

2002
Description of Institutional
Animal Care and Use Program

Oak Ridge National Laboratory
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Prepared by the
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Oak Ridge, Tennessee 37831-6122
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UT-BATTELLE
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"Description of Institutional Animal Care and Use Program"
Oak Ridge National Laboratory
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ACRONYMS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AALAS	American Association for Laboratory Animal Science
ACLAM	American College of Laboratory Animal Medicine
ALAT	Assistant Laboratory Animal Technician
ALD	Associate Laboratory Director
AVMA	American Veterinary Medical Association
CEO	Chief Executive Officer
CSD	Chemical Sciences Division
DOE	Department of Energy
DSO	Division Safety Officer
DTO	Division Training Officer
DACLAM	Diplomate, ACLAM
DVM	Doctor of Veterinary Medicine
ENU	N-ethyl-N-nitrosourea
EPA	Environmental Protection Agency
ESD	Environmental Sciences Division
ESTD	Engineering Science and Technology Division
EWSD	Environmental and Waste Services Division
GET	General Employee Training
HEPA	High Efficiency Particulate Air
IACUC	Institutional Animal Care and Use Committee
IBC	Institutional Biosafety Committee
IH	Industrial Hygiene
IO	Institutional Official
ISMS	Integrated Safety Management System
LAR	Laboratory Animal Resource
LATG	Laboratory Animal Technologist
LLC	Limited Liability Corporation
LSD	Life Sciences Division
LSS	Laboratory Shift Superintendent
MHV	Mouse Hepatitis Virus
NIH	National Institutes of Health
NSTD	Nuclear Science and Technology Division
OLAW	Office of Laboratory Animal Welfare
ORNL	Oak Ridge National Laboratory
ORNL-ACUC	Oak Ridge National Laboratory – Animal Care and Use Committee
OSSD	Operational Safety Services Division
PCR	Polymerase Chain Reaction
PHS	Public Health Service
PI	Principal Investigator
PSS	Plant Shift Superintendent
PVC	Polyvinylchloride
QA	Quality Assurance
R&D	Research and Development
RCT	Radiation Control Technician
RSS	Research Safety Summary

RWP	Radiation Work Permit
SBMS	Standards Based Management System
SOP	Standard Operating Procedure
SPF	Specific Pathogen Free
TMGC	Tennessee Mouse Genome Consortium
USDA	United States Department of Agriculture
UT	University of Tennessee
VMT	Veterinary Medical Technician

I. Introduction

A. Name of Program Unit

UT-Battelle LLC manages the U.S. Department of Energy's Oak Ridge National Laboratory (ORNL). Included in the Program Unit are the Life Sciences Division (LSD) and Environmental Sciences Division (ESD) where all permanently maintained animal usage occurs. Additionally, there is some animal usage in Chemical Sciences Division (CSD), Engineering Science and Technology Division (ESTD), and Nuclear Science and Technology Division (NSTD). The ORNL Animal Care and Use Committee (ORNL-ACUC) also reviews protocols for wildlife activities for ORNL's Environmental and Waste Services Division (EWSD).

B. Overview and Purpose

The Oak Ridge National Laboratory is a multi-program science and technology laboratory managed for the U.S. Department of Energy (DOE) by UT-Battelle LLC. In support of the Department's missions, ORNL conducts basic and applied research and development (R&D) to create scientific knowledge and technological solutions that strengthen the nation's leadership in key areas of science; to increase the availability of clean, abundant energy; to restore and protect the environment; and to contribute to national security. The primary research focus utilizing animals at ORNL occurs in the Mammalian Genetics and Functional Genomics Groups of LSD. The research effort utilizes chemical mutagenesis and transgenic and targeted mutation technology, coupled with a variety of phenotype screens, to map and evaluate gene function in mice. ESD use of animals represents the next largest animal usage. ESD and the EWSD perform environmental assessment studies primarily utilizing fish. Nuclear medicine studies in the NSTD make up a smaller, but important, research effort at ORNL. Collaborative efforts between LSD and ESTD are focused on development of instrumentation to facilitate rapid, large-scale phenotyping of mice generated in LSD.

C. Description of the Organization

W. F. Harris, Ph.D., Associate Laboratory Director for Biological and Environmental Sciences, serves as the Institutional Official (IO) for ORNL (UT-Battelle). He reports directly to and was appointed as Institutional Official by the UT-Battelle CEO, Dr. William Madia. The veterinarian, as Institutional Veterinarian, reports directly to the IO, and has oversight of all animal care and use at ORNL. ORNL presently has part-time subcontracted services of a board-certified laboratory animal veterinarian, Dr. Dorcas P. O'Rourke. She also has management responsibilities associated with LSD's Laboratory Animal Resources Group, which provides animal care for the large conventional mouse facility in Bldg 9210. Other animal facilities at ORNL are a one-room satellite rodent facility, two one-room temporary rodent housing facilities, and fish housed and used at ESD. Research staff provides all animal care for these animals, with oversight by the Institutional Veterinarian.

See Appendix 1 for organization charts of units within ORNL using animals in research.

D. Key Institutional Representatives

W. F. Harris, Ph.D., Institutional Official
Associate Laboratory Director for Biological and Environmental Sciences

B. R. Beatty, B.S., Chairperson
ORNL-Animal Care and Use Committee (ORNL-ACUC)
Life Sciences Division

D. P. O'Rourke, M.S., D.V.M., DAACLAM, ORNL Veterinary Consultant,
ORNL Interim Institutional Veterinarian
College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee

W. F. Harris, Ph.D., Acting Division Director
Life Sciences Division

S. G. Hildebrand, Ph.D., Division Director
Environmental Sciences Division

B. A. Berven, Ph.D., Acting Group Leader (also LSD Director of Operations)
Laboratory Animal Resources Group, Life Sciences Division

K. A. Kerber, A. S., VMT, LATG
Laboratory Animal Resources Group Veterinary Technician, Life Sciences Division

H. G. Hodge, Supervisor
Laboratory Animal Resources Group Supervisor, Life Sciences Division

P. E. Barker, Research Technician
Assistant Facility Manager, Life Sciences Division

D. G. Edds, Operational Safety Services Division Representative/
LSD Safety Office, Operations Group, Life Sciences Division

M. S. Greeley, Jr., Ph.D., Vice-Chairperson
ORNL Animal Care and Use Committee
Environmental Sciences Division

Billy Stair, Director
ORNL Communications and Community Outreach Directorate

E. Accreditation History

ORNL Biology Division (in February 1997 merged with Health Sciences Research Division to form Life Sciences Division) was first accredited on May 5, 1967. The Health Sciences Research Division was first accredited in 1987. The Environmental Sciences Division animal facilities were added to the accredited unit at the 1999 site visit, which resulted in full accreditation. Suggestions for improvement in 1999 included increase of cage top sanitation to biweekly, improvement in water bottle sanitation, separation of clean and dirty sides of cage

wash facilities, inclusion of QA testing in the re-derivation process, and cessation of multiple, incompatible activities in animal housing areas in Buildings 4500S and 4501.

The following are the major changes in the program and facilities since the last site visit, including those reported in annual reports made to the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Status of Suggestions for Improvement from the 1999 AAALAC Site Visit
(AAALAC comments in Italics)

1. *Cage sanitation practices were generally satisfactory. The following areas need attention:*

a. *Frequency of cage top changes needs to be increased from once monthly to twice monthly (Bldg 9210).*

Cage tops are sanitized twice monthly (every other week). Frequency of cage top changing is included in a monthly QA report; evaluated performance level for the 2001 calendar year averaged 94%.

b. *Procedures for sanitizing water bottles should be reviewed and modified as needed to minimize the spread of pathogens (Bldg 9210).*

Based on review of QA culture sample results, bottles were adequately sanitized 92% of the time over the 2001 calendar year.

c. *Efforts to separate clean/dirty activities in cage wash areas need to be implemented.*

Separation of clean and dirty cage wash activities has been implemented. Practices to work "clean" to "dirty" continue to be broadly applied, and include the establishment of 5 SPF and 7 "one-way" rooms. Covered supply carts are in use for storage of clean cage supplies.

d. *Planning and QA measures to ensure pathogen elimination with re-derivations planned for a move to a barrier facility need to be made.*

PCR analysis results of frozen embryo and sperm samples (planned for use in future re-derivations) confirmed that infectious agents were detected in no embryos, with mouse parvo virus detected in 1 of 20 sperm samples. This indicates there is little risk of transmitting disease agents from our conventional colony to the re-derived colony, where re-derivations will be done only by embryo transfer and never via sperm. The Cryopreservation Laboratory Program Description, which serves as the QA Plan for the cryopreservation/re-derivation program was formally reviewed in 2001 and suggestions for improvement were implemented. Over the past year, 144 lines of mice needed for a large research project have been successfully re-derived by our staff (using embryos) into an SPF facility located at the University of Tennessee. This is further confirmation that our re-derivation processes have been successfully developed.

e. *Separation of housing and sanitation and procedure activities, and improved environmental controls for Buildings 4500S and 4501 are needed.*

In 2001, renovations to Building 4500S were completed to allow separation of housing and sanitation and procedure activities. Building 4501 was converted to a temporary housing facility, where animals are only housed for short periods of time, and there are sufficient supplies available so that no sanitation procedures are carried out when animals are present. The ORNL-ACUC has defined criteria for acceptable temperature and humidity ranges and periods for holding animals in temporary housing facilities. Conditions in both facilities are monitored via regular veterinary rounds.

1999 Changes

Organizational Changes

- Addition of half-time ORNL-ACUC Coordinator position.
- Addition of one new animal care technician position.

Programmatic Changes

- Cage top changing frequency increased to twice monthly.
- Fourth SPF room put in place in Bldg 9210
- Times of euthanasia recorded on log sheet to ensure timely euthanasia of animals collected at euthanasia stations.
- An SOP documenting risk associated with rodent research added to Laboratory Animal Resources Manual. Material is reviewed with all new animal care staff.
- ORNL introduced an Integrated Safety Management System, which emphasizes individual responsibility for safety, environment, health, and quality.
- Preliminary groundwork for separating clean/dirty activities implemented (educational efforts, team building with unionized staff, trials in new methodology, and ergonomic evaluations).

2000 Changes

Organizational Changes

- The Department of Energy changed contractors for management of ORNL. Effective April 1, 2000, UT-Battelle, LLC, became the new management contractor for ORNL.
- Effective April 1, 2000, W. F. Harris replaced D. E. Reichle, as the Institutional Official at ORNL.
- Effective April 1, 2000, W. J. Madia replaced A. Trivelpiece as CEO.
- Addition of a full-time veterinary technician for Building 9210.

Programmatic Changes

- PCR analysis of frozen embryo and sperm samples completed. Infectious agents detected in zero embryos and in 1 of 20 of sperm samples. Poster presented at National AALAS.
- QA Plan for Cryopreservation/Re-derivation Program reviewed in independent self-assessment.

- Renovations to separate housing, sanitizing, and procedure areas in Building 4500S animal facility started.
- Building 4501 animal room re-classified to a temporary animal housing facility. Animals housed for short periods of time; sanitizing procedures performed when animals are not present.
- ORNL-ACUC defines criteria for acceptable temperature and humidity ranges and periods for holding animals in temporary housing facilities.
- Set up of a mock-up room in Bldg 9210 using Thoren ventilated racks with automatic watering for the purpose of evaluating equipment for the new defined flora SPF barrier facility.

2001 Changes

Organizational Changes

- C. F. Foltz, D.V.M., DACLAM, ORNL institutional veterinarian and LSD Laboratory Animal Resources Group Leader, left the staff of ORNL at the end of November 2001
- D. O'Rourke, D.V.M., DACLAM, Director of Laboratory Animal Care at the University of Tennessee, was appointed as interim attending veterinarian, to serve in this capacity until a permanent replacement for Dr. Foltz is hired.
- B. A. Berven, Ph.D., Director of Operations for LSD, is serving as Acting Group Leader for the Laboratory Animal Resources (LAR) Group of LSD until an animal facility manager is recruited and hired. He is assisted by a management team consisting of: the veterinary technician, the animal caretaker supervisor, the LSD Quality Assurance Coordinator, the ORNL Animal Care and Use Committee Coordinator, and an LSD assistant animal facility manager.
- R. C. Mann, Ph.D., Director of LSD, left the staff of ORNL at the end of September 2001. W. F. Harris, Ph.D., ORNL's Institutional Official, is serving as Acting Division Director until a new LSD Director is appointed. (All of ORNL's mice are housed in LSD facilities.)
- ORNL's facility operations management system was re-organized in 2001. Complex Managers were assigned for various ORNL areas, both at the ORNL site and at the Y-12 Site. ORNL animal facilities are located in 3 different Complex Areas. The Complex Manager acts as the "landlord" and has responsibility for scheduling and coordinating maintenance and repairs to buildings and utilities. Complex Managers do not have responsibilities for activities occurring in their buildings. Complex Managers interface with a Facilities Operations Manager in each ORNL division to facilitate needed maintenance and repairs.

Programmatic Changes

- Establishment of 5 SPF and 7 "one-way" animal rooms (for major production stocks/strains) in Building 9210 facility (SPF Room 213 with microisolator cages has remained MHV negative to date).
- Covered clean equipment stored in Building 9210 hallways on 3-sided carts.
- Use of a dedicated cage wash crew in Building 9210.
- 144 lines of mice re-derived by ORNL staff using embryos, at an SPF facility located at the University of Tennessee.
- Renovations completed to Building 4500S rodent facility to allow separation of housing, sanitation, and procedure activities.

- Design of a new LSD defined flora, specific pathogen free (SPF) barrier facility completed.

2002 Changes

Programmatic Changes

- Total funding for construction of SPF barrier mouse facility approved.
- Construction of SPF barrier mouse facility begun in June 2002.
- Decision made to outsource all animal care for the new SPF barrier mouse facility, to include a board-certified laboratory animal veterinarian, animal facility manager, veterinary technician, secretary, and animal care technicians.
- All information regarding care and use of laboratory animals at ORNL is now incorporated in ORNL's official Standards Based Management System (SBMS) as: "Subject Area: Animal Research."
- ORNL will fund travel for one additional ORNL-ACUC member per year to attend a national animal care and use conference.

F. Nature of Research, Testing, and Teaching Programs

The major types of research and testing programs at ORNL involving animals are summarized in the table below. The only teaching activities involving animals are covered in one protocol for veterinary training on animal procedures.

Type of Research, Testing, Teaching	Number of PIs	Number of Protocols	Protocol Numbers
Fish Behavior	2	3	0279, 0285, 0299
Chemical Mutagenesis (mice)	1	2	0260, 0289
Targeted Mutagenesis (mice)	2	2	0261, 0273
Radiation Effects (mice)	1	2	0276, 0288
Phenotype Screening (mice)	2	2	0259, 0289
Cryopreservation	1	1	0289
Immunotherapy (rats, mice)	1	2	0243, 0256
Colony Health Surveillance	1	1	0284
Development of Microsensors, CT Scanners (mice)	1	1	0286
Genotype, Phenotype Characterization and Analysis (mice)	4	6	0240, 0241, 0261, 0263, 0273, 0281
Lipid/Fatty Acid Distribution (mice, rats)	2	2	0239, 0241
Structural Nucleosomes (chicken)	1	1	0272
Environmental Monitoring (fish, geese, reptile)	4	6	0264, 0266, 0269, 0270, 0287, 0292
Breeding (fish)	1	2	0247, 0248
Toxicity Testing (fish, minnow)	1	3	0249, 0274, 0283

larvae)			
Display Aquaria (fish, reptiles, amphibians)	1	1	0280
Fertilization Study (mice)	1	1	0285
Functional Genomics – Vitronectin (mice)	1	1	0277
Investigator Training on Animal Procedures (mice)	1	1	0258
High Throughput RNA Production (mice)	1	1	0245
Gene Regulation	1	1	0293
Animal Work Contracted at Other Institutions* (All are PHS-Assured)	4	5	0246, 0278, 0282, 0292, 0291

*Uniformed Services University of the Health Sciences (Bethesda, MD), University of Pittsburgh, Texas A & M University, University of Southern California (Los Angeles), University of Tennessee (Knoxville). In all cases, animals are owned by the institutions where the work is performed and are covered by IACUC-approved protocols at these institutions. The ORNL-ACUC assigns a “tracking” protocol number to these projects in order to confirm at 1-year intervals that the work is continuing to be performed under approved protocols at the institutions.

Additional information is provided in:

- Appendix 2: List of current protocols
- Appendix 3: Daily average inventory and annual use by species
- Appendix 4: Animal Usage Form

G. Research Funding Source(s)

Note that some protocols are listed twice, indicating that different tasks or portions of the protocol are financed by different sources.

Funding Source	Number of Protocols	Protocol Numbers
DOE	27	0241, 0243, 0247, 0248, 0256, 0259, 0261, 0264, 0266, 0269, 0270, 0273, 0274, 0276, 0278, 0279, 0280, 0282, 0283, 0284, 0287, 0287, 0289, 0282, 0295, 0299, 0300
NIH	10	0240, 0243, 0260, 0273, 0285, 0289, 0290, 0294, 0296, 0298
LDRD (ORNL Laboratory Director's Research & Development Seed Money)	8	0244, 0245, 0247, 0249, 0277, 0288, 0291, 0293
NASA	1	0272

Department of Defense	1	0269
American Diabetes Association	1	0281
American Heart Association	1	0263
LSD Lab Animal Resources Group, Fee for Service	1	0258
Uniform Services University of the Health Sciences	1	0246
Work for Others (WFO) Pharmactinium	1	0246

H. Summary of Facilities

The large conventional mouse facility is located in Bldg 9210 at the Y-12 site, 7 miles east of the ORNL site where all other animals are housed. Please refer to maps included in Appendix 5A for information on location of facilities relative to one another. Floor plans for animal areas in each of the buildings are included in Appendix 5B.

Bldg #	Site/Division	Type	Animal Space (sq. ft)	Service Space (sq. ft)	Total Space (sq. ft)
9210	Y-12/LSD	Rodent/ Conventional/ SPF	18,600	21,909	40,509
4500S	ORNL/LSD	Rodent/SPF	231	55	286
4501	ORNL/NSTD	Rodent/SPF	184	184	368
3500	ORNL/ESTD	Rodent/ Conventional	100	NA	100
1504	ORNL/ESD	Fish	8,000	NA	8,000
1505	ORNL/ESD	Fish	531	642	1,173
Total Square Footage					50,436

Bldg 9210, consisting of three floors, is a conventional facility that houses approximately 19 inbred strains and 250 hybrid or mutant stocks used for genetic and mutational studies. It includes extensive numbers of mutant mouse lines, which for the most part are maintained as closed colonies

The single animal room in Bldg 4500S is an SPF housing facility for mice used in nuclear medicine/immunotherapy studies. Mice utilized are from commercial or occasionally inter-institutional sources, are used for acute or chronic non-survival studies, and are not bred in-house. Mice are housed in ventilated microisolator caging.

The single animal room in Bldg 4501 is an SPF temporary housing facility for rats and mice used in nuclear medicine studies. Animals utilized are from commercial or occasionally inter-institutional sources, are used for acute or chronic non-survival studies, and are not bred in-house. Animals are housed for short periods of time (up to 3 weeks) in open or bonneted caging in a ventilated rack (mice and rats are housed on separate shelves and handled separately).

The single animal room in Bldg 3500 is a satellite facility used only for temporary housing of animals from either Bldg 9210 or other institutional sources for imaging and microelectronic implant studies. Nearly all studies at this time are non-survival acute procedures.

Building 1504 houses large living streams, an aquatic toxicology laboratory, several tanks of fish, amphibians, and reptiles for display, and fish for experimental purposes.

Building 1505 has a single animal room for housing fish and a laboratory used for terminal studies in collected specimens.

I. Other Units not Included in This Description

No other housing areas are present at ORNL. ESD performs wildlife studies but no animals other than fish are housed for research. Chemical Sciences Division occasionally performs acute non-survival procedures utilizing rodents from outside sources (animals are not housed longer than 12 hours).

J. Contract Facilities

Not applicable.

K. Other Relevant Background

A Memorandum of Cooperation for the Tennessee Mouse Genome Consortium has been established between ORNL and the University of Tennessee (Knoxville and Memphis), the University of Memphis, Vanderbilt University, St. Jude Children's Research Hospital, Meharry Medical College, and East Tennessee State University for the purpose of characterizing mutants generated at ORNL. All institutions have PHS Assurances and all are AAALAC-accredited with the exception of the University of Memphis.

II. Description

A. Institutional Policies and Responsibilities

1. Monitoring the Care and Use of Animals

a. Institutional Animal Care and Use Committee (s) (IACUC)

1) Who appoints Committee/Who is Institutional Official

The Institutional Official appoints the members of the ORNL-ACUC. W. F. Harris, Ph.D., Associate Laboratory Director for Biological and Environmental Sciences, is the ORNL Institutional Official.

2) Composition/Frequency of Meetings/Responsibilities of the Committee

The ORNL-ACUC meets monthly and is responsible for protocol review, semi-annual program review and facility inspection, providing reports to the Institutional Official, review of concerns involving the care and use of animals at ORNL, and establishing institutional policy and guidelines for animal use and training. Once appointed, new members participate in an orientation meeting with the ORNL-ACUC Chairperson and are provided with printouts or web access for the following materials:

- The 2000 ORNL Assurance of Compliance with the Public Health Service, plus annual updates
- The 2000 Report of the AVMA Panel on Euthanasia
- The Public Health Service Policy on Humane Care and Use of Laboratory Animals
- OLAW's Institutional Animal Care and Use Committee "Guidebook"
- Access information for the ORNL-ACUC's internal Home Page
- A copy of the ORNL-ACUC protocol form
- The 1996 "*Guide for the Care and Use of Laboratory Animals*"

Committee Members:

Barbara Beatty, B.S., Chairperson
Research Associate, Operations Group, LSD

David Edds, M.S.
Industrial Hygienist, Operational Safety Services Division, and
Operations Group, LSD

Mark S. Greeley, Jr., Ph.D., Vice Chairperson
Research Scientist, Environmental Health and Risk Analysis
Group, ESD

H. Gerald Hodge, *ex officio* (non-voting)
Animal Caretaker Supervisor, Laboratory Animal Resources
Group, LSD

Joan F. Hughes, B.S.
Group Leader, Environmental Protection and Waste Services
Division

Dabney K. Johnson, Ph.D., *ex officio* (non-voting)
Group Leader, Mammalian Genetics Group, LSD

Kristen A. Kerber, A. S., VMT, LATG
Veterinary Technician, Laboratory Animal Resources Group, LSD

Edward J. Michaud, Ph.D.
Group Leader, Functional Genomics Group, LSD

Darla R. Miller, B.S.
Coordinator, Tennessee Mouse Genome Consortium, Mammalian
Genetics Group, LSD

Rev. Terry Mosley
Pastor, Free Will Baptist Church, Oak Ridge, Tennessee

Dorcas P. O'Rourke, D.V.M., DACLAM
Interim ORNL Attending Veterinarian, Interim ORNL Institutional
Veterinarian, Director of the Office of Laboratory Animal Care,
University of Tennessee College of Veterinary Medicine

J. Warren Webb, Ph.D.
Wildlife Administrator and Research Scientist, Environmental
Health and Risk Analysis Group, ESD

3) Frequency of Committee Review

The ORNL-ACUC reviews the animal care and use programs and inspects animal facilities twice a year, at six month or shorter intervals (See Appendix 6 for most recent Semi-Annual Report). The committee meets monthly for protocol review and discussion of pertinent business. Approved minutes from the last two monthly meetings are located in Appendix 7.)

4) USDA Inspection Report Responses

ORNL facilities are USDA registered but not subject to inspections. ORNL submits an Annual USDA Report.

5) Other Monitoring Procedures

Contacts for anonymously reporting animal welfare concerns are posted in all facilities.

b. Animal Care and Use Protocol Review and Approval

All protocols are reviewed at scheduled ORNL-ACUC meetings and are approved for a period of three years (see Protocol Form, Appendix 8). Annual renewals (see form, Appendix 8) are required and are reviewed and administratively approved by the Chairperson unless significant changes are made. In the absence of the Chairperson, the Vice-Chairperson is fully authorized to act as Chairperson. Amendment requests are made via an amendment form (see form, Appendix 8) and require full committee review if any significant changes are made. If full Committee review of a protocol or amendment results in conditional approval, the Committee members present (a quorum) authorize the Chair or an appropriate subcommittee to review the response to conditions, to request additional information if needed, and to approve the proposed project or modification. All administratively approved annual renewals and amendments are summarized in the ORNL-ACUC minutes. The committee takes responsibility for scientific review if no outside peer review is performed and no outside or internal review independent of the committee is currently required. Protocols involving more than momentary pain and distress require specific and clear monitoring and endpoint descriptions to be specified in the protocol. If the procedures have not been performed previously, veterinary oversight is required, and pilot studies are generally recommended.

c. Physical Restraint

A “loose” restraint (~50mm diameter x 200mm length cylinder) is used for imaging mice in one protocol. The tube is ventilated and an infrared camera monitors the animal’s physical activity. Animals are conditioned to accept confinement in the tube through a series of increasingly longer confinements until restraint is accepted without distress. Maximum period of confinement is 2 hours.

A second protocol has use of an 8-minute mouse restraint for behavior testing. The Lucite chamber is approximately 50mm x 200 mm.

Additionally, brief restraint in a mouse-sized ventilated Lucite cylinder is used for tail vein injections.

d. Multiple Major Surgical Procedures

Not applicable.

e. Food or Fluid Restriction

Not applicable.

2. Veterinary Care

a. Institutional Arrangement

ORNL has made the decision to outsource all animal care staff for the new SPF rodent facility, which will include services of a full-time board-certified laboratory animal veterinarian who will also serve as ORNL's Institutional veterinarian. In the interim, services of a part-time consultant Institutional Veterinarian, Dorcas O'Rourke, D.V.M., DACLAM, have been obtained under subcontract. Her time is 20% (one day per week) committed to oversight of animal care and use at ORNL. Dr. O'Rourke's responsibilities are outlined below:

Institutional Veterinarian Job Description: Qualifications: Doctor of Veterinary Medicine; Diplomate of American College of Laboratory Animal Medicine; knowledge of AAALAC International accreditation and regulatory requirements for animal care and use; experience with rodent facility management.

Veterinary Duties: Oversight of methods to improve health status of conventional mouse colony; membership on the ORNL-ACUC; regular animal health surveillance; clinical veterinary treatment of research animals as appropriate; development of animal care and use guidelines and policies; serve as a resource, providing veterinary expertise for protocols for new and ongoing research; oversight of research animal training program for research staff members; oversight of research animal training program for animal care staff; oversight of husbandry requirements and performance; contribution to and review of annual report to AAALAC International; contribution to and review of program description for triennial AAALAC International site visit.

Dorcas O'Rourke, M.S., D.V.M., DACLAM, serves on the ORNL-ACUC and provides veterinary care for fish housed at ESD. Dr. O'Rourke visits the ESD facilities approximately monthly. Back-up veterinary services are provided by clinical veterinarians from the University of Tennessee College of Veterinary Medicine, under the direction of Dr. O'Rourke.

Management Duties: Weekly interaction with the Laboratory Animal Resource Supervisor, Veterinary Technician, and staff of 19 animal care technicians; input for future animal facility equipment and operations needs; consultant for scheduling for animal husbandry and sanitation activities; input for selection and purchase of supplies and equipment for the animal facilities; input for determination of maintenance needs. Dr. O'Rourke also provides regularly scheduled AALAS certification-training sessions to the animal care staff in Bldg 9210.

b. Others' Roles

Kristen Kerber, Veterinary Assistant, performs weekly rounds of all rodent animal rooms, responds to daily health checks, provides treatment under veterinary oversight, performs re-derivations of ORNL mice into an SPF facility at the University of Tennessee, performs environmental monitoring and sanitation QA, provides training to animal care and research staff, and performs sentinel

screening and health surveillance. Gerald Hodge performs supervision of Bldg 9210 animal care staff. Bldg 9210 animal care staff are trained to identify animals requiring veterinary attention.

3. Personnel Qualifications and Training

a. Animal Resources Professional/Management/Supervisory Personnel

Dorcas O'Rourke, M.S., D.V.M., DAACLAM: Sixteen years experience in laboratory animal medicine and wildlife management and medicine. ACLAM continuing education requirements current, AAALAC Council Member, Attends ACLAM meetings, makes presentations and attends national AALAS meetings, attended and presented at 2002 Charles River Short Course, is on SCAW Board of Trustees, is author of book chapters on laboratory animal medicine.

Barry Berven, Ph.D., LSD Director of Operations, Acting Group Leader for Laboratory Animal Resources Group: Over 20 years experience in science management, 7 years experience in environmental health physics research.

Gerald Hodge, LSD: Greater than 30 years experience as an animal care technician, 7 years supervisory experience. Is a member of and has attended Appalachian Branch and National AALAS meetings for the last 5 years, is a member of Laboratory Animal Management Association, has certificates of completion from AALAS Leadership Academy and Management Course, and attends monthly animal care staff training. Past member of Board of Directors, Appalachian Branch AALAS.

Kristen Kerber, A.S., LATG, Licensed Veterinary Medical Technician: 2 years experience as a research technician, 5 years experience in laboratory animal field. Is a member of and attends Appalachian Branch and National AALAS meetings, Attended 2002 Charles River Short Course.

Gene Barker, LSD: Thirteen years experience as a research technician in mouse transgenic and breeding colony management. Is a member of the Appalachian Branch AALAS.

Steve Kennel, Ph.D., LSD: Supervises, in collaboration with Dr. O'Rourke, animal care for Bldg 4500S. Dr. Kennel has greater than 20 years experience in animal based research.

Russ Knapp, Ph.D., NSTD: Supervises, in collaboration with Dr. O'Rourke, animal care for Bldg 4501, has greater than 30 years experience working with mice and rats.

Mike Paulus, Ph.D., ESTD: Supervises, in collaboration with Dr. O'Rourke, animal care for temporary animal housing facility in Bldg 3500 (no permanent housing), has received required training for working with mice and has received one-on-one training from Dr. C. Foltz, previous ORNL Institutional Veterinarian.

Mark Greeley, Ph.D., ESD: Researcher and Director of the Aquatic Toxicology Laboratory, supervises, in collaboration with Dr. O'Rourke, animal care for Bldg 1504 and 1505. He has 25 years experience in maintaining fish colonies for research purposes, is a long-standing ORNL-ACUC member, participates in ORNL-ACUC continuing education, serves as Vice Chairperson of the ORNL-ACUC, and is a member of the Appalachian Branch AALAS.

Mike Ryon, M.S., ESD: Research scientist, over 16 years experience in Biological Monitoring and Abatement Programs, supervises care for display aquaria in Bldg 1504.

Barbara Beatty, B.S., LSD: Quality Assurance Coordinator, 9 years QA experience, 4 years research technician experience, 7 years research associate experience, is a member of and attends Appalachian Branch and National AALAS meetings, attends national animal care and use conferences, ORNL-ACUC member and Chair since October 1996, past member of Board of Directors and past Secretary/Treasurer, Appalachian Branch AALAS.

Diane Embleton, B.S, LSD: ORNL-ACUC Coordinator for 2 years, is a National AALAS Member, member of and attends Appalachian Branch AALAS meetings, attends national animal care and use conferences.

David Edds, M.S., LSD: Operational Safety Services Division (OSSD) Representative, 13 years experience, ORNL-ACUC member for 7 years, member of and attends Appalachian Branch AALAS meetings, and participates in safety training for LSD investigative and animal care staff.

b. Animal Care Personnel

Nineteen bargaining unit employees (18 full-time, 1 part-time) are part of the Laboratory Animal Resources (LAR) Group of LSD at the Bldg 9210 mouse facility. Employees have from over 30 years to less than 1 year of experience working with mice. Continuing education opportunities have included rotating attendance at Appalachian Branch AALAS meetings over the last 5 years, required monthly training classes performed in-house, weekly safety meetings, and attendance of several staff members at the District IV AALAS meetings. The animal care staff are also presently attending weekly on-site ALAT training sessions provided by the University of Tennessee Office of Laboratory Animal Care. The ALAT CD-ROM, manuals, and workbooks are being provided by ORNL. Two animal care technicians currently have AALAS technician certification at the ALAT level. On-the-job training includes working with an experienced animal care technician for at least a 2-week period prior to beginning independent animal care responsibilities. In addition, new animal care staff members are required to review the LAR Section Standard Operating Procedures (SOPs), are trained on new and revised SOPs, and receive all other mandatory institutional and divisional training.

The veterinarian, veterinary technician, and 9210 animal staff supervisor regularly attend National AALAS and other appropriate regional and national meetings. The veterinary technician has certification at the LATG level and is a Licensed

Veterinary Medical Technician. ORNL supports travel to one national animal care and use conference each for the ORNL-ACUC Chairperson, the ORNL-ACUC Coordinator, and for one additional ORNL-ACUC member.

Animal care staff members not included in the LAR Group are part-time effort positions (4500S - research assistant; 4501 – research assistant with 15 years experience in animal care, also served on the ORNL-ACUC for a 2 year period; 1504 & 1505 – students and technicians perform animal care under the direct supervision of the responsible scientist). All staff members working with vertebrate animals are required to view the video “Animal Care Matters” and are required to take a web-based course and test on using the mouse in research or a web-based course on use of fish and wildlife. Currently, no formal continuing education opportunities are offered to these part-time and student care staff.

c. Research Staff

All new animal research staff members listed on ORNL-ACUC protocols are required, through the division’s training office, to view the videotape, “Animal Care Matters.” In addition, all staff using mice are required to take a web-based training for use of mice in research. An open book test with a passing grade of 80% correct is also required. A similar web-based training for the use of fish and wildlife is provided to staff members using these species. One-on-one training is provided for all new personnel through the responsible principal investigator (PI) or the veterinary staff, as appropriate. One-on-one training is documented by the PI or by the veterinary staff member performing the training. All personnel listed on a protocol are required to read and sign that they have read the protocol. Supervisors of staff utilizing mice in Bldg 9210 attend a monthly meeting with the Laboratory Animal Resource Management Team; current issues and new and revised SOPs are reviewed at this meeting. As additional training, all 9210 research technicians using animals are currently reviewing the ALAT CD-ROM training materials.

4. Occupational Health and Safety of Personnel

a. Hazard Identification and Risk Assessment

The OSSD provides industrial hygiene services, which usually consists of matrixing an industrial hygiene representative to each division. These individuals provide, in conjunction with their parent group, guidance for ensuring a safe work environment at ORNL. Each division at ORNL is required to have a safety officer and an OSSD representative (this may be the same individual). The division is responsible for ensuring a safe work environment. ORNL Medical Division evaluates the health of all UT-Battelle employees and administers individual employee health reviews based on the occupational risks determined by Industrial Hygiene and the OSSD representative.

The division OSSD representative is in charge of monitoring the use of hazardous agents and must approve of and set the conditions for use of any hazardous agents before they can be used in animals or otherwise. OSSD representatives have access to a variety of equipment for evaluating environmental conditions that

might impact the health and safety of personnel. The OSSD representative for LSD is David Edds, who has a master's degree in industrial hygiene and 13 years experience. Mr. Edds also is a member of the ORNL-ACUC. OSSD representatives for ESD and Engineering Science and Technology Division (ESSD) are Marwan Bader (10 years experience) and John Czachowski (15 years experience) respectively. Division Safety Officers for ESD and ESSD are Monty Ross (5 years experience) and Bill Langford (10 years experience) respectively.

b. Personnel Training

1) Description of Special Qualifications and Training for Work with Hazardous Agents in Animals

All new employees at ORNL must receive the following training: General Employee Training, Division Specific Training (general safety, contact with supervision, emergency information, work area safety, vehicular safety, electric safety, mentoring, animal use, and training requirements), and Site-Specific Training (which can include radiation training, hazardous chemical training, respirator training, animal exposure, etc.). All training is tracked through the Division Training Office, also a requirement for each division.

