pocket mice are gentle laboratory subjects and would make good species for use in experiments. However, the difficulty of breeding them in captivity detracts from their suitability.

Ecological Alternatives -

Other rats, mice.

Rodentia: Cricetidae, Muridae - Wild Rats

Introduction -

The wild rats of North America are from two rodent families, the Cricetidae and the Muridae, and four genera, Rattus, Neotoma, Sigmodon, and Oryzomys. Members of all four have been used in some way in the laboratory.

Neotoma, the woodrats or packrats, is a widespread genus ranging from the east coast forests to the deserts of the west. There are eight species, all easily distinguished by their hairy tails.

Sigmodon, the cotton rats, are represented by S. hispidus, which is abundant in grassland habitats throughout the southern U. S. Two minor species are found in the foothills of northern Mexico, southern Arizona, and New Mexico.

Oryzomys palustris, the rice rat, occurs in marshy areas in the southeastern U. S. It is a semi-aquatic species that usually nests under debris above high-water level.

The Rattus species have been introduced by man and are adapted to living in and around man-made structures, particularly where food is stored.

Rattus and Neotoma weigh about six to 12 ounces and their body length is from seven to nine inches. Sigmodon and Oryzomys are smaller weighing between two and six ounces and measuring between four to seven inches in body length (412). All have long tails, are typically rodent-like in appearance, nocturnal, and omnivorous. They are relatively abundant locally within their ranges.

The multimammate mouse (Rattus natalensis), an African wild rodent, has been introduced to the U. S. for laboratory testing and is laboratory-adapted (461).

Caging/Lab Conditions -

Wire mesh, glass, and sheet metal are acceptable materials for cages (453, 462). Wooden enclosures should be avoided. The dimensions of cages in use range from 27 x 17 x 14 inches for a mesh cage with a galvanized removable floor tray, to 16 x 15 x 15 inches for a sheet metal cage with a wire mesh door (463). A 3 x 8 x 15 foot enclosure with

28 compartments has been used to house a colony of wild rats (462). Nesting materials such as cotton, excelsior, and twigs are necessary (464, 465) as are nest boxes (453, 463).

Conditions under which Central American cotton rats, Sigmodon, were successfully maintained in a lab are 15 to 16°C, 75 to 80 percent relative humidity, and 24 hours of light per day (466).

Male rice rats were kept in a common enclosure while females were housed individually and no serious confrontations or fights were reported (467).

Nutrition -

The rice rat has been successfully maintained on a diet of small grains, whole or rolled oats, greens, fruit and raw vegetables, plus dog food fortified with cod liver oil (453, 468).

Recommended diets for Neotoma floridana include carrots, sunflower seeds, peanuts, stale bread, tomatoes, oatflakes, berries, hard and fresh corn (463, 464).

Cotton rats have been fed combination diets of sunflower seeds, whole wheat, oatmeal, cod liver oil, corn, and powdered protein (meat scrap) with periodic greens and apple or tomato supplements (463).

Diets for Rattus are not available, but it is likely that combinations of the above would prove suitable because the feeding habits of all the wild rats are comparable.

Water should be provided ad libitum for all wild rats.

Breeding/Rearing -

The breeding and rearing characteristics of the wild rats are summarized in Table 13.

Behavioral Restrictions -

Researchers who have kept Sigmodon hispidus, the cotton rat, report that cage mates are likely to fight, and that the cotton rats do not tame easily or lend themselves to handling (453, 463). Rice rats are docile and handle easily (469). The eastern woodrat (Neotoma floridana) fight when caged in numbers, but usually becomes habituated to others and ceases hostilities (464). Wild populations are

Table 13. BREEDING AND REARING CHARACTERISTICS OF THE WILD RATS

Group	Litters/ Years	Gestation	Time of Estrous*	Young/ Litter	Age at Maturity
Sigmodon (463, 470)	3-6	27 days	Feb. to Nov.	3-6	50 days
Rattus (412, 467)	12	21-22 days	Year-round	8-10	90 days
Neotoma (464, 465, 471, 472, 473)	?	30-36 days	March to Nov.	3-4	60-90 days
Oryzomys (467, 468)	3	25 days	Feb. to Oct.	3-4	50 days

*The wild rats are polyestrous in captivity.

somewhat colonial and should adapt well to lab conditions. Species of Rattus which have been bred for laboratory animals, will be treated elsewhere.

Ecological Role -

All wild rats are ominivorous. The woodrats (Neotoma) and Old World rats (Rattus) feed more on animals than the cotton rats (Sigmodon) and rice rats (Oryzomys) which favor green vegetation (412).

All wild rats are potential and often valuable sources of food for predatory snakes, birds, and mammals (412). Sigmodon hispidus affects grassland bird populations by eating eggs and are agricultural pests in certain areas (463).

Longevity -

Desert woodrats, Neotoma lepida, have lived over five years in captivity and the other species of wild rats probably are capable of rather long lives in captivity (412).

Problems with Mass Culture -

Woodrats have a musky odor that may become rather unpleasant to lab workers (465) and are very suitable hosts for fleas, mites, etc. Care should be taken to eliminate parasites from new individuals received from natural populations (471, 474).

Toxicity Testing -

The genus Rattus has been used extensively to determine the feasibility of using U-5897, a male chemosterilant, to control pest populations of wild rats. Single oral doses ranging from 0 to 300 mg/kg were administered to males of four species of Rattus with mixed results (475, 476).

Other rodenticide studies have employed Norway and Black rats with a variety of administration methods, primarily forced ingestion of food additives (477, 478, 479).

General Suitability -

The suitability of wild rats for lab research is good due to their ability to reproduce in large numbers, their small size, and their relatively inexpensive diets. Oryzomys palustris is recommended by labs that use it (469).

Ecological Alternatives -

Cotton rat alternatives include voles, Microtus, and rice rat (412).

The eastern woodrat could be replaced by other woodrats and by the Norway and Black rat (412).

The rice rat alternatives are the Norway rat, packrat, and cotton rat (412).

The genera of the wild rats are somewhat ecologically interchangeable.

Rodentia: Sciuridae - Ground Squirrels

Introduction -

Ground squirrels (Spermophilus, Ammospermophilus), are rodents that inhabit openlands of many types and are limited to the midwestern and plains region of North America. They are diurnal, burrow dwellers, and hibernate during adverse seasons. Their average body length is five to ten inches, and they possess long furry tails. Adult body weights in this group range from six to 38 ounces (144).

Caging/Lab Conditions -

Wooden cages will be gnawed through by ground squirrels (453). However, 12 cubic feet packing crates surrounded with wire mesh with a sand floor have served as suitable housing (480). Metal suspension cages from four to ten cubic feet have been used (481). Litter composed of pine shavings and burlap sacking has been used (481).

The California ground squirrel, Spermophilus beecheyi, has been housed and bred in 4 x 8 x 2 feet enclosures of 1/2 x 1 foot welded wire. A nest box, 12 x 12 x 12 inches should be included (481).

Nutrition -

Ground squirrels are omnivorous and have been successfully sustained on combinations of seeds, greens, fresh vegetables, dog food, raw meat, bone meal, and cod-liver oil (853). Purina lab show has proven adequate for Spermophilus (=Citellus) parryi (480). Ground squirrels like to hoard food in nest boxes even if it is supplied ad libitum and the nest boxes should be cleaned daily (482).

Breeding/Rearing -

The gestation period for the Columbian ground squirrel (Spermophilus columbianus) is 24 days (453). The young of the thirteen-lined ground squirrel (S. tridecemlineatus) are weaned at 30 days, but those of the spotted ground squirrel (S. spilosoma) are not weaned until about 48 days after birth (483).

Various ground squirrels have been bred successfully in captivity, including the golden-mantled ground squirrel, Ammospermophilus lateralis) the antelope squirrel (A. leucurus) for three generations, the Columbian ground squirrel (S. columbianus) the thirteen-lined ground squirrel, S. tridecemlineatus (453), and S. beechayii (481). Green vegetable material (i.e., lettuce) given before spring breeding (60 to 70 g per day) may influence the reproductive performance in ground squirrels (481).

Behavioral Restrictions -

Ground squirrels are solitary in nature and present problems when confined in small areas. When housed together they fight, except for a few weeks during the breeding season (484). The arctic ground squirrel S. parryi is cannibalistic in captivity (480) and there is evidence that other species will eat their young when caged (484). While single individuals adapt well to cage living, they do not become docile and cannot be handled easily. Ammospermophilus from the Mojave Desert makes a good cage squirrel and becomes fairly domesticated (485).

Ecological Role -

Ground squirrels are primarily herbivores though certain species rely heavily on insects during dry seasons in arid habitats. They are a food source for raptors and large carnivorous mammals.

Longevity -

The life span of ground squirrels in nature is quite variable, but in captivity they have been known to live up to eight years (453).

Toxicological Testing -

Bishydroxycoumarin has been successfully administered to the California ground squirrel via an oral administration tube and later assayed from liver and fecal samples. A dose of 20 mg/kg body weight was given every 24 hours to adult squirrels. Differences in plasma dicoumarol were reported, the concentration depending on differences in diet (486).

General Suitability -

There are problems with breeding wild caught ground squirrels. Successes with S. beecheyi are encouraging and makes this species a prime choice for future use.

Ecological Alternatives -

Chipmunks, prairie dogs.

Rodentia: Scuridae - Chipmunks and Squirrels

Introduction -

The chipmunks are rodents of the squirrel family and closely related to ground dwelling squirrels. There is one species common throughout eastern North America, Tamias, and sixteen western species, Eutamias. They are from three to seven inches in length, excluding their long, furry tails. They weigh from two to five ounces, the eastern chipmunk being larger than the western forms.

Chipmunks occur in all types of habitats from woodlands in eastern North America to rocky slopes, high deserts and conifer scrubland in the Rockies. They are diurnal, primarily ground dwellers, and omnivorous although they can exist for long periods on seeds alone.

These small squirrel-like creatures are very active and alert. They require facilities with adequate room for exercise.

Tree squirrels (Sciurus , Tamiasciurus) and flying squirrels (Glaucomys) are well studied in the wildlife literature, but there appears to be very little maintenance information available.

Caging/Lab Conditions -

Glass or metal cages 2 x 2 feet with tops of fine wire mesh have been successfully used to house chipmunks in the lab. A substrate of dead leaves or another adequate substance should be used (453). An exercise wheel would probably increase the acceptability of the enclosure.

Nutrition -

Chipmunks are omnivorous, but feed mainly on fruits, nuts, seeds, and invertebrates. Their natural diets conform to the seasonal availability of foods. In the laboratory, mixtures of seeds (canary and sunflower) with fresh greens, fruits, chopped raw meat and cod liver oil have been successful diets (453). The average daily water intake for Tamias is about 33 ml. (487), and it has been found that Eutamias need less water per gm of body weight than its eastern cousin (488).

Tree squirrels are primarily consumers of nuts and these dietary items are suggested as food for captive individuals (489).

Breeding/Rearing -

Chipmunks are not readily bred in captivity, but the long-eared chipmunk (Eutamias quadrimaculatus) has been successfully bred in a large, screened, outdoor cage in California (453). Pregnant females brought into a lab may eat their young (487) or refuse to care for them (490).

The gestation period is 31 days for all chipmunks. Tamias striatus produces two litters each year of four to six young each (491), while Eutamias breed but once in the spring and average five young per litter (490).

The gestation period in Sciurus is about 44 days, in Tamiasciurus about 38 days, and for Glaucomys about 40 days. They all breed once a year and give birth in late spring or early summer. The number of young varies from two to eight (412). There is little known about captive breeding in these genera.

Behavioral Restrictions -

Because chipmunks and other squirrels are solitary in natural situations, there may be difficulty in keeping more than one individual in each cage. Their social intolerance is well documented (453, 492). Various species of Eutamias have been housed in groups with no fighting; however, most chipmunks and squirrels are non-social and territorial in nature and would probably be difficult to house in numbers.

Longevity -

Chipmunks have been known to live up to eight years in captivity. Because they adapt well to conditions of captivity, their mortality is minimal in laboratory situations (453).

Squirrels will live from 10 to 15 years in captivity (412).

Toxicity Testing -

Other than for simple residue analysis, squirrels and chipmunks have not been used in toxicological testing.

General Suitability -

Chipmunks and squirrels are difficult to breed and maintain in a laboratory. Suggested species from this group are the eastern chipmunk (T. striatus) because of its abundance in the eastern United States and its size, and the least chipmunk (E. minimus) for similar reasons, though its range is northern and western. These species, and Glaucomys are available from some dealers.

Ecological Alternatives -

Mice, ground squirrels.

Rodentia: Cricetidae - Voles and Allies

Introduction -

The microtines include the lemmings (Dicrostonyx, Lemmus) the red-backed voles (Clethrionomys) and other basic grassland dwellers (Phenacomys, Microtus).

This group includes both woodland, grassland and tundra species. All occupy similar niches in these habitats, being consumers of green vegetation with dentition specifically adapted for crushing grasses, sedges, seeds, bark, etc. They are mouse-like in form and size.

Because species of Microtus for example, M. pennsylvanicus, M. ochrogaster, are the most utilized in laboratory work, and most of the available information refers to these forms, the following comments are generally restricted to them.

Caging/Lab Conditions -

Two basic sizes of enclosures have been used to house these small rodents. Smaller cages of various sizes and materials have been used to house breeding pairs; 12 x 8 x 5 inch metal cages with lift off lids, (493 , 494) 12 x 11 x 6 inch plastic cages with wire mesh tops 12 x 18 x 11 inch stainless steel, 18 x 7 x 4 inch fiberglass (496); 29 x 19 x 13 inch polypropylene cages (496). A 36 x 30 cm cage for mother and young has been used (496). Substrates of peat moss (493, 494), and sand (497) have been used. It has been reported that wire mesh flooring is unsatisfactory (495).

Large enclosures for colonies have been used by some labs. A 6 x 25 x 2 foot galvanized metal cage with 48, 2 x 3 x 6 inch nest boxes, has been used for studies on adrenocortical activity in confined populations (498).

Feeders have been used of 6 x 2-1/4 x 9/16 inch dimensions with a 2 x 2-1/4 x 1 inch catch basket for overspill (398).

Bedding materials are necessary, and some that have been used include cotton, corn husks (499), dried grass, hay and wood shavings (496).

Nutrition -

Corn, hay, wheat, oats, mixed vitamized olive oil and excess hay (493,500) are some foods administered to Microtus in the lab. Others include Rockland mouse diet supplemented with fresh greens (lettuce and cabbage) (498) though one report states green vegetable material is not necessary and when given causes diarrhea and undue mess (495). One lab, whose feed is given in Table 14, reports that M. ochrogaster is quite sensitive to deficiencies of any sort (495). Other feeds can be found in references 499, 501 and 496. All sources state that water should be available ad libitum.

Breeding/Rearing -

Microtus breeds well under laboratory conditions. The gestation period is 21 days (453, 493, 502) and M. agrestis averages 3.7 pups per litter. M. oregoni averages four or five litters each year under natural conditions. Gestation is about 25 days in this species (503). M. ochrogaster pups are weaned at 21 days, females reach maturity at 35 to 40 days and males at 42 to 45 days (495). Grasshopper mice, Onychomys, are seasonally polyestrous in the wild with single females producing three to six litters each year for two or three years (497).

Female pine voles, M. pinetorum, usually have two to four litters each year, but could produce eight (504).

The effect of ambient temperature and light conditions on the reproductive ability of M. ochrogaster is shown in Tables 15 and 16.

Special Needs -

Overcrowding Microtus impairs their reproductive potential and sociability (498). Acceptable population optimum densities are not known. Small cages are sufficient for one breeding pair and a single litter (495).

Grasshopper mice (Ocychomys) should be supplied with a substrate suitable for dust baths (497).

Pine voles (M. pinetorum) are inhabitants of the cooler woodland floor and are sensitive to high temperature (504).

Table 14. COMPONENTS OF THE STANDARD LABORATORY FEED USED FOR MAINTAINING MICROTUS OCHROGASTER IN THE LABORATORY

Ingredient*	Weight (kg.)
Meat scrap	12
Fish meal	4
Soybean meal	13
Dried skim milk	4
Dried brewers yeast	3
Alfalfa meal (17% protein)	5
Yellow corn meal	10
Corn flakes	15
Rolled oat groats	15
Wheat middlings	15
Corn or cotton seed oil	2
Wheat germ oil	0.3
Steamed bone meal	1
Salt	1
Vitamin D_3 (15,000 ICU/gm)	0.02
Aurofac 10	0.35

*Ingredients were thoroughly mixed, then pelleted through 3 cm dies. The general analysis has been maintained at about 21% protein, 8% fat, 9% ash.

Source: 495 (Gier and Cooksey, 1967)

TABLE 15. REPRODUCTION OF M. OCHROGASTER IN THE LABORATORY AT DIFFERENT TEMPERATURES

Temperature* Night	Day	Number of Days	Pairs	Reproductive Period	No. Young Born	Weaned	Percent Survival
25	30	160	16	38	3.6	3.3	91
20	24	120	15	34	3.7	3.4	92
10	15	102	12	31	4.0	3.5	88
5	9	60	16	24	4.6	3.5	82
0	4	48	20	27.5	4.4	3.0	67

*There was no consistent shift from one temperature to another, with some animals spending several months at the same temperature. Light was maintained above 20 foot candles for 14 hours.

TABLE 16. REPRODUCTION OF M. OCHROGASTER IN THE LABORATORY ON DIFFERENT LIGHT SCHEDULES

Hours* Light	Number of Days	Pairs	Average reproductive Period	% Bred 3 Days Postpartum	Average No. Young	Reproductive Efficiency
14	60	16	24	86	4.57	93
12	35	25	36	43	4.2	73
10	32	26	48	39	3.7	46
11	37	28	59	40	3.7	38
12	38	30	28	83	4.55	84
13	32	31	23.5	88	4.6	94

*The colony was stabilized at 14 hours light, 10 hours dark, then shifted down to 10 hours light by the steps and periods listed below, with approximately 50 cycles accumulated in each stage. Temperature was maintained at 9 C day and 5 C night, on 12-12 hour changes.

Source: 495

Behavioral Restrictions -

Fighting is frequently reported among the microtine rodents (412) and aggressiveness, litter mortality and infectious disease have been shown to increase as the density increases (498).

Ecological Role -

The small herbivorous rodents are important components of nearly all natural terrestrial communities. They are a primary food source of smaller predacious reptiles, birds, and mammals.

Longevity -

Members of this group have a life-time of about one year (503).

Problems with Mass Culture -

Parasites can be a problem (497, 504).

Toxicity Testing -

The use of Microtus in behavioral studies of overcrowding is well documented (498, 505) and their reactions to certain pesticides (e.g., endrin, dieldrin) have also been studied. The substances were administered orally in a corn mixture and the subjects individually caged (6). The effect of endrin on an enclosed outdoor population was studied by periodic trapping following administration of the chemical by spraying. The result was an immediate decline in numbers and no recovery in numbers for years after (506). Similar studies with endrin administered orally to pine voles were conducted (507). Rodenticide studies have also utilized voles (508, 509).

General Suitability -

They are small animals, easily and inexpensively kept. M. pennsylvanicus, for example, is plentiful, has a large range, and is easily bred in captivity. These are very suitable animals, in general.

Ecological Alternatives -

Other voles (412), Onychomys (grasshopper mice), Clethrionomys (red-backed voles).

Rodentia: Cricetidae - Wild Mice

Introduction -

The mice of the genus Peromyscus and allies, Onychomys, Reithrodontomys, Zapus, are by far the most abundant and widespread native mammal group in North America. The white-footed mice are medium-sized mice with grey to brown backs and sides and a very light underbody. There are about 16 species in the United States and representatives of the genus can be found in all types of habitat. The most widespread species, P. maniculatus, ranges through North America with the exception of the southeastern U.S., the tundra of northern Canada, and the hot deserts of Mexico.

All members of the genus are nocturnal; they are generally ground dwellers, though some species nest in trees. In nature, they rely heavily on seeds for their food, but eat invertebrates readily. Species that have been used for laboratory research include the white-footed mouse (P. leucopus), the deer mouse (P. maniculatus), the golden mouse (Peromyscus = Ochrotomys nuttali), and the cotton mouse (P. gossypinus).

Caging/Lab Conditions -

Lab enclosures that have proved satisfactory for housing wild mice include a 15.5 x 14.2 x 12 inch cage of 1-1/4 inch wire mesh with baking pans for the tops and bottoms, quart fruit jars with cotton for nesting and a sawdust floor (510); a 9 x 7 x 5 inch wire mesh cage with removable wood top and a metal pan for the floor and cotton for nesting (511); large aquariums or wire cages with solid bottoms covered with a layer of sand or wood chips and nest material in small nest boxes (512). For numbering systems and data card see reference 494.

Nutrition -

Successful diets include frisky dog crackers and lettuce (510); rolled oats, dry meat scraps, dry skim milk, whole wheat, wheat germ, cod liver oil, sunflower seeds, hemp seeds, canary seeds, millet, iodine salt and lettuce (494, 513); and commercial mouse chow supplemented with rolled oats, whole corn, sunflower seeds, nuts, greens, and fruits (512). Food and water are always supplied ad libitum.

Breeding/Rearing -

The members of this group that have been used in labs, for the most part, breed year round and have three to six young per litter. Gestation last from 23 to 30 days, the young are weaned at about 25 days and achieve sexual maturity at about 55 days (510, 511, 514). P. maniculatus females mature faster than males (514).

P. gossypinus is known to hybridize with P. leucopus in captivity and produce fertile offspring. Cotton mice (P. gossypinus), are somewhat colonial in captivity and apparently reach sexual maturity later than some other wild mice (approximately 70 days) (510). This species usually nests off the ground in nature (511).

Golden mice (O. nuttali) share communal nest boxes in the lab. In nature, they do not breed from November to February.

Special Needs -

Nest boxes are better for litter rearing than open cage floors.

Ecological Role -

Mice are small omnivores that are an important food source for small carnivorous reptiles, birds, and mammals. They are found in almost all habitats throughout the world and are very plentiful in many areas. In some cases they are significant dispersers of seeds.

Longevity -

It is very unlikely that the average life span of wild mice in nature exceeds two years, but in captivity, a life span of five to eight years is not unusual (412).

Problems with Mass Culture -

There are many known parasites of mice, e.g., nematodes (510, 515), fur mites, and fleas (516), lice (494), and these should be controlled. Otherwise, wild mice (Peromyscus) seem quite suitable for mass culture.

Toxicity Testing -

Wild mice, (Peromyscus maniculatus, P. leucopus), have been used in studies of the effects of DDT (167) and Endrin (348, 506, 517) both in the lab and under natural conditions. Mouse populations declined after the ground application of endrin by spraying (eight ounces per acre) and when fed endrin with their food in the laboratory, showed adverse effects (348).

Experiments on the effects of sodium flouracetate - the rodenticide was fed with bait in a natural environment - have also been performed (518).

General Suitability -

This group in general is very easily bred and maintained in the lab. P. maniculatus is the most abundant and widespread species, but others are just as suitable for research.

Ecological Alternatives -

Other mice (412).

Rodentia: Muridae - Lab Rats and Mice

Introduction -

The choice of research animals in the past has been based on availability and ease of maintenance. No group of animals was more available than the old world rats (Rattus norvegicus) and mice (Mus musculus) which have adapted so well to living with man. The white lab rats and mice of today are albino strains of these species that have been selectively bred for research purposes.

Caging/Lab Conditions -

There are numerous suitable cages available commercially for housing laboratory animals, largely designed for lab mice and rats. Unique apparatuses for restraining, anesthetizing, bleeding, and feeding them are available, and are specially designed for these gentle, docile, easily manipulated species.

Lab rats and mice can be housed in nearly any enclosure including fruit jars (519), screened cages (520), metal or plastic cages (521, 522). Overcrowding should be avoided. Typical mice cages are 18 x 12 x 6 inches and typical rat cages are 24 x 20 x 10 inches (289).

The type of bedding seems to be more important than the cage. Pregnant mice produced larger litters, and a greater percentage of their progeny was successfully weaned on sawdust than on a commercial deoderized cellulose (523). Mice housed on sawdust bedding also outproduced those on corn cob bedding (524). Other bedding suggestions can be found in references 525, 526 and 527. Other environmental factors found to affect the performance of lab mice are ambient light conditions (528, 529), noise (530), feeding schedules (531), isolation and stress (532), NH_3, dust, and relative humidity (521). A thorough maintenance procedure for mice is presented in reference 533.

Sawdust bedding for rats is considered inadequate due to the intestinal obstruction it causes. When it is screened of small particles it provides a better substrate (534). Ventilation of the lab facilities is a proven necessity (535).

Nutrition -

Commercially prepared lab diets are used exclusively by many institutions and together with water and a few minor additions in specific cases, is supplied ad libitum (519, 522, 536, 537, 538, 539, 540, 541). There are food tube dispensers available for accurate dispensing of certain diets. Feeding frequencies have been shown to affect growth in young rats (543). There are controls for diarrheal disease available (544).

The suggested ration for adult mice is four to five gms per day and for adult rats, 12 to 15 gms per day (289).

Breeding/Rearing -

The breeding of lab rats and mice is very easily accomplished if they are allowed space enough. The mouse gestation period lasts an average of 19 days; the litter size averages 10 to 12 young and they are weaned at 16 to 21 days of age. They are sexually mature at 60 days. Females can produce up to ten litters in a lifetime and the male breeding life lasts about 18 months. One male to three females is a suggested ratio for colonies (289).

Albino rats are sexually active for about one year. Females gestate for 20 to 22 days and eight to 12 young per litter can be expected. They are weaned at about 21 days and sexually mature at 100 days (289).

Behavioral Restrictions -

Overcrowding should be avoided.

Ecological Role -

Lab rats and mice are bred for man's uses only and have no ecological role in the wild.

Toxicity Testing -

Albino rats and mice have been the most used subjects to date in toxicity testing. LD_{50}'s for large numbers of compounds are available (545). (Testing methodologies, it should be noted, vary greatly). Behavioral tests have also been developed. Reviews of these methods and their validity may be found in references 546 and 547.

General Suitability -

Laboratory bred rats and mice are readily available and easily maintained. However, because they have been selectively bred for laboratory work and long removed from the genetic influences of wild-types, their relationship to natural fauna is indefinable, and the results of studies using them may not be extrapolable to populations in the wild.

Ecological Alternatives -

Wild strains of rats and mice.

Rodentia: Castoridae, Cricetidae, Erethizontidae, Sciuridae, Capromyidae - Large Herbivores

Introduction -

The beaver (family Castoridae), the muskrat (family Cricetidae), the porcupine (family Erethizontidae), the yellow-bellied marmot, the woodchuck (family Sciuridae), and the nutria (family Capromyidae) are large herbivores not often used in laboratories, In nature the beaver (Castor canadensis) is distributed locally throughout North America, utilizing streams and ponds with nearby cottonwoods, alders, aspens, and so on (412). Both the muskrat (Ondatra zibethica) and the nutria (Myocastor coypus) are inhabitants of marshes, swamps, and bayous. The muskrat ranges throughout most of North America. The nutria occurs only in the southern U.S. and Mexico, having been originally a South American form. The porcupine (Erethizon dorsatum) is a heavy bodied inhabitant of forested areas of the western mountains and Canada. The woodchuck (Marmota monax) occurs throughout the boreal Canadian forests and down into the eastern U.S. It prefers open woods, and brushy , rocky ravines (412). The yellow-bellied marmot, (Marmota flaviventris) is an inhabitant of talus slopes, valleys, and foothills throughout the Rocky Mountains (412).

These large herbivores measure from 20 inches in total length (muskrat) to 45 inches (beaver). They feed on all types of vegetation - leaves, bark, twigs, and roots.

Caging/Lab Conditions -

Woodchucks were caged outdoors in 5 x 20 x 5 feet cages made of wire mesh which was extended into the ground to prevent escape by burrowing (548). Porcupines (2-3) have been housed in 3 x 6 x 6 foot wire mesh (549), and a pair of nutria in 3 x 2 x 1 foot metal cages with sawdust bedding (550).

Nutrition -

Captive woodchucks were fed on ear corn and smoked herring (548), porcupines on lawn grass, hay, fruits, oats, and lab chow (549), and nutria on sugar beets and grasses (550).

Breeding/Rearing -

Woodchucks breed in February and March and average 3.5 young per litter (551,552). Marmots breed about the same time as woodchucks and produce from three to six young per litter. The young emerge from the burrow after about 30 days.

The colonial beaver has one litter each year which averages three young. They are born between April and July. Female beavers do not breed until they are 2 1/2 years old (412).

The gestation period for porcupines is seven months and usually there is one young each time. Breeding takes place in the fall and birth in spring. There has been some success breeding porcupines in captivity (549).

Nutria gestate for 120 to 139 days (the average is 128 days) and average five young per litter. There is post-partem estrous and two litters a year seems to be the norm. In captivity the sexual life of nutria lasts to five years. Large cages are needed for breeding (550).

Special Needs -

Fresh food for the beaver, if needed, would probably be difficult to provide, and its enclosure would have to have a large water supply, a necessity for the grooming and cleaning of their oily fur.

Fresh food for the woodchuck and marmot, which should include fresh greens, might also prove difficult and expensive to gather.

Outdoor cages might be required for some species in this groups.

Behavioral Restrictions -

Woodchucks and marmots are solitary, territorial creatures which do not readily accept other individuals of their species in their cages (548). They also hibernate for months during the winter.

None of these species can be maintained in wooded enclosures for all of them would find no difficulty in gnawing their way out.

Ecological Role -

The beaver is a furbearer; its dams create lakes and ponds. Like the marmot and woodchuck, it is a primary consumer. Nutria and muskrats are also classified as furbearers. The porcupine feeds on bark and twigs in forested regions of western U.S. and Canada. All provide food resources for the large carnivores.

Longevity -

Beaver have been known to life up to 11 years in the wild and to 19 years in captivity. The woodchuck's life span is four to five years (412). The life span of marmots is unknown, but is probably very similar to that of the woodchuck. Nutria will live 12 years in captivity (412).

Problems with Mass Culture -

Space will be a limiting factor for these large rodents (551). The Sciurid forms will not readily accept confinement in colonies. The large rodents are hosts of the usual external parasites including ticks, fleas, etc.(551).

Porcupines are covered with quills and may prove to be difficult to handle.

Toxicity Testing -

Though no references were located dealing with the use of the beaver, marmot, or woodchuck in toxicity testing, marmots and woodchucks have been used frequently for physiological studies of hibernation.

General Suitability -

These rodents are probably too large to handle easily. In addition, they require rather large enclosures. Nutria are used by some researchers who consider them quite suitable subjects (550).

Ecological Alternatives -

Rice rat, voles.

Carnivora: Canidae - Large Species

Introduction -

Large carnivores have been decreasing in number as their habitats have decreased in size. Wolves (*Canis lupus*, and *C. niger*) have lost large amounts of habitable area in the U.S. and are practically extirpated from this country except from Alaska (Canada still has many wolf packs).

The common fox species are the red fox (*Vulpes vulpes*) and the gray fox (*Urocyon cinereoargenteus*). Both species are locally common throughout North America, the red fox occurring in open woodlands north of Mexico and the gray fox in woodlands south of Canada (412).

The coyote (*Canis latrans*) is an inhabitant of open brushland prairies and deserts throughout central and southern North America.

The wolf and coyote are dog sized and the foxes generally smaller (412).

Large cats are too rare to consider for mass culture and no lab work on bears (Ursidae) is known.

Caging/Lab Conditions -

Most cages for large carnivores are outdoors and large.

Nutrition -

Foxes have been maintained on fish and cooked cereals (553) In the wild the large carnivores are predators and eat most animal matter, as well as some berries and grasses.

Breeding/Rearing -

Wolves are generally social creatures and live in closely knit packs. Their breeding is tied to their social structure; they probably produce only the number of offspring needed to keep their pack viable. Gestation is nine weeks long (554).

Foxes breed from February to May, depending on latitude and local conditions, and produce from one to seven young per litter (555,556,557). Their gestation period is 52 days and the young breed at one year (558,559).

Coyotes have a gestation period of 64 days (554). Five to ten young are born in April or May and mature in one year.

Data on the breeding of large carnivores in captivity is negligible, but in large enough enclosures there should be no problem.

Special Needs -

Large carnivores prefer a den to sleep (556). They probably need large areas for exercise and high protein diets.

Ecological Role -

The smaller forms are predators on rabbits and rodents. The wolves generally prey on larger animals. Infrequently, livestock is taken by all of these species. Of economic importance to man is the fur of these species (412,443).

Longevity -

Fox have a ten to fifteen year life span in captivity (555).

Problems with Mass Culture -

Canids are very susceptible to external parasites such as ticks, lice, etc., as well as tapeworms and roundworms (443).

Toxicity Testing -

In general, there is very little data concerning wild Candis and toxicity testing. The effect of diethylstilbestrol, a synthetic estrogen, on red foxes has been studied with good success. The foxes were force-fed the substance (560).

Coyotes have been subjected to the management of their numbers by the administration of diethylstibestrol in bait (561).

General Suitability -

Due to their size, the large carnivores are probably less suitable than smaller ones for laboratory research. However, fox have been commercially reared by the fur industry and are breedable in captivity.

Due to their dwindling population and large size, wolves are considered very unsuitable for lab testing.

Coyotes are more plentiful than wolves and probably more available to researchers.

Ecological Alternatives -

Domestic dog.

Carnivora: Procyonidae, Mustelidae

Introduction -

The small carnivores include the family Procyonidae which has one representative in North America, the raccoon (Procyon lotor) which occurs throughout the U.S. with the exception of the high Rockies. The species of Mustelidae are numerous: the marten (Martes americana), the long-tailed weasel (Mustela frenata), the mink (Mustela vison), the badger (Taxidea taxus), the striped skunk (Mephitis mephitis), and the European ferret (Mustela putorius). The ring-tail (Bassariscus astutus), the only North American member of the Bassariscidae, has not been extensively used in lab work, but could prove very suitable. The following information refers to those species of small carnivores which have been selected because of previous laboratory use.

Caging/Lab Conditions -

Mink, weasels, and ferrets have been maintained under lab conditions. A good enclosure for weasels is an 11 x 9 x 8 inch wooden nest box connected by a doorway at one end of the box with a runway of 1/2 inch galvanized wire mesh (18 x 9 x 8 inches) where feeding and watering take place. The nest box is provided with a removable plywood lid held in place by a fastening device. The lid must be water right if the box is used outdoors. A false top is placed underneath the lid, consisting of a frame filled with 1/2 inch galvanized soldered wire, resting on two cleats and held in position by two wire finishing nails pushed through holes in the sides of the box. Shavings and excelsior are adequate nesting materials. Great care must be taken to secure all edges and joints because weasels are superb escape artists (562).

The proper housing of mink can be readily learned from mink ranches. European ferrets have been successfully housed in rabbit cages with a 12 x 6 x 6 inch nest box (563) and in double compartment cages similar to those described for weasels (564). They have been domesticated for at least 2,000 years and their exact genetic ancestry is quite nebulous. A natural photoperiod is necessary to effect breeding in this species (565).

Nutrition -

In the wild, the small carnivores feed on crayfish, small birds, insects, chipmunks, rabbits, mice, and shrews (566). Diets for weasels in captivity include hamburger, mice, fish, bread, milk, and ground liver (567). Ferrets eat similar foods as well as dry dog food and Gerber's Meat Base for Babies (563,564). Bottles containing milk may be placed in the cage to facilitate serving that item (562).

Mink are fed cotton rats, raw meat (568), mice, fish, muskrats, and frogs (569). For the fur trade, a special mink food is prepared and sold commercially.

Breeding/Rearing -

The breeding and rearing characteristics of the Mustelidae are summarized in Table 17.

Raccoons produce one litter each year of two to seven young which are born in April or May about 65 days after conception. They are weaned at about two months (412, 554).

The ringtail also produces one litter each year of three to four young that are born in May or June and develop at a rate comparable to that of the raccoon (412).

Captive weasels breed readily but about 58 percent of females bred in captivity fail to produce young and the number whelped in captivity is often less than the four to nine average whelped in the wild (560). The family Mustelidae has delayed implantation which makes gestation times difficult to determine (570).

Special Needs -

Weasels need dark sleeping chambers (562,564) and escape-proof cages (562). A natural photoperiod is essential for successful ferret breeding (565). The diets of the small carnivores should include a substantial proportion of meat (563).

Behavioral Restrictions -

All mustelids have musk glands which are responsible for their peculiar odors. The best known example is the skunk (571, 572). These glands can be removed. The weasels, ferrets, and mink are nervous and frequently vicious animals (443, 474, 563). Skunk nest boxes should be left undisturbed while young are present (573).

Table 17. REPRODUCTION IN MUSTELIDAE UNDER NATURAL CONDITIONS

Species	Litters/ Year	Gestation*	Time of Estrous	Young/ Litter	Age at Maturity
Martin (412)	1	8-9 months	July - August	?	4-12 months
Weasel (412, 574)	1	8-9 months	June - July	4-8	Females - 3 months Males - 1 year
Mink (412, 443 575)	1	40-75 days	March - April	2-10	1 year
Ferret (563, 565)	1-2	42 ± 2 days	April - September	2 +	9-12 months
Skunk (412, 443, 554, 571, 572)	1	63 days	February - May	2-16	1 year
Badger (412, 443)	1	6-9 months	August-September	1-7	1 year

*Many Mustelids exhibit delayed implantation.

Glass, wood, or any other material that can be gnawed or broken may be hazardous (562).

Ecological Role -

The small carnivores are predators on many smaller vertebrates and invertebrates. Many also eat seeds, fruits, and certain herbaceous material. Skunks and raccoons are often scavengers and will readily feed on carrion and human garbage.

Longevity -

Ferrets have lived to nine years (563) and the life spans of other mustelids are probably comparable; smaller forms will have shorter life spans.

Problems with Mass Culture -

Skunk parasites are numerous and many of them are easily transmitted (515, 576, 577, 578). Influenza, canine distemper, and tuberculosis are a few diseases reported in captive ferrets (563); however, distemper can be treated.

The small carnivores are usually solitary in nature and there are reports of individuals caged together displaying great animosity towards their cage mates (574).

All of these species are nocturnal by habit; scheduling, and so on, must be made to fit their cycle (571, 572).

Toxicity Testing -

Mink, which are farmed by the pelt industry and readily available, have been used in testing of psychoactive compounds, These were administered orally to adult males in capsule form by means of a hollow wooden rod. Direct observations of the males' behavior followed. Their innate aggressiveness made them appropriate models for this work (579). Wild mink have been trapped, killed and tested for DDT residues in body tissues (580) and used in lab feeding experiments with heavy metals, PCB's, etc. (581, 582, 583).

Wild trapped raccoons were examined for incidence of DDT DDD, and dieldrin in a Missouri study (584).

In Great Britain a study was conducted of pesticide levels in the European badger (Meles meles), found dead along roadways from 1964 to 1968 (585).

General Suitability -

Small carnivores are relatively unavailable in large numbers with the exception of mink that have been used in psychological behavior testing and judged quite suitable for such research (579). Culls are available from some ranchers. Raccoon and skunks are locally abundant, but large-scale trapping could decimate populations. European ferrets are an exotic domestic species and their suitability in toxicity testing of wild species is questionable.

Ecological Alternatives -

Domestic cats.

Carnivora: Canidae, Felidae - Domestic Species

Introduction -

This subgroup of carnivores includes the dog (Canis familiaris) and the cat (Felis domestica), both familiar animals in the laboratory. They are adapted to living wit man, being handled, etc., and are very suitable for lab work.

Caging/Lab Conditions -

A 36 x 16 x 18 inch cage of any easily cleaned and structurally sound material is adequate for the housing of cats Breeding cages are larger, 36 x 24 x 30 inches and both require litter (289).

Dogs have been housed in 4 x 4 foot pens with water and fo containers and resting platforms. These are connected to outside running pens ranging in length from 12 to 20 fe Inside pens 6 x 8 feet will house five adults (289). Con- crete floors in dog kennels provide easy cleaning (289).

Nutrition -

Commercially available diets with cooked horsemeat or brot and additional vitamins are used for dogs. Cats are also maintained on commercial foods. Water should be provided ad libitium for both (586, 587).

Suggested dog rations are one-half ounce dry dog chow per one pound of body weight. Adult cats should be fed four percent of their body weight each day. Twice this ration should be given to growing animals (289).

Breeding/Rearing -

Dogs vary somewhat in their breeding schedule, so the beagle is chosen as the pattern subject because of its size, availability, and adaptability to lab conditions. Beagle females undergo estrous every 150 to 200 days and apparently can produce only one litter each year. Gestation lasts 63 days and four to eight young are born per litter. They are weaned in six to eight weeks and are sexually mature in one year. The breeding life of female beagles is six to ten years and that of males a little longer. One male can service about 60 females in a breeding colony though hand-mating may be required (289).

Domestic cats can breed a number of times each year. The estrous cycle is intermittent and irregular. Gestation lasts an average of 62 days and litter size is from one to six with an average of four. Weaning takes place in six to eight weeks and the young are sexually mature in six months. The breeding life of females is four to five years and males generally can breed a few years longer. In a breeding colony, one male for six females is suggested (289).

Special Needs -

Dogs require no special bedding but a sleeping platform raised from the floor. Cats should be provided bedding and litter of wood shavings, etc.

Pregnant female dogs near term should be provided with whelping boxes lined with shredded paper. Pregnant female cats near term should be provided with nest boxes. They should always be separated from other animals (289).

Behavioral Restrictions -

Both dogs and cats are easily handled and should present no behavioral problems in the lab.

Ecological Role -

The ecological role of carnivores similar to the dog and cat is discussed in the section on large carnivores.

Longevity -

Domestic dogs are productive for six to 14 years, cats for four to seven years (289).

Problems with Mass Culture -

There are no apparent problems associated with mass culture of domestic dogs and cats.

Toxicity Testing -

The use of domestic cats and dogs in research is quite extensive. Tests and methods from many disciplines including physiological, immunological, biomedical, and toxicological areas are available for both species.

General Suitability -

This group is available in large numbers and adapted to laboratory life. The animals are handled with ease and are most suitable subjects for experimental work. How suitable they are for toxicity testing is difficult to say because their gene pools have been manipulated by man and, in some important ways, have been free for many years from natural selection pressures.

Ecological Alternatives -

Mink, European ferret.

Ungulates: Equidae, Bovidae

Introduction -

The domestic ungulates that have been used for scientific testing other than the swine and allies are the donkey, mule, horse, pony, and cattle. They are all large herbivores, eating a variety of plant foods.

Caging/Lab Conditions -

The enclosures to contain domesticated ungulates must be of suitable dimensions to accommodate their large size. Access to an outdoor lot is desirable. No better description of suitable enclosures is available, probably because the maintenance of these domestic animals is so routinely accomplished.

Special Needs -

Salt licks are necessary for all these animals.

Behavioral Restrictions -

All these animals may be difficult to handle and control because of their large size. The biting and kicking of donkeys and mules is well known.

Ecological Role -

The ungulates are grazing and browsing animals which rely entirely on plant material for their food.

Problems with Mass Culture -

The space needed to house these animals is considerable. Likewise, the food expense is much greater than for other groups.

Toxicity Testing -

Because these animals are of economic importance, they have been used quite extensively in many types of experimental work, particularly in the study of the effects of pesticides on their bodily functions. Chemicals tested on cattle include heptachlor (588), DDT, BHC, lindane, methoxychlor, chlordane, toxaphene, aldrin, dieldrin, endrin (589, 590) and others (591, 592). These chemicals are

administered in foods and through capsules. The testing was conducted primarily to assay chemical residues in animal products consumed by man. Substances that have been studied for their effects on cattle are nicotinic acid (593), dimethyformamide (594), arsenic, flourine, lead, salt, selenium (595), iodine (596), and molybdenum (597).

Calves have been used in studies of taste reception; the peripheral nerves were successfully exposed and impulses monitored (598, 599).

Horses, donkeys, and mules have also been subjects in studies of such pesticides as organophosphorous and carbomate pesticides (591), DDT, toxaphene, and chlordane (592).

General Suitability -

The large size and domestic status of this group reduces its suitability for the laboratory. Smaller ungulates (sheep, miniature swine) may be better subjects.

Ecological Alternatives -

Deer, sheep.

Ungulates: Suidae, Tayassuidae

Introduction -

Because of their smaller size, the swine (*Sus scrofa*) and javelina (*Tayassu tajacu*) are used more in research than other domestic ungulates (600). The special breeding of miniature swine in the last decade or so has been very extensive and their availability is increasing. (For a review of breeding stock and strains developed, see reference 600). While miniatures possess all the characteristics of standard swine, they are far smaller and weigh from 70 to 90 kg, about as much as the average man. Their size and availability have led to their increasing use in biomedical research (600). The javelina, or collared peccary, is a native North American animal found commonly in Texas, New Mexico, Arizona, and Northern Mexico. It belongs to the family Tayassuidae. Another closely related species, the white-lipped peccary (*T. Pecari*), occurs from Central America to Paraguay. This swine-like animal weighs between 16 and 20 kg, and averages about 90 cm in length with a shoulder height of approximately 54 cm. The ears are small and erect, and the hair is long, bristly and dark in color (601).

The following discussion is restricted to the miniature swine unless otherwise indicated.

Caging/Lab Conditions -

Housing for miniature swine is generally a large community cage of pipe and fencing. From one to ten pigs, depending on age, are housed in 4 x 4 x 8 feet indoor cages with concrete floors and adequate ventilation. Outdoor cages 8 x 16 feet with earthen floors and a shelter roof with sawdust bedding at one end of the pen housed an unknown number of animals (602). Old dog kennels are apparently quite suitable enclosures (603). The transport apparatus, observation and holding pens used at the Sinclair Research Farm are shown in Figures 15, 16, and 17 respectively.

Techniques for handling miniature swine for laboratory procedures are presented, with diagrams, in reference 604.

Pentobarbital sodium is a recommended anesthesia at 50 mg/4 kg body weight administered via the anterior vena cava. Blood collecting has been accomplished from the same vessel using 19 or 20 guage needles. Tranimul (600) and a spinal two percent procaine (603) have proven acceptable tranquilizers.

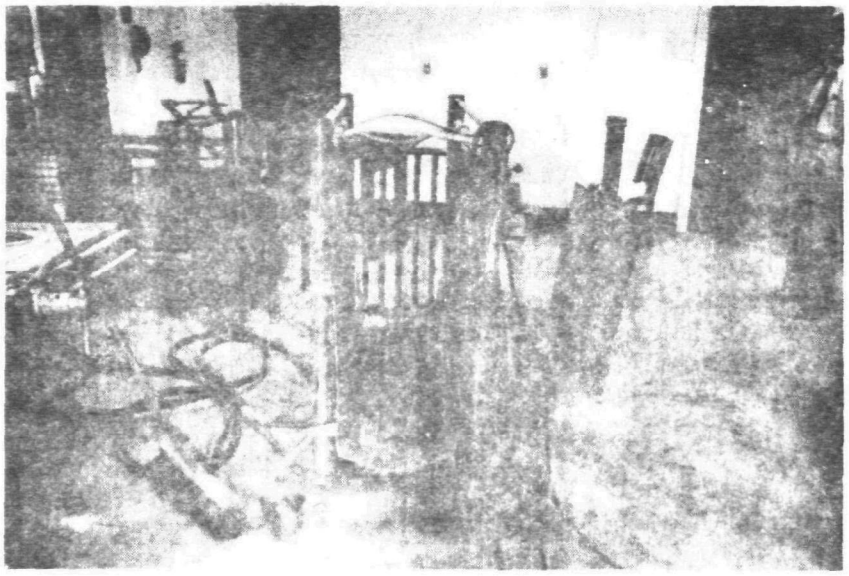


Figure 15. Miniature swine transport apparatus. Sinclair Comparative Research Farm, University of Missouri, Columbia, Mo. 65201

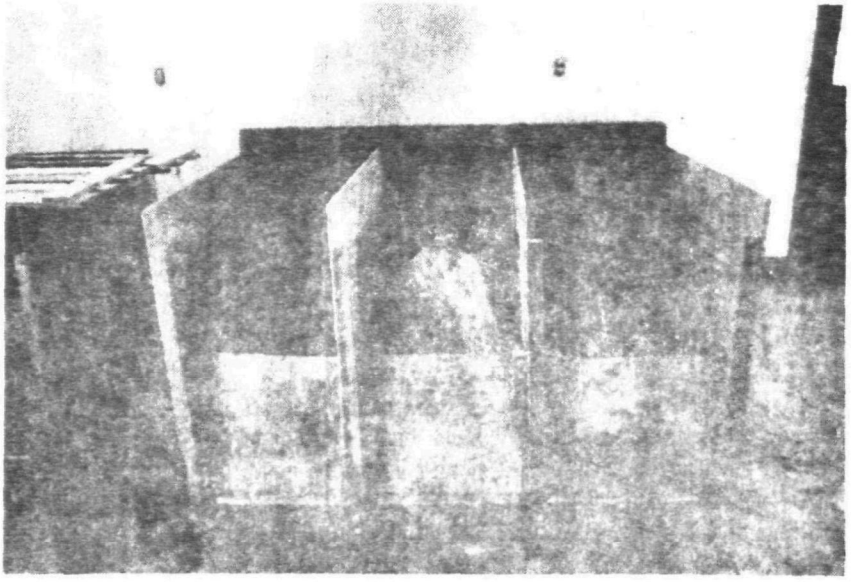


Figure 16. Miniature swine observation enclosures (20" x 30" floor area). Sinclair Comparative Research Farm

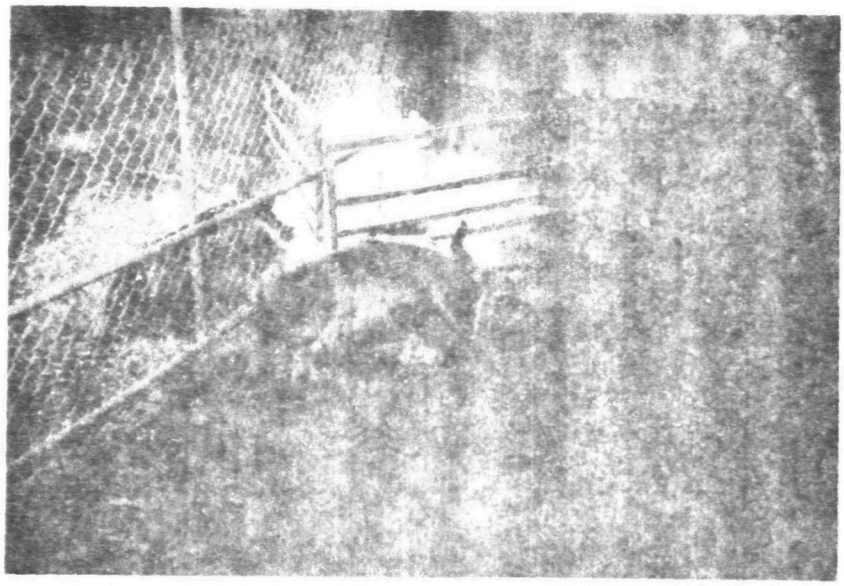


Figure 17. Miniature swine in holding pen (8' x 12' floor area). Sinclair Comparative Research Farms

Nutrition -

Commercial pelleted hog feed given twice daily is a suitable diet (602). Water should be supplied ad libitum. A small container for the water should be used to prevent the swine from playing in it (600). The Hormel Institute has utilized four different pig diets: pig starter, growth ration, gestation rations, and maintenance ration, containing about 19, 15, 16, and 13 percent protein, respectively. The Institute has also developed an artificial diet for baby pigs. These four diets are given in Tables 18 through 21.

Breeding/Rearing -

With a regimen of full feeding, an average of 5.5 pigs per litter are obtained by the Minnesota Hormel Institute. A piglet weighs 750 to 800 gms at birth, grows to 18 kg at 140 days, and is full grown and about 90 kg at one year (600). The Battelle Northwest Biology Laboratory uses Pittman-Moore miniature swine, but limits the feeding regimen of adult swine to 900 gms of feed per day. Their swine average six young per litter but do not reach adult weight until they are two to three years old. Gestation ranges 112 to 115 days (603) for the miniature swine. For the javelina which averages about three young per litter, the period ranges from 142 to 148 days. Some females have two litters each year (601).

The Oregon Dental School, Portland, has maintained Pittman-Moore swine for some years, and reports that young were weaned at 42 days and all members of the same age group housed together for four months, segregated according to sex; this communal housing effort tends to increase the sociability of the swine (602).

Special Needs -

Pen-to-pen contact between individuals should be limited to prevent transmission of disease (603). New arrivals should be temporarily quarantined; vaccinations for certain diseases are available (603).

Swine housed outdoors need protection from the weather and should always be provided shelter from direct sunlight. Concrete floors are recommended to control parasites (603). Exercise areas are needed if swine are housed indoors (603).

Table 18. GROWTH RATION
(Fed to Pigs from 56 to 112 Days of Age)

Item	Amount per 100 lbs	Protein (percent)	Calcium (percent)	Phosphorous (percent)
Corn, ground	35	2.98	0.007	0.088
Oats, ground	30	3.60	0.030	0.108
Wheat Standard Middlings	15	2.40	0.015	0.135
Dehydrated Alfalfa Meal 17%	10	1.70	0.150	0.020
Fish Meal	4	2.40	0.200	0.120
Soybean Oil Meal 44$	2	0.88	0.005	0.013
Tankage 60%	2	1.20	0.120	0.060
Dicalcium Phosphate	0.5		0.135	0.090
Salt	0.5			
Hi-zinc Trace Mineral Mix	0.5			
Vitamin A + D Oil	0.2			
Vitamin Supplement	0.1			
Aurofac	0.05			
Irradiated Yeast (FIDY-9)	0.25			
	99.875	15.16	0.662	0.634

SOURCE: (605).

Table 19. GESTATION RATION
(Fed to Bred Gilts until their Litters are Weaned)

Item	Amount per 100 lbs	Protein (percent)	Calcium (percent)	Phosphorous (percent)
Corn, ground	30	2.55	0.006	0.008
Oats, ground	15	1.80	0.015	0.054
Dehydrated Alfalfa Meal 17%	15	2.55	0.225	0.030
Sun-cured Alfalfa Meal 17%	15	2.55	0.225	0.030
Wheat Bran	10	1.40	0.010	0.120
Soybean Oil Meal 44%	5	2.20	0.010	0.032
Tankage 60%	3	1.80	0.180	0.090
Distillers' Dried Solubles	2	0.56	0.008	0.030
Dried Fish Solubles	2	1.00	0.003	0.020
Dried Whey	2	0.24	0.020	0.016
Dicalcium Phosphate	0.5		0.135	0.090
Salt	0.5			
Irradiated Yeast	+			
Aurofac	+			
Vitamin A + D Oil	+			
	100.0+	16.65	0.937	0.520

SOURCE: (605).

Table 20. MAINTENANCE RATION
(Fed to Culls until Marketing)

Item	Amount per 100 lbs	Protein (percent)	Calcium (percent)	Phosphorous (percent)
Oats, ground	37	4.40	0.033	0.159
Corn, ground	25	2.12	0.005	0.068
Dehydrated Alfalfa Meal 17%	20	3.40	0.300	0.040
Wheat Standard Middlings	15	2.64	0.015	0.135
Soybean Oil Meal 44%	1	0.44	0.003	0.007
Tankage 60%	1	0.60	0.060	0.030
Hormel Mineral	0.5		0.140	0.030
Salt	0.5			
Irradiated Yeast	+			
Vitamin A + D Oil	+			
	100.0+	13.60	0.556	0.469

SOURCE: (605).

Table 21. ARTIFICIAL DIETS FOR BABY PIGS

Per Quart Homogenized Cow's Milk

One Whole Egg Plus

5 ml Salt Mixture Consisting of:

49.80 g	$FeSO_4$	7 H_2O
3.90 g	$CuSO_4$	5 H_2O
3.60 g	$MnCl_2$	4 H_2O
0.26 g	KI	

200 ppm Aureomycin on Solid Basis plus water to make one liter solution.

SOURCE: (605).

Pregnant females should be separated from other swine on the 109th day of gestation, washed and cleaned of parasites, and housed in a disinfected cage with clean straw bedding (see 603 for a plan for farrowing pens).

Behavioral Restrictions -

Sexes should be separated and sows with a new litter should be handled with care because they can be quite dangerous (603).

Ecological Role -

In the wild, pigs are omnivorous, feeding on nuts, fruits, leaves, cacti, grubs, etc. (412). Javelina inhabit areas of rather dense vegetation (chaparal, mesquite, brushlands) and are usually localized around a waterhole (412).

Longevity -

Miniature swine are known to live for more than six years (600).

Problems with Mass Culture -

Although miniature, these swine are still large as lab animals (600, 602). Maintenance is expensive and the food costs may be limiting (606). Swine are subject to a host of diseases, some of which are curable (603).

The javelina is rather new to the laboratory world and little is known about its diseases and parasites (601).

Toxicity Testing -

Miniature swine are used in biomedical research (607, 608, 609, 610, 611, 612)and other fields as well. The Sinclair Farms, University of Missouri, uses them extensively as test animals (613).

Toxicity testing has been done for a variety of substances, including manganese chloride (614) and selenium (615). Pesticide effects have been investigated by scores of workers (152, 590, 591, 592, 616, 617, 618, 619, 620, 621).

Javelinas have been used in cardiovascular research (601).

General Suitability -

Swine are domestic animals. Their availability and the vast amount of knowledge we possess about their breeding and maintenance are their greatest advantages as lab animals. As test animals they have been used successfully in research in immunology, nutrition, dental, radiocardiology, cardiovascular physiology, and pathology (600, 606, 622). Their suitability as representatives of a complex, natural ecosystem is unknown. The javelina, however, is a wild form and would be quite suitable for toxicity testing, if available in the future in large numbers.

Ecological Alternatives -

Sheep, cattle.

Ungulates: Bovidae

Introduction -

Sheep and goats are grazing and browsing herbivores. There are both domestic and wild species but the native members of this group - Dall's sheep (Ovis dalli) the bighorn sheep (Ovis canadensis), the mountain goat (Oreamnos americanus), the Bison (Bison bison) and the musk ox (Ovibos moschatus) are rare species and not suitable for use as test animals.

Caging/Lab Conditions -

Cages should be dry and well ventilated and of sufficient size to allow free movement, with access to sizable runs. Both sheep and goats can be communally housed, though the uncastrated males should be limited in their access to other animals during the rutting season, which occurs in the fall, because they become aggressive and produce a great odor (623).

The layout of an operating suite for sheep (at the Agricultural Research Council Institute of Animal Physiology) is detailed in reference 624.

Nutrition -

Mature goats do well on sun cured 15 percent protein alfalfa pellets and block salt with the addition of one pound of an inexpensive roughage such as hay or straw. Does used for reproductive purposes should receive from one to 1.5 pounds per day of a mixture of choice legume hay and 14 to 16 percent quality grain. Clean water should be available at all times (623)..

Breeding/Rearing -

Goats ordinarily breed in the fall of the year and give birth from February to June. The kids need milk for six weeks and then should be weaned on a high protein dry food diet. They reach maturity at three years and weigh 120 to 150 pounds as adults (623).

Ecological Role -

Goats and sheep are herbivores and feed on a great variety of plant materials. The wild forms are high mountain animals.

Problems with Mass Culture -

The long generation time and large size of these animals makes mass culture expensive.

Toxicity Testing -

Pesticide residues in free-roving mountain goats have been determined (625) by standard tissue analysis. Domestic stock have been utilized for many types of research including toxicity studies of various substances (590, 592, 616, 617, 618, 619, 620, 626, 627). Noteworthy is the Veterinary Diagnostic Laboratory at the Iowa State University which have been working with long-term effects of pesticides on domestic sheep (628, 629).

General Suitability -

The suitability of all domestic animals as subjects in toxicity testing is suspect because of their close association with man's world, and because they have been so long removed from the selection pressures of their natural environment. However, the sheep is probably more suitable in this respect than other domestic species (613, 630).

Handling of these domestic creatures is not difficult. Their suitability for surgical methods, etc., is discussed at length by Fletcher, et al., 1964 (623).

Ecological Alternatives -

Other ruminants.

Ungulates: Cervidae, Antilocapridae

Introduction -

The deer and allies are grazing and browsing animals from two families. The Cervidae are the deer-like ungulates: elk, moose, mule deer, white-tailed deer. The Antilocapridae have a single representative in the U.S., the pronghorn, which inhabits open prairies and sagebrush plains of the west central U.S. Unlike Cervids, in which only males have deciduous antlers, both sexes of pronghorn possess true horns that are never shed.

The weight of the adult male elk (Cervus canadensis) and the moose (Alces alces) is from 800 to 900 pounds; female weights are about 200 pounds less. They stand five to six feet high and the males of both species have magnificent racks. The moose antlers are broadly palmate and elk, antlers are morphologically similar to those of native deer, but considerably larger.

The mule deer (Odocoileus hemionus) and the white-tailed deer (O. virginianus) are smaller than elk, standing between three and four feet high and weighing between 150 and 400 pounds. The mule deer ranges throughout western North America in many habitat types. The white-tail is an eastern species inhabiting all types of forest and brushland (412).

Pronghorn (Antilocapra americana) stand about three feet high and weigh between 75 and 130 pounds (412).

Caging/Lab Comditions -

Any large outdoor enclosure of adequate areal extent and height is suitable, but cover is essential both from the sun and the cold in northern climates. An outdoor holding pen used at the Denver Wildlife Research Center is shown in Figure 18.

In the wild, deer eat twigs, shrubs, acorns, grass farm crops, and certain fungi (412, 443). Elk and pronghorn have similar diets. The moose inhabits swampy, marshy areas and feeds particularly on aquatic vegetation, but also on shrubs and saplings. White-tailed deer were maintained on Ralston Purina Dairy chow for three years during a pesticide study (631).

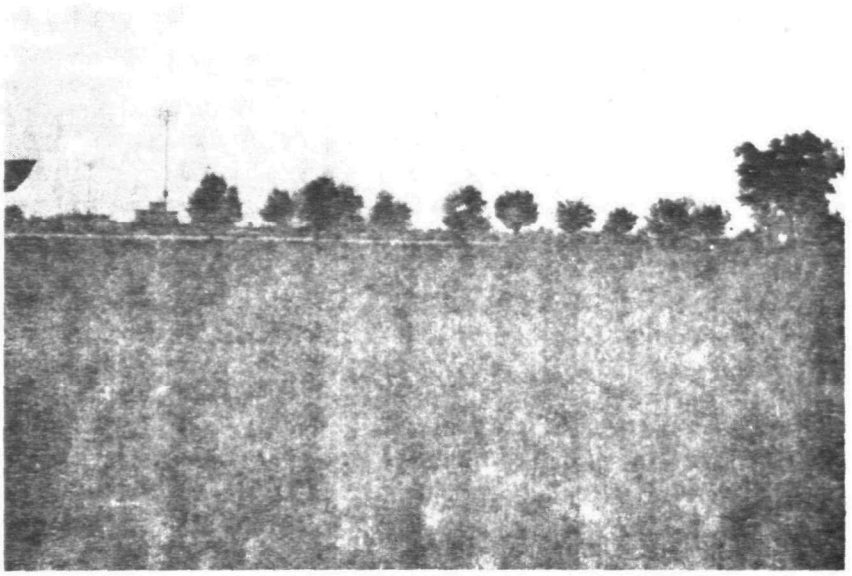


Figure 18. Deer holding pen. Denver Wildlife Research Center, Federal Center, Denver, Colorado

Water in large quantities is essential to all of these animals (412, 443, 631).

Breeding/Rearing -

White-tail deer have been bred and reared in captivity, producing one or two fawns each year. Females mature in their second year (631). In nature, all the ungulates breed once a year and produce one or two fawns (412). Gestation for the pronghorn, moose, and elk is about eight months and for the deer species from six to seven months (412).

Special Needs -

Moose prefer aquatic habitats for feeding and wading during the summer months and may require water facilities for such activity in captivity. All ungulates require mineral (salt) licks (443).

Behavioral Restrictions -

Many species have a migratory tendency and may become quite restless at certain times during the year (412, 443).

Ecological Role -

The ungulates are rather strict herbivores and graze on many plant foods (412). They are prey for large predators, particularly wolves. They also constitute the major big-game animal group in North America. In some agricultural localities, they can be serious pests of crops (412).

Longevity -

Elk have lived to 25 years in captivity. In the wild their life span is probably less. Deer live to 16 years in the wild, moose to 20 years, and pronghorn have been known to reach 14 years (412).

Problems with Mass Culture -

The cost of maintaining a heard of large animals is high and the reproductive rates are rather slow. A deer, for instance, gives birth to one or two fawns each year.

In enclosed situations, deer chew each others tails and ears. Mortality due to the "white muscle disease" can be significant (631).

Toxicity Testing -

DDT residues have been assayed from samples of free-running pronghorn, deer, elk, and moose (625). Radioiodine has been sampled from thyroid tissues of free running deer and in Colorado following underground explosions in Nevada (632). Strontium-90 has been assayed from the mandibles of free running mule deer (633).

One study of the effects of dieldrin on reproduction, mortality, etc., in white-tail deer in Missouri was conducted under controlled conditions on captive subjects (631).

General Suitability -

Limited reproduction and large size problems probably exclude the large ungulates from mass culture and extensive use in toxicity testing. The white-tail deer or mule deer would be the most likely candidates from this group for use in toxicity testing.

Ecological Alternatives -

Sheep.

Primata

Introduction -

Because of their close relationship to man, non-human primates have been used in many fields of research. Primates are omnivorous animals that have acquired the use of their hands and often their feet as effective grasping organs through the development of opposable digits. This makes them particularly adapted for climbing, and most primate species are arboreal.

They are found in the Old and New World tropical and subtropical latitudes. The species used in research in North America come from South America, (Platyrrhines), Africa (Papio, Cercopithecus, Erythrocebus, Theropithecus) and Asia (Macaca), (634). Recent restrictions on the capture and shipment of primates may affect availability in the future.

The most studied primate species other than man is the Rhesus monkey (Macaca mulatta). Adults weigh 20 to 25 pounds and their life spans are much longer than those of the other mammalian groups. The comments below refer to this species, but many are applicable to other species as well (i.e., squirrel monkeys, Saimiri).

Caging/Lab Conditions -

Cages for adult Rhesus monkeys should be at least 24 x 24 x 28 inches and house no more than a pair while breeding or a mother and her infant. Smaller juveniles can be kept in 14 x 12 x 18 inch cages (289). Cages of various dimensions can be used to house large numbers of primates. They are social animals and intra-specific contact is desirable. Enclosures should be provided with suitable props for use in climbing and exercise. Cages used at Iowa State University are shown in Figures 19, 20 and 21.

Nutrition -

Primates cannot metabolize ascorbic acid, so a daily ration must be provided. After weaning, all ages of monkeys can be sustained on commercially prepared monkey chow (289). In nature they are omnivorous, feeding primarily on fruits. Supplements to the ration of chow can include all types of fruit and vegetable foods. Daily rations should be four percent of body weight, and in communal cages a number of

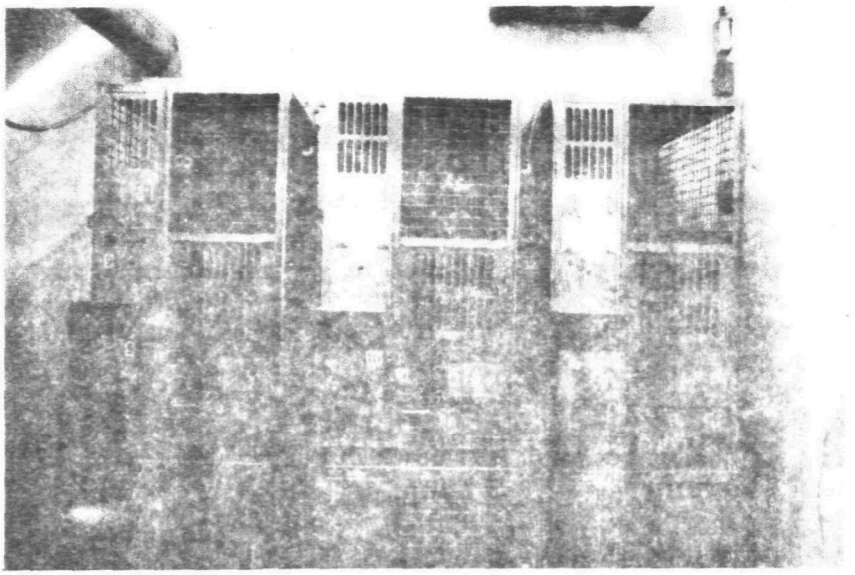


Figure 19. Primate cages - squirrel monkeys - metal - commercially available. Iowa State

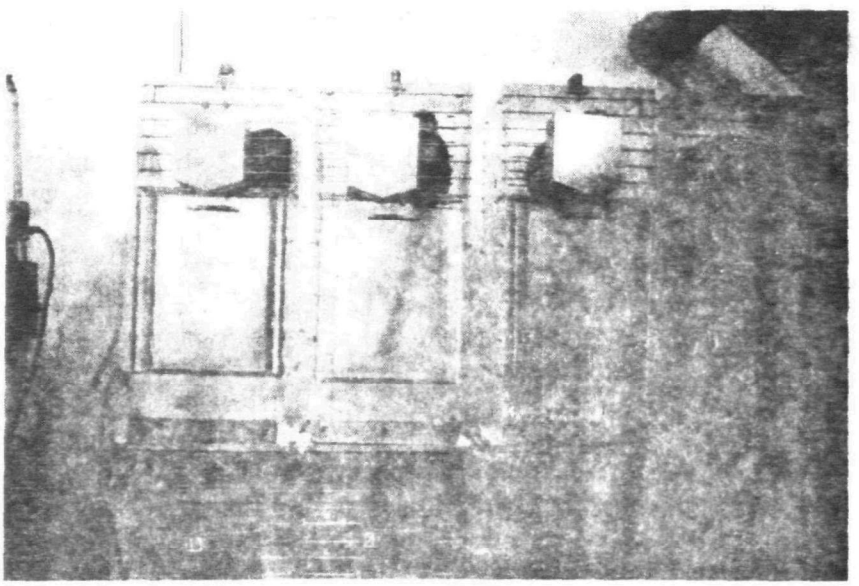


Figure 20. Plexiglas and metal - fabricated at Iowa State. (For dimensions see Figure 21.)

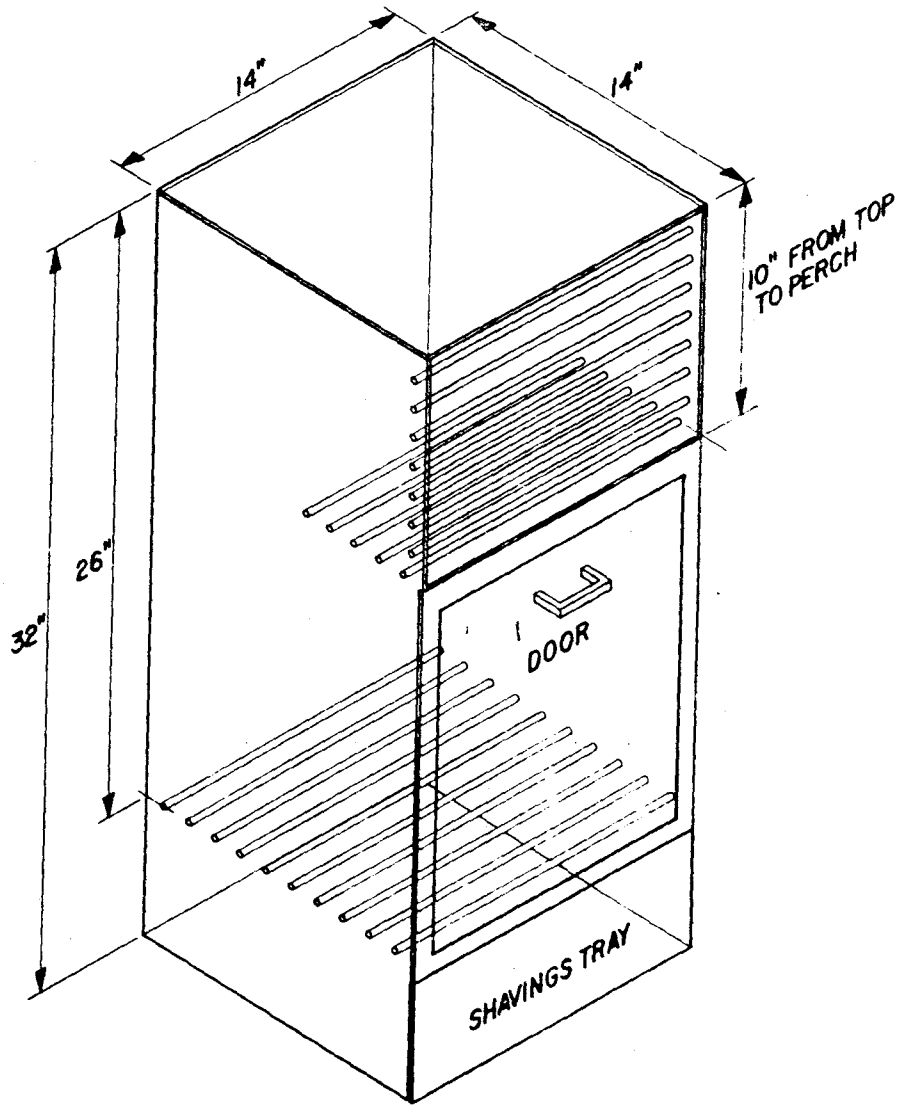


Figure 21. Plexiglas cage, fabricated at Iowa State University.

feeding areas should be established because dominant individuals will monopolize food sources. Water should be supplied ad libitum (289).

Breeding/Rearing -

Gestation for Rhesus monkeys averages 165 days, and there is usually only one young born at a time. They are frequently removed from the mother soon after birth and reared in a separate nursery. This procedure decreases infant mortality. After three to six months, the young will eat solid foods. Sexual maturity is achieved at five years for females and six years for males. The breeding life of Rhesus monkeys is 12 to 15 years and females are receptive periodically throughout the year. The breeding colony sex ratio can be one male for up to ten females.

Special Needs -

Vitamin C is a diet requirement for primates (289).

Behavioral Restrictions -

Monkeys are often difficult to handle and unpredictable. Special training of prospective handlers should be considered for their safety (289). A review of physical and chemical restraints is presented in reference 635.

Ecological Role -

Non-human primates are omnivorous animals of tropical and sub-tropical regions of the world. Their staple foods are fruits, leaves, roots, insects; they frequently consume larger animals. They are prey for large predators.

Longevity -

Life spans of 15-20 years are not uncommon for Rhesus monkeys (289).

Problems with Mass Culture -

The generation time for primates is probably too long to allow extensive use of this order of mammals when large numbers are required.

Toxicity Testing -

Because of the long life spans of primates, they are invaluable in long-term research. The close relationship of

monkeys to man is also an inducement to their use in research. Experimental work in which primates have been subjects include pesticide toxicity work (272, 620, 636, 637), inhalation of various gases (638), and other toxicity studies (104, 639, 640, 641).

General Suitability -

Non-human primates are desirable as experimental subjects because of their close relationship to man. Their long life spans and specialized behavior also make them very suitable for certain types of studies (630). However, extensive use of primates in research is impossible because of their slow rate of reproduction. Recent export restrictions may reduce their availability in the future and increase cost.

Ecological Alternatives -

Fruit-eating bats, man.

Marsupiala

Introduction -

The only marsupial native to North America is the opossum (Didelphis marsupialis) which exists throughout North America except for the higher Rockies and the plains of the Dakotas. It is probably limited in the north by the low winter temperatures.

The opossum is an unspecialized mammal with a prehensible tail. Its pelt is of poor quality. It is primarily nocturnal and omnivorous. Farming areas and disturbed woodlands are preferred habitats (412).

Adult opossums are about 18 inches in length with a tail of equal length and weigh between nine and 13 lbs. (412). The pouchless opossum (Marmosa mitis) has only recently been used in the laboratory.

Caging/Lab Conditions -

Cages for breeding pairs are 20 x 22 x 15 inches of galvanized metal and 32 x 26 x 24 inches of galvanized metal for individuals (642).

Large outdoor cages seem to better the reproductive performance in opossums. Large wooded areas (150 x 100 feet) were closed off by chain link fence eight feet high with an eighteen inch wide metal strip around the top. Nest boxes 5 x 5 x 3 feet were provided (642). A 16 x 16 feet enclosure of poultry netting surmounded by tin strips with walls extending two feet in the ground has been used (643). Breeding pens 20 x 40 feet are large enough to accommodate ten adults (644). Other suitable nest boxes include doghouse type structures with removable roofs (645) and barrels with straw bedding (644).

In one lab, relative humidity under 50 percent resulted in swollen and cracked tails (646).

Marmosa have been kept in 8 x 8 x 11 inch cages with a 4 inch metal can secured to a cage top for a nest facility. Colonial metal enclosures of 2 x 2 x 3 feet have also been used (647).

Nutrition

Opossums do well on simple diets such as dog pellets supplemented twice a week with canned dog food and some fresh fruit or vegetables (645).

Other supplements include raw meat scraps, bone meal (644), cat chow, and mealworms (642).

Water is supplied ad libitum in most labs.

Breeding/Rearing -

Marsupial reproduction differs from that of other mammals in one unique way: the young are born after a very short gestation period (12-13 days for the opossum [643]) and crawl to a pouch where further development takes place. There are usually 13 teats in the mothers pouch. This can be a limiting factor in litter size. The litter average for 17 females in one colony was 6.7 (644). There are usually two litters each year (648).

Breeding in captivity is apparently best achieved in large outdoor pens (642, 645). In some cases, in smaller confines the nest boxes were removed during the mating period.

One lab team removed the young from the mothers pouch and, in one case, the female produced a second litter of 12 only 16 days later (644).

Special Needs -

Nest boxes are considered necessary (647) as is suitable bedding changed weekly--straw, shredded paper (642, 643, 644, 645, 649). Litter pans should be changed daily (642).

Behavioral Restrictions -

Some reports of pair incompatibility (647, 649) suggest social problems in keeping opossums.

Another lab reported a very high mortality rate (to 75 percent) in colonial housing, although a great decrease in this rate was shown when individuals were caged separately and sanitary practices strictly enforced. (The high mortality was due mainly to infectious diseases) (642). The nocturnal habits of opossums may pose problems in their handling and use.

Ecological Role

The opossum will eat any food, plant or animal. Insects make up a large portion of their diet during certain seasons of the year. Other food items include bird eggs, birds, snails, earthworms and carrion (413). The opossum is a food source for large raptors and carnivorous mammals (412).

Longevity -

The opossum has been known to live up to four and one-half years in captivity (646). Mortality rates in captured individuals are very high in some cases (642).

Problems with Mass Culture -

Parasites are probably the greatest deterrent to the maintenance of large colonies (647, 649, 650, 651, 652, 653, 654). The parasite load is very great in captive individuals, and the colony must be cleaned up every six months (645).

There is evidence that litter size decreases in captivity and that breeding takes place more frequently (644).

The opossum does best in outdoor enclosures because it need large spaces. This kind of caging also eliminates the need to change litter pans daily (642, 643, 644, 655).

Toxicity Testing -

Though little toxicological testing has been done with opossums, the ready accessibility of relatively undeveloped young offers a unique possibility for work with developing organisms. (See reference 656 for a successful technique for the removal of "embryos" from the teat and subsequent handling procedures.)

General Suitability -

Because of the accessibility of its young and its ability to breed in captivity, the opossum seems well suited for toxicological testing.

Ecological Alternatives -

Other omnivores.

Edentata

Introduction -

The armadillo is a member of a tropical family (Dasypodidae) that have degenerate teeth and a horny protective body covering resembling a plated coat of armor. One species (Dasypus novemcinctus) has established itself in the central southern U.S. and is commonly seen at night within its range. Armadillos feed on the plant and animal material which they unearth with their specially adapted, well-clawed forefeet. The average adult is about 16 inches in length and has a 15-inch tail. They weigh from eight to 17 pounds (412).

Caging/Lab Conditions -

A single L-shaped unite consisting of two pens separated by a removable plywood divider placed diagonally through the center of a wading pool is described as suitable for armadillos. The first pen in the L-shaped structure is 9 x 4 x 4 feet. The second is 18 x 4 x 4 feet. Fir boards and plywood were used for the walls and floor and lined with two or three layers of 44 inch wide fiberglass cloth and treated with a resin compound and surface curing agent. (Enclosures of metal or wood proved inadequate because of injury to the animals or maintenance costs). In the pool, the bottom was covered with gravel. Cedar shavings were used as floor bedding (657). Smooth two-foot metal plates covered with wire netting (658) and tile-covered brick walls four feet high in an enclosure 26 x 15 x 6 feet (659) are other cages that have been used to house armadillos.

Nutrition -

Armadillo diets vary, but a combination of animal and vegetable material is the rule. Canned dog food, raw beef, milk, eggs, liver and fruits are all desirable components. One diet consisted of 1 1/2 cups water, 1/2 cup milk, three hard-boiled eggs, two to three chopped bananas, one chopped apple, one cup cat chow, one pound ground meat, and three T multivitamins (657). Other possible additions to the general diet are tomatoes, cod-liver oil, mink Developer Chow, molasses, honey and potatoes (660).

Water should always be available.

Care should be taken to provide sufficient vitamin K. K-Sol powder from Vet-a-mix, Inc., Shenandoah, Indiana is one possible source (660).

Breeding/Rearing -

The gestation period of armadillos is difficult to determine because the implantation of the blastocyst is delayed for a variable length of time. Polyembryony has also been reported in armadillos. One to twelve young are born, usually in the spring (658, 661).

Only a few armadillos have been reared under zoological garden conditions, but the Apelt Armadillo Farm at Comfort, Texas has bred the nine-handed armadillo in some numbers (658).

Special Needs -

Armadillos need an abundant floor covering of materials like wood shavings, straw and sand, or an earth floor (657, 658, 659). Logs and large rocks allow for scratching and diggin which alleviates localized pyogenic infection of claws and footpads (657). Concrete floors are not desirable (658).

Vitamin K is necessary to prevent major blood loss from minor, superficial lacerations and can be supplied by the inclusion of K-Sol powder in the diet (see Nutrition).

Armadillos are tropical animals and seem to be adversely affected by ambient temperatures of less than 50°F (659).

A large source of water is recommended (657, 658, 659, 660).

Behavioral Restrictions -

Small wooden cages cause undue stress, and metal enclosures are inadequate because of injury caused when the armadillo digs at the sharp corners (657).

Armadillos feed at night in nature and remain nocturnal in captivity (659). Red lights may be needed in the lab.

Ecological Role -

Armadillos are secondary consumers and scavengers. They consume many insects and other terrestrial invertebrates (412). Their meat is edible, but predators other than men have a difficult time overcoming their armor.

Longevity -

Nine-banded armadillos have lived over six years in captivity. Other species have life spans of 10 to 15 years (658).

Problems with Mass Culture -

Armadillos require special caging and a lot of space (657). They often injure themselves during shipping and are very susceptible to pyogenic infection of claws and footpads. It is difficult to cleanse them thoroughly because of their dermal plates and they are therefore more susceptible to parasites.

Toxicity Testing -

Although toxicity studies with armadillos as subjects are unknown, physiological research has been conducted which may indicate the suitability of armadillos for such testing. This research includes general hematological studies (662, 663, 664, 665, 666).

General Suitability -

Depending on the caging facilities available, the armadillo is more or less suitable for toxicological research. Surgical techniques are seriously hindered by the dermal skeleton (659).

Ecological Alternatives -

Moles
Six-banded armadillo: Euphractus sexcinctus
La Plata three-banded armadillo: Tolypeutes matacus
Bolivian hairy armadillo: Chaetophractus nationi
Small hairy armadillo: Chaetophractus vellerosus
Pichiy: Zaedyus pichiy

Exotic Species

Introduction -

There are a number of exotic species used in research in the U.S. Some are recent introductions and some have been used for years. The guinea pig (Cavia) has been used in medical research because of its immune responses to foreign proteins and other somewhat unique metabolic characteristics. Adults weigh between 850 to 1200 gms and are gentle animals that are very easy to handle. Golden hamsters (Mesocricetus) and gerbils (Meriones) are closely related to rats and mice (family Cricetidae) and somewhat similar in size and appearance to smaller rodents, with a body length of about five inches. The lab rats and mice are of European origin and are treated elsewhere. Hamsters are natives of temperate Europe and Asia. There are three varieties used in research, the Golden, European and Chinese hamsters, but the Golden is the most prominent in research. Gerbils are of the same size as hamsters and of two varities, African and Mongolian. The latter is more prominent in research.

Other exotic species that have been utilized as test animals are the hedgehog, the chinchilla, and the sand rat (Psammomys obesus). Because of the more extensive use of hamsters, gerbils and guinea pigs, the following text will concern itself primarily with these three animals.

Caging/Lab Conditions -

A cage 30 x 24 x 12 inches is ample space for a breeding pair of guinea pigs and their unweaned offspring. Smaller enclosures (18 x 12 x 12 inches) are suitable for two cagemates of any age. Suggested bedding for guinea pigs is coarse wood shavings or shredded beet pulp. Recommended temperatures and relative humidity for the animal enclosures are 68° to 72°F and 45 to 55 percent (289).

Hamster and gerbil breeding pairs and young do well in 20 x 10 x 6 inch cages made of almost any material. A 24 x 24 x 8 inch cage is adequate space for eight to ten adults. Bedding of white pine shavings or some commercially available substance is suggested and should be changed frequently. Other possible bedding material includes corn cobs, beet pulp, and peat moss about 65° to 75°F and 55 to 65 percent humidity are very suitable conditions for the animal enclosure (289, 667). A good rule of thumb for housing these animals is that 200 square inches of floor space

should be provided for a breeding pair and their litter. In a communal situation, 15 square inches per individual is adequate (668).

Nutrition -

A daily source of ascorbic acid (Vitamin C) is required by guinea pigs, and it is reported that this species has a high requirement for folic acid, thiamine, arginine and potassium (669). Commercial chow (35 gms/day) is an adequate diet for guinea pigs if the above additions are made (289).

Adult hamsters need a daily ration of eight to ten gms of food (which is commercially available), but females should receive an extra two to four gms during pregnancy and lactation (289). Gerbil requirements are similar. They have been maintained on a diet of rolled oats, sunflower seeds, and guinea pig chow supplemented with fresh vegetables (667). Both hamsters and guinea pigs need a constant supply of water, but gerbils use very little (668).

Breeding/Rearing -

The gestation period of guinea pigs is 59 to 67 days. Three to four litters per year, averaging three offspring per litter, are possible (289). The young can be weaned at 10 days. Sexual maturity occurs at about five months; the breeding life of guinea pigs lasts for up to five years.

The hamster's gestation period lasts 15 to 18 days, and the average litter size is about seven young. Females are breedable immediately following parturition (289). In eight to ten weeks they are sexually mature and can produce offspring for about one year.

For gerbils, the gestation period is 24 to 26 days, and the mean litter size is 4.5. Sexual maturity is reached at about 85 days (667). Best reproductive results occur when pairs of adults are kept separately (668).

Special Needs -

Guinea pigs require Vitamin C daily, but this is included in special guinea pig chow available commercially (669).

Ecological Role -

Guinea pigs, hamsters and gerbils are all herbivores. Guinea pigs are basically grass eaters; hamsters and gerbils are seed eaters. All are possible prey species for predators of many kinds.

Longevity -

Guinea pigs live to about five years. Gerbils and hamsters live from two to three years in captivity.

Toxicity Testing -

Substances have been tested on all of these species through food additives, vapor inhalation and topical application (449, 621, 670, 671, 672, 673, 674, 675, 676). Substances tested include pesticides (670, 672, 677, 678, 679, 680, 681), heavy metals (673, 674), carcinogens (682, 683) and gases of various types (626, 684, 685).

General Suitability -

Guinea pigs, hamsters and gerbils are routinely maintained in laboratories and are very suitable as experimental animals. A number of qualities make some of them suitable for toxicity testing. For instance, inbred guinea pigs exhibit very homogeneous sexual behavior, while heterogeneous animals show great individual variations (686) making possible quantitative studies of behavioral responses to toxic substances. It should be noted that the homogeneity of the genetic background of these individuals could bias such testing. Gerbils are 10 times more sensitive, in terms of avoidance responses, than lab rats (668) and behavioral changes may be more discernible in gerbils than in other lab animals. None of these species, however, is native to North America; they have been selectively bred to various extents for use as lab animals. Therefore, they are probably less suitable than native species in studies which hope to extrapolate results to wild populations.

Ecological Alternatives -

Voles, mice.

SUGGESTED SPECIES

Synopsis sheets of the suggested species from each animal subgroup have been prepared for use as quick reference sources. These sheets can be found in Appendix D. The information was largely taken from references used in the text or from the references enumerated in the synopses themselves. Much general breeding and life history data was acquired from texts concerned with specific animal groups in the wild when there was no information available from laboratory colonies (57, 133, 144, 196, 221, 310, 321, 330, 687, 688, 689, 690). A summary list of suggested species from terrestrial animal groups is found on Table 23.

Final List

A further distillation has been made from the suggested species for each group in order to identify terrestrial animals that are best suited for use in laboratory experiments. These are recommended for use in cases where ecological role, phylogenetic relationships and habitat preferences are not of significant importance for certain types of testing. They represent good, all-around test animals and are the most likely selections for preliminary studies. Most are frequently used species in many labs at the present time. The list of suggested species is shown below in Table 22.

Table 22. FINAL LIST OF SUGGESTED SPECIES

Gryllus sp.	field cricket
Drosophila sp.	fruit fly
Musca domestica	house fly
Tenebrio sp.	mealworm
Rana pipiens	leopard frog
Anas platyrhynchos	mallard duck
Columba livia	pigeon
Phasianus colchicus	ringnecked pheasant
Colinus virginianus	bobwhite quail
Coturnix coturnix	Japanese quail
Gallus gallus	domestic chicken
Mus Musculus	house mouse - lab variety
Rattus norvegicus	norway rat - lab variety
Canis familiaris	domestic dog
Microtus pennsylvanicus	meadow vole
Peromyscus maniculatus	deer mouse

TABLE 23. LIST OF SUGGESTED SPECIES FROM SELECTED TERRESTRIAL ANIMAL GROUPS

INVERTEBRATES

Lumbricus terrestris
Helix aspersa
Milax sp.
Araneus diadematus
Gryllus pennsylvanicus
Praying mantids
Blissus leucopterus
Leafhoppers
Tribolium sp.
Tenebrio sp.
Carpocapsa pomonella
Agrotis ypsilon
Musca domestica
Drosophila melanogaster
Apis melifera
Isopods
Centipedes

HERPETOFAUNA

Rana pipiens
Ambystoma mexicanum
Terrapene sp.
Chelydra serpentina
Anolis carolinensis
Phrynosoma cornutum
Thamnophis sirtalis
Elaphe sp.

AVES

Phalacrocorax auritus
Podilymbus podiceps
Anas platyrhynchos
Branta canadensis
Puffinus griseus
Casmerodius albus
Fulica americana
Philohela minor
Larus argentatus
Melanerpes erythrocephalus
Falco sparverius
Tyto alba
Columba livia
Colinus virginianus
Phasianus colchicus
Dendragupus obscurus
Melospiza melodia
Richmondena cardinalis
Turdus migratorius
Sturnus vulgaris
Passer domesticus
Gallus gallus
Coturnix coturnix
Melopsittacus undulatus

MAMMALS

Blarina brevicauda
Eptesicus fuscus
Sylvilagus floridanus
Dipodomys ordi
Neotoma lepida
Oryzomys palustris
Spermophilus beecheyi
Eutamias minimus
Tamias striatus
Microtus pennsylvanicus
Peromyscus maniculatus
Mus musculus
Rattus norvegicus
Myocaster coypus
Vulpes vulpes
Mustela vison
Mustela putorius
Canis familiaris
Miniature swine*(Sus)*
Ovis sp.
Odocoileus virginianus
Odocoileus hemionus
Saimiri sciureus
Didelphis marsupialis
Dasypus novemcinctus

Selection of Animal Species for Testing

In toxicity testing, habitat and ecological role are often considered important in the selection of species. The Trophic Triangle shown in Figure 22 represents the basic food chain, an important factor in determining the ecological role of a potential subject species. Decomposers are associated with the substrate, which is an accumulation of organic detritus. At this level, waste products, and dead plant and animal material are decomposed and their nutrients made available to the primary producers, the vegetation. From the plant level, herbivores, omnivores, secondary and top carnivores, in turn, utilize foods from their own level or the ones below. This triangle shows the trophic level and the general habitats of the suggested species on that level.

Suggested species, their place in the Trophic Triangle, and preferred habitat are shown in Table 24. This table can be of particular importance in the selection of species for use in multispecies test systems. For instance, if it is desired to assess the impact of a chemical which has contaminated a forest, forest species can be selected from each food chain and animal group (if suitable species from each are identified).

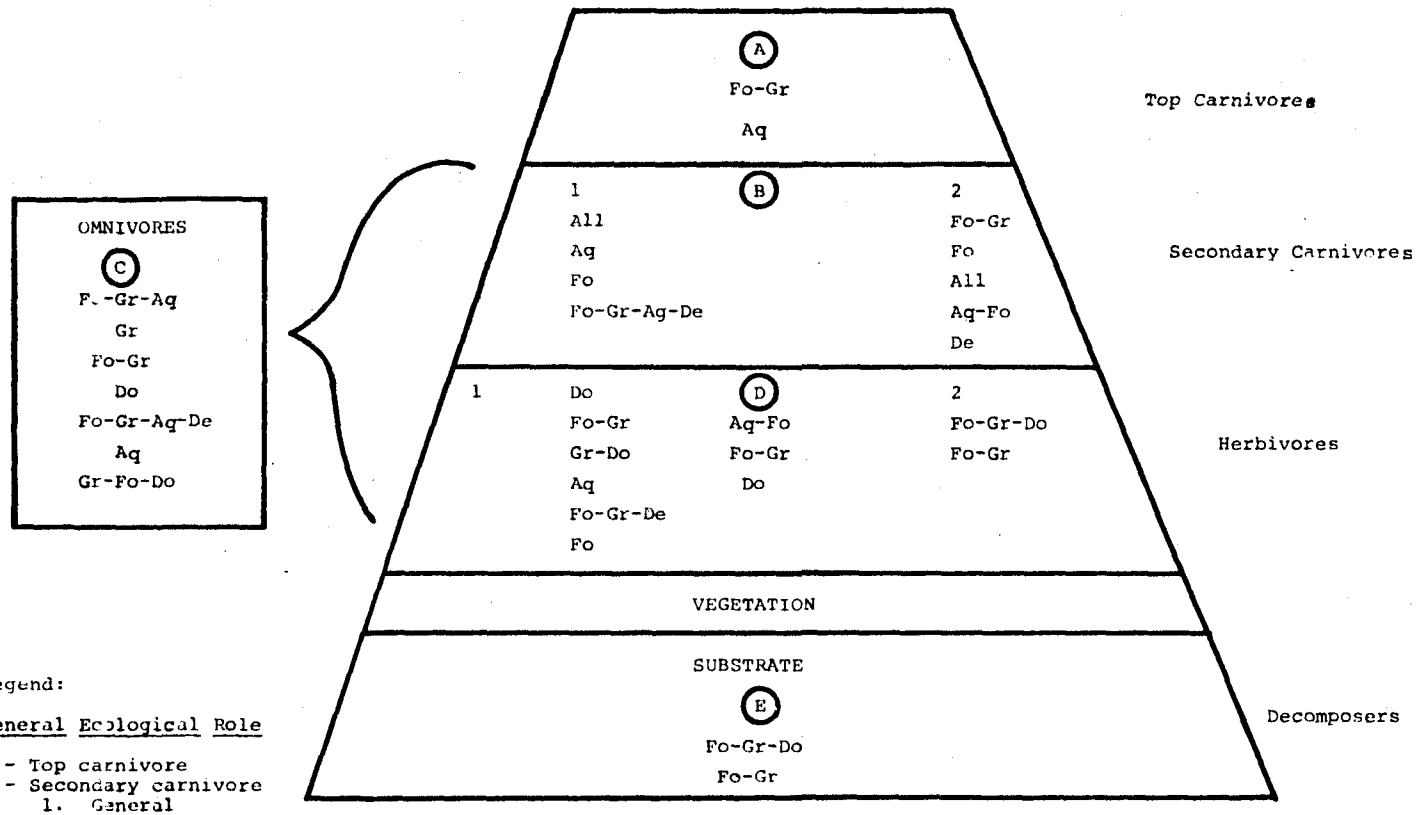
The following is a hypothetical system constructed in this fashion.

A Possible Multispecies System

Top Carnivore	Vulpes vulpes Falco sparverius
Secondary Carnivore	Mustela vison Melanerpes erythrocephalus praying mantids Rana pipiens
Omnivore	Didelphis virgenianus Sturnus vulgaris
Herbivore	Oryzomys palustris Colinus virginianus Terrapene Helix aspersa
Decomposer	Lumbricus terrestris Isopods

Of course, in order to achieve this level of testing much

FIGURE 22. TROPHIC TRIANGLE



Legend:

General Ecological Role

A - Top carnivore
B - Secondary carnivore
    1. General
    2. Insectivore
C - Omnivore
D - Herbivore - General
    1. Seed, fruit eater
    2. Grass foliage eater
E - Decomposers

Habitat Preference

Fo Forest
Gr Grassland
Do Desert
Aq Aquatic

TABLE 24. ECOLOGICAL ROLES OF SUGGESTED SPECIES

SECONDARY CARNIVORES

TOP CARNIVORES

Aq

Phalacrocorax auritus (Double-crested cormorant)
Podilymbus podiceps (Pied-billed Grebe)
Puffinus griseus (sooty shearwater)

Fo-Gr

Vulpes vulpes (red fox)
Falco sparverius (sparrow hawk)
Tyto alba (barn owl)

GENERAL

Aq

Fulica americana (coot)

All

Thamnophis sirtalis (garter snakes)
Elaphe sp. (rat snakes)

FO

Philohela minor (woodcock)

B: Fo-Gr-Aq-De

Mustela vison (mink)

INSECTIVOROUS

Fo-Gr

Blarina brevicauda (shrew)
Araneus diadematus (orb-weaving spider)
Turdus migratorius (robin)
praying Mantids
Anolis carolinensis (green anole)

De

Phrynosoma cornutum (horned lizards)

Aq-Fo

Chelydra serpentina (snapping turtle)

All

Rana pipiens (leopard frog)

Aq

Ambystoma mexicanum (axolotl)

Fo

Melanerpes erythrocephalus (red-headed wookpecker)
Eptesicus fuscus (big brown bat)

Centipedes

OMNIVORES

Fo-Gr

Eutamias minimus (least chipmunk)
Didelphis marsupialis (opossum)
Dasypus novemcinctus (armadillo)

Gr

Spermophilus beecheyi (California ground squirrel)

Do

Mus musculus (house mouse)
Rattus norvegicus (Norway rat)
Canis familiaris (dog)

Fo-Gr-Aq-De

Mustela putorius (European ferviet)

Aq

Anas platyrhynchos (mallard)
Larus argentalus (herring gull)

Gr-Fo-Do

Sturnus vulgaris (starlings)
Passer domesticus (house sparrow)

HERBIVORES

SEED, FRUIT EATERS

Fo

Melanerpes erythrocephalus (red-headed woodpecker)
Dendragapus obscurus (blue grouse)

Ag

Branta canadensis (Canada goose)

Gr-Do

Columba livia (pigeon)

Fo-Gr

Colinus virginianus (Bobwhite)
Phasianus colchicus (ring-neck pheasant)
Melospiza melodia (song sparrow)

Do

Gallus gallus (domestic chicken)

Coturnix coturnix (Japanese quail)

Fo-Gr-De

Peromyscus maniculatus (deer mouse)
P. luecopus (white footed mouse)

GENERAL

Aq-Fo

Terrapene sp. (Box turtle)

Go-Gr

Richmondena cardinalis (cardinal)

Do

Melopsittacus undulatus (Australian parakeet)
Ovis (Domestic sheep)
Saimiri sciureus (squirrel monkey)

FOLIAGE EATERS

Fo-Gr

Sylvilagus floridanus (Cottontail)
Microtus pennsulvanicus (Meadow vale)
Odocoileus virginianus (whitetail deer)
O. hemionus (mule deer)
Helix aspersa (land snail)
Milax sp. (slug)
Blissus leucopterus (chinch beetle)
Leafhoppers
Carpocapsa pomonella (codling moth)
Agrotis ypsilon (noctuid moth)
Gryllus pennsylvanicus (cricket)
Tribolium sp. (flour beetle)
Tenebrio sp. (darkling beetle or mealworm)

Fo-Gr-Do

Musca domestica (house fly)
Drosophila melanogaster (fruit fly)
Apis mellifera (honey bee)

Fo-Gr-Aq

Sigmodon hispidus (Hispid cotton rat)
Oryzomys palustris (rice rat)

DECOMPOSERS

E: Fo-Gr

Lumbricus terrestris (earthworm)

E: Fo-Gr-Do

Isopods

Legend

Prey Preference

preliminary work on lab maintenance must be done on many species. For example, in Table 24, few carnivores are listed. They are generally large animals and difficult to feed in captivity, and very few attempts have been made to use them in any type of testing. Traditionally, omnivores and herbivores are most often used as test subjects, and their numbers are greater both in the laboratory and in nature. See Appendix C for suppliers of some suggested species. (Considerable changes may have occured in this list as it dates to 1971.)

It is suggested that testing in the near future utilize the List of Species, shown on Table 24, but that serious research be initiated to develop other potentially valuable species, in order that an efficient, ecologically meaningful, multispecies test program can be initiated. The state of toxicity testing cannot develop as it should without research of this kind.

SECTION V

METHODOLOGIES

INTRODUCTION

Webster defines scientific method as "a systematic procedure, technique, or mode of inquiry" employed by the scientific community. It goes on to add that it is a "way, technique, or process of or for doing something". A standard procedure and methodology are tools which help the experimenter achieve reproducible results. In this section, the methodologies available for use in toxicological testing will be reviewed and an assessment will be made of the suitability of each for use in toxicology studies. Previous use and results obtained, applicability to wild North American animal species, experts opinions, and expense are the fundamental criteria used for determining suitability. Suggestions for further use are often included in the text.

General Scientific Procedure

Toxicological experimental procedures consist of two basic steps: exposure of the test animal to the test substance and analysis of the results. Exposure techniques, though they may seem straightforward to the layman, have an important effect on results. Some standard, specific routes of exposure for laboratory research include:

a) injection

1) intravenous (i.v.)
2) intra peritoneal (i.p.)
3) intra muscular (i.m.)
4) into egg yolk (birds)

b) oral exposure

1) blended in food
2) in loaded food items
3) in water
4) intubation (force feed)

c) topical application

1) dermal
2) ocular
3) in bedding

d) in air (vaporized)

The route of exposure should be chosen on the basis of the test animal's size and anatomy, and in accordance with the manner in which the animal will possibly encounter the substance under natural conditions. (Dosages are also discussed under Section III, Special Problems).

The second major step in testing is analysis of the results. Analytical procedures available to the scientist are discussed under two catagories: Physical/Chemical and Behavioral Methodologies.

Selection of Categories

Selection of these two categories was a difficult task. As the reader will realize, there is considerable overlapping between categories; however, the divisions allow for a more thorough treatment of all possible methods and also facilitate use of tables which describes the methodologies available (see Table 25). See Figure 23 for interrelationships of Physical/Chemical methods.

Another problem in organization was that certain methodologies are specific technical procedures (e.g.Chromatography, Histology) while others are general methods of scientific experimentation (e.g. Physiology, Population Dynamics) in which the specific techniques can be used. Further distinctions on this point can be found in the text for each category.

Physical/Chemical Tables

There is a natural division between physical and behavioral test methods. For this reason, and because of the volume of information, it seemed advisable to separate the literature into physical and behavioral methodologies. In the case of stress, an entry has been made in both tables because it affects both physical and behavioral aspects of animals. (See Behavioral Methods for further discussion.)

The tabular presentation of methodologies shown in Table 25 has been developed for a number of reasons. First, it simplifies the presentation of a great number of methodologies, and pinpoints areas of concentrated study by showing the amount of information available for each area. It also suggests which methods are not frequently used for a given group. References were gathered with toxicological testing as the primary objective, and the methodologies selected reflect this.

However, the selection was not limited to toxicological testing, because certain "unrelated" methodologies may prove quite suitable for such work.

TABLE 25. METHODOLOGY: PHYSIOLOGICAL

INVERTEBRATES	Ciliar Transport	Stress	[1]Respiration	Enzymes	Anesthesia	Telemetry	[2]Observe/ Monitor	[3]Population Dynamics
Worms							27,30 34,35, 39,54, 130,336, 691,692, 693	32
Snails and Allies		905					39	50
Spiders and Allies							695	54,56,696, 697,698,699
Insects		112		63, 64			122,700	35,39,50,72,7 76,78,84,85, 96,108,115, 123,124,129, 336,701,702, 703,704,705, 706
General Arthropods								50,130,707, 708,710
HERPS								
Snakes					141, 176, 177, 711, 712, 713		714, 715	174
Lizards					141, 712		148	174,716
Alligators								
Turtles			717, 718		141, 744		148	
Amphibians							139,148, 719,720, 721,722	
BIRDS								
Pelican and Cormorant								
Loons and Grebes								185
Waterfowl		195					199,200, 203,209 211,283, 723	193,197,201, 276,283,724, 725,726,727
Shearwaters and Petrets								
Herons and Ibis								185,222
Cranes, Rails, and Coots								
Woodcock								38,229,230
Gulls and Terns							235,236	185,231
Woodpeckers								238

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Ciliar Transport	Stress	[1]Respiration	Enzymes	Anesthesia	Telemetry	[2]Observe/ Monitor	[3]Population Dynamics
Raptors								
Doves and Pigeons				728	729, 730			
Bobwhite Quail							283	276,282,283, 286,368,731
Pheasant		302				732, 733	283,293 314	282,283,287, 290,291,301, 303,734,735, 736
Miscellaneous Galliformes						311, 315, 643	199,314	308,312
Perching Granivores								
Perching Insectivores							37	38,238,333, 337,737
Perching Omnivores		256					199,347, 738,739, 740	225,238,339, 741
Domestic Galliformes	358, 359	374, 742, 743					283,744, 745	201,283,368, 369,370,371, 746,747,748, 749,750,751
Japanese Quail		394		389			283,293	283
Exotics					262			
MAMMALS								
Moles and Shrews								88,423,752,753
Bats							438	430,435
Rabbits and Hares			754	755, 756	757		447,448, 452,580, 677,758	445,759,760
Desert Rodents								
Wild Rats								
Ground Squirrels								
Chipmunks, Tree Squirrels								
Voles and Allies							460	493,498,517, 761,762
Deermice and Allies		348						332,517,763
Lab Mice and Rats	331, 985	256, 532, 766, 767, 768, 769, 770	682, 771, 772, 773, 774, 775	64, 393, 776, 777, 778, 779, 780, 781, 782	783		447,448 536,540, 545,677, 681,758, 760,784, 785,786, 787,788, 789,790, 791,792, 793	5,19,445,543, 761,774,794, 800,801,802, 803,804,805, 806,807,808, 809,810,811, 812

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Ciliar Transport	Stress	[1]Respiration	Enzymes	Anesthesia	Telemetry	[2]Observe/ Monitor	[3]Population Dynamics
Large Herbivores						813		
Large Carnivores							814	
Samll Carnivores							574,581, 583,815, 816,817	
Domestic Carnivores	818	770	754		819		545,820, 821	445,522,805, 822
Horse and Allies		770, 823		619		824, 825, 826		597,619
Swine and Allies				619				
Sheep and Allies		823			628			
Deer and Allies					826	211		631,827
Primates		770	626, 635, 682, 772	619	262			
Opossum					655		828	
Armadillo			622	829				
Exotics			754				677,681, 830,831	683,832,833

[1]This includes anoxia and inhalation

[2]This includes LD_{50}

[3]This includes fecundity, reproduction, mortality, growth rates, ecology

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

INVERTEBRATES	Chromatography	Residues	Bioassays	Metabolism	[4]Genetics	Anticholinesterase	Hepatic Studies	Endocrinology	[5]Anatomy	[6]Physiology
Worms	77	29, 77	49, 834, 835							
Snails			834					836		46,47,48, 837,838
Spiders and Allies										
Insects	77, 234, 839	80, 125, 126	71,83, 111, 834, 840, 841, 842	67,69, 73,74, 82,94, 95, 108, 131, 741, 843, 844, 845, 846, 847, 848	12, 116, 117, 119, 128, 849, 850, 851, 852, 853, 854, 855, 856, 857	65, 127, 841, 858, 859, 860, 861	113			70,862
General Arthropods		54, 147								
HERPS										
Snakes									863	
Lizards								864	863	
Alligators										
Turtles									863	
Amphibians		147, 865	150, 155, 866, 867, 868, 869		12, 154				870, 871	871,872, 873,874, 875,876
BIRDS										
Pelican and Coromorant		185, 249, 877								
Loons and Grebes		185								
Waterfowl	234, 276	276	207, 212, 877, 878		198, 879		113			
Shearwaters and Petrels		216, 877	218				217			
Herons and Ibis		185								

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Chromatography	Residues	Bioassays	Metabolism	[4]Genetics	Anticholinesterase	Hepatic Studies	Endocrinology	[5]Anatomy	[6]Physiology
Cranes, Rails, and Coots										
Woodcock		27, 226								
Gulls and Terns	220, 234, 880	185, 210						233		
Woodpeckers										
Raptors	220	242								
Doves and Pigeons		253, 881	267, 273	882, 883				202	251, 259, 323	884,885
Bobwhite Quail	276, 277	276, 278, 279	277	274						
Pheasant		287, 298, 304, 305		294, 886					306	296
Miscellaneous Galliformes										887
Perching Granivores										319,884
Perching Insectivores		335, 338, 888	889							
Perching Omnivores		210, 341, 739, 740, 890	335	256, 342						328
Domestic Galliformes		891	670, 756, 892, 893	894, 895	360, 361, 362, 367, 896, 897, 898	364	113, 401, 899			350, 900,901
Japanese Quail		397, 881, 902	390, 670, 903	393			391, 393, 401, 404, 405	385		385
Exotics										
MAMMALS										
Moles and Shrews				904						905,906,907
Bats		908								439

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Chromatography	Residues	Bioassays	Metabolism	[4]Genetics	Anticholinesterase	Hepatic Studies	Endocrinology	[5]Anatomy	[6]Physiology
Rabbits and Hares		452	626, 909, 910	640	911, 912, 913		152, 914		754, 915, 916	608,917, 918,919, 920,921
Desert Rodents										
Wild Rats			475	466				477, 479		
Ground Squirrels							486			922
Chipmunks, Tree Squirrels										488,907
Voles and Allies				466	6, 507			498, 762, 923, 924	502	
Deermice and Allies										907
Lab Mice and Rats	619	925	386, 522, 541, 926, 927, 928, 929	96, 256, 393, 617, 640, 679, 767, 930, 931, 932, 933, 934, 935, 936, 937, 938, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951	9,116 519, 538, 805, 912, 952, 953, 954, 955, 956, 957, 958, 959	858, 969, 961	113,387, 401,557, 784,792, 914,948, 951,952, 961,962, 963,964, 966,967, 968,969, 970,971, 972,973, 974	477, 479, 791, 975, 976, 977, 978	754, 979, 980, 981, 982, 983, 984	831,848, 919,920, 935,946, 985,986, 988,989, 990,991, 992,993, 995,996, 997,998
Large Herbivores										
Large Carnivores		625						560, 561		
Small Carnivores		582		999				1000		

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Chromatography	Residues	Bioassays	Metabolism	[4]Genetics	Anticholinesterase	Hepatic Studies	Endocrinology	[5]Anatomy	[6]Physiology
Domestic Carnivores				935, 937, 942, 1001	805, 822, 958		1002, 1003			628,917, 919,991, 1004,1005, 1006,1007, 1008,1009, 1010,1011, 1012,1013
Horse and Allies	591, 619	590, 610		589, 593, 617, 631, 937, 1014, 1015, 1016		858, 1017	113			596,920, 1018,1019
Swine and Allies	591, 619	590		617, 620			152, 113		1020	596,601, 607,609, 610,1010, 1021,1022
Sheep and Allies	619	590, 625	1023	617, 620			113			610,629, 920
Deer and Allies		625		1024						
Primates	619		910	620, 640, 1025	1026					637,990, 1027
Opossum										626,651, 1028
Armadillo									1030	1030
Exotics			621, 670	679	674, 675, 1031		113, 401, 935		585	667,685, 868,1032

[4]This includes teratology and tumors

[5]This includes all studies of organs not covered in other categories

[6]This includes neurophysiology

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

INVERTEBRATES	Histology	Pathology	Pharmacology	Hematology	[7]Oology	[8]Embryology	[9]Other
Worms							
Snails and Allies			1032				36
Spiders and Allies							
Insects	66					114, 844, 1033	36,89, 861,1034
General Arthropods							
HERPS							
Snakes		1035					178,179,1036
Lizards	172	169,1035					1036,1037
Alligators				1038			1039
Turtles		1035		165			1036
Amphibians		151,873		872		140, 1040, 1041	
BIRDS							
Pelicans and Cormorants	186				187,188, 232,400, 1042,1043		
Loons and Grebes							
Waterfowl	192,205			205,213			
Shearwaters and Petrels					217,219		
Herons and Ibis		202			1044		
Cranes, Rails and Coots					223		
Woodcock					227	226	
Gulls and Terns				188,232, 237			
Woodpeckers							
Raptors	240,247, 1045	202			188,236, 237,243, 244,388, 1046, 1047, 1048, 1049		

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Histology	Pathology	Pharmacology	Hematology	[7]Oology	[8]Embryology	[9]Other
Doves and Pigeons	251,258, 259,260 323				257,260, 1050		
Bobwhite Quail							1051
Pheasant		295	300		294,299		1051,1052
Miscellaneous Galliformes							1053,1054
Perching Granivores							
Perching Insectivores							
Perching Omnivores	328				188		
Domestic Galliformes	372	1055		351	355,356, 371,399, 405,1056, 1057,1058, 1059,1060, 1061,1062, 1063,1064	363,365, 366,1065	
Japanese Quail	399			399	382,389, 392,396, 400,736, 1066, 1067		
Exotics							
MAMMALS							
Moles and Shrews							1068,1069
Bats							
Rabbits and Hares	446,449, 450,451, 671, 1070,1071			918,919, 1072		1073	442,831, 1051,1074
Desert Rodents							
Wildrats							
Ground Squirrels							1075,1076
Chipmunks, Tree Squirrels							

TABLE 25. METHODOLOGY: PHYSIOLOGICAL
(CONTINUED)

	Histology	Pathology	Pharmacology	Hematology	[7]Oology	[8]Embryology	[9]Other
Voles and Allies				1077			
Deermice and Allies							
Lab Mice and Rats	387,522, 775,805, 925, 1066, 1078, 1079, 1080	544, 775, 831, 1006, 1081, 1082, 1083, 1084, 1085, 1086, 1087	869,956	539,805, 919,1088, 1089, 1090, 1091, 1092,		538,1093	626,685, 1094,1095, 1096
Large Herbivores							1097
Large Carnivores							33,1098,1099
Small Carnivores				1100		570	33
Domestic Carnivores	110,369, 780,805	586,1006		805,819, 919,1083			
Horse and Allies				608			33,594
Swine and Allies	601,621			608,611, 614,615, 1103			604
Sheep and Allies				608		1104	
Deer and Allies	631,1105						1106,1107, 1108,1109
Primates		634	1110			641	635,773,831
Opossum	654	652,1111		651,1028, 1112,1113			655,656,1114
Armadillo				663,664, 665,666			
Exotics	449,671, 1115	1055, 1116		608,1072, 1113,1117		674	677,831,1115, 1118

[7]This includes shell thinning, hatchability, growth rate, mortality.

[8]See also Oology.

[9]This includes handling, immobilizing, application techniques, methods of capture, techniques for assessment, clean-up procedure and instrumentation.

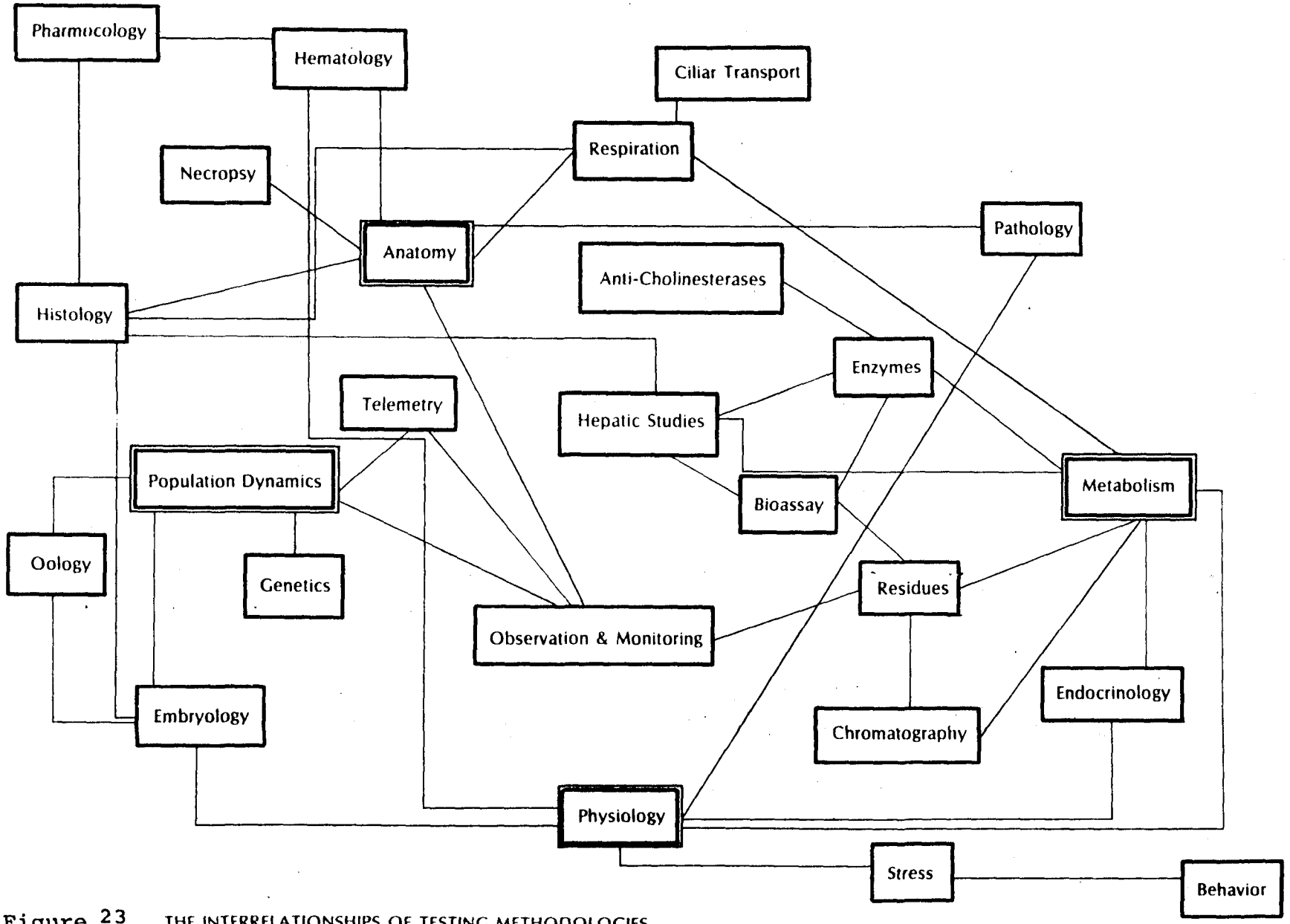


Figure 23. THE INTERRELATIONSHIPS OF TESTING METHODOLOGIES

PHYSICAL/CHEMICAL METHODS

Ciliary Transport

The structure and function of cilia are generally consistent. They are usually engaged in the transport of minute particles or secretions. For instance, in the respiratory tract of higher animal groups, the cilia transport mucous which serves as a lubricant and a natural defense mechanism.