2) Description of Educational Programs

All ORNL employees are required to attend regularly scheduled safety meetings. In addition, monthly in-house training for Bldg 9210 animal care staff includes both laboratory animal science and safety training topics. Occupational health and safety issues are reviewed at approximately half of these training sessions. The Division Safety Officer provides an additional weekly safety-oriented training meeting for Bldg 9210 animal care staff. Staff members performing work considered hazardous are required to take ORNL training for these specific activities, which include use of fork lifts, dust masks, hoods, hazard identification, etc.

Surveillance for potential zoonotic agents transmitted by rodents (LCMV, Hantavirus) indicates that these agents are not present in our colonies. Information sheets on risks associated with rodent-based research are provided to rodent users. The LSD Division Training Office has copies available as well. *Mycobacterium fortuitum* has been identified in aged fish in Bldg 1505. The PI has discussed the zoonotic potential with staff, the OSSD representative has reviewed appropriate precautions, and an information sheet on precautions is posted in the laboratory.

c. Personal Hygiene and Protection

1) Personal Protective Equipment/Work Clothing Provided

Animal care personnel are supplied with a sufficient number of clean company-provided coveralls, khakis, surgical scrubs, or lab coats for a

daily change of clothing, or dedicated field clothing as appropriate for field studies. A commercial laundry launders the clothing. Company-provided safety shoes and safety glasses are made available to all employees. Protective gloves are required for all animal manipulations and for filling of water bottles. Hearing protection is required for cage washing operations. Radiation protective clothing remains in the radiation containment area and gloves used to mix chemicals are disposed of by double bagging.

2) Shower/Change Facilities

In Bldg 9210, sinks for hand washing are available in water stations and bathroom areas, and shower areas for changing are provided. Sinks are present in animal rooms located in Bldgs 4500S, 4501, 3500, 1504, and 1505. Shower facilities are available in all ORNL facilities, although they are not dedicated for use by part-time animal care staff in other facilities housing animals.

3) Eating, Drinking, and Smoking Policies

Eating, drinking, applying cosmetics, and smoking are not allowed except in designated areas. Lounges for Bldg 9210 animal care staff are located in rooms 9207-128 and 9210-125A mezzanine. There are no designated lounges for part-time animal care staff in other facilities housing animals, however, general use lounges are available in all facilities (laboratory coats would not be worn in these areas).

There are two research staff members in Bldg 9210 who have medical conditions that require frequent drinking of water. They have been medically evaluated by the Director of ORNL's Health Services Division. Upon his recommendation, the ORNL-ACUC granted approval for these two individuals to drink water from covered containers while working in animal rooms.

d. Medical Evaluation and Preventive Medicine for Personnel

1) Description of Program; Personnel Included

All UT-Battelle employees are included in the occupational health program. This includes a complete pre-employment physical examination including chest radiograph, electrocardiogram, pulmonary function, eye test including tonometry, hearing test, and blood and urine tests. Tetanus vaccinations are made available to all employees. Tuberculin testing and rabies vaccinations are available when indicated. The same physical examination is repeated on each employee at least every 24 months. Animal care staff in Bldg 9210 receive physicals every 12 months because this frequency is mandatory for employees in the Hearing Conservation Program. ORNL's Health Services Division also handles on-the-job injuries and illnesses. The Health Services Division is responsible for administering the medical evaluation and preventative medicine program. Students, contract, and subcontract workers do not receive

physicals but are included in all other medical and support services (e.g., Site Specific training).

2) **Aspects Relating to Hazardous Agents**

All animal care personnel are furnished with safety glasses and are required to wear them in designated areas. Individuals use hearing protection devices when operating cage washers. Dust masks are required for dumping of soiled rodent bedding and are encouraged for use in cage changing. All personnel, both technical staff and caretakers, who work with animals are advised to report any bites or possible allergic reactions to their supervisors. Any potential safety concern can be reported to the OSSD Representative at any time and the right to stop work under any circumstance that is a potential safety risk is strongly encouraged and a part of daily work practices at ORNL. Anyone who is thought to have been exposed to a hazardous agent will be examined and tested by the Health Services Division. In the case of possible exposure to radionuclides, personnel are tested by Dosimetry Services and by Health Services Division.

3) **Special Precautions for Primate Users**

Not applicable.

e. **Animal Experimentation Involving Hazards**

1) **Description of Institutional Policies**

Use of biohazardous agents must be approved by the ORNL Institutional Biosafety Committee (IBC), which sets the conditions for such use. The IBC serves ORNL in providing review and approval of research involving the use of recombinant DNA organisms and/or biohazardous agents in accordance with the directives of the NIH Guidelines for Research Involving Recombinant DNA Molecules (January 2001). Subject matter experts assigned to each division oversee all work using radiation or chemical hazards. Each division has a radiation control officer, a chemical hygiene officer, a safety officer and/or an OSSD representative who provide oversight and expertise in the use of hazardous agents. In addition, ORNL has a comprehensive Environment, Safety, and Health Program that includes written procedures to address a variety of health and safety concerns, including staff exposures to and interactions with chemical and physical agents.

2) **Description of Oversight Process and Husbandry Practices**

The OSSD representative for LSD serves on the ORNL-ACUC, and his evaluation of use of potentially hazardous agents is included in protocol review. All experiments that involve the use of hazardous or potentially hazardous agents must first be detailed in an ORNL on-line Research Safety Summary which details all hazards associated with a project, and

the controls in place to address such hazards. The Research Safety Summary is reviewed and approved by applicable subject matter experts and division management before work on the project is authorized to begin. In LSD's Bldg 9210, procedures are in place to identify animal rooms where hazardous agents are in use, and such rooms are labeled with a standard sign which lists the following: name of agent(s), potential hazards, precautions to be taken in the room, ORNL-ACUC protocol number, and two contact persons (principal investigator and OSSD Representative). The principal investigator attaches the proper notices, marked prominently with a "Bull's Eye," on the animal room door and notifies the LAR supervisor. Precautions to be taken by animal care technicians when handling animals treated with hazardous agents are described in an SOP, "Bull's Eye Cages" (Appendix 9).

In Bldgs 4500S and 4501, where radioisotopes are used, animal housing areas are located within radiation containment areas and are subject to compliance with ORNL Health Physics procedures.

Bldgs 9210 and 3500 each have a miniature X-ray computed tomography system (microCT) for scanning mice. The scanning equipment is protected with an interlock system that will shut the unit down if opened while the unit is in operation (this design protects personnel from accidental exposure). A representative from the Radiological Surveillance Section performs an annual inspection.

If questions are raised concerning possible contamination of animals or animal-related materials with hazardous agents, the Operational Safety Services Division (OSSD) will test the site.

3) Containment of Hazardous Agents

Mixing of hazardous chemicals and use of radionuclides are performed in exhaust hoods. Please refer to "Bull's Eye" SOP attached as Appendix 9 for details on containment of chemically treated rodent cages. All work with radionuclides is monitored by trained Radiological Control Technicians (RCTs) from the OSSD. Radiological work is confined to radiological areas and work is performed under an ORNL radiological work permit that is established and monitored by the area RCT. Radioactive-contaminated cages are first decontaminated with a commercial cleaning solution, followed by use of a hand counter to verify absence of radioactivity. If the cages are found to be contaminated with radioactivity, then they are treated as radiological waste. The cages, animal bedding, and any supplies found to be radioactive are segregated and discarded into compatible radioactive waste containers and disposed of according to ORNL waste acceptance criteria. Radioactive animal carcasses are accumulated in a freezer located on the dock of Bldg 4501 and are disposed of according to ORNL waste acceptance criteria. Waste generators receive training in categorization and proper disposal of all hazardous wastes.

Non-contaminated fish carcasses are disposed of via a fish grinder in Bldg 1505, Rm 153. Potentially contaminated fish or wildlife may be stored or archived for a period of time in locked freezers in Rm 153, then are disposed of at the Y-12 Landfill, in compliance with ORNL waste management procedures. Mouse carcasses contaminated by chemical agents are segregated and labeled, frozen, and transported to the UT College of Veterinary Medicine carcass incinerator.

4) Scavenging of Anesthetic Gases

Use of anesthetic gases at ORNL is currently limited to use of isoflurane delivered via isoflurane precision vapor delivery equipment. A small remaining amount of in-date Metofane© is used only in certified hoods. No procedures requiring anesthesia are performed in animal rooms. The OSSD representative is available to measure possible exposure to anesthetic gases.

5) List of Approved Hazardous Agents

a) Biologic Agents

Protocol	Agent	Concentration	Route of Administration	Duration of Exposure	Species
0249	Green fluorescent protein (GFP) reporter gene	NA- minimal volume	micro-injection fish eggs (embryos)	Life of embryo	Zebrafish Medaka
0297	Tumor Cells	0.5 ml	IP	Life of animal	Mice & Rats
	Tumor Cells	0.2 mls	IV, SC	Life of animal	
0241 exp. 8/18/02	Tumor cells MKN74-PRC/CMV-E4 cells	1 X 10 ⁶ cells/ 0.5 ml	SC	4 months (until euthanized)	Mice
	MKN74-PRC/CMV-FHIT cells	1 X 10 ⁶ cells/ 0.5 ml	SC	4 months (until euthanized)	
0273	Recombinant DNA constructs	NA in ES cells	Via ES cells into blastocysts	Life of animal	Mice

b) Chemical Agents

Protocol	Chemical	Hazard Level	Route & Dosage	Duration of Exposure	Species
0289	N-ethyl-N-nitrosourea (ENU)	Mutagen, carcinogens	IP, 50-250 mg/kg	single dose	mice
			IP, 85 mg/kg	single dose once per week (2-4 wks)	
0283	Effluent water samples, environmental contaminants	Possible trace amounts of toxic chemicals or carcinogens	Submerged exposure in water, trace amounts	up to 7 days	fish (larvae)
0274	Wastewater discharges Oak Ridge Y-12 Plant	Possible trace amounts of toxic chemicals or carcinogens	Submerged exposure in water, trace amounts	up to 21 days	fish (embryos)
0281	Streptozotocin (STZ) to induce diabetes	Carcinogen	IP, 10mg/100g body weight	3 times, life span of animal	Mice
0260	N-ethyl-N-nitrosourea (ENU)	Mutagen, carcinogens	IP, 150 mg/kg	single dose	Mice
			IP, 85 mg/kg	single dose once per week for 3 wks	
0287	Possible environmental contaminants	Possible heavy metals	Accumulated in living environment	Life span until capture	Northern water snake fish
0294	Formalin	Carcinogen	Terminal Perfusion, 200 gms/200ml	10 min.	rat

For standard mutagenesis, only males are routinely injected with ENU, and they are kept for various times after injection. They remain fertile for seven weeks, and then enter a period of sterility for 1-3 months, depending on dose. After they regain fertility, they are actively bred to untreated females. Males that continue to be healthy and to breed successfully can be kept up to one year or more.

Bull's Eye procedures are employed in Bldg 9210 when these chemicals are in use. Caretakers must wear gloves and dust masks, which are disposed of in a plastic bag before leaving the room. Caretakers are also required to wash hands immediately after leaving the room.

c) Physical Agents

Protocol	Agent	Hazard Level	Concentration & Route	Half Life	Species
0259	<u>MicroCT Scanner</u>	X-Ray Ionizing Radiation	Whole body exposure	NA	Mice
0297	<u>Radioisotopes</u> Iodine 125 Indium 111 Rhenium 188 Yttrium 90 Bismuth 213 Actinium 225	Ionizing Radiation	100µCi / 0.2mls IV 50µCi / 0.2mls IV 50µCi / 0.2mls IV 100µCi / 0.2mls IV 200µCi / 0.2mls IV 10µCi / 0.2mls IV	I-125/60.14 d In-111/2.8 d Re-188/17 h Y-90/2.67 d Bi-213/45.5 min Ac-225/10 d	Mice
0256	<u>Radioisotopes</u> Iodine 125 Iodine 131 Rhenium 188 Yttrium 90 Bismuth 212 Bismuth 213 Actinium 225 Technitium 99m Holmium 166 Astatine-211 Thulium-170 Dysprosium-166 Platinum-195	Ionizing Radiation	in PBS solution 100µCi / 0.2mls IV 300µCi / 0.2mls IV 200µCi / 0.2mls IV 100µCi / 0.2mls IV 300µCi / 0.2mls IV 300µCi / 0.2mls IV 10µCi / 0.2mls IV 50µCi / 0.2mls IV 50µCi / 0.2mls IV 25µCi / 0.2mls IV 50µCi / 0.2mls IV 100µCi / 0.2mls IV 100µCi / 0.2mls IV	I-125/60.14 d I-131/8.02 d Re-188/17 h Y-90/2.67 d Bi-212/45.5 min Bi-213/45.5 min Ac-225/10 d Tc-99m/6.01 h Ho-166/26.8 h At-211/7.214 h Tm-170/128.6 d Dy-166/81.6 h Pt-195/4.02 d	Mice
0239	<u>Radioisotopes</u> Iodine 123 Iodine 125 Iodine 131 Carbon 14 Fluorine 18	Ionizing Radiation	in 6% Bovine solution 20-30µCi / 0.2mls IV 10-20µCi / 0.2mls IV 10-20µCi / 0.2mls IV Not used currently 100µCi / 0.2mls IV	I-123/13.27 h I-125/60.14 d I-131/8.02 d C-14/5730 y F-18/109.77 min	Mice & Rats
0293	X-Ray	X-Ray Ionizing Radiation	> or = 20 cGy whole body exposure	NA	Mice
0286	MicroCT Scanner	Ionizing Radiation	whole body exposure 20 minutes to 2 hours	NA	Mice
	<u>Radioisotopes</u> Iodine 125 Iodine 131 Technitium 99m Thulium 170		maximum curie amounts 300µCi / 0.2mls IV 300µCi / 0.2mls IV 300µCi / 0.2mls IV 300µCi / 0.2mls IV	I-125/60.14 d I-131/8.02 d Tc-99m/6.01 h Tm-170/128.6 d	
0276	X-Ray	Ionizing Radiation	> or = 10 cGy (up to 600 cGy) whole body exposure	NA	Mice
0288	X-Ray	Ionizing Radiation	> or = 10 cGy (up to 600 cGy) whole body exposure	NA	Mice
0289	MicroCT Scanner	X-Ray Ionizing Radiation	whole body exposure	NA	Mice
0241	Argon Laser Beam (to induce laser fluorescence)	Laser-visible light range via fiber optic	Small targeted area, 1x/wk, 12-16 wks	NA	Mice
0296	MicroCT Scanner	Ionizing Radiation	Whole body exposure 20 minutes to 2 hours	NA	Mice
	<u>Radioisotopes</u> Iodine 125 Iodine 131 Technitium 99m		Maximum curie amounts 300µCi / 0.2mls IV 300µCi / 0.2mls IV 300µCi / 0.2mls IV	I-125/60.14 d I-131/8.02 d Tc-99m/6.01 h	

f. **Facilities, Procedures, and Monitoring**

1) **Description of Requirements for Showers and Change Facilities**

All full-time animal care personnel have access to shower facilities and are given time to shower before leaving at the end of the day. Showers for men in Bldg 9210 are located in Rooms T-22 and T-30. Women's showers in Bldg 9210 are located in T-10 and T-31. Additionally, shower facilities are available for part-time employees in all facilities housing animals.

2) **Description of Procedures that Reduce Potential for Injury**

Personal protective equipment includes: protective gloves, company-purchased clothing, safety shoes, safety glasses, hearing protection devices, and dust masks. Instructions provided to Bldg 9210 animal care staff include personal consultation, mandatory safety training classes, and review of our SOPs. Additionally, managers for all operations and projects at ORNL complete a web-based Research Safety Summary (RSS) that identifies all hazards and measures taken to address them. The RSS is reviewed by health and safety subject matter experts and approved by division management before work is authorized to proceed.

3) **Description of Special Facilities**

In Bldg 9210 animals exposed to hazardous (chemical) agents are housed within conventional animal rooms. In Bldgs 4500S and 4501 animals exposed to hazardous (radioisotope) agents are housed in positively ventilated caging.

4) **Description of Housing and Care for Animals Exposed to Hazardous Agents**

Animals exposed to hazardous (chemical) agents in Bldg 9210 are handled according to the Bull's Eye SOP provided in Appendix 9. Briefly:

- Animal Care Technicians and Research Technicians will wear dust masks and disposable latex, plastic, or rubber gloves while handling mice in Bull's Eye cages.
- Bull's Eye cages should be changed/handled last after all unmarked cages in the room are changed/handled.
- Feed must be added to Bull's Eye cages with a scoop. Feed on the cages or in the dispenser is not handled with gloved hands.
- Carcasses discovered in Bull's Eye cages must be bagged separately and disposed of in the specially marked container in the west freezer on the back dock.
- Gloves should be removed and thrown into the trashcan in the animal room before leaving the room.

- Animal Care and Research Technicians should wash hands after completing work with chemically-treated mice.
- Bull's Eye cages should be stacked separately and washed last. The machine should be acid washed after washing these cages.

Procedures for handling animals exposed to hazardous (radioisotope) agents in Bldgs 4500S and 4501 are, briefly:

- Individuals working in a Radiological Area or with radioactive material are required to complete ORNL Radiological Worker Training prior to performing any radiological work. Technicians providing husbandry for such animals have completed all required ORNL training, and all caging and husbandry activities are restricted to areas posted per ORNL Radiation Protection Procedure Guidelines. Such areas have a Radiation Work Permit (RWP) posted that lists the requirements to work in the area. Staff members are required to frisk with radiation monitoring equipment while working with radioisotopes and when exiting radiological areas. Radiological workers are enrolled by the area Radiological Control Technician (RCT) in a monitoring program, which includes dosimetry monitoring (thermoluminescent detectors, finger ring detectors, *etc.*) and bioassay (urine analysis, whole body count, *etc.*). The RCT routinely surveys these Radiological Areas for contamination, and any items removed, such as cages or animal bedding, are surveyed before release.
- All injection/administration of radionuclides is done in a HEPA-filtered Type C hood, which is approved for use of radioisotopes.
- Animals injected with radioactive materials are housed in a designated facility with procedures for entry and exit limited to specially trained individuals as defined by ORNL Radiation Protection Procedure Guidelines.
- Caging is changed and cleaned by the Principal Investigator or a single research assistant who has received appropriate training and certification as defined by ORNL Radiation Protection Procedure Guidelines. Radioactive-contaminated cages are first decontaminated with a commercial cleaning solution, followed by use of a hand counter to verify absence of radioactivity.

Radioactive waste disposal is performed using guidance provided in ORNL's Standards Based Management System "Subject Area: Solid Radioactive Waste Management." ORNL requires logging of all potentially radioactive waste, holding of waste for the predicted life of the radioisotope, and evaluation of the waste for radioactivity prior to disposal. Training is also required to dispose of waste, with the required waste training based on the categories of wastes being handled.

B. Animal Environment, Housing, and Management

1. Physical Environment

a. Housing

1) Primary Enclosures

Mice and rats are group-housed in solid-bottom plastic cages with stainless steel wire mesh or wire bar lids. Ventilated racks with caging and shelving are used for housing mice and rats in Bldgs 4500S and 4501. Conventional open caging is used for mice housed in Bldgs 9210 and 3500 (when animals are present in this temporary holding facility). In Bldg 9210, Rm 115 contains mice housed in solid-bottom plastic cages in ventilated racks with automatic watering. This room was set up to evaluate caging systems for the new SPF barrier facility that will replace Bldg 9210 in the summer/fall of 2003. Select groups of mice and rats have cage enrichment by way of PVC tubing or nestlets added to their cages.

Fish are housed in rectangular glass aquaria of various sizes and fiberglass tanks of various sizes and shapes.

For both rodents and fish, compatible animals are group housed whenever possible to allow for normal social interaction.

2) Sheltered or Outdoor Housing

Not applicable.

3) Naturalistic Environments

Not applicable.

b. Animal Space Provisions

1) Description of Sources/Process for Determining Cage/Pen Size

The "*Guide*" is used for determining the density of housing for mice and rats used at ORNL. Allowable animal densities are posted in all rodent animal rooms.

The "*Guide*" does not specifically address cage sizes for fish. EPA Guidelines for medaka culture suggest a range of 4-5 adult medaka per gallon. However, flow-through systems, which provide large volumes of fresh water, permit even higher stocking densities. Water quality, fish health, and degree of aggressive behavior (which can be associated with crowding) determine tank population numbers.

2) **Description of Exceptions to the “Guide” and Other Applicable Regulations**

The ORNL-ACUC approved an alternate breeding scheme for selected cages in Bldg 9210 that includes slightly more than the “Guide”-recommended mouse cage weight limits for short periods of time. The reason for requesting the alternate scheme was to permit increased production of animals for a large re-derivation effort. Because ammonia levels in some cases were measured to increase to unacceptable levels by day 6, cages are changed twice weekly to accommodate the housing exception.

c. **Temperature and Humidity**

Temperature minimum and maximum for each animal room is measured daily. Humidity minimum and maximum is measured daily for Bldg 4500S and for representative rooms in Bldg 9210. Additionally, a monitor for light levels, temperature, and humidity is placed in animal rooms on a weekly rotational basis in Bldg 9210. The veterinarian reviews documents generated from this monitoring process. Equipment being used to monitor temperature and humidity are validated by comparison to calibrated and certified instruments provided by the OSSD Representative.

Display tanks of native fish and brood fathead minnows are maintained at or slightly above ambient water temperatures. Research tanks of medaka, fathead minnows, and zebrafish are maintained at species-specific holding and spawning temperatures by the use of semi-submersible aquarium heaters or combination heating elements/thermistors/electronic controllers. Temperature of medaka brood stock tanks is monitored and archived electronically, with pager notification to the Principal Investigator of out-of-range conditions. Temperatures in all other tanks are hand-measured and recorded daily. Air temperatures are centrally regulated to remain within normal office temperatures. Humidity guidelines are not applicable to fish.

d. **Ventilation**

1) **Description of Performance Aspect**

The OSSD Representative on an annual basis (see Appendix 10) performs monitoring of ventilation and air balance. Air exchanges are measured using a balometer and air balance is evaluated based on manometers located throughout Bldg 9210 that monitor zones of animal rooms. Air balances in Bldgs 4500S and 4501 are positive (ventilated racking) and negative in Bldg 3500 where temporary housing of animal cages is in an exhaust hood.

Because air is constantly infused into tanks, each fish housing unit functions as a positive pressure room. Consequently, room ventilation rates do not impact animals as significantly as in terrestrial systems.

Airflow in rooms is checked periodically and documented (see Appendix 10).

2) Description for Special Primary Enclosure

There are 6 ventilated racks in Bldg 9210 (Rm 115), which were set up for evaluation and then subsequently selected for use in the planned new SPF barrier facility. They are double-sided Thoren racks, 3 holding duplex cages and 3 holding single cages. They are equipped with Edstrom Watering System. The air exchange rate is set at 50 changes per hour, and the racks are under positive pressure. The exhaust magnahelic gauge is set at .25 and the supply is set at .32. The exhaust prefilter is checked monthly and the supply prefilter is checked quarterly.

Ventilated racks are used in both Bldgs 4500S and 4501. Bldg 4500S has an Allentown Model MD7115UHT96MK individual cage ventilation system. A magnahelic pressure meter is used to monitor air exchange and the rate of exchange is maintained at 86.8 exchanges per hour. Prefilters are sanitized weekly. Bldg 4501 has two ventilated shelf racks, one Lab Products and one Allentown (model #RS10198UHT25MF). The Allentown rack is HEPA filtered. Air exchange is measured using a hand held velometer and the rate of exchange is maintained at 50-90 exchanges per hour. Roughing filters are changed two times per year or more often if needed. Conventional cages housed in Bldg 3500 are placed in an exhaust hood.

3) Description of Filtration/Treatment of Recycled Air

The Bldg 4500S ventilated rack vents to the animal room. The room itself has 100% fresh air exchange and prefilters and exhaust filters on the rack are HEPA. The Bldg 4501 animal room receives 100% fresh air, and one rack is hard-ducted to the exhaust system and the other has room air exhausting through a HEPA filter back into the room.

e. Illumination

For rodent facilities a 12:12, 14:10, or 10:14 light cycle is used depending on research needs. Light intensity varies from 5 to 80 Lumens (100-861 lux) at the cage level depending on location in the room (light intensity is monitored on a regular, rotational, basis). Previous measurements made at 1 meter above the floor for representative rooms in Bldg 9210 averaged 305 lux. Lighting fixtures are fluorescent and are controlled by automatic timers located outside the room door; the lights are not sealed. No ORNL animal rooms have windows that allow natural light exposure; most animal rooms do have windows opening to the corridor.

A 16:8 light cycle is used for illumination in facilities housing fish. Illumination in room 163 of Bldg 1505 ranges between 485-595 lux and room 263 in Bldg 1505 ranges between 430-920 lux, depending on location in room. Illumination in Bldg 1504 ranges between 380-860 lux. High intensity lighting (32,500 lux) is used over

the spiraling streams in that facility. Aside from the high intensity lighting, which is incandescent, all lights are fluorescent, non-sealed fixtures.

f. **Noise**

1) **Design Features and Control Methods**

One room used for behavior testing in Bldg 9210 has sound-absorbing panels covering the walls..

2) **Background Noise**

Radios, alarms, and other sound generators are not allowed in animal rooms. Activities in rodent and fish rooms do not generally generate excessive noise.

2. **Behavioral Management**

a. **Structural Environment**

Conventional rodent caging is used. Selected groups of animals receive cage enrichment in the form of PVC tubing or nestlets. Virgin pulp bedding used in Bldgs 9210 and 3500 provides opportunity for nesting and burrowing.

PVC pipes, rocks, or other types of cover are placed in community fish tanks to provide retreats for species of fish that require such cover. Other tanks are partially covered with opaque plastic covers to provide alternating protective shaded areas within the tanks.

b. **Social Environment**

1) **Physical Contact**

Mice and rats are, for the most part, group housed.

Fish species are group-housed in tanks, and are free to engage in normal behavioral and reproductive activities. Fish are not individually housed.

2) **Isolated/Individually House Animals**

No special arrangements are in place to address enrichment for singly housed rodents.

c. **Activity**

1) **Provisions for Species-Typical Activities**

Rodents and fish are allowed to exhibit species-typical behaviors such as exploration and foraging in their home cages and aquaria.

2) **Forced Activities**

Not applicable.

3. **Husbandry**

a. **Food**

1) **Type and Source**

Natural ingredient diet, Purina 5001, is generally used in Bldgs 9210, and Purina high fat diet 5020, is used for selected groups of animals to enhance production. Oatmeal or Lafeber Laboratories Bite-A-Berries are used as a supplement in some cases. Gamma-irradiated Pico-Lab Rodent Diet 20 (Purina, #5053) is used in Bldgs 4500S and 4501. Bldg 3500 receives animals from Bldg 9210 and houses those animals temporarily; therefore, food is the same as for Bldg 9210.

Experimental fish (other than fathead minnow fry) are fed a flake food from Ziegler Bros., Inc., Fish Diet /Aquatox flakes, and are also fed brine shrimp, freshly-hatched (in-house) from San Francisco Bay Brand artemia eggs supplied by Inve Aquaculture, Inc. and Salt Lake Brine Shrimp from Brine Shrimp Direct. Fathead minnow fry are fed newly hatched brine shrimp from another source (Avocet Artemia Inc., Salt Lake City, Utah). Display fish are fed frozen San Francisco Bay Brand brine shrimp or earthworms.

2) **Storage Facilities of Vendors**

For Bldgs 9210 and 3500 feed is shipped directly from the manufacturer or, for the Pico-Lab Diet, shipped to a distributor who in turn overnight ships to our laboratory. Animals housed in Bldgs 4500S and 4501 use Purina 5053 irradiated PicoLab Rodent Diet 20, which is ordered through Green Farm and Garden Supply in Oliver Springs, TN (15 miles away). Green's delivers directly to ORNL receiving and they deliver the feed to 4500S. Research staff members transport feed to the Bldg 4501 temporary housing facility on an as-needed basis.

Aquatox flake food is produced on demand and shipped immediately from the vendor in plastic bags purged with nitrogen. Brine shrimp cysts are packaged in vermin-proof cans at the originating companies and stored at approximately 4 degrees C.

3) **Storage in Animal Facilities**

Food for the large mouse colony in Bldg 9210 is purchased directly from the manufacturer by the truckload. An environmentally controlled storage room (Room 101) capable of holding this large quantity of feed is used for storage. Temperature and humidity levels are periodically measured by the OSSD Representative and maintained between 70-74 degrees F and below 50% humidity. Pest control is provided by a contract service and various pest-monitoring devices are used to ensure vermin are not present. Food for Bldgs 4500S and 4501 is stored in 4500S, Room F-147, an environmentally controlled storage room in which the temperature and humidity are monitored periodically. Live traps for rodents and sticky traps for roaches are used in the storage room to monitor for vermin. No food is stored in Bldg 3500.

Brine shrimp cans and stock bags of flake fish food are stored in the dark at less than 4 degrees C as per the vendor's instructions. Working stocks of flake food are stored temporarily (for approximately 1-2 weeks) in sealed jars within the laboratories where fish are located. Frozen brine shrimp are shipped on dry ice and maintained frozen until use. Worms are maintained in culture in Bldg 1504 or purchased from local bait shops.

4) Storage in Animal Rooms

In all facilities, food is stored in sealed containers in animal rooms. In rodent facilities, the container is lined with plastic (Bldg 9210) or sanitized each time it is refilled (Bldgs 4500S, 4501, and 3500). In Bldg 9210, the feed bag plus feed is placed in the container; the plastic liner and feed are replaced quarterly. A milling date is recorded on any interim container.

Fish are housed in laboratory areas; therefore, food storage is as described above in Storage in Animal Facilities.

5) Food Preparation Areas

Not applicable for rodents.

Food preparation for fish occurs in the laboratories where the fish are located.

6) How Food is Provided

Food is provided *ad libitum* to all rodents in wire mesh or wire bar cage top feeders.

Crushed flakes are provided *ad libitum* on tank surfaces one to three times per day. Brine shrimp solution (1-5 ml) is poured into tanks, generally twice daily. Frozen brine shrimp for display tank fish are thawed in small amounts of tank water prior to feeding. Earthworms are chopped and dumped in tanks several times weekly.

7) Quality Control Procedures

All rodent feed is used within six months of the milling date, with all feed typically replaced quarterly. The oldest feed is used first, and any feed remaining from a previous shipment is moved to the front of the newly arrived feed. Feed for Bldg 9210 is generally ordered every 3 months, and feed for cage tops, located in supply barrels in mice rooms, is changed on a quarterly basis to ensure freshness and nutritive quality or more frequently to ensure use within the milling date. Monitoring of feed quality is limited to that done by the manufacture (QA documents from the manufacturer are reviewed by the veterinarian).

Aquatox flake fish food is formulated as a complete balanced diet with ingredients and handling maximized for low contamination. Ingredient and nutrient components are kept constant to minimize nutrient variability. Brine shrimp are the highest quality provided by vendors. Amounts of food purchased are calculated so that foodstuffs are consumed within the viability dates as provided by the manufacturers or vendors. Out-of-date food is discarded.

b. Water

1) Source, Treatment and How Provided

Potable water comes from the City of Oak Ridge water treatment plant. Water used in Bldg 9210 is hyperchlorinated to 15-20 ppm of free chlorine using sodium hypochlorite in Edstrom Industries chlorine injector stations. Water provided via Edstrom automatic watering in Room 115 is chlorinated at 5-7 ppm. Water in Bldgs 4500S and 4501 is autoclaved. Bldg 3500 receives animals from Bldg 9210 and houses those animals temporarily; therefore, water is the same as for Bldg 9210. In the event that a water bottle would need to be refilled tap water would be used.

Water used in the aquatic facilities in ESD originates as "potable" water from the City of Oak Ridge water treatment plant. Water is dechlorinated (using activated charcoal) and filtered before use at the ESD complex. Water for the spiraling streams in Bldg 1504 originates at the spring-fed headwaters of 1st Creek on the Oak Ridge Reservation.

2) Quality Control Methods

Potable water is analyzed for chemical contaminants and water quality daily (at pumping station). ORNL has the capability to store 7.5 million gallons of potable water. To ensure integrity of ORNL's water distribution system, ORNL takes monthly samples of their water supply at 12 points through out the distribution system (bacterial counts, chlorine concentration, etc.).

In Bldg 9210 chlorine levels delivered by the Edstrom Industries chlorinators are monitored on a monthly basis, and monthly samples are

collected for culturing of bacterial levels. Aside from monitoring autoclave function, evaluation of effectiveness of water sterilization in Bldgs 4500S and 4501 are not routinely performed.

Water for fish tanks is analyzed for chemical contaminants and water quality daily (at pumping station). Chlorine levels are constantly monitored at ESD before the water is routed to fish tanks. Periodic cultures of incoming water are used to check for bacterial contamination.

c. Bedding

1) Type, How Used, and How Selected

Carefresh© virgin pulp shredded paper contact bedding, provided by Absorption Corp., is used for mice housed in Bldg 9210 and Bldg 3500. Carefresh© bedding was selected based on absorptive qualities, burrowing and nesting enhancement, and virgin (not recycled) paper source. Hardwood chip contact bedding is used in Bldgs 4500S and 4501, selected for its absorptive qualities, cost, and consistency of quality. Bldg 3500 receives animals from Bldg 9210 and houses those animals temporarily; therefore, bedding is the same as for Bldg 9210.

In Bldg 1504, substrates are used in display tanks for decorative, enrichment, and water quality purposes. Aquarium gravel, plants, and rocks are used in these cases.

2) Storage Facilities/Vermin Control

Rodent bedding is stored on pallets in all facilities with monitoring devices (rodent and insect monitors) to ensure the absence of vermin. Storage is in Rooms 190 (9210) and 147 (4500S). No bedding is stored in Bldg 3500 or in Bldg 4501.

3) Quality Control Procedures

Monitoring of fungal levels in bedding dispensers is performed monthly in Bldg 9210 (Bldg 3500 utilizes bedding from this facility), and review of manufacturer QA reports constitute quality control for bedding. Review of manufacturer QA reports constitute quality control for bedding for Bldgs 4500S and 4501.

d. Miscellaneous Animal Care and Use Equipment

1) Motorized Vehicles

Company vans or trucks, environmentally controlled but not limited to use for animal transport, are available for transport between ORNL sites and for transporting animals from wildlife field sites to the lab. Cages of mice are placed in a closed ventilated sanitizable container during transport. An environmentally controlled van, dedicated for rodent transport, is used to

transport mice to other TMGC sites in Tennessee. Cages of mice are contained in a sanitizable compartment in the TMGC van.

2) Other Equipment

- Two forklifts are used for moving palletized feed and bedding (Bldg 9210).
- Chlorinators for filling water bottles (7 total, 5 in use) (Bldg9210).
- Chlorinator (1) for automatic watering system in animal room 115 (Bldg 9210).
- Autoclaves (3) located in Bldgs 9210, 4500S and 4501.
- Tunnel washers (4) located in Bldg 9210.
- Ventilated racks (3) located in Bldgs 4500S and 4501, and (6) located in Bldg 9210.
- Temperature monitors for fish tanks in Bldg 1505, with pager notification of PI

e. Sanitation

1) Bedding Change

a) Frequency of Contact and Non-Contact Bedding Changes

Consistent with guidelines, solid bottom caging is changed once weekly. Contact bedding is provided for all rodents. Twice weekly bedding changes are made for trio matings with a litter in Bldg 9210 and for SCID mice housed in Bldg 4500S. In Bldg 9210, bedding is changed on a 14-day schedule for animals in ventilated racks and for one group of mice in solid bottom conventional open cages (see Exceptions to “*Guide*” below).

b) Exceptions to “*Guide*” (or Regulations) Recommended Frequencies

The ORNL-ACUC has approved an animal cage change frequency of 14 days for one Bldg 9210 rodent animal room containing ventilated racks. Ammonia levels inside cages were measured and were acceptable at 14 days.

The ORNL-ACUC also approved an animal cage change frequency of 14 days for one subspecies stock of mice in Bldg 9210 (castaneous mice, CAST/Ei stock from the Jackson Laboratory, *Mus musculus castaneus*). Breeding of this stock is very difficult and extension of the cage change frequency has allowed breeding pairs to be established. Ammonia levels inside cages were measured and were acceptable at 14 days.

c) Location Where Soiled Bedding is Removed

In Bldg 9210, dumping stations are present in all (3) cage-washing rooms, with one located on each floor of the 3-story building. Cages are filled at the off-end of the tunnel washer. There is separation of clean/dirty activities in the cage wash rooms via floor markings, although these rooms are large open rooms where both activities are performed.