Studies of cilia involve a determination of the rate of flow in "normal" tissue and of the changes that may occur under the influence of an irritant, e.g. gas. Both *in vivo* and *in vitro* experiments have been devised (359, 765) and model systems have been developed for mucous flow and ciliary activity (764). The procedures used are either surgical, to view *in vivo* tissue, or extration of epithelial tissue for an *in vitro* analysis. These studies yield reliable measures of the effects of gaseous toxicants or "caustic" liquid.

In previous work (358, 765, 818) chickens and domestic lab mammals have been used, but because cilia are found in various systems in many animals, this method could conceivably be utilized on other forms.

Evaluation - Changes in ciliar transport mechanisms can directly affect necessary functions of related systems (immunology, food transport) and, if they can be correlated with reduced vitality, increased illness, reduction in growth rates, etc. they can be considered significant.

Stress

In a sense, every environmental or physiological alteration is a stress. Physiological systems (e.g. adrenocortico - thyroid system in mammals) have been developed by animals to cope with stress, modifying physiological functions by feedback in order to cope with the change (770). They can be affected by many factors (including toxic chemicals) and the result is often a "less fit" organism. It is this reasoning that has led some researchers to test the effects of stress on subjects previously exposed to a toxic substance. The effects of stress on exposed animals when compared to controls is a valid measure of toxicity.

An advantage of stress testing is that it gives results which can be extrapolated to a population in nature exposed to changes in temperature, food availability, day length, disturbances, social stresses, molting, migration patterns, etc. (256). Stress testing does not require expensive equipment nor advanced technology.

Stress testing has used starvation on exposed lab rats (768), (Peromyscus maniculatus) (348), (Coturnix) (394), chickens (374, 742, 743), and pheasants (302). Temperature stress has also been used (694, 767, 769). These two methods are the most frequently employed; however, isolation stress (532), revolving drum stress (766), and the introduction of pathogens to exposed subjects (195) have also been used. The possibilities are innumerable.

Evaluation - Stress testing offers a look at the effects of a toxic substance on animals under conditions that might be met in nature. It is applicable to all animals that can be maintained in the lab. (See Behavioral Methods - Stress). (For a review of stress - physiology see reference 770).

Respiration

In respiration experiments where study of ciliar transport is not the objective, the study generally focuses on the toxicity of gaseous substances measured by physiological and anatomical parameters. Vapors are usually introduced into a closed chamber containing the test subject by passing a controlled amount of air over the emitting liquid. Periodic sampling/monitoring of the inner atmosphere verifies the gas concentration. Apparatuses are all of the same basic plan (626, 773), but highly complex equipment has been designed for specific uses (such as the study of turtle respiration - described in reference 718). Long-term, repeated, or continuous exposure is used with less toxic substances or with those usually encountered in low concentrations (771, 772, 773).

Postexposure analysis can include examination of the gross anatomy of lungs and other body organs (682, 773), histopathologic scrutiny of the respiratory tract (773), serum and liver enzyme analyses (773), and observation of behavioral changes (626, 682).

Previous subjects in respiration experimentation have been typical lab species--mice, rats, guinea pigs, rabbits, dogs, goats, pigs, monkeys--but the technique should prove easily

adaptable to all forms of animal life, even those organisms which respire largely through their outer integument (i.e. Helminths).

The methods described here are limited to the testing of gaseous or airborn substances. In the case of particulate matter, mists or aerosols can be used. Determination of the concentration of the test substance in the air is made difficult when particles of varying sizes occur in the test atmosphere. Particles of unit density are considered in these instances. (A caution concerned with interpretation of results - gases and particulate matter can be inhaled and swallowed as well.) (803).

Evaluation - Respiration experiment techniques are an excellent route of exposure and can be used to dose animals for further physical and behavioral tests.

Enzymes

Enzymes are organic catalysts which, like their inorganic counterparts, function in "the lowering of the activation energy of a reaction (resulting in) a faster attainment of the equilibrium point. Presumably all reactions of any degree of complexity occurring in living cells are catalyzed by a specific enzyme. Thus the integrity of the enzyme population of a cell or organism is paramount to its well being" (1119). Enzymes occur in most living tissue, but those of the liver have received special study because most metabolism of ingested materials takes place in this organ (393, 778).

Almost any enzyme level can be determined in any tissue if its activity can be measured under controlled conditions. Review articles on liver microsomal enzymes are available (777, 780) and entire books have been written on enzyme methodology. However, biochemical procedures are often exacting and require an experienced technician. Likewise, the necessary equipment is expensive. While the results of enzyme testing give an exact measure of the direct effect of the test substance, interpreting the indirect effects may be more difficult. For instance, the stimulation of an enzyme may cause biochemical side effects that are not directly related to the test substance. Enzyme inhibition can do the same. In order to evaluate the activity of an enzyme and determine the effect of any change in concentration, it is necessary to know the activity of its products and their subsequent reactions.

Evaluation - Enzyme studies can show variations in levels and activity, but their real value can only be realized when it is possible to correlate enzyme changes with changes in vital metabolic processes.

Anesthesia

The administration of anesthesia to animals can be risky, especially when little is known about the effects of an anesthetic on a relatively new species of animal in the lab. The effects of an anesthesia might change as a result of the subjects exposure to a toxic substance.

Evaluation - Anesthesia/narcosis can be a good test method in itself when used as a stress but unless the compound used is potentially an environmental contaminant the results would not be directly applicable to natural stresses. However, reduced tolerance induced by a toxic substance to any anesthetic may be an indication of reduced tolerance to other man-made chemicals possibly found in nature.

Telemetry

The monitoring of radio tagged animals has been used for some years by wildlife researchers to determine the movements and body temperatures of free ranging individuals. Grouse (311, 315), pheasants (732, 733), ducks (825), and large mammals (824, 826) have been studied in this way.

Evaluation - Radio work can be done with many species, but its applicability to toxicity testing is unknown because of the lack of control in open fields. The success at monitoring body temperature (813, 824) suggests that heart rate and respiration could also be monitored with radio equipment. Equipment for large scale programs will be rather costly; single transmitters can cost $500 to $1000 and receivers, depending on the size and capabilities, cost upwards of $500.

Observe/Monitor

This general category includes the simple observation of exposed animals, for a dosage - mortality correlation, "control" studies in which a toxic substance was tested for its destructive ability on a given species or larger group of animals, residue analyses (seperately listed in the tables for quick reference), and studies which report general changes in an animals appearance or activity after

exposure to drugs or other substances. (For studies on specific behavioral aspects see Behavioral Methods).

General population counts on invertebrates (336), and soil invertebrates in particular (1047,130,54), have been conducted Earthworm populations have been monitored in many studies of toxic substances (30, 34, 35, 37, 692). Other terrestrial species that have received attention are frogs (721), snakes (148), pheasants and Japanese quail (293), and monkeys (831). Most of these investigations have been performed under lab conditions.

Reports of the general appearance of exposed creatures are limited in number, but external lesions and other morphological changes have been reported in frogs (139, 1120).

Of special note are the experiments in which brain wave patterns have been routinely monitored by Iowa State researchers by using surgically implanted electrodes (628). This procedure is used with primates as well, and could be applied to many larger animals. It may be more widely used than we have been able to detect.

Evaluation - Observation of mortality rates and general activity of the subject, as well as the palatability of test substances, is an excellent method for screening potential compounds. Survival studies are essential for determining the gross effects of exposure on animals. However, more detailed biochemical tests, such as enzyme analysis, and histopathological examination of subjects are required to elucidate mechanisms of action and the direct causes of mortality. Behavioral testing is recommended for sublethal poisoning.

Population Dynamics

Population dynamics is the study of numbers of organisms, their reproductivity, growth, and survival. It is concerned with the factors that control the abundance and the distribution of a species. The effects of toxic chemicals on the reproductivity of animal population is thought by many researchers to be a key point of concern in population studies (20). There has been a large amount of work on population dynamics (241). Such research can conceivably be performed with any species desired. Aspects of such research include fertilization, embryo viability, egg hatchability, growth rates, and overall survival. Experiments are designed around the life history and reproductive modes of the test animal. For instance, birds are particularly suited for embryological studies in which the egg yolk is injected with the test substance, a route of exposure difficult to employ on mammals.

A basic protocol consists of dosing the test animal and then scrutinizing parameters that may affect reproduction or reproductivity itself. There are acute studies in which immediate mortality is a sure measure, and chronic studies which are concerned with more subtle, long-term effects due to single or repeated exposures.

While Population Dynamics is closely related to many other methods (Observe/Monitor, Embryology, Oology) and uses many procedures from other fields,it is distinguished here to emphasize its importance. Viable populations are ecologically important for genetic, social and often economic reasons, and though the test animals are individuals, it is the integrity and viability of the entire gene pool that determines the fate of a species.

Evaluation - Population studies can be performed on entire populations, or on a few individuals from whom extrapolations can be made. The methods deal with effects on whole organisms or populations and are not directly concerned with individual effects or mechanisms. Population studies are recommended as screening methods to assess overall effects, but must be followed by more exact tests if the goal is to determine mechanisms and causes.

Chromatography

Chromatography is the set of methods used by chemists to identify organic substances by color and by their positions relative to each other on chromatographs. Chromatography is based on the theory that solutes, such as amino acids, will tend to be distributed between two immiscible liquids according to their relative solubility in each. There are many ways of creating this separation. For example, gas chromatography vaporizes the mixture by heating it and blows it over a column of material with a high melting point. The molecules of the mixture will partition themselves between the gas and liquid according to their relative solubilities in the two phases.

Chromatography can determine the presence of chemicals tested for and a quantitative result can be obtained from some types of procedures. This method is very useful in metabolism, residue, and degradation studies where data on chemical presence and integrity are desired. It is a standard procedure in many biochemical laboratores, and exact methods are available in most tests on lab methods.

Evaluation - By itself, chromatography is not a very useful technique for toxicological testing but used as part of larger scale testing, such as metabolism studies (591, 619) and residue analyses (77, 220, 276) it is a most useful tool.

Residues

Residue analysis is simply the determination of the concentration of toxic substances in a given place at a given time. It can apply to soils, plants, animals, or atmosphere - any part of the environment. However, the analysis of animal tissues is of specific concern here.

Animal tissues are analyzed for residues through the use of basic laboratory techniques. Extraction - clean up - gas liquid chromatography is an oft-used flow in such studies (29). The results can give a quantitative measure of the test substance if conditions are right. The significance of the residue or its effects on the animal is another problem and not as easily determined. Bioassay, as used in this report, is similar to residue analysis, but is concerned with the relative strength of a substance and its gross effect on the test organism. Residue Analysis, Observe/Monitor (LD_{50}) and Assay methods are interrelated.

Evaluation - Residues in themselves do not show any adverse effects on the animal. They must be correlated with their effects on the subject species.

Bioassay

An assay is a chemical analysis to determine the presence, absence, or quantity of one or more components of a substance. In the sense it is used here it is practically synonymous with residues, but is concerned more with the relative strength of a substance in specific body tissues and the effect of that substance on the host tissue. This is usually achieved by measuring the activity of the substance or related enzyme. (910, 927). Assay is used routinely in drug research and methods applied in that field are frequently suitable for toxicological testing.

Evaluation - As with the presence of residues, presence of a toxic chemical does not give any indication of its effects on the host animal and therefore is of use only if levels can be correlated with decreased survival, reproductive success, behavioral changes, etc.

Metabolism

Metabolism research is concerned with the study of the way living systems handle substances. It is a very broad biochemical category that analyzes the actual breakdown, storage, re-use, and clearance of test substances and their effects on metobolic pathways, including enzyme inhibition and altered drug metabolism (941, 947).

Radio-isotope labelling is used in many metobolic studies, particularly those in which the toxic substance is an organic compound and specific pathways are of interest. In this test the products resulting from the chemical breakdown in the system can be located and analyzed.

Metabolism studies are closely related to many categories in this section. (See Figure 23, The Interrelationship of Methodologies.) In these investigations, the chemical fate of toxic substances and their effects on the life processes of test organisms are the primary objectives and they often explain the gross effects readily visible by general observation. Metabolism studies are at the biochemical level and can be performed on all animals.

Evaluation - Metabolic studies are important in toxicity testing only if alterations at the biochemical level can be correlated with a reduction in viability of the test animals. For instance, interference with certain metabolic pathways can reduce the ability of animals to utilize food efficiently, defend itself against disease, etc.

Genetics

Genetics is a broad subject which deals with, among other things, the transfer of information from one generation to the next. Therefore, embryology, reproductivity, mutagenicity, and resistance are related to heredity. In fact, because all characteristics of living systems are more or less expressions of genetic material, this category should be the largest. However, we have generally limited it to those studies mentioned above.

The references under "Genetics" are primarily concerned with long-term reproductivity (822, 958) carcinogenic studies (1031, 1081) and resistance (see below). Probably because so little is known concerning specific genes in most animals, much of the work of this nature is restricted to Drosophila (117, 119, 849).

Resistance to an environmentally selective factor like a pesticide has been reported in insects (12, 128, 854, 855, 856, 857), frogs (12, 154), and mice (6, 9, 507, 519), and presents a particular problem in the testing of toxic chemicals (see Special Problems).

The reproductivity of species has received a rather large amount of interest because many environmental toxicologists believe it to be one of the best ways to study the overall effect of toxic substances on animal life (see Population Dynamics). Some of the specific methods involved in these studies are included above.

Evaluation - Mutagenic effects on animals as a result of exposure are considered undesirable, often affecting its survival. Genetic studies are limited to a few species at present (e.g. Drosophila, mice, frogs) and may be difficult to perform on other species less genetically known.

Anticholinesterases

Acetylcholine is a basic impulse transmitter between nerve cells outside the central nervous system. The enzyme which destroys the transmitter is a cholinesterase. The action of many organic insecticides is in effect to create anticholinesterases which restrict the enzyme and may cause muscular fasciculations in the affected animal. Cholinesterase is also present in red blood cells, which are more easily monitored than nerve endings.

Purification of the insect cholinesterase for in vitro experiments is available (860). Anticholinesterases is separated from enzymes because of the work with neurotoxic insecticides.

Evaluation - Anticholinesterases can be routinely tested on all mammals and birds. A cross section of animals should be used because of variations among species. Though a very specific test, it is valuable and can show direct effects on animal survival.

Hepatic Studies

The vertebrate liver has received much attention in toxicological testing because it serves as a "detoxification center" where a variety of injurious chemical compounds are converted by liver enzymes or where vital enzyme systems such as carbohydrate metabolism can be adversely affected by toxic substances.

Evaluation - The liver is an excellent organ for the study of enzyme action and histopathology.

Endocrinology

Traditionally, endocrinology has been the study of the ductless glands and the physiological adjustments that their special products facilitate. Because the glands themselves, and their products can be affected by the introduction of foreign substances into the system, they have received some attention by pharmacologists who have been involved in studying the effects of drugs (978). Contro programs of many types have used synthetic estrogens (e.g. diethylstilbestrol) as possible control agents (477, 479, 1000), and toxicologists have monitored endocrine levels and gland size to determine the effects of toxic substances on rats (977).

Lab methods are based on blood chemistry in which the amount of a specific endocrine is determined in order to compare the measurement to a standard. Organ weights and size can also be compared.

The endocrine system is regulated by many factors, among which the autonomic nervous system has the primary role. All types of stresses (drugs, tension, sickness) can affect the production of endocrines. Certain endocrine levels are also affected by circadian rhythms, such as breeding cycles, hibernation, etc. These natural and "unnatural" variations must be considered in endocrinology work.

Background endocrine information is available for many mammalian species (762, 923, 924).

Evaluation - The endocrine system is very important in all animals. Breeding, molting, hibernation, activity, are a few functions mediated by endocrines. Any test which shows alterations in levels of endocrines, especially those related to breeding (e.g. FSH, estrogen,) due to exposure to toxic chemicals is of value.

Anatomy

This category includes the general anatomical and morphologic work in which gross (e.g. hyperplasia-hypoplasia) or cellular changes have been reported after exposure to a test substance. The studies included here often overlap other areas. To avoid redundancy, anatomy is restricted here to include only those papers dealing mainly with anatomical features and general subjects that can not be

included under a different methodology. Rodent muscular efficiency (982), necropsy (585, 863, 984) uterotrophy (983), heart cells (980), bone marrow (979), and pulmonary changes (754) have all been studied in conjunction with exposure to a wide range of toxic substances. General nervous system changes (981), and kidney lesions (915) have been reported after exposure to toxic chemicals in rats and rabbits.

Taxonomists have developed comparative methods of analysis (e.g. morphometric, 118) which could prove applicable to the comparisons of exposed vs. control subjects in toxicological testing.

Evaluation - General anatomical studies can show changes due to exposure which are unimportant to a species: for example, hyperplasia-hypoplasia, or microscopic alterations in tissue organization (see Histology). However, if survival or longtime reproductivity are affected by the changes, then anatomical studies can be valuable in determining the effects of toxic chemicals. General anatomical analysis is part of post mortem work and should be included in that phase of testing.

Physiology

Physiology is the study of function, whether it be on the cellular or organ level, in any part of an animal's body. This very broad category includes the toxicology of the nervous system (48,872,991,992,1011), circulatory system (920, 921, 1018), respiratory system (685), muscles (629), auditory system (1008, 1012), renal system (587, 1084), general studies (70, 596, 1006), and many more.

The methods used are as varied as the subjects included under Physiology, and there are literally shelves of texts and lab manuals available which deal with all aspects of the science. (The major reference is Handbook of Physiology American Physiological Society 1959, 8 Vols.)

Evaluation - See specific methods for further information and recommendations.

Histology

The study of tissues, their structure and organization, is called histology. It overlaps anatomy, physiology, morphology and other fields, but is strictly defined as the microscopic studies of tissue arrangements and form. In this report, histology includes most tissues studies,

including carcinogenic work. Methods employed are microscopy as well as gross inspection of tissue reactions. (Technical help in tissue preparation may be necessary for microscopy).

Dermal reactions to toxic substances can be performed on many animals(449,450,1070,1071,1102). The method is a simple topical application and succeeding analysis of the exposed tissue. (A review of the method is given in reference 671).

Almost all animal tissues can be analyzed for the effects of toxic substances if a route of exposure is available. Inhalation studies of the respiratory tract (775, 1080) have been discussed under respiration. I.V. and I.P. injections and oral dosing can indirectly expose inner organs (kidney, 446; muscle,1101;; nervous tissue, 66; liver, 387, see Hepatic Studies).

Evaluation - Histology can be effectively used in the analysis of effects of exposure to toxic chemicals on all animal tissues. Comparisons of dosed animals to controls or standards will give some indication of direct effects on the anatomy of test animals. An evaluation of what these alterations mean in terms of function, survival etc. should then be made. (Major reference: Bloom, W. and Fawcett, D.W. 1968 A Testbook of Histology, 9th ed. Saunders)

Pathology

Pathology is the study of disease and the structural and functional changes caused by disease. Study of this sort can utilize methods such as histology and physiology. Disease can be used as a stress to test the effects of a substance on affected individuals. Exposed animals can be treated with a pathogen (195). Standard microbiological methods can be obtained from a great number of lab tests, though their applicability to toxicity testing is limited. Most of the references under Pathology do not deal directly with toxicological testing, but the information on diseases has potential use in dealing with maintenance problems or in selecting stressors.

Evaluation - The use of disease as a stress is quite suitable for toxicity testing. It is a natural stress, one that must frequently be confronted by a species in natural environments.

Pharmacology

Pharmacology is the science of drug action. Many methods used in this field are equally applicable to toxicology because both deal with foreign substances that animals must cope with. Drug testing performed by the drug manufacturer is expected to result in knowledge of the drugs action as well as its safety for animal use. Toxicological testing must do the same. Drugs can also be used as stressors in toxicity testing or in studies of their synergistic effects if there is a chance the two could be used together.

A review of methods for the use of primates in pharmacological and toxicological tests is available (1110).

Evaluation - The use of drugs as stressors is recommended only in cases where the animals would be exposed to the drug, such as domestic species that may receive veterinary care.

Hematology

Blood is the aqueous suspension of cells that is the circulating fluid in most animals. It is a very remarkable body tissue that functions as a medium for the transport of nutrients, as a regulator of metabolism, as protective tissue, etc. Its elaborate composition is too complex to describe. It differs from species to species, often remarkably.

Blood is easily obtained from most animals without requiring their death and techniques for bleeding mice (1091), swine (615), mink (1100), and other animals (1072) are published. Basic blood chemistry is known for most mammals and some other vertebrates (611, 651, 663, 664, 1038, 1088).

Total serum protein, urea nitrogen levels, glucose levels and cholesterol levels have been affected by food additives in mice (539). Anticholinesterase changes are determined from blood levels (608). Other parameters that can be measured routinely are hematocrit, leucocyte levels, enzyme levels, and hemoglobin activity.

Evaluation - Blood studies are efficient and valuable because of the many meaningful measurements that can be taken without sacrificing the test animal. Though larger species (e.g. nutria, sheep, galliformes, turtles, ducks) are recommended, tests on smaller species are possible, and insect hematology is fast accumulating background information that can prove useful for comparison.

Oology

There are two terrestrial animal groups that produce large eggs which lend themselves to experimentation: birds and reptiles. Their eggs are a unique vehicle for developmental and embryonic study. In this report Oology has been separated from Embryology to accentuate and more suitably treat the toxicity studies, all avian, which are concerned with egg production (399, 405), egg shell characteristics (392, 1063), egg viability and hatchability. (194,400,1056,1067), and ovulation lag (736). Actual development of the embryo and unique responses to chemical exposures are treated in Embryology.

There are basically two approaches that can be taken in Oology - Embryology research. The adult organism can be treated and reproductive/developmental success monitored, or the embryo itself can be dosed. The second approach is simple in oviparous groups (See Embryology). Parental exposure can be accomplished by a variety of routes (see introduction to Methodologies). Correlations between brain residue of female parents and egg production have been reported (396) and ovulation lag in Japanese quail is reviewed in reference (1107).

Eggshell thinning in larger birds has been reported since the early 1960's and been given much attention. A good review of that phenomenon can be found in reference 1064.

Evaluation - Oological studies of toxic chemicals are very important because of the direct effect on reproductivity that can be estimated from the results. It is limited to oviparous species at present.

Embryology

The study of developmental biology has utilized frogs' eggs and birds' eggs in a great many ingenious fashions. Researchers have studied pesticide distribution in exposed embryos (1057), teratogenic effects (226, 363, 641, 1073,1093), fine structural changes (365), and tissue residues (1062) Plancental transfer of C^{14} labeled Parathion in sheep (1104)and eggshell thickness related to embryological development (1063) are other previous investigations.

Exposure of the embryo is accomplished in bird studies by injecting the yolk sac via the air sac (355, 356). The direct exposure, quantification of dose, and quality control make this method scientifically desirable.

Some invertebrate eggs may be used in much the same way as bird eggs to perform statistically valid experiments. However, the route of exposure must be different from that used with bird eggs due to the small size of the eggs. Topical application (844, 1033), and exposure of adults (114), are possible routes.

Evaluation - Embryos and their development are directly related to the survival and reproductive potential of a species. These studies are recommended for all potentially exposed animal species.

Other

Under this heading are included techniques for handling and testing animals, such as restraints (442, 604), drug immobilization (33, 655, 1098, 1106, 1107, 1108, 1109), and hypnosis (1074). Special apparatuses for use in metabolic cages (1096), for dusting insects (1034), for feeding (656, 831), and for measuring activity (1094, 1097) have been used for experimental purposes. More sophisticated devices available include electroantennegrams for insects (89), body capacitor - olfactometers for small rodents (1075, 1076), and a respiratory apparatus (773) (see Respiration).

Clean-up procedures are necessary for laboratory work such as chromatography and the literature has much information available for specific analytical methods (e.g. 1095).

This listing is a general one, because detailed techniques and methods are quite varied, are often restricted to specific types of analytical procedures, and may be valid only for a given species.

BEHAVIORAL METHODS

Introduction

The central premise upon which behavioral toxicology rests is that an animal's reactions to enviornmental stimuli (activity, habits, instinct, learning) can be affected by alterations in its internal environment and that these changes can be measured, thus directly indicating levels of toxicity. This branch of toxicology is important because changes in behavioral patterns affect an animal's interrelationships with its environment, thereby affecting its ability to perform tasks necessary for survival. For instance, a reduced reaction rate in avoidance responses indicates a loss of awareness which would make the individual less capable of escape from a predator. Reduced awareness and chance of survival go hand in hand in a natural environment. Behavioral testing is one method of measuring subacute toxicity which can be directly related to an animal's overall fitness. A summary of references available on various behavioral methodologies is found in Table 26.

Repellents

In this discussion, a repellent means any chemical compound that protects a potential food source; it can cause side effects such as fright, convulsions, decreased productivity, sterility and death. Known concentrations of repellents can be field sprayed in solid or liquid form to protect plants from predators such as slugs (1121) and deer (1182). Sprayed plots were noticeably more productive than control plots. Grain baits were used on rodents to measure repellent effectiveness (508, 509, 1147) or prevent pregnancy (478). Treated seed, either fed directly or grown and then fed, has been used with numerous birds to determine its potential for reducing pest reproductivity (266). Encapsulated rodent baits were fed to rats as sterilants (1146) and to domestic farm animals to measure the dangers of oral exposure (1177). A toxic, fright-producing chemical was sprayed on plots in fields and kept blackbirds away from the whole field (349). With the exception of 349, these repellents are not species-specific and could probably be used on invertebrates and vertebrates alike.

Repellents can be used as a behavioral test, comparing the reactions to those of dosed and control animals.

TABLE 26. METHODOLOGY: BEHAVIORAL

INVERTS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Worms								42
Snails and Allies	1121	1122		1123		55	53,1124	
Spiders and Allies								
Insects			112	91,1125, 1126, 1127, 1128, 1129, 1130, 1131				
General Arthopoids								

[1]This includes food preference.

[2]This includes conditioned response and avoidance.

TABLE 26. METHODOLOGY: BEHAVIORAL
(CONTINUED)

BIRDS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Perching Omnivores	285,349, 1137		1138					
Domestic Galliformes		1139	374,742, 743				1135	
Japanese Quail			394			384,398, 403		309
Exotics								

[1]This includes food preference.

[2]This includes conditioned response and avoidance.

TABLE 25. METHODOLOGY: BEHAVIORAL
(CONTINUED)

HERPS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Snakes						1132		
Lizards								
Alligators								
Turtles								
Amphibians		1138						

[1]This includes food preference.

[2]This includes conditioned response and avoidance.

TABLE 26. METHODOLOGY: BEHAVIORAL
(CONTINUED)

BIRDS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Pelican and Cormant								
Loons and Grebes								
Waterfowl			195			194		
Shearwaters and Petrels								
Herons and Ibis								
Cranes, Rails and Coots								
Woodcock								
Gulls and Terns								
Woodpeckers								
Raptors								
Doves and Pigeons	266	270				254		263,264, 269,1134
Bobwhite Quail	285	280					1135	
Pheasant	285,1136	734	302				1135	
Miscellaneous Galliformes								
Perching Granivores								
Perching Insectivores								

TABLE 26. METHODOLOGY: BEHAVIORAL
(CONTINUED)

MAMMALS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Moles and Shrews			1140,1141			904,1142		
Bats			1141		437			
Rabbits and Hares					599,1143, 1144	1145		
Desert Rodents			457					
Wild Rats	478,1146, 1147							
Ground Squirrels			482,1148, 1149					
Tree Squirrels and Chipmunks	1147							
Voles and Allies								
Deermice and Allies	508,509	1150,1151	348			511,1152, 1153		
Lab Mice and Rats	509,1094, 1146		256,532, 766,767, 768,799		1143,1144	520,1094, 1154,1155, 1156,1157, 1158,1159, 1160		547,1161,1162, 1163,1164, 1165,1166, 1167,1168, 1169,1170, 1171,1172, 1173,1174

TABLE 26. METHODOLOGY: BEHAVIORAL
(CONTINUED)

MAMMALS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Large Herbivores						1097		
Large Carnivores								
Samll Carnivores			1140,1175			579,584		
Domestic Carnivores					527,1007, 1009,1011, 1012,1144, 1176			
Horse and Allies	1177				598,599, 628,1178			
Sheep and Allies			823		627,628			1178,1179, 1180,1181
Deer and Allies	1182							
Primates			770		599			104,547, 1183,1184, 1185

TABLE 26. METHODOLOGY: BEHAVIORAL
(CONTINUED)

MAMMALS	Repellents	[1]Discrimination	Stress	Pheromones	Neurophysiology	Other	Instinct	[2]Learning
Opossums		1186				1188		1189,1190
Armadillo								
Exotics			1187		1143	482,686, 1191		1166

[1]This includes food preference.

[2]This includes conditioned response and avoidance.

Evaluation - The use of repellents to measure behavioral reactions altered by a second chemical is quite feasible for all species. They can also be used to test avoidance reaction to evaluate the sensitivity of a test species to a hazard.

Discrimination

Discrimination is the ability to distinguish one thing from another by means of one or more of the five senses. Most laboratory studies have dealt with visual and olfactory cues.

Olfactory tests done with rodents have demonstrated the importance of odor in food detection and palatability (1150, 1151).

Newts can discriminate visually between dosed tadpoles and controls, attacking the dosed animals far more frequently (1133). This type of test could be done with any predatory animal that hunts by sight.

Bobwhite quail have been trained in a Skinner box, fed a toxic substance for a few weeks, and then retested. Their performance was much lower in the retests (280). This type of testing could be done on all animals.

Pigeons (270) and opossums (1186) have been tested for visual acuity but this type of test could be done on any vertebrate species and could readily be adapted to toxicity testing. For a diagram of the apparatus used see Figure 24.

Behavioral tests of depth perception have been done using a visual cliff, with young cornish hen chicks (1139) and first generation offspring of pheasants fed a toxic substance (734). Movements of the young chicks when placed in the middle of the visual cliff were recorded. In both cases, movements were considered partially a produce of the chick's previous environment, but the offspring of the dosed parents differed significantly in performances from the controls. For a diagram of the apparatus used in these tests, see Figure 25.

Primates have been tested for visual discrimination and were rewarded when they made a correct choice (630). For an idea of the chamber used, see Figures 26 and 27.

A general discussion of chemoreception in gastropods may also be classified under the category of discrimination (1122).

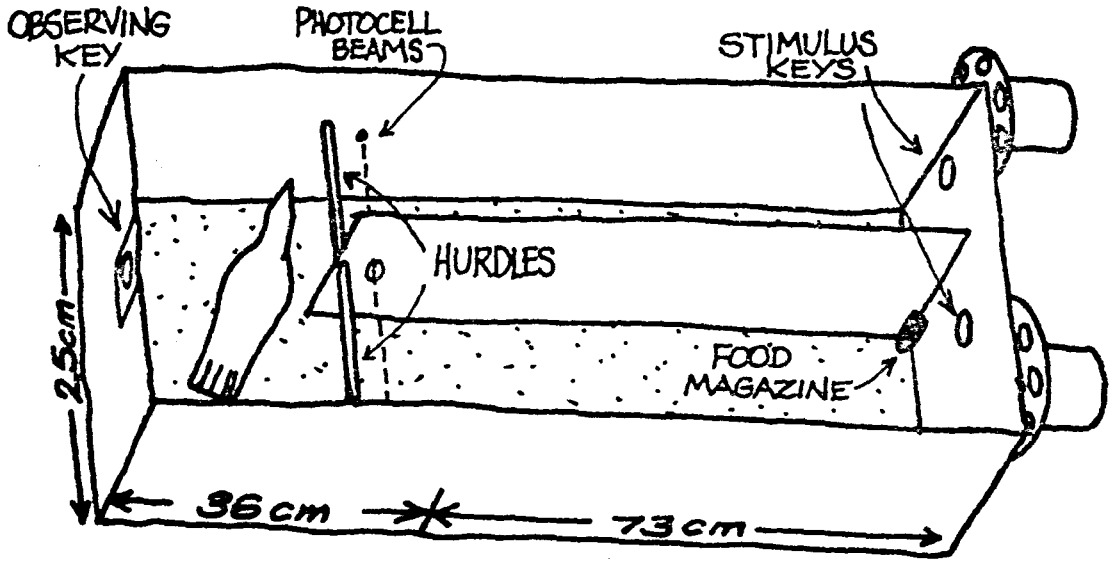


Figure 24. Diagram of visual acuity chamber

A partition divides the chamber into two alleys, entrances to which are marked by hurdles mounted two inches above the floor. Photocells, illuminated by a white 28V. 0.1-ampere lamp, record entrances into the alleys. The plastic observing key and the glass stimulus keys are one inch (2.5 cm) in diameter and mounted approximately 10 inches (25 cm) above the floor.

Source: (270) Copyright 1971 by the Society for the Experimental Analysis of Behavior, Inc.

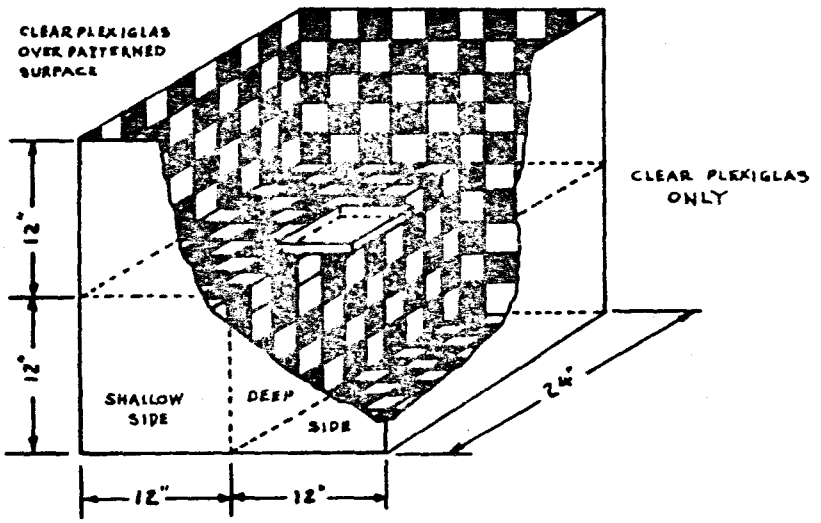


Figure 25. Cutaway of a visual cliff used as a test apparatus.

Source: (1139) Copyright 1964 by the American Psychological Association. Reprinted by permission.

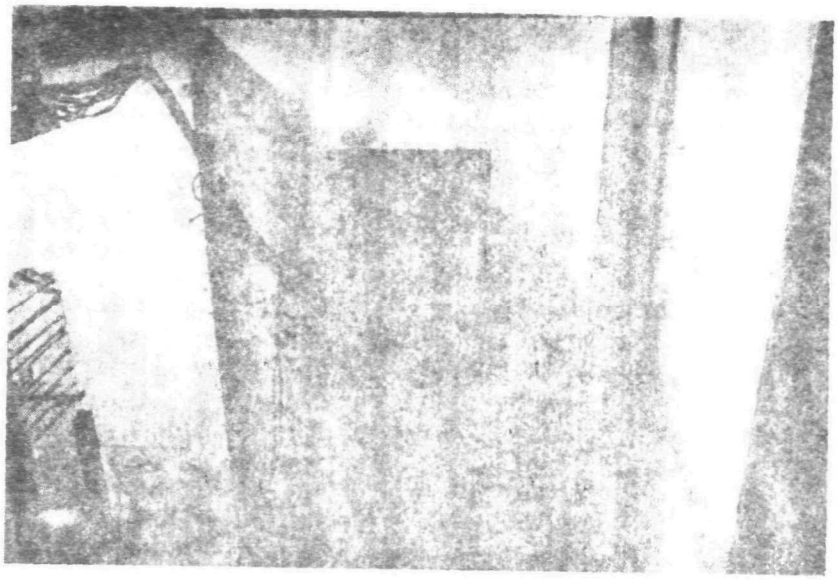


Figure 26. Discriminatory device used in primate testing

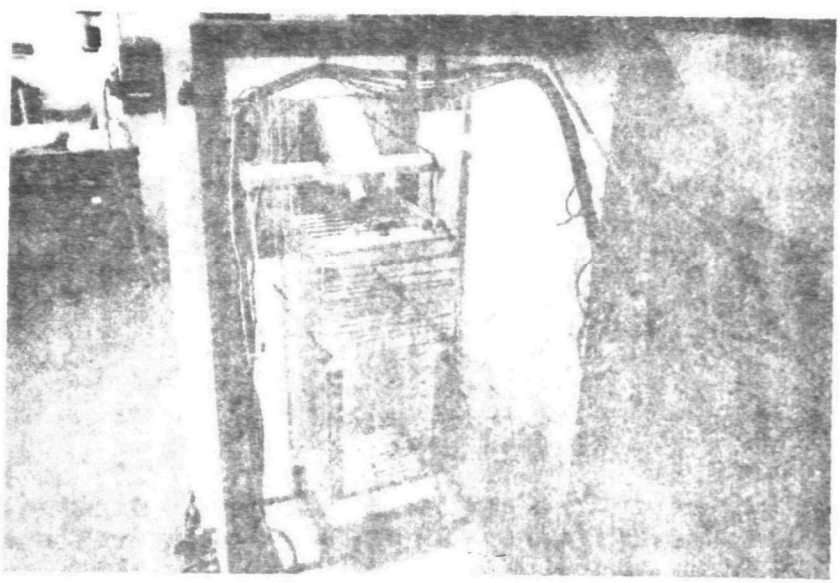


Figure 27. Inside of visual discrimination chamber for primate testing

Source: (630)

Evaluation - Discrimination tests, comparing control and dosed organisms, may be directly extrapolated to species in natural environments. The "less aware" individual, for example, is more susceptible to predation and other environmental hazards.

Stress

Included under the general category of stress are such things as infections, intoxicants, trauma, nervous strain, heat, cold, muscular fatigue and irradiations. In most stress studies, a toxic substance is fed, the animals are subjected to an additional stress, and then survial rate (348, 394, 742, 743, 1178), reproductive rate (112, 348) and tissue levels of the substance (374, 743, 768) were studied. Painful or emotional stimuli have been used to study renal response in large mammals (823). Isolation stress has been studied in lab mice (770). Temperature gradients have been used to study growth in snails (694). Mallards have been fed toxic substances and then exposed to a virus (195). They were less resistant than the control birds. Rats have been drugged and exposed to cold and other stresses. Drug action and metabolism were affected (767) and drug tolerance levels were lowered (766).

Hibernation has been tested as a stress factor. General field studies have been done on skunks (1140, 1175), and lab observations on using the bat and hedgehog (1141). It has been found that hibernation effects oxygen consumption and temperature regulation in ground squirrels (1149) and hamsters (1187). The transition periods at the beginning and end of hibernation have been studied in pocket mice (457), ground squirrels and hamsters (482).

Evaluation - Animals are stressed in their natural environments by many factors, for example, disease, temperature, food shortages, etc. (See Physical/Chemical Methods - Stress) and their adaptations for coping with seasonal and daily stresses are of physiological importance. A test which can compare the reactions of control and dosed organisms to stress situations might be extrapolated to quantify the effect of toxic substances on the reactions of wild population. For instance, hibernation occurs in many mammals (e.g. daily torpor in shrews) and, a few birds, and diapause occurs in insects. These natural occurrances are necessary for the survival of many species and alternations in this behavior will affect the viability of the organism during a period of stress. Isolation, disease, and fatigue are stresses directly applicable to natural situations and

are suggested parameters for testing a species reaction to stress after exposure to toxic chemicals.

Pheromones

Pheromones are chemical compounds produced by animals to attract mates and communicate sexual receptiveness. They are reported to occur in many groups of animals, but are best known in insects. Since their discovery, they have been used as a means of controlling and eliminating insect pest species. Methods of extraction, purification, and use as masks for attractants have been studied in the cockroach (1125) and many Lepidopterans (91, 1130). A general discussion of the physical structure, commercial use, and technology of pheromones is given in reference 1129. The chemical basis of insect sociality (1128) and the use of moths (1126) have been studied. The Red-Banded leaf roller has been used to determine the structural features necessary for a compound to elicit synergistic or inhibitory activity (1127). The purification and evaluation of synthetic attractants for insects has also been studied (1131). Snail trails as cueing devices have also been studied (1123).

Pheromones might be used in mammalian studies, as mammals are known to use olfactory cueing.

Evaluation - Pheromones are effective means of insect control, can be combined with toxic chemicals (1129) and could be used as a method of collection of insects for lab use. It is possible to use pheromones in behavioral testing in which the reactions of dosed animals and control animals to pheromones is compared.

Neurophysiology

Neurophysiological studies encompass taste reception and auditory studies, drugs, induced convulsions, EEG's and EMG's.

Taste reception studies range from analysis of the potentially important consequences of taste reception (599) to mammalian species differences in taste sensitivity (1143, 1144), the electroneural response spectrums of taste fibers in the domestic cat (1011), electrophysiological studies in the ungulate (589, 1178), dog (1176), bat and opossum (437), and electrophysiological studies of synergistic effects of taste reception in the dog (1009).

The auditory responses of sheep have been measured before and after dosing with a toxic substance (627). Cats have been given toxic substances in an effort to inhibit cochlea potential response (1012) and dogs have been used in similar studies (1007).

Mice have been raised on different types of bedding and then administered a convulsion producing drug. It was found that drug thresholds correlated with the type of bedding used (527).

Sheep have been given toxic substances orally followed by EEG's. Gross behavioral observations were also made (628).

Although mammals have been the principal subjects used in neurophysiological studies, birds, herptiles, and some of the larger invertebrates could be used. The limiting factor for invertebrates is the lack of appropriate electrophysiological equipment.

Evaluation - EEG and other electrophysical methods are excellent techniques for recognizing changes due to exposure to toxic substances. However, data on mortality, impaired functioning, and altered reproduction potentials or behavior are necessary before altered electrical responses can be correlated with harm or potential detriment to the test species.

Other

This category includes general behavior, sleep, circadian rhythms, and photoperiodism as parameters that can be important for toxicity testing.

Mites have been exposed to a toxic substance to observe its effects on their dispersal and oviposition rates (55). The aggregating behavior of Natrix (water snake) (1132) might be used in toxicity testing. Metabolism, food capacity and feeding differences between species have been studied in shrews (904). Social (511, 1152) and sexual (1153) behavior have been studied in the genus Peromyscus. A method of recording burrowing activity (see Figure 28) has been devised and used on the mountain beaver (1097).

A means has been worked out for quantifying general activity in mice (1094).

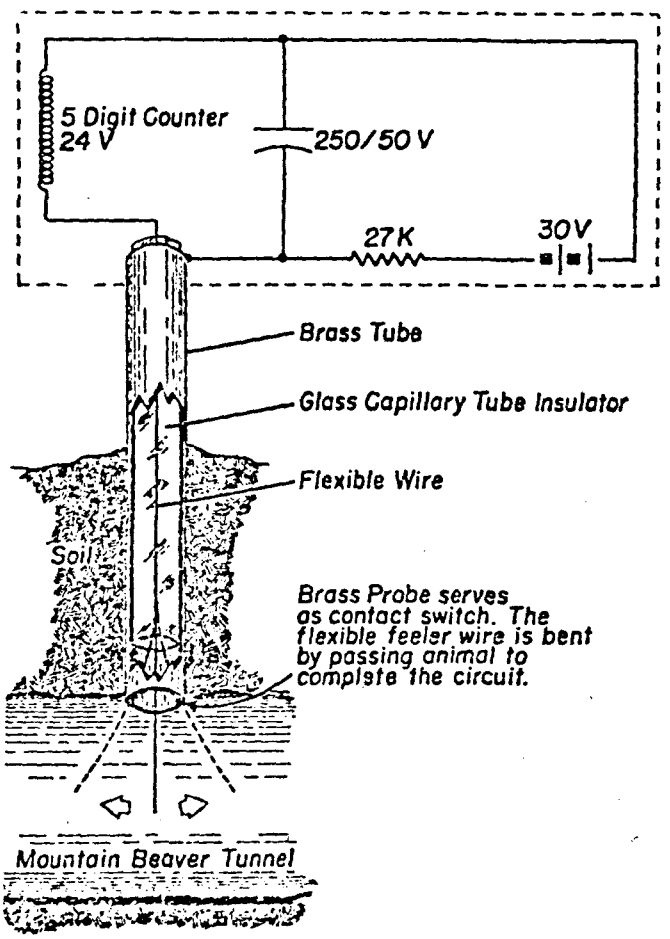


Figure 28. Wiring diagram of traffic counter. Mountain beaver burrow with pictorial drawing of burrow probe in place.

: (1097)

Various methods of dosing Coturnix with toxic substances have been used to determine (a) the effects on sleeping time and (b) if the route of exposure made any difference in sleeping time (384, 398, 403).

Mallard and black ducks, though closely related species, have been shown to have striking differences in their responses to different photoperiods (194). Differences due to circadian rhythms have been demonstrated in drug-induced mortality (1159) and heart rate (1158) in mice and rats. The effects of photoperiodism on reproductive cycles have been studied in birds (254).

Most of these methods did not deal directly with toxicity testing. However, they point out various aspects of animal behavior that should be taken into account when an animal is used in such testing. Even though these methods were used on a limited number of species, there seems to be no reason why they could not be applied to all vertebrate species and some invertebrates.

Evaluation - The general behavior of most species can be monitored and specific alterations caused by toxic substances can be measured. The results may be extrapolated to wild populations to evaluate the effect of the toxic substances on their viability.

Instinct

Instinct is defined as behavior that is innate, not learned. Because of its universality and its uniformity within a species, instinct can be a useful parameter in measuring the effects of toxic substances.

The reaction of Gallinaceous chicks to frightening stimuli has been measured in terms of the distance and duration of the chicks' movement away from the stimuli (1135). For pictures of the apparatus used, see Figure 29. Another test of Gallinaceous chicks exposed the chicks to a tape recording of a hen's call and measuring their speed of response (1135). For pictures of this apparatus, see Figure 30.

Spiders have also been used in studies of instinct. Their webs were photographed, measured and compared by computer to controls. /For pictures of the examination table used, see Figure 31. For pictures of the photographic setup used, see Figure 32. A general discussion of methods of preparing webs for photography and of calculating the

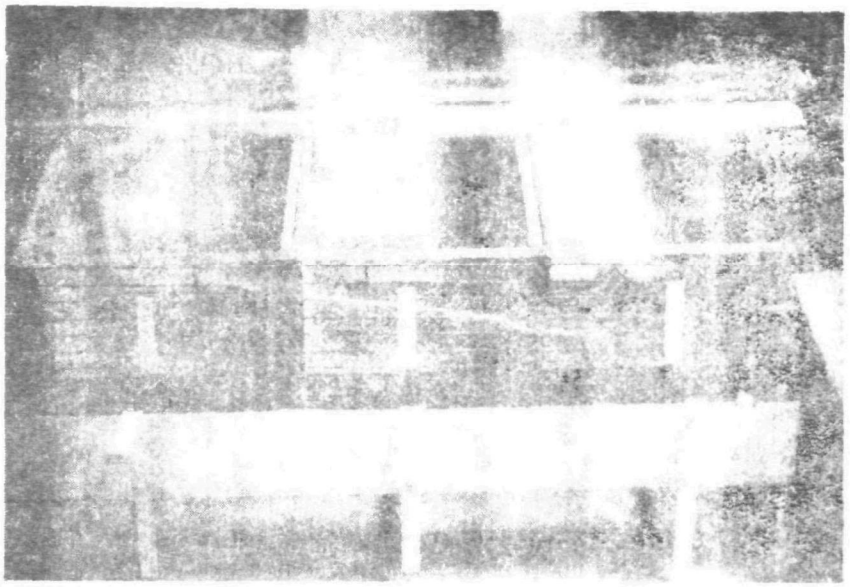


Figure 29(a). Runways with frightening device at one end.

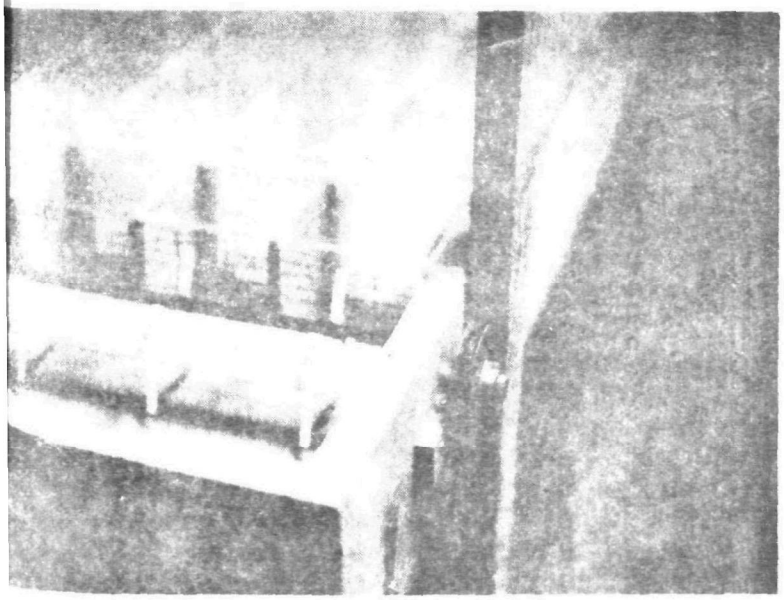
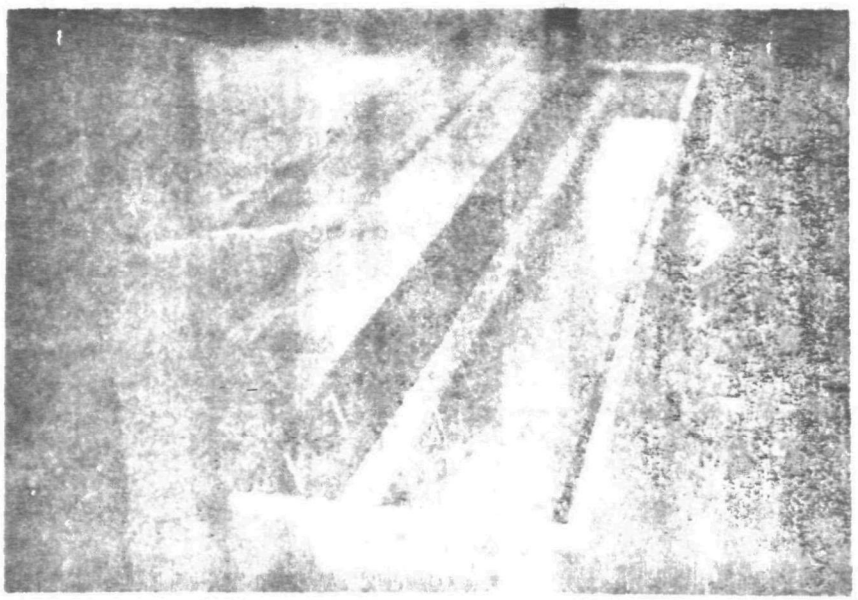


Figure 29(b). Runway mechanism for operating frightening device.



igure 29(c). Runways, rightening device, and amera used to record hick's response.

ource: (1135)

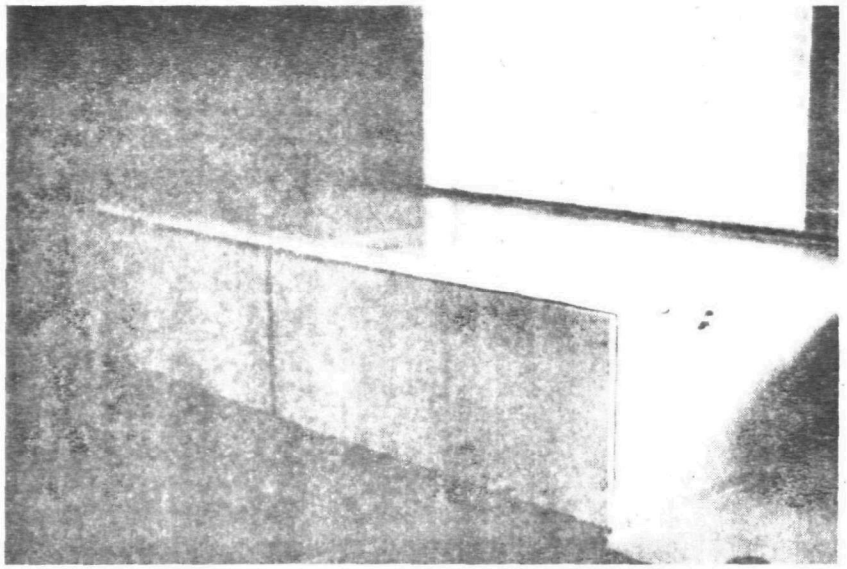


Figure 30(a). Enclosure for 10 runways.

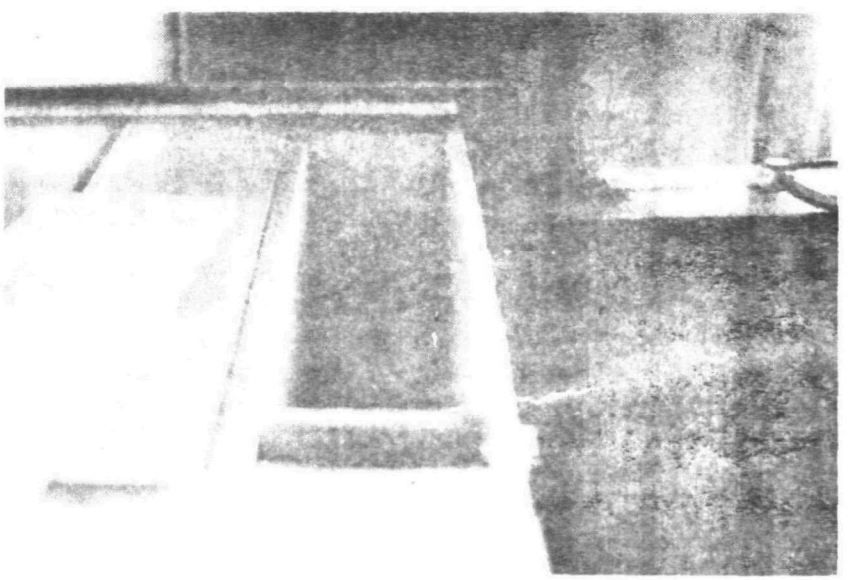


Figure 30(b). Runway with speaker at far end.

Source: (1135)

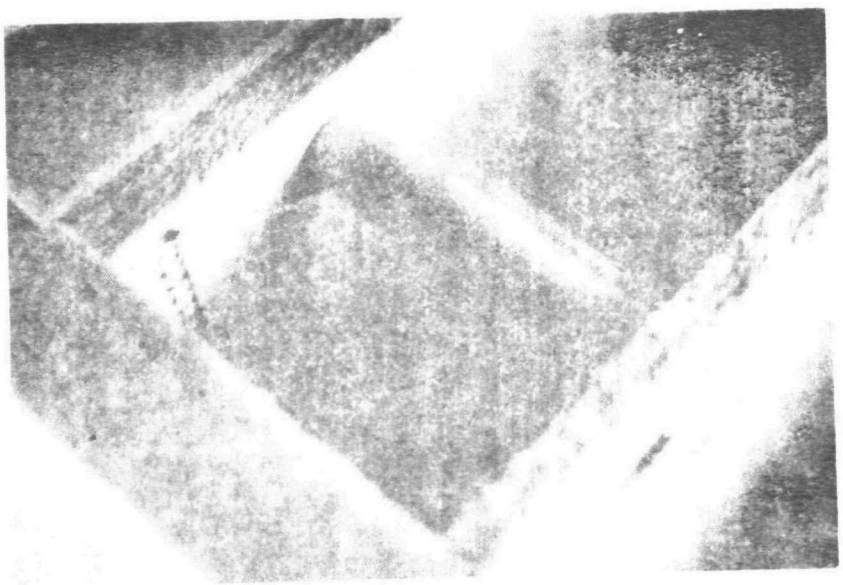


Figure 30(c). Holding area - point of release.

Source: (1135)

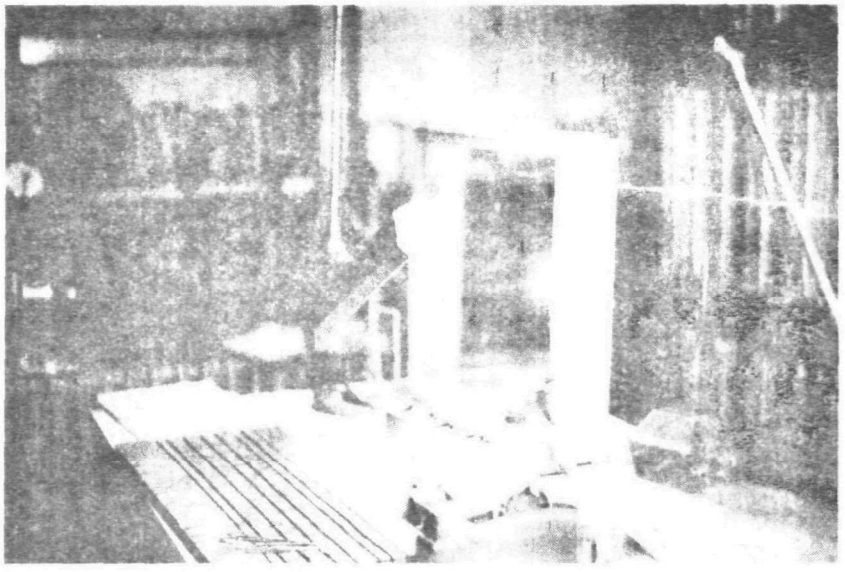


Figure 31(a). Examination table prior to photographing web.

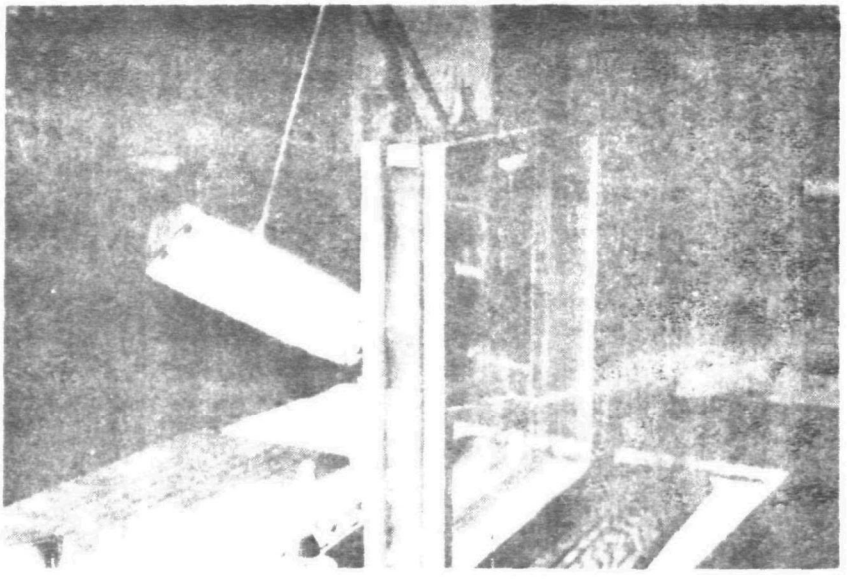


Figure 31(b). Examination table prior to photographing web.