The Bldg 4501 temporary housing facility consists of a single-room facility with all husbandry performed in the animal room during periods when animals are absent. Cages are dumped in the room, hand washed, air-dried, clean bedding put in, and then autoclaved. The Bldg 4500S animal facility has a separate room where cages are dumped, hand washed, air dried, clean bedding put in, and then autoclaved. In Bldg 3500 cages are dumped, rinsed, bagged, and returned to Bldg 9210 for sanitation.

2) Cleaning and Disinfection of Primary Enclosures

a) Washing/Sanitizing Frequency For:

i. Solid Bottom Cages

Cage inventories for all facilities are listed in Appendix 11.

Most rodent cages are washed once weekly. There are some groups of cages washed twice weekly; cages in ventilated racks and one small group of cages are washed every 14 days. The 14-day cage changing has been approved by the ORNL-ACUC.

In Bldg 1505, fish tanks are emptied and sanitized between groups of fish (approximately every 18 months for flow-through systems). Approximately two thirds of the recirculating tank water is replaced with fresh water on a weekly basis.

In Bldg 1504, glass display tanks contain fish year round, have a gravel substrate, and are supplied continuously with water. Algae are scraped from the rocks and glass, and organic matter and wastes are siphoned off about once every 2-3 weeks. Once a year, the water level is lowered, all the rocks are removed and rinsed, the tank inside is scraped, and the outside of the tank is cleaned. Fish remain in the tank during these procedures, so they are never completely emptied and sanitized. If disease or fungus occurs, they are treated *in situ*.

In Bldg 1504, experimental fish tanks include round and square fiberglass enclosures without substrate but with continuous water flow. These tanks are cleaned at the end of an experiment. The tanks are drained, algae are scraped off the sides and bottom, and the tank is treated with a mild bleach solution.

ii. Suspended Wire Bottom/Slotted Floors

A small number of Lucite metabolic cages with slotted floors are used under one protocol. They are sanitized before and after each experiment, at a frequency of 7 days or less.

iii. Cage Tops

- Bldg 9210- Twice monthly.
- Bldgs 4500S and 4501- Every two weeks.
- Bldg 3500- No long-term animal housing.

iv. Cage Racks and Shelves

- Bldg 9210- Shelf bars are cleaned quarterly, and ventilated racks in use in Rm 115 are wiped down every 14 days.
- Bldgs 4500S and 4501- Once weekly.
- Bldg 3500- Exhaust hood used for temporary holding is sprayed and wiped with a quaternary disinfectant between groups of animals.

v. Cage Pans Under Suspended Cages

Not applicable.

vi. Playpens, Floor Pens, Stalls, etc.

Not applicable.

vii. Corrals/Outdoor Paddocks

Not applicable.

b) Cage Washing/Sanitizing Procedures

In Bldg 9210, tunnel washers are located on each floor of the 3-story mouse facility (Industrial model M105690, Consolidated model CWTSB 30-26, Girton model G1536S). Cages are washed in the 2nd and 3rd floor tunnel washers, with the 1st floor washer

serving as back-up. A fourth tunnel washer (MTP series 2000) is located on the 2nd floor and is used to wash cage tops and miscellaneous small equipment. The 1st floor cage washer is used as back-up only (Industrial model M105690).

In Bldgs 4500S and 4501, cages are dumped, rinsed, and allowed to soak with quaternary ammonia disinfectant, then rinsed, dried, filled with bedding, and autoclaved. Radioactive cages are first decontaminated with a commercial cleaning solution. Caging used in Bldg 3500 is returned to Bldg 9210 for sanitizing.

Flow-through aquaria holding research stocks of fish are partially cleaned at least once a week by siphoning fish wastes and brine shrimp shells. Every other week, the sides of the aquaria are wiped to remove algae. These tanks are broken down and sanitized only when populations are rotated, or approximately every 18 months. In static renewal tanks of research fish, approximately 1/3 of the water is changed weekly while siphoning waste, and aquarium walls are wiped down as needed to remove algae. Static renewal tanks are completely broken down and disinfected between uses. Display tanks are supplied continuously with water and are periodically (~ every 2-3 weeks) siphoned to remove excess wastes, algae are scraped from rocks, and the inside of the tank is scraped as needed to remove algae. Otherwise, these flow-through display systems and biological filters are not disturbed. Supplies used in short-term fathead minnow fry toxicity tests are disinfected between every use.

c) Cleaning/Sanitizing Agents Used

In the rodent facilities, Pharmacal detergent (Clout©), acid (Acid Power©), and quaternary ammonia (Quatricide PV©) are the primary cleaning agents used. Additional agents used include 8.5% sodium hypochlorite (Bleach), phosphoric acid cleaning agents (Lime Away), and a glassware detergent (Alconox©).

Fathead minnow caging is acid-sanitized between groups of fish (Bldg 1504). Bleach (5%) is used for washing equipment and supplies for the animal room in both the aquatic toxicology lab in Bldg 1504 and in the animal room in Bldg 1505. Equipment is rinsed and allowed to sit for 24 hours prior to use.

d) Exceptions to “Guide” (or Regulations) Recommended Sanitation Intervals

The “Guide” recommends weekly intervals for cleaning solid-bottom cages. Fish in aquaria, although technically housed in solid-bottom cages, are in a much more complex environment that is easily disrupted by weekly cleaning. This is particularly true of recirculating systems, where bacterial populations necessary for

biological filtration take some time to establish. Additionally, physically netting and removing fish from their aquatic environment is stressful and can be especially disruptive to breeding populations. The fish in Buildings 1504 and 1505 are housed in either well-filtered or high volume flow through systems, which effectively prevent toxin and waste build-up. Routine cultures monitor bacterial populations. Cultures were taken from both newly set up tanks and well-established systems, and colony counts were comparable in both cases. Based on this information and the previous justification, the ORNL-ACUC has approved this practice.

3) **Cleaning and Disinfection of Secondary Enclosures**

a) **Animal Room Cleaning Frequency, Procedures, Methods and Agents**

Animal rooms in Bldgs 9210, 4500S, and 4501 are cleaned and sanitized at least once a year or between groups of animals as needed. Rooms are emptied of animals, all surfaces are pressure sprayed with a quaternary ammonia disinfectant, and wiped dry. All supplies (feed barrels, brooms, and dust pans) are sanitized. Feed and bedding barrels in Bldgs 4500S and 4501 are sanitized quarterly in addition to the annual room sanitization. HVAC vents in animal rooms in Bldg 9210 are cleaned quarterly. Temporary holding space in Bldg 3500 is an exhaust hood that is sanitized after each use with a quaternary ammonia disinfectant.

Weekly cleaning activities in occupied rodent rooms include sweeping, mopping with a quaternary ammonia disinfectant, and use of a quaternary disinfectant to clean barrel lids and door knob area of animal room doors. Investigators also sweep rooms they have been working in.

Bimonthly cleaning in Bldg 1505 includes bleach sanitization of working surfaces, and use of Kelsan or Sporidicin disinfectants for floors. In Bldg 1504 in the fathead minnow area working surfaces are bleach sanitized before and after a test.

b) **Corridor and Support Area Cleaning**

Bldg 9210 -- Corridors and support areas are dust mopped daily and wet mopped weekly with a quaternary ammonia disinfectant. Corridors are waxed quarterly.

Bldgs 4500S, 4501, 3500, 1504, and 1505 -- The animal room is used for support activities.

c) Implements

Bldg 9210 -- Mop heads are rinsed and left to air dry after use daily and are changed monthly. The mop heads are laundered commercially. Mop buckets are cleaned once monthly in the tunnel washer used for cleaning cage tops. Brooms and dustpans are sanitized as needed.

Bldg 4500S and 4501 -- Sponge mop heads are replaced every few months. Brooms and dustpans are sanitized as needed. In Bldg 3500 sweeping and mopping in animal room is performed by bargaining unit janitors and changing of mop heads and sanitation of implements are not monitored by animal care program personnel.

In Bldg 1505 nets and equipment are dedicated to large tanks and are sanitized using bleach when tanks are broken down. Recirculating tanks share some supplies, which are sanitized between tanks. For fathead minnows in Bldg 1504 no nets, *etc.* are used.

d) Separation of Cleaning Implements By Room

Bldg 9210 -- Brooms, mops, and dustpans are labeled for dedicated use in specific animal rooms. Mop buckets (using fresh water and quaternary disinfectant for each room) are used in multiple animal rooms.

Bldgs 4500S and 4501 -- A single animal room is located in each facility.

Cleaning implements are not shared among rooms in Bldgs 1504 and 1505. In Bldg 1505 large tanks have dedicated implements while smaller tanks do share implements.

4) Sanitation of Cage Equipment

a) Procedures and Frequency for Feeders

Not applicable.

b) Procedures and Frequency for Watering Devices

For Bldgs 9210, 4500S, and 4501 water bottles and sipper tubes are changed weekly. In Bldg 9210 used bottles are placed in mobile wagons with a tub for holding the water bottles. Bottles are taken from the animal room, sipper tubes and stoppers are removed, the bottles are dumped and rinsed with water, then filled and stoppered with stoppers that have been heat sanitized using boiling water. Sipper tubes and stoppers are boiled for 10 minutes.

Water bottles are de-scaled quarterly using an acid wash. In Bldgs 4500S and 4501 bottles, stoppers, and sipper tubes are hand washed in quaternary ammonium solution, rinsed, bottles filled with distilled water, stoppered, and autoclaved.

c) Procedures and Frequency for Enrichment Devices

PVC tubing used for enrichment is removed from the cage and sanitized every other week. Nestlets are removed with each cage change unless a new litter is present, in which case the nesting would be removed the following week. PVC tubing in Bldg 9210 is washed in the tunnel washer; in Bldgs 4500S and 4501 they are hand washed in a quaternary ammonium solution, rinsed, and autoclaved.

PVC and other types of breeding habitats or enrichment structures for fish in Bldgs 1504 and 1505 are removed and sanitized with bleach when tanks are disassembled between groups of animals.

5) Sanitation of Transport Cages, Equipment, and Vehicles

In Bldg 9210, animals are transported in the primary cage within the building. Animals are sometimes transported to Bldg 3500. After completion of studies at that site the cages are dumped, rinsed with a quaternary ammonium solution, bagged, and returned to the 9210 facility where they are treated as dirty equipment. Transportation is via passenger vehicle, which has a sanitizable closed container for the transportation of animals. An SOP for transporting animals between facilities is provided for users.

Animals are transported between Bldgs 4500S and 4501. Mice are transported in microisolator cages to 4501, housed there temporarily (up to 24 hours) in standard caging in a ventilated shelf rack, and returned to 4500S in clean microisolator cages. Transportation is via passenger vehicles, which are not specifically sanitized for the transportation of animals.

Re-derived ORNL mice are housed at UT College of Veterinary Medicine SPF facilities; they are transported by ORNL staff to other member institutions of the Tennessee Mouse Genome Consortium (TMGC), which are located across the State of Tennessee. The dedicated transport van is specially equipped with a stainless steel insert to make it easily sanitizable. A tension pole (similar to a shower curtain rod) is used to keep cages in place during transport. Mice are generally in sealed microisolators. The van is also used to transport carcasses to the University of Tennessee College of Veterinary Medicine incinerator. The van is sanitized with Quatricide after every use.

Transport of live field-collected fish to laboratories at ESD is by passenger vehicles, which are not specifically sanitized for the transportation of animals.

6) How Effectiveness of Cage Sanitation is Monitored

In Bldg 9210, monthly culture samples are taken from caging supplies on all floors. In addition, temperature tapes are run weekly for each tunnel washer, and for the 3 cage washers the starting and ending temperatures for each run are recorded on a log sheet. Log sheets are reviewed monthly and a monthly QA Report is generated by the Quality Assurance Coordinator and reviewed by the veterinarian. Animal care staff are advised that the cage washers should not be run if temperatures are not at least 180 degrees. The supervisor reviews recorded activity tape printouts for the newer tunnel washer.

All caging supplies in Bldgs 4500S and 4501 are autoclaved. Use of temperature-sensitive tape and review of activity tape printouts ensure that autoclaves are operating properly.

For Bldgs 1504 and 1505 random swabs are taken on a quarterly basis for microbiological monitoring after fish tanks and implements are washed.

f. Waste Disposal Methods

1) Soiled Bedding and Refuse

In Bldg 9210, bedding dumped at the dumping stations is transported via conveyor systems directly to dumpsters. Dumpsters are emptied according to need, generally more than once weekly. Dumpsters are emptied at an approved sanitary landfill.

In Bldgs 4500S and 3500, non-radioactive bedding is bagged and disposed of with regular trash. In Bldg 4501 bedding not subjected to radiological contamination is disposed in regular trash after being checked by a Health Physics Technician and being certified as uncontaminated. Radiologically contaminated bedding is disposed of as radioactive biological waste. Radioactive-contaminated cages in 4500S and all cages in Bldg 4501 are first decontaminated with a commercial cleaning solution, followed by use of a hand counter to verify absence of radioactivity. If the cages are found to be contaminated with radioactivity, then they are treated as radiological waste. Cages, animal bedding, and any supplies found to be radioactive are segregated and discarded into compatible radioactive waste containers and disposed of according to ORNL waste acceptance criteria. Waste generators receive training in categorization and proper disposal of all hazardous wastes.

In Bldgs 1504 and 1505 water and waste from fish tanks is flushed down the drain.

2) Animal Carcasses

Animal carcasses from Bldg 9210 are collected and placed temporarily in carcass refrigerators located in Rms. 232 and 328, or directly into freezers on the north loading dock. Carcasses are removed from refrigerators and transferred to freezers daily. Frozen carcasses are transported for incineration at the University of Tennessee College of Veterinary Medicine incinerator once weekly.

In Bldgs 4500S and 4501, all animal carcasses are sent to 4501 for disposal and are disposed of as radioactive biological waste. Radioactive animal carcasses are accumulated in a freezer located in Bldg 4501 and are disposed of (incinerated) according to ORNL waste acceptance criteria. Carcass waste is double-bagged, packaged in Department of Transportation (DOT) approved boxes, are DOT-labeled, are checked by a Health Physics Technician, and the appropriate waste disposal documentation is prepared.

In Bldg 3500, uncontaminated carcasses are taken to 9210 for incineration. Carcasses from radiolabeled animals are transferred to Bldg 4501 to be disposed of with their radiologically contaminated carcasses.

Fish carcasses known to be uncontaminated are ground and flushed down the drain using a fish grinder located in Bldg 1505, Rm 153.

3) Hazardous Wastes

Carcasses of chemically treated mice from Bldg 9210 are bagged and labeled, placed in a dedicated freezer, and then incinerated at the University of Tennessee College of Veterinary Medicine incinerator once weekly. No infectious or radioactive hazards are used in animals in this facility.

In Bldgs 4500S and 4501, all animal carcasses are sent to 4501 for disposal and are disposed of as radioactive biological waste. Radioactive animal carcasses are accumulated in a freezer located in Bldg 4501 and are disposed of (incinerated) according to ORNL waste acceptance criteria. Carcass waste is double-bagged, packaged in Department of Transportation (DOT) approved boxes, are DOT-labeled, are checked by a Health Physics Technician, and the appropriate waste disposal documentation is prepared.

In Bldg 3500, carcasses from radiolabeled animals are transferred to Bldg 4501 to be disposed of with their radiologically contaminated carcasses.

Potentially contaminated fish carcasses may be stored or archived for a period of time in freezers in Bldg 1505, Rm 153, then are disposed of at the Y-12 Landfill, in compliance with ORNL waste stream requirements.

g. Pest Control

1) **Program**

The pest control program for Bldg 9210 is administered by contract service through Russell's Pest Control Co., Inc. All service portions of the building and outside dock areas are inspected and treated monthly, more often if needed, by licensed pest control technicians. Agents used include baits, traps, and chemicals (Dursban, Empire, BP-100, Max Force bait, Avert, cmpui20). Administration of chemical agents is limited to service areas, dock, and laboratories. Insecticides are changed at the discretion of the pest control technician, and new chemicals must be approved by the veterinarian prior to use. All pest control activities provided by the contractor are recorded in a log maintained in the veterinarian's office. This log provides Material Safety Data Sheets (MSDS's) for all agents used. In addition to contracted services, monitors are placed strategically for roach detection by the veterinary staff, and snap traps are used to control escaped rodents.

Bldgs 4500S, 4501, and 3500 have live rodent traps and roach monitors in place. To date there has been no indication of vermin present in those facilities.

In Bldgs 1504 and 1505 rodent traps and roach monitors are strategically placed and monitored by research staff.

2) **Notification of Animal Users**

Concerns for the use of insecticides and their potential impact on mutagenesis studies performed in Bldg 9210 would require investigator review of all chemicals used. The Mammalian Genetics Group Leader would be contacted or a building-wide e-mail notification would be generated describing any plans to utilize chemicals (this has not occurred within the last 3 years; baits rather than chemicals are utilized).

h. **Provisions for Emergency, Weekend, and Holiday Care**

1) **Procedures for Weekend/Holiday Care**

Bldg 9210 -- Weekend care is provided by research staff (investigators and technicians) and the veterinary staff, on a rotational basis. The veterinary staff or the animal care staff supervisor provides Holiday duty. Each room is checked as described by SOP for food, water, wet cages, water bottles not working, sick or dead animals, lighting, and temperature.

Bldgs 4500S and 4501 -- Weekend and holiday care is provided, as described above, by the investigative staff of Drs. Kennel and Knapp. If animals are held over a weekend or holiday in Bldg 3500 the investigative staff provide care.

In all cases documentation of weekend and holiday checks are made on animal room door sheets. In addition, a logbook is filled out in Bldg 9210 by the on-duty personnel for communicating any information to the next person on duty or to responsible staff following the weekend or holiday.

ESD animal care staff check all fish daily including weekends and holidays. A local log of these activities is maintained for each group (aquatic toxicity laboratory, display tanks, the 1505 fish holding room).

2) **Procedures for Contacting Responsible Animal Care and/or Veterinary Personnel**

The names of several responsible individuals with their pager, home, and work telephone numbers are posted at the entrances to each rodent and fish facility. The veterinarian is available by beeper if questions or problems arise. In addition, the Plant Shift Superintendent's (PSS) at Y-12 or the Laboratory Shift Superintendent's (LSS) office at ORNL are fully staffed emergency and operations centers that can be contacted and have contact information for the Facility Manager, the Assistant Facility Manager, the Veterinarian, and appropriate Principal Investigators.

3) **Procedures for Monitoring Animal Facility Mechanical Systems**

In Bldg 9210, utilities operators are on duty 24 hours a day, 7 days a week and check the animal facility twice every 12 hours. The Y-12 utilities operations office is alerted when selected animal room temperatures are below 66 degrees and above 74 degrees Fahrenheit. There are alarms for all AC units and exhaust fans. Monitoring is also done manually by monitoring minimum/maximum temperatures, and using monitoring devices to measure temperature, humidity, and light levels. In Rm 115, the automatic watering system is monitored via the Edstrom Watchdog System. It alarms if there is any water flow problem. For after-hour alarms, the system is programmed to call the Y-12 Plant Shift Superintendent's Office, which has contact names and numbers. The Thoren ventilated racks in Rm 115 have magnahelic gauges, which show if there is any problem with airflow, but they are not equipped with, alarms.

Bldg 4500S, 4501, and 3500 steam and power are monitored by the ORNL utilities office.

Temperatures in fish streams in Bldg 1505 are monitored and alarmed (notifies the PI of a variation outside of 20-30 degrees Centigrade). Humidity sensors on the floor alarm and notify the PI of leaks. Chlorine levels are monitored constantly and alarm locally and at the LSS office (PI would be notified of any variation in chlorine levels). The ORNL utilities office monitors steam and power.

4) **Brief Description of Disaster Plan**

There are existing well-established emergency and disaster organizations in place at both ORNL and Y-12 to address adequate provisions for facilities and personnel. Such activities are coordinated through the offices of the Laboratory Shift Superintendent (LSS) at ORNL and the Plant Shift Superintendent (PSS) at Y-12. Both the LSS and the PSS organizations are staffed by professionals who are responsible for and experienced and trained in coordinating all emergency actions at the extensive U.S. Department of Energy facilities. DOE-provided in-plant services of fire, security, and medical personnel and equipment are available on a 24-hour basis.

These emergency organizations form the foundation for ensuring responsible care for ORNL's animals in the case of a disaster. Names of key animal personnel, who are most knowledgeable about the animals' needs and are qualified to make decisions about their care according to conditions at hand, are on the priority emergency call list for the LSS and PSS as follows:

- Bldg 9210 (at Y-12) - Institutional Veterinarian, Veterinary Technician, Lab Animal Resources Group Leader, Mammalian Genetics Group Leader, Complex Manager, Facility Operations Manager, Assistant Facility Manager, Animal Caretaker Supervisor, LSD Safety Officer/OSSD Representative.
- Bldgs 4500S, 4501, 3500 (at ORNL) - Institutional Veterinarian, Veterinary Technician, Principal Investigator or head technician for animals in each building, Facility Operations Manager, and the LSD, Nuclear Science and Technology Division, and the Engineering Science and Technology Division Safety Officers and OSSD Representatives.
- Bldgs 1504, 1505 (at ORNL) - Institutional Veterinarian, Principal Investigators with animals in each building, Complex Manager, Facility Operations Manager, ESD Safety Officer.

4. Population Management

a. Identification and Records

1) Methods for Identification of Each Species

Cage cards identify all rats and mice. In the Bldg 9210 facility most mice are ear notched at weaning as needed for identification purposes; one investigator uses ear tags. Cage cards contain genetic information, identification numbers of animals in the cages (where necessary), date of birth, mating (when appropriate), any experimental manipulation or pertinent information, and, in some cases, a cage number. PI and protocol information are located on the animal room door sheet in Bldgs 9210 and 4500S, and on the cage card in Bldg 4501.

Each fish tank has a card identifying the species housed within. Cage cards also contain the protocol number, investigator's name, and telephone number.

2) Procedures for Maintaining Individual Records

Individual records in the form of breeding cards, pedigrees, and computer records for selected lines of mice housed in Bldg 9210 are maintained by research staff and are available for review by the veterinarian or ORNL-ACUC by contacting the lead research technician. No individual animal health records are maintained, but health check cards are maintained with cages of animals when appropriate and reviewed by the veterinarian or veterinary technician and filed by room number once removed from a cage. Individual records are not kept for fish.

b. Genetics and Nomenclature

1) Program for Advising Investigators on Selection of Animals Based on Genetics

Mice in Bldg 9210 have been used for genetics research for over 50 years, and investigators utilizing them are knowledgeable of the genetic issues potentially influencing their research. Dr. Kennel and Dr. Knapp (who is not a part of the Mammalian Genetics Group) are not located in Bldg 9210 and have ongoing studies with established model systems. For these reasons there is no active program for educating investigators utilizing rodents at ORNL about choice of animals for model systems (although the veterinarian is available for providing relevant information). Investigators typically employ their own experience in the generation of genetic models (that is one of the major foci of the Mammalian Genetics Program at ORNL), as well as information in Genetic Variants and Strains of the Laboratory Mouse, Third Edition (Lyon, Rastan, and Brown, eds., Oxford University Press, 1996), and phenotype information found in the scientific literature and now on Web-based databases (e.g., the Jackson Laboratory's Bioinformatics site <http://www.informatics.jax.org>). Additionally, the ORNL-ACUC considers issues such as appropriateness of model in the protocol review process.

In cases where a mouse model for a human genetic disease is specifically created, or is sought from among a potentially mutant population, biochemical, behavioral, or morphological parameters known to be relevant in humans are used as guides for assessing the appropriateness of the model or expression of the known human abnormality in the mouse.

2) Program for Advising Investigators on Use of Standard Nomenclature

Until 1999, this had been an informal procedure in which investigators sought advice about nomenclature from Drs. L. B. Russell and E. M.

Rinchik. Dr. Russell, now retired, has had a 50-year distinguished career in mouse genetics, and has served in the past on the International Committee for Standardized Nomenclature for the Mouse. Dr. Rinchik, currently Mouse Genetics and Genomics Program Leader at ORNL, has had over 20-years' experience in mouse genetics and is currently a member of the International Committee on Standardized Genetic Nomenclature for Mice of the International Mammalian Genome Society.

At present, if they do not already know the mouse nomenclature rules, all senior investigators are made aware that they are published on the Mouse Genome Informatics Web Site at the Jackson Laboratory (<http://www.informatics.jax.org/mgihome/nomen/>). Internal peer review of manuscripts before submission to journals has generally insured that nomenclature rules are followed when genetic data are published by ORNL staff.

With publication of ORNL mutant mouse stocks on the Web, as well as with the intimate involvement of ORNL's Mouse Genetics Program in the Tennessee Mouse Genome Consortium (TMGC), a more rigorous review of nomenclature for mutant stocks advertised on the ORNL Mutant Mouse Database (<http://public.ornl.gov/Mouse/SearchFoRmcfm>) is now routine. Before any mutation is listed on this ORNL website, a committee composed of Drs. Rinchik and Russell, as well as Ms. Darla Miller, Oak Ridge administrator for the TMGC, review the data and nomenclature for each stock. Both senior investigative and technical staff have been notified in writing of this more rigorous nomenclature standard.

3) **Breeding Colony Maintenance**

a) **Species, Stocks and Strains**

Only mice are bred in Bldg 9210. Except for one non-inbred stock of *Mus spretus* continually bred, and various *M. musculus/spretus* F1 hybrids sometimes produced, all mice are *Mus musculus*. A number of inbred strains (some maintained here since the late 1940s, and others imported over the years) are maintained, as well as a number of segregating-inbred and congenic strains. The current collection of strains can be found in ORNL's Mutant Mouse Database (<http://bio.lsd.ornl.gov/mouse/>).

b) **Breeding Scheme**

Each mouse of an inbred strain or a non-inbred mutant stock receives a unique number, which is ledgered, and can be traced back to the original animal in the colony, even in strains whose founders were established here in the 1940s. For inbreds, we currently set up brother X sister pairs directly from the breeding pen at weaning. Small colonies of standard inbreds are usually kept no larger than 6-8 duplex pens, and maintainers are instructed to never go more than three generations before the colony is cut

back to a single line (even when there are 20-50 pens). Some investigators are more rigid in their inbreeding, bottlenecking each generation. Historically, large production colonies (e.g., C3H/RI strain) have been cut back to a single line every 10 years. However, owing to significant production problems, the 101/RI strain, a former large production inbred, has not been cut back for at least 25 years.

There is only one production colony (the T stock, homozygous for seven recessive mutations manifesting externally visible phenotypes), which has been maintained in a non-inbred state. The stock was very large (300 breeding pens), but is now only at 50 pens and is also cryopreserved. Typically, one female from a given pen is set aside to be a future breeder. She is mated with a male weaned from a different pen. Never are more than two animals from any one pen set up as breeders for the next generation. In the past when a suspected spontaneous recessive mutation was traced back to the T stock, it has been difficult to find common ancestry even after going back 10-20 generations.

c) Genetic Monitoring Program

All mice within an inbred strain are earmarked at weaning and ledgered (see Section 3b above). Maintainers are required to report any visible variants immediately. Any variant detected in an inbred strain is tested genetically to determine mode of inheritance. If a recessive mode is indicated, pedigrees are traced back and breeding of the inbred is continued from lines least closely related to the line in which the variant appeared.

Large inbred production colonies of similar outward appearance (e.g., C3Hf/RI, 101/RI, and their F1 hybrids, which are all full-colored agouti in appearance) are kept in separate rooms. There is heavy reliance on coat color and texture, external morphology, and behavior as monitors of genetic contamination, since routine molecular, biochemical, or immunological monitoring of inbred strains is not done. For example, contamination of full-colored agouti C3H stock with any other strain carrying any recessive coat-color mutation would eventually be evident in inbred descendants and would be revealed by the occurrence of variants. If an albino variant were found in C3H/RI, for example, the genotype of molecular background markers for this variant would be determined to ascertain whether they were of C3H origin. If C3H background genes were indicated in the variant, the variant would be classified as a coisogenic mutation. Contamination of inbred strains homozygous for recessive coat-color markers would be easier to detect, unless the contaminating strain either had exactly the same coat-color genotype or carried coat-color mutations that were recessive to each coat-color marker carried in the strain of question. Like-colored inbred strains live in separate rooms to

minimize the probability of strain contamination, and the same rules for reporting variants apply as described above. In ORNL's 50-year history, there has not been an obvious contamination as defined by these detection criteria.

One colony that has been monitored genetically is the T stock, which is homozygous for seven recessive visible markers (six coat-color markers, as well as an externally visible ear-length marker). This stock has been critically important for mutation-rate experiments, and there is an active program to periodically progeny-test representatives from this stock to ensure that it is still homozygous for each of the six recessive coat-color markers (homozygosity for the ear mutation is directly observable in each mouse in every generation).

Most current breeding activities involve either the recovery or maintenance of new, heritable mutations. Genetic monitoring of the considerable number of mutant lines is a *de facto* consequence of the breeding protocol required to recover or maintain each mutation. Some breeding protocols provide for the identification of the mutant genotype directly from phenotype in each generation; in other cases, a test-cross or progeny test is done every generation, when necessary, or, when closely linked and/or flanking visible genetic markers are used, every third or fourth generation. The cryopreservation program freezes gametes early in the history of a mutant pedigree to insure against accidental loss of a mutation.

d) **Use of Substrain Designation When Reporting Experimental Results**

Currently, there are seven registered substrain designations associated with the Oak Ridge Mammalian Genetics and Genomics Program: **R**, for Oak Ridge National Laboratory; **RI**, for Drs. W. L. and L. B. Russell; **Rn**, for Dr. E. M. Rinchik, **Gso** for the retired Dr. W. M. Generoso, **Dkj**, for Dr. D. K. Johnson, **Pp** for the retired Dr. Ray Popp and Diana Popp, and **Bd** for Biology Division, keyed to a former ORNL veterinarian, Dr. V. Godfrey. Of these seven substrain designations, only three (**R**, **RI**, and **Rn**) are in active use. Investigative staff have been informed that they should always use a complete substrain designation for inbred and mutant stocks at least once in their published papers. Special care is taken to describe substrain history in its nomenclature as much as possible (e.g., C57BL/6JRn is ORNL's substrain of C57BL/6J imported to Oak Ridge in 1987 and inbred by Rinchik).

ORNL's participation in large-scale mutagenesis experiments supported by the Department of Energy and by the National Institutes of Health provided the impetus for Dr. Rinchik to adopt a more generic registered substrain designation (**R**) that is keyed to

the entire Oak Ridge program rather than to one investigator (similar to **J** for the Jackson Laboratory and **H** for the MRC Mammalian Genetics Unit in Harwell, UK). Thus, the majority of new mutants or new substrains developed at ORNL will carry this generic **R** substrain designation in stock, locus, or allele designations.

C. Veterinary Medical Care

1. Animal Procurement and Transportation

a. Methods for Evaluating Quality of Animals

Rodents being considered for import into ORNL animal facilities are classified as originating from Approved Vendors or Atypical Vendors. Approved (commercial) Vendor health reports are monitored on a regular basis by the veterinarian, and if vendors provide animals of consistent and reliable health status the animals may enter our facilities without a quarantine period. Approved Vendors include Taconic, Jackson Labs, some areas of Charles River and some areas of Harlan Sprague Dawley. Atypical (non-commercial or other institution) Vendor health reports are reviewed and animals quarantined and tested upon arrival at ORNL facilities.

High quality fish embryos are procured from reliable vendors. Only healthy specimens are collected for the fish, amphibian, and reptile display tanks.

b. How Animals are Transported

All rodents imported to ORNL are shipped via commercial carriers except for animals from Harlan Sprague Dawley, which are transported by vendor owned and operated ground vehicles. Currently, all transport of animals within the institution and to the Tennessee members of the TMGC is via company owned vehicles.

Fish embryos from commercial vendors are shipped via commercial transport companies. For display tanks in ESD, wild caught fish specimens are transported in aerated plastic containers in company vehicles. Wild caught reptiles and amphibians are transported in aerated plastic containers or snake containers, as appropriate, in company vehicles.

2. Preventive Medicine

a. Quarantine, Stabilization, and Separation

1) Receiving and Initial Evaluation Procedures

Newly arrived rodents are removed from shipping cartons and put in regular caging by the investigator. Any illness or death among the shipped animals is reported to the veterinarian.

Quarantine is not applicable for fish embryos used in ESD. Display tank specimens are monitored daily until it is clear they have adapted to their new environment and are flourishing.

2) Quarantine Facilities and Procedures for Purpose Bred Animals

Animals from Approved Vendors are not quarantined, although a 7-day stabilization period is required by ORNL-ACUC Guidelines (Guideline ORNL-ACUC 005). Atypical Vendor animals are quarantined in an isolated room (or ventilated cage shelf), handled last, and tested following arrival for agents for which the colony (whether SPF or conventional) is known to be free. Studies of an acute nature are performed in Bldg 4501 and mice from Atypical Vendors have been allowed to enter the facilities based on health reports from the sending institution and without serologic testing to confirm the absence of possible disease agents (parasite exams are performed).

Purpose-bred fish obtained from vendors or other facilities are housed separately from other animals within the room. Cohorts are always housed separately so new arrivals would not be mixed with established tanks. No preventative treatments are performed.

3) Quarantine Facilities and Procedures for Random Source Animals

Not applicable for rodents at ORNL.

For wild-caught fish (wild-caught darters and minnows for display tanks in Building 1504), it is less stressful for the fish to be introduced directly into the display tanks (where they can learn captive feeding behaviors from long-term residents and where conditions are optimal for their survival). Only the healthiest captives are introduced and this is an infrequent event.

4) Isolation Facilities and Procedures for Ill Animals

Cages of sick mice in Bldg 9210 may be identified to handle last; in some cases this may apply to an entire room of animals which will be changed only after other animals and with husbandry practices that minimize the possibility of disease spread to other cages or rooms. There has been no outbreak of infectious disease in Bldgs 4500S or 4501 that have warranted implementation of procedures for disease containment.

In Bldgs 1504 and 1505 the entire tank is treated in the event of a disease outbreak. If diseased fish are identified within a tank in Bldgs 1504 and 1505, the entire tank is treated. Treatments include salt baths, antifungal agents, and other appropriate medications..

5) Periods for Physiologic, Psychologic, and Nutritional Stabilization

As per ORNL-ACUC Guideline 005, all animals entering the facility undergo a 7-day stabilization period.

6) **Program for Separation of Animals by Species, Source, and Health Status**

Only mice are housed in Bldgs 9210 and 4500S. In Bldg 4500S all animals utilized come from a single commercial vendor and no breeding occurs in this facility. In Bldg 4501 rats and mice are occasionally co-housed. In this case animals are housed on separate shelves of the ventilated rack, and husbandry is performed separately. In Bldg 9210 there are 5 SPF rooms (Rms. 111, 112, 115, 206, 213) and 7 “one-way” production rooms (Rms. 109, 110, 201, 202, 203, 204, 205) where animals can move out of but not into the room. These rooms are generally grouped together, entrance to the rooms is limited to necessary personnel only, and protective clothing and dedicated supplies are used in these rooms. These rooms are not entered if an individual has been in contact with any other animals, and additional husbandry procedures are in place to minimize the possibility of exposure to disease agents present in the remainder of the Bldg 9210 mice. Animals from approved vendors may enter these rooms. Animals from atypical vendors that have gone through quarantine can enter these rooms. In the occasional event that animals from a conventional room need to be transferred to an SPF room, a “burnout” period, consisting of cessation of breeding and anti-parasitic treatment, is used. All other mice in Bldg 9210 are considered to be of the same health status and no further separation of animals by health status is practiced, aside from those described above for sick animals.

Fish are generally separated by species and source. Mixing of compatible species only occurs in the display tanks in Bldg 1504, where natural community ecosystems are used for educational purposes.

b. Surveillance, Diagnosis, Treatment, and Control of Disease

1) Program

a) Daily Observation of Animals

All rats and mice are observed daily by experienced research technicians. In Bldg 9210 two SOPs, "Daily/Weekend/Holiday Check of Animal Facility" and "Debilitating Phenotypes and Body Condition Scoring" describe procedures for checking rooms and assessing the condition of animals. Green health check cards are used for identifying cages with sick animals; these are in turn logged on a room activity sheet that creates a record of health related activities in that room. Room sheets are checked daily by veterinary staff for any new health checks. In addition, verbal or e-mail notification of the veterinarian or veterinary technician is an option. In Bldgs 4500S, 4501 and 3500 research staff notify the veterinarian or veterinary technician by phone, beeper, or e-mail of any animal health related concerns they might have.

Fish are observed daily by investigators or members of their research staff. Each Principal Investigator has a minimum of 10 years experience caring for and working with fish; staff are trained by investigators to recognize abnormal behavior or signs of illness. Sick animals in Bldgs 1504 and 1505 are reported to the PI who contacts the consulting veterinarian by telephone, pager, or email.

b) Procedure for Providing Veterinary Medical Care

In Bldg 9210 the veterinary technician, under supervision from the veterinarian, checks daily for any new health check cards. Health check cards are placed by anyone having concerns about the health of the animals (animal care or investigative staff). Bldgs 4500S and 4501 investigators or research technicians contact the veterinarian or veterinary technician directly by phone, beeper, or e-mail.

Sick fish in ESD are reported to the PI who will contact the consulting veterinarian by telephone, e-mail, or pager. Depending upon urgency, the veterinarian will visit the facility, have sick animals transported to the University of Tennessee, or provide telephone consultation.

c) Medical Records Maintenance Procedures

For rodents, health check cards are maintained on the cage until the health concern is resolved or until the animal is euthanized. Health check cards are then filed by room number along with filled room activity sheets. These files are maintained in the veterinarian's office. Additional records related to animal health

including necropsy reports, bacteriology, parasitology and health surveillance information, quality assurance testing results, etc., are maintained in the veterinarian's office. The veterinarian or veterinary technician maintains these records.

In ESD routine tank treatments such as salt baths are recorded in daily records. Diagnostic laboratory reports, necropsy results, and other medical records are kept in the consultant veterinarian's file in Building 1505, Room 370.

d) Preventative Medicine Programs for Each Species

Health reports for Approved Vendors (Jackson, Taconic, Charles River, and Harlan) are reviewed by the veterinary staff on a regular basis. Observation of rodents upon arrival, quarantine of Atypical Vendor source rodents, and the 7 day holding period constitute preventative medicine programs for most incoming rodents. Cessation of breeding and treatment with an anti-parasitic agent is performed on animals that need to be moved to an SPF room from a conventional room (usually for breeding purposes).

Reliability of vendors, segregation, and observation of fish upon arrival constitute preventative medicine programs for fish.

e) Animal Health Monitoring

In Bldg 9210 procedures for health are described in SOP, "Health Surveillance of Bldg 9210 Mouse Colonies." The majority of mouse colonies maintained in Bldg 9210 are currently "conventional" and known to be infected with a number of mouse pathogens. Current plans for the colonies include rederivation to specific pathogen free (SPF) status in association with populating new rodent facilities in autumn of 2003. Additionally there are 5 SPF rooms (Rms. 111, 112, 115, 206, 213) and 7 "one-way" production rooms (Rms. 109, 110, 201, 202, 203, 204, 205) where animals can move out of but not in to the room. The SPF and "one-way" rooms in Bldg 9210 have sentinel animals, exposed to dirty bedding, that are tested quarterly. Additionally sentinels are placed in 2 conventional rooms, rotating to different conventional rooms each quarter.

Bldg 4500S- Sentinel mice are exposed to dirty bedding from animals housed for experimental purposes as described in SOP, "Maintaining Health Surveillance in Specific Pathogen Free (SPF) Animal Rooms." Sentinel animals are submitted annually for a comprehensive panel of agents serologically. Quarterly samples are submitted for basic serology, *Salmonella sp* and *Citrobacter rodentium* culture, and internal and external parasite exams.

Bldg 4501- Rats from Approved Vendors are housed in this temporary housing facility and held for relatively brief periods of

time on an all-in-all-out basis. Mice from Bldg 4500S are occasionally held briefly in this facility with practices in place to minimize the likelihood of cross-transmission of agents between these groups of animals. No health surveillance is performed in this temporary housing facility.

Bldgs 1504 and 1505- Daily observation of fish and quarterly random tank cultures are taken to monitor tank health in fish colonies.

2) **Diagnostic Resources**

a) **Clinical Laboratory**

The University of Tennessee College of Veterinary Medicine provides clinical pathology, microbiology, and parasitology diagnostic support for rodent and fish colonies. Monthly sanitation QA and quarterly health surveillance samples from Bldg 9210 as well as miscellaneous diagnostic samples comprise the majority of samples tested at UT. Support services for the ESD facilities for the last year included quarterly microbiological samples. Some in-house diagnostics are available for rodents including CBC and blood chemistry. These newly acquired capabilities are not yet utilized on a regular basis.

b) **Necropsy/Histopathology**

The veterinary technician performs necropsies on sentinel rodents. All other rodent necropsies are sent to the University of Tennessee College of Veterinary Medicine pathologists for necropsy and histopathology, including any abnormal sentinels identified. The consulting veterinarian performs fish necropsies, and the University of Tennessee College of Veterinary Medicine examines histological samples.

c) **Use of Available Diagnostic Resources Including Commercial Labs**

The University of Tennessee College of Veterinary Medicine and the University of Missouri Veterinary and Laboratory Animal Diagnostic Laboratories as described above are used for rodents and fish. Serologic testing for rodent disease agents is performed at the University of Missouri.

d) **Radiology**

X-ray diagnostics are not available. If necessary, MicroCT scanning is available in Bldg 9210 for clinical use.

3. **Surgery**

a. **Surgical Monitoring**

Rodent surgery is performed by investigative staff in areas of the laboratory dedicated to that purpose (Bldg 9210, Rms. 134, 136, 138). Guidelines for Survival Surgery in Rodents have been established by the ORNL-ACUC and are reviewed at the time of protocol submission as well as at semi-annual facility and laboratory inspections. Guidelines advise use of appropriate anesthesia, monitoring of the rodent's respiration and response to stimulus, keeping the rodent warm during surgery and recovery, assessment for post-operative pain, use of analgesics if indicated, and recording of surgical procedures. No surgical procedures are performed in fish.

b. **Presurgical Planning**

Presurgical planning procedures are described in individual ORNL-ACUC protocols, in ORNL-ACUC Guidelines ("Survival Surgery in Rodents"), SOPs ("Analgesics and Anesthetics" and "Rodent Aseptic Surgical Technique/Surgical Monitoring"), and in the ORNL-ACUC Training Course, "Use of the Mouse in Research."

c. **Training Program**

Training for surgical procedures in mice is included in the web-based training course for mouse users. Topics included in the testable material include the NIH Guidelines for aseptic surgery of rodents (asepsis of surgery, facilities, preparation of the surgical site, preparation of surgical instruments and sutures, preparation of the surgeon, and analgesia and environmental modification). Additional information is provided in the following SOPs, "Analgesics and Anesthetics" and "Rodent Aseptic Surgical Technique/Surgical Monitoring." Hands-on mentoring/training is provided by investigative and veterinary staff.

d. **Major and Minor Procedures**

1) **Criteria for Differentiation**

Defined by the "*Guide*": Major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, craniotomy, joint replacement, and limb amputation). Minor survival surgery does not expose a body cavity and causes little or no physical impairment (such as wound suturing; peripheral-vessel cannulation).

2) **Practices Employed During Nonsurvival Procedures**

There are currently two protocols approved as non-survival surgical procedures; one is for a terminal bleed and the other is for a terminal perfusion. The non-survival surgical procedures in this case are performed by clipping and cleaning the surgical site, the surgeon wears gloves, and the instruments are clean.

e. Aseptic Procedures

1) Procedures and Equipment

The following recommendations are excerpted from the SOP, "Rodent Aseptic Surgical Technique/Surgical Monitoring."

- After induction of anesthesia, clip, shave, or pluck hair from the surgical site.
- Prepare the skin with betadine solution. Use a cotton tipped applicator and scrub in a circular pattern, beginning in the center and spiraling outward. Do NOT wet the mouse with an excessive amount of solution.
- Follow betadine prep with two 70% alcohol preps, using the same scrubbing pattern described in the previous step.
- Place a sterile drape over the rodent (preferred).
- Use sterile instruments. For multiple procedures, instruments should be autoclaved prior to the initial surgery, stored on a sterile drape, and the tips sterilized between each use in a glass bead sterilizer.
- The surgeon should wear sterile gloves.
- Suture material, wound clips, and anything that touches the surgical site must be sterile.
- Non-absorbable sutures/clips should be removed in 7 to 14 days. Exceptions to this (such as post-laparotomy pregnant females which will be terminated within a few weeks) must be approved by the ORNL-ACUC.

2) Methods Used to Sterilize Instruments and Protective Clothing

Instruments are autoclaved prior to rodent surgery and sterilized between animals using a hot glass bead sterilizer.

f. Surgical Facility Use

There are no dedicated surgical facilities for rodents at ORNL. Surgeries are performed in dedicated areas of investigator laboratories (Bldg 9210, Rms. 134, 136, 138). These laboratories are also used for harvest of embryos for targeted mutagenesis and cryopreservation. The number of rodent surgeries performed annually is 336. The lab bench area is cleaned at the beginning and end of the surgical procedures with alcohol or Quatricide© and covered with a moisture

proof backed absorbent pad. The absorbent pad is discarded at the end of each surgical session.

g. Postsurgical Actions

Investigative staff members oversee and provide post-surgical care and records for the surgical procedures performed in rodents. The veterinarian is available to provide guidance if necessary. Post-surgical care can include provision of analgesics and keeping the mice in a warm environment isolated from any non-anesthetized cage mates. Once animals are recovered from anesthesia they are returned to their home cages.

h. Mammalian Survival Procedures (non-rodent)

Not applicable.

1) Where Performed; Professional Supervision

2) Description of the Program

3) Major Support Equipment Available

4) Training and Experience of Personnel Performing Surgery

i. Rodent Survival Procedures

1) Facilities for Survival Rodent Surgery

The following survival surgeries in rodents are performed at ORNL. Persons performing surgeries are trained on specific procedure(s) by veterinary staff or by researchers with demonstrated proficiency in the procedure(s).

Surgery	Number Performed /Year	9210 Room #s	Protocol	Responsible Professional
Hemicastration	None*	NA	0259	Johnson
Vasectomy	36	136	0261	Michaud
Embryo Transfer	100			
Embryo Transfer	100	134	0273	You
Hemicastration	None*	NA	0276	Rinchik
Splenectomy	None*			
Vasectomy	None*	NA	0285	Culiat
Embryo Transfer	None*			
Vasectomy	30	138	0289	Rinchik
Embryo Transfer	20			
Hemicastration	None*			
Splenectomy	None*			
Nephrectomy	None*			
Ovary Transplant	50			

*These surgeries are approved under these protocols; none have been performed to date.

2) **Types of Procedures Performed**

See table listed above.

3) **Techniques Used to Prevent Infection**

Described above in SOP, "Rodent Aseptic Surgical Technique/Surgical Monitoring."

j. **Farm Animal Procedures**

Not applicable.

1) **Where Performed; Professional Supervision**

2) **Description of Program**

3) **Major Support Equipment Available**

4) **Training and Experience of Personnel Performing Surgery**

k. **Survival, Non-Mammalian Procedures**

Not applicable.

- 1) **Facilities for Non-Mammalian Surgery**
- 2) **Types of Procedures Performed**
- 3) **Techniques Used to Prevent Infection**

l. **Nonsurvival Procedures**

Not applicable.

- 1) **Facilities for Nonsurvival Procedures on Nonrodent Mammals**
- 2) **Description of Program**
- 3) **Major Support Equipment Available**
- 4) **Training and Experience of Personnel Performing the Procedure**

4. **Pain, Distress, Analgesia, and Anesthesia**

a. **How and by Whom are Levels of Pain and Distress Assessed and Categorized**

Investigators in conjunction with the veterinarian assess pain and distress. Criteria for monitoring pain and distress and appropriate intervention are described in the ORNL-ACUC protocol. In addition, the SOP, "Debilitating Phenotypes and Body Condition Scoring," describes criteria for monitoring of animal well-being, pain, and distress.

b. **IACUC Guidelines for Avoiding Unnecessary Pain or Distress**

ORNL-ACUC Guidelines for potentially painful or distressful procedures in rodents include, "Rodent Toe Clipping for Identification" (not recommended), "Survival Surgery in Rodents," "Stabilization Period for Imported Animals" (one week), "Retro-Orbital and Cardiac Bleeding Policy" (anesthesia required, cardiac is a terminal procedure), "Mouse Tail Clip Policy" (anesthesia required in mice >4 weeks of age or 18 grams), and "Transport of Rodents within ORNL" (transportation in environmentally controlled vehicles in dedicated sanitizable containers). Additional recommendations are included in the ORNL-ACUC Mouse Training Course and include the following for tumor endpoints and monoclonal antibody production.

The ORNL-ACUC has specific considerations when reviewing protocols in which tumors will be induced. It is recommended that special sense organs be avoided (e.g. retro-orbital injection of tumors is inappropriate). Intramuscular administration of tumors is discouraged due to pain associated with expansion of tumors as they grow along muscle planes. Tumor implantation in footpads also is inappropriate as the animal experiences pain when tumors are injected at this weight-bearing surface. Subcutaneous implantation in the flank, intraperitoneal, and intravenous implantation are recommended. Guidelines for tumor burden and study duration are that tumors not interfere with ambulation, eating, drinking, defecation or urination. Tumors should not exceed 10% of the body weight, should not be ulcerated, or cause excessive (>20%) loss of body weight (excluding tumor mass). Most tumors reaching 1 cm diameter are in excess of 10% body weight. The average mouse weighs 25-30 grams so a 20% weight loss would be 5-6 grams, therefore, an adult mouse weighing 20-22 grams should be humanely euthanized. Body condition is a useful alternative to weight loss for the assessment of endpoints. Death as an endpoint is unacceptable.

Specific ORNL-ACUC considerations for monoclonal antibody production procedures include the observation of the mice at least once daily. Tapping should be done before there is severe abdominal distention. The number of taps should be limited to no more than 3; however, if the animal is in poor condition the mouse should be humanely euthanized prior to 3 collections. Consideration should be given to the animal that will be injected (strain, age and sex), to characteristics of the tumor line, and to whether the tumor line is from the same strain as the mouse being injected. Some tumors are more aggressive than others. Alternatives to the use of monoclonal antibody production are required in Europe and are strongly encouraged by the PHS. Investigators are advised to call the staff veterinarian or the ORNL-ACUC Chairperson or Coordinator for further information when considering this experimental procedure.

NOTE: For both tumor induction and monoclonal antibody production tumor lines must be MAP tested.

c. Agents Used for Each Species

Anesthetic agents for use in rats and mice include: Avertin (1.25% tribromoethanol), Isoflurane, Sodium Pentobarbital, and Ketamine/xylazine. A small remaining in-date stock of Metofane© is also used. Analgesic agents for use in rats and mice include Buprenorphine and Butorphanol. Dosages are available in the existing SOP, "Analgesics and Anesthetics." Environmental modifications include providing a warm comfortable environment (e.g.- nestlet) with palatable food readily available (e.g.- moist diet placed on the cage floor). Behavioral modification might include simple management techniques such as not overcrowding, providing a compatible cage mate, or removal of an aggressive cage mate.

Tricaine methane sulfonate is the anesthetic of choice for use in fish.

d. How Veterinarian Provides Input to Choice and Use of Drugs

The veterinarian reviews protocols during the ORNL-ACUC meetings and decides on appropriateness of anesthetics and analgesics used. She is also available to assist in protocol development and selection of appropriate anesthetics and analgesics.

e. How Use of Anesthetics and Analgesics is Monitored

The investigator is responsible for monitoring his/her animals. Guidelines for monitoring anesthesia and use of analgesics are included in SOPs (“Analgesics and Anesthetics” and “Rodent Aseptic Surgical Technique/Surgical Monitoring”), and in the ORNL-ACUC Mouse Users Training Course. Review of laboratory records by the ORNL-ACUC visitors during a semi-annual review constitutes the formal mechanism for reviewing the use of anesthetics and analgesics. Informal evaluation of the use of anesthetics and analgesics consists of veterinary oversight of the primary animal facility (Bldg 9210) and mentoring by experienced and trained research staff members.

f. Training and Experience of Personnel Performing Anesthesia

The responsible PI or the veterinarian supervises administration of anesthesia in rodents or fish until the trainee develops proficiency in performing the procedure.

g. Description of Safety Procedures for Using Volatile Anesthetics and for Gas Scavenging

Inhalant anesthetics are used either in fume hoods or have exhaust scavenger systems. The OSSD Representative performs monitoring of exposure levels. Use of ether for anesthesia is forbidden.

5. Euthanasia

a. Methods for Each Species

Mice:	Carbon dioxide Anesthetic overdose Cervical disarticulation w/o anesthesia (scientific justification) Cervical disarticulation with anesthesia Decapitation with anesthesia Decapitation without anesthesia (young pups, RNA) Exsanguination under anesthesia
Fish:	Anesthetic overdose (MSS-222) Cervical transection Stunning blow to head (large fish, EPA Protocol) Asphyxiation (smaller fish, EPA Protocol) Decapitation
Chicken:	Exsanguination under anesthesia
Geese:	Injection/commercial veterinary euthanasia solution
Rats:	Anesthetic overdose with decapitation
Snakes:	Anesthetic overdose (pentobarbital)

b. Training and Experience of Personnel

Most of the euthanasia of mice performed with carbon dioxide is done by animal care staff in Bldg 9210, who have been trained by the veterinarian and supervisor. Euthanasia performed by the research staff is done by the individuals listed under the ORNL-ACUC protocol. Training in euthanasia methods for the research staff is via one-on-one mentoring by the PI or designated experienced staff.

6. Drug Storage and Control

a. General Storage Arrangements

Non-controlled drugs are stored in the veterinarian's office (or in Bldg 1505 in PI's office and in a locked drawer in Rm 263 for ESD). Controlled drugs are maintained in a locked drawer in the locked veterinarian's office in Bldg 9210. ORNL has a subcontract with a clinical Oak Ridge veterinarian licensed in the State of Tennessee, who dispenses the controlled drugs when prescribed by the consultant Institutional Veterinarian. Since our Institutional Veterinarian is on site only one day per week, this arrangement allows wider coverage for timely dispensing of controlled substances as needed.

b. Recordkeeping Procedures

For controlled drugs, a log is maintained in the veterinarian's office in Bldg 9210 to record inventories, and amounts withdrawn, for what purpose, and by whom.

c. Ensuring Drugs and Supplies are Within Date of Expiration

For non-controlled and controlled drugs the veterinarian or the veterinary technician regularly check expiration labels. Outdated drugs and supplies are discarded. The ORNL-ACUC also checks expiration labels during the semi-annual animal facility inspections.

D. Physical Plant (Rodent Facilities- Buildings 9210, 4500S, 4501, and 3500)

1. Functional Areas

a. Overview of General Arrangement and Condition of Facility - Location and General Arrangements

1) Location of Animal Facility

Bldg 9210 is located in the Life Sciences Complex of the Y-12 site. The building is a 3-story facility with animal rooms on all floors. The building was originally built for weapons production/research in 1945. Currently, 52 of the 56 animal rooms located in this building are used as animal holding space. One room (Rm 115) is set up with ventilated racks, similar to those that will be used in the new SPF barrier facility.

Animal holding space in Bldg 4500S is a single room (F-149) with a single ventilated rack for animal housing. This satellite housing facility is located at the ORNL site and animal space is on the 1st floor of the building.

Animal holding space in Bldg 4501 is a single room (317B) with two ventilated racks for animal housing. This temporary housing facility is located at the ORNL site and animal space is on the 3rd floor of the building.

Animal holding space in Bldg 3500 is a single room (C-15) with temporary animal holding space in an exhaust hood. This facility is located at the ORNL site and animal space is on the 2nd floor of the building.

2) Physical Relationship to Research Labs

Bldg 9210 -- Laboratories and offices are co-located in the facility, generally peripherally located to the animal space on each floor.

Bldgs 4500S -- A separate wash, autoclave, and storage room is contiguous with the single animal housing room.

Bldgs 4501, 3500 -- Laboratories are contiguous with animal space in these one-room facilities.

3) General Arrangement of Animal Facility

In all rodent facilities there are common corridor systems. Bldgs 9210 and 3500 are conventional facilities while Bldgs 4500S and 4501 are maintained as SPF facilities. Building 9210 has 5 rooms maintained as SPF; 3 are on a single first floor corridor, and 2 are on separate corridors on the second floor. Bldg 9210 also has 7 rooms maintained as one-way rooms; 5 are on a common second floor corridor and two are located on one first floor corridor. These special rooms require special practices, which are posted on each animal room door.

4) Specialized Animal Housing Systems

Bldgs 9210 and 3500 utilize open caging systems. Bldg 9210 has one room (115) set up with 6 Thoren ventilated racks and automatic watering. Bldgs 4500S and 4501 utilize one ventilated cage rack and two ventilated shelf racks, respectively. The entire animal housing and use area in Bldg 4501 is designated as a radiation containment area. The Bldg 4500S animal facility has one section of its single ventilated rack designated as a radiation containment area.

5) Cubicle Availability and Design

Not applicable.

b. Functional Space

1) **Total Square Feet and Number of Animal Housing Rooms (Traditional Biomedical Facilities)**

a) **Indoor space, A/C and heated**

Building	Number of Animal Rooms	Animal Space (Sq. Ft)
9210	52	18,600
4500S	1	231
4501	1	184
3500	1	100
		Total: 19,115

b) **Indoor space, heated, no A/C**

Not applicable.

c) **Indoor space, not environmentally controlled**

Not applicable.

d) **Outdoor facilities**

Not applicable.

2) **Total Square Feet and Number of Animal Housing Areas ("Farm" Facilities)**

Not applicable.

a) **Indoor Space, A/C and Heated**

b) **Indoor Space, Heated, No A/C**

c) **Indoor Space, Not Environmentally Controlled**

d) **Outdoor Facilities**

e) **Acreage and Location**

c. **Support Areas**

1) **Total Square Feet and Number of Support Area Rooms**

a) **Receiving**

None.

b) **Rodent/Rabbit Quarantine**

In Bldg 9210 an empty available animal holding room (~300 Sq. Ft) is designated for receiving and quarantine when necessary. No specific animal room is used; use for quarantine depends on what is currently vacant. In Bldgs 4500S and 4501 space is not available for quarantine, however, animals are housed separately in the ventilated racks in the event that Atypical or non-Approved Vendor animals are held. No animals are quarantined at the Bldg 3500 temporary housing facility.

c) **Random Source Animal Quarantine**

Not applicable.

d) **Isolation for Sick Animals**

In Bldg 9210 an empty animal holding room may be designated for isolation of sick animals when necessary. Also, sick animals housed in a room with other animals are isolated by using a "Change Last" sign and by placing them on a separate shelf. In Bldgs 4500S and 4501, space is not available for isolation of sick animals, however, animals are housed separately in the ventilated racks.

e) **Aseptic Surgery**

Surgery is only performed in rodents. Dedicated areas of laboratories used for rodent surgery include: Bldg 9210-Rooms 134,136, and 138.

f) **Necropsy**

Diagnostic necropsies are usually performed in Rm 135 of Bldg 9210 (216 sq. ft). They are also occasionally done in Rm 125 (262 sq. ft).

g) **Radiology**

Diagnostic radiology is not available. MicroCT scanning is performed in Bldgs 3500 and 9210.

h) Diet Kitchen

None.

i) Food Storage

Building	Room	Sq. Ft
9210	101	669
4500S	F-147	55
4501	no storage	
3500	no storage	

j) Bedding Storage

Building	Room	Sq. Ft
9210	190	1,310
4500S	F-147	55
4501	301	attic annex
3500	no storage	

k) Cage Wash

Building	Room	Sq. Ft
9210	128	480
	130	946
	231	216
	233	675
	235	1,680
	330	600
	334	190
	335	540
		Total: 5,327
4500S	F-147	55
4501	317B	in animal room
3500	no cage wash facility	Cages returned to Bldg 9210

l) Clean Cage Storage

Building	Room	Sq. Ft
9210	103	305
	104	305
	107	
	341	305
		600
		Total: 1,515
4500S	F-147	55
4501	317B	in animal room
3500	no storage of cages	
Storage Containers*	ORNL site	720

*Contain new Thoren cages for new SPF barrier facility.

m) Incinerator/Waste Disposal

Freezers for carcass disposal and 5 dumpsters (3 for bedding, 1 for trash, 1 for glass) are located at the back dock of Bldg 9210. Carcasses treated with hazardous chemicals are labeled and segregated from untreated carcasses. Carcasses are incinerated at the University of Tennessee. Carcasses are stored in a freezer in room 317 of Bldg 4501 for disposal by burial at the ORNL landfill site (Bldgs 4500S, 4501, 3500). The bedding is disposed of as radioactive waste (Bldg 4501) or regular trash (Bldg 4500S and 3500).

n) Diagnostic Lab

All diagnostic procedures, aside from some necropsies performed in Bldg 9210, are out-sourced.

o) Animal Procedural Space

All procedural space is in labs contiguous with animal space in each facility.

p) **Personnel Facilities**

Building	Room	Sq. Ft
9210	130A	216
	144&145	188
	240&241	263
	239	247
	339	247
	338	232
		Total: 1,393
4500S	no dedicated space	
4501	no dedicated space	
3500	no dedicated space	

q) **Service Corridors**

Building	Location	Sq. Ft
9210	1 Floor	4,016
	2nd Floor	4,275
	3rd Floor	3,404
		Total: 11,695
4500S	single room	
4501	single room	
3500	single room	

r) **Lounge Facilities**

Two lounge spaces are available for animal care staff in Bldg 9210, room 125A mezzanine and room 128 (Bldg 9207). There is a total of ~600 sq. ft combined space. No other facilities have dedicated lounge space.

s) **Administrative Space**

Administrative space for the Laboratory Animal Resources Section in Bldg 9210 includes rooms 100A, 122, 122A, 122B, and 147. The combined square footage of this space is ~846. Offices of the ORNL-ACUC Chair and Coordinator are located in Bldg 4500S, Rms. H-162 and S-118, with a total area of 298 sq. ft

t) **Mechanical**

Mechanical space in Bldg 9210 includes 1,296 sq. ft on the 1st floor, 864 sq. ft on the 2nd floor, 2,064 sq. ft on the 3rd floor for a total of 4,224 sq. ft. There is no dedicated mechanical space for one room animal facilities (4500S, 4501, and 3500); the HVAC is part of the system for the entire building. Ventilated racks or an exhaust hood provides the microenvironment for the animals.

2. **Construction Guidelines**

a. **Corridors in Animal Facility**

1) **Composition, Dimensions, Protection**

Bldg 9210- Corridors on the first and second floors have water based epoxy painted block walls; the third floor corridor walls are made of glazed block. Corridors are between 8 and 10 feet wide. There are no protective materials applied to walls and corners.

2) **Transport of Animals/Caging Equipment Through Common Use Corridors or Elevators**

Bldg 9210- Animals transported through the corridors in this conventional facility are in open caging on laboratory carts. Caging moved for room sanitation purposes is placed on racks covered with washable nylon covers. Animals being maintained as SPF generally do not enter the corridors except for non-return trips to the laboratory or for annual sanitation of the room (the caging is covered with washable nylon covers during that time). In certain circumstances some animals are transported from SPF rooms to the laboratory and returned (e.g., implantation of embryos in recipient females) in which case the mice are transported inside an autoclaved steel flask covered with aluminum foil.

b. **Animal Room Doors**

Bldg 9210- Doors on the 1st floor are water-based epoxy painted wood with view panels and are 42" x 84". The 2nd floor doors are water-based epoxy painted metal with view panels and are 42" x 84". Third floor doors are water-based epoxy painted metal with view panels and are 42" x 78". Bldg 4500S- Door is water-based epoxy painted metal, with a covered view window and a digital door lock, and measures 41" X 82". Bldg 4501- Door is water-based epoxy painted metal, with a view window, and measures 36" X 80". Bldg 3500- Door is water-based latex painted metal, with covered view window, and measures 40" x 84".

c. **Exterior Windows**

None in any animal rooms in any of the animal holding areas.

d. Floors

Bldg 9210- Floors of corridors, support space, and animal rooms are sealed concrete, with the exception of 3 painted floors (Rooms 125A, 312, and 328). Bldgs 4500S, 4501, 3500- Floors are tile or linoleum.

e. Drainage and Plumbing

Bldg 9210- Six-inch floor drains are located in each of the cage or bottle washing rooms; there are no floor drains in any animal rooms. Animal rooms in this facility are not equipped with sinks. Bldgs 4500S, 4501, 3500- There are no floor drains in any of these facilities. There are double sinks in 4500S and 4501, and a single sink in 3500.

f. Walls

Bldg 9210- Animal rooms are water-based epoxy painted plaster on blocks and wallboard. In areas for sanitizing, 1st and 2nd floors have water based epoxy painted block walls, the 3rd floor walls are made of glazed block in service areas. Bldgs 4500S, 4501, 3500- Animal rooms are water-based epoxy painted plaster on blocks and wallboard.

g. Ceilings

Bldg 9210- Ceilings in all animal rooms are water-based epoxy painted plaster. Ceilings in areas for sanitizing are unfinished. Bldgs 4500S, 4501, 3500- 4500S has a suspended water resistant panel ceiling, 4501 has a water-based epoxy painted plaster ceiling, and 3500 has a suspended polyfiber ceiling.

h. Heating, Ventilation, and Air Conditioning

All air systems that supply the animal facilities use 100% fresh air. All air exchanges are within "Guide"-lines except for 2 areas in ESD where room air exchanges are 9 exchanges per hour (tank forced air obviates the need to meet the air exchanges recommended by the "Guide" in this case). See Appendix 10 for detailed HVAC performance criteria.

i. Power and Lighting

1) Physical Aspects

The electrical outlets in all animal rooms and support areas are equipped with ground fault interrupters, or waterproof or water resistant covers.

2) Provision of Emergency Power

Bldg 9210- An emergency generator provides for exhaust fans and emergency lighting during power outages. Bldgs 4500S, 4501, 3500- In

the event of a power outage Pls are notified by the Laboratory Shift Superintendent and if temperature extremes exist the animals are transported to another area (e.g., from 4501 to 4500S).

3) History of Power Failure

ELECTRICAL POWER FAILURES

Building 9210

11/10/1999 -- 4-hour planned outage for power system preventative maintenance. Emergency generator power on AC fans; work scheduled when outside temperature conditions favorable. No impact on colony.

7/13-14/2000 -- 1-hour outage from switchgear failure. No actions taken due to short duration. No impact on colony.

11/11/2000 -- 4-hour planned outage for power system preventative maintenance. Emergency generator power on AC fans; work scheduled when outside temperature conditions favorable. No impact on colony.

2/10/2001 -- 9-hour planned outage for power system reconfiguration. Emergency generator power on AC fans; work scheduled when outside temperature conditions favorable. No impact on colony

2/17/2001 -- 8-hour planned outage for power system reconfiguration. Emergency generator power on AC fans; work scheduled when outside temperature conditions favorable. No impact on colony.

3/31/2002 -- There was an unplanned chilled water outage (not electrical power-related). A planned outage for work in another building disrupted the chiller supplying 9210. Temperatures in 9210 began to rise, and the work was suspended in order to restore chilled water supply to 9210. No impact on the colony.

4/27-28/2001 -- A 3-hour steam (not electrical) outage was planned for maintenance activities on 4/28. Instead, steam was interrupted on the previous evening, resulting in building temperatures in the low 60s by morning. Rising daytime temperature prevented further cooling, and the job was completed as scheduled. There appeared to be no impact on the colony, but animal rooms were below acceptable temperatures for several hours. The root cause of the early steam outage was determined to be a communications problem, which was addressed and corrected at a Maintenance Priority Meeting on 5/3/2001.

6/26/2001 -- 38-minute unplanned outage caused by circulating current encountered during planned switching. No actions taken due to short duration. No impact on colony.

1/17/2002 -- 17-minute unplanned outage caused by circulating current. No actions taken due to short duration. No impact on colony.

Building 4500S

11/06/00 -- The building had an unplanned power outage (mower cut a guy wire) resulting in loss of power from 9:21AM to 9:56AM (less than 30 minutes). The duration was brief and there were no effects on the animals.

03/01/02 -- The building had an unplanned power outage (relay setting/switching error) resulting in loss of power from 9:08AM to 9:32AM (less than 30 minutes). The duration was brief and there were no effects on the animals.

Building 4501 -- Temporary housing area, no animals present at times of outages.

11/06/00 -- The building had an unplanned power outage (mower cut a guy wire) resulting in loss of power from 9:21AM to 9:56AM (less than 30 minutes). The duration was brief and there were no animals present.

03/01/02 -- The building had an unplanned power outage (relay setting/switching error) resulting in loss of power from 9:08AM to 9:32AM (less than 30 minutes). The duration was brief and there were no animals present.

Building 3500 -- Temporary housing area, no animals present at times of outages.

11/06/00 -- The building had an unplanned power outage (mower cut a guy wire) resulting in loss of power from 9:21AM to 9:56AM (less than 30 minutes).

03/01/02 -- The building had an unplanned power outage (relay setting/switching error) resulting in loss of power from 9:08AM to 9:32AM (less than 30 minutes).

4) Animal Loss or Health Problems Resulting From Power Failure

None.

j. Storage Areas

Bldg 9210- Clean cages, water bottles, *etc.*, are stored in designated areas of sanitation areas. Additional storage space is available in Rooms 103, 104, 107, and 341. Supplies are stored in Rooms 233 and 130. The Laboratory Animal Resources Group stores no flammable substances.

Bldgs 4500S, 4501, 3500- In 4500 storage of clean cages, water bottles, *etc.* is in Room F-147, in the animal room in 4501, and there is no storage space in 3500.

k. **Noise Control**

There are no special features for reducing noise in the animal facilities. In Bldg 9210, Rm 306 animal room has been converted to an animal behavior-testing lab. This room has noise-absorbing panels added to it.

l. **Cage Sanitation Facilities**

1) **Location**

Bldg 9210- Cage wash rooms are available on each floor (1st floor cage washer is used as back-up only); dirty caging is transported to the cage wash area via common use corridor systems and elevators.

Bldg 4501- Caging is washed by hand in sinks in the animal room and then autoclaved, also in the animal room, prior to use. Cage washing and autoclaving are not done when animals are present.

Bldg 4500S- Caging is washed by hand in sinks in Rm F-147 adjacent to the animal room (washed caging is then autoclaved, also in F-147, prior to use).

Bldg 3500- There are no cage washing facilities at this site. Caging is dumped, rinsed, and bagged for transport to Bldg 9210 where it is treated as dirty caging.

2) **General Features**

In Bldg 9210 dirty caging enters the large cage wash rooms via doors at the dirty end of the tunnel washer. Clean procedures are at the opposite end of the room but are not physically separated. There is no attempt to separate clean and dirty activities in Bldgs 4500S or 4501.

3) **Equipment**

Bldg 9210- Four tunnel washers are available for cage and cage top sanitation. Three of the 4 tunnel washers are equipped with dumping and conveyor stations for bedding removal. The 1st floor cage wash room is out of service, and is maintained as back-up only. An autoclave is used for sterilizing some caging accessories (sipper tubes, stoppers, and cage tops) for housing SPF mice. Bldgs 4500S and 4501- Autoclaves are available for sterilizing caging supplies in both facilities. Ventilated cage and shelf racks are used for housing rodents. In Bldg 4500S a hood is available for cage changing.

3. **Non-Rodent Mammalian Aseptic Surgery**

Not applicable.

a. **Facilities for Aseptic Surgery**

- 1) **Location of Support Functions**
- 2) **Construction Features of Operating Room**
 - a) **Interior Surfaces**
 - b) **Ventilation System**
 - c) **Lighting**
 - d) **Outlets**
 - e) **Scavenging**
 - f) **Fixed equipment**

- b. **Contamination Control**
- c. **Surgical Support Areas**
- d. **Post-operative Recovery Area**

4. **Farm Animals or Field Studies**

Not applicable.