Source: (53)

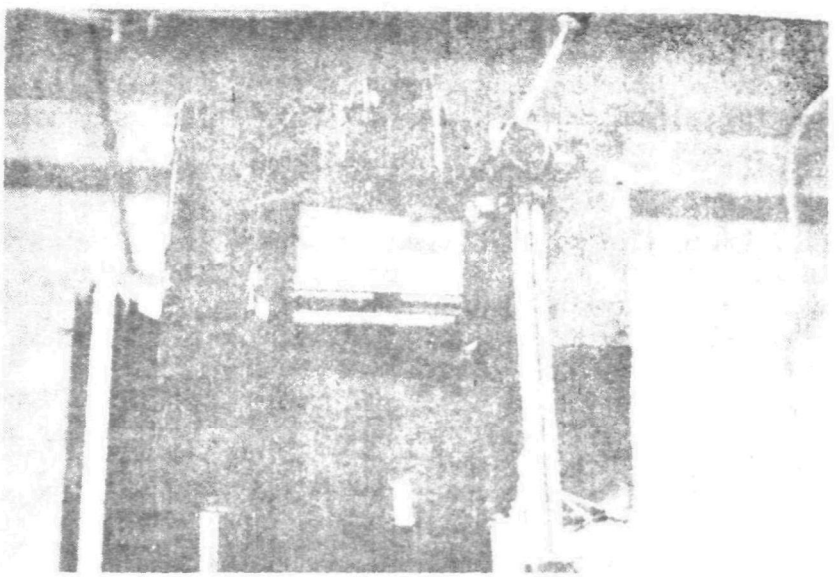


Figure 32. Apparatus for photographing web.

Source: (53)

changes in structure of the webs is found in 1124. A discussion of the variables that affect web building and web building's applicability to pharmacological testing is also included. Reference 53 describes a study of spiders exposed to drugs and allowed to spin webs.

Evaluation - Innate behavior is uniform in a species and universal in the animal kingdom. It can be used to test an animal's viability when exposed to toxic substances. For example, behavior related directly to mating (e.g., courtship displays in grouse, ducks) could be monitored (perhaps in cage field enclosures). Instinctual behavior related to nesting (all birds), migration (ducks), territoriality (Dipodomys), maternal care of young (birds and mammals), and breeding (all species) could also serve as parameters.

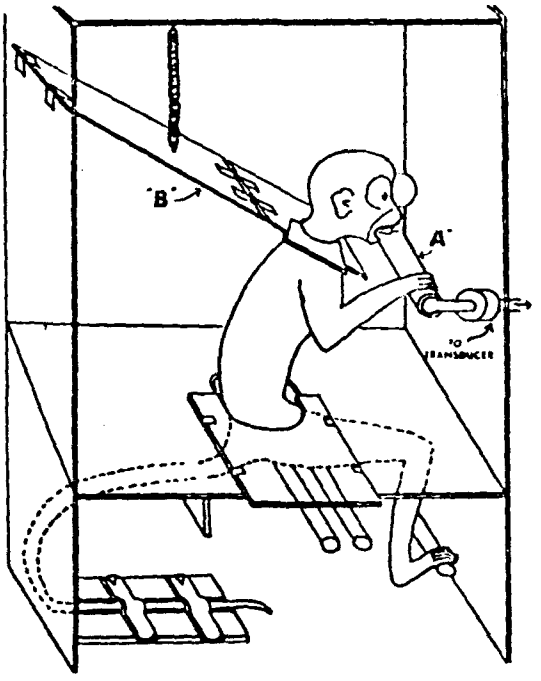
Learning

In the laboratory, learning is studied by teaching tasks or skills to a subject, then testing its ability to accomplish them. Data are available on learning for many species and experiments concerned with the effects of toxic substances on learning are easily carried out.

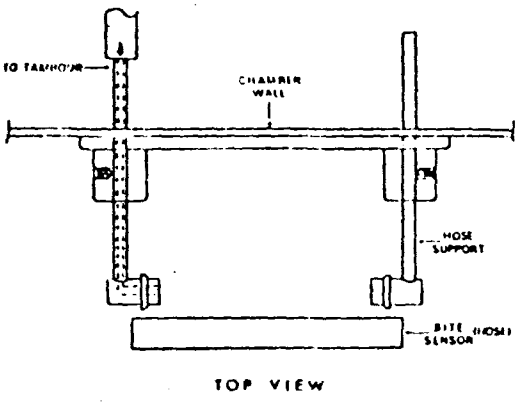
The effects of various drugs on mice have been studied via the learned skill of walking a revolving rod (1173). Rats have been tested in a Skinner box, drugged, then retested (547, 1165, 1171).

Rats and mice have been trained and then tested to determine their reaction to increasing amounts of toxic vapor (1168). Rats have also been drugged, taught and then retaught without drugs and their performances compared (1164). Rats have been injected with an endotoxin and a dose-dependent decrease in activity noted (1163). Pavlovian procedures have been used to produce aggression in rats as a response to a tone stimulus (1162). Gerbils have been taught discriminated avoidance response to a Skinner box (1166). Opossums have been taught to solve the Guthrie-Horton puzzle box (1189). They were also taught to give an avoidance response (1190). For an automated method for studying aggression in primates (1185) see Figure 33. Primates were also trained, given toxic substances, and impaired operant behavior was noted in all cases (104, 547, 1184). Sheep were trained, then electrodes were surgically implanted in their skulls and they were orally dosed with a capsule of toxic material daily. Their general behavior and results of post-operation tests were evaluated (1179). Coturnix chicks were fed toxic substances and changes in their

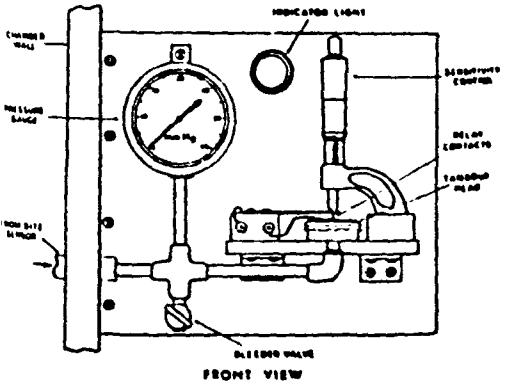
Figure 33. An automated method for studying aggression in primates.



a. Bite Sensor and Hinged Neck Yoke



b. Bite Sensor Assembly



c. Biting Transducer Assembly

The biting sensor is a piece of pure gum rubber, general purpose pressure-vacuum laboratory hose (E. H. Sargent and Co., Catalogue No. S-73515, size F). Its outer diameter is 5/8 in. and the wall thickness is 1/8 in. The ends of the hose were forced over 1/2 in. diameter brass nipples, which were soldered at right angles to the 1/4 in. brass hose supports. These hose supports were long enough to allow the hose assembly to be moved toward or away from the monkey's face. One of the support arms was drilled out to permit transmission of pressure changes to the biting transducer.

Source: 1185 Copyright 1966 by the Society for the Experimental Analysis of Behavior, Inc.

avoidance responses were noticed (380). The effects of drugs on mixed reinforcement tests were studied in pigeons and it was found that endogenous stimuli were affected (264). The effects of an amnesia producing drug on repeated reversals of discriminatory testing were also studied in the pigeon (1134). A general study of conditioned and unconditioned aggression without the use of toxic substances was also undertaken with the pigeon (269). A general study of factors that influence learning has been done with land snails (42).

Evaluation - Learning involves the acquisition of a task or skill or other capability on the part of the subject. The ability to respond or to perform the task can be hindered by exposure to toxic chemicals and the degree of confusion or change. Also, the ability to learn new tasks can be tested on dosed subjects and controls and the effects of exposure on the learning process can be evaluated.

This type of testing can reveal an animal's ability to cope in its natural environment and can conceivably be performed on any animal that can learn.

SUGGESTED METHODOLOGIES

Introduction

Scientific experimentation is designed to produce results that prove or disprove a hypothesis. The general hypothesis in toxicity testing is very often that the test chemical is adversely affecting an animal or animal species. Consequently, the methods used must distinguish toxic effects.

After years of study, many environmental toxicologists have concluded that any change caused by a chemical that alters, injurs, reduces, or otherwise affects the immediate or long term reproductivity of a species is a significant toxic effect; reproductive fitness is the major factor determining the success of a species (20, 822). The parameters used to determine the effect of toxic substances on reproductivity can include decreased reproductive life span, sterility, reduced litter/clutch size, early mortality, behavioral changes affecting intra and interspecific communication and so on.

A distinction must be made between toxic effects which can be correlated with decreased reproductivity and those which are not or cannot. For example, residue analyses and bioassays are useless unless they can be correlated with acute toxicity, chronic toxicity or decreased reproductivity. LD_{50}'s often do not reflect environmental conditions because concentrations of the test chemical do not match those found in nature nor do the routes of exposure used fit the exposure routes found in natural environments.

Suggested Approaches to Toxicity Testing

The methods selected in this report yield effects that are directly or indirectly related to the reproductive fitness of a species. The list does not include all possible test methods, but was assembled using the information found in the literature and the recommendations of environmental toxicologists.

A list of selected toxicity testing methods is presented in Table 27. (See evaluations of each method in previous section for further discussion.) To obtain comprehensive results, most methods should be used with both adults and juveniles. Some methods, however, are suited for embryos; this is specified under the heading "Routes of Exposure." Routes of exposure were selected to best reflect those that

Table 27. SELECTED APPROACHES TO TOXICITY TESTING

Suggested Test Method	Suggested Species	Suggested Route of Exposure	Level of Toxic * Response
Physical/Chemical			
Respiration - Ciliar Transport	all species	vapor	C
Population Dynamics - Observe/Monitor	all species	oral-topical	A,B
Genetic	Drosophila, mice Rana pipiens	all routes (adults & embryos)	D
Anticholinesterases	all species	oral	D
Metabolism - Enzymes	all species	oral-topical	D
Endocrinology	all species	oral-topical	C,D
Anatomy	all species	oral-topical	C
Hematology	all species	oral-inject	C,D
Histology	all species	all routes	C,D
Embryology - Oology	oviparous species opossum	inject-oral (adults & embryos)	A,B,C
Behavioral			
Discrimination	vertebrate species	oral-topical vapor	A,B
Stress			
isolation	social species (e.g. mice,bees)	oral-topical vapor	A,B
temperature	all species	oral-topical	A,B

Table 27 (cont.). SELECTED APPROACHES TO TOXICITY TESTING

Suggested Test Method	Suggested Species	Suggested Route of Exposure	Level of Toxic* Response
Stress (cont'd)			
disease	all species	oral-topical	A,B
food shortage	all species	oral-topical	A,B
Instinct	all species	oral-topical vapor	A,B
Learning	vertebrate species	oral-topical vapor	A,B

*See Figure 34

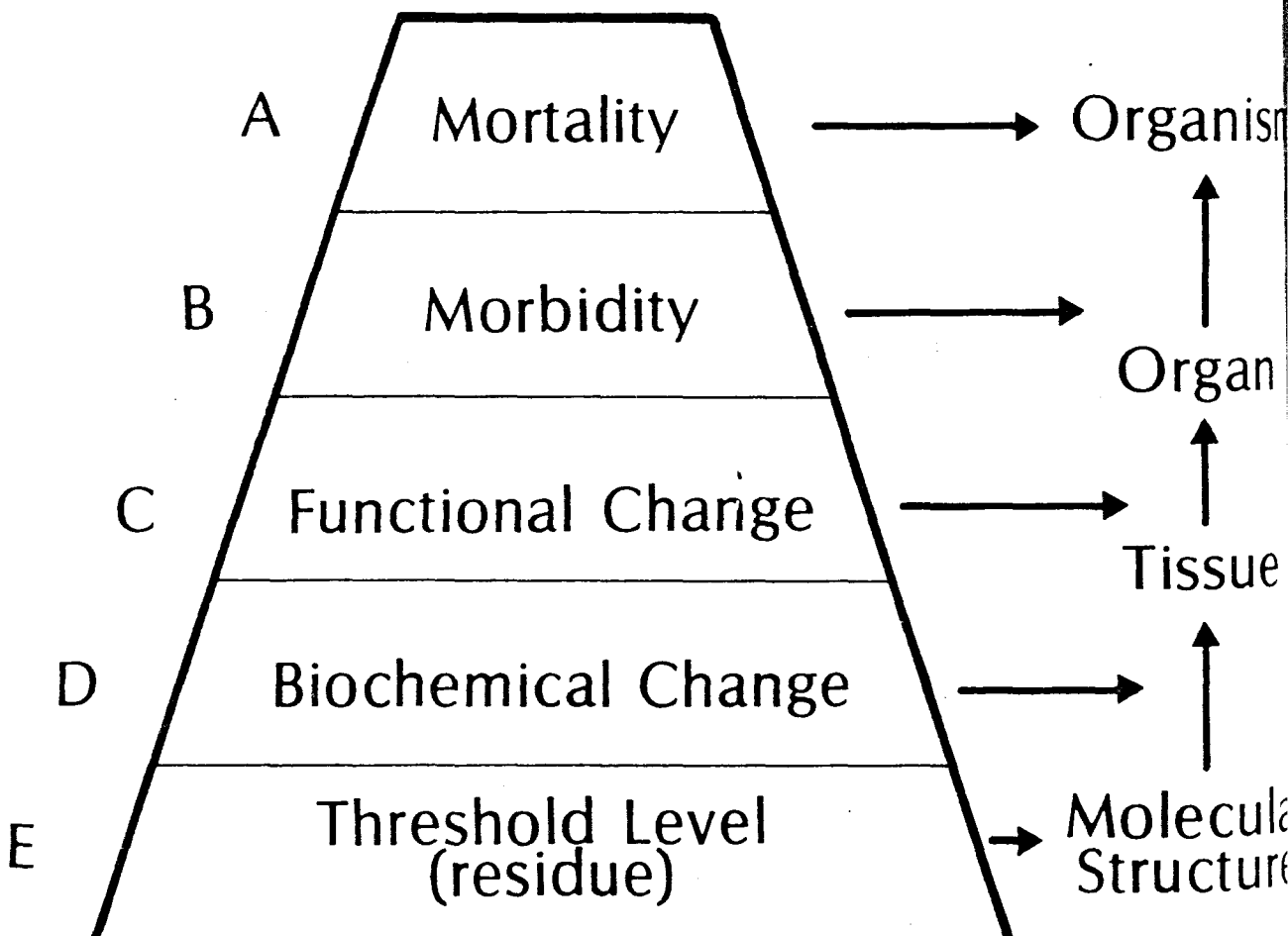


FIGURE 34

Succession of Toxic Response

may occur naturally. Figure 34. Succession of Toxic Responses, is keyed to Table 27 to show which methods are best suited for investigating toxic effects at each level of response.

General Studies

Long term generation studies can use aspects of all selected methods, and can be conducted on any species that is readily bred under laboratory conditions (See final list in Suggested Species). When short generation times are desired, insects (Drosophila, Musca), mice (Mus, Peromyscus), voles (Microtus), rice rats (Oryzomys), and Japanese quail are recommended. Generation studies are important for toxicity testing because their results are directly applicable to the long term reproductivity of animals. The costs and time involved are considerable, but the results are well worth the effort. Previous experimental work can be used as a guide to further study (see reference 822).

The Selection of Methods for Toxicity Testing

There are two important decisions to make in testing of toxic chemicals: (a) choosing the test that will tell the most about the specific chemical substance; and (b) choosing the animals that will be used for subjects. These decisions are frequently interrelated.

Selection of test method should be made in light of previous work with chemicals of the same class as the test substance. The test selected should give preliminary information. For example, chemical A is a member of a class of compounds which are known or suspected of being neurotoxic, but the biochemical mechanism is unknown. These chemicals are also contaminants of forested lands and are taken into plants in large quantities. Initial testing should then utilize herbivores of forest inhabitats and, because chemical A may be neurotoxic, behavioral tests applicable to these species should be used in conjunction with neurophysiological-biochemical lab work to determine the mechanism of action.

Certain approaches to toxicity testing require basic knowledge about the test animal and its special characteristics (see Table 27). For example, mutagenicity testing can most easily be done on genetically well-known animals (e.g. Drosophila) while dosing embryos in teratogenic experiments is best accomplished with easily accessible embryos (e.g. those of oviparous species). On the other hand, if a route of exposure is available, most tests can be performed on any species.

A description of the levels of toxicity testing is provided in Figure 34. The organism as a whole and survival are of primary importance while morbidity research, functional studies, biochemical analyses and residue determinations are sublevels of interest. The survival of each individual is dependent on what occurs at each lower level and testing at each one is a way of obtaining information about the whole organism. All information which can be related to the survival of individuals can be a means of assessing what will happen at the population level.

The level selected for testing should be chosen on the basis of known information about the chemical and the suitable species.

State-of-the-Art

Feelings are mixed throughout the scientific world about the state-of-the-art of toxicity testing. Some experts in environmental toxicology feel that the field is on a par, scientifically and technically, with most of the biologically related disciplines. On the other side, there are scientists who feel that present efforts fall short of sufficiency and that more areas of testing should be introduced to the field. They cite the lack of background residue data with which most testing is done as one example of inadequate testing. They advocate the routine inclusion in preliminary work of total in - total out, total residue and clearance work and 100 percent of the dose accounted for after exposure. Other suggestions and criticisms follow. They summarize the general feelings we have encountered in the literature and from contact with scientists.

1. testing juveniles as well as adults (all age classes)

2. more tests required on species actually affected by the test chemical in natural situations, not just lab animals

3. increase federal funds for environmental toxicology research and education programs

4. more long term generation, chronic, and subacute, studies for wild species

5. poor knowledge of test animals' background (i.e. possible previous exposure) before testing

6. most tests performed in the past were not sensitive to

the effects of test chemicals on the animal (e.g. LD_{50}, residue) and prove little more than exposure

7. failure of test methods, especially routes of exposure, to coincide with actual concentrations and rates encountered in nature

The Future

At the present time there is research taking place in an attempt to develop a computerized methods of quantitatively analyzing the biochemical structure of drugs and predicting from that structure, the mechanism of their action, without microscopic knowledge of complex processes occurring in vivo. This is theoretically possible because all compounds have a chemical structure which determines their physical activity or reactions with other compounds. If The Hansch Approach, as this is called, ever becomes a usable system, it could concievably be adapted for use with whatever class of chemicals one desires, including toxic chemicals. However, the difficulties in the development and operation of such a system are immense and much further research is necessary before the Hansch approach can be applied.

Another approach to testing which appears to have greater feasibility is the use of model ecosystems for toxicity testing. The EPA labs in Corvallis, Oregon, are putting forth a major effort to build systems that can be readily set up for widespread use. These systems are being designed for specific problems or for areas where certain chemicals are applied (e.g. agricultural systems and pesticides). The problems associated with model ecosystems and toxicity testing are presented in the Special Problems Section of this report.

Many environmental toxicologists feel that the future of toxicity testing should include a greater standardization of testing methods/procedures and a greater emphasis on the effects of chemicals on natural animal populations. LD_{50} and residue studies are of preliminary importance, but the subacute physical, chemical and behavioral effects of realistic doses administered under conditions found in the environment on animals that are actually affected are now in order and have been initiated in various institutions around the country and the world. We must learn the sublethal effects of chemicals in order to determine the overall hazard they present and to quantitatively and realistically predict the level of toxicity of any chemical that is applied to our environment.

APPENDIX A

CONTACTS AND CONSULTANTS

CONTACTS AND CONSULTANTS

	EXPERTISE	ADDRESS
James Akerman	Wildlife Biologist	EPA Ecological Effects Branch Washington, D.C.
Richard Aulerich	Mustelid Toxicology	Department of Poultry Science Michigan State University East Lansing, MI
Mr. Woodrow Benson	Pesticide Monitoring	Director Idaho Community Pesticide Project State House Boise, Idaho 83720
Joel Bitman	Avian Toxicology	Agricultural Research Station USDA Beltsville, MD
Woodrow Benson	Pharmacology	Professor Department of Physiology College of Veterinary Medicine Washington State University Pullman, Washington
William Buck	Behavioral Toxicology	Director Behavioral Toxicology Laboratory Veterinary Medicine School Iowa State University Ames, Iowa 50010
Ron Borchard	Psychologist-Herpetologist	University of Tennessee Knoxville, TN

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
James Cannelli	Avian Toxicology	Massachusetts Coop Wildlife Research Unit Amherst, MA
Charles Cargille	Model Ecosystems	NICH NIH Bethesda, MD
Helene C. Cecil	Avian Toxicology	Agricultural Research Station USDA Beltsville, MD
Richard Crawford	Pathology/ Epidemiology	Professor Diagnostic Pathology College of Veterinary Medicine Washington State University Pullman, WA
Cirpriano Cueto	Toxicologist	National Center of Toxicology Research Pine Bluff, AR
Armondo de la Cruz	Effect of Pesticides on isopods & grasshoppers	Professor Department of Zoology Mississippi State University State College, MS 39762
William Diechman	Beagle Toxicology	University of Miami Medical School Coral Gables, FL
Richard Dorn	Veterinarian Toxicology/ Epidemiology	Professor Department of Veterinary Micro Veterinary Medicine School University of Missouri Columbia, MO

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
Virgil Freed	Environmental Toxicologist	Environmental Health Science Center Oregon State University Corvalis, OR 97331
Milton Friend	Avian Toxicology	Denver Wildlife Research Center Federal Center Denver, CO
A. R. Gaufin	Limnology	Professor Department of Zoology University of Utah Salt Lake City, UT
Jerry B. Graves	Avian Toxicology	Louisiana State University Baton Rouge, LA
D. Gerberg	Insect Toxicology	Insect Control and Research Baltimore, MD
Yvonne Greichus	Biochemistry/ Wildlife Toxicology	Experimental Station South Dakota University Brookings, SD 57006
Max A. Haegele	Avian Toxicology	Denver Wildlife Research Center Federal Center Denver, CO
R. L. Harris	Veterinary Toxicology/ Pesticides	Research Leader Veterinary Toxicology and Entomology Laboratory USDA P. O. Box GE College Station, TX 77840

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
Dale Hattis	Genetic Toxicology	Case Western Reserve University Cleveland, OH
Robert Heath	Avian Toxicology	Patuxent Wildlife Research Center Laurel, MD
Glenn A. Hood	Rodenticide, Repellent and Chemosterilant Development	Denver Wildlife Research Center Federal Center Denver, CO
Robert Horton	Senior Research Health Advisor	EPA Research Triangle Park, NC
Howard L. Hunt	Insect Toxicology	EPA ARC-East Beltsville, MD
David Hutchenson	Nutrition/ Toxicology	Associate Professor Veterinary Medical School Sinclair Research Farm University of Missouri Columbia, MO
Harold Kaplan	Toxicology/ Physiology	Southern Illinois University Carbondale, IL
Philip Kearny	Avian Toxicology	Agricultural Research Station USDA Beltsville, MD
Irving Klaus	Avian Toxicology	Patuxant Wildlife Research Center U.S. Fish & Wildlife Laurel, MD

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
George Levinskas	Avian Toxicology	Monsanto Corporation St. Louis, MO
Mark Lewis	Behavioral Toxicologist	FAA Civil Aeromedical Institute AAC - 1186 6 P.O. Box 25082 Oklahoma City,OK 73125
Ray Linder	Effects of Pesticide Residues on Pheasant Population	South Dakota Cooperative Wildlife Unit South Dakota State University Brooking, SD 57006
Keith R. Long	Environmental Toxicology	Director Institute of Agricultural Medicine University of Iowa Iowa City, IA 52241
William Lower	Genetics	Environmental Trace Substance Center University of Missouri Columbia, MO
John Luteman	Supervisor or Director - Beltsville Laboratories	Building 402 ARA - East EPA Beltsville, MD
John McCanne	Rodenticide Research	EPA Beltsville, MD
R. L. Metcalf	Pesticide & Aquatic Model Ecosystems	Professor Department of Entomology University of Illinois Urbana, IL 61801

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
David Mick	Environmental Toxicologist	Department of Environmental Quality State of Iowa Des Moines, IA
Howard L. Morton	Toxicologist-Honeybees	Agricultural Research Station USDA Tucson, AZ
John Mulder	Director, Animal Laboratory Resources	Michigan State University East Lansing, MI
L. D. Newsom	Environmental Toxicology	Louisiana State University Baton Rouge, LA
John J. Raisweiler	Bats - Breeding & Maintenance	Department of Medicine Columbia University New York, NY
Jane F. Robens	Toxicologist	Hoffman-LaRouche Nutley, NJ
John Santolucito	Behavioral Toxicity	National Environmental Research Center EPA Research Triangle Park, NC
Peter Savarie	Pest Control Evaluation	Denver Wildlife Research Center Federal Center Denver, CO
Ed Schafer	Bird Damage Control and Chemical Development	Denver Wildlife Research Center Federal Center Denver, CO

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
Dale Schwindamer	USDA Registry List of Animal Care Facilities	USDA Washington, D.C.
Joan Spyker	Rodent Toxicology	University of Virginia Medical School Charlottesville, VA
William Stebbins	Oto Toxicologist	Kresge Hearing Research Institute University of Michigan College of Medicine University of Michigan Ann Arbor, MI 48104
Lucille Stickel	Avian Toxicology	Patuxant Wildlife Research Center U.S. Fish & Wildlife Laurel, MD
William Stickel	Avian Toxicology	Patuxant Wildlife Research Center U.S. Fish & Wildlife Laurel, MD
Gary Van Gelder	Behavioral Toxicology	Chairman Department of Anatomy and Physiology Veterinary Medical School University of Missouri Columbia, MO
S. Bradleigh Vinson	Entomologist	Texas, A & M College State, TX
Ralph C. Wandes	Director, Toxicity Information Center	National Academy of Science

CONTACTS AND CONSULTANTS
(continued)

	EXPERTISE	ADDRESS
Bernard Weiss	Behavioral Toxicology	University of Rochester Rochester, NY
Larry Wing	Wildlife Management/ Pesticide Residues	Associate Professor Department of Zoology Science Hall II Room 105 Iowa State University Ames, IA
Peter N. Witt	Spiders - Drug Testing	Division of Research North Carolina Department of Mental Health Raleigh, NC
Robert Wolf	Infectious Disease	Delta Primate Research Center Tulane University Covington, LA 70435
James C. Yarbrough	Effect of Pesticides on Aquatic Vertebrates	Professor Department of Zoology Mississippi State University State College, MS 39762
Robert Yeager	Executive Secretary of Animal Division	National Academy of Science Washington, D.C.
Ray Zimmerman	Avian Research	Ralston Purina Co. St. Louis, MO

APPENDIX B

LIST OF SUPPLIERS

SCIENTIFIC	COMMON	KEY
INVERTEBRATES		
Lumbricus terrestris	earthworm	CON, GEL, HER, LEM, NBL, STE
Helix sp	land snail	STE
Gryllus sp	cricket	LEM, MAZ, NBL, STE
Apis melliferra	honey bee	STE
Drosophila sp	fruit fly	CON, GEL, MAZ, NBL STE
Praying mantids		MAZ, STE
Tenebrio sp	mealworm	CON, GEL, LEM, MAZ, NBL, STE, TAR
Musca domestica	housefly	MAZ, STE
HERPETOFAUNA		
Rana pipiens	Leopard frog	BEI, CON, GEL, HOF, LAJ, LEM, MAZ, NBL, NIC, SHE, STE, TAR, WES
Ambystoma mexicanum	Mexican axolotl	HAC
Chelydra serpentina	Snapping turtle	LEM, MAZ, STE, ZOO
Terrapene sp	box turtle	LEM, MAZ, STE, TAR, ZOO
Anolis carolinensis	Anole	CHC, GEL, HER, LEM, MAZ, TAR
Phrynosoma cornutum	horned lizard	MAZ
Thamnophis sirtalis	garter snake	CHC, MAZ, PET, SAI, STE, TAR, WHI
Elaphe sp	rat snake	PET, WHI, ZOO
BIRDS		
Anas platyrhynchos	mallard	ADV, MAZ, TRS
Branta canadensis	Canada goose	ADV, MAZ, TRS
Ardeola sp	egret	SAI
Tyto alba	barn owl	CHC, MAZ
Columba sp.	pigeon	ANM, LEM, MAZ, RAI, RID, STE, TOT, TRS
Colinus virginianus	bobwhite	BRN, MAX, THR, TRS
Phasianus sp	pheasant	RED
Sturnis vulgaris	starling	BRN

Source: 7

(CONTINUED)

SCIENTIFIC	COMMON	KEY
BIRDS (continued)		
Passer domesticus	house sparrow	MAZ
Gallus domesticus	chicken	BAB, COF, HAB, MAZ, RED, THR, TOW
MAMMALS		
Eptesicus fuscus	big brown bat	MAZ, NIC, ZOO
Sylvilagus sp.	cottontail	ANM, MAZ
Dipodomys sp.	kangaroo rat	PET, PFI, RID, WHI
Neotoma lepida	pack rat	MAZ
Sigmodon hispidus	cotton rat	PET, RID
Spermophilus beecheyi	California ground squirrel	LOK, MAZ, RID, TOT
Tamias striatus	Eastern chipmunk	RID, TOT, WHI, ZOO
Eutamias sp.	Western chipmunk	ZOO
Peromyscus maniculatus	deer mouse	MAZ, PET
Myocaster coypus	nutria	ANM, CHC, MAZ
Mustela vison	mink	mink ranches
Ovis sp.	sheep	ADV, BAR, BUB, CLA, STM
Sus scrofa	swine, miniature	HRL, LIT, MAZ, TRF, VIV
Saimiri sciureus	squirrel monkey	BRP, FDI, PRL, RID, SDA, WAC, WHI, ZOF, ZOO
Didelphis virginianus	opossum	ANM, MAZ, RID, SAI
Daysypus novemcinctus	armadillo	ANM, BRN, CHC, FRE, LOK, MAZ, PFI, RID, SAI, TAR, ZOO

(CONTINUED)

ADV Adventure Hill
Edith Tramutola
R.F.D.
Clinton, New Jersey 08809

ANM Animals, Inc.
Dr. Jon Kenneth Ferguson
Route 1, Box 395
Wharton, Texas 77488

BAB Babcock Poultry Farm, Inc.
Dr. Jack F. Hill
P.O. Box 280
Ithaca, New York 14850

BAR Bar-Wan Rabbitry & Kennels, Inc.
L. M. Barnfield
Route 1, Box 60
Crocker, Missouri 65452

BEI Beaumanor Farms
Thomas G. Herrick
1712 Sheridan Road
Cleveland, Ohio 44121

BRN Bronson Tropical Birds
Julien Bronson
70 Nagle Avenue
New York, New York 10040

BRP Blue Ribbon Pet Farm, Inc.
John H. Marolf
8772 S.W. 131st Street
Miami, Florida 33158

BUB Richard Bubolz
Route #2
Rio, Wisconsin 53960

CHC Charles P. Chase Company, Inc.
Charles P. Chase
7330 N.W. 66th Street
Miami, Florida 33166

CLA George Clauss
18-19 Saddle River Rd.
Fairlawn, New Jersey 07410

COF Colonial Poultry Farms, Inc.
M. R. Irwin
Pleasant Hill, Missouri 64080

CON Connecticut Valley Biological
Supply Co., Inc.
Michael L. Taylor
Valley Road
Southampton, MA 01073

FDI Fauna Distributors, Inc.
Miguel A. Campo
2286 N.W. 36th Street
Miami, Florida 33142

FRE Alton V. Freeman
Spruce Pine, NC 28777

GEL CCM: General Biographical, Inc.
Order Desk
8200 South Hoyne Avenue
Chicago, Illinois 60620

HAB Hall Brothers Hatchery, Inc.
Mrs. A. B. Hall
200 Cook Hill Road
Wallingford, Connecticut 06492

HAC Hacienda Aquatics
John A. Kopec
P.O. Box 218
La Puente, CA 91747

HER Hermosa Reptile and Wild Animal
Farm, Inc.
Charles McClung
P.O. Box 182
219 Pacific Coast Highway
Hermosa Beach, CA 90254

HOF E. G. Hoffman & Son
Earl A. Hoffman
P.O. Box 815
Oshkosh, Wisconsin 54901

HRL The Hormel Institute, University
of Minnesota
J. Hobart Belknap
801 16th Avenue N.E.
Austin, Minnesota 55912

LEM The Lemberger Company
Sheldon Antall
1222 W. South Park Avenue
Oshkosh, Wisconsin 54901

LIT Norman B. Little
P.O. Box 382
Rocky Hill, Connecticut 06067

LOK Otto Martin Locke
P.O. Drawer 731
New Braunfels, TX 78130

MAZ George Mazur Enterprises, Inc.
Ben Sherman
77 Eye Street, S.E.
Washington, D.C. 20003

NBL National Biological Laboratories, Inc.
Frank S. Blasdell
P.O. Box 511
236 Dominion Road
Vienna, Virginia 22180

NIC J. C. Nicholls, Jr.
Biological Collector
Murphy, NC 28906

PET The Pet Corral
Norman Nye
4146 Oracle Road
Tucson, Arizona 85705

PFI Pet Farm, Inc.
Dr. B. M. Levine
3310 NW South River Drive
Miami, Florida 33142

PRL Primelabs, Inc.
Dr. James H. Vickers
Monmouth County Airport
Farmingdale, NJ 07727

(CONTINUED)

RAI Research Animals, Inc.
Norman or Pete Weissman
Box 405
Braddock, PA 15104

RED Redwood Game Farms and Laboratories
David G. or Norma S. Lewis
1955 North Redwood Road
Salt Lake City, UT 84116

RID Rider Animal Company, Inc.
V. D. Rider
R.R. #2, Box 270
Brooksville, FL 33512

SAI Safari Animal Imports, Inc.
Frederick W. Thorpe
7703 N.W. 36th Avenue
Miami, FL 33147

SDA San Diego Animal Supply
James M. Robinson, Jr.
P.O. Box 544
Lemone Grove, CA 92045

SHE J. R. Schettle Biologicals
J. R. Schettle
P.O. Box 184
Stillwater, MN 55082

STM Stumbo Farms
Donald L. Stumbo
O'Neil Road
Lima, NY 14485

TAR Tarpon Zoo, Inc.
Trudie Jerkins or Fred Penman
P.O. Box 847
Tarpon Springs, FL 33589

THR Three Springs Kennel Company, Inc.
W. J. or Paul R. Haas
R.D. #1
Zelienople, PA 16063

TOT Tote Em In Zoo
Mr. Tregembo
Route 2, Box 368
Wilmington, NC 28401

TOW Town Line Poultry Farm
Henry E. Geerlings
4198 96th Avenue
Zeeland, MI 49464

TRF Thompson Research Foundation
Crosby L. Brownson
Box 97, Postal Route #1
Monee, IL 60449

TRS Truslow Farms, Inc.
John U. Truslow
Chestertown, MD 21620

VIV Vita Vet Laboratories
Forest E. Conder
1600 West 26th St.
Marion, IN 46952

WAC Woodard Asiatic Corporation
Dr. Geoffrey Woodard
12310 Pinecrest Toad
Herndon, VA 22070

WES Weston Research Laboratories, Inc.
Fritz Oyen
Route 1, Box 33
Purcellville, VA 22132

WHI White Animal Farm
Helen D. Perley
Seavey's Landing Road
West Scarboro, ME 04074

ZOF Zoological Fauna
J. Patrick McHale
1526 West Highland Avenue
Chicago, IL 60626

ZOO Zoological Center, International
Ray Pawley
15W506 W. 63rd Street (Burr Ridge)
Hinsdale, IL 60521

APPENDIX C

SUGGESTED SPECIES SYNOPSIS SHEETS

LUMBRICUS TERRESTRIS

Description Segmented, cylindroid invertebrate with body length from 6 to 12".

Availability Found in most moist soil areas of the world; readily available from biological supply houses and bait dealers.

Features Readily available; breeds easily in lab; easy and cheap maintenance; ecologically important.

Laboratory Light - natural photoperiod; temperature - 50°F for breeding.

Cage Any size container having drainage holes covered with screen and filled with gravel, dung, soil, and peat moss is sufficient for a limited number of worms; 4 x 8 x 2' bin can support 50,000 worms.

Food Peat moss, manure, leaf mold, sod, decaying organic material, chick starter, cornmeal; special needs include moisture and substrate mixture of pH 7.

Water Moisture necessary at all times.

Parasites & Disease

Breeding & Rearing Breeds well in loose substrate of one part dung, 3 parts soil, and 5 parts peat moss if space, moisture & temperature is optimal; eggs deposited in small cocoons throughout substrate.

Special Costs

Restrictions

Toxicological Studies 27, 28, 29, 30, 32, 33, 34, 35, 36, 37, 38, 39

Ecological Alternatives Snails.

Physiological Parameters

HELIX ASPERSA

Description Land snail; commonly called the garden snail; life expectancy-unknown.

Availability Found in temperate areas of world; relatively abundant in most moist areas.

Features Mainly nocturnal; can be bred and reared successfully in the laboratory.

Laboratory Temperature, 23°C; constant moisture required by active snails.

Cage Colonies kept in wood boxes, 55x40x28cm; 5cm layer of damp soil; box covered with aluminum screen; quart fruit jars with 5cm of damp soil in bottom can be used for breeding.

Food Lettuce and fresh vegetables.

Water Sufficient moisture must be present at all times.

Parasites & Disease

Breeding & Rearing Sexual maturity-4 months; avg. number of eggs laid-53 (about 10 eggs survive hatching); eggs are laid in 5cm of soil in bottom of jar and are 3mm in diameter; usually laid during wet season; photoperiod important in egg laying.

Special Costs

Restrictions

Toxicological Studies 42, 46, 47, 48, 49, 50

Ecological Alternatives Earthworms, some herbivorous insects.

Physiological Parameters

MILAX sp.

Description Shell-less mollusc, body 1/2 to 2" long.

Availability Some suppliers.

Features Snail-like, active day and night, produces large amounts of mucous.

Laboratory Light-natural photoperiod; temperature - should fluctuate around 20°; relative humidity - over 70%.

Cage Material - all glass aquaria, any large waterproof container; substrate - peat-soil.

Food Vegetables, such as carrots, potatoes.

Water Unchlorinated, unpolluted.

Parasites & Disease

Breeding & Rearing Reproduction stimulated by fluctuating temperature around 20°C.

Special Costs

Restrictions

Toxicological Studies

Ecological Alternatives Snails.

Physiological Parameters

ARANEUS DIADEMATUS

Description Small orb-weaving spiders about 2/5 to 1" long.

Availability Common orb-weaver of the Eastern U.S.; most dependable source of spiders is Mr. Leonard Pankhurst, 204 Stroud St., Canastota, NY, 13034 (52) .

Features Easy maintenance; ecologically important; web-building is a useful experimental tool.

Laboratory Light - 13L:11D photoperiod; temperature - temperature change important (colder at night).

Cage 50 x 50 x 10cm aluminum enclosures with removable glass windows suitable to study web patterns (52).

Food Flies, mealworms.

Water Provided by syringe administration or saturated cotton ball.

Parasites & Disease

Breeding & Rearing Minimal breeding in lab; cocoons of fall may be hatched in the lab under correct conditions.

Special Costs

Restrictions

Toxicological Studies 53, 1124

Ecological Alternatives Centipedes.

Physiological Parameters

GRYLLUS PENNSVLVANICUS

Description Dark-colored insect with an adult body length of 1/2" large hind legs adapted for jumping (57).

Availability Found in pastures, meadows, roadsides, fields and homes all over the U.S. (316); readily available through comercial suppliers and bait dealers.

Features Readily available; easy maintenance and breedability in lab.

Laboratory Light - 16L:8D photoperiod; temperature - 24°C; relative humidity - 60 to 65% (58).

Cage 42 x 18 x 18" stock aluminum cages for 250 adults; 11 x 7 x 8" glass aquarium for 500 nymphs; (Note: moist sand substrate should be avaiblable for oviposition).

Food Mixture of biscuit meal, dried milk, Bemax and dried yeast in ratio 50:5:40:5; greens should be supplied once a week (62).

Water Constant supply.

Parasites & Disease

Breeding & Rearing Some crickets breed all year while others breed once a year (58); oviposition should occur in moist sand; hatching may occur in petri dishes; incubation - 15 days at 24.0°C; hatching takes place after 3 month diapause period at 4.0°c (62).

Special Costs

Restrictions

Toxicological Studies 71

Ecological Alternatives Roaches, grasshoppers, praying mantids, predaceous beetles and other herbivorous insects.

Physiological Parameters

STAGMOMANTIS CAROLINA - praying mantid

Description Large, elongate, slow-moving insects with modified front legs (57); body length - about 2".

Availability Found mainly in the Southern U.S., readily obtainable from commercial suppliers.

Features Fairly available, good breedability in lab (61).

Laboratory Light - natural protoperiod; temperature - 75 to 80°F; relative humidity - 50 to 70%.

Cage Glass jar or box closed with small mesh screen and provided with small sticks and a potted house plant is sufficient for a pregnant female (61).

Food Any living insect of appropriate size, for example, live house flies, blue-bottle flies, grasshoppers, grubs.

Water Should be given to mantid everyday with medicine dropper.

Parasites & Disease Host for certain parasitic flies and some mites.

Breeding & Rearing Mantids lay a dozen to 400 eggs enclosed in ootheca; trees are ootheca deposition sites; eggs over winter and hatch in spring and early summer; eggs may be hatched in lab under correct light, temperature, and nutrition conditions.

Special Costs

Restrictions Cannabalistic tendencies.

Toxicological Studies

Ecological Alternatives Crickets, roaches, grasshoppers, other herbivorous insects, predaceous beetles.

Physiological Parameters

BLISSUS LEUCOPTERUS

Description Pest insect with body length of 3.5mm (57); nonflying.

Availability Found in wheat, corn rye and barley fields in U.S. with some abundance.

Features Fairly abundant; economically important; probability of easy maintenance and breedability in lab is good.

Laboratory Light - natural photoperiod; temperature - "room".

Cage No special cage requirement; Blissus may be reared in small glass tubes (as in done with milkweed bugs).

Food Organic plant fluids (57).

Water With food.

Parasites & Disease

Breeding & Rearing Eggs laid in May in ground; several hundred eggs hatch a week to 10 days after laying; nymphs mature at 4 to 6 weeks.

Special Costs

Restrictions

Toxicological Studies 72

Ecological Alternatives Milkweed bugs and other herbivorous invertebrates.

Physiological Parameters

EMPHOASA FABAE, XEROPHLOEA MAJOR - leafhoppers

Description Small hopping insects about 1/4 to 1/2" in length (57).

Availability Found all over U.S. on all types of plants; easy to capture with insect net. (Note: use only one abundant species specific to geographical area in testing (121).

Features Abundant; high probability of ease of maintenance and breedability in lab; economically important pest.

Laboratory Light - natural photoperiod; temperature - "room".

Cage An aquarium of sufficient size with small mesh screen roof; soil, water, and specific food of selected leafhopper should be available.

Food Specific food plant depends on leafhopper used (for example, Erythroneura prefers apple leaves (121).

Water Available with food.

Parasites & Disease

Breeding & Rearing Most have one generation per year; some have two or three generations per year.

Special Costs

Restrictions

Toxicological Studies

Ecological Alternatives Tree hoppers, froghoppers, planthoppers.

Physiological Parameters

TRIBOLIUM sp.

Description Flour beetle; length-to 5mm.

Availability Abundant in flour waste; suppliers.

Features

Laboratory Light-unknown; temperature-21 to 27°C; relative humidity-unknown.

Cage Glass jars.

Food Flour, dog food, fruits.

Water Obtained from food.

Parasites & Disease

Breeding & Rearing 100 adults in 2 liter jar with flour; life cycle completed in 8 weeks.

Special Costs

Restrictions

Toxicological Studies 80

Ecological Alternatives Other granivorous beetles.

Physiological Parameters

TENEBRIO sp.

Description Mealworms; length 13 to 17 mm; dark color-larvae yellow

Availability Very abundant around food stores, suppliers.

Features

Laboratory Light-natural photoperios; temperature-over 30°C; relative humidity-70%.

Cage Size-60x50x50cm; material-anything easy to clean ventilation necessary.

Food Grain, dog food, fruits.

Water Obtained in moist food.

Parasites & Disease

Breeding & Rearing Sexual maturity-depends on temp. and r.h.; life cycle-complete in 4 to 6 months; breed year-round.

Special Costs

Restrictions

Toxicological Studies

Ecological Alternatives Other phytophagous beetles.

Physiological Parameters

CARPOCAPSA POMONELLA

Description Small pest moth (57).

Availability Found all over U.S., usually on foliage, fruits or nuts (57).

Features High potential for easy breeding and maintenance in lab; economically important pest.

Laboratory Careful balance of temperature and humidity (87, 88).

Cage 53.3 x 30.5 x 7.6cm stainless steel trays with 10-13mm layer of burrowing medium on bottom (87).

Food Wheat germ, soybean meal, rice flour, pinto beans, alfalfa meal, cottonseed meal, corn meal, carrot powder (87).

Water Should be available in food.

Parasites & Disease

Breeding & Rearing Larvae may be reared in lab from eggs in 6 oz. wax paper cups with cardboard lids (88).

Special Costs

Restrictions Cannibalistic larvae.

Toxicological Studies

Ecological Alternatives

Physiological Parameters

AGROTIS YPSILON

Description Heavy-bodied moths having a wingspan of 1 to 2 inches (57).

Availability In the largest family of lepidopterans; attracted to lights at night for easy capture.

Features Have potential for routine breeding and rearing in lab; economically important.

Laboratory Light-light: dark photoperiod; temperature - 80±2°F.

Cage 20 adults held in 12 x 12 x 16" glass panel cages.

Food Rearing medium includes lima beans, pinto beans, brewer's yeast, ascorbic acid, agar & water (88); dental cotton plugs soaked in 10% dextrose solution is good nutrient (86); larvae fed red clover or tobacco.

Water Available in food.

Parasites & Disease

Breeding & Rearing Gallon jars with moist paper serve as laying environments; larvae may be placed in 12 x 8 x 2" pyrex trays with 1 inch of moistened soil; post fifth instar larvae placed in small flint jars with 1.5" layer of substrate containing 6% moisture (86).

Special Costs

Restrictions Larval forms are cannibalistic.

Toxicological Studies

Ecological Alternatives Other noctuid moths.

Physiological Parameters

MUSCA DOMESTICA

Description Common house fly with body length of 1/2"; longevity may be one year.

Availability Sometimes considered the most common animal known to man.

Features Easy maintenance and breedability; veteran of previous research; economically important.

Laboratory Light - natural photoperiod; temperature - 27.5±1°C; relative humidity - 50 to 55%.

Cage 45 x 45 x 45cm wooden cage with screen side for easy cleaning (98). .

Food Adults - shallow dish of sugar; larvae - dog bisguits, fish, wheat bran, alfalfa meal, baker's yeast, malt extract, manure, sugar and water.

Water Water-soaked cotton balls should be available at all times.

Parasites & Disease Serve as vectors for numerous diseases (57).

Breeding & Rearing Adults transferred to gauge-lined plywood cage; milk-water soaked cotton balls are placed in cage for oviposition site; egg-laden cotton balls placed in muslin-covered jars where hatch larvae pupate; pupae placed in adult cage to emerge (98).

Special Costs

Restrictions

Toxicological Studies 80, 108, 109, 111, 113, 114, 115, 618

Ecological Alternatives Blowflies, stable flies, other suitable diptera.

Physiological Parameters

DROSOPHILA MELANOGASTER

Description Small flies about 3 to 4mm in body length (57).

Availability Common throughout U.S.; college campuses use cultures for genetic demonstrations; Contact: Drosophila Information Science Department of Biology, University of Oregon, Eugene, Oregon 97430.

Features Easy to breed and maintain in lab; veteran of previous research; good model for population studies.

Laboratory Light - normal photoperiod; temperature - "room".

Cage Drosophila can be raised in baby food jars with no problems.

Food A banana-agar or banana-meal mixture in the bottom of a jar is suitable nutrition (102).

Water

Parasites & Disease

Breeding & Rearing Place adults in baby food jar with correct medium and culture becomes self-pertuating

Special Costs

Restrictions

Toxicological Studies 116, 117, 118, 119, 618

Ecological Alternatives Shore flies, other small dipterans.

Physiological Parameters

APIS MELLIFERA

Description Social insect having a body length of approximately 3/4".

Availability Only honeybee to occupy the U.S.; readily obtainable from beekeepers throughout the U.S. (57).

Features Easy to keep and work with; good model for study of the effects of chemicals on social organizaiton, popuation dynamics and behavior; ecologically important.

Laboratory Light - natural photoperiod; temperature - ambient.

Cage 6 x 6 x 2" cage for 100 bees (121) in field or lab.

Food 60% sucrose and pollen (121).

Water Available in food.

Parasites & Disease

Breeding & Rearing Self-perpetuating in most cases for at least a season when established outdoors; reproductive uncertainty arises when colonies are brought into the lab; nest boxes are necessary for colony (57).

Special Costs Special bee handling equipment.

Restrictions

Toxicological Studies 115, 122, 123, 124, 126, 128, 129

Ecological Alternatives Bumblebee.

Physiological Parameters

ISOPODA

Description Gray to black crawling crustaceans; doros-ventrally flattened body; length, 5 to 15 cm (687).

Availability 4000 marine species; only pill bugs or wood lice are truly terrestrial; wood lice found in great numbers in moist areas under boards and in logs.

Features Some terrestrial isopods have ability to roll up in ball for protection and prevention of water loss (687).

Laboratory Light-natural photoperiod; temperature "room".

Cage Terreriums of any size filled with moist soil and having abundant detritis.

Food Algae, moss, bark, decaying vegetable and animal matter; some terrestrial species are parasitic on ants, and some are carnivorous.

Water Moist areas; organisms react to water loss by rolling into ball (687).

Parasites & Disease

Breeding & Rearing Eggs brooded in a marsupium after fertilization postlarvae hatching stage with last pair of legs incompletely developed (687).

Special Costs

Restrictions

Toxicological Studies 131

Ecological Alternatives Millipede, termite.

Physiological Parameters

CHILOPODA - centipedes

Description Wormlike, or heavy-bodied; length from 1' in tropics to 1" or 2" in North America (687).

Availability Most are confined to the moist environment beneath stones and wood and in soil; found the world over (687).

Features Poison gland in claws; only active on surface of ground at night; slow but prolific reproducers (687).

Laboratory

Cage Terrariums of any size, filled with moist soil, having plenty of cover (rocks, wood) and small arthropods.

Food Small arthropods mainly; will eat toads, small snakes and small mice (687).

Water

Parasites & Disease

Breeding & Rearing Sexes distinguishable; some species lay and brood their eggs, others simply lay the eggs; years are required to reach sexual maturity.

Special Costs

Restrictions

Toxicological Studies 130

Ecological Alternatives Spiders.

Physiological Parameters

RANA PIPIENS

Description leopard frog, head and body 2" to 5"; life expectancy - 2 to 3 years.

Availability Numerous suppliers; ponds, quiet waters throughout U.S.

Features Amphibian, insectivorous; reacts to movement.

Laboratory Light-regular photoperiod; temperature of water, 18 to 20°C.

Cage Large tanks with aquatic and terrestrial areas (22"x14"x14" for 25 adults for short periods).

Food Cooked lettuce, cooked greens, trout chow, liver bits, pond plankton, larvae, mosquitoes, flies, crickets.

Water Unchlorinated, unflouridated, unpolluted; moist conditions necessary.

Parasites & Disease Many natural internal and external parasites. New arrivals should be quarantined and checked.

Breeding & Rearing External insemination in water, embryos in shallow enamel pans; 100's of eggs from each female; generation time is 13 to 15 months.

Special Costs Large operation may need special water treatment.

Restrictions No spinach in diet (may cause kidney stones); clean water necessary.

Toxicological Studies 147, 148, 149, 150, 151, 152, 153, 154, 155, 758

Ecological Alternatives Turtles, lizards, toads, salamanders.

Physiological Parameters

AMBYSTOMA MEXICANUM

Description Partially or completely neotonous slamander having a length of 30cm (142).

Availability Found in nature only in Mexico; may be obtained from commercial suppliers (142); being bred at University of Michigan Amphibian facility (137); veteran of scientific research.

Features Fairly available; breedable in lab (artificial fertilization); veteran in lab situation (142).

Laboratory Light - natural photoperiod; temperature - water temperature of 14° to 18°C.

Cage 50 x 100 x 35cm asbestos cement container houses 10 adults; no sand, gravel or plants are needed (142).

Food Beef heart, liver; vitamins (142).

Water Unchlorinated water necessary (142).

Parasites & Disease Fungal infections (142).

Breeding & Rearing Sexual maturity reached in one year; breeding season from December to June; eggs laid vary between 200 to 2,000 per spawning; "hatching" occurs after 2 weeks (142); usually 2 spawnings per year.

Special Costs

Restrictions

Toxicological Studies 160, 162

Ecological Alternatives Other Ambystoma.

Physiological Parameters

TERRAPENE SP.

Description Dry land-turtles with body lengths of 4 to 6.5"; ages of 30 to 40 years are common; some live to be a century old (133).

Availability Found all over most of the U.S. in woodland areas; available in pet stores.

Features Available; long lived for chronic testing; hardy, maintains extremely well in captivity.

Laboratory Light - natural photoperiod; temperature - "room".

Cage Dry tanks (130 x 72cm) with water available for drinking (165); terraria with dirt for turtle to dig in, and wate for occasional immersion.

Food Gound horsemeat and lettuce(165); raw hamburger and table scraps (133).

Water Ad libitum

Parasites & Disease Tuberculosis, Salmonella, assorted parasites.

Breeding & Rearing Spring mating is usually the case; 3 to 6 leathery eggs are laid in June or July; eggs hatch in September (1192); eggs may be collected from natural nest sites for rearing.

Special Costs

Restrictions Breeding in captivity difficult.

Toxicological Studies 167, 180

Ecological Alternatives Species of Clemmys.

Physiological Parameters

CHELYDRA SERPENTINA

Description Large, short-tempered turtle; 8 to 12" body length; 10 to 35 lbs. (133).

Availability Southern Canada to Gulf of Mexico; Atlantic Ocean to Rockies; generally occupies a permanent body of fresh water (133).

Features Hardy; good test animal when young; easy to maintain under lab conditions (175); economically important (133).

Laboratory Light - natural photoperiod; temperature - "room".

Cage Enclosed outdoor area (5 x 8') with shallow stone or concrete pond (12 to 15" deep) and shelters in the form of logs or stumps (133).

Food Insects, small fish, earthworms, dog food, vegetation (175).

Water Ad libitum

Parasites & Disease Tuberculosis; Salmonella; assorted parasites (166).

Breeding & Rearing Eggs laid in June; clutch size between 20 and 30 eggs; eggs hatch in September and October (1192).

Special Costs

Restrictions Breeding in lab is questionable; handle with care.

Toxicological Studies 147, 175, 180

Ecological Alternatives Macroclemys temmincki (alligator snapping turtle).

Physiological Parameters

ANOLIS CAROLINENSIS

Description The false chameleon with a body length of 5 to 7 1/2" (133).

Availability Common in Southeastern U.S.; may be purchased in pet stores (133).

Features Available, easy to maintain in lab.

Laboratory Light - natural photoperiod; temperature - "room".

Cage Standard aquarium filled with sandy soil, some plants and small branches (133).

Food Mealworms, wide variety of insects.

Water Spray cage with water everyday to supply lizards with water.

Parasites & Disease

Breeding & Rearing 1 to 2 eggs per clutch; 6 to 9 clutches per season (1193).

Special Costs

Restrictions Do not breed well in captivity.

Toxicological Studies 175, 180, 148

Ecological Alternatives Eumeces, toads, snakes.

Physiological Parameters

PHRYNOSOMA CORNUTUM

Description Spiny lizard; body length - 2.5 to 4" (133).

Availability Found in parts of Kansas, Texas, Arizona and northern Mexico (133) can be bought from pet stores.

Features Available; easy to maintain under lab conditions.

Laboratory Light - natural photoperiod; temperature -"room".

Cage Aquarium filled with sandy soil, some plants, small branches, rock pile, and water dish (133).

Food Spiders, sowbugs, insects (133).

Water Ad libitum

Parasites & Disease

Breeding & Rearing 14 to 37 eggs per clutch; 1 or more clutches per year (1193).

Special Costs

Restrictions Poor breedability in lab.

Toxicological Studies

Ecological Alternatives Toads, snakes, Eumeces.

Physiological Parameters

THAMNOPHIS SIRTALIS

Description Striped, thin-bodied snake, 18 to 26 inches long (133).

Availability Eastern half of U.S. (133); found in a wide variety of habitats in the city and country.

Features Available; easy to maintain and handle in lab; breeds well in captivity giving birth to many young at one time (175).

Laboratory Light-natural photoperiod, needed for heating purposes; temperature - 26° to 36°C; relative humidity - 33 to 66% (171).

Cage Terrariums with tightly sealed lids; pea gravel or artificial grass substrate; rock or branches necessary for ecdysis (133, 171, 173).

Food Frogs, toads, salamanders, fish tadpoles, earthworms, chopped raw fish (133).

Water ad libitum in open bowls.

Parasites & Disease Ectoparasites common.

Breeding & Rearing Sexual maturity reached in 1 to 2 years; 3 to 85 young per clutch per season (1193).

Special Costs

Restrictions

Toxicological Studies 148, 175, 715

Ecological Alternatives Lizards, turtles.

Physiological Parameters

ELAPHE spp.

Description Large, constricting, handsome snakes having body lengths ranging from 24 to 72 inches (133).

Availability Found throughout most parts of U.S. in trees, cliffs, fields, etc. (133).

Features Wide distribution; maintains extremely well in captivity.

Laboratory Light-natural photoperiod, needed for heating purposes, temperature - 26° to 36°C, relative humidity - 33 to 60% (171).

Cage Terrarium with tightly sealed lids; pea gravel or artificial grass substrate; rocks or branches necessary for ecdysis (133, 171, 173).

Food Mice, rats, small birds, lizards, frogs (133).

Water ad libitum in open bowls.

Parasites & Disease Ectoparasites common.

Breeding & Rearing 1 to 44 eggs per clutch.

Special Costs

Restrictions Breedability in lab questionable.

Toxicological Studies 175, 180, 715

Ecological Alternatives Lizards, turtles.

Physiological Parameters

PHALACROCORAX AURITUS

Description Large, dark, water birds with long necks and tails; 30 to 35" body length (221).

Availability Common on coasts, inland lakes and rivers, generally in the Eastern half of the U.S. (221).

Features At top of their food chain; intelligent; good short-term test bird; easy to obtain adults, young and eggs; typical of fish-eating birds (181).

Laboratory Light - natural photoperiod; temperature - young birds kept warm with heating lamps (181).

Cage 6 x 6' cage made of wood, cement, chicken wire and indoor-outdoor carpet will house 6 cormorants; cage should be equipped with tree trunks and large water tanks for bathing and drinking (181).

Food Fish (181, 182) and vitamin B supplement and ferrous iron supplement; grit required as a digestive aid and mineral supplement (182).

Water Ad libitum (See "Cage").

Parasites & Disease

Breeding & Rearing Clutch size - 2 eggs; one clutch per year; colonial nesters (182, 183).

Special Costs Food costs high due to bird's appetite.

Restrictions Somewhat hard to breed in captivity; takes a long time to reach sexual maturity; large bird with enormous appetite.

Toxicological Studies 188, 189

Ecological Alternatives Gulls and terns.

Physiological Parameters

PODILYMBUS PODICEPS

Description A duck-like diver; body length - 12 to 15" (221).

Availability Found throughout the U.S. and Canada, near or in ponds, creeks and marshes (221).

Features Diurnal and migratory; fairly abundant.

Laboratory Light - natural photoperiod; temperature - ambient.

Cage Mallard specifications.

Food In nature, they eat vertebrate and invertebrate aquatic organisms; grit needed as a digestive aid and mineral supplement.

Water Ad libitum.

Parasites & Disease Worms, ticks, fleas, lice, botulism.

Breeding & Rearing 2 eggs per clutch; one clutch per year; young are precocial and nidifugous (183).

Special Costs

Restrictions Poor breeders in captivity.

Toxicological Studies 185

Ecological Alternatives Diving ducks.-

Physiological Parameters

ANAS PLATYRHYNCHOS

Description A familiar surface-feeding duck; body length 20 to 28"; weight 2 1/2 to 3 1/2 lbs.; life expectancy - 20 years in captivity, 16 years in wild.

Availability Found in wooded swamps, marshes and ponds throughout mos of the U.S. (221); legislation exists which may limit wild bird testing.

Features Good availability; breeds easily in captivity; large enough for large samples; representative waterfoul; adap well to small areas; healthy, stable and vigorous; (381) important in sports and recreation.

Laboratory Light - 10D:14L photoperiod (207); temperature - "room"

Cage 8 x 14 x 7' open wire mesh cage for natural environment testing, 20 to 25 birds per cage (381); 30 x 15 x 6' recommended pen size for 5 adult birds, must be provided with water area of 10" depth (192);30 x 15 x 6' pen with nest boxes, 250 gallon water troughs and feeders, used t breed mallards (194).

Food Natural-grain, seeds, roots, water plants, snails, insect small aquatic organisms (1194); lab-commercial game bird ration usually supplemented with millet, cracked corn, wh milo and barley; grit needed as digestive aid and dietary supplement (191, 192).

Water Water available at all times for drinking and recreation.

Parasites & Disease Flies, true bugs, lice, protozoans, worms, flukes; botulism.

Breeding & Rearing Sexual maturity - 1 to 2 yrs.; 5.1 to 9.3 eg per clutch; renesting probability high; incub tion period of 21 to 28 days; 49 to 60 day fl ing period; newly-hatched young display impri behavior; easy breeders in captivity (381).

Special Costs Cages have to be cleaned on a regular basis; cial floor covering materials keep cleaning fr quency and expense minimal (381).

Restrictions Territorial during breeding; crowding should avoided; migration restlessness during certa periods of year.

Toxicological Studies 184, 192, 195, 197, 198, 199, 200, 201, 203, 206; 208, 209, 210, 213, 1195

Ecological Alternatives Other waterfowl.

Physiological Parameters

BRANTA CANADENSIS

Description Largest of wild geese; length - 35 to 43"; weight - 8 to 13 pounds; (1194); Longevity - 33 years in captivity, 23 years in wild.

Availability Found in wet lands in most of North America during some part of the year (221).

Features Canada geese keep well in captivity (1196).

Laboratory See mallard (Anas platyrhynchos) synopsis.

Cage See mallard (Anas platyrhynchos) synopsis.

Food Natural food - grain, grasses, green parts of plants and small bits of animal matter (1194); Lab food - commercial game bird ration; grit needed for digestive aid and mineral supplement.

Water Should be available at all times for drinking or bathing.

Parasites & Disease Flies, true bugs, lice, worms and flukes (1194); may be susceptable to botulism, fowl cholera, avian tuberculosis, and malaria.

Breeding & Rearing Becomes sexually mature in 2 to 3 years; Nest in early spring, usually mid-April; 4 to 7 eggs laid per nesting; 28 day incubation period; male and female guard nest; 63 to 86 day fledging period; young take to water immediately (1194).

Special Costs

Restrictions Migratory restlessness; territorial during mating season; protected by legislation.

Toxicological Studies 205,206,210,213, 248 (Note: See mallard (Anas platyrhynchos) section for similar references).

Ecological Alternatives Other waterfowl of the world.

Physiological Parameters

PUFFINUS GRISEUS

Description Gull-like sea bird; body length 16 - 18".

Availability Sea bird found in Northern and Southern hemisphers along Atlantic, Gulf and Pacific coasts; most abundant in Pacific area (221, 1196).

Features Migratory and diurnal; nasal salt glands allow bird to convert sea water to fresh water.

Laboratory Sea birds do not do well in the laboratory.

Cage Cage provisions have not been fully documented for sea birds.

Food In nature, they feed on fish, crustacea, cephalopods and macrophyton; grit needed in diet as a digestive aid and mineral supplement.

Water Salt or fresh water should be provided at all times.

Parasites & Disease Ticks, worms, mites, fleas, lice.

Breeding & Rearing Colonial nesters; usually one egg laid per year; incubation - 6 to 11 weeks; 13 to 14 week fledging period; sexual maturity usually reached by second year; renesting possibiltiy high (183, 215); young are altrical and nidicolous.

Special Costs

Restrictions Not easily maintained or bred in captivity slow reproducers.

Toxicological Studies 218

Ecological Alternatives Gulls or terns.

Physiological Parameters

CASMERODIUS ALBUS

Description Wading birds with long necks and legs and pointed bills; body length of 37-40" (221).

Availability Found in marshes, coastal areas and rivers of the Eastern half of the U.S. (221).

Features Fairly easy to maintain in captivity.

Laboratory Light - natural photoperiod; temperature - "room".

Cage Outdoor duck pens of sizable dimensions may be used for holding Egrets.

Food Feed on both vertebrate and invertebrate aquatic organisms.

Water Ad libitum.

Parasites & Disease Ticks, worms, mites, fleas, lice.

Breeding & Rearing 3 eggs per clutch; 1 clutch per year; young altricial and nidiculous, young fledge at 5 weeks of age (183, 222) colonial nesters.

Special Costs

Restrictions Slow breeder, large size, protected by law.

Toxicological Studies 185

Ecological Alternatives Cranes, rails, coots.

Physiological Parameters

FULICA AMERICANA

Description Gray duck-like bird (221); body length - 15" (1196).

Availability Found in marsh areas mainly in the Eastern U.S. (221).

Features Readily available for study.

Laboratory Light - natural photoperiod; temperature - ambient.

Cage Mallard cage specifications.

Food Carnivorous in wild, need grit for digestive aid and mineral supplement.

Water Ad libitum

Parasites & Disease

Breeding & Rearing Young are precocial and nidifugous.

Special Costs

Restrictions Protected by legislation.

Toxicological Studies

Ecological Alternatives Herons, ibis, ducks.

Physiological Parameters

PHILOHELA MINOR

Description A large, husky, neckless, long-billed brown bird; lenght of body - 10 to 12" (221).

Availability Found in swamps, wetlands and thickets throughout most of the U.S.

Features Migratory and territorial; available; socially tolerant of one another; hardy, withstands heat and cold well (225).

Laboratory Light - natural photoperiod; temperature - "room".

Cage 20 x 40 x 8' field enclosure; 4 x 4 x 5' ground cage; 22 x 29 x 30" steel cage for lab (225). All cages are provided with food and water; they should be covered with burlap to prevent flushing and injury to the bird.

Food In the lab they feed on earthworms, mealworms, fly larvae placed in peat moss in food pans at all times; in nature, they feed on invertebrates near lakes, ponds and streams.

Water Ad libitum.

Parasites & Disease Ticks, mites, fleas, lice, worms.

Breeding & Rearing

Special Costs Cages must be kept clean to prevent disease.

Restrictions Nervous and therefore prone to injure themselves in captivity.

Toxicological Studies 27, 226, 227, 228, 229, 230

Ecological Alternatives Snipe.

Physiological Parameters

LARUS ARGENTATUS

Description Known as common seagull; body length - 23 to 26" (221).

Availability Ranges in most of the Eastern half of the U.S. near the coast and along river banks (221).

Features Easy to obtain eggs and adult birds; good subjects for short term studies and oological studies because of colonial nesting; migratory and diurnal.

Laboratory Light - natural photoperiod; temperature - "room".

Cage Cage dimensions used for ducks may be suitable for the herring gull.

Food Invertebrate and vertebrate aquatic life should be supplied at all times; grit should be supplied as a digestive aid and mineral supplement (182).

Water Ad libitum.

Parasites & Disease Fleas, mites, ticks, lice.

Breeding & Rearing 4 eggs per clutch; 1 clutch per year; high re-nesting potential; incubation period - 27 days; young precocial, nidicolous, fledge at 43 days (183, 184, 231).

Special Costs

Restrictions Hard to maintain in lab; protected by legislation.

Toxicological Studies 188, 231, 232, 235, 236, 249

Ecological Alternatives Terns, other gulls, pelicans.

Physiological Parameters

MELANERPES ERYTHROCEPHALUS

Description Chisel-billed tree-climbers; stiff, spiny tails; 8-8 1/2 body length; head completely red (221).

Availability Found throughout U.S. in woodlands (221).

Features Diurnal and migratory; hole-nesters.

Laboratory Light - natural photoperiod; temperature - "room".

Cage Bobwhite quail cages with modifications.

Food In nature - seeds and terrestrial insects - grit needed in diet to aid in digestion and supplement minerals.

Water

Parasites & Disease Hosts of fleas, mites, lice.

Breeding & Rearing 2 to 3 eggs per clutch; 12 day incubation period; young are nidicolous and altricial; 24 day fledging period.

Special Costs

Restrictions

Toxicological Studies 238

Ecological Alternatives Brown creeper, nuthatch.

Physiological Parameters

FALCO SPARVERIUS

Description Small sparrow-like falcon; body length - 9 to 12"; long life spans.

Availability Most abundant hawk found throughout North America (241).

Features Available, fairly easy to maintain; good indicators of pesticide level in the environment, small and fairly easy to handle in comparison with larger raptors.

Laboratory

Cage Outdoor pen - 8 x 12 x 7' enclosure with roof made of 1 x 1 1/2" mesh with box 10 x 10 x 15" with 3" diameter opening; larger dimensions (50 x 20 x 6') can house multiple pairs of hawks (239); alternate cage - 6 x 6 x 8' solid wire cage for a pair of sparrow hawks.

Food Dead cockerels, ground beef, vitamins, minerals, and turkey breeder crumbs (239, 240); a combination of hamsters and chicken heads and necks may be substituted for ground beef; roughage in diet is needed for formation of fecal pellets.

Water Ad libitum.

Parasites & Disease Hosts for external parasites to a limited extent (182).

Breeding & Rearing Sexual maturity - end of first yr.; clutch size - 3 to 7, average 4 or 5 eggs; incubation period - usually 30 days; fledging period - 30 days; nesting success - 3.88 young hatched per nest and 3.81 young fledged per nest (215, 241).

Special Costs Equipment for handling, fairly expensive diet

Restrictions Violence during handling; slow reproducer requiring large pens.

Toxicological Studies 184, 188, 243, 249

Ecological Alternatives Kites, shrikes, buteos, accipiters and falcons

Physiological Parameters

TYTO ALBA

Description Long-legged, light-colored owl with while, heart-shaped face; body length - 15 to 20"; claws and beak modified to handle prey (221).

Availability Found mainly in the Eastern half of the U.S., particularly near barns and old buildings (221)..

Features Fairly good availability; fairly easy to maintain; good indicator of pesticide levels in environment; nocturnal.

Laboratory Light - natural photoperiod; temperature - "room".

Cage 6 x 10 x 50' chicken wire and wood frame cage with porches and nest box for one breeding pair (20).

Food Carnivorus habits in nature and in lab; small whole rodents - especially while brooding (20); roughage needed for fecal pellet formation.

Water Ad libitum

Parasites & Disease

Breeding & Rearing 5 to 11 eggs per clutch (1196).

Special Costs

Restrictions Hard to tame and breed in captivity; capable of causing injury to handler.

Toxicological Studies 245, 248

Ecological Alternatives Kites, shrikes, buteos, accipiters and falcons.

Physiological Parameters

COLUMBA LIVIA

Description Domesticated bird; body length about 13", weight - about 450g.; sexual monomorphic (221, 1196) .

Availability Common throughout U.S. in association with man; captured with traps or mist nets; can be raised in captivity or obtained commercially.

Features Large enough for good samples; can be breed easily in captivity; easily housed and caged; good temper ment, can be conditioned and trained (182, 267, 272).

Laboratory Light - 14L:10D photoperiod; temperature - 20°±1°C (251).

Cage 20 x 29 x 18cm steel rat cage houses one bird (250) 40 x 64 x 40cm cage houses one bird (251) ; 45.7cm^2 cage made of 7.3 x 2.5cm gage welded wire with perch, food can automatic watering device and nest box should house a pai (252) 115 x 134 x 229cm cage houses a pair of birds (25

Food Commercial pellet diet or a mixture of wheat, maize, and tick beans (251); grit should be available to birds at all times (182); eats seed and grain in the wild.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Breeding season - all year; clutch size - 2 eggs; laying interval between eggs - 1.5 to 2 days; incubation period - 15 days; fledging period - 17 days; fertility - 83.3 to 92.9% eggs; (215, 252).

Special Costs

Restrictions Protected by legislation in some areas.

Toxicological Studies 152, 199, 252, 255, 259, 260, 261, 262, 263, 264, 266, 267, 268, 269, 270, 271, 272, 273, 885, 1134

Ecological Alternatives Ring dove, mourning dove.

Physiological Parameters

COLINUS VIRGINIANUS

Description Chicken-like bird; body length 8.5 - 10.5".

Availability Eastern Farmlands from Gulf of Mexico to Ontario.

Features Breeds well in captivity; requires minimal housing space; readily available and easy to maintain.

Laboratory Light - natural photoperiod; temperature - "room".

Cage 6 x 10 x 8" cage constructed of 1" wire mesh;
4 x 6 x 1.5' pen designed for a large number of birds;
3 x 6' outdoor pens made of wire net (276).

Food F & M game bird chow, turkey starter mash, Purina pigeon chow (274, 277, 278); grit needed to aid digestion and to supply dietary minerals (182).

Water Ad libitum.

Parasites & Disease Fleas, mites, ticks and lice.

Breeding & Rearing 25 day incubation period; young are precocial and nidfugous.

Special Costs

Restrictions Cannibalism in crowded quarters; protected by legislation.

Toxicological Studies 202, 274, 275, 276, 278, 279, 280, 281, 282, 283, 284, 285, 286

Ecological Alternatives Coturnix, pheasant, grouse.

Physiological Parameters

PHASIANUS COLCHICUS

Description Large gallinaceous bird with long pointed tail; body length - male, 33-36"; female - 20 1/2" (221).

Availability Found in farming country north of Mason-Dixon line.

Features Breeds easily in captivity; easily maintained; migratory and diurnal.

Laboratory Light - normal photoperiod; temperature - "room".

Cage Pen complex 22 x 6.1 x 1.7m. can be partitioned into 27 individual cages with dirt runways and plywood shelters (287).

Food Diet recommended in Poultry Formula guide (288); grit needed (182).

Water Ad libitum

Parasites & Disease Ticks, lice, mites, fleas.

Breeding & Rearing Precocial and nidifugous young.

Special Costs

Restrictions Cannabalistic in crowded quarters; protected by law.

Toxicological Studies 210, 248, 281, 282, 290, 291, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 734, 735, 1197, 1198

Ecological Alternatives Grouse, turkey.

Physiological Parameters

DENDRAGAPUS OBSCURUS

Description Plump-breasted, short-winged birds approximately 17-21" long (1196).

Availability Lives in coniferous forests of humid Pacific coast and mountains (1196).

Features Easy to maintain and breed in captivity; useful in field and lab telemetric studies due to large size.

Laboratory Light - natural photoperiod; temperature -"room'.

Cage 24.5 x 2.5 x 3.25' concrete tank with wire mesh top;
20 x 10 x 6' pen made of fish netting and steel posts;
8.1 x 16.2' pen with turf floors and mesh roof;
1 x 1 x 2' wire cage used for the field.

Food Game bird chow in pellet form (308); grit needed as a digestive aid and mineral supplement (182).

Water Ad libitum.

Parasites & Disease Ticks, mites, fleas, worms.

Breeding & Rearing Sexual maturity reached in 112 days (309); young are nidifugous and precocial (183).

Special Costs

Restrictions Cannabalistic in crowded quarters; protected by legislation.

Toxicological Studies 312, 313

Ecological Alternatives Coturnix, bobwhite quail, pheasant.

Physiological Parameters

MELOSPIZA MELODIA

Description Sparrow; length - 5 to 6 3/4"; short, stout bill used for seed cracking; longevity of approximately 2 years (221).

Availability Found throughout most of the U.S. in woods and non-aquatic areas (221).

Features Readily available in the wild.

Laboratory Light - natural photoperiod; temperature -"room".

Cage 120 x 60 x 60cm cage made of wood and 12mm mesh; single bird cage of 41 x 61 x 38cm; 3 x 3 x 2' cages and 6 x 5 x 2' cages used.

Food Lab food - pigeon chow, canary or millet seed, or a mixture of biscuit meal, dried milk, soya bean meal and maw seed; Natural food - seeds; cuttle bone necessary for beak trimming purposes, and grit should be provided as a digestive aid and mineral supplement.

Water Should be available at all times.

Parasites & Disease Lice, flies, mites and ticks.

Breeding & Rearing Usually four nestings a season; 2 to 5 eggs per nesting; incubation period of 12 to 13 days; young leave nest after 9 to 11 days; young fledged after 45 days; difficult to breed in captivity.

Special Costs

Restrictions Highly territorial and migratory; hard to breed in captivity; subject to parasitism.

Toxicological Studies 275

Ecological Alternatives Blackbirds.

Physiological Parameters

RICHMONDENA CARDINALIS

Description Solid red-crested bird about 8 to 9 inches long (221). life span of 3 to 6 yrs. in nature (321).

Availability Found in U.S. east of plains near towns, farms, roads, swamps, etc. (221).

Features Readily available; one of the best "model" granivores.

Laboratory Light-natural photoperiod; temperature-room.

Cage 120x60x60cm wood and 12 mm mesh cage (317); 3x3x2' to 6x5x2' cages have been used with enclosed nest box (318); 61x41x38cm cage has been used for single bird (319).

Food Pigeon chow (275) canary or millet seed (319); bisquit meal, dried milk, soya bean meal, maw seed (319); cuttle bone needed for beak trimming and grit needed also (182, 319).

Water Ad libitum.

Parasites & Disease Lice, fleas, mites, and ticks (321).

Breeding & Rearing Cardinals become sexually mature at one year; 2 to 5 eggs per clutch; 4 to 5 clutches per year (241); 12 to 13 day incubation; fledging period is 45 days (288).

Special Costs

Restrictions Highly territorial and protected by legislation; does not adapt well to lab.

Toxicological Studies

Ecological Alternatives Blackbirds.

Physiological Parameters

TURDUS MIGRATORIUS

Description Long-legged, short-billed bird; body length - 8.5 - 10.5" (221); longevity in wild - 1 to 3 years; longevity in captivity - 12 years.

Availability Found throughout United States (221).

Features Song bird; monogamous mating; migratory and diurnal.

Laboratory Light - 15L:9D photoperiod; temerature - 70° to 80°F.

Cage 9 x 12 x 7' communal cage for 20-70 birds;
20 x 15 x 22" cage for 10 birds;
9.75 x 7.0 x 8.0" metabolism cage for one bird.

Food Beetles, true bugs, spiders, caterpillars, ants, small hymenoptera are major food items; grasshoppers, crickets, craneflies, moths, millipedes and snails are minor food i

Water Should be available at all times.

Parasites & Disease Hosts for mites and ticks.

Breeding & Rearing 3 to 4 eggs per clutch; usually 3 clutches pe year (241); 13 to 16 day incubation period; young are altricial and nidicolus; fledging takes 13 days on the average.

Special Costs

Restrictions Territorial, hard to breed and maintain in lab; protected by legislation.

Toxicological Studies 37, 326, 333, 334, 335, 336, 337, 338

Ecological Alternatives Vireos, warblers, goatsuckers.

Physiological Parameters

STURNUS VULGARIS

Description Similar in shape to a Meadowlark; 7 1/2-8 1/2" body length (221).

Availability Common resident species throughout continental U.S.; readily captured in Japanese mist nets.

Features Mean annual adult mortality in wild, 51-58% (344); largely insectivorous in the wild; not protected by law.

Laboratory Temperature -"room", 70°-80°F (329); light-natural photoperiod.

Cage Outdoor individual holding cages 2x1x1'; (342) Metabolism cages 9 3/4x7x7" (329).

Food Chick mash, ad libitum (329)

	Protein	Fiber	Fat	NFE	Ash	ad lib. consumption (dry wt. (g)/day)	% highly digestable
pellets:	23.9%	5.6%	3.4	57.2	10%	18gm	3.4%
mealworms:	50.4%	4.9	34.9	6.7	3.2%	6gm	85%

Water Ad libitum (329).

Parasites & Disease

Breeding & Rearing Young altricial (344) ;first clutch 5.5±0.9, second clutch 4.1±0.96 (344); Incubation - 12 days (344) ;Fledging - 21st day after hatching (344).

Special Costs

Restrictions Highly territorial; require separate cages for each breeding pair (182).

Toxicological Studies Used in nationwide monitoring of levels of certain pesticides and pollutants (342, 347, 739, 740).

Ecological Alternatives Red-winged Blackbird, Eastern Meadowlark.

Physiological Parameters

PASSER DOMESTICUS

Description 5 to 6 1/4" body length (221); life expectancy - 3 years with exceptions up to 12 years (331).

Availability Found in great numbers around cities, towns, and farms in the U.S. and Canada (221).

Features Familiar bird, distributed where man lives.

Laboratory Light - natural photoperiod; temperature - "room".

Cage 7 x 7 x 7' cage - 50 to 100 birds for 4 to 6 weeks; 2 x 2 x 2' cage - 7 to 10 birds for 3 days; 6 x 9 x 6' cage - individual test cage; all cages made of 1" mesh poultry netting.

Food Pigeon chow checkers and chick starter mash; grit needed as a digestive aid and mineral supplement.

Water Ad libitum

Parasites & Disease Lice, flies, mites and ticks; serves as a carrier of encephalitis.

Breeding & Rearing 3 to 6 eggs per clutch; 11 day incubation period, 14 day fledging period; 2 to 4 clutches per breeding season.

Special Costs

Restrictions Birds are highly territorial and require separate cages for each breeding pair.

Toxicological Studies 210, 275, 335, 339, 342

Ecological Alternatives Meadowlarks.

Physiological Parameters

GALLUS GALLUS

Description The common domestic chicken.

Availability Can be obtained in large numbers from commercial breeders in any part of the country.

Features Large yet easily handled; easily obtainable; continuous nesters; can be housed in limited space and cheaply.

Laboratory Light - 14L:10D photoperiod; temperature - 23.9°C; Humidity - 45 to 78% depending on age of bird.

Cage 30 x 20x 8" cage for 10 to 20 chicks; 15 x 10 x 8" experimental cage for 10 to 50 chicks; individual and brooder units with shavings, peat moss or commercial litter are available commercially.

Food Commercial chow - ad libitum; grit needed for mineral requirements.

Water Ad libitum.

Parasites & Disease Newcastle disease, bronchitus, fowl pox, coccidiosis, other parasites common.

Breeding & Rearing Sexual maturity - 20 to 22 weeks; continuous laying throughout most of year; incubation period - 21 to 23 days.

Special Costs

Restrictions Cannabalistic tendencies.

Toxicological Studies 350, 355, 356, 358, 359, 360, 361, 362, 363, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 466, 619, 1199

Ecological Alternatives Jungle fowl, wild turkey.

Physiological Parameters Spontaneous motility in chick embryos (1200); heart rate of chick embryo (357); monitoring system for cardiac and gross motor activity (378).

COTURNIX COTURNIX

Description Small quail; weight - male, 110g. (90-120g.), female 130g. (110-150g.) (383).

Availability Exotic species; easy to breed in laboratories.

Features 6 week reproductive cycle; cheap to breed and raise in laboratory; small for easy handling in lab yet large enough for good sample (406); representative of upland game birds (199, 406).

Laboratory Light - 14L:10D photoperiod; relative humidity - 60 to 70%.

Cage 3 x 6 x 1.5' with 1/2" mesh breeder cage for 5 males and 15 females (379); 12 x 12 x 7" cage by GOF Mfg. Co., P.O. 8152, Savannah, GA 31400, recommended for breeding.

Food Seeds, commercial game bird chow.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Sexual maturity - 6 weeks; recommended sexual ratio- 1 cock:3 hens, 2 cocks:7 hens; laying schedule - continuous all year approximately 200 to 300 laid per year with artificial light source; incubation time and temperature - 16 days±8 hours at about 100°F; Fertility - 90% after 50 days (379); up to 4 generations per year.

Special Costs

Restrictions Migrating restlessness observed in April and September under natural conditions.

Toxicological Studies 199, 249, 255, 293, 326, 371, 380, 382, 384, 385, 386, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 406

Ecological Alternatives Bobwhite quail.

Physiological Parameters

MELOPSITTACUS UNDULATUS

Description Small, slender bird; about 4 - 6" long.

Availability Native to tropical Australia; commercially available at pet stores and distributors.

Features Easy to breed and maintain in the laboratory.

Laboratory Light - natural photoperiod; temperature - "room".

Cage Standard breeding cages, nest boxes and bowls are available commercially.

Food Commerical chows; sunflower seeds supplement diet; iodine necessary in diet; tree branches necessary for bill trimming purposes.

Water Ad libitum.

Parasites & Disease Fleas, ticks, lice and mites.

Breeding & Rearing Sexually mature at 6 months; will breed all year round; 3 to 4 eggs per clutch, can have as many as 6 eggs per clutch; 18 to 23 day incubation period; fledged at 6 to 9 weeks.

Special Costs

Restrictions Very noisy, sanitation problems common.

Toxicological Studies

Ecological Alternatives Parrot.

Physiological Parameters

BLARINA BREVICAUDA

Description large shrew, head and body 3-4 inches; adult females 16.5 g, males 18. g (416) life expectancy: 20 months (414).

Availability common in eastern U.S.A.; pit traps; some dealers.

Features Food and water must be constantly available. Active day and night. Individual variation to temperment. Odor may be problem. Possess poison glands (saliva).

Laboratory: Light - ?; Temperature - "room'; Realtive humidity - ? +70%; Air quality - watch amnonia and musk levels; Sound - may be sensitive.

Cage Density - 1, more with care; Size - variable; don't jump, do dig; Material - variable, will climb screen; Nest - provide container and lining or allow soil chamber and provide lining; Bedding - sphagnum or soil, 3-6 inches deep; Sanitation - ?; Furniture - ?.

Food Ground horse or beef; canned dog food; earthworms; mice; mealworms. CAUTION: Very sensitive to food shortages. Feed ad libitum.

Water CAUTION: Sensitive to water shortages. Water ad libitu

Parasites & Disease Sensitive to exposure, especially getting fur wet. Mites.

Breeding & Rearing Sexual maturity at 1-2 months. Gestation about 21 days. Post-partum estrous - (?); Litter size 5-7; Litter number 2, possible 3 per year. Season - peak in April. Weaned - about 22 days.

Special Costs Initial trapping program and transport, or suppl

Restrictions Consume less than 1/2 body weight in food/day. Difficult to maintain in lab.

Toxicological Studies

Ecological Alternatives Cryptotis parva , Sorex cinereus.

Physiological Parameters 906

EPTESICUS FUSCUS

Description Large bat, head and body 3" to 4" forearm 1" to 2"; weight 10 to 16 g; life expectancy unknown.

Availability Available locally throughout U.S.; collected with mist nets.

Features Nocturnal; usually solitary, sometimes colonial.

Laboratory Light - 13L:11D photoperiod; temperature, 21 to 28°C; relative humidity, 55-92%.

Cage Density - 5 to 10 per cage; size is variable, 20 x 20 x 25 cm to 80 x 92 x 138 cm with roosting box 80 x 92 x 57 cm; material - wood, hardware cloth (bats hang from hardware cloth ceiling).

Food Young birds, mealworms and other insects, high vitamin-protein mixtures.

Water Ad libitum.

Parasites & Disease Mites, fleas.

Breeding & Rearing Sexual maturity - 1 year; gestation - unknown (delayed implantation); post-partum estrous - no; litter size - usually two; litter number - one per year; season - May through June

Special Costs Initial supply.

Restrictions Breedability in captivity unknown.

Toxicological Studies 438

Ecological Alternatives Other insectivorous bats.

Physiological Parameters

SYLVILAGUS FLORIDANUS
(S. AUDUBONI)

Description Cottontail rabbit; head and body 12 to 17"; tail 1 to 2"; weight, 1.5 to 4 lbs; life expectancy - over 2 yrs.

Availability Abundant throughout brush, grasslands in Eastern and Southern U. S. (deserts and foothills in Southwestern U. S.).

Features Active day and night, game animal.

Laboratory Light-"natural photoperiod"; temperature-"room"; relative humidity - over 50%.

Cage Density - over 4; Size - variable (minimum 1 sq. ft. per animal); Material - wood, plastic, metal, fiberglass.

Food Green vegatation, commercial rabbit diet.

Water Ad libitum.

Parasites & Disease Tularemia.

Breeding & Rearing Sexual maturity - about 1 yr.; Gestation 26 to 30 days; Postpartumestrous - ?; Litter size - 2 to 7; Litter number - 2 to 4 per year; Season - spring to fall.

Special Costs Initial supply.

Restrictions

Toxicological Studies

Ecological Alternatives Hares, voles.

Physiological Parameters

DIPODOMYS ORDI

Description Large kangaroo rat, head and body 4" to 5"; weight, 43 to 70g; light-colored desert rodent with large head and long tail, 5" to 6"; front legs weak, saltatorial; fur-lined cheekpouches (412).

Availability Common in sandy soils throughout western plains and deserts of U.S.; some dealers.

Features Nocturnal, active throughout year, stores seeds, burrows, temperament varies individually, often docile and easy to handle.

Laboratory Light- 12L:12D; temperature- 24 to 28°C; relative humidity- very low (as in natural environment).

Cage Density- 1, but depends on cage size; size- variable, 10 x11 x16" to 2 x2 x3'; breeding cage- 5'x7'x2'; must be covered; material- variable, glass or metal (will gnaw wood); nest- mailing tube, small boxes; bedding, dried leaves, grass; substrate- dried leaves; sand important for cleaning pelage.

Food Cereals, ground meat meal, various seeds; greens may be important nutritionally and for proper breeding.

Water Water requirements negligible though may increase if kangaroo rats become accustomed to drinking.

Parasites & Disease

Breeding & Rearing Sexual maturity 90 days; gestation- 29 to 32 days; post-partum estrous, unknown; litter size- 2 to 5; litter number- 1 to 2 per year; season, May through June and late summer- weaned 20 to 25 days.

Special Costs Initial trapping program and transport; supply.

Restrictions Heteromyid rodents are very territorial; colonial breeding may be a problem.

Toxicological Studies

Ecological Alternatives Dipodomys sp.; Perognathus hispidus; Perognathus flavus.

Physiological Parameters 454, 457

NEOTOMA LEPIDA

Description Desert wood rat, head and body 6" to 7", tail, hairy, 4.5" to 6.5"; weight, 100-170g.; life expectancy - 5 yea

Availability Locally common on southwestern deserts and rocky slopes, some dealers.

Features Generally nocturnal.

Laboratory Light - normal photoperiod; temperature - 23°C+; relativ humidity - 60 to 70%.

Cage Density - depends on cage size; size - variable; materia metal, glass, wire mesh; nest - cotton, grass, etc.

Food Seeds, fruits, acorns, cactus, food scraps.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Sexual maturity - 60 days; gestation - 30 to 36 days; post-partum estrous - unknown; litter size - 1 to 5 (avg. 3); litter frequency - over 4 per year; season - warm months; weaned - 15 da

Special Costs Initial acquisition of breeding stock.

Restrictions

Toxicological Studies

Ecological Alternatives Other woodrats.

Physiological Parameters

ORYZOMYS PALUSTRIS

Description Small rat; head and body 4.5" to 5.5", tail 4.5" to 7"; weight 40 to 75g.; life expectancy - unknown.

Availability Common in marshlands in Southeastern U. S.

Features Nocturnal, semiaquatic.

Laboratory Light- natural photoperiod; temperature- "room"; relative humidity- unknown.

Cage Density- depends on cage size; size variable- 27x17x14" to 3'x8'x15" cage with smaller compartments; material- wire mesh.

Food Green vegetation, seeds, dog food.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Sexual maturity - 50 days; gestation - 25 days; post-partum estrous - unknown; litter size - 1 to 7 (avg. 3.5); litter frequency - 3 to 5 per year; season - warm months; weaned at 13 days.

Special Costs Initial trapping program.

Restrictions

Toxicological Studies

Ecological Alternatives Any small herbivore.

Physiological Parameters

SPERMOPHILUS BEECHEYI

Description Large ground squirrel, head and body 9" to 11"; tail 1.25" to 2.5"; weight 45-70 g.; life expectancy-unknown.

Availability Locally abundant along Pacific coast.

Features Diurnal burrowing rodent, openland inhabitant.

Laboratory Light-normal photoperiod; temperature-"room"; relative humidity-over 50%.

Cage Density-depends on cage size; size-variable, 12 ft.^{3} packing crates with wire mesh, 4 to 10 ft^3 metal suspension cages; material-metal, wire mesh (wood not suitable); nest-boxes; bedding-cotton; substrate-burlap sacks, wood shavings; furniture-exercise wheels; sanitation-daily; breeding cage-4x8x2' of 1/2" wire mesh nest box, 12x12x12".

Food Seeds, greens, fresh vegetables, dog food, raw meat, lab chows.

Water Ad libitum.

Parasites & Disease Mites.

Breeding & Rearing Sexual maturity-1 year; gestation-25 to 30 days; post-partum estrous-unknown; litter size-4 to 15 (avg. 7); litter frequency-1 year; season-usually March to April; weaned-35 to 40 days.

Special Costs Initial trapping program.

Restrictions Possible social problems in colonies.

Toxicological Studies

Ecological Alternatives Other ground squirrels, Eutamias.

Physiological Parameters 1148, 1149

EUTAMIAS MINIMUS

Description Small chipmunk, head and body 3.5" to 4.5"; tail, bushy, 3" to 4.5"; weight, 30-60g.; life expectancy-5 to 8 years.

Availability Common throughout various habitats of west central lowlands and upland U.S. and across most of Canada.

Features Diurnal; very active, alert, agile; climbs readily; lives in burrows; stores seeds.

Laboratory Light-normal photoperiod; temperature-"room"; relative humidity-over 25%.

Cage Density-pair or more; size-variable, 2'x2'x2' must be covered; material-glass, metal, wood may be unsatisfactory; nest-small box, mailing tube; bedding-dried leaves, small twigs, grass; substrate-soil paper, wire mesh; furniture-exercise wheel.

Food Fruits, nuts, seeds insects, greens, chopped raw meats, cod liver oil.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Sexual maturity-1 year; gestation-30 days; post-partum estrous-no; litter size-2 to 6; litter number-1 per year; season-late spring; weaned-25 days.

Special Costs

Restrictions Poor previous success at breeding in captivity.

Toxicological Studies

Ecological Alternatives Tamias striatus; Spermophilus sp.; Peromyscus sp.

Physiological Parameters

TAMIAS STRIATUS

Description Woodland terrestrial squirrel; head and body 5" to 6"; tail bushy, 3" to 4"; weight, 65 to 130g.; life expectancy-8 years.

Availability Locally common throughout deciduous forests of eastern North America.

Features Diurnal, quick, alert, hibernates in winter.

Laboratory Light-500 to 2400 lux; temperature-20° to 25°C; relative humidity-70%.

Cage Density-1, possible more; size-2x2x2'; material-glass, metal, wire mesh; nest-box, flower post, mailing tube; bedding-cotton, grass dried leaves; furniture-exercise wheel, climbing poles.

Food Seeds, corns, fruits, insects.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Sexual maturity-6 months to 1 year; gestation-31 days; post-partum estrous-no; litter size-2 to 8 (avg. 4.5); litter frequency-2 per year; season-April and August.

Special Costs Initial trapping or supply.

Restrictions Unsociable habits may cause difficulty in group cages.

Toxicological Studies

Ecological Alternatives Tree squirrels.

Physiological Parameters

MICROTUS PENNSYLVANICUS

Description Meadow vole; head and body 3.5" to 5"; tail 1.5" to 2.5"; weight 30 to 70g.; life expectancy-unknown.

Availability Abundant in low moist areas, grasslands of all types, orchards, etc. across northern U. S. and Canada.

Features Active day or night.

Laboratory Light-natural photoperiod; temperature-"room"; relative humidity-over 10%.

Cage Density-depends on cage size; size-12x8x5" for breeding pair, 6x25x2' with 48 2x3x6" nest boxes; material-wire mesh, metal, plastic, fiberglass; nest-box 2x3x6"; bedding-cotton, fine grass; substrate-peat moss, sand wood shavings, corn husks; furniture-exercise wheel

Food Grasses, seeds, bark; greens may cause diarrhea.

Water Ad libitum.

Parasites & Disease Mites, fleas.

Breeding & Rearing Sexual maturity-40 to 50 days; gestation-21 days; post-partum estrous-yes; litter size-1 to 9 (avg. 3 to 5); litter frequency-monthly; weaning-21 days.

Special Costs Initial supply.

Restrictions

Toxicological Studies

Ecological Alternatives Other voles.

Physiological Parameters

PEROMYSCUS MANICULATUS

Description Typical mouse, brown to gray back and sides, white underparts; head and body 3" to 4"; tail 2" to 5"; weight 19 to 35g.; life expectancy-5 to 8 years.

Availability Common to abundant throughout the U. S. except the Southwest; numerous dealers.

Features Primarily nocturnal, some diurnal activity; limited climbing.

Laboratory Light-unknown; temperature-"room"; relative humidity-over 10%.

Cage Density-dependent on cage size; size-variable, 9x7x5", or 15x14x12"; material-wire mesh, wood, metal; nest-fruit jar on its side; bedding-cotton, dried leaves; substrate-woodchips, sand; furniture-exercise wheel.

Food Commercial diets available, lettuce, rolled oats, meat scraps, seeds, fruit.

Water Ad libitum.

Parasites & Disease Mites, ticks, fleas, nematodes.

Breeding & Rearing Sexual maturity-60 days; gestation-25 days; post-partum estrous-yes; litter size-3 to 6; litter frequency-5 to 9 per year; season-year round; weaned-25 days.

Special Costs Initial supply.

Restrictions

Toxicological Studies 167, 348, 506, 517

Ecological Alternatives Any small ominovorous rodent.

Physiological Parameters

MUSTELA VISON

Description Carnivorous mammal with long slender bodies and short legs; usually dark brown with white throat patch; total length 20" to 25" (Male) (1201); 17" to 20" (Female); weight-1-1/2 to 3 lbs. (Male); 1-1/4 to 2-2/5 lbs. (Female); life expectancy-3 years.

Availability Available from pelt industry farming; numerous mink ranches in U.S. raising 5000 or more animals per year (1202); have been trapped by professional trappers; range entire N. American continent except southeastern U.S.

Features Aggressive; chiefly nocturnal; at home on land or in water; usually lives in banks of stream or lake or under tree stumps or logs; usually solitary.

Laboratory Light-natural photoperiod; temperature-"room"; relative humidity-unknown.

Cage Density-1; observation cage-40x60x40cm.

Food Cotton rats; raw meat; mice; fish; muskrat; frogs; special mink food when sold commercially mixed diet of horsemeat, bonefish, ocean fish, liver and cereal.

Water Ad libitum.

Parasites & Disease Aleutian disease (viral disease causing lesions); Chediak-Higashi syndrome, leucodystrophy, hemivertebrae, Ehlers-Danlos syndrome, encephalopathy.

Breeding & Rearing Sexual maturity-1 year; gestation-40 to 75 days; litter size-2 to 10 per year; litter frequency-2 per year; season-March to April.

Special Costs Initial supply.

Restrictions Very nervous; can be vicious, will fight cage-mates.

Toxicological Studies 579, 580

Ecological Alternatives Other Mustelidae.

Physiological Parameters

MYOCASTOR COYPUS

Description Muskrat-like; head and body 22 to 25"; tail 12" to 17"; weight 15 to 20 lb.; hind feet webbed; life expectancy - 12 years.

Availability Locally abundant in southern Mississippi River Valley, Texas.

Features Nocturnal, feeder on aquatic plants, burrows in banks.

Laboratory Light- natural photoperiod; temperature- about 70°F; relative humidity- over 50%.

Cage Density - 2; size - variable (3x2x1'); material - metal, hardware cloth; nest (for breeding) 20x20cm; bedding - straw; substrate - sawdust.

Food Sugarbeets, carrots, cabbage, grass, bread, cereals.

Water Ad libitum.

Parasites & Disease Salmonella, tuberculosis, tularaemia, ticks, fleas (550).

Breeding & Rearing Sexual maturity - 5 to 9 months; gestation - avg. 128 days; post-partum estrous - yes; litter size - 2 to 11; litter number - over 2 per yr.; season - year round; weaned - 24 hours.

Special Costs Initial supply, metal cages.

Restrictions Metal cages only; ponds for swimming may be necessary.

Toxicological Studies

Ecological Alternatives Voles, muskrats.

Physiological Parameters

MUSTELA PUTORIUS

Description Carnivore; head and body about 20", tail about 10", bushy; weight 400-3,500g; life expectancy - 3 to over 5 years.

Availability Suppliers.

Features Domestic form, long fur, easily handled in lab.

Laboratory Light - natural photoperiod; temperature - 10° to 30°C.

Cage Density - dependent on cage size; size - variable ($60cm^2$ for 3 females or 2 males; material - wood metal, concrete; bedding - wood shavings.

Food Meat, bread, milk, commercial diets.

Water Ad libitum.

Parasites & Disease Pneumonia, gastric ulcers, virae, bacteria, mites, ticks.

Breeding & Rearing Sexual maturity - 4 to 5 mos.; gestation - 51 to 43 days; post-partum estrous - unknown; litter size - 3 to 15; litter number - 2 per yr.; season - spring to July, August.

Special Costs Initial supply.

Restrictions

Toxicological Studies

Ecological Alternatives Weasels, mink.

Physiological Parameters

VULPES VULPES

Description Red fox; head and body 22-25"; tail 14-16", wt. 30-80 lb.

Availability Most of U. S., fur ranchers.

Features Doglike animal, reddish fur; bushy tail, active day and night.

Laboratory Light-natural photoperiod; Temperature - "room"; Relative humidity - 50%.

Cage Density-variable; size-large enough for exercise; Material-wood, metal, wire mesh; Nest-dog house; Bedding-straw.

Food Fish, cooked cereals, berries, dog food.

Water Ad libitum.

Parasites & Disease Ticks, fleas, rabies.

Breeding & Rearing Sexual maturity - 1 yr.; Gestation - about 51 days; Postpartumestrous - No; Litter size 4 to 9: Litter number - 1/yr. Season - March to April.

Special Costs

Restrictions

Toxicological Studies 560

Ecological Alternatives Coyotes.

Physiological Parameters

CANIS FAMILIARIS

Description Domestic dog; head and body 12 to 45"; tail 5 to 25", life span - to 15 years.

Availability Dog pounds, breeders, supply houses.

Features Docile, domestic.

Laboratory Light-"natural photoperiod"; temperature-"room", relative humidity-60-80%.

Cage Density 1 to 5; size-depends on size (from 1to3 sq. ft. per individual); material-wood, metal, concrete; bedding-shavings, rugs; furniture-nest box for mothers.

Food Commercial dog food.

Water Ad libitum.

Parasites & Disease Ticks, fleas, tapeworms, heartworms.

Breeding & Rearing Sexual maturity 1 yr.; gestation-9 wks.; post partem estrous-no; litter size-3 to 10; litter number-1 to 2 per year; weaned-about 28 days.

Special Costs Purebred animals are expensive.

Restrictions

Toxicological Studies 822

Ecological Alternatives Domestic cats.

Physiological Parameters

SUS SCROFA - miniature swine

Description Small pig; weight - 100 to 200 pounds (1203).

Availability Through Sinclair Comparative Research Farm, University of Missouri, Columbia, MO 65201; $70-$100 per animal.

Features Breeds and maintains fairly easily in lab; good "model" animal; economically important (1203); veteran test animal.

Laboratory Light - natural photoperiod; temperature - "room".

Cage 8 x 12' fenced-in holding pens, 3 to 4 sq. ft. per animal; 6 animals per cage(1203); experiment enclosure 2 x 3.5' (1203).

Food Various mixtures of cornstarch, sugar, soybean meal, corn ground shell, meat and bone meal, alfalfa meal, wheat shorts, dical phosphate, limestone, salt, vitamin premix once a day (1204);

Water Should be available at all times.

Parasites & Disease

Breeding & Rearing 114 day gestation period; 3 litters per year.

Special Costs Cage cleaning.

Restrictions

Toxicological Studies 612

Ecological Alternatives Dog, other pigs.

Physiological Parameters

OVIS - domestic sheep

Description Domestic ungulate wool producer.

Availability Has wide distribution in U.S.; found in almost all agricultural areas.

Features Plentiful; adapts to lab easily; not too expensive; model ruminant; can tolerate surgical procedures; economically important; easy to maintain and breed; behaves well; good animals for physiological and behavioral studies (613).

Laboratory Light-natural photoperiod; temperature-"room".

Cage Any type of large outdoor or indoor pen is sufficient as long as it gives each animal 64 sq. ft. of territory (613).

Food Hay supplemented by corn and protein (613).

Water Ad libitum

Parasites & Disease Much information available; wide variety of parasites and diseases.

Breeding & Rearing Usually breed in fall and give birth in early spring to early summer; 1 to 2 offspring per yr. (613).

Special Costs

Restrictions

Toxicological Studies 590, 592, 613, 616, 617, 618, 619, 620, 627, 628, 629

Ecological Alternatives Other ruminants.

Physiological Parameters

ODOCOILEUS sp.

Description Deer; height 3" to3.5'; weight-females 100 to 250 lbs., males, 75-400 lbs.; carry antlers during part of year, large tail; life expectancy-15 years.

Availability White-tail deer found through N. America except Calif., Nev., Utah; mule deer found in N. America west of Great Plains; both species abundant locally.

Features Active day and night, seen often in herds; very alert creatures; generally found in forested regions.

Laboratory Outdoor enclosures most suitable; cover from sun and cold essential.

Cage Any enclosure of suitable areal extent and height will suffice; walls-over 8 feet.

Food Twigs, berries, acorns, apples, cereals, dairy chow.

Water Ad libitum.

Parasites & Disease

Breeding & Rearing Sexual maturity-1 year; gestation-6 to 7 months; post-partum estrous-no; litter size-1 (sometimes 2); litter number-1 per year; season-breed in fall; birth in spring.

Special Costs Large enclosures, initial supply.

Restrictions Large size, long generation time.

Toxicological Studies 625, 631

Ecological Alternatives Rabbits, woodchuck.

Physiological Parameters

SAIMIRI SCIUREUS

Description Small New World monkey; prehensile tail; head and body 9 to 12"; weight - 2 to 4 lbs.

Availability Imported from Colombia, S.A.; available through Primate Imports Corporation, 34 Munson St., Port Washington, L.I., N.Y. (613, 1205).

Features

Laboratory Light-natural photoperiod; temperature - 74°F (1205).

Cage 15x20x30" individual cage (about the same price as a dog cage (613); 24x24x28" metal and plastic test cage (1205); 14x19x24" individual maintenance cages (1205).

Food Commercially prepared chow plus oranges, fresh fruit and other supplements (613).

Water Ad libitum

Parasites & Disease Worm parasites common; enteric and respiratory problems common (613).

Breeding & Rearing 160 to 170 day gestation period.

Special Costs

Restrictions Animals fairly expensive and rare; will bite.

Toxicological Studies 613, 1205

Ecological Alternatives

Physiological Parameters

Special Reference The Squirrel Monkey, Cooper and Rosenblum.

DIDELPHIS MARSUPIALIS

Description Marsupial; head and body 15" to 20"; tail 9" to 20"; weight, 9 to 13 lbs.; life expectancy-over 7 years.

Availability Locally abundant throughout most of U. S.

Features Nocturnal, slow moving, will act dead if molested, odorous prehensile tail and opposable first digits.

Laboratory Light-natural photoperiod; temperature-over 15°C; relative humidity-over 50%.

Cage Density-2; size-20x22x15" and larger; material-galvanized metal; nest boxes-5x5x3' in large outdoor cages.

Food Fruits, vegetables, eggs, carrion, dog pellets, table sc

Water Ad libitum.

Parasites & Disease Many and varied diseases (641, 642, 647, 649, 650, 651, 653).

Breeding & Rearing Sexual maturity-1 year; gestation-13 days, followed by 2 months in pouch of mother; post partum estrous-no; litter size-up to 14; litt number-2 per year possible; season-warm month weaned-2 months; best results in large, outdoor pens.

Special Costs Initial supply.

Restrictions Opossums are very susceptible to disease.

Toxicological Studies

Ecological Alternatives Marmosa mitis.

Physiological Parameters 651, 1028, 1112

DASYPUS NOVEMCINCTUS

Description Armadillo; armored body; head and body 16"; tail 15"; wieght 8 to 17 lbs.; life expectancy 6 years.

Availability Southern U. S., Texas area.

Features Nocturnal, digger, polyembryonous.

Laboratory Light-natural photoperiod; temperature-over 20°C; relative humidity-unknown.

Cage Density-unknown; size-9x9x4' and 18"x4'x4' compartments in one cage; material-concrete floor plywood walls; nest-unknown; substrate-cedar shavings; furniture-pool of water, logs for scratching.

Food Berries, fruits, roots, dog food, raw beef, milk, eggs, liver.

Water Ad libitum.

Parasites & Disease Infection beneath dermal plates is common.

Breeding & Rearing Sexual maturity-unknown; gestation-(delayed implantation); post-partum estrous-no; litter size-1 to 12; litter number-1 per year; season-breed in fall.

Special Costs Initial supply; special caging.

Restrictions Armadillos easily injure themselves on metal, screen, etc., and armor may restrict certain methods.

Toxicological Studies

Ecological Alternatives Other armadillo species.

Physiological Parameters 662, 663, 664, 665, 666

SECTION VII

BIBLIOGRAPHY

1. Rudd, R. L. 1964. Pesticides and the Living Landscape. The University of Wisconsin Press, Madison, Wisconsin

2. Animal Welfare Act, Statutes at Large, 80 (1966) PL 89-543

3. Animal Welfare Act, Statutes at Large, 84 (1970) PL 91-579

4. Reitz, A. W., Jr. 1972. Environmental Law, North American International, Washington, D. C.

5. Chance, M. R. A. 1947. Factors influencing the toxicity of sympathomimetic amines to solitary mice. J. Phar. and Exp. Ther. 89:289

6. Webb, R. E., Hartgrove, R. W., Randolph, W. C., et al. 1973. Toxicity studies in endrin-susceptible and resistant strains of pine mice. Tox. and App. Phar. 25:42-47

7. National Academy of Sciences. 1971. Animals for Research. Institute of Laboratory Animal Resources, National Research Council, Washington, D. C.

8. Kendeigh, S. C. 1964. Animal Ecology. Prentice-Hall, Englewood Cliffs, N. J.

9. Ozburn, G. W., and Morrison, F. O. 1962. Development of a DDT-tolerant strain of laboratory mice. Nature 196:(4858):1009

10. Georghiou, G. P. 1972. The evolution of resistance to pesticides, pp 133-167 in Annual Review of Ecology and Systematics, Vol. 3, ed. by R. F. Johnston. Annual Reviews, Inc. Palo Alto

11. Hart, L. G., et al. 1963. Stimulatory effects of chlordane on hepatic microsomal drug metabolism in the rat. Tox. App. Pharm. 5:371

12. Cory, L., Fjeld, P., and Serat, W. 1971. Environmental DDT and the genetics of natural populations. Nature 229:128-130

13. Sterburg, J., and Kearns, C. W. 1952. The presence of toxins other than DDT in the blood of DDT-poisoned roaches. Science 116:144

14. Sternburg, J., et al. 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. J. Econ. Entom. 43:214

15. O'Brien, R. D. 1967. Insecticides Action and Metabolism. Acad. Press

16. Miyake, S. S., et al. 1957. Distribution of DDT-dehydrochlorinase in various tissues of DDT-resistant house flies. J. Econ. Entom. 50:359

17. Lovell, J. B., and Kearns, C. W. 1959. Inheritance of DDT-dehydrochlorinase in the house fly. J. Econ. Entom. 52:931

18. Huxley, J. 1943. Evolution, The Modern Synthesis. Harper and Bros. New York

19. Kapoor, I. P., et al. 1970. Comparative metabolism of methoxychlor, methiochiochlor and DDT in mice, insects and in a model ecosystem. J. Agr. Food Chem. 18(6):1145-1152

20. Stickel, W. 1974 - Patuxant Wildlife Research Center, U.S. Fish and Wildlife Service, Laurel, Maryland, Personal Communication

21. Woodwell, G. M. 1967. Toxic substances and ecological cycles. Sci. Am. 216(3):24-31

22. Crosby, D. G. 1973. The fate of pesticides in the environment. Ann. Rev. of Plant Physiology 24:467-492

23. Gunther, F. A. 1966. Advances in analytical detection of pesticides. In: Scientific Aspects of Pest Control, Publ. 1402, National Acad. Sci., National Research Council, Washington, D. C.

24. Buchsbaum, R. 1948. Animals Without Backbones. U. of Chicago Press, Chicago

25. Ball, R. C. and Curry, L. L. 1956. Culture and agricultural importance of earthworms. Cir. Bull. 222. Michigan State U., Agr. Expt. Sta.

26. Ashby, G. J. 1972. Earthworms. The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ch 53:610-613 ed. U.F.A.W. staff, Churchill Livingstone Edinburgh and London

27. McLane, M. A. R., et al. 1971. Organochlorine pesticide residues in woodcock, soils, and earthworms in Louisiana, 1965. Pest Mon. J. 5(3):248-250

28. DeWitt, J. B., et al. 1960. Pesticidal residues in animal tissue. Trans. Twenty-Fifth North American Wildlife Conference:277-285

29. Wheatley, G. A., and Hardman, J. A. 1968. Organochlorine insecticide residues in earthworms from arable soils. J. Sci. Fed. Agr. 19:219-225

30. Doane, C. C. 1962. Effects of certain insecticides on earthworms. J. Econ. Entomol. 55:416-418

31. Satchell, J. E. 1955. The effects of B.H.C., DDT and parathion on soil fauna. Soils and Fertilizer 18:279-285

32. Thompson, A. R. 1971. Effects of nine insecticides on the numbers and biomass of earthworms in pasture. Env. Contam. and Tox. Bull. 5(6):577-586

33. Bailey, T. N. 1971. Immobilization of bobcats, coyotes and badgers with phencyclidine hydrochloride. Journal of Wildlife Management 35(4)

34. Lipa, J. J. 1958. Thermocline off the coast of Tropical West Africa. Nature 181:863

35. Polivka, J. B. 1951. Effect of insecticides upon earthworm populations. Ohio J. Sci. 51(4):195-196

36 Davis, B. N. K. 1968. The soil macrofauna and organochlorine insecticide residues at twelve agricultural sites near Huntingdon. Ann. App. Bio. 61:29-45

37. Dimond, J. B., et al. 1970. DDT residues in robins and earthworms associated with contaminated forest soils. Can. Entomol. 102(9):1120-1130

38. Jefferies, D. J., and Davis, B. N. K. 1968. Dynamics of dieldrin in soil, earthworms and song thrushes. Journal of Wildlife Management 32(3):441-456

39. Davis, B. N. K., and French, M. C. 1969. The accumulation and loss of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields. Soil Bio. and Biochem. 1:45-55

40. Davey, S. P. 1963. Effects of chemicals on earthworms: a review of the literature. U.S.F.W.S. Special Scientific Report 74:1-18

41. Wright, C.A. 1967. Land and freshwater Molluscs, the U.F.A.W. Handbook on the Care and Management of Laboratory Animals, ch 52:604-609 ed. U.F.A.W. staff E.S. Livingstone, ltd., Edinburgh and Scotland

42. Stephens, G. J., and McGaugh, J. L. 1972. Biological factors related to learning in the land snail (Helix aspersa Muller). Anim. Behav. 20:309-315

43. Herzberg, F., and Herzberg A. 1962. Observations on reproduction in Helix aspersa. Am. Midland Nat. 68:297-306

44. Herzberg, F. 1965. Crowding as a factor in growth and reproduction of Helix aspersa. Am. Zoo. 5:254

45. Stephens, G. J. and Stephens, G. C. 1966. Photoperiodic stimulation of egg laying in the land snail Helix aspersa. Nature 212:1582

46. Walker, R. J. 1967. Certain aspects of the pharmacology of Helix and Hirudo neurons. Symp. on Neurbiol. of Invert. Ed: J. Salanki, 1967:227-253

47. Kerkut, G. A., and Walker, R. J. 1962. The specific chemical sensitivity of Helix nerve cells. Comp. Biochem. Physiol. 7:277-288

48. Kerkut, G. A., and Walker, R. J. 1961. The effect of drugs on the neurones of the snail, Helix aspersa. Comp. Biochem. Physiol. 3:143-160

49. Kerkut, G. A., and Leake, L. D. 1966. The effect of drugs on the snail pharangeal retractor muscle. Comp. Biochem. Physiol. 17:623-633

50. Menhinick, E. F. 1962. Comparison of invertebrate populations of soil and litter of mowed grasslands in areas treated and untreated with pesticides. Ecology 43(3):556-561

51. Barnett, S. F. 1972. Ticks. The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed U.F.A.W. Staff, Churchill Livingstone, Edinburgh and London

52. Witt, P. N. 1971. Instructions for working with web-building spiders in the laboratory. BioSci. 21(1):23-25

53. Witt, P. N. 1974. North Carolina Dept. of Mental Health, Raleigh N.C., Personal Communication

54. Griffiths, D. C., Raw, F., and Lofty, J. R. 1967. The effects on soil fauna of insecticides tested against wireworms (Agriotes sp.) in wheat. Ann. App. Bio. 60:479-490

55. Pielou, D. P. 1960. The effect of DDT on oviposition and on behavior in the European red mite, Panonychus ulmi (Koch). Can. J. Zoo. 38:1147-1151

56. Lord, F. T. 1956. The influence of spray programs on the fauna of apple orchards in Nova Scotia IX. Studies on means of altering predator populations. Can. Entomologist 88:129-137

57. Borror, D. J., and DeLong, D. 1954. An Introduction to the Study of Insects. Holt, Rinehart & Winston Co., New York

58. Harris, C. R., and Svec, H. J. 1963. Mass rearing of the common field cricket, Gryllus pennsylvanicus for use as a test insect in toxicological studies. Bulletin Entom. Res. 54:805

59. Rochford, P. J. 1972. The American cockroach. The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed U.F.A.W. Staff, Churchill Livingstone, Edinburgh and London

60. Ashby, G. J. 1972. Locusts. The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed. U.F.A.W. Staff, Churchill Livingstone, Edinburgh, and London

61. Gurney, A. B. 1950. Praying mantids of the United States, native and introduced. Ann. Report: Smith. Inst. 339-362

62. Ashby, G. J. 1972. The house cricket, Chapter 49, pp 588-589 in The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed. by U.F.A.W. Staff, C. Livingstone, Edinburgh and London

63. Brindley, W. A., and Dahm, P. A. 1970. Miscroscopic examination of parathion activating microsomes from American cockroach fat body. J. Econ. Entomol. 63:31-38

64. Meksongsee, B., et al. 1967. Effect of inhibitors and inducers of microsomal enzymes on the toxicity of carbamate insecticides to mice and insects. J. Econ. Entom. 60(5):1469-1471

65. Nakatsugawa, T., and Dahm, P. A. 1965. Parathion activation enzymes in the fat body microsomes of the American cockroach. J. Econ. Entom. 58(3):500-509

66. Twarog, B. M., and Roeder, K. D. 1957. Pharmacological observations on the desheated last abdominal ganglion of the cockroach. Ann. Entom. Soc. Amer. 50:231-237

67. Hamilton, E. W. 1971. Metabolism of aldrin and dieldrin by the American cockroach, Periplaneta americana. Ph.D. Thesis, Iowa State University

68. Menzer, R. E. and Casida, J. E. 1965. Nature of toxic metabolites formed in mammals, insects and plants from 3-(Dimethoxy-phosphenyloxy)-N, N-Dimethyl-cis-crotonamide and its N-methyl analog. J. Agr. Food Chem. 13:102-112

69. O'Brien, R. D. 1957. Properties and metabolism in the cockroach and mouse of malathion and malaoxon. J. Econ. Entomol. 50(2):159-164.