5. **Security**

The Bldg 9210 mouse facility is located on the grounds of a DOE weapons plant that has a high level of security. A high chain link fence surrounds the Life Sciences complex and a badge is required for entry on site. Additionally, entry to the animal facility requires admittance via a proximity card reader or by a guard. Entrances to the area are guarded twenty-four hours per day. Bldgs 4500S, 4501, and 3500 are within the secured area at the ORNL site. A pass is required for entry and entrances to the ORNL are guarded twenty-four hours per day.

D. **Physical Plant (ESD Fish Housing Facilities, Buildings. 1504 and 1505)**

1. **Functional Areas**

a. **Overview of General Arrangement and Condition of Facility Location and General Arrangements**

1) **Location of Animal Facility**

ESD buildings 1504 and 1505 are located at the west end of the ORNL site in the Marilyn Lloyd Environmental and Life Sciences Complex (also the future location of the new SPF mouse facility and Life Sciences Division laboratories and offices). The two buildings are across the street from one another. Bldg 1504 is a one-story building with a large open area

housing living streams and display tanks. A separate aquatic toxicology laboratory is located in this facility where fathead minnow studies are periodically conducted. Bldg 1505 is a three-story building of offices and laboratories. Two animal rooms are located in this building; one (Rm 263) houses medaka and fathead minnows (occasionally zebra fish) in both static and flow-through tanks. The other room (Rm 153) is used for terminal procedures for specimens collected in the field.

2) Physical Relationship to Research Labs

Fish are housed within research areas in both buildings 1504 and 1505.

3) General Arrangement of Animal Facility

Facilities in 1504 and 1505 are conventional.

4) Specialized Animal Housing Systems

Not applicable.

5) Cubicle Availability and Design

Not applicable.

b. Functional Space

1) Total Square Feet and Number of Animal Housing Rooms (Traditional Biomedical Facilities)

a) Indoor space, A/C and heated

The total square footage of facilities in the ESD complex is 9,173. All of the space is conditioned space.

b) Indoor space, heated, no A/C

Not applicable.

c) Indoor space, not environmentally controlled

Not applicable.

d) Outdoor facilities

Not applicable.

2) Total Square Feet and Number of Animal Housing Areas ("Farm" Facilities)

Not applicable.

c. **Support Areas**

1) **Total Square Feet and Number of Support Area Rooms**

a) **Receiving**

None.

b) **Rodent/Rabbit Quarantine**

Not applicable.

c) **Random Source Animal Quarantine**

Not applicable.

d) **Isolation for Sick Animals**

Not applicable.

e) **Aseptic Surgery**

Not applicable.

f) **Necropsy**

Not applicable.

g) **Radiology**

Not applicable.

h) **Diet Kitchen**

Not applicable.

i) **Feed Storage**

No dedicated space.

j) **Bedding Storage**

Not applicable.

k) **Cage Wash**

Not applicable.

l) **Clean Cage Storage**

Maintained in housing area.

m) **Incinerator/Waste Disposal**

Not applicable.

n) **Diagnostic Lab**

Not applicable.

o) **Animal Procedural Space**

Laboratory and housing areas are combined.

p) **Personnel Facilities**

No dedicated space but showers and lounges are available.

q) **Service Corridors**

Not applicable.

r) **Lounge Facilities**

Available but not dedicated to animal care personnel.

s) **Administrative Space**

Not applicable.

t) **Mechanical**

In Bldg 1504 the mechanical space is approximately 600 sq. ft
Building mechanical space for 1505 is not independent of the
building systems.

2. **Construction Guidelines**

a. **Corridors in Animal Facility**

Not applicable.

1) **Composition, Dimensions, Protection**

2) **Transport of Animals/Caging Equipment Through Common Use
Corridors or Elevators**

b. **Animal Room Doors**

All doors except the entrance door to Bldg 1504 are water-based epoxy painted metal doors with view windows. The entrance door to Bldg 1504 is full-length glass with a painted metal frame. Doors to Bldg 1504 are all 36" X 84." The doors to Rooms 153 and 263 in Bldg 1505 are 47" X 85."

c. **Exterior Windows**

Not applicable.

d. **Floors**

Finished concrete in both rooms in Bldg 1505. Sealed and non-slip surfaced concrete in Bldg 1504.

e. **Drainage and Plumbing**

Floor drains are present in all ESD animal areas and are 2 inches in diameter (drain to 6" pipe).

f. **Walls**

Walls in both buildings consist of water-based epoxy treated concrete block.

g. **Ceilings**

Ceilings in Bldg 1504 are water-based epoxy painted metal and suspended fiberboard (aquatic toxicology lab). Ceilings in Bldg 1505 are water-based epoxy treated cement.

h. **Heating, Ventilation, and Air Conditioning**

Buildings are all environmentally controlled. Air exchanges do not have a direct impact to the aquatic species used in these areas as tanks receive forced air. However, two rooms do have air exchange rates slightly below those recommended by the "Guide" (9 air exchanges per hour). HVAC function performance is reported in Appendix 10.

i. **Power and Lighting**

1) **Physical Aspects**

Fluorescent lighting is used in both buildings with the exception of the incandescent high intensity lighting located over the spiraling living streams located in Bldg 1504. Lights are not sealed; outlets are both open and covered. All outlets have ground fault interruption protection.

2) **Provision of Emergency Power**

There is an emergency generator for Bldg 1505 and a battery powered backup for Bldg 1504. There is a battery back-up for the temperature monitoring system in Bldg 1505. Heat is steam dependent and not power dependent, air is maintained with outages. There are emergency power outlets available for powering pumps or portable filters if needed in the event of an emergency.

3) **History of Power Failure**

11/06/00 – There was an unscheduled outage power outage (mower cut guy wire) from 9:21AM to 9:56AM (less than 30 minutes). The outage was

brief enough that there was no effect on the fish housed in Bldgs 1504 and 1505 at that time.

4) Animal Loss or Health Problems Resulting From Power Failure

None have ever occurred.

j. Storage Areas

Laboratory, storage and animals space are co-located.

k. Noise Control

No loud equipment is allowed in close proximity to fish holding areas. No loud noises are generated by these species.

l. Cage Sanitation Facilities

Laboratory, storage and animals space are co-located.

1) Location

2) General Features

3) Equipment

3. Facilities for Aseptic Surgery

Not applicable.

4. Farm Animals or Field Studies

Not applicable.

5. Security

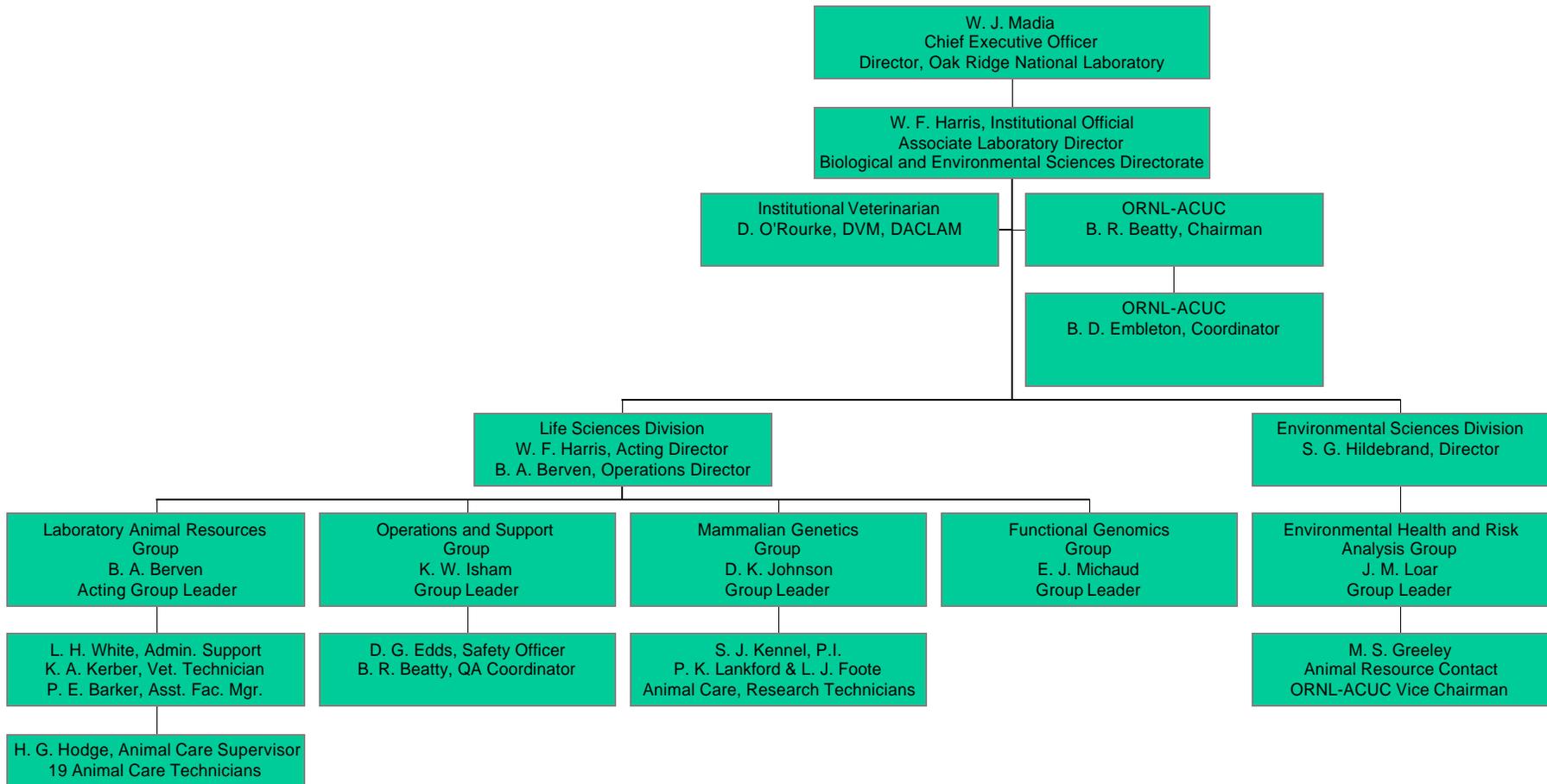
The ESD facilities located at the ORNL site are on the west end of the complex and located inside the secured area. All ESD buildings have card key access. After hour guard protection is in place.

III. Appendices

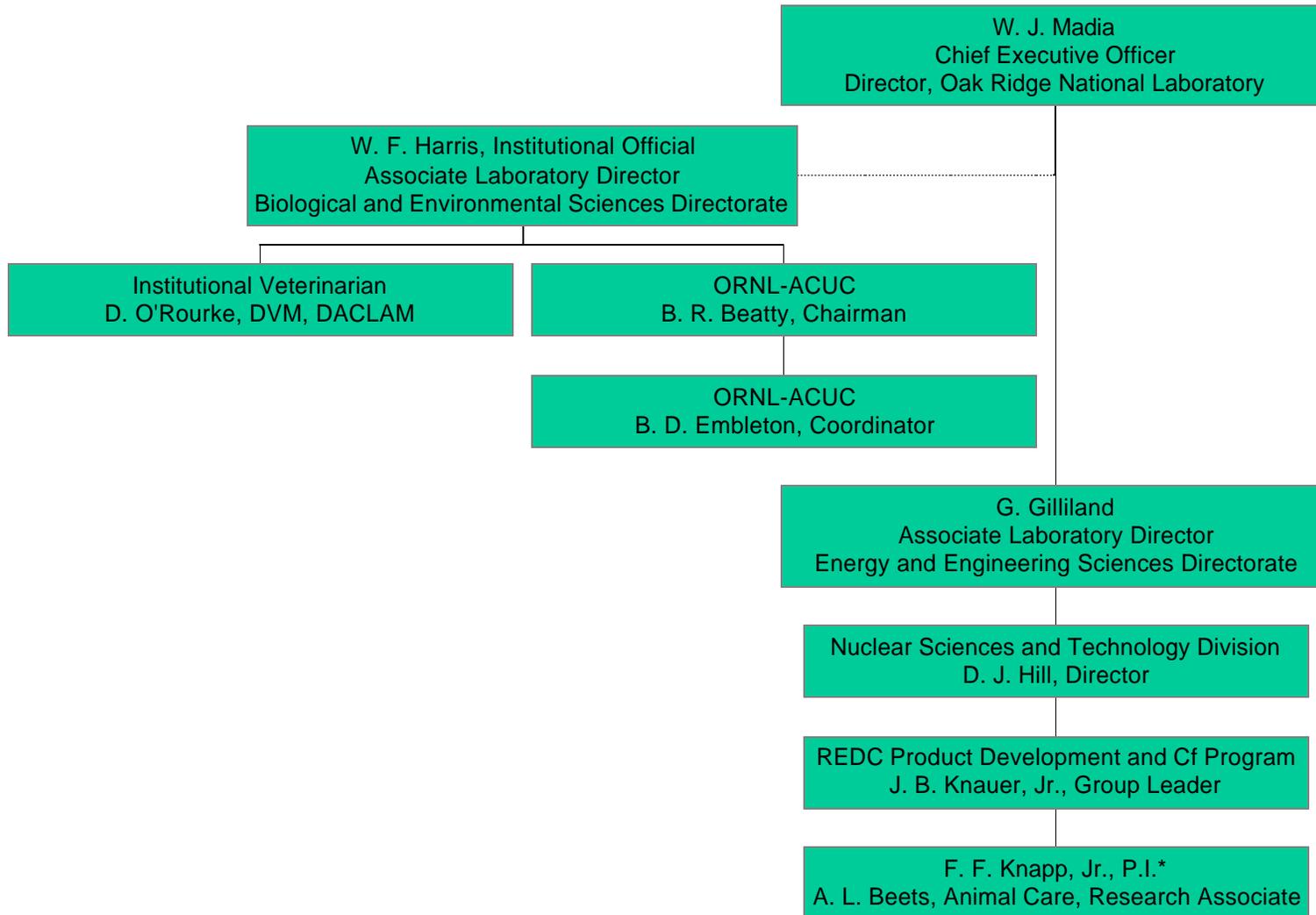
Appendix 1

Organization Charts

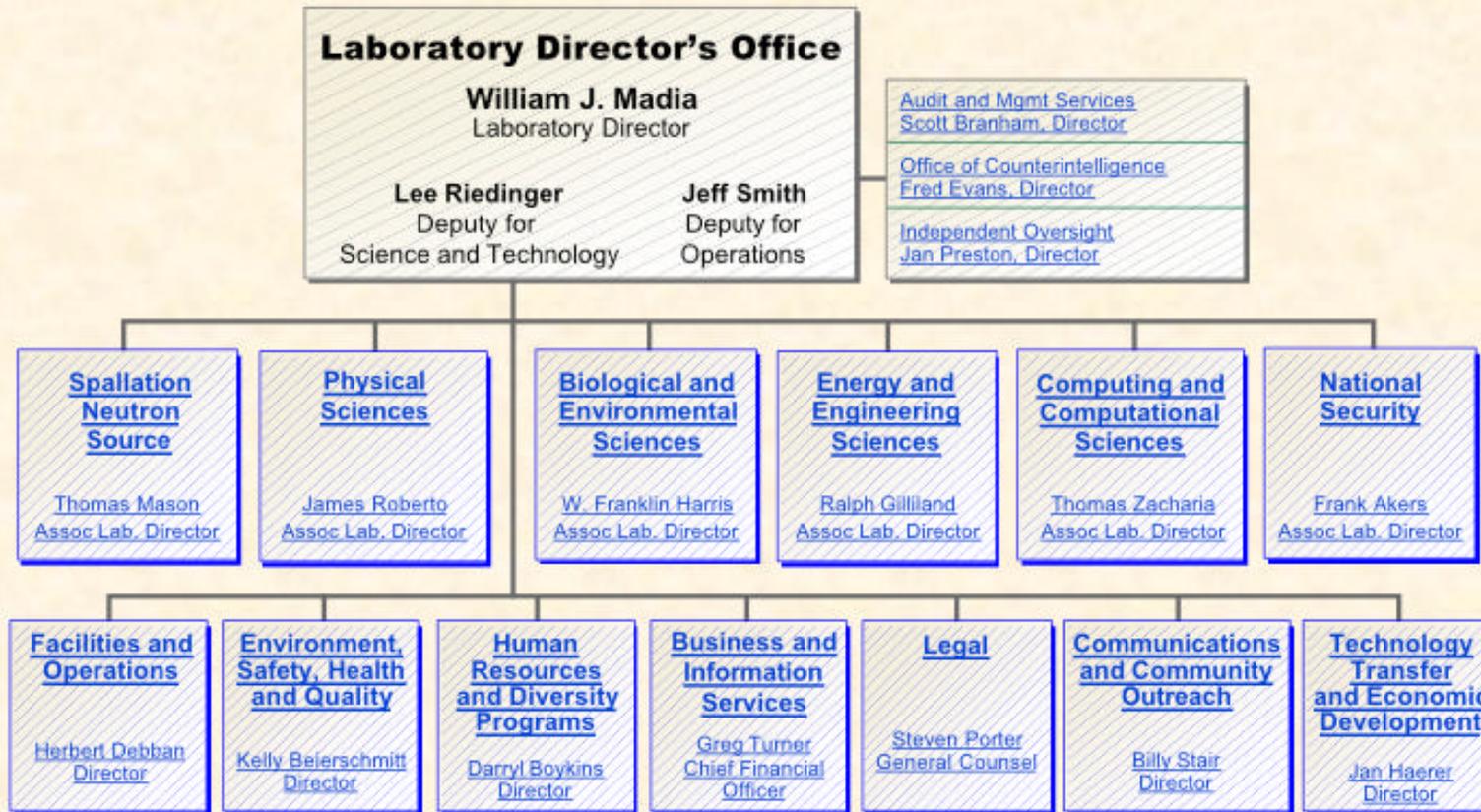
ORNL Animal Care and Use Program



ORNL Animal Care and Use Program



Oak Ridge National Laboratory



OAK RIDGE NATIONAL LABORATORY
U. S. DEPARTMENT OF ENERGY

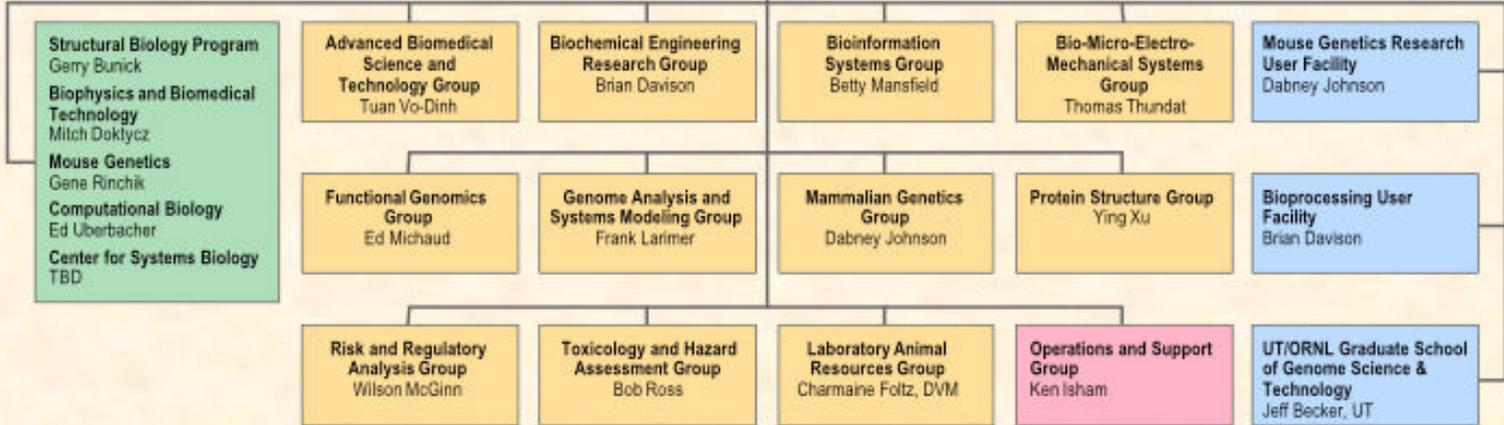


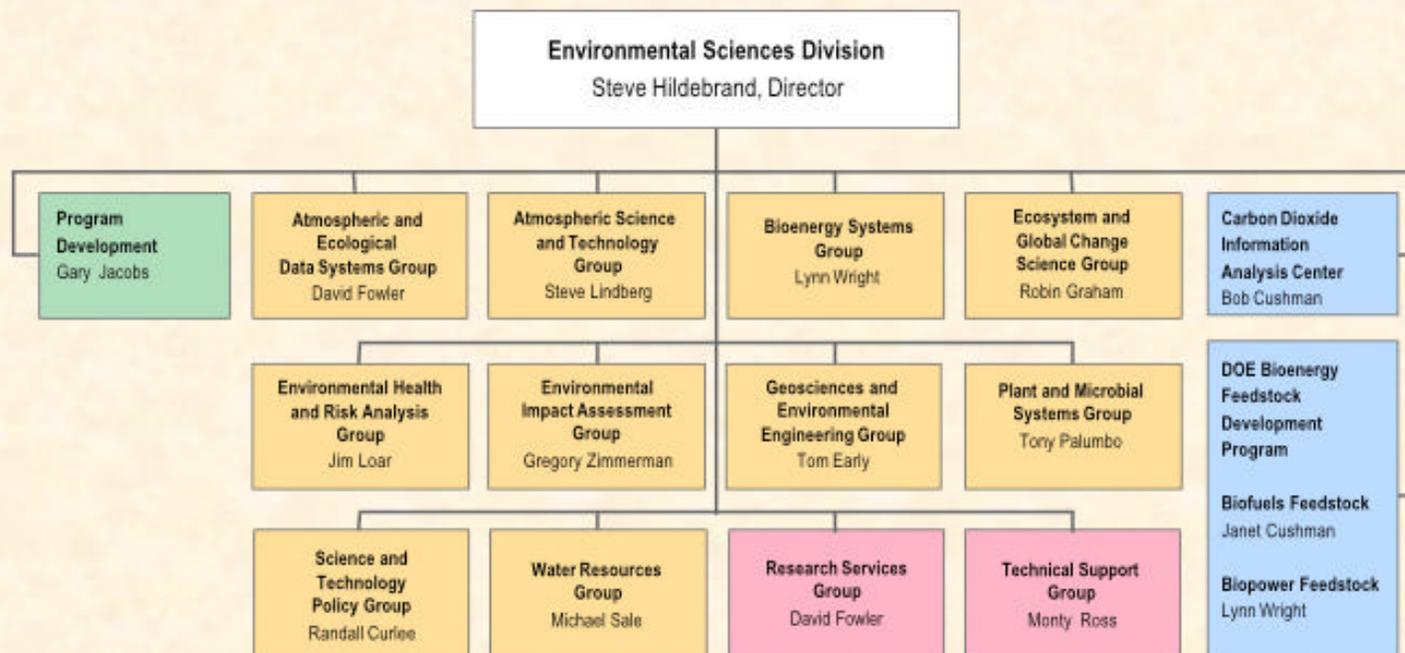
11/16/01

Effective 10/1/01

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Life Sciences Division
Frank Harris, Acting Director





Appendix 2

List of Active Protocols

Active Protocols

Principal Investigator	Protocol #	Species	Protocol Title
Bevelhimer, M.	0295	Fish	Fish Guidance by Turbulence
Bevelhimer, M.	0299	Fish	Effects of Sound on Fish at Hydropower Dams
Bunick, G.	0272	Chicken	Structural Studies of Nucleosomes
Culiat, C.	0285	Mice	Genetic Variation In and Role of Egg ECM in Fertilization
Doktycz, M.	0277	Mice	Understanding Vitronectin Through Functional Genomic Approaches
Donaldson, T.L.	0291	Rats	Tracking - Pre-Symptomatic Diagnostic Method for Infection by Pathogens
Ericson, N.	0278	Rats	Tracking - Implantable Micro-Instrumentation for Real-Time Tissue Perfusion Monitoring
Greeley, M., Jr.	0244	Fish	Molecular Basis for Molecular Resistance to Low-Level Ionizing Radiation in Mosquitofish
Greeley, M., Jr.	0247	Fish	Maintenance and Breeding of the Zebrafish, <i>Brachydanio rerio</i>
Greeley, M., Jr.	0248	Fish	Maintenance and Breeding of the Fathead Minnow, <i>Pimephalus promelas</i>
Greeley, M., Jr.	0249	Fish	Integrated Complex Pathways of Endocrine Disrupting Compounds in Zebrafish and Other Fish Models
Greeley, M., Jr.	0270	Fish	Biological Indicators Assessment of Fish Health and Exposure to Environmental Contaminants
Greeley, M., Jr.	0274	Fish	Development and Applied Use of a Fish Embryo-Larval Toxicity Test for Environmental Monitoring Purposes
Greeley, M., Jr.	0283	Fish	Applied Use of The Fathead Minnow Larval Survival and Growth Test for Estimating The Chronic Toxicity of Effluents and Receiving Waters
Greenbaum, E.	0282	Rats & mice	Tracking - High-Density Microelectronic-Tissue Hybrid Sensor for Imaging
Hoyt, P.	0245	Mice	Automated High-Throughput Production of RNA from Mouse Tissues
Hughes, J.	0264	Geese	Environmental Surveillance Program, Annual Goose Roundup
Hughes, J.	0292	Fish	Environmental Surveillance, Fish Sampling Program
Johnson, D	0298	Mice	Characterization of the Phenotypic Variation in Expression of the orpk Mutation
Johnson, D	0259	Mice	A Mouse House Screenotype
Johnson, D	0263	Mice	Regulation of a Putative Adipocyte

Principal Investigator	Protocol #	Species	Protocol Title
			Aminophospholipid Translocase-a Prime candidate gene for Increased Body Fat on Mouse Chromosome 7
Johnson, D	0281	Mice	Regulation of a Putative Human Aminophospholipid Translocase Gene by Insulin, Carbohydrates, Obesity and Diabetes
Kennel, S.	0297	Mice & Rats	Production of Immune Reagents to Endothelium
Kennel, S.	0256	Mice	Vascular Targeted Radioimmunotherapy
Knapp, F.	0239	Mice & Rats	Biological Evaluation of Radiolabeled Fatty Acid Analogues
Liu, Y.	0293	Mice	Genetic Analysis of Gene(s) Regulating Mammalian Telomeres and Cell Cycle
Michaud, E.	0261	Mice	Functional Analysis of Complex Biological Systems in the Skin of Mice
O'Rourke, D.	0258	Mice	Investigator Training and Services Provided by Laboratory Animal Resources
O'Rourke, D.	0284	Mice	Health Surveillance of Mouse Colonies Using Sentinel Mice
Paulus, M.	0286	Mice	Small Animal Imaging
Paulus, M.	0296	Mice	Micro-CT Identification and Monitoring of Prostate tumors in TRAMP Mice
Peterson, M.	0269	Fish	Monitoring and Assessment of Contaminant Accumulation in Aquatic Organisms
Rinchik, E.	0260	Mice	Mutagenesis of the Mammalian Imprinting Process(es)
Rinchik, E.	0276	Mice	Genetic Variation in Cellular Responses to Low-Dose Radiation
Rinchik, E.	0288	Mice	Pilot Molecular Phenotyping for Variability in Response to Low-Dose Radiation
Rinchik, E.	0289	Mice	Mouse Genetics and Mutagenesis
Ryon, M.	0266	Fish	Fish Community Studies Test of the Biological Monitoring and Abatement Program
Ryon, M.	0279	Fish	Development of a New Technique to Assess Susceptibility of Fish to Predation Resulting from Sublethal Stresses (Indirect Mortality)
Ryon, M.	0280	Fish	Live Animal Displays in Aquatic Ecology Laboratory, Building 1504
Ryon, M.	0287	Aquatic Wildlife, Snakes	Do Contaminants Released From the Oak Ridge Reservation Accumulate in Aquatic Snakes?
Todd, P.	0294	Rat	Organizational Distribution of Lipids and Fatty Acids Within the Rodent Using the Techniques of Secondary Ionic (SIMS) and Tandem (MS/MS) Mass Spectrometry
VoDinh, T.	0241	Mice	Assessment of Tumor Suppressor Activity of

Principal Investigator	Protocol #	Species	Protocol Title
			Fhit gene in vivo & in vitro; Phenotypic and Genotypic Responses of Over 500 Genes in Presence or Absence of the Fhit Gene
VoDinh, T.	0246	Pig, Sheep	Tracking - Acoustic Monitoring Technology
VoDinh, T.	0290	Canine	Tracking - Multi-Spectral Imaging
You, Y.	0273	Mice	Functional Analysis of the Distal Half of the Mouse Chr 15

Appendix 3

Daily Average Inventory and Annual Use by Species

Current Daily Average Animal Inventory and Annual Use

Species	Average Daily Inventory	Annual Use
Mice	60,000	216,300
Rats	23	53
Avian (non-domestic)	0	79
Chicken	0	12
Fish	2,500	116,300
Minnow Larvae	2,500	16,000
Amphibians	10	50
Reptiles	10	50
Aquatic Snakes	0	16

Appendix 4

Animal Usage Form

Animal Usage

Type of Procedure	Protocol #s	Species	Approx #/Year	Principal Investigator	Location of use Bldg/Rm	
Administration of mutagens, teratogens, carcinogens (ENU)	0260	Mice	100	Rinchik	9210	135
	0289	Mice	447	Rinchik	9210	135
Administration of Radioisotopes	0239/0300	Mice Rats	400 none to date	Knapp	4501	317A
	0243/0297	Mice Rats	20 none to date	Kennel	4501	317A
	0256	Mice Rats	200 none to date	Kennel	4501	317A
	0286	Mice	20	Paulus	4501	317A
	0296	Mice	none to date	Paulus	4501	317A
Antibody Production/ Freund-s/Ascites	0243/0297	Rats Mice	5 25	Kennel	4500S	F-149
Behavior Testing	0259	Mice	125	Johnson	9210	306 307 329
	0279	Fish	810	Ryon	1504	1
	0289	Mice	400	Rinchik	9210	306 307 329
	0295	Fish	300	Bevelhimer	Field study	
	0299	Fish	200	Bevelhimer	1504	tank room
Embryo transfer (via oviduct and uterus)	0261	Mice	100	Michaud	9210	136
	0273	Mice	100	You	9210	134
	0285	Mice	none to date	Culiat	9210	136
	0289	Mice	20	Rinchik	9210	138
Environmental toxicity testing & Biological Monitoring	0249	Zebrafish Medaka	500	Greeley	1505	263
	0266	Fish	47,000	Ryon	Field Study	
	0269	Fish Minnow Clams	213 143 500	Peterson	1505	153
	0270	Fish	257	Greeley	1505	153
	0274	Medaka (embryo- larvae)	2,000 5,000	Greeley	1505	263
	0283	Minnow larvae	6000	Greeley	1504	12
	0287	Snakes Fish	20 20	Ryon/Campbell	Field Study	
	0264	Geese	3	Hughes	Field Study	
Environmental Monitoring	0292	Fish	810	Hughes	Field Study	
Hemi-castration	0259	Mice	none to date	Johnson	9210	138
	0276	Mice	none to date	Rinchik	9210	138
	0289	Mice	none to date	Rinchik	9210	138
Nephrectomy	0289	Mice	none to date	Rinchik	9210	138
Ovary transplant	0289	Mice	50	Rinchik	9210	138
Restraint (Short Term & one 2hr confinement) (MicroCT Scan or X-Ray)	0276	Mice	60	Rinchik	9210	125
	0286	Mice	50	Paulus	9210 3500	125 C-15
	0288	Mice	32	Rinchik	9210	125

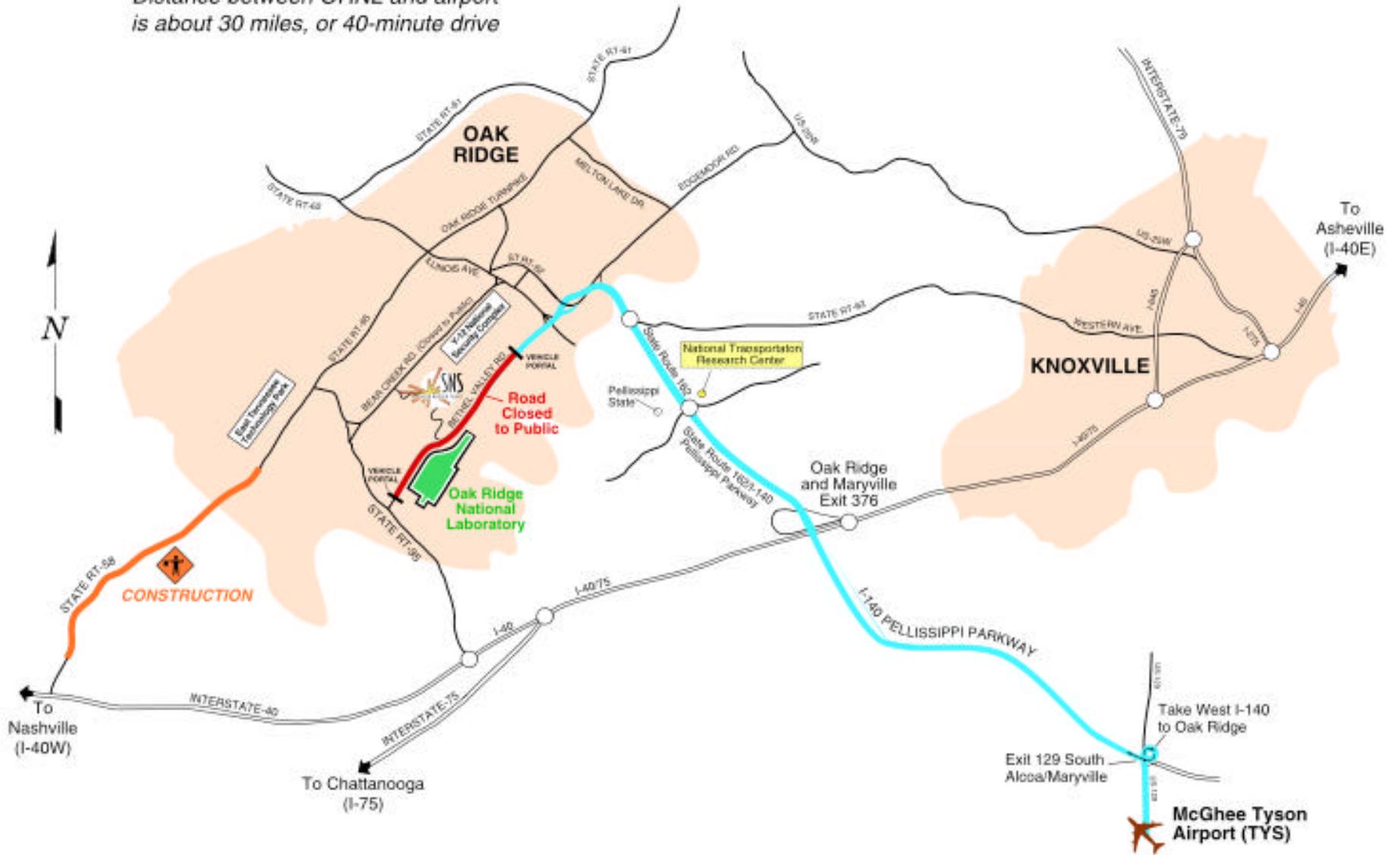
Type of Procedure	Protocol #s	Species	Approx #/Year	Principal Investigator	Location of use Bldg/Rm	
	0293	Mice	none to date	Liu	9210	125
Splenectomy	0289	Mice	none to date	Rinchik	9210	138
	0276	Mice	none to date	Rinchik	9210	138
Tumor generation	0241	Mice	6	Vo-Dinh	4500S	F-149
	0243/0297	Mice Rats	200 none to date	Kennel	4500S	F-149
	0256	Mice	200	Kennel	4500S	F-149
	0296	Mice	none to date	Paulus	9210 3500	na
Vasectomy	0261	Mice	36	Michaud	9210	136
	0285	Mice	none to date	Culiat	9210	136
	0289	Mice	30	Rinchik	9210	138
Wildlife trapping, whole body count and release	0264	Geese	105	Hughes	Field Study	
Wildlife trapping	0266	Fish	47,000	Ryon	Field Study	
	0269	Fish Minnow Clams	213 143 500	Peterson	1505	153
	0270	Fish	798	Greeley	1505	153
	0274	Fish	none to date	Greeley	1505	153
	0279	Fish	810	Ryon	1504	1
	0280	Fish, Reptiles, Amphibians	210	Ryon	1504	1
	0287	Snakes Fish	100 none to date	Ryon/Campbell	Field Study	
	0292	Fish	810	Hughes	Field Study	
0299	Fish	200	Bevelhimer	Field Study		

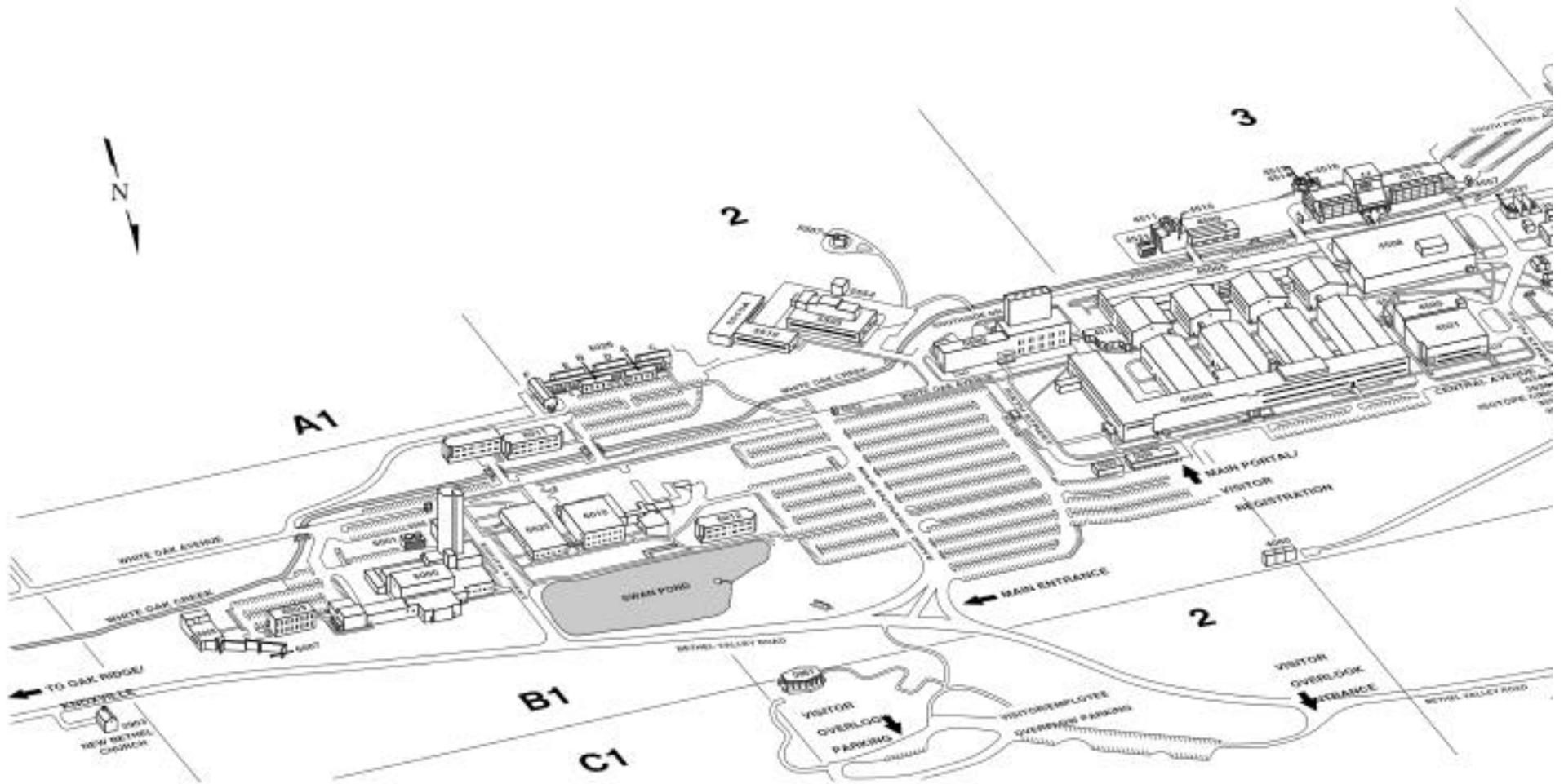
Appendix 5A

Maps

Oak Ridge/Knoxville Airport Route Map

Distance between ORNL and airport is about 30 miles, or 40-minute drive



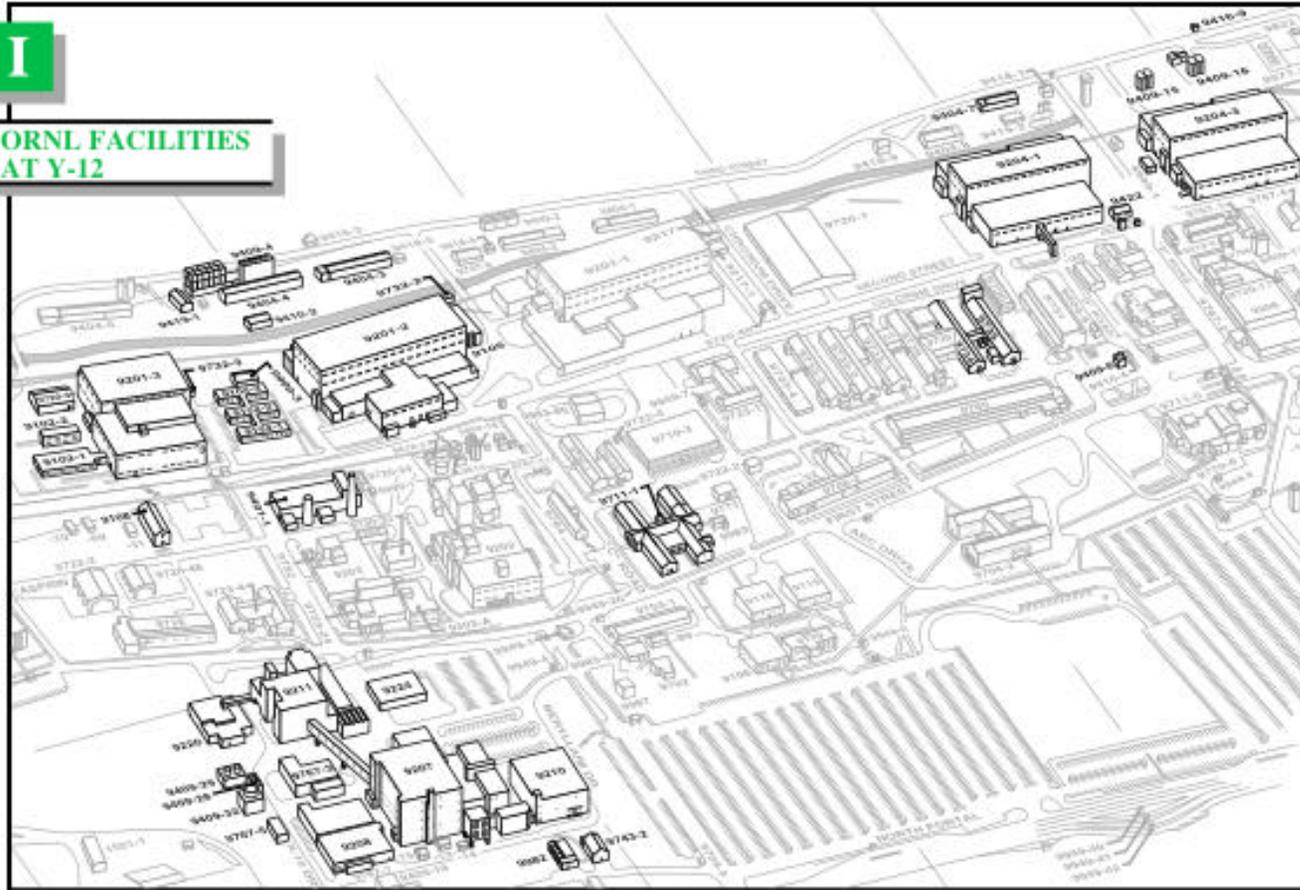


KEY

BLDG	LOC	FUNCTION	BLDG	LOC	FUNCTION	BLDG	LOC	FUNCTION
0502D	A1D	New Bethel Church	3003D	C4D	Fluor House Graphite Reactor	4300D	05D	13.8-kV Substation
0501D	C2D	Water Overhead	3007D	C4D	Sol. Mod. & Characterization Lab	4305D	C2D	High-S. Portal - Sentry Post 610D
1000D	06D	Engineering	3009D	C4D	Water Demineralizer	4500ND	05D	Control Rm. & Admin.-North
10524D	05D	Cont. Eng. Off. D	3009D	04D	Low-Intensity Testing Plant. (LUTP)	4500SD	05D	Control Rm. & Admin.-South
10600D	06D	Cover. Eng. Off. D	3009D	06D	Source-and Special Material Vault	4501D	06D	High-Level Radiochemical Lab
10544D	05D	Engr. Model Shop-TTC	3010D	C3D	Bulk Shielding Reactor	4502D	05D	Departmental Engineering
10548D	05D	Engr. Office-TTC	3012D	05D	Rolling Mill	4507D	05D	High-Rad. Level (HRL) Chem. D
1057D	C3D	100-m Metall. Tower	3013D	C4D	Waste Operations Annex	D	D	Des. Lab
1059D	06D	Health Effect Info Bldg D	3017D	C4D	Chemical Tech. Ctr. Annex	4508D	05D	Metals & Ceramics Laboratory
1061D	06D	Health Prot. Serv. Bldg D	3018D	06D	Exhaust Stack for Bldg 3030D	4509D	06D	Compressor House
1062D	06D	West Dr. Bldg. Lab Protection	3018ND	06D	Radiochem. Processing Pilot Plant	4510D	06D	Cooling Tower for 4509D
1063D	06D	Regional Sci. Ctr. Cont.	3018ND	06D	HRL Analytical Lab-N	4511D	06D	Cooling Tower for 4508D
1066D	05D	Prog. Sh.-W/P. Lab	3020D	04D	Exhaust Stack for Bldg 3018D	4512D	05D	Lab Emergency Response Ctr-D
1070D	06D	Plant Sciences Lab	3020D	04D	North Tank Farm	4513D	06D	Backup Substation for HRL
1074D	06D	Aqua. Res. Lab	3020ND	04D	Solid State Division Offices	4514D	06D	HRL Emergency Generator
1075D	06D	Environ. Sci. Lab	3022D	04D	MS/C Phys. Burnt. Hot Cell-A	4515D	06D	High-Temp. Mts Lab (H-TML)
1086D	06D	Env. Environ. & Atmos. Fac. D	3026D	06D	Radiois. Development Lab-ND	4516D	06D	Cooling Tower for H-TML
1077D	06D	Life Sci. Data Analysis	3028D	04D	Dismantling & Burn. Hot Cell	4521D	06D	Cooling Tower for 4505D
1090D	06D	Isot. Serv.	3027D	06D	Styl. Nucl. Materials (SRM) Vault	4567D	06D	S. Park. Lot Port. -see. Post 79D
1090D	05D	East Greenhouse	3029D	03D	Radioisotope Production Lab-A	5000D	05D	Main Portal-Sentry Post 1D
1091D	05D	West Greenhouse	3029D	03D	Radioisotope Production Lab-C	5002D	05D	Genet. Users-Human Res. Office
1094D	05D	West Office Trailer	3029D	03D	Radioisotope Production Lab-DE	5003D	06D	High-voltage Accelerator Lab
1095D	05D	East Office Trailer	3029D	03D	Radioisotope Production Lab-EE	5005D	06D	Transmutation Research Lab
1096D	05D	Sci. State Archival. Assurances	3029D	03D	Radioisotope Production Lab-ED	5006D	06D	E. Parking Lot Portal-Sentry
2001D	C4D	Health & Saf. Res. Annex	3034D	03D	Radio Area Services	D	D	Post 18D
2002D	C4D	Process Water Cov. Str. D	3036D	06D	Hot. Area Stor. & Sec. Fac. D	5007D	06D	Radionucl. Spectrometer Facility
2007D	04D	Health Phys. Ctr. Lab	3037D	03D	Environ. & Health Prot. Ctr. D	5010D	06D	Analytical Mass
2008D	06D	Health Phys. Int. Ctr. Lab	3038D	03D	Radioisotope Laboratory	D	D	Spectrometry Lab
2009D	04D	Cal. Cor. Bldg. D	3038D	04D	Stack. Gen. Rad. Gas Dispos. Fac. D	5010ND	06D	Inorg. Mass Spectrometer Lab
2010D	04D	Cafeteria	3042D	06D	O. Ridge Research Plant. (ORRP)	5050D	05D	White Oak E. Portal-Sentry
2011D	04D	Mech. Prop. Lab. No. 2D	3044D	06D	Special Materials Machine Shop	D	D	Post 19D
2012D	05D	W. Maint. Sec. Ctr. D	3047D	03D	Isotope Technology Lab	5054D	06D	Electrical Substation for Bldg. 5050D
2013D	06D	West Portal	3048D	04D	Interim Manipulator Rep. Facility	6000D	01D	Half-Sold House for Res. Fac. D
2016D	04D	Sec. & Air-cond. Sec. Ctr. D	3077D	C4D	Air-Cooler-Low Intensity Test	6014D	01D	Cooling Tower -- Bldg. 6000D
2019D	C3D	Solar Energy Laboratory	D	D	Plant. (LTPM)	6015D	01D	Modular Office Bldg. D
2024D	C4D	Info. Ctr. Computer	3080D	C3D	Reactor Experiment Control Room	6020D	01D	Gas Compressor Hall -- Bldg. 6000D
2025D	04D	High-Peak-Level Anal. Fac. D	3080D	C3D	Pumproom -- ORRP	6027D	01D	Joint Institute For Heavy D
2026D	C4D	Info. Ctr. Trailer Annex	3080D	C3D	Aux. Hot. ORP-C/No. 1D	D	D	Ion Research
2030D	C4D	Mobile Ctr. Unit-Trailer	3087D	C3D	Heat Exchanger -- ORRP	6080D	01D	Joint Inst. Ctr. & Lab For Heavy D
2030D	06D	Mass. & Controls Support Facility	3092D	04D	Off-Gas Facility-4000 (OFM)	D	D	Ion Research
2060D	05D	Change House	3092D	C4D	Reactor Area Equip. Bldg. D	6010D	01D	Oak Ridge Electron Lin. Accel. D
2060D	C4D	Environmental Storage	3100D	C4D	Material Storage Vault	6011D	01D	Computing & Telecom. Facility
2060D	05D	Gen. & Pre-Hg. D	3100D	C4D	ORP-Cooling Tur. 3D	6012D	06D	Computer Sciences Research Fac. D
2060D	04D	Rad. Sh. Trng. & PC-Sup. Ctr. D	3104D	03D	West Research Service Ctr. D	6020D	01D	Engineering Physics Ctr. & Lab Fac. D
2070D	04D	Res. Des. & Spn. Dept. Off. D	3106D	06D	Cal. Met. Rm. for Bldg. 4001 D	6029ND	06D	CRTO-User Services
2070D	05D	Plant & Equip. Ctr. Ctr. D	D	D	4505, 4507D	6029SD	06D	CRTO-System Dep. Tech. D
2074D	04D	Steam Plant	3110D	04D	Cal. Vent. Filters for Rad. Area	6029SD	06D	CR. C/ Compliance AC
2075D	04D	Sewage Treatment Plant	3112D	C4D	North-portal Sentry Post 600D	D	D	Documentation -- Documentation --
2076D	06D	Descont. Liquid	3114D	C3D	Hot Test Development Facility	D	D	Environmental Compliance
2076D	04D	Fabrication Dept. -- Shop A	3118D	C3D	Low-Temperature/Reactor Fac. D	D	D	Section
2076D	04D	Coal Research Laboratory	3117D	C3D	Cooling Tur. -bulk Shielding Plant-D	6030SD	06D	CRTO-User Services
2076D	04D	Rad Waste Evaporator Fac. D	3070D	06D	Storage Vault	6029SD	06D	Gen. Mgmt. - Ctr. C/ Guest
2076D	04D	Exp. Cool. Tur. for Bldg. 2071D	3028D	03D	Personal Monitoring Station	D	D	& User Interact. D
2080D	06D	Coal Lab. & Coal Pile Runoff	3030D	04D	Waste Operations Control	6029PD	AC	Train. Conference/Meetings
D	D	Ther. Fac. Bldg. D	D	D	Control	D	D	
2087D	04D	Exp. Serv. Trng. Ctr. Rm. D	3037D	04D	Surface Sciences Lab			
2090D	04D	Cool. Tur. Substn. for Bldg. 2071D	3038D	C3D	Post Terminal Research Center			
2090D	04D	Substation in Steam Plant	3044D	C3D	Buildings Technology Center			
2090D	06D	Aeration Pond-Gas	3047D	C3D	Office For Phys. & Renewable Res. D			
2094D	06D	Aeration Pond-Water	3050D	04D	Solid State Res. Facility			
2094D	06D	Coal Pile Settling Basin	3070D	C3D	Envelope Systems Research Center			
2094D	06D	Coal Pile Monitoring Facility	3050D	06D	Ch. & Support Fac. -- Energy Ctr. D			
2097D	04D	General Machine Shop	3050D	06D	H&C Div. Ctr. & Research Fac. D			
2098D	04D	Steam Plant Storage Building	3050D	06D	Gas Research Service Center			
2097D	04D	CRTO Support Group	3050D	04D	High-Rad. Level Chem. Engr. Lab			
2098D	04D	Bethel Valley Waste Ctr. Truck	3060D	04D	Oncogenesis Lab			
2098D	06D	West Portal-Sentry Post 3D	3060D	04D	Ration Prod. Dev. Lab Annex			
2098D	06D	Environmental Compliance	3060D	04D	Radioisotope Production Lab-CD			
D	D	& Documentation Group	3067D	04D	South Tank Farm			
2098D	06D	Res. Prot. And Storage	3068D	04D	Radio Service Center			
2098D	04D	5000-kVA Substation	3013D	04D	Setting Room			
2098D	04D	Steam Plant Scale House	3017D	04D	Ration Product Dev. Lab			
2098D	06D	S. Hl. Wtr. Gate-Sentry Post 4D	3018D	04D	Neutronization Facility			
2098D	06D	Coal Yard (Mtr. Gate-Sentry)	3022D	03D	Control Research Lab			
D	D	Post 60D	3022D	04D	Process Waste Systems Drain			
2098D	04D	S. End. 3rd St. Gate-Sent. Post 7D	3023D	04D	High-rad Level Form. Lab (HRLSLD)			
2098D	06D	Chlorinator Bldg. D	3024D	03D	Liquid Metal Cleaning Facility			
2098D	06D	Coal Pile Runoff Treatment Plant	3025D	06D	Hydrogen & Oxy. Gas. Station			
2098D	04D	Energy Generator Cool Handling	3026D	04D	Process Waste Pond No. 1-North			
2098D	06D	CRTO Contractor Facility	3026D	04D	Process Waste Pond No. 2-South			
2098D	06D	Res. Training & Test Facility	3040D	04D	Process Waste Treatment Plant			
2098D	04D	SW L/W-CAT Sys. Upgrade	3040D	03D	Instrument & Controls Div. D			
2098D	06D	Environ. & Health Prot. -D	D	D	Off. Annex			
D	D	Drink. Ctr. D	3050D	04D	Research Laboratory Annex			
2098D	06D	Applied HP-Field Survey	3067D	03D	Instrument Laboratory Annex			
2098D	06D	Environmental & Health	3068D	04D	Coal Conversion Facility			
D	D	Protection -- Mass. Assurance	3069D	04D	Waste Management Storage			
2098D	06D	Gen. Compliance Training Fac. D	3070D	06D	Hot Storage Garage			
2098D	C4D	13.8-kV Substation	3080D	04D	Instrument & Controls Div. D			
2098D	06D	Graphite Reactor	D	D	Off. Annex			
			3088	04D	Non-radio. Wastewater Tr. Plant			

I

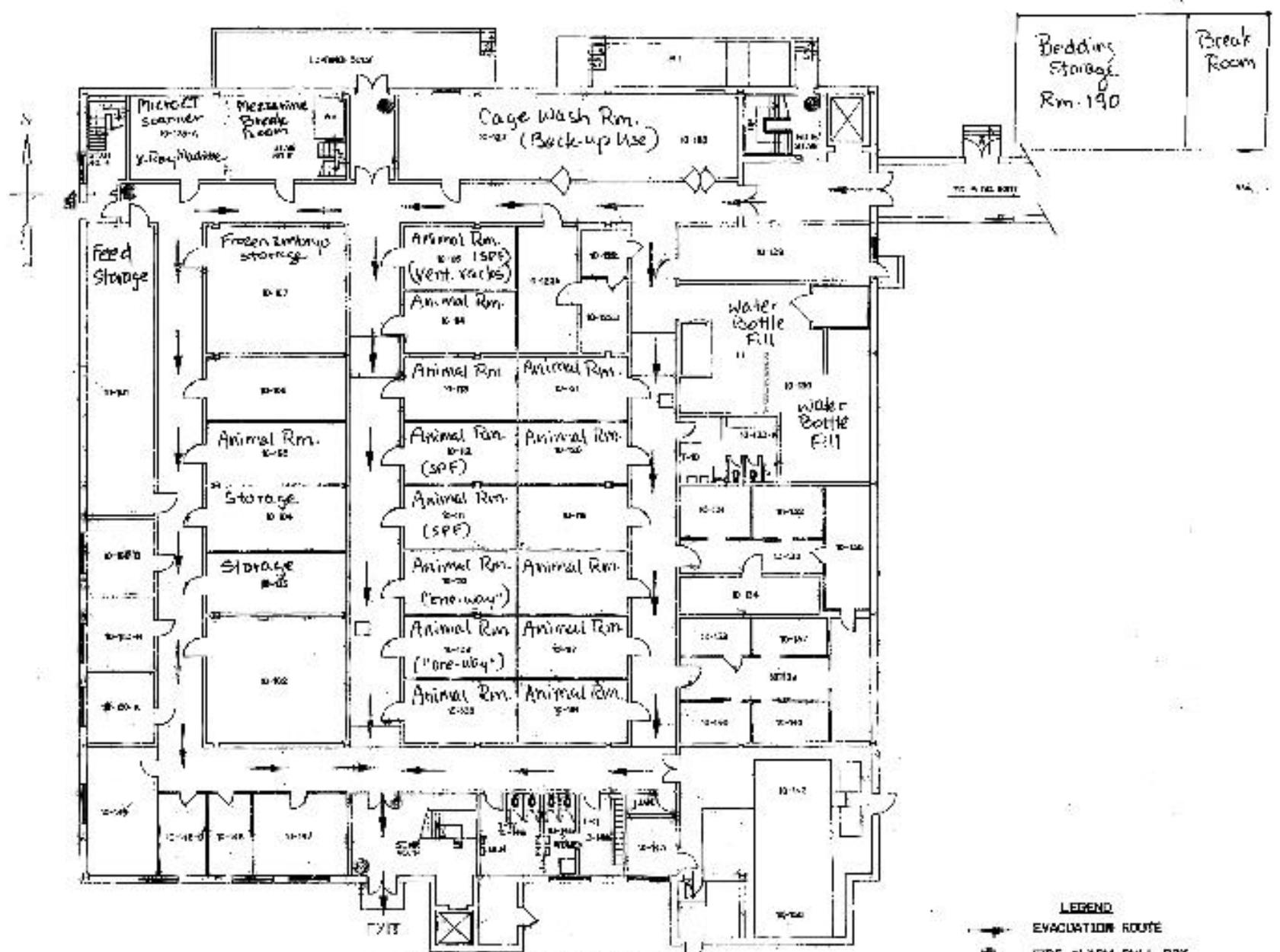
ORNL FACILITIES AT Y-12



- 9102-1 ETD-Office Building
- 9102-2 ETD-Office Building
- 9104-1 ETD-Office Building
- 9104-2 ETD-Office Building
- 9104-3 ETD-Office Building
- 9105 FED-Office Building
- 9108 ETD-Office Building
- 9201-2 FED-Admin./lab
- 9201-3 ETD-Admin./lab
- 9204-1 ETD-Ofc./lab
- 9204-3 CTD-Isotope Separations
- 9207 BD Admin. Lab
- 9208 BD Lab/engin.
- 9210 BD Mammalian Gen.
- 9211 BD Co-Carcinogenesis
- 9220 BD Virus Control Lab
- 9224 Cell Fractionation System
- 9401-1 ETD-Laboratory
- 9404-3 FED-Pumphouse
- 9404-4 FED-Pumphouse
- 9404-7 FED-Pumphouse Storage
- 9409-4 FED-Cooling Twr. for Bldg. 9201-2
- 9409-6 FED-Cool. Twr.
- 9409-15 Cool. Twr. for Bldg. 9204-3 (Isotope Separations)
- 9409-16 Cool. Twr. for Bldg. 9204-3
- 9409-28 BD-Cooling Tower
- 9409-29 BD-Cooling Tower
- 9409-32 BD-Cooling Tower
- 9416-9 CTD-Pumphouse
- 9419-1 ETD-Surplus
- 9422 FED-Helium Compres. Bldg.
- 9610-2 ETD Storage Bldg.
- 9711-1 Technical Library & Y-12 Fac.
- 9720-39 ETD-FED Storage
- 9732-2 Carpenter Shop
- 9732-3 Y-12 Paint Shop
- 9734 ACD-Spectrochemistry Lab
- 9735 ACD-Mass Spectrometer
- 9743-2 BD-Pigeon Quarters
- 9767-3 BD-Refrigeration Bldg.
- 9767-5 BD-Plant Air
- 9962 BD-Greenhouse
- 9999-1 CTD-motor Generator House
- 9999-3 FED-Power Supply

Appendix 5B

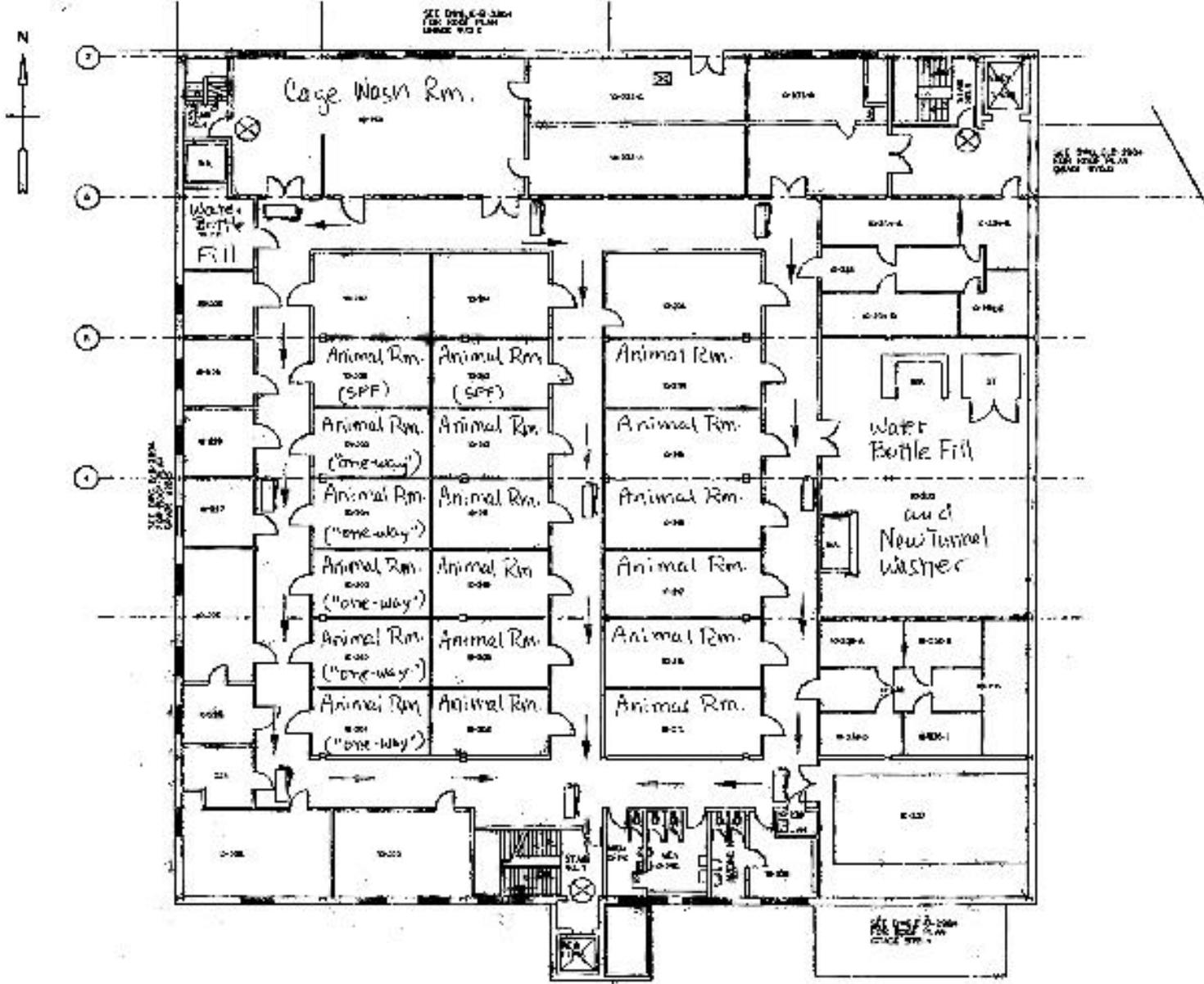
Floor Plans



BERYLLIUM DRIVE

9210 FIRST FLOOR EVACUATION PLAN

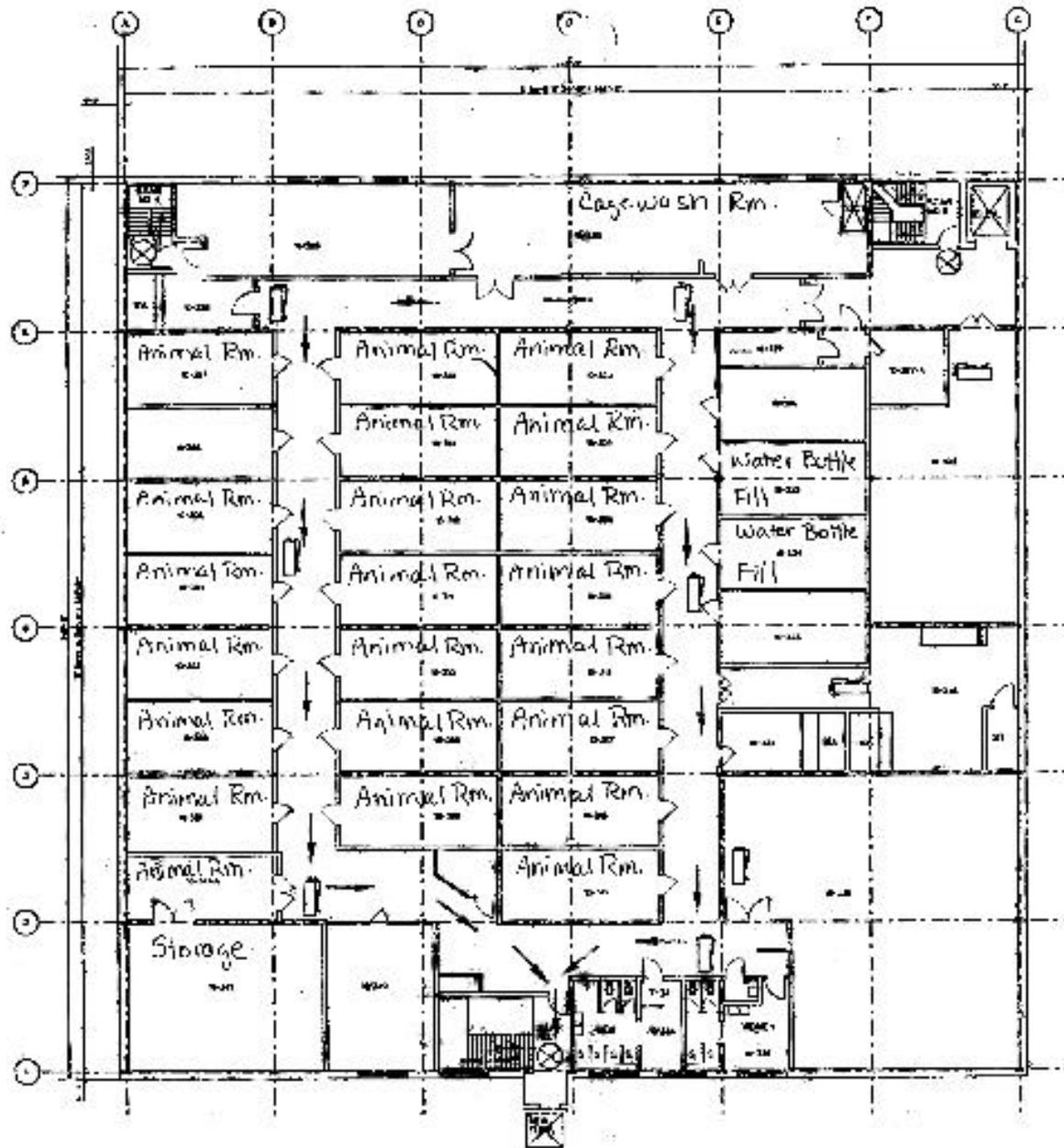
- LEGEND**
- EVACUATION ROUTE
 - FIRE ALARM PULL BOX



← BERYLLIUM DRIVE →

9210 SECOND FLOOR EVACUATION PLAN

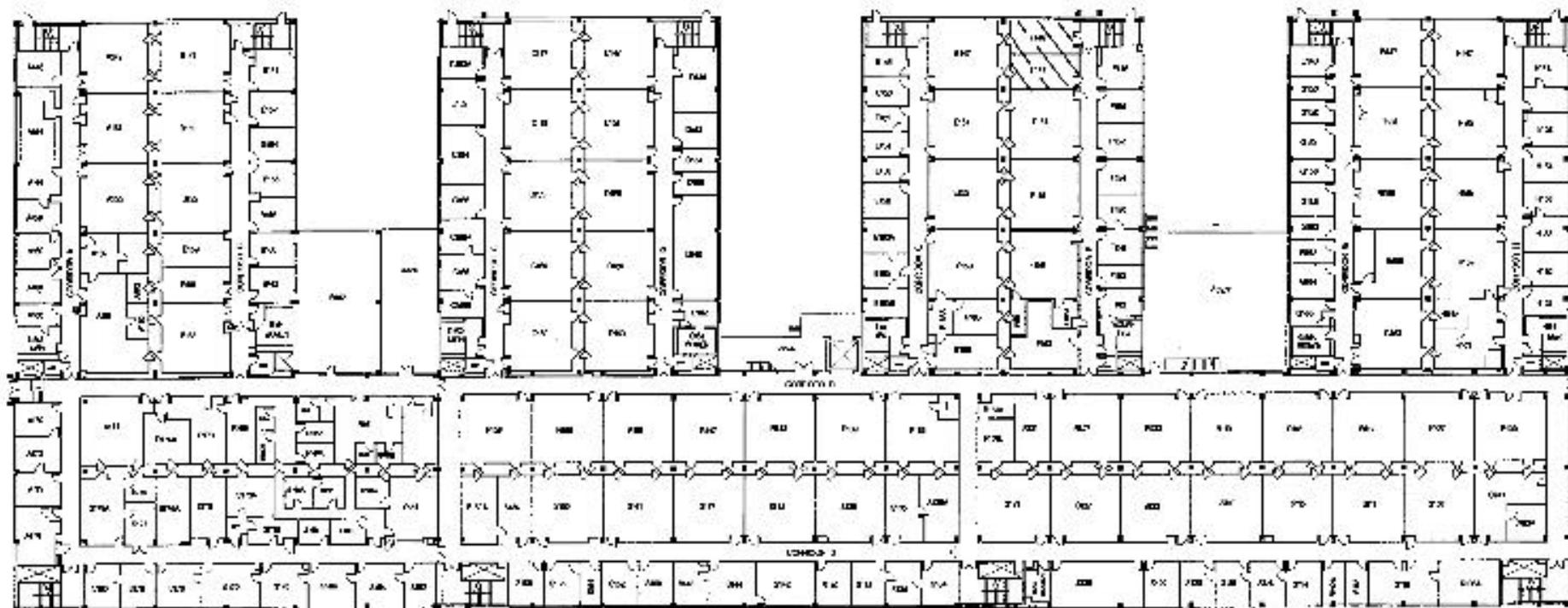
- LEGEND**
- EVACUATION ROUTE
 - ⊗ FIRE EXTINGUISHERS 9TYPE
 - ⊗ EXITS