70. Patel, N. G., and Cutkomp, L. K. 1967. Pysiological responses of cockroaches to immobilization, DDT, and dieldrin. J. Econ. Entom. 60(3):783-788

71. Harris, C. R. 1964. Influences of soil type and soil moisture on the toxicity of insecticides in soils to insects. Nature 202:724

72. Cruz, C. 1972. Chlordane effects on the hairy chinch bug. Ph.D. Thesis, Rutgers University, State University of New Jersey

73. Agosin, M., et al. 1965. Enhanced protein synthesis in Triatoma infestans treated with DDT. Esp. Parasitol 16:318-324

74. Agosin, M., et al. 1961. Intermediary carbohydrate metabolism of Triatoma infestans (Insecta; Hemiptera) - I. Glycolytic and pentose phosphate pathway enzymes and the effect of DDT. Comp. Biochem. Physiol. 2:143-159

75. Yothers, M. A., and Carlson, F. W. 1948. Effect of DDT on the green peach aphid and its Coccinellid predators. J. Econ. Entom. 41:515-516

76. Hintz, S. D. 1971. Herbicidal influence on cereal grain aphids in North Dakota. Ph.D. Thesis, North Dakota State University

77. Davis, B. N. K., and Harrison, R. B. 1966. Organochlorine insecticide residues in soil invertebrates. Nature 211:1424-1425

78. Moffitt, H. R., et al. 1972. Toxicity of several commonly used orchard pesticides to adult Hippodamia convergens. Env. Entomol. 1(1):20-22

79. Atallah, Y. H. and Newsom, L. D. 1966. Ecological and nutritional studies on Coleomegilla masculata De Geer (Coleoptera: Coccinellidae). III. The effect of DDT, toxophene, and endrin on the reproductive and survival potentials. J. Econ. Entomol. 59:1181-1187

80. Whitney, W. K. 1967. Laboratory tests with dursban and other insecticides in soil. J. Econ. Entomol. 60(1):68-74

81. Cole, J. H. 1972. Beetles. The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed U.F.A.W. Staff, Churchill Livingstone, Edinburgh and London

82. Dempster, J. P. 1968. The sublethal effect of DDT on the rate of feeding by the ground-beetle. Entomologia Experimentalis et Applicable 11:51-54

83. Fleming, W. E., et al. 1962. Bioassay of soil containing residues of chlorinated hydrocarbon insecticides. U.S. Dept. Agr. Tech. Bull. No. 1246

84. Fleming, W. E., and Hawley, I. M. 1950. A large-scale test with DDT to control the Japanese beetle J. Econ. Entomol. 43:586-590

86. Harris, C. R. et al. 1958. A laboratory method of mass rearing of the block cutworm, Agrotis ypsilon, for insecticide tests. Canadian Entomologist 90(6):328-331

87. Howell, J. F. 1967. Paraffin films to control dehydration of an artificial rearing medium for codling moth. J. Econ. Entom. 60(1):289-290

88. Shorey, H. H., and Hale, R. L. 1965. Mass-rearing of the larvae of nine Noctuid species on a simple artificial medium. J. Econ. Entom. 58(3):522-524

89. Roelofs, W. L., and Comeau, A. 1971. Sex pheromone perception: electroantennogram responses of the red-banded leaf roller moth. J. Insect. Physiol. 17:1969-1982

90. Roelofs, W. L. and Arn, H. 1968. Sex attractant of the red-banded leaf roller moth. Nature 219:513

91. Jacobson, M., et al. 1970. Sex pheromones of the Lepidoptera. Recent progress and structure-activity relationships, in: Chemicals Controlling Insect Behavior. Ed. M. Berosa, Acad. Press, New York

92. Berger, R. S. 1966. Isolation, identification, and synthesis of the sex attractant of the cabbage looper, Trichoplusia ni. Ann. Entom. Soc. America 59(4):767-771

93. Shorey, H. H. et al. 1965. Sex pheromones of Noctuid moths. VI. Absence of behavioral specificity for the female sex pheremones of Trichoplusia ni versus Autographa californica and Heliothis zea versus H. virescens (Lepidoptera:Noctuidae). Ann. of the Entomol. Soc. of America 58(5):600-603

94. Nasim, A., and Brindley, W. A. 1971. Effects of chlorcyclizine or phenobarbitol on in vitro detoxification activity by larvae wax moth gut homogenates. Tox. and App. Phar. 18:124-132

95. Ahmad, N., and Brindley, W. A. 1969. Modification of parathion toxicity to wax moth larvae by chlorcyclizine, aminopyrine or phenobarbital. Tox. and App. Phar. 15:433-440

96. Polles, S. G. 1970. The fate of C^{14}-labeled endrin in tobacco budworm (Heliothis virescens) (Fabricius) larvae. Ph.D. Thesis, Mississippi State University

97. Kishaba, A. N. et al. 1967. Laboratory technique for studying flight of Cabbage looper moths and the effects of age, sex, food, and tepa on flight characteristics. J. of Econ. Entomol. 60(2):359-366

98. Ashby, G. J. 1972. The house fly, Chapter 43, pp 549-553 in The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed. by U.F.A.W. Staff, C. Livingstone, Edinburgh and London

99. Stones, L. C. 1972. The stablefly, Chapter 45, pp 556-564 in The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed. U.F.A.W. Staff, Churchill Livingstone, Edinburgh and London

100. Bemrick, W. J. and Schlotthauer, J. C. 1969. Mosquito colonies in veterinary research. Lab. An. Care 19:186-190

101. Hilsenhoff, W. L. and R. P. Narf 1967. Colonization of Chironomus plumosus. Mosquito News 27:363

102. Wheeler, M. R. 1972. Fruitflies, Chapter 42, pp 543-548 in The U.F.A.W. Handbook on the Care and Management of Laboratory Animals, Ed. by U.F.A.W. Staff, 4th ed. C. Livingstone, Edinburgh and London

103. Ashby, G. J. 1972. Blowflies, The U.F.A.W. Handbook on the Care and Management of Laboratory Animals, ch 44:554-555. Ed. U.F.A.W. Staff, Churchill Livingstone, Edinburgh and London.

104. Reynolds, H. H., and Back, K. C. 1966. Effect of injected monomethyl hydrazine on primate performance. Tox. and App. Phar. 9:376-389

105. Biever, K. D. 1965. A rearing technique for the colonization of Chironomid nidges. Ent. Soc. America 58:135-136

106. Yeatman, F. R. and Hirsch, J. 1971. Attempted replication of and selective breeding for instrumental conditioning of Drosophila melanogaster. Animal Behav. 19:454-462.

107. Monteith, L. G. 1963. Habituation and associative learning in Drino bohemica Mesh (Diptera:Tachinidae). Can. Ent. 95:418-426

108. Benezet, H. J., and Forgash, A. J. 1972. Penetration and distribution of topically applied malathion in the house fly. J. Econ. Entom. 65:53-57

109. Gerolt, P. 1969. Mode of entry of contact insecticides. J. Insect Physiol. 15:563-580

110. Blend, M. J. 1970. In vivo and in vitro effects of low concentrations of dieldrin and steroid hormones on the dog prostate. Ph.D. Thesis, Cornell University, Ithaca, New York

111. Ilnytzka, S. 1970. Bioassay of insecticide residue in soils. Ph. D. Thesis, McGill University, Montreal, Canada

112. Sharma, N. 1973. Sublethal insecticide and its effect on reproduction in the housefly, Musca domestica L. and the yellow fever mosquito Aedes aegypti L. Ph.D. Thesis, Rutgers University, State University of New Jersey

113. Johnsen, R. E., and Dahn, P. A. 1966. Activation and degradation efficiencies of liver microsomes from eight vertebrate species, using organophosphates as substrates. J. Econ. Entomol. 59:1437-1442

114. Beard, R. 1965. Ovarian suppression by DDT and resistance in the house fly (Musca domestica L.) Entom. Exp. Appl. 8:193-204

115. Vinopal, J. H., and Johansen, C. A. 1967. Selective toxicity of four O-(methylcarbomyl) oximes to the house fly and the honey bee. J. Econ. Entom. 60(3):794-798

116. Bateman, A. J. 1966. Testing chemicals for mutagenicity in a mammal. Nature 210:205-206

117. Lüning, K. G., 1966. Drosophila-tests in pharmacology. Nature 209:84-86

118. Sokal, R. R. 1959. A morphometric analysis of strains of Drosophila melanogaster differing in DDT resistance. J. Kansas Entom. Soc. 32(4):155-172

119. Altenburg, L. S. 1958. The effect of photoreactivating light on the mutation rate induced in Drosophila by tertiary-butyl hydroperoxide. Genetics 43:662-664

120. Holm, S. N. 1966. The utilization and management of bumble bees for red clover and alfalfa seed production. Ann. Rev. Entomol. 11:155-182

121. Morton, H. L. 1974. Agricultural research service, USDA, Tucson, Arizona, Personal Communication

122. Morton, H. L., et al. 1972. Toxicity of herbicides to newly emerged honey bees. Env. Entomol. 1(1): 102-104

123. Morse, R. A. 1961. The effect of sevin on honey bees. J. Econ. Entomol. 54(3):566-568

124. Levin, M. D., et al. 1968. Impact on colonies of honey bees of ultra low-volume (undiluted) malathion applied for control of grasshoppers. J. Econ. Entom. 61(1):58-62

125. Waller, G. D. 1969. Susceptibility of an alfalfa leaf-cutting bee to residues of insecticides on foliage. J. Econ. Entom. 62(1):189-192

126. Anderson, L. D. 1952. Toxicity of several new insecticides to honey bees. J. Econ. Entom. 45(3): 466-469

127. Ahmad, Z. 1971. Selective toxicity of carbophenothion and trichlorfon to the honey bee, Apis mellifera Linnaeus and the alfalfa leaf cutter bee, Megachile rotundata Fabricius. Ph.D. Thesis, Washington State University

128. Graves, J. B., and Mackensen, O. 1965. Topical application and insecticide resistance studies on the honey bee. J. Econ. Entom. 58(5):990-993

129. Anderson, L. D., and Atkins, E. L., Jr. 1958. Toxicity of pesticides to honey bees in laboratory and field tests in southern California, 1955-1956. J. Econ. Entom. 51(1):103-108

130. Edwards, C. A., Dennis, E. B., and Empson, D. W. 1967. Pesticides and the soil fauna: effect of aldrin and DDT in an arable field. Ann. App. Bio. 60:11-22

131. Reichle, D. E. 1967. Radioisotope turnover and energy flow in terrestrial isopod populations. Ecology 48:351-366

132. Deoras, P. J. 1967. Handling of scorpions. Husbandry of Lab Animals:17-20

133. Connant, R. 1958. A Field Guide to Reptiles and Amphibians of the United States and Canada, East of the 100th Meridian. Boston, Houghton Mifflin

134. DiBerardino, M. A. 1967. Frogs. Methods in Developmental Bio. pp. 53-74

135. Schmidt, R. S., and Hudson, W. R. 1969. Maintenance of adult Anurans. Lab. An. Care 19(5):617-620

136. Shapiro, H. 1965. A plastic frog tank, with observations on survival. Lab. An. Care 15(6):422-425

137. Nace, G. W. 1968. The amphibian facility of the University of Michigan. BioSci. 18(8):767-775

138. Fankhauser, G. 1963. Amphibia. Animals for Research. Principles of Breeding and Management, 17:475-498, New York Academic Press

139. Kaplan, H. M., et al. 1964. Toxicity of fluoride for frogs. Lab. An. Care 14:185-188

140. Cameron, J. A. 1940. Effect of flourine on hatching time and hatching stage in Rana pipiens. Eco. 21(2):288-292

141. Kaplan, H. M. 1969. Anesthesia in amphibians and reptiles. Fed. Proc. 28(4):1541-1546

142. Boterenbrood, E. C. 1972. Urodeles, Chapter 39, pp 520-532 in The U.F.A.W. Handbook on the Care and Management of Laboratory Animals. Ed. by U.F.A.W. Staff, C. Livingstone, Edinburgh and London

143. Hirschfield, W. J., et al. 1970. Growth of larval and juvenile Rana pipiens on four laboratory diets. Am. Zool. 10(3):315

144. Berns, M. W. 1965. Research notes. Mortality caused by kidney stones in spinach-fed frogs. Bio. Sci. 15(4):297-298

145. Wright, P. A., and Flathers, A. R. 1961. Faciliation of pituitary-induced frogs ovulation by progesterone in early fall. Proc. Soc. Exp. Bid Med. 106:346-347

146. Gibbs, E. L., Gibbs, T. J., and VanDyck, P. C. 1972. Rana pipiens: health and disease. Lab. An. Care 16(2):142-160

147. Meeks, R. L., 1968. The accumulation of 36 Cl ring-labeled DDT in a freshwater marsh. J. Wildlife Mgmt. 32(2):376-398

148. Herald, E. S. 1949. Effects of DDT oil solutions upon amphibians and reptiles. Herpetologica 5:117-120

149. Korschgen, L. J. 1970. Soil-food-chain-pesticide relationships in aldrin-treated fields. Journal of Wildlife Management 34(1):186

150. Ferguson, D. E., and Gilbert, C. C. 1968. Tolerances of three species of Anuran amphibians to five chlorinated hydrocarbon insecticides. J. Minn. Acad. Sci. 13:135-138

151. Kaplan, H. M., and Overpeck, J. G. 1954. Toxicity of halogenated hydrocarbons insecticides for the frog, Rana pipiens. Herpetologica 20(3):163-169

152. Potter, J. L., and O'Brien, R. D. 1964. Parathion activation by livers of aquatic and terrestrial vertebrates. Science 144:55-57

153. Upshall, D. G., et al. 1968. Biochemical studies on the teratogenic action of bidrin and other neuroactive agents in developing hen eggs. Biochem. Phar. 17:1529-1542

154. Boyd, C. E., et al. Possible DDT resistance in two species of frogs. Copeia (2):426-429

155. Sanders, H. O. 1970. Pesticide toxicities to tadpoles of the western chorus frog, Psendacris triseriata, and Fowler's toad, Bufo woodhousii fowleri. Copeia (2): 246-251

156. Bucher, N. L. R. 1946. Effects of 2, 4-dichlorophenoxyacetic acid on experimental animals. Proc. Soc. Exp. Bio. Med. 63:204

157. Goldfischer, S. et al. 1970. Cooper in hepatocyte lysosomes of the toad, Bufo marinus L. Nature 288:172-173

158. Kilburn, K. H. 1967. Mucociliary clearance from bullfrog (Rana cantesbiana) lung. J. App. Physiol. 23:804-810

159. Bernfeld, P., Nixon, C. W., and Homburger, F. 1964. Studies on the effect of irritant vapors on ciliary mucus transport. Tox. and App. Phar. 6:103-111

160. Schlumberger, H. G., and Lucke, B. 1948. Tumors in fishes, amphibians and reptiles. Cancer Research 8:657-754

161. Elkan, E. 1963. Three different types of tumors in Salientia. Cancer Research 23, Nov. :1641-1645

162. Balls, M. 1962. Spontaneous neoplasms in amphibia: a review and descriptions of six new cases. Cancer Research 22:1142-1154

163. Van Der Steen, A. B. M., et al. 1972. Cutaneous neoplasms in the Leopard Frog (Rana pipiens). Lab. An. Sci. 22(2):216-222

164. Duryee, W. R. 1965. Factors influencing development of tumors in frogs. N. Y. Acad. Sci. 126:59-84

165. Gaumer, A. H., and Goodnight, C. J. 1957. Some aspects of the hematology of turtles as related to their activity. Am. Mid. Nat. 58:332-340

166. Doyle, R. E., and Moreland, A. F. 1968. Diseases of turtles. Lab. An. Digest 4:3-6

167. Stickel, L. F. 1951. Wood mouse and box turtle populations in an area treated annually with DDT for five years. Journal of Wildlife Management 15(2):161-164.

168. Benes, E. S. 1968. A study in laboratory maintenance of the Gila monster. Lab. An. Care 18(1):69-74

169. Boam, G. W. and Cowan, D. F., 1970. The pathogenicity for mice of two species of salmonella isolated from the green iguana. J.A.V.M.A. 157(5):617-619

170. Marcus, L. C. 1971. Infectious diseases of reptiles. J.A.V.M.A. 159(11):1626-1631

171. Wallach, J. D. 1971. Environmental and nutritional diseases of captive reptile. J.A.V.M.A. 159(11) 1632-1643

172. Johnson, T. S., et al. 1967. Cellular renewal of intestinal epithelium in the western fence lizard Sceloporus occidentalis. Can. J. Zoo. 45:63-71

173. King, F. W. 1971. Housing, sanitation, and nutrition of reptiles. J.A.V.M.A. 159(11):1612-1615

174. George, J. L., and Stickel, W. H. 1949. Wildlife effects of DDT dust used for tick control on a Texas prairie. Am. Midl. Nat. 42(1):228-237

175. Burghardt, G. 1974. Psychology Dept., University of Tennessee, Knoxville, Tennessee, Personal Communicatio

176. Karlstrom, E. L., and Cook, S. F., Jr. 1955. Herpetological notes. Copeia, 1955 (1):57-58

177. Brazenor, C. W., and Kaye, G. 1953. Anaesthesia for reptiles. Copeia, 1953 (3):165-170

178. Betz, T. W. 1962. Surgical anesthesia in reptiles, with special reference to the water snake, Natrix rhombitera. Copeia 2:284-287

179. Hackenbrock, C. R., and Finster, M. 1963. Fluorthane; a rapid and safe inhalation anesthetic for poisonous snakes. Copeia 2:440-441

180. Cosgrove, G. E. 1971. Reptilian radiobiology. J.A.V.M.A. 159(11):1678

181. Greichus, Y. A. 1974. Dept. of Biochemistry, South Dakota State University, Brookings, South Dakota, Personal Communication

182. Elder, W. H. 1972. Lecture notes on ornithology. Dept. of Zoology, University of Missouri, Columbia

183. Pettingill, O. S., Jr. 1970. Ornithology in Laboratory and Field, 4th edition. Burgess Publishing Company, pp 352-369

184. Prestt, I. 1971. Techniques for assessment of pollution effects on seabirds. Proc. Roy. Soc. Lond. 177:287-294

185. Keith, J. O. 1966. Insecticide comtaminations in wetland habitats and their effect on fish-eating birds. J. App. Ecology 3:71-85 (suppl.)

186. Hannon, M. R. 1972. Distribution and physiological effects of DDT,DDD and DDE in penned double-crested cormorants. Ph.D.Thesis,South Dakota State University

187. Anderson, D. W., and Hickey, J. J. 1970. Oological data on egg and breeding characteristics of brown pelicans. Wilson Bulletin 82, (1):14-28

188. Anderson, D. W., and Hickey, J. J. 1972. Eggshell changes in certain North American birds. Proc. of 15th Intl. Orno. Cong. 514-540

189. Anon. 1967. USDI Fish and Wildlife Serv. Res. Publ. 43:57-58

190. Blus, L. J. 1970. Measurements of brown pelican eggshells from Florida and South Carolina. BioSci. 20(15):867-869

191. Fredrickson, L. 1971. Lecture notes on waterfowl biology. Dept of Zoology, University of Missouri, Columbia

192. Grandy, J. W., Locke, L. N., and Bagley, G. E. 1968. Relative toxicity of lead and five proposed substitute shot types to pen-reared mallards. Journal of Wildlife Management 32(3):483-488

193. Longcore, J. R., et al. 1971. DDE thins eggshells and lowers reproductive success of captive black ducks. Bull. of Environmental Contamination and Toxicology 6(6):485-490

194. Winner, R. W. 1972. Activity of black and mallard ducks in a controlled environment. J. Wildlife Mgmt. 36:187-191

195. Friend, M., and D. O. Trainer 1970. Polychlorinated biphenyl: interaction with duck hepatitis virus. Science 170:1314-1316

196. Johnsgard, P.A. 1968. Waterfowl - their biology and natural history. U. of Nebraska Press, Lincoln

197. Hyde, K. M. 1972. Studies of the responses of selected wildlife species to mirex bait exposure. Ph.D. Thesis, Louisiana State University and Agricultural and Mechanical College

198. Bunch, T. D. 1971. Effects of dieldrin on chromosomes of bone marrow and lymphocytes of the semi-domestic mallard duck. Ph.D. Thesis, Utah State University.

199. Tucker, R. K., and Haegele, M. A. 1971. Comparative acute oral toxicity of pesticides to six species of birds. Tox. and App. Phar. 20:57-65

200. Heath, R. G. 1969. Nationwide residues of organochlorine pesticides in wings of mallards and black ducks. Pest. Mon. J. 3(2):115

201. Balasubromanion, A. 1971. Effect of certain organochlorines on reproduction in chickens and mallard ducks. Ph.D. Thesis Louisiana State University and Agricultural and Mechanical College

202. Wallach, J. D. and Flieg, G. M. 1969. Hyperparathyroidism in captive bonds. J.A.V.M.A. 155(7):1046-1051

203. Dindal, D. L., and Peterle, T. J. 1968. Wing and body tissue relationships of DDT and metabolite residues in mallard and lesser scaup ducks. Env. Contam. and Tox. Bull. 3(1):37-48

204. Johnson, L. G., and Morris, R. L. 1971. Pesticide and mercury levels in migrating duck populations. Env. Contam. and Tox. Bull. 6(6):513-516

205. Cook, R. S., and Trainer, D. O. 1966. Experimental lead poisoning of Canada geese. Journal of Wildlife Management 30(1):1-8

206. Trainer, D. O., and Hunt, R. A. 1964. Lead poisoning of waterfowl in Wisconsin. Journal of Wildlife Management 28:95

207. Vermeer, K., and Armstrong, F. A. J. 1972. Mercury in Canadian prairie ducks. J. Wildlife Mgmt. 36(1): 179-182

208. Curnow, R. D. 1970. Kinetics of 65-zinc in the mallard. Ph.D. Thesis, Colorado State University

209. Keith, J.O., and Mulla,M.S. 1965. Relative toxicity of five organophosporus mosquito larvicides to mallard ducks. Journal of Wildlife Management 29:553-563

210. Dustman, E.H., and Stickel, L.F. 1969. The occurence and significance of pesticide residues in wild animals. Annals of N.Y. Academy of Sciences 160(1):162-172

211. Springer, P.F. 1957. Effects of herbicides, fungicides on wildlife. N. Caro. Pest. Man. 1957:87-106

212. Hartung,R., and Hunt,G.S. 1966. Toxicity of some oils to waterfowl. Journal of Wildlife Management 30(3): 564-570

213. Barrett, M.W., and Karstad,L.H. 1971. A flourescent erythrocyte test for lead poisoning in waterfowl. Journal of Wildlife Management 35(1):109

214. Heath, R.G. et al. 1972. Comparative dietary toxicities of pesticides to birds. Special Scientific Report Wildlife No. 152, Washington, D.C.

215. Lock, D. 1968. The growth rate and related factors in Procellariiforms, Chapter 22, pp 267-283 in Eco. Adp. Breeding in Birds. Methune & Co. Ltd.

216. Wurster, C. F., Jr., and Wingate, D. B. 1968. DDT residues and declining reproduction in the Bermuda Petrel. Science 159:979-981

217. Robinson, J., et al. 1967. Organochlorine residues in marine organisms. Nature 214:1307-1311

218. Parslow, J. L. F., et al. 1972. Ingested pollutants in puffins and their eggs. Bird Study, March:18-33

219. Moore, N.W., and Tatton, J.O'G. 1965. Organochlorine insecticide residues in the eggs of sea birds. Nature 207:42-43

220. Homes, D.C., Simmons, J.H., and Tatton, J.O'G. 1967. Chlorinated hydrocarbons in British Wildlife. Nature 216:227

221. Peterson, R.T. 1934. A Field Guide to the Birds. Houghton Mifflin Company, Boston

222. Flickinger, E. L., and Meeker, D.L. 1972. Pesticide mortality of young white faced ibis in Texas. Bulletin Envir. Contam. Tox. 8(3):165-168

223. Fowler, J. F. 1970. Effect of dieldrin on egg hatchability and chick survival in purple and common gallinules. Ph.D. Thesis, The Louisiana State University and Agricultural and Mechanical College

224. Lamont, T., and Reichel,W. 1970. Organochlorine pesticide residues in whooping cranes and everglade kites. Br.Sport Fisheries and Wildlife 87(1):158-159

225. Stickel, W. H., et al., 1965. Care of captive woodcocks. J. of Wildlife Mgmt. 29(1):161-172

226. Wright,B.S. 1960. Woodcock reproduction in DDT sprayed areas of New Brunswick. J.W. Mgt. 24(4):419-420

227. Kreitzer, J.F. 1971. Thickness of the American woodcock eggshell. Bull. of Environmental Contamination and Toxicology 9(5):281-286

228. Wright, B.S. 1965. Some effects of heptachlor and DDT on New Brunswick woodcocks. Journal of Wildlife Management 29(1):172-185

229. Stickel, W. H., Hayne, D. W., and Stickel, L. F. 1965. Effects of heptachlor contaminated earthworms on woodcocks. Journal of Wildlife Management 29(1):132-146

230. Stickel,W.H., et al. 1965. Body condition and response to pesticides in woodcocks. Journal of Wildlife Management 29:147-155

231. Keith, J. A. 1966. Reproduction in population of herring gulls (Larus argentatus) contaminated by DDT. J. App. Ecology 3:57 (suppl.)

232. Zitko, V., and Choi, P.M.K. 1972. PCB and p,p'-DDE in eggs of cormorants, gulls and ducks from the bay of Fundy, Canada. Bulletin of Envir. Contam. Tox. 7(1):63-64

233. Jefferies, D.J., and Parslow,J.I.F. 1972. Effect of one polychlorinated biphenyl on size and activity of the gull thyroid. Env. Contam. and Tox. Bull. 8(5): 306-310

234. Koeman, J. H., et al. 1969. Chlorinated biphenyls in fish, mussels and birds from the River Rhine and the Netherlands coastal area. Nature 221:1126-1128

235. Zitko, V., et al. 1972. Polychlorinated terphenyls in the environment. Env. Contam. and Tox. Bull. 7(4):200-201

236. Hickey, J.J., and Anderson, D.W. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162:271-273

237. Anderson, D. W. 1970. Chlorinated hydrocarbons: their dynamics and eggshell effects in herring gulls and other species. Ph.D. Thesis, The University of Wisconsin, Madison, Wisconsin

238. Scott, T. G., et al. 1959. Some effects of a field application of dieldrin on wildlife. Journal of Wildlife Management 23:409-427

239. Porter, R. D., and Wiemeyer, S. N. 1970. Propagation of captive American kestrels. J. Wildlife Mgmt. 34(3):594-604

240. Lincer, J. L., and Peakall, D. B. 1970. Metabolic effects of polychlorinated biphenyls in the American kestrel. Nature 288:783-784

241. Henny, C.J. 1972. An analysis of population dynamics of selected avain species with special reference to changes during the modern pesticide era. Wildlife Res. Report 1:1-99, U.S. Fish and Wildlife Service

242. Fyfe, R.W., et al. 1969. Regional population declines and organochlorine insecticides in Canadian prairie falcons. Can. Field Naturalist 83:191-200

243. Porter, W.D., and Wiemeyer,S.N. 1972. DDE at low dietary level kills captive American kestrels. Bulletin of Envir. Contam. Tox 8:193-199

244. Wiemeyer, S.N., and Porter,R.D. 1970. DDE thins eggshells of captive American kestrels. Nature 227:737-738

245. Risebrough, R. W., et al. 1968. Polychlorinated biphenyls in global ecosystem. Nature 220:1098-1102

246. Bagley, G.E., Reichel, W.L., and Cromartie, E. 1970. Identification of polychlorinated biphenyls in two bald eagles by combined gas-liquid chromatography--mass spectrometry. J. AOAC 53(2):251-261

247. Finreite, N., and Karstad, L. 1971. Effects of dietary methyl mercury on red-tailed hawks. Journal of Wildlife Management 35(2):293-300

248. Moore, W.W., and Walker, C.H. 1964. Organic chlorine insecticide residues in wild birds. Nature 201:1072-1073

249. U. S. Bureau Fisheries and Wildlife 1966. Pesticide wildlife relationships. U.S. Dept. Int. Resource Public. 43

250. Haegele, M. A., and Hudson, R. H. 1973. DDE effects on reproduction of ring doves. Envir. Poll. 4:53-57

251. Jefferies, D. J., and French, M. C. 1972. Changes induced in the pigeon thyroid by p,p'-DDE and dieldrin. J. Wildlife Mgmt. 36:24-30

252. Sturtevant, J. 1971. The effects of mestranol containing synthetic grit on the breeding of pigeons. Tox. and App. Phar. 19:634-648

253. Lincer, J.L., and Peakall,D.B. 1973. PCB pharmacodynamics in the ring dove and early gas chromatographic peak diminution. Envir. Poll. 4:59-68

254. Jenner, C.E., and Engels, W.L. 1951. The period of darkness as the critical factor in the photoperiodic response of birds. Anat. Rec. 11:548

255. Robinson, J. et al. 1967. Residues of dieldrin (Heod) in the tissues of experimentally poisoned birds. Life Sci. 6:12-7-1220

256. Anon. 1965. USDI Fish and Wildlife Serv. Res. Publ. 23:50-51

257. Peakall, D. B. 1971. Effect of polychlorinated biphenyls (PCB's) on the eggshell of ring doves. Env. Contam. and Tox. Bull. 6(2):100-101

258. Locke, L. N., and Bagley, G. E. 1967. Lead poisoning in a sample of Maryland morning doves. Journal of Wildlife Management. 31(3):515-518

259. Jefferies, D. J., and French, M. C. 1969. Avian thyroid: effect of p,p'-DDT on size and activity. Science 166:1278-1280

260. Brown, V. K., et al. 1965. The effects of aldrin and dieldrin on birds. Food and Cosmetic Tox. 3(4):675-679

261. Sturtevant, J. 1970. Pigeon control by chemosterilization: population model from laboratory results. Science 170:322-324

262. Mulder, J. B., and Brown, R. V. 1972. An anesthetic unit for small laboratory animals. Lab. An. Sci. 22(3):422-423

263. Dews, P.B. 1955. Studies on behavior I. Differential sensitivity to pentobarbital of pecking performance in pigeons depending on the Schedule of Reward. J. Phar. and Exp. Ther. 113:393-401

264. Dews, P.B. 1950. Effects of chlorpromazine and promazine on performance on a mixed schedule of reinforcement. J. Exp. Anal. of Behav. 1(1):73-82

265. Dews, P.B. 1957. Studies of behavior III. Effects of scopolamine on reversal of a discriminatory performance in pigeons. J. Phar. and Exp. Ther. 115:343-353

266. Sturtevant, J., and Wentworth, B. C. 1970. Effect on acceptability and fecundity to pigeons of coating SC 12937 bait with zein or ethocel. Journal of Wildlife Management 34(4):776-782

267. Blough, D. S. 1958. New test for tranquilizers. Science 127:586

268. Dews, P.B. 1956. Modification by drugs of performance on simple schedules of positive reinforcement. N.Y. Acad. Sci., Ann. 65:268-281

269. Reynolds, G. S., Catania, A. C., and Skinner, B. F. 1963. Conditioned and unconditioned aggression in pigeons. J. Exp. Anal. of Behav. 6(1):73-74

270. Blough, P.N. 1971. The visual acuity of the pigeon for distant targets. J. Exp. Anal. of Behav. 15(1):57-67

271. Hanson, H.M., Witoslawski, J.J., and Campbell, E.H., 1964. Tox. and App. Phar. 6:690-695

272. Kelleher, R. T. 1966. Conditioned reinforcement in second-order schedules. J. Exp. Anal. of Behav. 9(5):475-485

273. Tansy, M.F., and Roth, R.P. 1970. Pigeons: a new role in air pollution. J. Air Poll. Control Assoc. 20: 307-309

274. Haynes, R.J. 1972. Effects of DDT on glycogen and lipid levels of bobwhites. J. Wildlife Mgmt. 36(2): 518-523

275. Hill, E.F. 1971. Toxicity of selected mosquito larvicides to some common avian species. Journal of Wildlife Management 36(4):757-762

276. Heath, R. G., and Spann, J. W. 1973. Reproduction and related residues in birds fed mirex, pp 421-435 in Pesticides and the Environment: A Continuing Controversy, ed. by W. B. Deichman. Intercontinental Medical Book Corp., New York

277. Bagley, G. E., and Cromartie, E. 1973. Elimination pattern of aroclor 1254 components in the bobwhite. J. Chromatog. 75:219-226

278. Hill, E. F., Dale, W. E., and Miles, J. W. 1971. DDT intoxication in birds: subchronic effects and brain residues. Tox. and App. Phar. 20:502-514

279. Bergstrand, J., and Klimstra, W. D. 1962. Toxicity of "dybar" to bobwhite quail. Journal of Wildlife Management 26(3):325-327

280. James, D., and Davis, K. B. 1965. The effect of sublethal amounts of DDT on the discrimination ability of the bobwhite, Colinus virginianus (Linnaeus). Am. Zool. Abstracts 5:229

281. DeWitt, J.B. 1955. Effects of chlorinated hydrocarbon insecticides upon quail and pheasants. Agr. and Food Chem. 3(8):672-676

282. DeWitt, J. B. 1956. Chronic toxicity to quail and pheasants of some chlorinated insecticides. J. Agr. Food Chem. 4(10):863-866

283. Heath, R. G., et al. 1972. Effects of polychlorinated biphenyls on birds. Proc. of the 15th International Ornithological Congress

284. Percival, H. F. 1972. Concentrations of selected chlorinated hydrocarbons in bobwhite quail and natural quail foods with notes of effects on reproduction. Ph.D. Thesis, Clemson University

285. Schafer, E. W., and Brunton, R. B. 1971. Chemicals as bird repellents: two promising agents. Journal of Wildlife Management 35(3):569-572

286. Rosene, W. 1965. Effects of field application of heptachlor on bobwhite quail and other wild animals. Journal of Wildlife Management 29(3):554-580

287. Barrett, M. W., and Bailey, E. D. 1972. Influence of metabolizable energy on condition and reproduction of pheasants. J. Wildlife Mgmt. 36:12-23

288. Summers, J. D., et al. 1966. Poultry feed formulas. Ontario Agri. Coll. Uni. of Guelph

289. Ralson Purina Company. 1961. Manual for Laboratory Animal Care. Ralston Purina Company

290. Atkins, T. D., and Linder, R. L. 1967. Effects of dieldrin on reproduction of penned hen pheasants. Journal of Wildlife Management 31:746-753

291. Genelly, R. E., and Rudd, R. L. 1956. Effects of DDT, toxaphene and dieldrin on pheasant reproduction. Auk. 73:529-539

292. Spann, J. W., et al. 1972. Ethyl mercury p-toluene sulfonanilide: Lethal and reproductive effects on pheasants. Science 175:328-331

293. Kreitzer, J. F., and Spann, J. W. 1973. Tests of pesticidal synergism with young pheasants and Japanese quail. Bulletin Envir. Contam. Tox 9(4):250-256

294. Dahlgren, R. B., Greichus, Y. A., and Linder, R. L. 1971. Storage and excretion of polychlorinated biphenyls in the pheasant. Journal of Wildlife Management 35(4):823-828

295. Burrage, R. H., and Saha, J. G. 1972. Insecticide residues in pheasants after being fed on wheat seed treated with heptachlor and 14C-Lindane. J. Econ. Entom. 65:1013-1017

296. Azevedo, J. A., et al. 1965. Physiological effects of DDT on pheasants. Calif. Fish and Game 51:276-293

297. Lamb, D. W., Linder, R. L., and Greichus, Y. A. 1967. Dieldrin residues in eggs and fat of penned pheasant hens. Journal of Wildlife Management 31(1):27-27

298. Dahlgren, R. B., et al. 1972. Residue levels and histopathology in pheasants given polychlorinated biphenyls. J. Wildlife Mgmt. 36(2):524-533

299. Dahlgren, R. B., and Linder, R. L. 1970. Eggshell thickness in pheasants given dieldrin. Journal of Wildlife Management 34(1):226-228

300. Lamb, D. W. 1969. Pharmacodynamics of dieldrin-C^{14} in pheasants. Ph.D. Thesis, South Dakota State University

301. Baxter, W. T., Linder, R. L., and Dahlgren, R. B. 1969. Dieldrin effects in two generations of penned hen pheasants. Journal of Wildlife Management 33(1):96-102

302. Dahlgren, R. B., et al. 1972. Effects of stress on pheasants previously given polychlorinated biphenyls. J. Wildlife Mgmt. 36(3):974-978

303. Dahlgren, R. B., Linder, R. L., and Ortman, K. K. 1970. Dieldrin effects on susceptibility of penned pheasants to hand capture. Journal of Wildlife Management 34(4):957-959

304. Hall, J. E., et al. 1971. Effects of aldrin on pen-reared pheasants. J. Wildlife Mgmt. 35(3):429

305. Linder, R. L., and Dahlgren, R. B. 1970. Occurrence of organochlorine insecticides in pheasants of South Dakota. Pesticides Monitoring Journal 3(4):227-232

306. Thill, R. E., et al. 1972. Effects of aldrin on young pheasants under semi-natural conditions. Env. Contam. and Tox. Bull. 7(2/3):188-192

307. Mussehl, T.W., and Schladweiler, P. 1969. Forest grouse and experimental spruce budworm insecticide studies. Montana Fish and Game Dept. Tech. Bull. No. 4

308. Neill, D.D., et al. 1971. Pesticide effects on the fecundity of the gray partridge. Bulletin of Environmental Contamination and Toxicology Vol. 6(6):546-551

309. Wing. L., et al. 1944. Brood habits and growth of blue grouse. Auk 61(3):426-440

310. Bent, A.C. 1965. Life History of North American Blackbirds, Orioles, Tanagers and Allies. Dover Publ. Inc., New York

311. McEwen, L.C., and Brown, R.L. 1966. Acute toxicity of dieldrin and malathion to wild sharp-tailed grouse. Journal of Wildlife Management 30(3):604-611

312. Finley, R. B. 1965. Adverse effects on birds of phosphamidon applied to a Montana forest. Journal of Wildlife Management 29(3):580-591

313. Mussehl, T. W., and Finley, R. B., Jr. 1966. Residues of DDT in forest grouse following spruce budworm spraying. Journal of Wildlife Management 30:270

314. Janda, J., and Bösseová, M. 1970. The toxic effect of zinc phosphide baits on partridges and pheasants. Journal of Wildlife Management 34(1):220-223

315. Boag, D. A. 1972. Effect of radio packages on behavior of captive red grouse. J. Wildlife Mgmt. 36:511-518

316. Brander, R. B. 1968. A radio-package harness for game birds. J. Wildlife Mgmt. 32(3):630-632

317. Evans, S. M., and Patterson, G. R. 1971. The synchronization of behavior in flocks of estrildine finches. Anim. Behav. 19:429-438

318. Moynihan, M., and Hall, M. F. 1953. Hostile, sexual and other social behavior patterns of the spice finch (Lonchura punctulata) in captivity. Behavioral 7:3-74

319. Jefferies, D. J. 1967. The delay in ovulation produced by pṕ-DDT and its possible significance in the field. Ibis 109(2):266-272

320. Power, D. 1974. Museum of Natural History, Santa Barbara, California. Personal communication

321. Bent, A. C., et al. 1968. Life history of North American cardinals, grosbeaks, bunting, towhees, finches, sparrows and allies. Bulletin 237, part 1, Smith. Inst., Washington D. C.

322. Deevey, E. S., Jr. 1947. Life tables for natural populations of animals. Quar. Rev. Bio. 22:283-314

323. Jefferies, D. J., and Walker, C. H. 1966. Uptake of pṕ-DDT and its post-mortem breakdown in the avian liver. Nature (London) 212:533-534

324. Oppenheim, R. W. 1972. Prehatching and hatching behavior in birds: a comparative study of altricial and precocial species. Anim. Behav. 20:644-655

325. Aschoff, J. Adaptive cycles: their significance for defining environmental hazards. Int. Jour. of Biometerol. 11:255-278

326. Stickel, L.F., and Stickel, Wm.H. 1970. Distribution of DDT residues in tissue of birds in relation to mortality, body condition, and time. Inter-American Conference on Toxicology and Occupational Medicine: August 1970, 57-64

327. Starr, R. I., et al. 1964. A laboratory method for evaluating chemicals as bird repellents. Agr. and Food Chem. 12(4):342-344

328. Benjamin, C.L. 1972. The physiological and histological effects of 20, 25-diazacholesterol dihydrochloride (SC-12937) on the reproduction of the male red-winged blackbird (Agelaius phoeniceus) Ph.D. Thesis, Ohio State University

329. Thompson, R. D., and Grant, C. Y. 1968. Nutritive value of two laboratory diets for starlings. Lab. An. Care 18(1):75-79

330. Bent, A. C. 1964. Life History of North American Nuthatchers, Wrens, Thrashes and their Allies. Dover Publ. Inc., New York

331. Summers-Smith, O. 1963. The House Sparrow. Collins, London, xvi + x69

332. Jackson, William B. 1952. Populations of the wood mouse (Peromyscus leucopus) subjected to the applications of DDT and parathion. Ecological Monographs 22(4):259-281

333. Mehner, J. F., and Wallace, G. J. 1959. Robin populations and insecticides. Atl. Naturalist 14:4-9

334. Wurster, D.H. et al. 1965. Bird mortality following DDT spray for Dutch elm disease. Ecology 46(4):488-499

335. Bernard, R. F. 1963. Studies on the effects of DDT on birds. Michigan State University Mus. Bio. Series 2(3):155-192

336. Barker, R. J. 1958. Notes on some ecological effects of DDT sprayed on elms. Journal of Wildlife Management 22:269-274

337. Johnson, E. V. 1969. Robin-pesticide ecology on a commercial fruit farm. Ph.D. Thesis, Cornell University, Ithaca, New York

338. Hunt, L. B., and Sacho, R. J. 1969. Response of robins to DDT and methoxychlor. Journal of Wildlife Management 33(2):336-345

339. Snyder, D. B. 1961. Strychnine as a potential control for red-winged blackbirds. Journal of Wildlife Management 25(1):96-99

340. Van Velzen, A. C., et al. 1972. Lethal mobilization of DDT by cowbirds. J. Wildlife Mgmt. 36:733-739

341. Stickel, L. F., and Stickel, W. H. 1966. Residues of DDT in brains and bodies of birds that died on dosage and in survivors. Science 151:1549-1551

342. Harvey, J. M. 1967. Excretion of DDT by migratory birds. Can. J. Zoo. 45:629-633

343. Mitchell, C. J., and Hayes, R. O. 1973. Breeding house sparrows, Passer domesticus, in captivity. Ornithol. Monographs 14:39-48

344. Kessel, B. 1957. A study of the breeding biology of the European starling (Sturnus vulgaris L.) in North America. The Am. Midland Naturalist 58(2):257-331

345. Anderson, T. R. 1973. A comparative ecological study of the house sparrow and the European tree sparrow near Portage des Sioux, Michigan. Uni. Micro. Inc., Ann Arbor, Michigan, 148

346. Will, R. 1969. Fecundity, density, and movements of a house sparrow population in Southern Illinois. Uni. Micro. Inc., Ann Arbor, Michigan

347. Schafer, E. W. 1972. The acute oral toxicity of 369 pesticidal, pharmaceutical and other chemicals to wild birds. Tox. and App. Phar. 21:315-330

348. Morris, R. D. 1968. Effects of endrin feeding on survival and reproduction in the deer mouse, Peromyscus maniculatus. Can. J. Zoo. 46:951-958

349. DeGrazio, J. W., et al. 1971. Use of 4-aminopyridine to protect ripening corn from blackbirds. Journal of Wildlife Management 35(3):565-569

350. Flick, D. F., O'Dell, R. G., and Childs, V. A. 1965. Studies of chicken edema disease. Poultry Science 44(6):1460-1465

351. Katz, D. 1969. A restrainer for simplifying cardiac blood collection from chickens. Lab. An. Care 19: 869-870

352. Zimmerman, R. 1974. Ralston Purina, St. Louis, Mo., Personal Communication

353. Hughes, B. O., and Wood-Gush, D.G.M. 1971. A specific appetite for calcium in domestic chickens. Anim. Behav. 19:490-499

354. Dilger, W. C. 1969. Caging and environment, Chapter 2 in: Diseases of Cage and Aviary Birds, ed. by M. Petrak. Philadelphia, Lea & Febiger

355. McLaughlin, J., Jr., et al. 1963. The injection of chemicals into the yolk sac of fertile eggs prior to incubation as a toxicity test. Tox. and App. Phar. 5:760-771

356. Walker, N. E. 1967. Distribution of chemicals injected into fertile eggs and its effect upon apparent toxicity Tox. and App. Phar. 10:290-299

357. Cain, J. R., et al. 1967. Heart rate of the developing chick embryo. Proc. Soc. Exp. Bio. Med. 126: 507-510

358. Battista, S.P., Denine, E.P., and Kensler, C.J. 1972. Restoration of tracheal mucosa and ciliary particle transport activity after mechanical denudation in the chicken. Tox. and App. Phar. 22:59-69

359. Battista, S.P. and Kensler, C.J. 1970. Use of the nonimmersed in vitro chicken tracheal preparation for the study of ciliary transport activity. Arch. Env. Health 20:318-325

360. Greenberg, J., and LaHam, Q.N. 1970. Reversal of malathion-induced teratisms and its biochemical implications in the developing chicks. Can. J. Zoo. 48:1047-1053

361. Walker, N. E. 1971. The effect of malathion and malaoxon on esterases and gross development of the chick embryo. Tox. and App. Phar. 19:590-601

362. Cecil, H. C., et al. 1974. Embryotoxic and teratogenic effects in unhatched fertile eggs from hens fed PCB. Bulletin Envir. Contam. 11(6)

363. Gilani, S. H. 1973. Cardiovascular malformations in the chick embryo induced by thalidomide. Tox. and App. Phar. 25:77-83

364. Miyazaki, S., and Hodgson, G. C. 1972. Chronic toxicity of Dursban and its metabolite, 3, 5, 6-Trichloro-2-pyridinol in chickens. Tox. and App. Phar. 23:391-398

365. Messier, P. E. 1973. Effects of LSD on the development, histology and fine structure of the chick embryo. Tox. and App. Phar. 25:54-59

366. Pagnini, G., et al. 1972. Comparative evaluation of the influence of some tetracyclines on chick embryo. Tox. and App. Phar. 22:427-431

367. Landauer, W. 1953. On teratogenic effects of pilocarpin in chick development. J. Exp. Zoo. 122(3): 469-483

368. Naber, E. C., and Ware, G. W. 1965. Effect of kepone and mirex on reproductive performance in the laying hen. Poultry Science 44:878-880

369. Lillie, R. J., et al. 1974. Normal reproductive performance of mature cockerels fed Aroclor 1248. Poultry Sci. 53(4):1-10

370. Lillie, R. J., et al. 1974. Differences in response of caged white leghorn layers to various polychlorinated biphenyls (PCBs) in the diet. Poultry Sci. 53:726-732

371. Lillie, R. J. et al. 1973. Methoxychlor in chicken breeder diets. Poultry Sci. 52(3):1134-1138

372. Whitehead, C. C., and Pettigrew, R. J. 1972. The subacute toxicity of 2,4-dichlorophenoryacetic acid and 2,4,5-trichlorophenoxyacetic acid to chicks. Tox. and App. Phar. 21:348-354

373. Greenberg, J., and LaHam, Q. N. 1969. Malathion-induced teratisms in the developing chick. Can. J. Zoo. 47(4):539-542

374. Ecobichon, D. J., and Saschenbrecker, P. W. 1969. The redistribution of stored DDT in cockerels under the influence of food deprivation. Tox. and App. Phar. 15(2):420-432

375. Bossen, E. H., et al. 1972. The effects of chloroquine on chick skeletal muscle in vitro. Tox. and App. Phar. 24:197-205

376. Kasuya, M. 1972. Effects of inorganic, aryl, alkyl and other mercury compounds on the outgrowth of cells and fibers from dorsal root ganglia in tissue culture. Tox. and App. Phar. 23:136-146

377. Paulson, G. D. 1969. An improved method for separate collection of urine, feces and expiratory gases from the mature chicken. Poultry Science 48:1331-1336

378. Rogallo, V. L., et al. 1968. A piezoelectric transducer for measuring cardiac and gross motor activity of small organisms. NASA Tech. Note D-4590

379. Padgett, C.A., and Ivey,W.D. 1959. Coturnix quail as a laboratory research animal. Science 129:267-268

380. Kreitzer, J. F., Heinz, G. H. 1974. The effect of sublethal dosages of five pesticides and a polychlorinated biphenyl on the avoidance response of Coturnix quail chicks. Envir. Poll. 6:21-29

381. Haegele, M. A. 1974. Denver Wildlife Research Center, Fed. Cen. Bldg. 16, Denver, Colorado, Personal Communication

382. Cecil, H. C. 1971. Effects of dietary p,p-DDE on egg production and eggshell characteristics of Japanese quail receiving on adequate calcium diet. Poultry Sci. 50:657-659

384. Cecil, H. C., et al. 1973. Effects of different classes of pesticides on pentobarbital anesthesia and toxicity in Japanese quail. Agr. Food Chem. 21(1): 114-117

385. McFarland, L. Z., and Lacy, D.B. 1969. Physiologic and endocrinologic effects of the insecticide kepone in Japanese quail. Tox. and App. Phar. 15:441-450

386. Gillett, J. W., et al. 1966. Interactions between DDT analogs and microsomal epoxidase systems. J. Agr. Food Chem. 14(6):540-545

387. Annau, E. A., et al. 1952. Biochemical and histological changes in the liver of mice following feeding of the insecticide aldrin. Can. J. Med. Sci. 30(6): 463-470

388. Peakall, D. B., and Lincer, J. L. 1972. Methyl mercury: its effect on eggshell thickness. Bulletin Envir. Contam. Tox 8:89-90

389. Bitman, J., et al. 1970. DDT-induced inhibition of avian shell gland carbonic anhydrase: a mechanism for thin eggshells. Science 168:594-596

390. Bunyan, P. J., Davidson, J., and Shorthill, M. J. 1970. Hepatic glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase levels in Japanese quail following the ingestion of p,p'DDT and related compounds. Chem. Bio. Interac. 2:175-182

391. Sell, J. L., et al. 1972. Decreased aniline hydroxlase activity in Japanese quail due to dietary DDT. J. Agr. Food Chem. 20(3):553-557

392. Bitman, J., et al. 1969. DDT induces a decrease in eggshell calcium. Nature 224:44-46

393. Bitman, J., et al. 1971. Comparison of DDT effect on pentobarbital metabolism in rats and quail. J. Agr. Chem. 19(2):333-338

394. Poonacha, K. B. 1972. Studies of genetic resistance to DDT and effects of environmental stress in the Japanese quail (Coturnix coturnix japanica) fed a diet containing DDT. Ph.D. Thesis, Univ. of Wisconsin

395. Creger, C. R., and Kubena, L. F. 1971. Lipotropic agents and DDT residue in birds and eggs. Env. Contam. and Tox. Bull. 6(5):453-459

396. Call, D. J. 1972. Effects of dieldrin and selected polychlorinated biphenyls upon Japanese quail. Ph.D. Thesis, University of South Dakota

397. Stickel, W. H., et al. 1969. Residues of dieldrin in relation to mortality in birds and mammals. Chap. 8:174-204 in: Chemical Fallout, Current Research on Persistent Pesticides, ed. by M. W. Miller and G. G. Berg, Charles C. Thomas, Publisher, Springfield, Ill.

398. Cecil, H. C. 1972. Effects of a single oral injection of polychlorinated biphenyls and terphenyls and a polybrominated biphenyl on pentobarbital sleeping times of Japanese quail. 165th ACS Meeting Dallas, Texas

399. Vos, J. G., et al. 1971. Toxicity of hexachlorobenzene in Japanese quail with special reference to porphyria, liver damage, reproduction and tissue residues. Tox. and App. Phar. 18:944-957

400. Shellenberger, T. E., et al. 1966. Cholinesterase inhibition and toxicologic evaluation of two organophosphate pesticides in Japanese quail. Tox. and App. Phar. 8:22-28

401. Hitchcock, M., and Murphy, J. D. 1971. Activation of parathion and guthion by mammalian, avian and piscine liver Homogenates and cell fractions. Tox. and App. Phar. 19:37-45

402. Wentworth, B. C., et al. 1968. Sterility induced in Japanese quail by spray treatment of eggs with mestranol Journal of Wildlife Management 32(4):879-887

403. Cecil, H. C. 1973. Effects of different classes of pesticides on pentobarbital anesthesia and toxicity in Japanese quail. J. Agr. Food Chem. Vol. 21(1)

404. Cecil, H. C., et al. 1974. Effects of nonpersistent pesticides on liver weight, lipids and vitamin A of rats and quail. Bulletin Envir. Contam. 11(6)

405. Cecil, H. C., et al. 1973. Polychlorinated biphenyl-induced decrease in liver vitamin A in Japanese quail and rats. Bulletin Envir. Contam. Tox. 9(3):179-185

406. Schafer, E. D., 1974. Denver Wildlife Research Center, Personal Communication

407. Shellenberger, T. E. 1968. Biological studies utilizing Japanese quail. Lab. An. Care 18(2):244-250

408. Dilger, W. C. 1969. Common types of cage birds, Chapter 1 in: Diseases of Cage and Aviary Birds, ed. by M. Petrak. Philadelphia, Lea & Febiger

409. Dilger, W. C. 1969. Behavioral aspects, Chapter 3 in: Diseases of Cage and Aviary Birds, ed. by M. Petrak, Philadelphia, Lea & Febiger

410. Tollefson, C. I. 1969. Nutrition, Chapter 7 in: Diseases of Cage and Aviary Birds, ed. by M. Petrak. Philadelphia, Lea & Febiger

411. Crandall, L. S. 1964. Family Soricidae, Family Talpidae, Family Tupaiidae, pp. 49-55 in: Management of Wild Mammals in Captivity. University of Chicago Press, Chicago, Illinois

412. Burt, W. H., and Grossenheider, R. P. 1964. A Field Guide to the Mammals, 2nd ed. Houghton Mifflin Co., Boston

413. Hamilton, W. J., Jr. 1929. Breeding habits of the short-tailed shrew, Blarina brevicauda. J. Mamm. 10:125-134

414. Dapson, R. W. 1968. Reproduction and age structure in a population of short-tailed shrews. J. Mamm. 49(2):205-214

415. Blair, W. F. 1940. Notes on home ranges and populations of the short-tailed shrew. Ecology 21(2): 284-288

416. Rood, J. P. 1958. Habits of the short-tailed shrew in captivity. J. Mamm. 39:499-507

417. Blus, L. J. 1971. Reproduction and survival of short-tailed shrews in captivity. Lab. An. Sci. 21(6):884-891

418. Mann, P. M. 1956. Keeping short-tailed shrews in captivity. J. Mamm. 37(2):274-275

419. Doremus, H. M. 1965. Heart rate, temperature and respiration rate of the short-tailed shrew in captivity. J. Mamm. 46(3):424-425

420. Whitaker, J. O., Jr., and Ferraro, M. G. 1963. Summer food of 220 short-tailed shrews from Ithaca, N. Y. J. Mamm. 44:419

421. Pearson, O. P. 1950. Keeping shrews in captivity. J. Mamm.31(3):351-352

422. Hamilton, W. J., Jr. 1949. The reproductive rates of some small mammals. J. Mamm. 30(3):257-260

423. Pearson, O. P. 1944. Reproduction in the shrew (Blarina brevicauda) An. J. of Anat. 75:39-80

424. Burt, W. H. 1940. Territorial behavior and populations of some small mammals in southern Michigan. Mus. Zoo. University of Michigan 45:7-58

425. Williamson, P. and Evans, P. R. 1972. Lead: levels in roadside invertebrates and small mammals. Bull. Envir. Contam. Tox. 8:280-288

426. Rasweiler, J. J., IV, and De Bonilla, H. 1972. Laboratory maintenance methods for some nectarivorous and frugivorous Phyllostomatid bats. Lab. An. Sci. 22(5):658-663

427. Constantine, D. G. 1952. A program for maintaining the free-tail bat in captivity. J. Mamm. 33(3):395-397

428. Mohos, S. C. 1961. Bats as laboratory animals. Anatom. Rec. 139:369-377

429. Gates, W. H. 1936. Keeping bats in captivity. J. Mamm. 17(3):268-273

430. Novick, A. 1960. Successful breeding in captive Artibeus. J. Mamm. 41(4):508-509

431. Orr, R. T. 1958. Keeping bats in captivity. J. Mamm. 39(3):339-344

432. Wimsatt, W. A., and Guerriere, A. 1961. Care and maintenance of the common vampire in captivity. J. Mamm. 42(4):449-454

433. Crandall, L. S. 1964. Order Chiroptera, pp. 59-65 in: Management of Wild Mammals in Captivity. University of Chicago Press, Chicago, Illinois

434. Rasweiler, J. J. 1974. Personal communication. Dept. of Anatomy, Columbia University, New York, N. Y.

435. Pearson, O. P., et al. 1952. Reproduction of the lump-nosed bat (Corynorhinus rafinesquei) in California. J. Mamm. 33(3):273-320

436. Racey, P. A. 1972. Bats. The U.F.A.W. Handbook on The Care and Management of Laboratory Animals, ed U.F.A.W. staff, Churchill Livingstone, Edinburgh and London

437. Tamar, H. 1961. Taste reception in the opossum and the bat. Physiol. Zoology. 34:86-91

438. Luckens, M. M., and Davis, W. H. 1964. Bats: sensivity to DDT. Science 146:948

439. Harris, P. D., et al. 1969. Effects of ethyl alcohol on subcutaneous microcirculation. Tox. and App. Phar. 14:6-12

440. Matthews, L. H. 1956. Breeding hares in captivity. Proc. Zoo. Soc. (London)

441. Crandall, L. S. 1964. Order Lagomorpha, pp. 201-205 in: Management of Wild Mammals in Captivity. University of Chicago Press, Chicago, Illinois

442. Obeck, D. K. 1973. A collapsible apparatus for cardiac bleeding of rabbits. Lab. An. Sci. 23(2): 276-278

443. Schwartz, C. W., and Schwartz, E. R. 1964. The Wild Mammals of Missouri, 2nd ed. University of Missouri Press

444. Fox, R. R., and Guthrie, D. 1968. The value of creep feed for laboratory rabbits. Lab. An. Care 18(1): 34-38

445. Treon, J. F., and Cleveland, F. P. 1955. Toxicity of certain chlorinated hydrocarbon insecticides for laboratory animals, with special reference to aldrin and dieldrin. Agr. and Food Chem. 3(5):402-408

446. Vos, J. G., and Beems, R. B. 1971. Dermal toxicity studies of technical polychlorinated biphenyls and fractions thereof in rabbits. Tox. and App. Phar. 19:617-633

447. Weil, C. S. 1971. Correlation of 4-hour vs. 24-hour contact skin penetration...formulation. Tox. and App. Phar. 18:734-742

448. Antonides, H. J. 1962. Toxicologic studies on armazide. Tox. and App. Phar. 4:44-54

449. Roudabush, R. L., et al. 1965. Comparative acute effects of some chemicals on the skin of rabits and guinea pigs. Tox. and App. Phar. 7:559-565

450. Guess, W. L. 1970. Tissue reaction to 2-chlorethanol in rabbits. Tox. and App. Phar. 16:382-390

451. McElligott, T. F. 1972. The dermal toxicity of paraquat: differences due to techniques of application. Tox. and App. Phar. 21:361-368

452. Allen, S. H. 1968. A thesis presented to the faculty of the graduate school, University of Missouri Department of Zoology Co-op Wildlife Res. Unit

453. Crandall, L. S. 1964. Order Rodentia, pp. 207-268 in: Management of Wild Mammals in Captivity. University of Chicago Press, Chicago, Illinois

454. Haley, T. J., 1964. Experimental usefulness of the kangaroo rat. Lab An. Care 14(2):95-102

455. Chew, R. M. 1958. Reproduction by Dipodomys merriami in captivity. J. Mamm. 39(4):597-598

456. Gisler, D. B., et al. 1964. The use of Perognathus flavus in research. Lab. An. Care 14(4):283-286

457. Bartholomew, G. A., and Cade, T. J. 1957. Temperature regulation, hibernation, and aestivation in the little pocket mouse, Perognathus longimembris. J. Mamm. 38:60-71

458. Butterworth, B. B. 1961. The breeding of Dipodomys deserti in the laboratory. J. Mamm. 43(3):413-414

459. Day, B. N., et al. 1956. Ord kangaroo rat in captivity. Science 124:485-486

460. Schroeder, M. H. 1966. Gophacide, a candidate for control of Ord's kangaroo rat. Journal of Wildlife Management 30:339-341

461. Davis, D. H. S., and Oettle, A. G. 1958. The multimammate mouse Mastomys natalensis Smith: a laboratory-adopted African wild rodent. Proc. Zoo. Soc. Lond. 131-293-299

462. Marsh, R. E. 1968. A colony nest box for rearing wild rats. Lab. An. Care 18(6):639-641

463. Meyer, B. J., and Meyer, R. K. 1944. Growth and reproduction of the cotton rat, Sigmodon hispidus hispidus under laboratory conditions. J. Mamm. 25(2)

464. Hamilton, W. J., Jr. 1953. Reproduction and young of the Florida wood rat, Neotoma floridana floridana. J. Mamm. 34(2):180

465. Svihla, A., and Svihla, R. D. 1933. Notes on the life history of the woodrat, Neotoma floridana rubida Bangs. J. Mamm. 14:73-75

466. Underhill, A. 1973. Use of neotropical rodents in protein efficiency studies. Lab. An. Sci. 23(4): 499-503

467. Conaway, C. H. 1954. The reproductive cycle of rice rats (Oryzomys palustris palustris) in captivity. J. Mamm. 35:263-266

468. Svihla, A. 1931. Life history of the Texas rice rat (Oryzomys palustris texensis). J. Mamm. 12:238-242

469. Hamilton, W. J., Jr. 1946. Habits of the swamp rice rat, Oryzomys palustris palustris. Am. Midland Nat. 36:730-736

470. Svihla, A. 1929. General notes on Sigmodon hispidus hispidus. J. Mamm. 10:352-353

471. Egoscue, H. J. 1957. The desert woodrat: a laboratory colony. J. Mamm. 38(4):477-481

472. Wood, F. D. 1935. Notes on the breeding behavior and fertility of Neotoma fuscipes macrotis in captivity. J. Mamm. 16(2):105-109

473. Chamberlain, E. B. 1928. The Florida wood rat in South Carolina. J. Mamm. 9:152-153

474. Lay, D. W., and Baker, R. H. 1938. Notes on the home range and ecology of the Attwater wood rat. J. Mamm. 19:418-423

475. Kennelly, J. J. et al. 1970. Laboratory studies of the effect of U-5897 on the reproduction of wild male rats. Journal of Wildlife Management 34(3):508-513

476. Durham, W. F., and Williams, C. H. 1972. Mutagenic, teratogenic and carcinogenic properties of pesticides. Ann. Rev. of Entomol. (1972):123

477. Brooks, J. E., and Bowerman, A. M. 1971. Estrogenic steroid used to inhibit reproduction in wild Norway rats. Journal of Wildlife Management 35(3):444-449

478. Gwynn, G. W., and Kurtz, S. M. 1970. Acceptability and efficacy of an antifertility agent in wild Norway rats. Journal of Wildlife Management 34(3):514-519

479. Marsh, R. E., and Howard, W. E. 1969. Evaluation of mestranol as a reproductive inhibitor of Norway rats in garbage dumps. Journal of Wildlife Management 33(1):133-138

480. Musacchia, X. J. 1954. Cannibalism and other observations of captive ground squirrels. J. Mann. 35(3):445-447

481. Marsh, R. E., and Howard, W. E. 1971. Care of ground squirrels (Spermophilus sp.) in captivity. Lab. Animal Sci. 21(3):367-371

482. Lyman, C. P. 1954. Activity, food consumption and hoarding in hibernators. J. Mamm. 35(4):399-406

483. Blair, W. F. 1942. Rate of development of young spotted ground squirrels. J. Mamm. 23:342-343

484. Selle, R. M. 1939. Golden-mantled ground squirrels raised in captivity. J. Mamm. 20(1):106-109

485. Ross, R. C. 1930. California Sciuridae in captivity. J. Mamm. 11(1):76-78

486. Colvin, H. W., Jr., Skaife, D. D., and Morrison, J.T. 1971. Liver and fecal bishydroxycoumarin following repeated toxic oral bishydroxycoumarin administration in ground squirrels. Tox. and App. Phar. 19:155-163

487. Panuska, J. A., and Wade, N. A. 1957. Field Observations on Tamias striatus in Wisconsin. J. Mamm. 38(2):192-196

488. Forbes, R. B. 1967. Some aspects of the water economics of two species of chipmunks. J. Mamm. 48(3): 466-468

489. Baumgras, P. 1944. Experimental feeding of captive fox squirrels. J. Wildlife Mgmt. 8(4):296-300

490. Condrin, J. M. 1963. Observations on the seasonal and reproductive activities of the eastern chipmunk. J. Mamm. 17:231-234

491. Schooley, J. P. 1934. A summer breeding season in the eastern chipmunk. Tamias striatus. J. Mamm. 15:194-196

492. Blair, W.F. 1947. Size of home range and notes on the life history of the woodland deer mouse and eastern chipmunk in northern Michigan. J. Mamm. 23:27-36

493. Ranson, R. M. 1941. Prenatal and infant mortality in a laboratory population of voles. Proc. Zool. Soc. 3:46-57

494. Dice, L. R. 1929. A new laboratory cage for small mammals, with notes on methods of rearing Peromyscus. J. Mamm. 10(2):116-124

495. Gier, H.T. 1967. Microtus ochrogaster in the laboratory. Trans. Kan. Acad. Sci. 70(2):256-265

496. Richmond, M. 1969. Management, breeding, and reproductive performance of the vole, Microtus ochrogaster, in a laboratory colony. Lab. An. Care 19(1):80-87

497. Egoscue, H. J. 1960. Laboratory and field studies of the northern grasshopper mouse. J. Mamm. 41(1):99-110

498. Louch C. D. 1956. Adrenocortical activity in relation to the density and dynamics of three confined populations of Microtus pennsylvanicus. Ecology 37(4):701-713

499. Storm, G. L., and Sanderson, G. C. 1968. Housing and reproductive performance of an outdoor colony of voles. J. Mamm. 49(2):322-324

500. Poiley, S. M. 1949. Raising captive meadow moles (Microtus pennsylvanicus pennsylvanicus). J. Mamm. 30(3):317-318

501. Selle, R. M. 1928. Microtus californicus in captivity J. Mamm. 9(2):93-98

502. Quiring, D. P. 1951. A comparison between Microtus drummondi (Aud. and Bach.) and Microtus pennsylvanicus. Growth 15:101-120

503. Cowan, I. M., and Arsenault, M. G. 1954. Reproduction and growth in the creeping vole, Microtus oregoni serpens Merriam. Can. J. Zoo. 32:198-208

504. Benton, A. H. 1955. Observations on the life history of the northern pine mouse. J. Mamm. 36(1):52-62

505. Christian, J. J. and Davis, D. E. 1966. Adrenal glands in female voles (Microtus pennsylvanicus) as related to reproduction and population size. J. Mamm. 47(1):1-18

506. Morris, R. D. 1969. Effects of endrin on small mammal populations. Ph.D. Thesis, University of Saskatchewan

507. Webb, R. E., and Horsfall, F. 1967. Endrin resistance in the pine mouse. Science 156:1762

508. Libby, J. L., and Abrams, J. 1966. Anticoagulant rodenticides in paper tubes for control of meadow mice. Journal of Wildlife Management 30(3):512-518

509. Marsh, R. E., et al. 1967. Laboratory tests on the effectiveness of prolin mouse tubes. Journal of Wildlife Management 31(2):342-344

510. Pournelle, G. H. 1952. Reproduction and early post-natal development of the cotton mouse, (Peromyscus gossypinus gossypinus). J. Mamm. 33(1):1-20

511. Nicholson, A. J. 1941. The homes and social habits of the wood mouse, (Peromycus leucopus noveboracensis). Am. Midland Nat. 25:196-223

512. Layne, J. N. 1966. Postnatal development and growth of Peromyscus floridanus. Growth 30:23-45

513. Dice, L. R. 1934. An improved Peromyscus ration. J. Mamm. 15(2):160-161

514. Clark, F. H. 1939. Age of sexual maturity in mice of Genus Peromyscus. J. Mamm. 19:230-234

515. Tiner, J. 1953. Fatalities in rodents caused by larval Ascaris in the central nervous system. J. Mamm. 34(2):153-167

516. Goodpaster, W.W., and Hoffmeister, D.F. 1954. Life history of the golden mouse, Peromyscus nuttalli, in Kentucky, J. Mamm. 35(1):16-27

517. Morris, R.D. 1970. The effects of endrin on Microtus and Peromyscus. I. Unenclosed field populations. Can. J. Zoo. 48(4):695-708

518. Tevis, L., Jr. 1956. Behavior of a population of forest mice when subjected to poison. J. Mamm. 34(3):358-370

519. Ozburn,G.W., and Morrison,F.O. 1964. The selection of a DDT-tolerant strain of mice and some characteristics of that strain. Can. J. Zoo. 42:519-526

520. Yen, H. C. Y., et al. 1970. Effects of various drugs on 3,4-dihydroxyphenylalanine (DL-DOPA)-induced excitation (agressive behavior)in mice. Tox. and App. Phar. 17:597-604

521. Murakami, H. 1971. Differences between internal and external environments of the mouse cage. Lab. An. Sci. 21(5):680-684

522. Huber, J. J. 1965. Some physiological effects of the insecticide kepone in the laboratory mouse. Tox. and App. Phar. 7:516-524

523. Johnston, R. F. and Rudd, R. L. 1957. Breeding of the salt marsh shrew. J. of Mammalogy 38:157-163

524. Port, C. D., and Kaltenback, J. P. 1969. The effect of corncob bedding on reproductivity and leucine incorporation in mice. Lab. An. Care 19:46-49

525. Ferguson, H. C. 1966. Effect of red cedar bedding on hexobarbital and pentobarbital sleep time. J. Pharm. Sci. 55:1142-1143

526. Vessell, E. S. 1967. Induction of drug-metabolizing enzymes in liver microsomes of mice and rats by softwood bedding. Sci. 157:1057-1058

527. Pick, J. R., and Little, J. M. 1965. Effect of type of bedding material on thresholds of pentylenetetrazol convulsions in mice. Lab. An. Care 15(1):29-33

528. Spalding,J.F., et al. 1969. Influence of the visible color spectrum on activity in mice. Lab. An. Care. 19(1):50-53

529. Spalding,J.F., et al. 1969. Influence of the visible color spectrum on activity in mice II. Influence of sex, color, and age on activity. Lab. An. Care 19(2):209-213

530. Iturrian, W. B., and Fink, G. B. 1968. Effect of noise in the animal house on seizure susceptibility and growth of mice. Lab. An. Care 18(5):557-560

531. Welch, J. G. 1968. Effect of intermittent feeding on body composition of mice. Lab. An. Care 18(6):596-601

532. Weltman, A. S., et al. 1968. Effects of isolation stress on female albino mice. Lab. An. Care 18:426-435

533. Palmes, E. D. and Del Pup, J. 1971. Procedure for the maintenance of stable laboratory mouse populations. Lab. An. Sci. 21(6):932-936

534. Smith, P. C., et al. 1968. Intestinal obstruction and death in suckling rats due to sawdust bedding. Lab. An. Care 18(2):224-228

535. Winter, C. A., and Flataker, L. 1962. Cage design as a factor influencing acute toxicity of respiratory depressant drugs in rats. Tox. and App. Phar. 4:650-655

536. Saunders, D. R., et al. 1972. Chlorpromazine-ultraviolet interaction on mouse ear. Tox. and App. Phar. 21:260-264

537. Klaassen, C. D. 1973. Comparison of the toxicity of chemicals in newborn rats to bile duct-ligated and sham-operated rats and mice. Tox. and App. Phar. 24:37-44

538. Budreau, C. H., and Singh, R. P. 1972. Teratogenicity and embryotoxicity of demeton and fenthion in CF#1 mouse embryos. Tox. and App. Phar. 24:324-332

539. Ewing, K. L., and Tauber, O. E. 1964. Blood chemistry changes in mice fed high levels of polyoxyethylene sorbitan derivatives. Tox. and App. Phar. 6(1964): 442-446

540. Bruce, D. W., et al. 1963. The acute mammalian toxicity of rare earth nitrates and oxides. Tox. and App. Phar. 5:750-579

541. Epstein, S. S., et al. 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Tox. and App. Phar. 23(1972):288-325

542. Morello, N., and Nicholas, J. 1969. A plastic food tube for accurately dispensing powdered diets to mice. Lab. An. Care 19(1):406-407

543. Wood, J. D. 1971. The influence of feeding frequency upon several aspects of the growth of rats. Ph.D. Thesis, Cornell University

544. Kraft, L. M., et al. 1964. Practical control of diarrheal disease in a commercial mouse colony. Lab. An. Care 14:16-19

545. Goldenthal, E. I. 1971. A compilation of LD_{50} values in newborn and adult animals. Tox. and App. Phar. 18:185-207

546. Cook, L., and Kelleher, R. T. 1963. Effects of drugs on behavior. Ann. Rev. of Phar. 3:205

547. Brady, J. V. 1956. Comparative approach to the evaluation of drug effects upon affective behavior. Ann. N.Y. Acad. Sci. 64:632-643

548. Grizzell, R. A., Jr. 1955. A study of the southern woodchuck, Marmota monax monax. Am. Midland Nat. 53(2):257-293

549. Shadle, A. R. 1950. Feeding, care, and handling of captive porcupines (Erethizon). J. Mamm. 31(4): 411-416

550. Davis, R. A. and Shillito, E. 1967. The coypu or nutria. The U.F.A.W. Handbook on the Care and Management of Lab Animals. Chapter 27:457-467. Ed. U.F.A.W. staff. E. S. Livingstone, Ltd., Edinburgh and Scotland.

551. Twichell, A. R. 1939. Notes on the southern woodchuck in Missouri. J. Mamm. 20:71-74

552. Snyder, R. L., and Christian, J. J. 1960. Reproductive cycle and litter size of the woodchuck.Ecology 41:647-656

553. Green, R. G., Carlson, W. E., and Evans, C. A. 1942. The inactivation of vitamin B_1 in diets containing whole fish. J. Nutrition:23:165

554. Brown, C. E. 1936. Rearing wild animals in captivity, and gestation periods. J. Mamm. 17(1):10-13

555. Sheldon, W. G. 1949. Reproductive behavior of foxes in New York State. J. Mamm. 30(3):236-246

556. Sullivan, E. G. 1956. Gray fox reproduction, denning, range, and weights in Alabama. J. Mamm. 37:346-357

557. Schoonmaker, J. W. 1938. Notes on mating and breeding habits of foxes in New York State. J. Mamm. 19:375-376

558. Pearson, O. P., and Enders, R. K. 1943. Ovulation, maturation and fertilization in the fox. Anatom. Record 85(1):69-83

559. Pearson, O. P. 1946. Certain aspects of reproduction in a head of silver foxes. American Naturalist 80(786):45-67

560. Linhart, S. B., and Enders, R. K. 1964. Some effects of diethylstilbestrol on reproduction in captive foxes. Journal of Wildlife Management 28(2):358-363

561. Balser, D. S. 1964. Management of predator populations with antifertility agents. Journal of Wildlife Management 28(2):352-358

562. Bissonnette, T. H., and Bailey, E. E. 1940. Den and runway system for weasels and other small mammals in the laboratory. Am. Midland Nat. 24:761-763

563. Willis, L. S., and Barrow, M. V. 1971. The ferret (Mustela putorius furo L.) as a laboratory animal. Lab. An. Sci. 21(5):712-716

564. Aldous, S. E. 1940. Notes on a black-footed ferret raised in captivity. J. Mamm. 21(1):23-26

565. Hammond, J. Jr., and Chesterman, F. C. 1972. The ferret. The U.F.A.W. Handbook on the Care and Management of Laboratory Animals ch 29:354-363. ed U.F.A.W. staff, C. Livingstone, Edinburgh and London

566. Errington, P. L. 1936. Food habits of a weasel family. J. Mamm. 17:406-407

567. Sanderson, G. C. 1949. Growth and behavior of a litter of captive long-tailed weasels. J. Mamm. 30(4):412-415

568. Svihla, A. 1931. Habits of the Louisiana mink (Mustela vison vulgivagus) J. Mamm. 12:366-368

569. Hamilton, W. J., Jr. 1936. Food habits of the mink in New York. J. Mamm. 17:169

570. Wright, P. L. 1942. Delayed implantation in the long-tailed weasel (Mustela frenata), the short-tailed weasel (Mustela cicognani) and the marten (Martes americana). Anat. Rec. 83:341-353

571. Cuyler, W. K. 1924. Observations on the habits of the striped skunk (Mephitis mesomelas varians). J. Mamm. 5:180-189

572. Allen, D. L. 1939. Winter habits of Michigan skunks. J. Wildlife Mgmt. 3:212-288

573. Stegeman, L. C. 1937. Notes on young skunks in captivity. J. Mamm. 18:194-202

574. Wright, P. L. 1948. Breeding habits of captive long-tailed weasels (Mustela frenata). Am. Midland Nat. 39:338-344

575. Petrides, G. A. 1950. The determination of sex and age ratios in fur animals. Amer. Midland Nat. 43:355-382

576. Allen, D. L., and Shapton, W.W. 1942. An ecological study of winter dens, with special reference to the eastern skunk. Ecology 23:59-68

577. Bill, J. F., and Chalgren,W.S. 1943. Some wildlife diseases in the eastern United States. J. Wildlife Management 7:311-317

578. Hamilton, W. J., Jr. 1936. Seasonal food of skunks in New York. J. Mamm. 17:240

579. Bauen, A., and Possanza, G. J. 1970. The mink as a psychopharmacological model. Arch. Int. Phar. 186: 133-136

580. Sherburne, J. A., and Dimond, J. B. 1969. DDT persistence in wild hares and mink. Journal of Wildlife Management 33(4):944-948

581. Aulerich, R. J., et al. 1973. Reproductive failure and mortality in mink fed on Great Lakes fish. F. Reprod. Fert., Suppl. 19, pp 365-376

582. Aulerich, R. J., et al. 1972. Rate of accumulation of chlorinated hydrocarbon pesticide residues in adipose tissue of mink. Can. J. of Zoo. 59(9):1167-1173

583. Aulerich, R. J., et al. 1971. Effects of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. of Zoo. 49(5):611-616

584. Morris, J. E. 1968. Insecticide residues in raccoons and some effects of dieldrin on raccoon reproduction. Master's Thesis, University of Missouri, Department of Zoology

585. Jeffries, D. J. 1969. Causes of badger mortality in eastern counties of England. J. Zool., London 157:429-436

586. Balazs, T., and Payne,X.J. 1971. Myocardial papillary muscle necrosis induced by hypotensive agents in dogs. Tox. and App. Phar. 20:442-445

587. Chapel-Edwards,K., et al. 1971. Hematological changes observed in female beagle dogs given ethynylestradiol. Tox. and App. Phar. 20:319-326

588. Hardee, D. D., et al. 1964. Residues of heplachlor epoxide and telodrin in milk from cows fed at ppb insecticide levels. J. Econ. Entomology 56(3):404

589. Williams, S., et al. 1964. Residues in milk of cows fed rations containing low concentrations of five chlorinated hydrocarbon pesticides. J. of A.O.A.C. 47(6):1124-1128

590. Carter, R. H., et al. 1956. Pesticide residues in animal products. The Yearbook of Agriculture, 1956-Animal Diseases, USDA, Washington, G.P.O.

591. Mendoza, C. E., and Shields, J. B. 1971. Esterase specificity and sensitivity to organophosphorous and carbamate pesticides: factors affecting determination by thin layer chromatography. J. AOAC 54:507-511

592. Radeleff, R. D., Woodard, G. T., and Bushland, R. C. 1956. Toxicity of insecticides. The Yearbook of Agriculture, 1956, USDA, Washington, G.P.O.

593. Waterman, R. A. 1972. Effects of nicotinic acid or methionine administration on lipid metabolism of Ketotic cows. Ph.D. Thesis, University of Wisconsin

594. De Faubert Maunder, M. J. et al. 1964. Clean-up of animal fats and dairy products for the analysis of chlorinated pesticide residues. Analyst 89(1056) 168-174

595. Binns, W. 1956. Chemical poisoning, in: The Yearbook of Agriculture, USDA, Washington, G.P.O.

596. Newton, G. L. 1972. Iodine toxicity. Physiological effects of elevated dietary iodine on calves and pigs. Ph.D. Thesis, North Carolina State University, Raleigh, North Carolina

597. Thomas, J. W., and Moss, S. 1951. The effect or orally administered molybdenum on growth, spermatogenesis, and testes histology of young dairy bulls. J. Dairy Sci. 34:929-934

598. Bernard, R. A. 1964. An electrophysiological study of taste reception in peripheral nerves of the calf. Am. J. Physiol. 206:827-835

599. Kare, M. A. 1963. Comparative studies on the sense of taste. Olfaction and Taste II:285-292, ed. by Y. Zatterman, Pergamon Press, New York

600. Bustad, L. K., and McClellan, R. O. 1968. Minature swine: development, management, and utilization. Lab. An. Care 18(2):280-287

601. Schilling, P. W., and Stone, H. L. 1969. Comparative cardiovascular measurements in the javelina (Tayassu tajacu). Lab. An. Care 19(3):331-335

602. Weaver, M. E., et al. 1965. Minature swine as laboratory animals. Lab An. Care 15(1):49-56

603. Earl, F. L. 1968. Housing and handling of minature swine. Lab. An. Care 18(1):110-115

604. Tumbleson, M. E., et al. 1968. Techniques for handling minature swine for laboratory procedures. Lab. An. Care 18(5):584-587

605. Dettmers, A. 1968. On nutrition of the miniature pigs at the Hormel Institute. Lab. An. Care 18(1): 116-119

606. Crossland, J. 1973. Man's best friends. Envir. 15(7):6-12, 28-30

607. Duke, T. W., and Szabuniewicz, M. 1969. The electrocardiogram of conventional and miniature swine. Can. J. Comp. Med. 33:118-127

608. Ecobichon, D. J., and Comeau, A. M. 1973. Pseudocholinesterases of mammalian plasma: physicochemical properties and organophosphate inhibition in eleven species. Tox. and App. Phar. 24:92-100

609. Larks, S. D., et al. 1971. Electrocardiographic studies of miniature swine: normal values. Lab. An. Sci. 21(4)

610. Planter, W. S. 1948. Electrocardiograms of mules, horses, cattle, sheep, swine and goats. University of Missouri Agricultural Experiment Station Research Bulletin 419:1-12

611. Tumbleson, M. E., et al. 1969. Serum biochemic and hematologic parameters of Hormel miniature swine from four to nine months of age. Lab. An. Care 19(3): 345-351

612. Tumbleson, M. E. 1971. A selected list of references on the use of swine in biomedical research. Dept. of Veterinary Physiology and Pharmacology, University of Missouri, Columbia

613. Van Gelder, G. 1974. CHMN. Dept. of Anatomy/ Physiology, School of Vet. Med., University of Missouri, Columbia, Personal Communication

614. Wade, D. A. 1972. Effects of manganese chloride on gravis gilts and newborn pigs. Ph.D. Thesis, South Dakota State University

615. Huhn, R. G., et al. 1969. Application of the orbital sinus bleeding technique to swine. Lab. An. Care 19(3):403-405

616. Hodge, H. C., et al. 1967. Toxicology and no-effect levels of aldrin and dieldrin. Tox. and App. Phar. 10:613-675

617. Quaife, M. L., et al. 1967. Survey of quantitative relationships between ingestion and storage of aldrin and dieldrin in animals and man. Fed. Cosmet. Tox. 5:39-50

618. Bann, J. M., et al. 1956. The fate of aldrin and dieldrin in the animal body. J. Agr. Food Chem. 4(11):937-941

619. Mendoza, C. E., et al. 1970. Evaluation of esterases from livers of beef, pig, sheep, monkey and chicken for detection of some pesticides by thin-layer chromatographic-enzyme inhibition technique. Analyst 94:805-810

620. Knaak, J. B., et al. 1968. Metabolism of carbaryl in man, monkey, pig and sheep. J. Agr. Food Chem. 16(3):465-470

621. Uzoukwn M. 1970. Studies in experimental dieldrin and nitrite toxicoses in swine and guinea pigs. Ph.D. Thesis, Michigan State University

622. McClellan, R. O. 1968. Applications of swine in biomedical research. Lab. An. Care 18(1):120-126

623. Fletcher, W. S., et al. 1964. The use of the goat as an experimental animal. Lab. An. Care 14:65-90

624. Harrison, F. A. 1972. Ungulates. Ch 30 pp 364-373 in The U.F.A.W. Handbook on the Care and Management of Laboratory Animals ed. U.F.A.W. staff, C. Livingstone, Edinburgh and London

625. Walker, K. C., et al. 1965. Residues of DDT in fatty tissues of big game animals in the states Idaho and Washington in 1962. USDA, ARS 33-105

626. Oberst, F. W., et al. 1970. Toxic effects of high concentration of bromobenzylnitrile in various animal species. Tox. and App. Phar. 16:66-72

627. Elsberry, D. D. 1972. Effect of dieldrin on auditory detection behavior and central auditory mechanisms in sheep. Ph.D. Thesis, Iowa State University

628. Van Gelder, G. A., et al. the effects of dieldrin and rulene exposure on experimental behavior and the electroencephalogram, in: Proceedings of the symposium on the biological impact of pesticides on the environment. Ed: J. W. Gillett, Environmental Health Sciences Series #1:125-133

629. Van Gelder, G. A., et al. 1969. Convulsive seizures in dieldrin exposed sheep during photic stimulation. Psycho. Reports 24:502

630. Buck, W. 1974. Personal Communication. Behavioral Toxicology Lab, College of Vet. Medicine, Iowa State University, Ames, Iowa

631. Murphy, D. A., and Korschgen, L. J. 1970. Reproduction, growth, and tissue residues of deer fed dieldrin. J. Wildlife Mgmt. 34(4):887-903

632. Whicker, F. W., Farris, G. C., and Dahl, A. H. 1966. Radioiodine in Colorado deer and elk thyroids during 1964-65. Journal of Wildlife Management 30(4):781-785

633. Longhurst, W. M., Schultz, V., and Connolly, G. E. 1967. Accumulation of strontium-90 in yearling Columbian black-tailed deer, 1960-67. Journal of Wildlife Management 32(3):621-623

634. Vickers, J. H. 1969. Diseases of primates affecting the choice of species for toxicological studies. Ann. N. Y. Acad. Sci. 162:659-672

635. Berendt, R. F. 1968. The effect of physical and chemical restraint on selected respiratory parameters of Macaca mulatta. Lab. An. Care 18(3):391-394

636. Moeller, H. C., and Rider, J. A. 1962. Plasma and red blood cell cholinesterase activity as indications of the threshold of incipient toxicity of ethyl-p-nitrophenyl thionobenzenephosphonate (EPN) and malathion in human beings. Tox. and App. Phar. 4:123-130

637. Santolucito, J. A., and Morrison, G. 1971. EEG of rhesus monkeys following prolonged low-level feeding of pesticides. Tox. and App. Phar. 19:147-154

638. Moss, G. F., et al. 1971. Plasma levels and urinary excretion of disodium cromoglycate after inhalation by human volunteers. Tox. and App. Phar. 20:147-156

639. Budny, J. A., and Arnold, J. D. 1973. Nitrilotriacetate (NTA): human metabolism and its importance in the total safety evaluation program. Tox. and App. Phar. 25:48-53

640. Michael, W. R., and Wakim, J. M. 1971. Metabolism of nitrilotriacetic acid (NTA). Tox. and App. Phar. 18:407-416

641. Banerjee, B. N., and Woodard, G. 1971. Teratologic evaluation of metaproterenol in the rhesus monkey (Macaca mulatta). Tox. and App. Phar. 20:562-564

642. Sherwood, B. F., et al. 1969. The opossum, Didelphis virginiana, as a laboratory animal. Lab. An. Care 19(4):494-499

643. Coghill, G. E. 1939. Studies on rearing the opossum (Didelphys virginiana). Ohio J. Sci. 39:239-249

644. Moore, C. R., and Bodian, D. 1940. Opossum pouch young as experimental material. Anat. Rec. 76:319-327

645. Krupp, J. H. 1964. A review of the use of the opossum for research, husbandry, experimental techniques and routine health measures. Lab. An. Care 14(3):189-194

646. Crandall, L. S. 1964. Order Marsupialia, pp. 19-23 in: Management of Wild Mammals in Captivity. Univ. of Chicago Press, Chicago, Illinois

647. Thrasher, J. 1969. Preliminary observations on mate compatability in Marmosa mitis. Lab. An. Care 19(1): 67-70

648. Wiseman, G. L., and Hendrickson, G. O. 1950. Notes on the life history and ecology of the opossum in southeastern Iowa. J. Mamm. 31(3):331-337

649. Thrasher, J. D., et al. 1971. The colony management of Marmosa mitis, the pouchless opossum. Lab. An. Sci. 21(4)

650. Flatt, R. E., et al 1971. Besnoitia darlingi in the opossum (Didelphis marsupialis). Lab. An. Sci. 21(1):106-109

651. Timmons, E. H., and Marques, P. a. 1969. Blood chemical and hematological studies in the laboratory confined, unanesthetized opossum, Didephis virginiana. Lab. An. Care 19:342-344

652. Potkay, S. 1970. Diseases of the opossum (Didelphis marsupialis): a review. Lab. An. Care 20:502-511

653. Barnes, R. D. 1968. Small marsupials as experimental animals. Lab. An. Care 18:251-257

654. Thrasher, J. D., et al. 1971. Natural variations in the S-phase of renewing epithelia of the pouchless opossum, Marmosa mites. Cell Tissue Kinet. 4:185-192

655. Feldman, D. B., and Self, J. L. 1971. Sedation and anesthesia of the Virginia opossum, Didelphis virginiana. Lab. An. Sci. 21(5):717-720

656. Jurgelski, W., Jr. 1971. Administration of test materials to the neonatal North American opossum (Didelphys marsupialis virginiana Kerr). Lab. An. Sci. 21(5):748-751

657. Gilbert, B. M., and Giacumetti, L. 1972. An environmental caging system and maintenance program for the nine-banded armadillo (Dasypus novemcinctus). Lab. An. Sci. 22(5):739-740

658. Crandall, L. S. 1964. Family Dasypodidae, pp. 190-194 in: Management of Wild Mammals in Captivity. Univ. of Chicago Press, Chicago, Illinois

659. Anderson, J. M., and Benirschke, K. 1966. The armadillo, Dasypus novemcinctus, in experimental biology. Lab. An. Care 16(3):202-216

660. Meritt, Dennis A., Jr. 1973. Edentate diets. I. Armadillos. Lab. An. Sc. 23(4):540-542

661. Talmage, R. V., and Buchanan, G. D. 1954. The armadillo (Dasypus novemcinctus): a review of its natural history, ecology, anatomy, and reproductive physiology. Pamphlet 41(2):1-135, The Rice Institute, Houston, Texas

662. Dhindsa, D. S. 1971. Respiratory functions of armadillo blood. Fed. Proc. 30:494

663. Lewis, J. H., and Doyle, A. P. 1964. Coagulation, protein and cellular studies on armadillo blood. Comp. Biochem. Physiol. 12:61-66

664. Ebaugh, F. G., Jr., and Benson, M. A. 1964. Armadillo hemoglobin characteristics and red cell survival. J. Cell. and Comp. Physiol. 64:183-192

665. Strozier, L. M., et al. 1971. Armadillos: basic profiles I. Serum chemistry values. Lab. Animal Sci. 21(3):399-400

666. Strozier, L. M., et al. 1971. Armadillos: basic profiles II. Serum proteins. Lab. An. Sci. 21(4)

667. Marston, J. H., and Chang, M. C. 1965. The breeding, management and reproductive physiology of the Mongolian gerbil (Meriones unguiculatus). Lab. An. Care 15(1):34-48

668. Schwentker, V. 1963. The gerbil, a new laboratory animal. The Illinois Veterinarian, 6:5-9

669. Durbin, D. G., and Robens, J. F. 1964. The use of laboratory animals for drug testing. Ann. N.Y. Acad. Sci. 3(2):696-711

670. Vos, J. G., et al. 1970. Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalene in two commercial polychlorinated biphenyls. Food Cosmet. Tox. 8:625-633

671. Draize, J. H., et al. 1944. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharm. and Exp. Therap. 82:377-390

672. Gustafson, C. G. 1970. PCB's--prevalent and persistent intensified research is needed to minimize their dangers. Envir. Sci. and Tech. 4(10):814-819

673. Ancill, R. J., and Richens, E. R. 1971. The effects of various hormones on the uptake and distribution of an organic mercurial compound in the guinea pig eye. Tox. and App. Phar. 20:206-215

674. Ferm, V. H., and Carpenter, J. J. 1970. Teratogenic and embryopathic effects of indium, gallium and germanium. Tox. and App. Phar. 16:166-170

675. Robens, J. F. 1970. Teratogenic activity of several phthalimide derivatives in the golden hamster. Tox. and App. Phar. 16:24-34

676. Anon. 1970. Another herbicide on the blacklist. Nature 226:309-311

677. Ambrose, A. M. 1963. Toxicologic studies on pyrethrin-type esters of chrysanthemumic acid. Tox. and App. Phar. 5:414-426

678. Sullivan, L. J., et al. 1972. In vitro vs. in vivo chromatographic profiles of carbaryl anionic metabolites in man and lower animals. Tox. and App. Phar. 22:161-174

679. Knaak, J. B., et al. 1965. The metabolism of carbaryl in the rat, guinea pig and man. J. Agr. Food Chem. 13(5):537-543

680. Lyon, J. P., et al. 1970. Repeated and continuous exposure of laboratory animals to acrolein. Tox. and App. Phar. 17:726-732

681. Fleisher, J. H., et al. 1970. Antagonism of sarin poisoning in rats and guinea pigs by atropine, oximes and mecamylamine. Tox. and App. Phar. 16:40-47

682. Adams, E. M., et al. 1952. Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. A.M.A. Arch. Ind. Hyg. and Occup. Med. 6:50-66

683. Shubik, P. 1972. The use of the Syrian golden hamster in chronic toxicity testing. Progr. exp. Tumor Res. 16:176-184

684. Jones, R. A., et al. 1970. Effects of long-term continuous inhalation of ozone on experimental animals. Tox. and App. Phar. 17:189-202

685. Murphy, S. D., et al. 1963. Altered function in animals inhaling conjugated nitro-olefins. Tox. and App. Phar. 5:319-330

686. Valenstein, E. S., Riss, W., and Young, W. C. 1954. Sex drive in inbred guinea pigs. J. Comp. Physio. Physchol. 47:162-165

687. Barnes, R. D. 1968. Invertebrate Zoology. W. B. Saunders Company, Philadelphia

688. Bent, A. C. 1940. Life history of North American cuckoos, goatsuckers, hummingbirds, and their allies. Bull. 176 Smithsonian Inst. U.S. Nat. Museum. USGPO, Washington, D. C.

689. Bent, A. C. et al. 1968. Life history of North American cardinals, grosbeaks, buntings, towhees, finches, sparrows and allies. Bull. 237, part III. Smithsonian Inst. Press, Washington, D. C.

690. Bent, A. C. et al. 1968. Bull. 237, part II. Smithsonian Inst. Press, Washington, D. C.

691. Korschgen, L. J. 1971. Disappearance and persistence of aldrin after five annual applications. Journal of Wildlife Management 35(3):494-500

692. Kring, J. B. 1969. Mortality of the earthworm, Lumbricus terrestris L., following soil applications of insecticides to a tobacco field. J. Scientific Notes 62:963

693. Giese, A. C., and Christensen, E. 1954. Effects of ozone on organisms. Physiol. Zoo. 27(2):101-114

694. Herzberg, F. and Herzberg, A. 1960. The effect of cold on growth of Helix aspersa. J. Exp. Zool. 145: 191-196

695. Hyche, L. L. 1956. Control of mites infesting earthworm beds. J. Econ. Entom. 49(3):409-410

696. Hagstrum, D. W. 1970. The ecological energetics of Tarentula kockii (Aranea, Lycosidae): its physiological basis, relation to microclimate and interaction with selected insecticides. Ph.D. Thesis, University of California, Riverside, California

697. Attiah, H. H., and Boudreaux, H. B. 1964. Influence of DDT on egg-laying in spider mites. J. Econ. Entomol. 57:50-53

698. Hueck, H. J., et al. 1952. The increase of egg production of the fruit tree red spider mite under influence of DDT. Physiol. Comp. Occol. 2:371-379

699. Camin, J. H., et al. 1964. Control of the snake mite Ophionipsus natricia (Gervais) in captive reptile collections. Zoologica 49(4):65-79

700. Hopkins, R., and Kirk, V. M. 1957. Effect of several insecticides on the English red worm. J. Econ. Entomol. 50:699-701

701. Ahmed, M. F., et al. 1954. The effect of systox on some common predators of the cotton aphid. J. Econ. Entom. 47:445-449

702. Atkins, E. L., Jr., and Anderson, L. D. 1954. Toxicity of pesticide dusts to honeybees. J. Econ. Entom. 47(6):969-972

703. Coaker, T. H. 1966. The effect of soil insecticides on the predators and parasites of the cabbage root fly (Erioischia brassicai (Bouche)) and on the subsequent damage caused by the pest. Ann. Appl. Biol. 57:397-407

704. Wright, D. W., et al. 1960. The effect of certain predators on the numbers of cabbage root fly (Eriorachia brassicae)(Bouche) and on the subsequent damage caused by the pest. Ann. App. Bio. 48:756-763

705. Cooper, E. L. 1965. A method of tissue grafting in the earthworm, Lumbricus terrestris. Am. Zool. 5:254

706. Cherry, E. T. 1970. The effects of carbofuran and disulfoton on certain beneficial and destructive insects occuring on burley tobacco. Ph.D. Thesis University of Tennessee

707. Way, M. J., and Scopes, N. E. A. 1968. Studies on the persistence and effects on soil fauna of some soil-applied systemic insecticides. Ann. App. Bio. 62:199-214

708. Edwards, C. A., and Dennis, E. B. 1960. Some effects of aldrin and DDT on the soil fauna of arable land. Nature 188(4752):767

710. Springer, P. E., and Webster, J. R. 1951. Biological effects of DDT applications on tidal salt marshes. Mosquito News 2(2):67-74

711. Hinsch, H., and Crandal, C. P. 1969. The effects of etorphine (M-99), oxymorphone hydrochlorine and neperidine hydrochloride in reptiles. Copeia 2:404

712. Calderwood, H. W. 1971. Anesthesia for reptiles. J.A.V.M.A. 159(11):1618-1625

713. Kramer, K. L., et al. 1965. Surgical anesthesia in snakes. Exp. Animal Anethesia, U.S.A.F. School of Aerospace Med. 374-378

714. Brock, E. M. 1965. Toxicological feeding trials to evaluate the hazard of secondary poisoning to gopher snakes, Pituophis catenifer. Copeia 1965 (2):244-245

715. Burghardt, G. M., and Abeshaheen, J. P. 1971. Responses to chemical stimuli of prey in newly hatched snakes of the genus Elaphe. An. Behavior 19:486-489

716. Saxon, J. G. 1970. The biology of the lizard Cnemidophorus tesselatus, and effects of pesticides upon the population in the Presidior Basin, Texas. Texas A&M University, University Microfilms; A^2 Michigan Order No. 70-22, 954

717. Johlin, J. M., and Moreland, F. B. 1933. Studies of the blood picture of the turtle after complete anoxia. J. Bio. Chem. 103:107-114

718. McCutcheon, F. H. 1943. The respiratory mechanism in turtles. Phys. Zoo. 16(3):255-269

719. Mulla, M. S. 1966. Toxicity of new organic insecticides to mosquito fish and some other aquatic organisms. Mosquito News 26(1):87-91

720. Mulla, M. S., et al. 1963. Field studies on the effects of insecticides on some aquatic wildlife species. J. Econ. Entomol. 56(2):184-188

721. Mulla, M. S. 1963. Toxicity of organochlorine insecticides to the mosquito fish Gambusia affinis and the bullfrog Rana catesbeiana. Mosquito News 23(4):299-303

722. Cooke, A. S. 1973. Response of Rana temporaria tadpoles to chronic doses of p,p-DDT, Copeia, No. 4 pp 647-652

723. Heath, R. G., and Prouty, R. M. 1967. Trial monitoring of pesticides in wings of mallards and black ducks. Env. Contam. and Tox. Bull. 2(2):101

724. Heath, R. G., et al. 1969. Marked DDE impairment of mallard reproduction in controlled studies. Nature 224(5214):47-48

725. Lehner, P. N., and Egert, A. 1969. Dieldrin and eggshell thickness in ducks. Nature 224:1218-1219

726. Muller, H. D., and Lockman, D. C. 1972. Fecundity and progeny growth following subacute insecticide ingestion by the mallard. Poultry Sci. 51:239-241

727. Heath, R. G., et al. 1972. Comparative dietary toxicities of pesticides to birds. Bureau Sport Fisheries Wildlife, Spec. Sci. Report Wildlife, 152

728. Peakall, D. B. 1967. Pesticide induced enzyme breakdown of steroids in birds. Nature 216:505-506

729. Bree, M. M., and Gross, N. B. 1969. Anesthesia of pigeons with CI 581 and pentobarbital. Lab. An. Care 19:500-502

730. Webster, D. M., and Hollard, V. D. 1973. A safe and simple injection anesthetic for birds. Physiol. Behavior 10:831

731. Kreitzer, J. F. and Spann, J. W. 1968. Mortality among bobwhites confined to a heptachlor contaminated environment. J. of Wildlife Mgmt. 32(4):874-878

732. Hessler, E. et al. 1970. A biotelemetery study of survival of pen-reared pheasants released in selected habitats. Journal of Wildlife Management 34(2):267

733. Kuck, T. L. et al. 1970. Movements and behavior of hen pheasants during the nesting season. Journal of Wildlife Management 34(3):626-630

734. Dahlgren, R. B., and Linder, R. L. 1971. Effects of polychlorinated biphenyls on pheasant reproduction, behavior and survival. Journal of Wildlife Management 35(2):315-318

735. Spann, J. W. et al. 1972. Ethyl mercury p-toluene sulfonanilide: lethal and reproductive effects on pheasants. Science 175:328-331

736. Jefferies, D. J. 1973. The effects of organochlorine insecticides and their metabolites on breeding birds. J. Reprod. Fert. (Suppl.) 19:337-352

737. Wurster, D. F., et al. 1965. Bird mortality after spraying for Dutch elm disease with DDT. Sci. 148: 90-91

738. Decino, T. J., Cunningham, D. J., and Schafer, E. W. 1965. Toxicity of DRC-1339 to starlings. Journal of Wildlife Management 29:249-253

739. Martin, W. E. 1972. Mercury and lead residues in starlings-1970. Pest. Mon. J. 6(1):27-32

740. Martin, W. E., and Nickerson, P. R. 1972. Organochlorine residues in starlings-1970. Pest. Mon. J. 6(1):33-40

741. Hill, E. F. 1972. Avoidance of lethal dietary concentrations of insecticide by house sparrows. J. Wildlife Mgmt. 36(2):635

742. Davidson, K. L., Sell, J. L., and Rose, R. J. 1971. Dieldrin poisoning of chickens during severe dietary restriction. Env. Contam. and Tox. Bull. 5(6):493-501

743. Seifert, J. H., et al. 1968. Effect of dietary restrictions upon dieldrin poisoning in chickens. Ann. Proc. of the North Dakota Acad. Sci 22:36-44

744. Sherman, M. W., et al. 1964. Acute and subacute toxicity of several organophophorus insecticides to chicks. Tox. and App. Phar. 6:147-153

745. Sherman, M., and Ross, E. 1961. Acute and subacute toxicity of insecticides to chicks. Tox. and App. Phar. 3:521-533

746. Ghadiri, M., et al. Biological effect of feeding malathion and carbaryl to laying hens and to roosters. Proc. Utah Acad. Sci.p. 185

747. Arnold, R. L. 1971. Effects of various levels of dietary selenium and arsenic on chickens. Ph.D. Thesis, South Dakota State University

748. Davison, K. L., and Sell, J. L. 1972. Dieldrin and p,ṕ-DDT effects on egg production and eggshell thickness of chickens. Bulletin of Envir. Contam. Tox. 7(1):9-18

749. Cecil, H. C. 1972. Dietary p,ṕ-DDT, o,ṕ-DDT or p,ṕ-DDE and changes in eggshell characteristics and pesticide accumulation in egg contents and body fat of caged white leghorns. Poultry Sco. 51:130-139

750. Sauter, E. A., and Steele, E. E. 1972. The effect of low level pesticide feeding on the fertility and hatchability of chicken eggs. Poultry Sci. 51:71-76

751. Lillie, R. J. et al. 1973. Dietary calcium, DDT source and age of hen on the reproductive performance of caged white leghorns fed DDT. Poultry Sci. 52(2):636-644

752. Conaway, C. H. 1958. Maintenance, reproduction and growth of the least shrew in captivity. J. Mamm. 39:507-512

753. Allanson, M., and Deanesly, R. 1934. The reaction of anoestrous hedgehogs to experimental conditions. Proc. of the Roy. Soc. Lond. B. 116:170-185

754. Hine, C. H., Megers, F. H., and Wright, R. W. 1970. Pulmonary changes in animals exposed to nitrogen dioxide, effects of acute exposures. Tox. and App. Phar. 16:201-213

755. Villeneuve, D. C., et al. 1971. Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Env. Contam. and Tox. Bull. 6(2): 120-128

756. Guilbault, G. G., Sadar, M. H., Kaun, S., and Casey, D. 1970. Effects of pesticides on liver cholinesterases from rabbit, pigeon, chicken, sheep and pig. Anal, Chim. Acta. 51:83-93

757. Gardner, A. F. 1964. The development of general anesthesia in the albino rabbit for surgical procedures. Lab. An. Care 14(3):214-225

758. Bucher, N.L.R. 1946. Effects of 24-Dichlorophenoxyaceti acid on experimental animals. Proc. Soc. Exp. Bid. Med. 63:204

759. Wright, P. A. 1960. Infertility in rabbits induced by feeding ladino clover. Soc. Exp. and Med. 105:428-430

760. Petty, C. W., and Karler, R. 1965. The influence of aging on the activity of anticonvulsant drugs. J. Pharm. Exp. Therap. 150(3):443-448

761. Good, E. E., and Ware, G. W. 1969. Communication. Tox. and App. Phar. 14:201-203

762. Christian, J. J., and Davis, D. E. 1966. Adrenal glands in female voles (Microtus pennsylvanicus) as related to reproduction and population size. J. Mamm. 47(1):1-18

763. Golley, F. B., et al. 1968. Components of growth in Peromyscus polionotus. Growth 32:103-111

764. Reid, L. 1970. Evaluation of model systems for study of airway epithelium, cilia and mucus. Arch. Intern. Med. 126:428-434

765. Dalhamn, T. 1956. Mucous flow and ciliary activity in the trachea of healthy rat and rats exposed to respiratory irritant gases. Acta. Physiol. Scand., 36 (supplement 123)

766. Goldberg, M. E., and Salama, A. I. 1969. Amphetamine toxicity and brain monoamines in three models of stress. Tox. and App. Phar. 14:447-456

767. Fuller,G.C., Bousquet, W.F., and Miza,T.S. 1972. Effect of cold exposure on drug action and hepatic drug metabolism in the rat. Tox. and App. Phar. 23:10-19

768. Brown, J. R. 1970. The effect of environmental and dietary stress on the concentration of 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane in rats. Tox. and App. Phar. 17:504-510

769. DeFreitas, A. S. W., et al. 1969. Chronic cold exposure and DDT toxicity. Chapter 18:361-367 in: Chemical Fallout, Current Research on Persistent Pesticides, ed. by M. W. Miller and G. G. Berg. Charles C. Thomas, Publisher, Springfield, Illinois

770. Adler, J. H. 1967. Aspects of stress in animals. Husb. Lab. An. 239-254

771. Siegel, J. 1971. Effects on experimental animals of acute, repeated and continuous inhalation exposures to dichloroacetylene mixtures. Tox. and App. Phar. 18: 168-174

772. Jenkins, L. J., Jr., et al. 1970. Long-term inhalation screening studies of benzene, toluene, oxylene, and cumene on experimental animals. Tox. and App. Phar. 16:818-823

773. Jenkins, L. J., Jr., et al. 1970. Repeated and continuous exposures of laboratory animals to trichlorofluoromethane. Tox. and App. Phar. 16:133-142

774. Durham, W. F. 1969. The influence of other factors on the toxicity of pesticides, Chapter 23:433-446 in: Chemical Fallout, Current Research on Persistent Pesticides, ed. by M. W. Miller and G. G. Berg. Charles C. Thomas, President, Springfield, Illinois

775. Meshorer, A., and Benhar, E. 1966. Accidental poisoning of inbred male mice by carbon tetrachloride. Lab. An. Care 16(3):198-201

776. Casterline, J. L., et al. 1971. The effect of 28-day pesticide feeding on serums and tissue enzyme activities of rats fed diets of varying casein content. Tox. and App. Phar. 18:607-618

777. Fouts, J. R. 1970. Some in vitro assay conditions that affect detection and quantitation of phenobarbital-induced increases in hepatic microsomal drug-metabolizing enzyme activity. Tox. and App. Phar. 16:48-65

778. Kay, K. 1966. Effect of pesticides on enzyme systems in mammals. Adv. Chem. Ser. 60:54-66

779. Thompson, W. R. 1947. Use of moving averages and interpolation to estimate median-effective dose. Bact. Rev. 11:115-143

780. Hart, L. G., and Fouts, J. R. 1965. Further studies on the stimulation of hepatic microsomal drug metabolizing enzymes by DDT and its analogs. Naunyn-Schmiedebergs Arch. exp. Path. U. Pharmak. 249:486-500

781. Bratkowski, T. A. 1970. Isolation and properties of a DDT-sensitive adenosine triphosphatase complex from rat brain. Ph.D., Thesis, University of Wisconsin, Madison, Wisconsin

782. Ball, W. L., et al. 1954. Modification of parathion's toxicity for rats by pretreatment with chlorinated hydrocarbon insecticides. Can. J. Biochem. Physiol. 32:440-445

783. Hagen, E. O., and Hagen, J. M. 1964. A method of inhalation anesthesia for laboratory mice. Lab. An. Care. 14:13-15

784. Alary, J. G., and Brodeux, J. 1970. Correlation between the activity of liver enzymes and the LD_{50} of parathion in the rat. Can. J. Physical. Pharmacol. 48(12):829-831

785. Gaines, T. B. 1960. The acute toxicity of pesticides to rats. Tox. and App. Phar. 2:88-99

786. Gaines, T. B. 1969. Acute toxicity of pesticides. Tox. and App. Phar. 14:515-534

787. Jacobson, K. H. 1972. Acute oral toxicity of mono and di-alkyl ring-substituted derivatives of aniline. Tox. and App. Phar. 22:153-154

788. Guthrie, F. E., et al. 1971. Response of the laboratory mouse to selection for resistance to insecticides. Tox. and App. Phar. 18:92-101

789. Apostolou, A., and Peoples, S. A. 1971. Toxicity of the avicide 2-chloro-4-acetoluidide in rats: a comparison with its nonacetylated from 3-chloro-p-toluidine. Tox. and App. Phar. 18:517-521

790. Al-Hachim, G. M., and Fink, G. B. 1968. Effect of DDT or arathion on condition avoidance response of offspring from DDT or parathion treated mothers. Psychopharmacologia 12:424-427

791. Triolo, A. J., and Coon, J. M. 1966. The protective effect of aldrin against the toxicity of organophosphate anticholinesterases. J. Pharm. and Exp. Therap. 154(3):613-623

792. Ortega, P. 1966. Light and electron microscopy of dichlorodiphenyltrichloroethane (DDT) poisoning in the rat liver. Lab. Invest. 15(4):657-679

793. Weaver, L. C., and Kerley, T. L. 1962. Strain difference in response of mice to d=amphetamine. J. Pharm. Ext. Therap. 135:240-244

794. Landauer, W., and Wakasugi, N. 1968. Teratological studies with sulphonamides and their implications. J. Emb. Exp. Morph. 20(3):261-284

795. Nelson,S.C. et al. 1956. Serum alkaline phosphatase levels, weight changes and mortality rates of rats fed endrin. J. Agr. and Food Chem. 4(8):696-700

796. Chance, M. R. A. 1946. Aggregation as a factor influencing the toxicity of sympathomimetic amines in mice. J. Phar. and Exp. Ther. 87:214

797. Bovard, E. W. 1958. The effects of early handling on viability of the albino rat. Psycho. Review 65(5):257-271

798. Oser, B. L., et al. 1963. Safety evaluation studies of calcium EDTA. Tox. and App. Phar. 5:142-162

799. Olson, K. J. 1972. Preface - series of papers relating reproductive activity to selected organosiloxane chemicals. Tox. and App. Phar. 21:12-14

800. Righter, H. F. 1972. Influence of age and sexual status on the development of toxic effects in the male rat fed aflatoxins. Tox. and App. Phar. 21:435-439

801. Deichmann, W. B., and Keplinger, M. L. 1966. Effects of combinations of pesticides on reproduction of mice. Tox. and App. Phar. 8:337-338

802. Palmes, E. D., and Del Pup, J. 1970. Mouse populations, biological systems for evaluating effects of environmental agents. Trans. N. Y. Acad. of Sci. 32:381-384

803. Barnes, J. M., and Denz, F. A. 1954. Experimental methods used in determining chronic toxicity. Phar. Rev. 6:191-242

804. Les, E. P. 1968. Environmental factors influencing body weight of C57BL#/6J and DBA/#2J mice. Lab. An. Care 18(6):623-625

805. Weil, C. S., and Carpenter, C. P. 1969. Abnormal values in control groups during repeated-dose toxicologic studies. Tox. and App. Phar. 14:335-339

806. Hadlow, W. J., et al. 1955. Stilbestrol-contaminated feed and reproductive disturbances in mice. Science 122:643-644

807. Les, E. P. 1968. Effect of acidified-chlorinated water on reproduction in C3H/HeJ and C57BL/6J mice. Lab. An. Care 18(2):210-213

808. Radovich, B. T. 1971. The effect of triethylene melanine upon the initial reproductive performance of the F_1 generation. Ph.D. Thesis, George Washington University.

809. Spyker, J. M. 1971. Methylmercury, mice and men. Ph.D. Thesis, University of Minnesota

810. Hadlow, W. J., and Grimes, E. F. 1955. Influence of stilbestrol-contaminated feed on reproduction in a colony of mice. Proc. An. Care Panel 6:19-25

811. Bernard, R. F. and Gaertner, R. A. 1964. Some effects of DDT on reproduction in mice. J. Mamm. 45(2):272-276

812. Bernard, R. F., and Gaertner, R. A. 1964. Some effects of DDT on reproduction in mice. J. Mamm. 45(2):272-276

813. Downhower, J. F., and Pauley, J. D. 1970. Automated recordings of body temperature from free-ranging yellow-bellied marmots. Journal of Wildlife Management 34(3):639-641.