← BERYLLIUM DRIVE →

9210 THIRD FLOOR EVACUATION PLAN

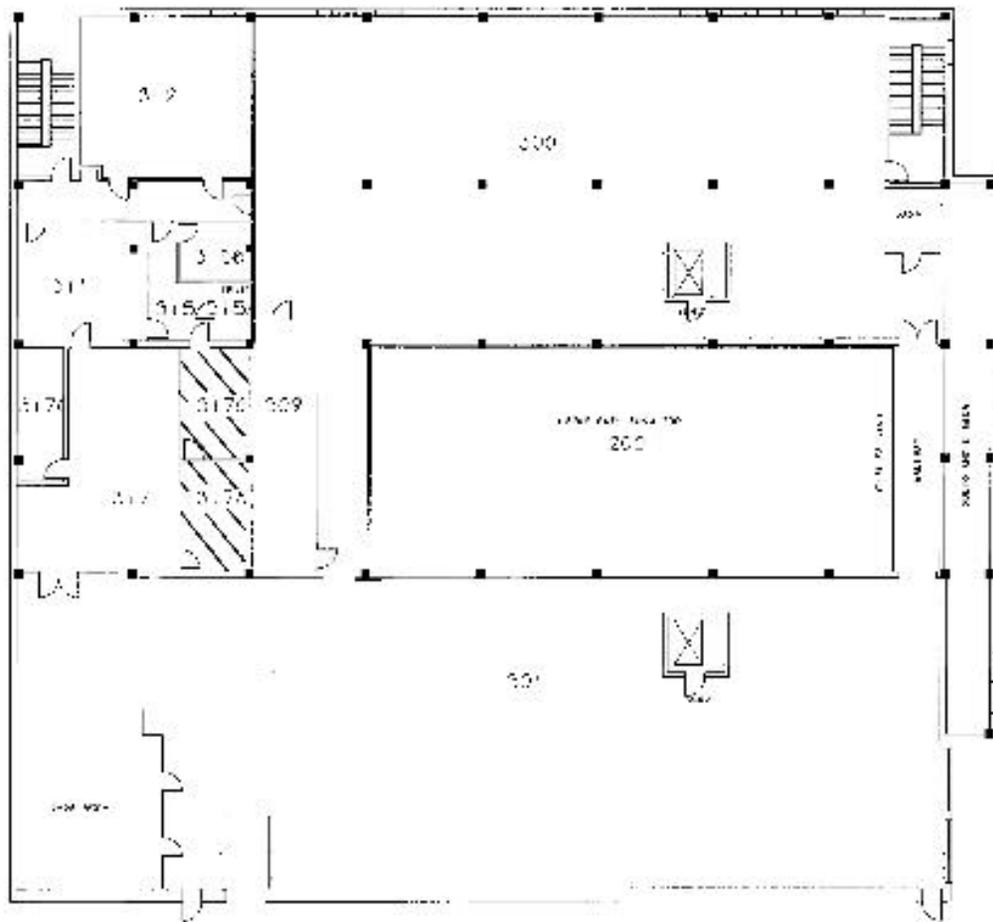
- LEGEND**
- EVACUATION ROUTE
 - ABC FIRE EXTINGUISHERS
 - X EXIT



FIRST FLOOR PLAN

Building 4500S, Room F147, Annual Room
 Room F149, Service Room (Cagewash, autoclave, storage)

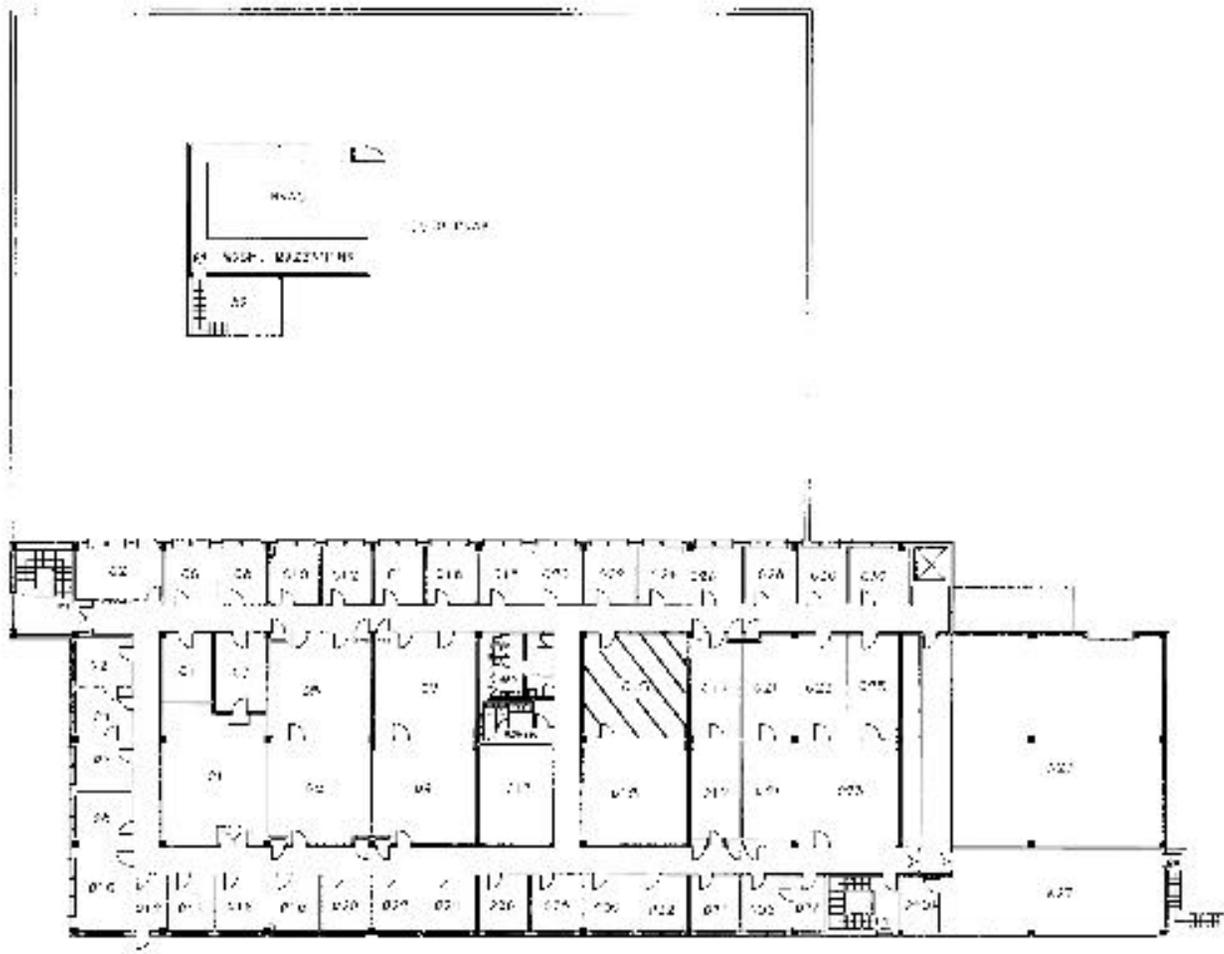




Third Floor Plan

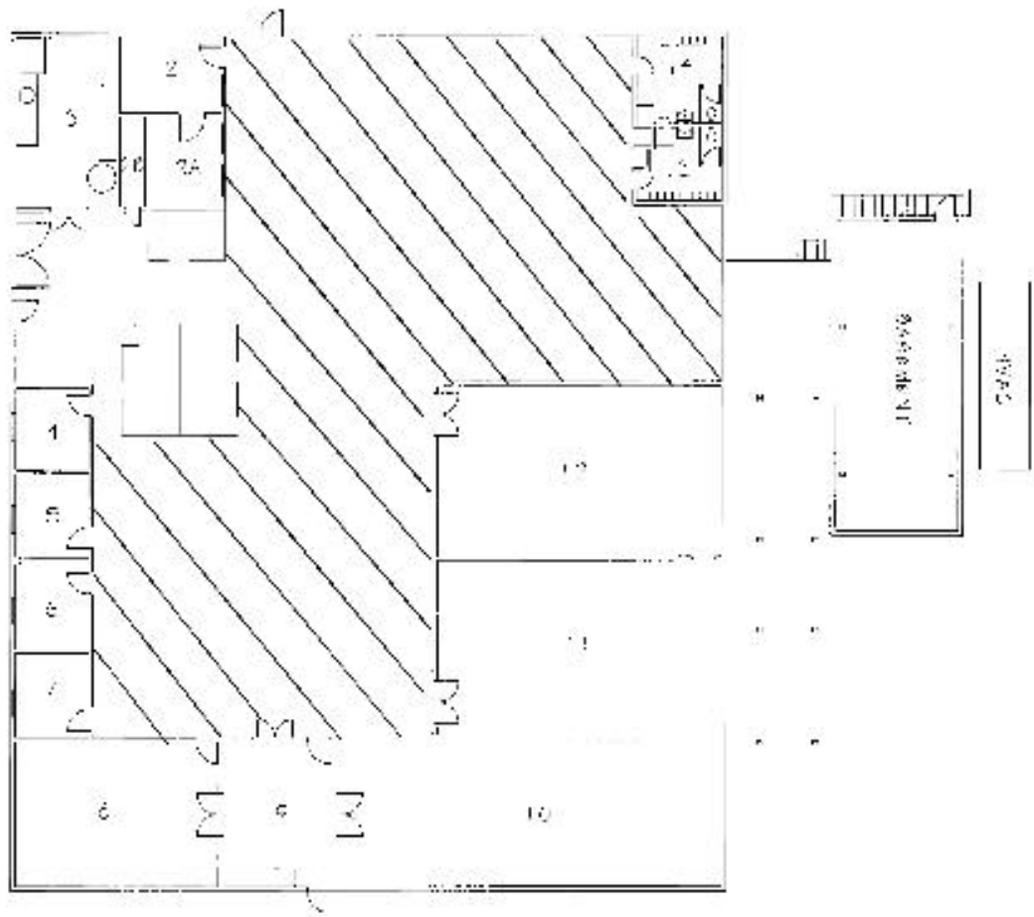
Building 4501, Room 317A, Animal Room
 Room 317B, Procedure/Lab Area





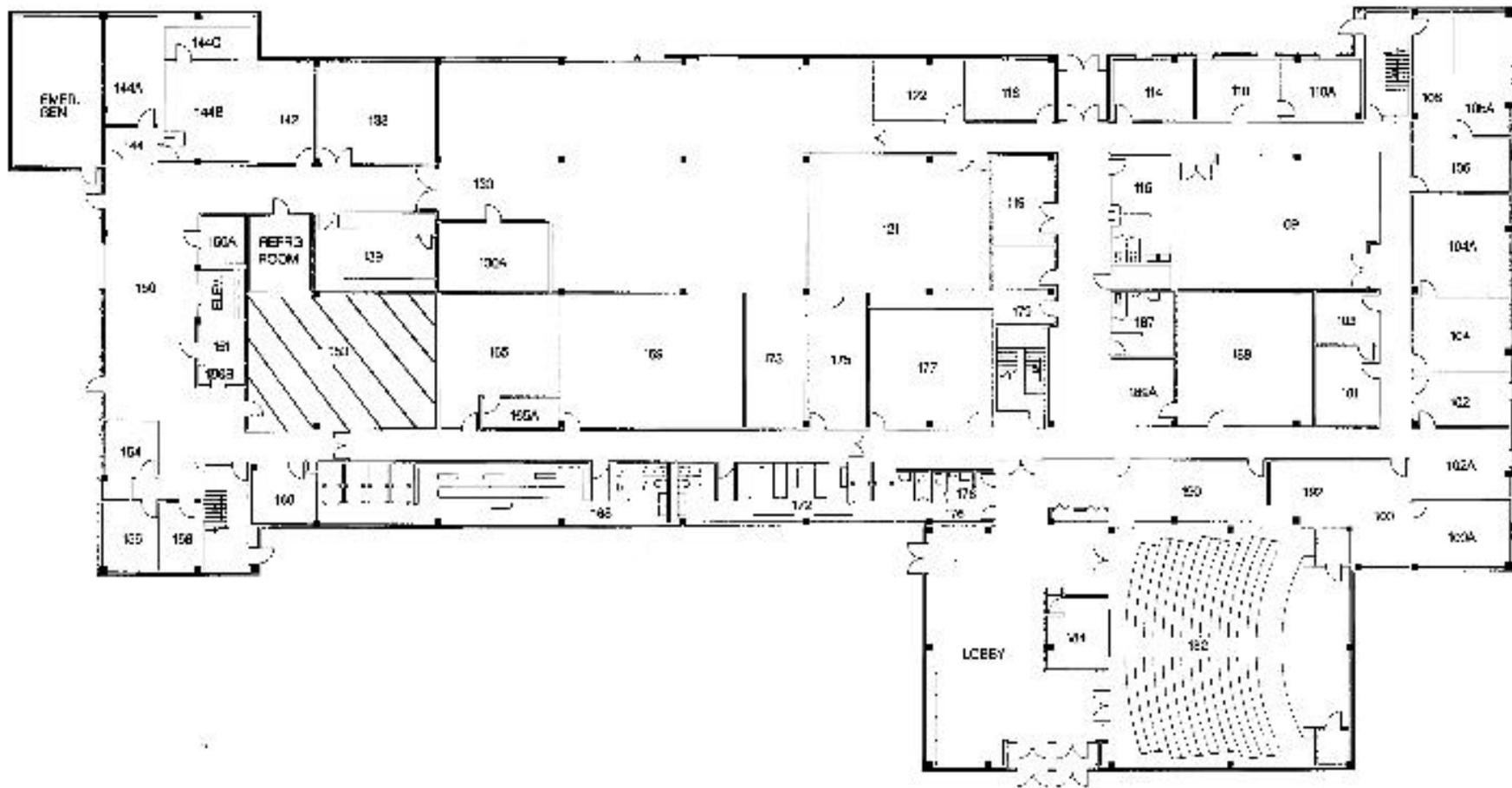
SECOND FLOOR PLAN

Building 3500, Room C15, Temporary animal housing in hood and small part of room



Floor Plan

Building 1504, Animal Room (fish tanks, spiraling stream)



First Floor Plan

Building 1505, Room 153, Field Work Station



Appendix 6

Semi-Annual Program Review

ORNL ANIMAL CARE AND USE PROGRAM**SEMIANNUAL REVIEW****April 24, 2002**

This represents the semiannual report of the Oak Ridge National Laboratory Animal Care and Use Committee (ORNL-ACUC), as required by the Public Health Service Policy on Humane Care and Use of Laboratory Animals and as a condition of this institution's Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare, and USDA Animal Welfare regulations, 9 CFR Chapter I, subchapter A, as applicable. All of ORNL's animal facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Evaluation of the Animal Care and Use Program

The ORNL-ACUC conducted its semiannual evaluation of the ORNL Animal Care and Use Program on April 24, 2002, using the *Guide for the Care and Use of Laboratory Animals*, and, as applicable, 9 CFR Chapter I, 2.31.

The following quorum of Committee members were present for this review: Barbara Beatty (Life Sciences Division-LSD), David Edds (Operational Safety Services), Mark Greeley (Environmental Sciences Division-ESD), Joan Hughes (Environmental Protection and Waste Services), Kristin Kerber (LSD), Dorcas O'Rourke (University of Tennessee), Darla Miller (LSD), Terry Mosley (community representative), and Warren Webb (ESD). Non-voting *ex officio* members G. Hodge (LSD) and D. Johnson (LSD) were present. Ed Michaud (LSD) was absent. This Semiannual Report has been approved and signed by a majority of the ORNL-ACUC.

The Committee used the Semiannual Program Review Checklist in the review process (attached). The following are the observations and the recommendations of the Committee's review.

Status of the Previous October 2001 Semiannual Program Review Results

One minor deficiency and two suggestions for improvement were identified during the October 2001 Semiannual Program Review.

- C **Minor Program Deficiency 01-02:** The only two approved methods for protocol and amendment approval are full Committee review or designated reviewer(s). The ORNL-ACUC's practice for conditional approval by the full Committee was for the attending members to designate the Chair or a subcommittee to approve responses to conditional approvals. This did not meet either of the options listed above. Any approval of conditional responses should be via full Committee review or via designated reviewer(s) with no Committee member requesting full Committee review of the response. This deficiency was corrected as of October 17, 2001.
- C **Suggestion for Improvement:** It was recommended that signature confirmation be added to the ORNL-ACUC protocol form for the principal investigator to attest that the animal activities described within the protocol submitted for IACUC review correspond with those described in any related grant, contract, or subcontract. Signature confirmation has been added to the protocol foRm

- C Suggestion for Improvement: With a recent consolidation of DOE funding for the Bldg. 9210 animal research activities, it was advised that protocols for the animal activities be re-evaluated for possible reorganization. Included in this recommendation was the recognition that a limited number of protocol approvals might need to be extended so that affected protocols could be rewritten and resubmitted to the ORNL-ACUC. Protocols have been reorganized and approved. Brief extensions of affected individual protocols were unanimously approved by the Committee to allow for uninterrupted coverage during the review period.

April 2002 Semiannual Program Review Results

This program review included review of four existing approved departures from the *Guide* and identified no program deficiencies and two suggestions for improvement.

- C Guide departure: The ORNL-ACUC approved animal cage change frequencies of 14 days for one Bldg. 9210 rodent animal room, Room 115, containing ventilated racks. Ammonia levels inside cages were measured and were at acceptable at 14 days.
- C Guide departure: The ORNL-ACUC approved an animal cage change frequency of 14 days for one subspecies stock of mice in Bldg. 9210 (castaneus mice, CAST/Ei stock from The Jackson Laboratory, *Mus musculus castaneus*). Breeding of this stock is very difficult and extension of the cage change frequency has allowed breeding pairs to be established. Ammonia levels inside cages were measured and were acceptable at 14 days.
- C Guide departure: The ORNL-ACUC approved an alternate breeding scheme for selected cages in Bldg. 9210 that includes slightly more than the recommended mouse cage weight limits for short periods of time. The reason for requesting the alternate scheme was to permit increased production of animals for a large re-derivation effort. Because ammonia levels do in some cases increase to unacceptable levels by day 6, cages are being changed twice weekly to accommodate the housing exception.
- C Guide departure: Two staff members requested a medical variance to the ORNL-ACUC policy for no drinking in animal rooms. The physician who is Director of ORNL Health Services Division evaluated each person, and his medical recommendation was that each person be permitted to drink water when working in animal rooms. The ORNL-ACUC approved variances for these two individuals.
- C Suggestion for Improvement: There are standard operating procedures (SOPs) in place for shipping and receiving of rodents in Life Sciences Division animal facilities. ORNL personnel outside LSD presently use the LSD SOP process for shipping and receiving of rodents. There is occasional ordering of fish by ESD investigators under oversight by the consultant ESD veterinarian, but the LSD SOP is not applicable to such ordering. The ORNL-ACUC should develop an ORNL-wide process for shipping and receiving of animals covered by ORNL-ACUC approved protocols. The process should be incorporated into the Standards Based Management System (SBMS) Subject Area, "Animal Research."
- C Suggestion for Improvement: Responsibilities and authorizations for review, revision, and additions to the LSD Laboratory Animal Resources (LAR) Manual should be clarified.

Inspection of Animal Facilities and Animal Use Areas

The ORNL-ACUC inspected the animal facilities and animal use areas on April 3, 9, and 23, 2002, using the *Guide*, and, as applicable, 9 CFR Chapter I, 2.31. The continuing and consistent maintenance of high sanitation and housekeeping standards in all ORNL animal facilities was noted during the Committee's inspections. The ORNL-ACUC lauds these pro-active efforts and commitments by the divisions to maintenance of quality standards in the animal facility operations.

Bldg. 4500S, Life Sciences Division at ORNL (Inspected April 23, 2002, by D. Edds and M. Greeley)

The facility consists of one animal room with a ventilated rack, and a separate room for cage wash, autoclave, storage, and animal procedures. The cages were clear, and very clean. The mice appeared to be in good health. The facilities were generally very clean and orderly. Log sheets were in use but did not include the year on the current sheet; some sheets in the logbook did have year information included. There were two suggestions for improvement and no deficiencies were cited.

- C Log sheets entries should consistently include the year.
- C A wall rack should be installed to hold mops and brooms off the floor.

Bldg. 4501 Temporary Housing Facility, Nuclear Science and Technology Division at ORNL (Inspected April 23, 2002, by D. Edds and M. Greeley)

This mouse and rat temporary housing facility consists of one animal room (317B) with two ventilated racks, cage washing and autoclave, and an adjacent room (317A) with storage area and hoods for isotope injection and procedures. There were mice present in one rack. The other rack contained experimental mice from isotope injection experiments underway at the time of inspection. The inspection team was unable to inspect room 317A because of this ongoing procedure. Portions of Room 317B that were not involved with the procedure activity were orderly and clean. The team had no recommendations and noted no deficiencies.

Bldg. 3500, Temporary Housing Facility, Engineering Science and Technology Division at ORNL (Inspected April 23, 2002, by D. Edds and M. Greeley)

There have been no animals in this satellite facility since June 2000. The facility should be thoroughly cleaned before it is again used with live animals. The inspection team had one recommendation and noted no deficiencies.

- C Since this facility has not been used in nearly two years, and since there are no active protocols associated with the facility, the team recommends it be re-inspected after cleaning and before live animal use.

Building 5510A, Rm 217, Chemical Sciences Division at ORNL (Inspected April 23, 2002, by D. Edds and M. Greeley)

This animal use area is restricted to a laboratory hood in Bldg. 5510A. The use is sporadic and infrequent (3 rats per year), and involves a terminal procedure. The hood is clean, but the surrounding laboratory area was cluttered. The

PIs indicated that they are preparing the laboratory for a move to another building. The team had no recommendations and noted no deficiencies.

Bldgs 1504 and 1505, Environmental Sciences Division at ORNL (Inspected April 23, 2002, by D. Edds and M. Greeley)

Bldg. 1504, Aquatics Lab: All display tanks and circular tanks were clean and well maintained, and the animals (fish, turtles, snakes, and frogs) appeared healthy. Current protocol numbers were displayed and logbooks were up to date. Log sheets were complete and maintained in binders. There were no suggestions for improvement and no deficiencies cited for the facility.

1505, Room 263: The fish facility was clean and orderly. Tanks were properly maintained and clean, and fish appeared healthy. Fish food was labeled and in date. Log sheets were detailed and complete. A mop was stored directly on the floor. The inspection team had one recommendation for improvement and noted no deficiencies.

C A wall rack should be installed to hold mops and brooms off the floor.

1505, Room 153 Field Work Station: This room is used for processing field fish samples, and usually contains no live animals. No animals were present at the time of inspection. The inspection team had no recommendations and noted no deficiencies.

Bldg. 9210 Laboratory Animal Use Areas, Life Sciences Division at Y-12 (Inspected April 3, 2002, by D. O'Rourke, J. Hughes, T. Mosley, and ORNL-ACUC Coordinator, D. Embleton)

Animal use areas in this large mouse facility are located in laboratories. All labs visited were clean and orderly. It was obvious that a facility-wide effort had been made to maintain housekeeping in these areas. All staff members interviewed were very courteous, knowledgeable, and cooperative. Several labs had specific areas where mouse surgery was performed, but the same areas were used for related tissue harvest. These areas were the only ones used for surgery and are disinfected prior to surgical use. The ORNL-ACUC recognizes this as adequate practice. Four recommendations for improvement and are listed below; no deficiencies were cited.

C There was a refrigerator used for food located in a hallway for a suite of laboratories. This refrigerator should be moved to an area where animal and laboratory work is not performed.

C One laboratory procedure area had personal items stored adjacent to surgical supplies. Personal items should be separated from surgical supplies.

C Copies of approved protocols are kept by individual PIs in their offices and are not readily available to Laboratory Animal Resources (LAR) staff. It is recommended that up-to-date copies of approved protocols be kept on file in the LAR office.

C The MicroCT scanner and X-Ray room is being used for several additional activities. Use of this area should be re-evaluated to decrease the number of activities taking place.

Bldg. 9210 Animal Facility, Life Sciences Division at ORNL (Inspected April 9, 2002, by B. Beatty, G. Hodge, K. Kerber, E. Michaud, D. Miller, and W. Webb)

This is a large conventional open-caging rodent facility, housing approximately 60,000 mice. The facilities were in clean and orderly condition. All Committee members noted and commented on the continued improvement in this facility. All animal rooms were very clean and orderly, no overcrowded cages were observed, no double litters or litters overdue for weaning were observed, the mice appeared healthy, and animal rooms were very quiet. Several rooms had special mating and weaning cards in use, which were very easy to interpret. Door sheet records were very good. There were numerous cloudy cages, but few cracked cages were observed and there is a system in place for culling of cages when mouse visibility is greatly impaired. The mouse transport vehicle was not available for inspection. Two suggestions for improvement and four minor deficiencies were noted.

- C Improvement in storage of items in several rooms was recommended. (Animal Rm112 had a cardboard box with cage tops stored; SPF Rm 206 had excessive items on shelves that should be in closed containers; Rm 231 water station had plastic bags stored on the floor; Rm 335 water station had loose gloves on racks that should be in closed containers.)
- C The barrier arrangement for one SPF and several one-way rooms on the second floor needs to be evaluated and approved by the veterinarian.
- C **(Minor Deficiency 02-01)** An animal facility worker was observed dumping soiled bedding without wearing a dust mask as described in Animal Resources Reference Manual SOP, VI Operations, J. "Manual Tunnel Washer Operations."
- C **(Minor Deficiency 02-02)** Mops, brooms, and dustpans are not being dedicated to specific animal rooms and service areas. Items are marked with room numbers but were observed in various areas not corresponding to the room numbers on the items.
- C **(Minor Deficiency 02-03)** Two animal rooms had notes and cage cards taped to walls (Rms.111, 203). Tape was observed on handles of several hand trucks. Use of tape can hinder sanitizing practices.
- C **(Minor Deficiency 02-04)** Animal rooms. 110 and 203 had strong odors. Ammonia levels and airflows should be evaluated for these rooms.

The deadline for correction of minor deficiencies is June 30, 2002. The ORNL-ACUC will track correction and closure of all cited deficiencies.

Signatures

There were no minority views to this report.

Signature **	Name (printed)
	David G. Edds
	Darla Miller
	Mark Greeley
	Kristen A. Kerber
	Dorcas O'Rourke
	J. Warren Webb
	Barbara R. Beatty

(6 signatures = majority of ORNL-ACUC voting members)

** Committee Signatures on file.

ORNL SEMIANNUAL PROGRAM REVIEW CHECKLIST INSTITUTIONAL POLICIES AND RESPONSIBILITIES

Date: April 24, 2002

**Members Present: B. R. Beatty, D. G. Edds, C., M. S. Greeley, Jr., H. G. Hodge,
J. F. Hughes, D. K. Johnson, K. A. Kerber, D. R. Miller,
R. T. Mosley, J. W. Webb**

*A M S

1. IACUC MEMBERSHIP AND FUNCTIONS			
- at least 5 members, appointed by CEO	X		
- members include veterinarian, scientist, non-scientist, and non-affiliated non-lab animal user	X		
- responsible for oversight and evaluation of institution's program	X		
- reports to Institutional Official (IO)	X		
- conducts semiannual evaluations of institutional animal care and use program	X		
- conducts semiannual inspections of institutional animal facilities	X		
- reviews and investigates concerns about animal care and use at institution	X		
- procedures for review, approval and suspension of animal activities	X		
- procedures for review & approval of significant changes to approved activities	X		
- policies for special procedures (e.g. restraint, multiple survival surgery, fluid restriction)	X		
2. IACUC RECORDS AND REPORTING REQUIREMENTS¹			
Reports to Institutional Official (IO)			
- reports of semiannual program reviews & facility inspections are submitted to IO	X		
- include minority IACUC views	X		
- describe departures from <i>Guide</i> or PHS Policy and reasons for departure <i>*Described in the Semi-Annual Program Review</i>	X		
- distinguish significant from minor deficiencies	X		
- include plan and schedule for correction of each deficiency identified	X		
Reports to Office of Laboratory Animal Welfare (OLAW)			
- reports include any minority IACUC views	X		
- annual report to OLAW documents program changes & dates of IACUC semiannual review	X		
- promptly advises OLAW of serious/ongoing <i>Guide</i> deviations or <i>PHS Policy</i> noncompliance	X		
- promptly advises OLAW of any suspension of activity by the IACUC	X		

Reports to United States Department of Agriculture (USDA)			
- annual report contains required information	X		
- reporting mechanism in place for IACUC-approved exceptions to the regulations and standards	X		
- reports within 15 days failure to adhere to timetable for correction of deficiencies	X		
- reports suspension of activity by the IACUC to USDA and any Federal funding agency	X		
Records			
- minutes of IACUC meetings and semiannual reports maintained for 3 years	X		
- IACUC review documentation maintained for 3 years after end of study	X		
- IACUC review of activities involving animals includes all required information	X		
3. VETERINARY CARE (See also next section - Veterinary Medical Care)			
- institutional arrangement for veterinarian with training or experience in lab animal medicine <i>*Current Veterinary Status: Consultant Veterinarian, ACLAM Diplomate</i>	X		
- veterinary access to all animals	X		
- provision for backup veterinary care	X		
- must provide guidance on handling, immobilization, sedation, analgesia, anesthesia, euthanasia	X		
- must provide guidance/oversight on surgery programs and oversight of postsurgical care	X		
- veterinary authority to oversee all aspects of animal care and use <i>*Suggestion for Improvement - Responsibilities and authorizations for review, revision and additions to the LSD Lab Animal Resources SOP Manual should be clarified.</i>	X		
4. PERSONNEL QUALIFICATIONS AND TRAINING			
- institution has established and implemented an effective training program	X		
- includes professional/management/supervisory personnel	X		
- includes animal care personnel	X		
- includes research investigators, instructors, technicians, trainees, students	X		
Training program content			
- humane practices of animal care (e.g. housing, husbandry, handling)	X		
- humane practices of animal use (e.g. research procedures, use of anesthesia, pre- & post-op care)	X		
- research/testing methods that minimize numbers necessary to obtain valid results	X		
- research/testing methods that minimize animal pain or distress	X		
- use of hazardous agents, including access to OSHA chemical hazard notices where applicable	X		
5. OCCUPATIONAL HEALTH AND SAFETY OF PERSONNEL			
Institutional program for a safe and healthy workplace			

- program is established and implemented	X		
- covers <i>all</i> personnel who work in laboratory animal facilities	X		
- based on hazard identification and risk assessment	X		
- personnel training (e.g. zoonoses, hazards, pregnancy/illness/immunosuppression precautions)	X		
- personal hygiene procedures (e.g., work clothing, eating/drinking/smoking policies)	X		
- procedures for use, storage & disposal of hazardous biologic, chemical, and physical agents	X		
- specific procedures for personnel protection (e.g., shower/change facilities, injury prevention)	X		
Program for medical evaluation and preventive medicine for personnel			
- pre-employment evaluation including health history <i>*Non-employees do not receive pre-employment physical, but are included in the Occupational Safety and Health Program</i>	X		
- immunizations as appropriate (e.g. rabies, tetanus) & tests	X		
- zoonosis surveillance as appropriate (e.g. Q-fever, tularemia, Hantavirus, plague)	X		
- procedures for reporting and treating injuries, including bites etc.	X		
Special precautions for personnel who work with primates (Not Applicable)			
- tuberculosis screening includes all exposed personnel			
- training and implementation of procedures for bites & scratches			
- education regarding <i>Cercopithecine herpesvirus 1</i> (Herpes B)			

*A = acceptable; M = minor deficiency; S = significant deficiency (is or may be a threat to animal health or safety)

ORNL SEMIANNUAL PROGRAM REVIEW CHECKLIST VETERINARY MEDICAL CARE

DATE: APRIL 24, 2002

*A M S

1. PREVENTIVE MEDICINE/ANIMAL PROCUREMENT & TRANSPORTATION			
- evaluation of animal vendors	X		
- procedures for lawful animal procurement, evaluation of animals, & transport <i>*Suggestion for Improvement - Development and inclusion of ORNL-level procurement in the Standards Based Management System subject area "Animal Research."</i>	X		
- procedures for quarantine, stabilization	X		
- policies on separation by species, source, health status	X		
- policies for isolation of sick animals	X		
- program of surveillance, diagnosis, treatment and control of disease	X		
- availability of diagnostic resources for preventive health program	X		
- provision for emergency, weekend and holiday veterinary care	X		
2. SURGERY			
- procedures for monitoring surgical anesthesia and analgesia	X		
- pre-surgical plan (e.g. identify space, supplies, conduct pre-op exam, define post-op care)	X		
- appropriate training or experience of personnel in surgery & anesthesia	X		
- major procedures distinguished from minor	X		
- use of effective aseptic procedures for survival surgery	X		
- implemented procedures for use of surgical facility	X		
- implemented procedures for using/scavenging volatile anesthetics	X		
- effective procedures for sterilizing instruments & monitoring expiration dates on sterile packs	X		
- documentation of post-operative monitoring and care	X		
3. PAIN, DISTRESS, ANALGESIA, AND ANESTHESIA			
- guidelines for assessment and categorization of pain	X		
- IACUC guidelines for avoiding unnecessary pain and distress	X		

- appropriate anesthetics, analgesics, tranquilizers used for each species	X		
- special precautions for the use of paralytics	X		
- veterinary input in the choice of drugs	X		
4. EUTHANASIA			
- compliance with current AVMA Panel on Euthanasia unless approved by the IACUC	X		
- guidance provided on appropriate methods for each species	X		
- training available for personnel in humane methods of euthanasia	X		
5. DRUG STORAGE AND CONTROL			
- safe, secure, storage arrangement	X		
- record keeping meets regulations	X		
- procedures exist for ensuring drugs are within expiration date <i>* Note: Minimal use of drugs; dates of drugs are monitored by the ORNL-ACUC during Semi-Annual ORNL-ACUC animal facility inspections.</i>	X		

***A** = acceptable; **M** = minor deficiency; **S** = significant deficiency (is or may be a threat to animal health or safety)

Notes:

Appendix 7

Minutes of Last Two ORNL-ACUC Meetings

- April 24, 2002
- May 22, 2002

Oak Ridge National Laboratory Animal Care and Use Committee (ORNL-ACUC) Meeting
Minutes of Wednesday
April 24, 2002

The ORNL-ACUC met in Rm 296 Conference Room, Building 1505, at 8:30 AM.