814. Blackmore, D. K. 1963. The toxicity of some chlorinated hydrocarbon insecticides to British wild foxes. J. Comp. Path 73:391-409

815. Bissonnette, T. H., and Csech, A. G. 1937. Modification of mammalian sexual cycles, VII-Fertile matings of raccoons in December instead of February induced by increasing daily periods of light. Proc. Roy. Sci. Lond. B 122(827):246-254

816. Ringer, R. K. et al. 1972. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. 164th Nat. Meeting of Amer. Chem. Soc., Vol. 12(2):149-154

817. Aulerich, R. J., et al. 1974. Effects of dietary mercury on mink. Archives of Envir. Contam. and Tox. Vol. 2(1):43-51

818. Asmundsson, T., and Kilburn, K. H. 1970. Mucociliary clearance rates at various levels in dog lungs. Am. Rev. Resp. Dis. 102:388-397

819. Mauck, H. P., Jr., et al. 1965. The level and stage of anesthesia: its influence on cardiac output and related hemodynamics. Tox. and App. Phar. 7:301-307

820. Harrison, D. L., Maskel, P. E. G., and Money, D. F. L. 1963. Dieldrin poisoning of dogs. New Zealand Vet. J. 11(2):23-31

821. Stowe, H. D., et al. 1973. Experimental oral lead toxicity in young dogs. Arch. Pathol. 95:106-116

822. Deichmann, W. B., et al. 1971. Subnormal reproduction in beagle dogs induced by DDT and aldrin. Ind. Med. and Surg. 40(2):10-20

823. Anderson, R. S. 1961. Diuresis due to stress in cattle. Nature 192:460

824. Lonsdale, E. M., Brapach, B., and Thorne, E. T. 1971. A telemetry system to determine body temperature in pronghorn antelope. Journal of Wildlife Management 35(4):747-751

825. Gilmar, D. S. 1971. A device for monitoring radio-marked animals. Journal of Wildlife Management 35(4):829-832

826. Van Ballenberghe, V., and Peek, J. M. 1971. Radio-telemetry studies of moose in northeastern Minnesota. Journal of Wildlife Management 35(1):63-71

827. Swiggart, R. C., et al. 1972. Wildlife kill resulting from the misuse of arsenic acid herbicide. Bulletin Envir. Contam. Tox. 8(2):122-128

828. Thrasher, J. D. 1970. The cell cycle and turnover times of the small intestinal epithelia of the pouchless opossum, Marmosa mitis. Experientia 26: 871-872

829. Peters, J. H., and Storrs, E. E. 1972. Amino acid levels and L-Asparanginase and Histaminase Activities in plasma of the armadillo, Dasypus novemcinctus. Lab. An. Sci. 22(2):233-235

830. Wilson, T. H., and Wiseman, G. 1954. The uses of sacs of everted small intestine for the study of the transference of substances from the mucosal to the serosal surface. J. Physiol. 123:116-125

831. McCollister, F. O., and Rowe, V. K. 1964. Toxicology of acrylamide. Tox. and App. Phar. 6:172-181

832. Keefe, T. J., et al. 1964. Ornithonyssus bacoti (Hirst) infestation in mouse and hamster colonies. Lab. An. Care 14(5):366-369

833. Wright, J. F., and Seibold, H. R. 1958. Nutrition: estrogen contamination of pelleted feed for laboratory animals, effect on guinea pig reproduction. J. Am. Vet. Med. Assoc. 132:258-261

834. Way, M. J., and Scopes, N. E. A. 1965. Side effects of some soil-applied systemic insecticides. Proc. of the Assoc. of App. Bio. 55:340-341

835. Gish, C. D., and Christensen, R. E. 1973. Cadmium, nickel, lead and zinc in earthworms from roadside soil. Envir. Sci. Tech. 7:1060-1062

836. Pelluet, D. 1964. The hormonal control of cell differentiation in the ovotestis of slugs (Gastero-poda: Pulmonata). Can. J. Zoo. 42:195-199

837. Louis-Ferdinand, R. T., and Fuller, G. C. 1970. The mechanism of the biologic activity of a series of nitroolefins. Tox. and App. Phar. 16:668-674

838. Duncan, C. J. 1961. Spontaneous activity in the isolated nerves of Pulmonate molluscs. Comp. Biochem. Physiol. 3:42-51

839. Derr, S. K. 1972. Biologically active compounds in the aquatic environment: a study of two environmental contaminants, DDE and Aroclor 1254, on the aquatic midge, Chironomus tetans. Ph.D. Thesis, Michigan State University

840. Brattsten, L. B. 1971. Role of mixed function oxidases of insects in their response to xenobiotics. Ph.D. Thesis, University of Illinois, Champaign-Urbana, Illinois

841. Vinopal, J. H. 1970. Selective toxicity and mode of action of phoxim and related esters in insects and mammals. Ph.D. Thesis, University of California, Riverside, California

842. Keppler, W. J., et al. 1965. Laboratory evaluation of certain larvicides against Culex pipiens, Linn, Anopheles albimanus Wied and Anopheles quadrimaculatus Say. Mosquito News 25:415-419

843. Hoskins, W. M. 1964. The metabolism of DDT in insects. World Rev. Pest Control 3:85-96

844. Agosin, M., et al. 1963. Intermediary carbohydrate metabolism in Triatoma infestans (Insecta; Hemiptera)-II. The metabolism of C14-glucose in Triatoma infestans nymphs and the effect of DDT. Comp. Biochem. Physiol. 8:311-320

845. Trehenre, J. E. 1957. Glucose absorption in the cockroach. J. Exp. Biol. 34:478-485

846. Georghiou, G. P. et al. Joint action of diquat and related one-electron transfer agents with propoxur and fenthion against mosquito larvae. J. Econ. Entomol. 67(2):184-186

847. Cohen, A. J., et al. 1960. Comparative detoxication: the enzymic conjunction of chloro compounds with glutathione in locusts and other insects. Biochem. J. 90:457

848. Sanchez, F. F. 1965. Factors affecting the latent toxicity of aldrin, DDT, and heptachlor to resistant and susceptible strains of the house fly. Ph.D. Thesis, Univ. of Hawaii

849. Kanehisa, T. 1957. Tests of chemical substances and specific genes for their effects on tumor incidence in D. Melanogaster. The Am. Nat. 41(861):393-396

850. Matsumura, F., et al. 1967. Effect of sesamex upon dieldrin resistance in the german cockroach. J. Econ. Entom. 60(4):942-944

851. Gadallah, A. J., et al. 1972. Effects of thiotepa on ribosomal protein of house fly eggs. J. Econ. Entomol. 65(4):1176-1178

852. Al-Adil, K. M., et al. 1973. Effects of orally ingested aflatoxin B_1 on nucleic acids and ribosomes of house fly ovaries. Tox. App. Pharm. 26:130-136

853. Al-Adil, K. M. et al. 1972. Biochemical effect of metepa on house fly eggs. J. Econ. Entomol. 65(2): 321-324

854. Atkins, E. L., Jr., and Anderson, L. D. 1962. DDT resistance in honey bees. J. Econ. Entom. 55:792

855. Sternburg, J., et al. 1954. DDT dehydrochlorinase, an enzyme found in DDT - resistant flies. Agr. Food Chem. 2(22):1125-1130

856. Oppenoorth, F. J. and Van Asperen, K. 1960. Allelic genes in the housefly producing modified enzymes that cause organophosphate resistance. Sci. 132:298

857. Brown, A. W. A. 1968. Insecticide resistance comes of age. Entomol. Soc. 14:3-9

858. Darlington, W. A., Partos, R. D., and Ratts, K. W. 1971. Correlation of cholinesterase inhibition and toxicity in insects and mammals. Tox. and App. Phar. 18:542-547

859. Guilbault, G. G., et al. 1970. Enzymatic methods of analysis trace ana. of various pesticides with insect cholinesterase. Anal. Chim. Acta. 52:75-82

860. Guilbault, G. G. 1970. Purification and properties of cholinesterases from honeybees. J. Agr. Food Chem. 18(4):692-697

861. Sadar, M. H., et al. 1970. Trace analysis of pesticides using cholinesterase from human serum, rat liver, electric eel, bean leaf beetle and white fringe beetle. Anal. Chem. 40(14):1770-1775

862. Harlow, P. A. 1958. The action of drugs on the nervous system of the locust (Locusta migratoria). Ann. App. Bio. 46(1):55-73

863. Dolensek, D. P. 1971. Necrospy techniques in reptiles. J.A.V.M.A. 159(11):1616-1617

864. Potter, R. L., and Hadley, M. E. 1969. Comparative effects of sulfhydryl inhibitors on melanosome movements within vertebrate melanophores. Experienta 26(5):536-538

865. Dimond, J. B., Kadunce, R. E., and Getchell, A. S. 1968. DDT residue persistence in red-backed salamanders in a natural enviornment. Env. Contam. and Tox. Bull. 3(4):194-202

866. Friess, S. L. 1972. Irreversibility in nerve node inactivation by teriary vs. quaternary aminoesters. Tox. and App. Phar. 22:208-212

867. Harris, A. J., and Miledi, R. 1971. The effect of type D botulinum toxin on frog neuromuscular junctions. J. Physiol. 217:497-515

868. Votava, Z. 1967. Pharmacology of the central cholinergic synapses. Ann. Rev. of Phar. 4:223-236

869. Camougis, G., and Davis, W. 1971. A comparative study of the neuropharmacological basis of action of pyrethrins. Pyreth. Post 11(1): 7-14

870. Pendleton, R., et al. 1968. The effect of a benzothiodiazide on the isolated toad bladder. J. Phar. and Exp. Ther. 164(2):348-361

871. Fingal, W., and Kaplan, H. M. 1963. Susceptibility of Xenopus laevis to copper sulfate. Copeia (1):155-156

872. Wilber, C. G. 1954. Toxicity of sarin in bullfrogs. Science 120:322

873. Joiner, G. N., and Abrams, G. D. 1970. Tuberculosis in the leopard frog (Rana pipiens). J. of Infectious Diseases 122(1 & 2)96-99

874. Setliff, J. A., et al. 1971. Effect of ciquatoxin on sodium transport across the frog skin. Tox. and App. Phar. 18:676-684

875. Ussing, H. H., and Zerahn, K. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta. Physiol. Scand. 23:110-127

876. Robertson, D. R. 1970. The ultimobranchial body in Rana pipiens XI. Response to increased dietary calcium - evidence of possible physiological function. Endocrinology 87(5):1041-1050

877. Woodwell, G. M., et al. 1967. DDT residues in an east coast estuary: a case of biological concentration of a persistent insecticide. Sci. 156:821-824

878. Dindal, D. L. 1968. Kinetics of CL^{36} DDT in wild waterfowl. Ph.D. Thesis, Ohio State University, final report on Contract 14-16-0008-685 between Dept. of Interior and O.S.U. Research Foundation

879. Khera, K. S., et al. 1965. Toxic effects in ducklings hatched from embryos inoculated with E.P.N. for systox. Fed. Cosmet. Tox 3:581-586

880. Switzer, B., and Lewin, V. 1971. Shell thickness, DDE levels in eggs, and reproductive success in common terns (Sterna hirundo) in Alberta. Can. J. Zool. 49;69-73

881. Bailey, S., and Bunyan, P. J. 1972. Interpretation of persistence and effects of polychlorinated biphenyls in birds. Nature 236:34-36

882. Findlay, G. M., and De Freitas, A. S. W. 1971. DDT movement from adipocyte to muscle cell during lipid utilization. Nature 229:63-65

883. Peakall, D. V. 1969. Effect of DDT on calcium uptake and vitamin D metabolism in birds. Nature 224:1219-1220

884. Jefferies, D. J., and French, M. C. 1971. The effect of p,ṕ-DDT on the rate, amplitude and weight of the pigeon and Bengalese finch. Br. Poult. Sci. 12:387-399

885. Revzin, A. M. 1966. Effects of endrin on telecephalic function in the pigeon. Tox. App. Pharm. 9(1):75-83

886. Gill, J. A. 1970. Toxicities of DDE and some other analogs of DDT to pheasants. Journal of Wildlife Management 34(1):223-226

887. Simpson, C. F., et al. 1972. Effect of long-term feeding of DDT to turkeys. Bulletin of Envir. Contam. Tox. 7(5):277-283

888. Bradfield, P. L. 1972. Robin population changes and pesticide transformation after methoxychlor replaces DDT for control of dutch elm disease. Ph.D. Thesis, Michigan State University, East Lansing, Michigan

889. Södergren, A., and Ulfstrand, S. 1972. DDT and PCB relocate when caged robins use fat reserves. Ambio. 1:36-40

890. Stickel, W. H., et al. 1973. Toxicity and persistence of mirex in birds, pp 437-467 in Pesticides and the Environment: A Continuing Controversy, ed. by W. B. Deichman. Intercontinental Medical Book Corp., New York

891. Beckman, H. et al. 1966. An improved method for the analysis of DDT and its metabolites in eggs. J. Assoc. official analytical chemists 49:996-999

892. Flick, D. F., and O'Dell, R. G. 1971. A chick bioassay for oral diuretics. Tox. and App. Phar. 19:29-36

893. Abou-Donia, M. B. and D. B. Menzel 1968. The metabolism in vivo of 1,1,1-trichloro-2,2-bis(P-chlorophenyl) ethane (DDT), 1,1-Dichloro-2,2-bis (p-chlorophenyle) ethane (DDD) and 1-dichloro-2,2-bis(p-chlorophenyl) ethane (DDE) in the chick by embryonic injection and dietary ingestion. Biochem. Pharm. 17:2142-2161

894. McChesney, E. W., and Banks, W. F., Jr. 1963. Toxicity and physiological dispostion of sodium-p-N-glycolylarsanilate. II. Absorption, excretion and metabolism in the chicken. Tox. and App. Phar. 5:702-718

895. Granett, S. E. S. 1972. Perturbations of metabolism in chick brain induced by hyperphenylolanemia and galactosemia. Ph.D. Thesis, Michigan State University.

896. Landauer, W. 1947. Insulin-induced abnormalitries of beak extremities and eyes in chickens. J. Exp. Zoo. 105(2):145-172

897. Landauer, W. 1952. Malformation of chicken embryos produced by boric acid and the probable role of riboflavin in their origin. J. Exp. Zoo. 120(3): 464-508

898. Landauer, W. 1948. The effect of nicotinamide and α-Ketoglutaric acid on the teratogenic action of insulin. J. Exp. Zoo. 109:283-290

899. Puyear, R. L., and Paulson, G. D. 1972. Effect of Carbaryl (1-Napththyl N-Methylcarbamate) on pentobarbital-induced sleeping time and some liver microsomal enzymes in white leghorn cockerels. Tox. and App. Phar. 22:621-627

900. Platonow, N. S., and Funnell, H. S. 1972. The distribution and some effects of polychlorinated biphenyls (Aroclor 1254) in cockerels during prolonged feeding trial. Can. J. Comp. Med. 36:89-93

901. Mull, R. L., et al. 1972. Effects of an acutely toxic dose of the avicide 3-chloro-p-toluidine in chickens. Tox. and App. Phar. 22:458-464

902. Richert, E. P., and Prahlad, K. V. 1972. Effects of DDT and its metabolities on thyroid of the Japanese quail, Coturnix coturnix japonica. Poultry Sci. 51: 196-200

903. Wright, P. L. 1947. The sexual cycle of the male long-tailed weasel (Mustela frenata) J. Mamm. 28(4):343-352

904. Buckner, C. H. 1964. Metabolism, food capacity, and feeding behavior in four species of shrews. Can. J. Zoo. 42:259-279

905. Morrison, P. R., et al. 1953. Physiological observations on a small shrew. Am. Physiol. Soc. Fed. Proced. 12:100-101

906. Morrison, P. R. 1948. Oxygen consumption in several mammals under basal conditions. J. Cell. Comp. Physiol. 31:281-291

907. Kendeigh, S. J. 1945. General notes. J. Mamm. 26: 86-87

908. Jefferies, D. J. 1972. Organochlorine insecticide residues in British bats and their significance. J. Zool., London 166:245-263

909. Souma, J. A., et al. 1972. The effect of cis- and trans-clomiphene citrate on the collagen content of fetal bone. Tox. and App. Phar. 23:339-343

910. Gordon, G. R., and Peters, J. H. 1967. Plasma histaminase activity in various mammalian species: a rapid method of assay. Soc. Exp. Bio. Med. 124: 399-404

911. Hollingsworth, R. L., et al. 1971. Fetal rabbit ductus arteriosus assessed in a teratological study on isoproterenol and metaproterenol. Tox. and App. Phar. 18:231-234

912. Schardein, J. L., et al. 1971. Teratogenesis studies with diphenhydramine HC_1. Tox. and App. Phar. 18: 971-976

913. Palmer, A. K. 1968. Spontaneous malformations of the New Zealand white rabbit: the background to safety evaluation tests. Lab. Animal 2:195-206

914. Capizzo, F., and Roberts, R. J. 1971. ∝-Napthyliso-thiocynate (ANIT)-induced hepatotoxicity and disposition in various species. Tox. and App. Phar. 19:176-187

915. Foulkes, E. C. 1971. Glomerular filtration and renal plasma flow in uranium-poisoned rabbits. Tox. and App. Phar. 20:380-385

916. McDonald, T. O., et al. 1972. Ocular toxicity of ethylene chlorohydrin and ethylene glycol in rabbit eyes. Tox. and App. Phar. 21:143-150

917. Gowdey, C. W., et al. 1954. The pharmacological properties of the insecticide dieldrin. Can. J. Biochem. and Physiol. 32:498-503

918. Pennington, K., and Fuerst, R. 1971. Biochemical and morphological effects of various gases on rabbit erythocytes. Arch. Env. Health 22:476

919. Goodwin, L. G., et al. 1957. The toxicity of diaminodiphenoxyalkanes. Brit. J. Pharm. 12:468-474

920. Luisada, A., et al. 1944. A comparative study of electrocardiogram and heart sounds in common and domestic mammals. Cardiologia 8:64-84

921. Banta, C. A., et al. 1972. A low-cost electronic cardiac auscultation device for monitoring rabbits under general anesthesia. Lab. An. Sci. 22(3):402-403.

922. Fritz, T. E., et al.1968. A central nervous system disorder in ground squirrels (Citellus tridecemlineatus) associated with visceral larva migrams. J.A.V.M.A. 153(7):841-844

923. McKeever, S. 1959. Effects of reproductive activity on the weight of adrenal glands in Microtus montanus. Anat. Rec. 135:1-5

924. Louch, C. D. 1958. Adrenocortical activity in two meadow vole populations. J. Mamm 39(1):109-116

925. Street, J. C., and Blau, A. D. 1966. Insecticide interactions affecting residue accumulation in animal tissue. Tox. App. Pharm. 8:497-504

926. Grant, D. L., Phillips, W. E. J., and Villeneuve, D. C. 1971. Metabolism of a polychlorinated biphenyl (Aroclor 1254)mixture in the rat. Env. Contam. and Tox. Bull. 6(2):102-112

927. Roszowski, A. P., and Kelley, N. M. 1963. A rapid method for assessing drug inhabition of feeding behavior. J. Phar. and Exp. Ther. 140:367-374

928. Gargus, J. L., et al. 1969. Utilization of newborn mice in the bioassay of chemical carcinogens. Tox. and App. Phar. 15:552-559

929. Dorfman, R. I., and Dorfman, A. S. 1955. Estrogen contamination in animal food. Proc. An. Care Panel 6:16-18

930. Cordes, C. L. 1971. Comparative study of the uptake, storage, and loss of DDT in small mammals. Ph.D. Thesis, North Carolina State University, Raleigh

931. Pekas, J. C. 1971. Uptake and transport of pesticidal carbamates by everted sacs of rat small intestine. Can. J. of Physiol. and Pharmacol. 49(1):14-21

932. Phillips, W. E. J. 1962. Low-temperature environmental stress and the metabolism of vitamin A in the rat. Can. J. Biochem. and Physiol. 40:491-499

933. Dale, W. E., Gaines, T. B., and Hayes, W. J. 1962. Storage and excretion of DDT in starved rats. Tox. and App. Phar. 4:89-106

934. McLean, H. E. M., and McLean, E. K. 1966. The effect of diet and 1,1,1-Trichloro-2,2-bis-(p-chlorophenyl)-ethane (DDT) on microsomal hydroxylating enzymes and on sensitivity of rats to carbon tetrachloride poisoning. Biochem. J. 100:564-571

935. Budny, J. A. 1972. Metabolism and blood pressure effects of disodium nitrolotriacetate (Na_2NTA) in dogs. Tox. and App. Phar. 22:655-660

936. Watrous, W. M., and Plaa, G. L. 1972. Effect of halogenated hydrocarbons on organic ion accumulation by renal cortical slices of rats and mice. Tox. and and App. Phar. 22:528-543

937. Aronson, A.L., and Ahrens,F.A. 1971. The mechanism of renal transport and excretion of ethylenediaminetetraacetate with interspecies comparisons. Tox. and App. Phar. 18:1-9

938. Epstein, S. S., et al. 1970. Carcinogenicity testing of selected food additives by parental administration to infant Swiss mice. Tox. and App. Phar. 16:321-334

939. Smyth, J. F., Jr., et al. 1970. Oral toxicity and excretion of four commercial polyoxyalkylene glycol compounds. Tox. and App. Phar. 16:675-680

940. Levander, O. A., Young, M. L., and Meeks, S. A. 1970. Studies on the binding of selenium by liver homogenates from rats fed diets containing either casein or casein plus linseed oil meal. Tox. and App. Phar. 16:79-87

941. Kolmodin-Hedman, B. 1971. Effect of exposure to lindane on drug metabolism decreased hexobarbital sleeping-times and increased antipyrine disappearance rate in rats. Tox. and App. Phar. 20:299-307

942. McChesney, E. W., et al. 1962. Toxicity and physiological disposition of sodium p-N-Glyoclylarsanilate. I. Observations in the mouse, cat, rat and man. Tox. and App. Phar. 4:14-23

943. Neal, R. A. 1967. Studies on the metabolism of diethyl 4-Nitrophenyl phosphorothionate (parathion) in vitro. Biochem J. 103:183-191

944. Balazs, T., et al. 1963. Renal tests in toxicity studies on rats. Tox. and App. Phar. 5:661-674

945. Ellis, R. W., and Fang, S. C. 1973. The metabolism of [^{14}C] lactate by rat kidney slices after acute mercurial intoxication. Tox. and App. Phar. 24: 230-238

946. Gemmill, C. L. 1965. The metabolism, pulse rate and body temperature of normal, thyroidectomized and hypermetabolic rats. Lab. An. Care 15(5):329-335

947. Conney, A. H., et al. 1967. Effects of pesticides on drug and steroid metabolism. Clinical Pharm. and Therap. 8(1): pt. 1:2-10

948. Laug, E. P., et al. 1950. Liver cell alteration and DDT storage in the fat of the rat induced by dietary levels of 1 to 50 ppm DDT. J. Pharm. and Exp. Ther. 98(3):268-273

949. Ludwig, G., et al. 1964. Excretion and distribution of Aldrin ^{14}C and its metabolites after orad administration for a long period of time. Life Sci. 3(2): 123-130

950. Nakatsugawa, T., et al. 1969. Degradation of parathion in the rat. Biochem. Pharm. 18, 1103-1114

951. Kato, R., and Gillette, J. R. 1965. Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharm. Exp. Ther. 150(2):279-284

952. Courtney, K. D., et al. 1970. Teratogenic evaluation of 2,4,5-T. Science 168:864-866

953. Epstein, S. S., and Shafner, H. 1968. Chemical mutagens in the human environment. Nature 219:385-387

954. Courtney, K. D., and Moore, J. A. 1971. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Tox. and App. Phar. 20:396-403

955. Posner, H. S., et al. 1969. Anomalies of the internal organs and diminished calcification of vertebrae in fetuses after benzhydrylpiperazine treatment of pregnant rats. Tox. and App. Phar. 17:76-82

956. Brown, A. M. 1965. Pharmacogenetics of the mouse. Lan. An. Care 15(2):111-118

957. Wallcave, J., Garcia, H., Feldman, R., et al. 1971. Skin tumorigenesis in mice by petrolium asphalts and coal-tar pitches of known polynuclear aromatic hydrocarbon content. Tox. and App. Phar. 18:41-52

958. Woodard, G., et al. 1973. Xanthan gum: safety evaluation by two-year feeding studies in rats and dogs and a three-generation reproduction study in rats. Tox. and App. Phar. 24:30-36

959. Thayer, P. S., and Kensler, C. J. 1973. Genetic tests in mice of caffeine alone and in combination with mutagens. Tox. and App. Phar. 25:157-168

960. Hagen, E. C., Jenner, P. M., Jones, W. I. 1971. Increased lethal effects of acutely administered anticholinesterases in female rats prefed with similar agents. Tox. and App. Phar. 18:235-237

961. Su, M. Q., et al. 1971. Comparative inhibition of aliesterases and cholinesterase in rats fed eighteen organophosphorus insecticides. Tox. and App. Phar. 20:241-249

962. Annau, E., Konst, H., and Plummer, P. J. G. 1956. Biochemical and historical changes in the liver of mice following feeding of the insecticide aldrin. Can. J. Med. Sci. 30:463-470

963. Rice, A. J., and Plaa, G. L. 1969. The role of triglyceride accumulation and of necrosis in the hemodynamic responses of the isolated perfused rat liver after administration of carbon tetrachloride. Tox. and App. Phar. 14:151-162

964. Williams, C. H. 1969. B-Glucuronidase activity in the serum and liver of rats administered pesticides and hepatoxic agents. Tox. and App. Phar. 14:283-292

966. Stock, B. H., and Fouls, J. R. 1971. The influence of in vivo administration of dimethyl sulfoxide on the in vitro metabolism of some monocyclic aromatic chemicals by hepatic microsomal preparations. Tox. and App. Phar. 18:859-868

967. Williams, C. H. 1970. B-Glucuronidase activity in the serum and liver of rats treated with parathion. Tox. and App. Phar. 16:533-539

968. Lal, H., et al. 1970. Impairment of hepatic drug metabolism by carbon tetrachloride inhalation. Tox. and App. Phar. 16:35-39

969. Slater, T. F., and Delaney, V. B. 1971. The effects of various drugs and toxic agents on bile flow rate and composition in the rat. Tox. and App. Phar. 20:157-174

970. Nishie, K., et al. 1972. Phototoxicity and differential hepatotoxicity as biological indicators of nitrosamine activity. Tox. and App. Phar. 23:680-691

971. Kutob, S. D., and Plaa, G. L. 1962. A procedure for estimating the hepatotic potential of certain industrial solvents. Tox. and App. Phar. 4:354-361

972. Hart, L. G., Shultice, R. W., and Fouts, J. R. 1963. Stimulatory effects of chlordane on hepatic microsomal drug metabolism in the rat. Tox. and App. Phar. 5:371-386

973. Evans, E. A., Eisenlord, A. G., and Hine, C. H. 1963. Studies in detoxication by means of the isolated perfused liver. Tox and App. Phar. 5:129-141

974. Kimbrough, R. D., et al. 1968. Combined effect of DDT, pyrethrum, and piperonyl butoxide on rat liver. Arch, Env. Health 16:333-341

975. Mischler, T. W., et al. 1971. Biological evaluation of two estrogenic steroids as possible rodent chemosterilants. Journal of Wildlife Mgmt. 35(3):449-454

976. Howard, W. E., and Marsh, R. E. 1969. Mestranol as a reproductive inhibitor in rats and voles. Journal of Wildlife Management 33(2):403-408

977. Clark, D. G. 1972. Effects of ethylene glycol dinitrate on pituitary-adrenocortical function in the rat. Tox. and App. Phar. 21:355-360

978. Szot, R. J., and Murphy, S. D. 1970. Phenobarbital and dexamethasone inhibition of the adrenocortical response of rats to toxic chemicals and other stresses. Tox. and App. Phar. 17:761-773

979. DiFazio, C. A., Green, C. D., and Smiddy, J. F. 1969. Comparative in vitro effects of nitrous oxide, halothane and cyclopropane on rat bone marrow oxygen consumption and anaerobic glycolysis. Tox. and App. Phar. 14:259-265

980. Wenzel, D. G., et al. 1970. Effects of nicotine on cultured rat heart cells. Tox. and App. Phar. 17: 774-785

981. Clark, D. G. 1970. The supersensitivity of the rat cardiovascular system to epinephrine after repeated injections of ethylene glycol dinitrate. Tox. and App. Phar. 17:433-442

982. Khairy, M. 1960. Effect of chronic diedrin ingestion on the muscular efficiency of rats. Brit. J. Indus. Med. 17:146-148

983. Tullner, W. W. 1961. Uterotrophic action of the insecticide methoxychlor. Science 133:647-648

984. Dale, W. E., et al. 1963. Poisoning by DDT: relation between clinical signs and concentration in rat brain. Science 142:1474-1476

985. Joyce, D., and Summerfield, A. 1966. Effects of drugs related to serotonin on water intake and defecation in rats. Implications for behavioral studies. Arch. Int. Phar. 161(2):489-494

986. Egle, J. L., Jr. 1972. Effects of inhaled acetaldehyde and propionaldehyde on blood pressure and heart rate. Rox. and App. Phar. 23(1972):131-135

987. Murphy, S. D., et al. 1964. Biochemical effects in rats from irritating air contaminants. Tox. and App. Phar. 6:520-528

988. Chevillard, L., et al. 1967. The influence of rearing temperature on some physiological characteristics of small laboratory animals (Homoeotherms). Husb. Lab. An. 395-445

989. Farkas, I., Desi, I., and Kemeny, T. 1968. The effect of DDT in the diet on the resting and loading electrocorticogram record. Tox. and App. Phar. 12:518-525

990. Crecitelli, F. N., and Gilman, A. 1946. Electrical manifestations of the cerebellum and cerebral cortex following DDT administrations in cats and monkeys. Am. J. Physiol. 147:127-137

991. Dési, I., et. al. 1962. Nervous system effects of a chemical herbicide. Arch. Env. Health 4:95-102

992. Steward, C. N., Coursin, D. B. and Bhagavan, H. N. 1972. Electroencephalographic study of L-glutamate induced sugars in rats. Tox. and App. Phar. 23: 635-639

993. Dési, I., et al. 1966. Changes of central nervous functions in response to DDT administration Acta. Physiol. Acad. Scientiarium Hungaricae Tomus 30(3-4): 275-282

995. Clark, R. 1969. A rapid method for testing anti-appetite drugs in mice. Tox. and App. Phar. 15: 212-215

996. Kaplan, M. L. 1972. Effect of acrylamide on rotarod performance and sciatic nerve B-Glucuronidase activity of rats. Tox. and App. Phar. 22:259-268

997. Hrdina, P. D., et al. 1973. Some neurochemical alterations during acute DDT poisoning. Tox. and App. Phar. 25:276-288

998. Woolley, D. E. 1973. Studies on 1,1,1-trichloro-2,2-bis-(p-chlorophenyl) ethane (DDT)-induced hyperthermia: effects of cold exposure and amino-pyrine injections. J. Pharm. and Exper. therapeutics 184(1):261-268

999. Gilbert, F. F. 1969. Physiological effects of natural DDT residues and metabolites on ranch mink. Journal of Wildlife Management 33(4):933-943

1000. Travis, H. F., and Schaible, P. J. 1962. Effects of diethylstilbestrol fed periodically during gestation of female mink upon reproductive and kit performance. Am. J. Vet. Res. 23(93):359-361

1001. Balazs, T., et al. 1971. Renal concentration test in beagle dogs. Lab. An. Sci. 21(4)

1002. Vogin, E. E., and Bokelman, D. L. 1971. The influence of experimentally induced hepatoxicity on the pressor responses to vasoactive substances in beagle dogs. Tox. and App. Phar. 20:291-298

1003. Henry, M. C., Davis, R. D., and Schein, P. S. 1973. Hepatotoxicity of 1-(2-chlorethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in dogs. Tox. and App. Phar. 25:410-417

1004. DeMarr, E. W. J. 1956. Effects of chlorpromazine on EEG and its activation. Fed. Am. Soc. for Exp. Bio., Fed. Proc. 15:416

1005. Funderburk, W. H., King, E. E., and Unna, K. R. Pharmacological properties of benzazole, III. Effect of 2-Aminobenzothiazoles on the electroencephalogram. J. Phar. and Exp. Ther. 108:94

1006. Beliles, R. P. 1971. The subchronic toxicity of 5-Benzyl-11-[4-(N-nathylpiperidylene)]-5,6-dihydromorphanthridine hydrogen maleate. Tox. and App. Phar. 18:451-456

1007. Goldman, W. J., Bielinski, and Mattis, P. A. 1973. Cochlear microphonic potential response of the dog to diuretic compounds. Tox. and App. Phar. 25:259-266.

1008. Daigneault, E. A. 1970. Influence of ototoxic drugs on acetylcholine-induced depression of the Cochlear N potential. Tox. and App. Phar. 17:223-230

1009. Funakoshi, M., and Zotterman, Y. 1963. Effect of salt on sugar response. Acta. Physiol. Scan. 57:193

1010. Liljestrand, G. and Zotterman, Y. 1954. The water taste in mammals. Acta. Physiol. Scand. 32:291-303

1011. Cohen, M. J., et al. 1955. The response spectrum of taste fibers in the cat: a single fibre analysis. Acta. Physio. Scand. 33:316-332

1012. Daigneault, E. A. 1972. Correlation of prolonged insecticide exposure and the activity of the inhibition of Cochlea N potential. Tox. and App. Phar. 21:495-502

1013. Owen, G., et al. 1970. Toxicity of some benzodaizepine compounds with CNS activity. Tox. and App. Phar. 16:556-570

1014. Fries, G. F., Marrow, G. S., and Gordon, C. H. 1972. Similarity of a polychlorinated biphenyl (Arochor 1254) and DDE in rate of elimination from cows. Env. Contam. and Tox. Bull. 7(4):252

1015. Harris, J. R., et al.1956. Effects of feeding dieldrin and heptachlor-treated alfalfa hay to dairy cows. J. Agr. Food Chem. 4(8):694-696

1016. Bruce, W. N., et al. 1965. Storage of heptachlor epoxide in the body fat and its excretion in milk if dairy cows are fed heptachlor in their diets. J. Agr. Food Chem. 13:63-67

1017. Kuhnen, H. 1971. Activating and inhibitory effects of bispyridinum compounds on bovine red cell acetylcholinesterase. Tox. and App. Phar. 20:97-104

1018. Hamlin, R. L., Smetzer, D. L., and Smith, C. R. 1965 Analysis of QRS complex recorded through a semi-orthogonal lead system in the horse. Am. J. Physiol. 207:325-333

1019. Garner, H. E., et al. 1972. Electrodes for recording cortical electroencephalograms in ponies. Lab. An. Sci. 22(2):262-265

1020. Herigstad, R. R. 1972. Pathology of inorganic and organic selenium toxicosts in young swine. Ph.D. Thesis, Michigan State University, East Lansing, Michigan

1021. Hamlin, R. L. 1960. The QRS electrocardiogram epicardiogram, vectorcardiogram and ventricular excitation of swine. Am. J. Physiol. 198:537-542

1022. Bay, W. W., et al. 1970. The experimental production and evaluation of drug-induced phototoxicity in swine. Tox. and App. Phar. 17:538-547

1023. Hebert, D. M., and Cowan, I. M. White muscle disease in the mountain goat. Journal of Wildlife Management 35(4):752

1024. Barber, T. A. 1971. Influence of pesticides on mule deer rumen bacteria. Ph.D. Thesis, Colorado State University

1025. Dalezios, J. I. et al. 1973. Excretion and metabolism of orally administered aflatoxin B_1 by rhesus monkeys. Fd. Cosmet. Toxicol. 11:605-616

1026. Vondruska, J. F., et al. 1971. An investigation into the teratogenic potential of captan, folpet and difolatan in nonhuman primates. Tox. and App. Phar. 18:691-624

1027. Werdegar, D., Johnson, D. G., and Mason, J. W. 1964. A technique for continuous measurement of arterial blood pressure in unanesthetized monkeys. J. App. Physiol. 19:519-521

1028. Rothstein, R., and Hunsaker, D., II. 1972. Baseline hematology and blood chemistry in the South American wooly opossum. Caluromys derbianus. Lab. An. Sci. 22(2):227-232

1029. Barnes, R. D., and Barthold, S. W. 1969. Reproduction and breeding behavior in an experimental colony of Marmosa mitis Bangs (Didelphidae). J. Reprod. Fert. Suppl. 6:447-482

1030. Szabuniewicz, M., and McCrady, J. D. 1969. Some aspects of the anatomy and physiology of the armadillo. Lab. An. Care 19(6):843-848

1031. Benitz, K. F., and Kramer, A. W., Jr. 1965. Spontaneous tumors in the Mongolian gerbil. Lab. An. Care 15(5):281-294

1032. Hill, R. B. 1958. The effects of certain neurohumors and of other drugs on the ventricle and radula protractor of Busycon canaliculatum. Biol. Bull. 115: 471-482

1033. Hartman, M. J. 1971. The effects of four anti-cholinesterase compounds on the embryology of Acheta domesticus. Ph.D. Thesis, Oregon State University

1034. Atkins, E. L., Jr., et al. 1954. Equipment and techniques used in laboratory evaluation of pesticide dusts in toxicological studies with honeybees. J. Econ. Entom. 47(6):965-969

1035. Clausen, H. J., and Duran-Reynals, F. 1937. Studies on the experimental infection of some reptiles, amphibia and fish with Serratia anolium. Am. J. Path. 13:441-451

1036. Cohen, N. 1971. Reptiles as models for the study of immunity and its phylogenesis. J.A.V.M.A., Vol. 159 (11):1662

1037. Gordon, R. E. and D. W. Tinkle 1959. A technique for collecting reptile eggs. Texas J. of Science 12(1-2): 14-16

1038. Greenfield, L. J., and Morrow, A. G. 1961. The cardiovascular hemodynamics of Crocodilia. J. of Surg. Rea. 1(2):97-103

1039. Brisbin, I. L., Jr. 1966. Reactions of the American alligator to several immobilizing drugs. Copeia (1): 129-130

1040. Hubbs, C. et al. 1960. Reduced survival of young etheostomatine fishes and Hylid tadpoles due to ammonia fumes produced by a rodent colony. Copeia (1)

1041. Cooke, A. S. 1973. The effects of DDT, when used as a mosquito larvicide, on tadpoles of the frog (Rana temporaria). Envir. Poll. 5(4):259-273

1042. Blus, L. J., et al. 1972. Logarithmic relationship of DDT residues to eggshell thinning. Nature 235: 376-377

1043. Blus, L. J. 1971. Eggshell thinning in the brown pelican: implication of DDT. Bio. Sci. 21:1213-1215

1044. Vermeer, K., and Risebrough, R. W. 1972. Additional information on eggshell thickness in relation to DDE concentrations in great blue heron eggs. Can. Field-Naturalist 86:384-385

1045. Borg, K. et al. 1970. Experimental secondary methyl mercury poisoning in the goshawk (Accipiter gentilis) Envir. Poll. 1-91-104

1046. Porter, R. D., and Wiemeyer, S. N. 1969. Dieldrin and DDT: effects on sparrow hawk eggshells and reproduction. Science 165:199-200

1047. Fox, G. A. 1971. Recent changes in the reproductive success of the pigeon hawk. Journal of Wildlife Management 35(1):122

1048. Cade, T. J. et al. 1971. DDE residues and eggshell changes in Alaskan falcons and hawks. Sci. 172:955-957

1049. Snyder, N. F. R., et al. 1973. Organochlorine, heavy metals, and biology of North American accipiters. Bio. Sci. 23(5):299-305

1050. Kreitzer, J. F. 1971. Eggshell thickness in mourning dove populations. J. Wildlife Mgmt. 35(3): 563-564

1051. Labisky, R. F. 1959. Night-lighting: a technique for capturing birds and mammals. Illinois Nat. Hist. Survey, Bio. Notes No. 40

1052. Klonglan, E. D., et al. 1956. Pheasant nest activity recording instrument. Journal of Wildlife Management 20(2):173-177

1053. Zwickel, F. C. and Bendell, J. F. 1967. A snare for capturing blue grouse. J. of Wildlife Mgmt. 31(1): 202-204

1054. Schladweiler, P. and Mussehl, T. W. 1969. Use of mist-nets for recapturing radio-equipped blue grouse. J. Wildlife Management 33(2):443-444

1055. Barnes, J. M. 1969. Toxic chemicals and peripheral neuropathy: experimental studies. Proc. Roy. Soc. Med. 62:205-208

1056. Dunachie, J. F., and Fletcher, W. W. 1966. Effect of some insecticides on the hatching rate of hen's eggs. Nature 212:1062-1063

1057. Guthrie, F. E., and Donaldson, W. E. 1970. Distribution of DDT and dieldrin in avian embryo. Tox. and App. Phar. 16:475-481

1058. Cecil, H. C. et al. 1973. Changes in eggshell quality and pesticide content of laying hens on pullets fed DDT in high or low calcium diets. Poultry Sci. 52(2):648-653

1059. Smith, S. I., et al. 1970. The effect of injection of chlorinated hydrocarbon pesticides on hatchability of eggs. Tox. and App. Phar. 16:179-185

1060. Marliac, J. P., and Mutchler, M. K. 1963. Use of the chick embryo technique for detecting potential effects of chemicals. Fed. Proc. Am. Soc. Exp. Bio. 22(1):188

1061. Thomas, F., and Medley, J. G. 1971. Effect on eggs of applications to poultry of pesticide formulations contaminated with chlorinated hydrocarbons. J. AOAC 54(3):681-684

1062. Thompson, E. M., et al. 1967. Methoxychlor residues in chicken eggs. J. Econ. Entom. 60(1):235-237

1063. Kreitzer, J. F. 1972. The effect of embryonic development on the thickness of the eggshells of Coturnix quail. Poultry Sci. 51:174-1765

1064. Stickel, L. F., and Rhodes, L. I. 1970. The biological impact of pesticides in the environment. Envir. Health Sci. Series No. 1

1065. Walker, N. E. 1967. Growth and development of chick embryos nourished by fractions of yolk. J. of Nutrition 92(1):111-117

1066. Stoewsand, G. S., et al. 1971. Eggshell thinning in Japanese quail fed mercuric chloride. Science 173: 1030-1031

1067. Jones, F. J. S., and Summers, D. D. B. 1968. Relation between DDT in diets of laying birds and viability of their eggs. Nature 217:1162-1163

1068. MacLeod, C. F., and Lethiecq. J. L. 1963. A comparison of two trapping procedures for Sorex cinereus. J. Mamm. 44(2):277-278

1069. Spencer, A. W., and Pettus, D. 1966. Habitat perferences of five sympatric species of long-tailed shrews. Ecology 47:677-683

1070. Weil, C. S., and Scala, R. A. 1971. Study of intra and interlaboratory variability in the results of rabbit eye and skin irritation tests. Tox. and App. Phar. 19:276-360

1071. Phillips, L., et al. 1972. A comparison of rabbit and human skin response to certain irritants. Tox. and App. Phar. 21:369-382

1072. Grice, H. C. 1964. Methods of obtaining blood and for intravenous injections in laboratory animals. Lab. An. Care 14(6):483-493

1073. Vogin, E. E., et al. 1970. Teratology studies in rats and rabbits exposed to an isoproterenol aerosol. Tox. and App. Phar. 16:374-381

1074. Klemm, W. R. 1965. Drug potentiation of hypnotic restraint of rabbits, as indicated by behavior and brain electrical activity. Lab. An. Care 15(2):163-167

1075. Marsh, R. E., et al. 1970. Effects of odors of rodenticides and adherents on attractiveness of oats to ground squirrels. Journal of Wildlife Management 34(4):821-825

1076. Howard, W. E., et al. 1969. A body capacitor-olfactometer for squirrels and rats. J. Mamm. 50(4): 771-776

1077. Dieterich, R. A. 1972. Hematologic values for five northern Microtines. Lab. An. Sci. 22(3):390-392

1078. Capizzo, F., and Roberts, R. J. 1970. Disposition of the hepatotoxin α-Napthylisothiocynate (ANIT) in the rat. Tox. and App. Phar 17:262-271

1079. Deichmann, W. B., et al. 1963. The hemopoietic tissue toxicity of benzene vapors. Tox. and App. Phar. 5:201-224

1080. Kociba, R. J., et al. 1973. Tissue response to ceramic foam dust following intratracheal and intraperitoneal administration. Tox. and App. Phar. 25:145-151

1081. Deichmann, W. B. 1970. Tumorigenicity of aldrin, dieldrin and endrin in the albino rat. Ind. Med. 39(10):37-45

1082. Munro, I. C., et al. 1971. Biochemical and pathological changes in the heart and liver of rats given brominated cottonseed oil. Tox. and App. Phar. 19:62-70

1083. Deichmann, W. B., et al. 1963. Adenocarcinoma in the lungs of mice exposed to vapors of 3-nitro-3-hexene. Tox. and App. Phar. 5:445-456

1084. Sharratt, M., and Frazer, A. C. 1963. The sensitivity of function tests in detecting renal damage in the rat. Tox. and App. Phar. 5:36-38

1085. Hoag, W. G., et al. 1965. Epidemiological aspects of the control of pseudomonas infection in mouse colonies. Lab. An. Care 15(3):217-223

1086. Flynn, R. J., et al. 1965. Pathogen status of commercially produced laboratory mice. Lab. An. Care 15(6):440-447

1087. Brown, D. R., and Murphy, S. D. 1971. Factors influencing dimethoate and triethyl phosphate-induced narcotics in rats and mice. Tox. and App. Phar. 18:895-906

1088. Williams, J. S., et al. 1966. Serum chemical components in mice determined by the use of ultramicro techniques. J. App. Physiol. 21:1026-1030

1089. Bergstrom, S. 1971. A simple device for intravenous injections in the mouse. Lab. An. Sci. 21(4)

1090. Puget, A. 1973. The afgan pika (Ochotona rufescens): a new laboratory animal. Lab. An. Sci. 23(2):248-251

1091. Cate, C. C. 1969. A successful method for exsanguinating unanesthetized mice. Lab. An. Care 19:256-258

1092. Heinecke, H. 1967. The experimental environment and its influence on the blood picture of laboratory animals. Husbandry of Laboratory Animals pp 447-457

1093. Weil, C. S., et al. 1972. Current status of tests of carbaryl for reproductive and teratogenic effect. Tox. and App. Phar. 21:390-404

1094. Tigner, J. R., and Bowles, W. A. 1964. Chloropicrin tested as an area repellent for house mice. Journal of Wildlife Management 28(4):748-751

1095. Salthouse, T. N., et al. 1973. Microspectrophotometry of macrophage lysosomal enzyme activity: a measure of polymer implant tissue toxicity. Tox. and App. Phar. 25:201-211

1096. Wittgenstein, W., and Rowe, K. W., Jr. 1965. A technique for prolonged infusion of rats. Lab. An. Care 15(5):375-378

1097. Lawrence, W. H., and Sherman, C. A. 1963. An electronic traffic counter for recording burrow activity of the mountain beaver. J. Mamm. 44(3): 399-405

1098. Pearson, A. M. et al. 1967. Evaluation of phencyclidine hydrochloride and other drugs for immobilizing grizzly and black bears. Journal of Wildlife Management 31:532-537

1099. Linhart, S. B., and Kennelly, J. J. 1966. Fluorescent bone labeling of coyotes with demethylchlortetracycline. Journal of Wildlife Management 31(2): 317-321

1100. Bergman, R. K., et al. 1972. A technique for multiple bleedings or intravenous inoculations of mink at prescribed intervals. Lab. An. Sci. 22(1): 93-95

1101. Freiss, S. L., et al. 1968. Changes in contractility of cat gastrocnemius tissues controlled by aryl ester infustion and stimulus burst frequency. Tox. and App. Phar. 13:99-107

1102. Smith, E. B., and Clark, D. A. 1972. Absorption of hydrazine through canine skin. Tox. and App. Phar. 21:186-193

1103. Earl, F. L., et al. 1971. Clinical laboratory values of neonatal and weaning miniature pigs. Lab. An. Sci. 21(5):754-759

1104. Villeneuve, D. C., et al. 1972. Placental transfer of ^{14}C-Parathion administered intravenously to sheep. Tox. and App. Phar. 21;542-548

1105. Barrier, M. J. 1970. Pesticide residues in selected tissues of the white-tailed deer, Odocoileus virginianus, L., in Calhoun County, South Carolina. Ph.D. Thesis, Clemson University

1106. Allen, T. J. 1970. Immobilization of white-tailed deer with succinychlorine chloride and hyaluronidae. J. of Wildlife Management 34(1):207

1107. Thomas, J. W., et al. 1967. Use of diazepam in the capture and handling of Cervids. Journal of Wildlife Management 31(4):686-692

1108. Behrend, D. F. 1965. Notes on field immobilization of white-tailed deer with nicotine. Journal of Wildlife Management 31(3):464-468

1109. Montgomery, G. G., and Hawkins, R. E. 1967. Diazepam bait for capture of white-tailed deer. Journal of Wildlife Management 31(3):464-468

1110. Thornett, H. D. et al. 1973. Methodology and management of simian toxicology studies with emphasis on special techniques. Laboratory Animal Science 23(3): 350-354

1111. Block, M. 1960. Wound healing in the new-born opossum. Nature 187:340-341

1112. Wolf, H. G., et al. 1971. Hematologic values for laboratory reared Marmosa mitis. Lab. An. Sci. 21(2):249-251

1113. Jones, J. B., et al. 1972. A comparison of hemoglobins of several laboratory animals. Lab. An. Sci. 22(4):532-537

1114. Walburg, H. E., et al. 1965. Microbiological evaluation of an isolation facility for the production of specific-pathogen-free mice. Lab. An. Care 15(3):208-216

1115. Verschuuren, H. G., et al. 1970. Influence of triphenyltin acetate on lymphatic tissue and immune responses in guinea pigs. Tox. and App. Phar. 16: 400-410

1116. Allen, R. C. 1946. Animal Health Program, Biology Division, Oak Ridge National Laboratory. Lab. An. Care 14(4):260-263

1117. Ruhren R. 1965. Normal values for hemoglobin concentration and cellular elements in the blood of Mongolian gerbils. Lab. An. Care 15(5):313-320

1118. Calhoon, J. R., and Matthews, P. J. 1964. A method for initiating a colony of specific-pathogen-free guinea pigs. Lab. An. Care 14(5):388-394

1119. Johnson, W. H., et al. 1966. Biology. Holt, Rinehart and Winston, New York

1120. Kaplan, H. M. 1962. Toxicity of chlorine for frogs. Lab. An. Care 12:259-262

1121. Miles, H. W., et al. 1931. On the ecology and control of slugs. Ann. Appl. Biol. 18:370-400

1122. Kohn, A. J. 1961. Chemoreception in gastropod molluscs. Am. Zool. 1:291-308

1123. Wells, M. J., and Buckley, S. K. L. 1972. Snails and trails. Anim. Behav. 20:345-355

1124. Witt, P. N., et al. 1968. A Spider's Web, Problems in Regulatory Biology. Springer-Verlag, New York

1125. Jacobson, M., and Smalls, L. A. 1966. Masking of the American cockroach sex attractant. J. Econ. Entomol. 59(2):414-416

1126. Roelofs, W. L. and Carde, R. T. 1971. Hydrocarbon sex pheromone in tiger moths (Arctiidae). Science 171(2):684-686

1127. Roelofs, W. L., and Comeau, A. 1971. Sex pheromone perception: synergists and inhibitors for the red-banded leaf roller attractant. J. Insect. Physiol. 17:435-448

1128. Blum, M. S. 1970. The Chemical Basis of Insect Sociality. Ed.: M. Berosa, Acad. Press, N.Y.

1129. Beroza, M. 1970. Current usage and some recent developments with insect attractants and repellants in the USDA. Chemicals Controlling Insect Behavior pp. 145-163 Acad. Press, New York

1130. Fukami, H. The feasibility of insect control utilizing a sex pheromone. Env. Toxicol.:571-586

1131. Gerger, R. S., and Canerday, T. D. 1968. Specificity of the cabbage looper sex attractant. J. Econ. Entomol. 61(2):452-454

1132. Tinkle, D. W., and Liner, E. A. 1955. Behavior of Natrix in aggregations. Field and Lab. 23(3-4):84-87

1133. Cooke, A. S. 1971. Selective predation by newts on frog tadpoles treated with DDT. Nature 229:275

1134. Dews, P. B. 1957. Studies of Behavior III. Effects of scopolancine on reversal of a discriminatory performance in pigeons. J. Phar. and Exp. Ther. 115:343-353

1135. Heinz, G. 1974. Patuxant Wildlife Research Center, Laurel, Maryland. Personal Communication.

1136. West, R. R. et al. 1969. Repelling pheasants from sprouting corn with a carbamate insecticide. Journal of Wildlife Management 33(1):216-219

1137. West, R. R., and Dunks, J. H. 1969. Repelling boat-tailed grackles from sprouting corn with a carbamate compound. Texas J. Sci. 21(2):231-233

1138. Simpson, C. F., et al. 1970. B-aminopropionitrile-induced dissecting aneurysms of turkeys: treatment with propranolol. Tox. and App. Phar. 16:143-153

1139. Tallarico, R. B., and Farrell, W. M. 1964. Studies of visual depth perception: an effect of early experience on chicks on a visual cliff. J. Comp. Physio. Phychol. 57(1):94-96

1140. Hamilton, W. J., Jr. 1937. Activity and home range of the field mouse, Microtus pennsylvanicus pennsylvanicus. Ecology 18:255-263

1141. Eliassen, E., and Leivestad, H. 1961. Sodium and potassium content in the muscles of hibernating animals. Nature 192, Nove. 4, 1961:459-460

1142. Eisenberg, J. F. 1964. Studies on the behavior of Sorex vagrans. American Midland Naturalist 72: 417-425

1143. Beidler, L. M., Fishman, I. Y., and Hardman, C. W. 1955. Species differences in taste responses. Am. J. Physiol. 181:235-239

1144. Pfaffmann, C. 1953. Species differences in taste sensitivity. Science 117:470

1145. Thacker, E. J., and Brandt, C. S. 1955. Coprophagy in the rabbit. J. Nutr. 55:375-385

1146. Ericsson, R. J. et al. 1971. Bait acceptance by rats of microencapsulated male sterilant alpha-chlorohydrin. Journal of Wildlife Management 35(3): 573-576

1147. Johnson, F. M., et al. 1962. Rodent repellent value of arasan-endrin mixtures applied to acorns. Journal of Wildlife Management 26:15-16

1148. Pengelley, E. J., and Fisher, K. C. 1957. Onset and cessation of hibernation under constant temperature and light in the golden-mantled ground squirrel. Nature 180:1371-1372

1149. Baldwin, F. M., and Johnson, K. L. 1941. Effects of hibernation on the rate of oxygen consumption in the thirteen-lined ground squirrel. J. Mamm. 22:180-182

1150. Howard, W. E., and Cole, R. E. 1967. Olfaction in seed detection by deer mice. J. Mamm. 48(1):147-150

1151. Howard, W. E., et al. 1968. Food detection by deer mice using olfactory rather than visual cues. An. Behavior 16:13-17

1152. Eisenberg, J. F. 1963. The intraspecific social behavior of some cricetine rodents of the genus Peromyscus. Am. Midland Nat. 69(1):240-246

1153. Clemens, L. G. 1969. Experimental analysis of sexual behavior of the deermouse Peromyscus maniculatus gambeli. Behavior 34:267-285

1154. Bindra, D., and Anchel, H. 1963. Immobility as in avoidance response and its disruption by drugs. J. Exp. Anal. of Behav. 6(2):213-217

1155. Sidman, M. 1955. Technique for assessing the effects of drugs on timing behavior. Science 22:925

1156. Mulder, J. B. 1971. Animal behavior and electro-magnetic energy waves. Lab. An. Sci. 21(3):389-393

1157. Khairy, M. 1959. Changes in behavior associated with a nervous system poison (DDT). J. Exp. Psychol. Quarterly 9:84-91

1158. Spoor, R. P., and Jackson, D. B. 1966. Circadian rhythms: variation in sensitivity of isolated rat atria to acetylcholine. Science 154:782

1159. Nelson, W., et al. 1971. Dose-response evaluations of a circadian rhythmic change in susceptibility of mice to ovabain. Tox. and App. Phar. 18:335-339

1160. Halberg, F., Visscher, M. B., and Bittner, J. J. 1953. Eosinophil rhythm in mice: range of occurance; effects of illumination feeding and adrenalectomy. Am. J. Physiol. 174:109-122

1161. Falk, J. L., and Hass, W. O., III. 1965. The control and recording of response force. J. Exp. Anal. of Behav. 8(3):195

1162. Vernon, W., and Ulrich, R. 1966. Classical conditioning of pain-ellicited aggression. Science 152:668

1163. Lal, H., and Brown, R. M. 1969. Effects of bacterial endotoxin on operant behavior of the rat. Tox. and App. Phar. 14:41-47

1164. Belleville, R. E.1964. Control of behavior by drug-produced internal stimuli. Psychopharm. 5:95-105

1165. Pradhan, S. N., and Roth, T. 1968. Comparative behavioral effects of several anticholinergic agents in rats. Psychopharm. 12:358-366

1166. Walters, G. C., et al. 1963. The gerbil as a subject in behavioral research. Psych. Reports 12:315-318

1167. Sidman, M. 1953. Avoidance conditioning with brief shock and no exteroceptive warning signal. Science 118:157-158

1168. Goldberg, M. E., Haun, C., and Smyth, H. F. 1962. Toxicologic implication of altered behavior induced by an industrial vapor. Tox. and App. Phar. 4:148-164.

1169. Heistad, G. T., and Torres, A. A. 1959. A mechanism for the effect of a tranquilizing drug on learned emotional responses. University of Minnesota Med. Bull. 30:518

1170. Sidman, M. 1956. Drug-behavior interaction. N.Y. Acad. of Sci., Ann. 65:282

1171. Pradhan, S. N., et al. 1967. Some behavioral effects of ditran (JB 329) in rats. Arch. Int. Pharmacodyn. 170(2):264-275

1172. Geller, I., and Seifter, J. 1962. The effects of mono-urethans, di-urethans and barbiturates on a punishment discrimination. J. Exp. Ther. 136:284-288

1173. Kinnard, W. J., Jr., and Carr, C. J. 1957. A preliminary procedure for the evaluation of central nervous system depressants. J. Phar. and Exp. Ther. 121:354-361

1174. Stein, L. 1963. Anticholinergic drugs and the central control of thirst. Science 139:46-48

1175. Selko, L. F. 1938. Hibernation of the striped skunk in Iowa. J. Mamm. 19:320-324

1176. Anderson, H. T., Funakoshi, M., and Zotterman, Y. 1962. Electrophysiological investigation of the gustatory effect of various biological sugars. Acta. Physiol. 56:362-375

1177. Robinson, W. H. 1970. Acute toxicity of sodium monofluoroacetate to cattle. Journal of Wildlife Management 34(3):647-648

1178. Sandler, B. E., et al. 1968. Effect of dieldrin on detour behavior in sheep. Psychological Reports 23, 451-455

1179. Sandler, B. E., et al. 1969. Dieldrin exposure and vigilance behavior in sheep. Psychon. Sci. 157: 1057-1058

1180. Van Gelder, G. A. 1969. Behavioral and electrophysiological effects of dieldrin in sheep. Industrial Medicine 38(3):67-67

1181. Sussman, D. E. 1967. The effect of exposure to dieldrin on avoidance response extinction and conditioned heart rate in sheep. Ph.D. Thesis, Iowa State University.

1182. Dietz, D. R., and Tigner, J. R. 1968. Evaluation of two mammal repellents applied to browse species in the Black Hills. J. of Wildlife Mgmt. 32(1):109-114

1183. Reynolds, H. H., et al. 1963. The effect of UDMH on learned behavior in the Java monkey. Aero. Med. 34:920-922

1184. Carter, V. L., et al. 1970. The effect of bromotrifluoromethane on operant behavior in monkeys. Tox. and App. Phar. 17:648-655

1185. Hutchinson, R. R., et al. 1966. An automatic method for the study of aggression in squirrel monkeys. J. Exp. Anal. of Behav. 9(3):223-237

1186. James, W. T. 1960. A study of visual discrimination in the opossum. J. Genetic Pyschol. 97:127-130

1187. Lyman, C. P. 1948. The oxygen consumption and temperature regulation of hibernating hamsters. J. Exp. Zoo. 109:55-78

1188. Friedman, H., and Marshall, D. A. 1965. Position reversal training in the Virginia opossum: evidence for the acquisition of a learning set. Quarterly J. Exp. Psycho. 17:250-254

1189. James, W. T. 1955. The behavior of the opossum in the Guthrie-Horton puzzle box. J. Genetic Psychol. 87:203-206

1190. James, W. T. 1958. Conditioned responses in the opossum. J. Genetic Psychol. 93:179-183

1191. Kaplan, H., and Hyland, S. O. 1972. Behavioral development in the Mongolian gerbil (Meriones unguiculatus). Anim. Behav. 20:147-154

1192. Smith, P. W. 1961. The amphibians and reptiles of Illinois. Illinois Natural History Survey Bulletin, Vol. 28, Article 1. State of Illinois, Dept. of Registration and Education, Natural History Survey Division

1193. Porter, K. R. 1972. Herpetology. W. B. Saunders Co., Philadelphia, Pennsylvania

1194. Musgrove, J. W., and Musgrove, M. R. 1953. Waterfowl in Iowa. State Conservation Commission, Des Moines, Iowa

1195. Graves, J. B. 1974. Entomology Dept., Louisiana State University, Baton Rouge, Louisiana. Personal Communication

1196. Rand, A. L. Birds of North America. Doubleday and Company, Inc., New York

1197. Linder, R. L., and Atkins, T. D. 1971. Effects of dieldrin Heterakis gallinarum of pheasants. Proc. S. D. Acad. Sci. 59:214-215

1198. Linder, R. L., et al. 1970. Residues in the brain of adult pheasants given dieldrin. J. Wildlife Mgmt. 34(4):954-956

1199. New, D. A. T. 1966. The Culture of Vertebrate Embryos. Logos Press, Academic Press Inc., N. Y.

1200. Hamburger, V., and Balaban, M. 1963. Observations and experiments on spontaneous rhythmical behavior in the chick embryo. Devel. Bio. 1:533-545

1201. Burt, W. H. 1957. Mammals of the Great Lakes Region. University of Michigan Press, Ann Arbor

1202. Padgett, G. A. et al. 1968. Mink as a biomedical model. Lab. Animal Care 18(2):258-266

1203. Hutchenson, D. 1974. Sinclair Research Farm, University of Missouri, Columbia. Personal Communication

1204. Brown, R. V., and Hutcheson, D. P. 1973. Nutrition and alcohol consumption in the Sinclair miniature pig. Quarterly Journal of Studies on Alcohol, Vol. 34(3):758-763

1205. Lewis, M. 1974. Personal Communication. FAA Civil Aeromedical Inst., ACC 1186G, P. O. Box 25082, Oklahoma City

1206. Steel, R. G. D. and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw Hill

1207. Loomis, T. A. 1968. Essentials of Toxicology. Lea and Febiger

1208. Goldstein, A. 1964. Biostatistics: An Introductory Text. The Macmillian Co.