Members Present:

Barbara Beatty (LSD), David Edds (OSSD), Mark Greeley, Jr. (ESD), Gerald Hodge, *ex officio* (LSD), Joan Hughes (EPWS), Dabney Johnson, *ex officio* (LSD), Kristen Kerber (LSD), Darla Miller (LSD), Terry Mosley (community representative), Dorcas O'Rourke (UTK Office of Laboratory Animal Care), Warren Webb (ESD)

Members Absent: Ed Michaud (LSD)

Non-Member Present: Diane Embleton, ORNL-ACUC Coordinator (LSD)

Guests Present: Sylvia Wolfe (DOE Research Program Coordinator) and four UTK Veterinary Students (Andrew Cruikshank, Todd Dolen, Melissa Dyson, Rachel Tuz)

Old Business:

1. Draft copies of the minutes of the 03/20/02 and 04/03/02 ORNL-ACUC meetings had been sent to the Committee members for review prior to the meeting. With minor corrections, the minutes were approved unanimously.
2. Conditional Approvals -
Protocol #0293, "Genetic Analysis of Gene(s) Regulating Mammalian Telomeres or Cell Cycle" was reviewed and conditionally approved by the Committee on 03/20/02. The investigator addressed the conditions cited by the Committee. The Committee reviewed and unanimously granted full approval to the revised protocol.
Protocol #0294, "Organizational Distribution of Lipids and Fatty Acids Within the Rodent Using the Techniques of Secondary Ionic (SIMS) and Tandem (MS/MS) Mass Spectrometry" was reviewed and conditionally approved by the Committee on 03/20/02. After reviewing the investigator's responses to the conditions cited by the Committee, the protocol was granted full approval by the Chair on 3/26/02.
3. Other Old Business
 - a) Protocol #0275, "Light Tags for Fish," submitted an amendment for full Committee review. A Committee meeting was convened on 4/3/02 to review the amendment request. The Committee unanimously granted full approval to the amendment with minor revisions.
 - b) A revision of the 2002 Disaster Plan for Animal Facilities was distributed in the pre-meeting package for review.
 - c) B. Beatty informed the Committee that the Standard Based Management System (SBMS) document for ACUC activities is in draft form and will be sent out for lab-wide review in the near future.
 - d) B. Beatty updated the Committee concerning a meeting held on 4/19/02 with Crystal Schrof of the ORNL legal department concerning Environmental Management Projects performed by Bechtel Jacobs Corporation (BJC) on the Oak Ridge Reservation that involve monitoring/sampling of wildlife in Melton Valley. C. Schrof's opinion was that UT-Battelle does not have responsibility under its contract with DOE for regulatory oversight of wildlife activities conducted by BJC on the Oak Ridge Reservation. Sylvia Wolfe said that she would follow up with DOE on this topic.

New Business:

1. A list of all currently approved protocols was distributed.
2. Annual Updates - No annual updates were received since the previous month's meeting.
3. Amendments/Addenda -The following amendment was received since the previous month's meeting.
Protocol #0294, an amendment request was made for "Organizational Distribution of Lipids and Fatty Acids Within the Rodent Using the Techniques of Secondary Ionic (SIMS) and Tandem (MS/MS) Mass Spectrometry" to change the breed/strain of rat being used. The Chair approved the amendment on 4/8/02 after conferring with the veterinarian.

4. Withdrawn or Expired Protocols -
Protocol #0251, "Radiotherapy for Viral Clearance; an HIV Model," was withdrawn by the PI.
5. New Protocol Submission -
Protocol #0295, "Fish Guidance by Turbulence" was reviewed and unanimously granted full approval by the Committee.
6. Meetings/Training - The following meetings were announced or discussed.
 - a) The Appalachian Branch of the American Association for Laboratory Animal Science (AALAS) will hold its spring quarter meeting on May 10, 2002, at the University of Tennessee (Knoxville campus).
 - b) The SCAW Conference will be held in Baltimore, Maryland on May 16-17, 2002.
 - c) ARENA in conjunction with OLAW and Charles River Laboratories (CRL) will sponsor the IACUC 101 course in Danvers, Massachusetts, on June 20, 2002.
 - d) The Fourth World Congress on Alternatives and Animal Use in the Life Sciences will hold a conference in New Orleans, Louisiana, August 11-15, 2002.
7. News/Handouts - No handouts were distributed.
8. The Semi-Annual Program Review was performed and the Semi-Annual Animal Facility Inspection Reports were reviewed and discussed. The members agreed on recommendations for deficiency citations and suggestions for improvement. The completed Semi-Annual Report will be distributed and reviewed for final approval at the May 22, 2002, ACUC meeting.
9. The approval process for LSD Lab Animal Resources (LAR) Manual of Standard Operating Procedures (SOPs) was discussed. B. Beatty informed the committee that it has been required that the ORNL Veterinarian review and approve all SOPs before they are updated in the LAR manual and since we now have an interim veterinarian it is not practical to require that interim veterinarian be required to do this. It was agreed by consensus that the veterinary technician be able to review and update the SOPs with guidance from the interim veterinarian.
10. Veterinarian's Report -
D. O'Rourke informed the Committee that she recently attended the ACLAM Forum Conference in Savannah, Georgia. The topic was "Genetics, Genomics, and Gene Therapy."

D. O'Rourke also reported that Walters Center at the University of Tennessee has had an outbreak of mouse MHV and MPV in hallway C, which is being evaluated by the veterinary staff. This is the hallway housing mice re-derived from ORNL's 9210 stocks.
11. The next ACUC meeting will be held on May 22 (Wednesday) in Building 1505, Room 296. The meeting was adjourned at 10:50 a.m.

Minutes submitted by B.D. Embleton and
Minutes approved by the ORNL-ACUC:

** Approval signature on file
Barbara Beatty Chairperson, ORNL-ACUC, MS-6122, 4500S

Oak Ridge National Laboratory Animal Care and Use Committee (ORNL-ACUC) Meeting
Minutes of Wednesday
May 22, 2002

The ORNL-ACUC met in Rm 296 Conference Room, Building 1505, at 8:30 AM.

Members Present:

Barbara Beatty (LSD), David Edds (OSSD), Mark Greeley, Jr. (ESD), Gerald Hodge, *ex officio* (LSD), Kristen Kerber (LSD), Darla Miller (LSD), Dorcas O'Rourke (UTK Office of Laboratory Animal Care), Warren Webb (ESD)

Members Absent: Joan Hughes (EPWS), Dabney Johnson, *ex officio* (LSD), Ed Michaud (LSD), Terry Mosley (community representative)

Non-Member Present: Diane Embleton, ORNL-ACUC Coordinator (LSD)

Guests Present: Robbyne Williams (UTK Veterinary Assistant)

Old Business:

1. Draft copies of the minutes of the 04/24/02 ORNL-ACUC meetings had been sent to the Committee members for review prior to the meeting. With minor corrections, the minutes were approved unanimously.
2. Conditional Approvals -No conditional approvals were reviewed since the previous month's meeting.
3. Other Old Business
 - a) Protocol #0294, "Organizational Distribution of Lipids and Fatty Acids Within the Rodent Using the Techniques of Secondary Ionic (SIMS) and Tandem (MS/MS) Mass Spectrometry," has incorporated the two refinement techniques recommended.
 - b) The ORNL-ACUC Coordinator is working with the Emergency Preparedness Office to determine how the ORNL 2002 Disaster Plan for Animal Facilities can best be incorporated into the emergency response system at ORNL.
 - c) B. Beatty informed the Committee that the Standard Based Management System document for ORNL-ACUC activities is in draft form and has been sent out for lab-wide review.
 - d) A meeting is scheduled with ORNL-ACUC members and management to discuss an investigator's point of view concerning ORNL-ACUC oversight of wildlife research, teaching, and testing.
 - e) ORNL researchers have further questioned the Bechtel Jacobs Corporation (BJC) activities to monitor/sample wildlife in the Melton Valley area. B. Beatty will follow up with W. F. Harris on this issue.
 - f) The Committee discussed and unanimously approved a process for updating the LSD Lab Animal Resources (LAR) Manual of Standard Operating Procedures (SOPs). The veterinarian or an individual designated by the veterinarian will be authorized to review, approve and update the SOPs. (Note: The interim veterinarian will provide guidance to the designee).
 - g) The Semi-Annual Program Review and Animal Facility Inspections Report was unanimously approved with minor corrections and signed by the Committee members present.

New Business:

1. A list of all currently approved protocols was distributed.
2. Annual Updates - The following annual updates were received since the previous month's meeting.
Protocol #0269, "Monitoring and Assessment of Contaminant Accumulation in Aquatic Organisms," was approved by the Chair with no changes.
Protocol #0270, "Biological Indicators Assessment of Fish Health and Exposure to Environmental Contaminants," was approved by the Chair with the addition of one person and the deletion of four persons.
3. Amendments/Addenda -The following amendments were received since the previous month's meeting.
Protocol #0289, "Mouse Genetics and Mutagenesis," amendment request to add one person was approved by the Chair on 5/21/02.

Protocol #0289, "Mouse Genetics and Mutagenesis," amendment request for decapitation of mouse pups for blood collection without anesthesia was unanimously approved by the Committee.

4. Withdrawn or Expired Protocols -
Protocol #0253, "The Maternal Genotype As a Major Factor in the Transmission of DNA Damage Incurred in Paternal DNA," was withdrawn by the PI.
5. New Protocol Submission -
Protocol #0296, "Micro-CT Identification and Monitoring of Prostate Tumors in TRAMP Mice" was reviewed and tabled by the Committee. If necessary due to time constraints, the Chair will convene an extra ORNL-ACUC meeting to review the investigator's response to requests for additional information.
6. Meetings/Training - The following meetings were announced or discussed.
 - a) The Appalachian Branch of the American Association for Laboratory Animal Science (AALAS) held its spring quarter meeting on May 10, 2002, at the University of Tennessee (Knoxville campus). P. Cope, D. Edds, D. Embleton, G. Hodge, K. Kerber, A. Toney and N. Wright attended from ORNL.
 - b) K. Kerber and D. O'Rourke will attend the Charles River Laboratories (CRL) Short Course on Laboratory Animals sponsored by ARENA in conjunction with OLAW and CLR. The conference will be held in Danvers, Massachusetts, on June 17-20, 2002.
 - c) The Fourth World Congress on Alternatives and Animal Use in the Life Sciences will hold a conference in New Orleans, Louisiana, August 11-15, 2002.
7. News/Handouts - The following handouts were distributed.
 - a) Two articles from the March 2002 Humane Society Pain and Distress Report, Volume 2, Issue 1, titled "IACUC Training Course" and "Use of Score Sheets for Welfare Assessment of Transgenic Mice."
 - b) An article from the 2002 Winter/Spring Issue of AAALAC International titled "Avoiding the Pitfalls of an Inadequate Semiannual Review."
8. The ACUC Coordinator requested that she be able to tape record the ACUC meeting for preparation of the minutes. She will check to see if there are any business or legal reasons to prohibit this.
9. The Committee discussed the need for an SOP for managing expired drugs. The Committee agreed by consensus that the process currently being performed was sufficient. Drug dates are checked during the Semi-Annual Review process.
10. B. Beatty updated the Committee on the AAALAC accreditation status of the University of Memphis. Memphis is not currently AAALAC Accredited. The Committee discussed the ramifications this might have with the mice in the Tennessee Mouse Genome Consortium and determined that since the University of Memphis is PHS-Assured this is not an issue.
11. The Protocol Annual Update Form was distributed and discussed. The Committee agreed by consensus to make minor correction to the foRm The form will be updated by the ORNL-ACUC Coordinator.
12. Veterinarian's Report - The following topics were discussed by D. O'Rourke:
 - a) An update was given on the outbreak of MHV in hallway C of the Walters Center at the University of Tennessee. The MHV room has been taken out of service and the etiology of the MHV is still unknown. The facility has been retested and results are negative. In order to better monitor the animals, the frequency and intensity of testing will be increased in the sentinel program. Also, the air flows will be checked in the facility.
 - b) Some of the C57BL/6 mice in Building 9210 are starting to develop skin lesions. The mice are currently being checked and evaluated to determine the cause of the lesions.
 - c) The fish housed in ESD are doing very well.
 - d) D. O'Rourke recently attended a SCAW Conference in which the philosophy of animal enrichment was discussed. SCAW hopes to have the meeting proceedings published for distribution.
 - e) The ORNL goose roundup is scheduled for June 20 and 21.

13. B. Beatty updated the Committee on the veterinarian search and staffing plans for the operation and management of the 9210 animal facility.
14. The next ACUC meeting will be held on June 26 (Wednesday) in Building 1505, Room 296. The meeting was adjourned at 10:10 a.m.

Minutes submitted by B.D. Embleton and
Minutes approved by the ORNL-ACUC:

** Approval signature on file
Barbara Beatty, Chairperson, ORNL-ACUC, MS-6122, 4500S

Appendix 8

Protocol Form

Protocol Amendment Form

Protocol Annual Renewal Form

<p>PROTOCOL FOR USE OF LIVE VERTEBRATES OAK RIDGE NATIONAL LABORATORY</p>
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The cover page, Part I, General Information and all applicable sections of Part II, Detailed Information must be completed for all research activities.

Guidance on research activities can be found at the following URLs:

- 1) ACUC home page (http://home.ornl.gov/divisions/life_sciences/acuc/index.shtm),
- 2) Laboratory Animal Resources Manual (<http://bio.lsd.ornl.gov/internal/manuals/animals.shtml>), and
- 3) Care and Use of Fish and Wildlife in Research, Testing and Teaching (<http://homer.ornl.gov/exams/wildlife/resources.html>).

Submit electronic copy to: Diane Embleton, ORNL-ACUC Coordinator
e-mail address: embletonbd@ornl.gov
 Building 4500S, MS-6122 , Phone: 574-0677

Title of Project:	TYPE IN THIS BOX
Principal Investigator (PI):	
Division:	
Phone:	
Address:	
Signature of Principal Investigator:	
Signature of Co-Investigator:	

Your signature as Principal Investigator or Co-Investigator on this application verifies that: (1) the information herein is true and correct and that you are familiar with and will comply with the legal standards of animal care and use established under federal and state laws and policies as well as laboratory policies; (2) the proposal has received or will receive approval for scientific merit by peer review; (3) the activities do not unnecessarily duplicate previous experiments; and (4) the activities described within this protocol are consistent with those described in the related grant, contract or subcontract.

Revised 06/02	
ORNL-ACUC USE ONLY	
ACUC No.:	Species:
Date Received:	USDA Category:
Full Review:	Approval Date:
Designated Review:	Conditional?
Deferred/Tabled:	Full Approval Granted:

Revised 06/02
ORNL-ACUC USE ONLY
Comment:

PART 1 GENERAL INFORMATION

A. Identification

1. This is a ___ **New** ___ **Continuation** ___ **Supplemental** application. If this is a Supplement or Continuation of a project previously approved by the committee, please indicate the ACUC number and title of the previous application:

TYPE IN THIS BOX

2. List funding source (actual or projected) with Grant or FWP number where applicable:

TYPE IN THIS BOX

- 3.

Anticipated start date:		Anticipated end date:	
-------------------------	--	-----------------------	--

B. Description of Animals

- 1.

Species	Breed/Strain	Maximum # maintained at any given time	Yearly total needed for this project	3 Year total for project

Comments:

- 2.

Source (Note: Animals received from outside sources must undergo a minimum 7 day stabilization period. See guidance at URL http://home.ornl.gov/divisions/life_sciences/acuc/guide5.shtm)	
<input type="checkbox"/>	Purchased from:

<input type="checkbox"/>	Bred or reared at the Oak Ridge National Laboratory
<input type="checkbox"/>	Captured from wild: if yes, given agency and permit number(s) and attach copy(ies)
<input type="checkbox"/>	Transferred from another study (* provide title & Principal Investigator of previous study)
<input type="checkbox"/>	Other (Please Describe):

3.

Sex:		Age/Weight:	
-------------	--	--------------------	--

4. Single or group housing (provide numbers per cage or pen):

TYPE IN THIS BOX

5. How will individual animals be identified?

collar; ear tag; ear notch; cage card (small rodents only); freeze brand; or list other:

TYPE IN THIS BOX

6. Are there special housing/environmental requirements?

TYPE IN THIS BOX

7.

Disposal of Animals After Completion of Activity:	
<input type="checkbox"/>	Return to breeding unit
<input type="checkbox"/>	Transfer to another research project (if yes, project title and investigator)
<input type="checkbox"/>	Euthanized
<input type="checkbox"/>	Other:

C. Non-Technical Summary

(A complete summary is required for conformance with PHS Policy). Summary must be written to ensure comprehension by non-scientists (for example, understandable at an 8th grade level). It must contain the following information and should not exceed two (2) pages:

- (1) Objectives of the research;
- (2) species and number of animals;
- (3) schedule procedures performed during each phase;
- (4) benefits, outcome and results expected.

TYPE IN THIS BOX

D. Flow Sheet

Pertinent information (i.e., numbers of animals, experimental manipulation) should be provided on a flow sheet. The

Committee members should be able to follow every manipulation of the animal from experiment initiation to completion (e.g., day one, group one, treatment; day two., etc).

TYPE IN THIS BOX

E. Qualifications of Personnel

New and more stringent regulations in both the PHS Policy and the Animal Welfare Act require that training be provided for investigators, graduate students and technicians. The Animal Welfare Act places responsibility with the ACUC for determining qualifications of individuals involved in animal research, teaching, and testing activities. Qualifications to perform specific procedures will be based upon information provided in this section.

Investigators are responsible for ensuring that all their personnel are trained and that employees perform proper animal procedures. Investigators, research technicians, and students who are not experienced with animal procedures required by the protocol should contact the ORNL Institutional Veterinarian

Please complete the following for people who will conduct procedures using animals (especially surgery, anesthesia, pre- or post-operative care, or euthanasia); the ORNL-ACUC will provide advice and training on all of these procedures if the personnel listed have no previous relevant experience. This page may be duplicated for projects in which a larger number of people will be working with animals.

1. The following have experience with the specific animals and specific procedures proposed in this study. They obtained this experience (How, When, and Where?)

TYPE IN THIS BOX

2. The following do not have experience with the animals and procedures to be used. (How will they be trained?)

TYPE IN THIS BOX

F. Duplication of Research

The Animal Welfare Act requires that the principal investigator give written assurance that the proposed research is not unnecessarily duplicative.

1. Does the proposed research duplicate any previous work? No Yes
2. ***If NO**, what procedures and sources did you use to determine that the proposed research does not duplicate previous work (e.g., Medline - <http://research.bmn.com/medline>)?

TYPE IN THIS BOX

3. ***If YES**, provide justification for the need to duplicate previous work.

TYPE IN THIS BOX

G. Alternatives to the Use of Animals

The Animal Welfare Act requires that the Principal Investigator must consider alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and provide a written narrative description of the methods and sources.

1. What is the justification for using live animals rather than alternative means of achieving research goals?

TYPE IN THIS BOX

2. What procedures and sources did you use to determine that non-painful alternatives were not available or appropriate? (e.g., Animal Welfare Information Center at <http://www.nal.usda.gov/awic/>, National Agricultural Library at <http://www.nal.usda.gov/>, or [301] 344-3212.)

TYPE IN THIS BOX

3. Why have you selected the particular species proposed in this project? (Include any biological characteristics essential to the proposed study)

TYPE IN THIS BOX

4. Provide an explanation of how the numbers of animals to be used were derived. If used in an experiment (test a hypothesis) numbers should be based on scientific and statistical requirements to achieve objectives.

TYPE IN THIS BOX

**PART 1
DETAILED INFORMATION**

Check and complete those sections A-E that are relevant to your project.

DELETE/REMOVE ALL SECTIONS NOT APPLICABLE TO YOUR PROPOSAL

- A. Non-Surgical Laboratory Procedures** (Pertains to any experimental procedure -- e.g., including non-surgical, pre-surgical and post-surgical procedures using animals, injections, tissue harvest, terminal perfusions, retro-orbital blood sampling, tail biopsy, behavior testing).

No <input type="checkbox"/>	Yes <input type="checkbox"/> Complete Section A
-----------------------------	---

- B. Surgical Procedures** (Pertains to any surgical procedure, including non-survival surgery. If other procedures are done on animals prior to or after surgery, **complete Section A and other applicable sections**).

No <input type="checkbox"/>	Yes <input type="checkbox"/> Complete Section B
-----------------------------	---

- C. Field Studies Involving Wild Animals** (this section includes non-surgical field procedures)

No <input type="checkbox"/>	Yes <input type="checkbox"/> Complete Section C
-----------------------------	---

- D. Hazardous Agents**

No <input type="checkbox"/>	Yes <input type="checkbox"/> Complete Section D
-----------------------------	---

- E. Euthanasia**

No <input type="checkbox"/>	Yes <input type="checkbox"/> Complete Section E
-----------------------------	---

Section A
Non-Surgical Procedures

1.

Sites of Study		
Site of housing prior to study:	Bldg.:	Room(s):
Site of experimental work:	Bldg.:	Room(s):

2.

Please check the following items that apply and fill out appropriate parts if this section.	
<input type="checkbox"/>	Antibody production
<input type="checkbox"/>	Blood withdrawal
<input type="checkbox"/>	Restraint with mechanical devices
<input type="checkbox"/>	Projects involving nutritional studies, tumor and disease models or models or toxicity testing
<input type="checkbox"/>	Anesthesia or analgesia (for non-surgical procedures)
<input type="checkbox"/>	Other (e.g., injections, tissue harvest, terminal perfusion, tail biopsy, behavior tests)

Antibody production

Describe procedure, antigen and adjuvants used, the ratio of antigen to adjuvant and routes of injection:.

Note: Vol. should be < 0.2 ml per injection site; subcutaneous route is recommended.

TYPE IN THIS BOX

Blood withdrawal

Describe method(s), amount(s) collected and frequency of collection:

Note: The volume of blood that can be collected cannot be more that 15% of the total blood volume in a 2 week period (for the mouse this is 0.3 ml for a 30 gram mouse or 0.25 fro a 25 gram mouse).

TYPE IN THIS BOX

Restraint with Mechanical Devices/Close Confinement

Describe device, duration of restraint or confinement, conditioning procedures and steps to assure comfort and well-being:

TYPE IN THIS BOX

Nutritional Deficiencies/Ascites Tumor/Monoclonal Antibody Production Disease/Neoplasia Models/Toxicity Testing

Describe methodology. State objective criteria used to assess physical condition, pain and distress during course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

TYPE IN THIS BOX

Anesthesia/Analgesia/Tranquilization (Other than Surgery Section B)

List procedures for which anesthetics, analgesic or tranquilizers are required.

TYPE IN THIS BOX				
Drug(s)				
Dose(s)				
Frequency				
Route(s)				

Injections

Describe injection route (intraperitoneal, intravenous, subcutaneous, intramuscular), volume(s) injected, and composition of substance injected. **Note:** Dose volumes appropriate for injection as defined by the Toxicology Subcommittee of the Association of the British Pharmaceutical Industry are: 20ml/kg for oral, subcutaneous and intraperitoneal routes (0.6 ml for a 30 gram mouse); 10 ml/kg by the intravenous route (0.3 gram mouse); intramuscular and intradermal routes are discouraged in the mouse.

TYPE IN THIS BOX

Tissue Harvest and Terminal Perfusion

Describe method of tissue harvest and tissues harvested and/or methods of terminal perfusion, to include confirmation that procedures are done under anesthesia and are terminal.

TYPE IN THIS BOX

Tail Biopsy

Describe amount of tail removed, method of removal, age of animals used, whether or not a second biopsy will be done, any coagulating agents used. Confirm that tail biopsies are performed in compliance with the ORNL Animal Care and Use Guidelines for this procedure (ORNL-ACUC-008: Mouse Tail Clip Policy, Rev.00, available at http://home.ornl.gov/divisions/life_sciences/acuc/guide8.shtm).

TYPE IN THIS BOX

Behavior Testing

Provide a description of test(s) administered, including equipment used, duration of test(s), and possible stress experienced by the animal. (Provide as an attachment if necessary.)

TYPE IN THIS BOX

3. Health Assessment:

How often will the clinical condition of animal subjects be assessed during experimental investigation? (e.g., how frequently will animals be observed? How often will weight, body temperature, and behavior be assessed?)

TYPE IN THIS BOX

Describe methods used to minimize degree and duration of discomfort. If drugs which might alleviate pain or distress will be withheld, provide humane endpoints (e.g., animals to be euthanized if weight loss exceeds 20% of normal weight; mice euthanized when tumors reach 1 cm. diameter or tumor ulcerates).

TYPE IN THIS BOX

SECTION B
Surgical Procedures

The attending veterinarian must be consulted on anesthetic regimens, surgical procedures and post-surgical care.

1.

<p>Survival Surgery: No <input type="checkbox"/> Yes <input type="checkbox"/> (See guidance at URL http://home.ornl.gov/divisions/life_sciences/acuc/guide2.shtm) <i>Survival surgical procedures (Pertains to any surgical procedure, including biopsies, where an animal is allowed to recover from anesthesia, however short the survival period). In non-survival surgery, the animal is euthanized while still anesthetized.</i></p> <p>Multiple survival surgery? No <input type="checkbox"/> Yes <input type="checkbox"/></p> <p><i>*If yes, provide justification for multiple survival surgical procedures:</i></p>
TYPE IN THIS BOX

2.

Surgeon:	
Describe surgeon's experience with procedures to be performed:	

3.

Location of housing and procedural areas: (Be as specific as possible, including building and room)	
Housing prior to and after surgery:	TYPE IN THIS BOX
Surgery Room:	
Recovery:	

4.

Briefly describe surgical procedure. (Include anticipated duration of procedure from start of anesthesia to recovery). Document steps taken to insure aseptic technique (autoclave instruments, prep skin, etc.)
TYPE IN THIS BOX

Statement on health status of animals:

a. How will health status of animals be assessed before initiation of procedure?

TYPE IN THIS BOX

b. Will animals be fasted prior to surgery? No Yes , *If YES, for how long?

TYPE IN THIS BOX

- c. Will water be withheld prior to surgery? No Yes , *If yes, for how long?

TYPE IN THIS BOX

NOTE: Water should not be withheld from rodents and should not be withheld for more than 12 hours from non-rodents.

5.

Anesthetic Protocol	
Anesthetist Name :	
Pre-anesthetic Agent(s):	Dose/Route:
Anesthetic Agent:	Dose/Route:
Describe indices and methods to be used to monitor level of anesthesia (e.g., blood pressure, heart rate, pedal reflex, jaw tone). All purposeful movement in response to painful stimuli, including toe pinch, must be abolished before surgery:	
TYPE IN THIS BOX	
Paralyzing Drugs: No <input type="checkbox"/> Yes <input type="checkbox"/>	Drug: Dose:
Justify the need to use paralyzing drugs:	

6. Post-operative care procedures (Survival surgery only)

- a. What is the anticipated duration for recovery from anesthesia?

TYPE IN THIS BOX

- b. How often will animal(s) be monitored during recovery? What specifically will be monitored?

TYPE IN THIS BOX

- c. After recovery and during experimental study, what criteria will be used to assess pain, distress and discomfort? (Post-surgical observations of behavior, appetite and body temperature should be recorded at least daily for three days postoperatively).

TYPE IN THIS BOX

- d. Analgesics: No Yes , routinely, Yes, only if indicated by clinical assessment.

***If yes**, specify pharmacological agent, dose (mg/kg), route and frequency of administration. If no, or not routinely, what are the criteria and who determines whether or not a given animal receives postoperative analgesia?

TYPE IN THIS BOX

Section C

Field Studies Involving Wild Animals

Note: The Animal Welfare Act requires that operative procedures at field be performed as aseptic procedures, i.e., surgical gloves, masks, sterile instruments and aseptic technique.

1. List any permits required (e.g., Tennessee Wildlife Resources Agency, Migratory Water Fowl, Endangered Species, etc.)

TYPE IN THIS BOX

2. List Study Site(s)

a. Location	
State:	County:

- b. Use street addresses, road intersections, property names, map coordinates, parks, state forests etc., to specifically locate each study site within the identified county and state; or attach a copy of a county map on which the study site is located. This is not necessary for state and federal parks and similar tracts.

3. Please check the following items that apply and fill out appropriate parts of this section.

<input type="checkbox"/>	Live capture and release. Complete section "a" below.
<input type="checkbox"/>	Non-survival collection. Complete 3.b. of this section.

- a. Live capture and release.

(1)

Describe method(s) of capture to be used including device(s) to be used, frequency with which these devices will be checked, and estimated maximum time animals will be restrained before release.
TYPE IN THIS BOX

(2)

What are the expected injury and/or mortality rates?
TYPE IN THIS BOX

(3)

What precautions will be used to minimize injury and/or mortality?
TYPE IN THIS BOX

(4)

In the event of injury or illness necessitating euthanasia, what method will be used and who will perform that procedure? Is the person performing the euthanasia experienced with the method?
TYPE IN THIS BOX

(5)

What precautions will be taken to reduce non-target captures?

TYPE IN THIS BOX

(6)

Describe marking procedures to be used.

TYPE IN THIS BOX

(7)

If a telemetry package is to be attached, describe the weight of the total package, type of antenna (including length), and method of attachment. Also, describe procedures for removal of the package from the animal.

TYPE IN THIS BOX

(8)

If blood or other tissue samples are to be taken, describe procedure(s) to be used, including number and weight or volume of sample(s) to be taken. Also, describe procedures to be taken to prevent infection and abscess formation at the sample site.

TYPE IN THIS BOX

(9)

Indicate where captured animals will be released. If the animals are transported indicate the method of transportation. If the animals are to be housed, indicate the type of housing and the length of time they will be housed. If release is at a site other than the site of capture, justify.

TYPE IN THIS BOX

(10)

If live animals are to be transported to ORNL housing, have arrangements been made for their housing? Where will they be housed?

TYPE IN THIS BOX

(11)

If drugs are to be used, indicate which drug, reason for its use, dosage.

TYPE IN THIS BOX

(12)

If drugs are not used, explain why drugs that might alleviate pain or distress will be withheld.

TYPE IN THIS BOX

(13)

Describe procedures for monitoring animal health and what parameters will be used to determine health status.

TYPE IN THIS BOX

(14)

Describe precautions taken to ensure the safety of personnel involved. (e.g., rabies immunization, tetanus immunization, and boating safety, etc.)

TYPE IN THIS BOX

b. Non-Survival Collection

(1)

Describe procedure(s) to be used.

TYPE IN THIS BOX

(2)

Describe precautions that will be taken to prevent non-target mortalities.

TYPE IN THIS BOX

(3)

How will carcasses be disposed of? Indicate the specific location where the collection(s) will be maintained.

TYPE IN THIS BOX

(4)

What paperwork will be maintained to document proper disposal and where will this paperwork be located?

TYPE IN THIS BOX

(5)

What precautions will be taken to ensure the safety of personnel involved? (e.g., rabies immunization, and boating safety, etc.)

TYPE IN THIS BOX

Section D
Hazardous Agents

1.

Will this project require the use of ionizing radiation? No Yes

*If Yes, list isotope(s) and/or describe irradiation procedure.

TYPE IN THIS BOX

2.

Will this project require the use of hazardous biologic agents (human/animal pathogens, tumor cells) or recombinant DNA? No Yes

TYPE IN THIS BOX

3.

If tumor cells will be used, have they been tested for contamination with human and/or animal viruses?

No Yes

TYPE IN THIS BOX

4.

Will this project involve the use of toxic chemicals or carcinogens? No Yes

TYPE IN THIS BOX

5.

Will this project involve the use of controlled substances? No Yes *If Yes, note the name of Division Responsible Officer dispensing controlled substance.

TYPE IN THIS BOX

6.

Describe special precautions for animal handlers.

TYPE IN THIS BOX

7.

Describe waste and animal disposal requirements.

TYPE IN THIS BOX

Section E
Euthanasia

1. What is/are the method(s) of euthanasia?

TYPE IN THIS BOX

a.

Chemical/Gas:		
Agent:	Dose:	Route:
Agent:	Dose:	Route:

b.

Physical:	
<input type="checkbox"/>	*Cervical Dislocation (Unless animal is sedated or anesthetized for this procedure, prior approval must be obtained from the ORNL-ACUC and be scientifically justified). Complete justification below.
<input type="checkbox"/>	*Decapitation (Unless animal is sedated or anesthetized for this procedure, prior approval must be obtained from the ORNL-ACUC and be scientifically justified.) Complete justification below.
<input type="checkbox"/>	Captive Bolt Pistol/Rifle
<input type="checkbox"/>	Exsanguination Under Anesthesia
<input type="checkbox"/>	Other:
*Must include <u>justification</u> below if the method is not recommended by the most recent AVMA Panel on Euthanasia (URL for 2000 AVMA: http://www.avma.org/resources/euthanasia.pdf)	
TYPE IN THIS BOX	

c.

Name and qualifications of person(s) performing euthanasia:
TYPE IN THIS BOX

d.

Method of disposal. Euthanized animal carcasses must be disposed of appropriately. Check the appropriate method:	
<input type="checkbox"/>	Incinerate
<input type="checkbox"/>	Carcass will contain radioactivity and will be disposed of in accordance with Biological Low-Level Radioactive Waste Regulations.
<input type="checkbox"/>	Carcass will contain hazardous agents and will be autoclaved prior to disposal.
<input type="checkbox"/>	Carcass will contain hazardous agents and will be segregated, and will be incinerated.
<input type="checkbox"/>	Other (please describe)

**PROTOCOL ADDENDUM/AMENDMENT FOR USE OF LIVE VERTEBRATES
OAK RIDGE NATIONAL LABORATORY**

Submit electronic copy to: **Diane Embleton, ORNL-ACUC Coordinator**
e-mail address: embletonbd@ornl.gov
 Building 4500S, MS-6122 , Phone: 574-0677

Protocol Number and Title:	TYPE IN THIS BOX
Principal Investigator (PI)/ Co-Investigator:	
Brief Title of Proposed Addendum/ Amendment:	

Note: As Principal Investigator or Co-Investigator you have verified that the activities described within this amended protocol are consistent with those described in the related grant, contract or subcontract.

Revised 12/02/01		
ORNL ACUC USE ONLY		
ACUC No.:	Full Reveiw:	Approval Date:
Date Received:	Designated Review:	Conditional?
Comment:		

1.

List funding source (actual or projected) with Grant or FWP number where applicable:
TYPE IN THIS BOX

2.

Does the proposed research duplicate any previous work? ? No <input type="checkbox"/> Yes <input type="checkbox"/> (If YES , provide explanation for why duplication is being done).
TYPE IN THIS BOX

3.

Anticipated start date:		Anticipated end date:	
-------------------------	--	-----------------------	--

4. **Description of proposed ADDENDUM/AMENDMENT**

(*must be written to ensure comprehension by non-scientists).

Describe objective of this addendum/amendment and schedule of procedures performed during each phase. Please address the following questions in your description (if procedure is detailed please include a flow chart).

- (1) Are additional animals required? Yes, No (If YES, provide species, breed/strain, maximum number of animals required at any given time, yearly total needed for this project and 3 year total for all animals.
- (2) Are additional painful/distressful procedures not addressed in the original protocol going to be used? Yes, No (If YES, please provide justification and identify individual who will be performing the procedures)
- (3) Is the method of euthanasia the same as described in the original protocol? Yes, No (If NO, please describe procedure, provide justification and identify individual who will be performing the procedures). For Guidance reference the AVMA Panel on Euthanasia (URL for 2000 AVMA: <http://www.avma.org/resources/euthanasia.pdf>)
- (4) Are personnel training qualifications for addendum/amendment addressed in the original protocol? Yes, No (If NO, please provide names and qualifications for individual performing new procedure(s)).
- (5) Explain why the change needs to be made and how this change pertains to the original protocol.
- (6) Describe how this change benefits or improves the expected outcome or results.

TYPE IN THIS BOX

ORNL-ACUC ANIMAL PROTOCOL ANNUAL UPDATE FORM

DATE: July 16, 2002

PROTOCOL NUMBER:

PRINCIPAL INVESTIGATOR:

DIVISION:

PROTOCOL TITLE: X

DATE OF ORIGINAL APPROVAL:

DATE OF LAST REVIEW:

DATE OF EXPIRATION:

The Animal Welfare Act requires that all research projects using animals be reviewed and approved annually by the Institutional Animal Care and Use Committee. This brief renewal application will provide the basis for an annual review for projects that have not changed or may have only minor modifications from year to year. A complete "Protocol for Animal Care and Use" must be submitted for new protocols, major changes in existing protocols, and for all protocols every three years. If you have any questions, please contact B.Diane Embleton at (865) 574-0677 or embletonbd@ornl.gov.

1. Please indicate the present status of your project by checking one of the statements below:

This project is no longer active, please withdraw.

This project is pending/active and there have been no changes in procedures with respect to animal use or personnel.

This project is active and there have been changes in the personnel or experimental procedures with respect to animals. (Describe any such changes below. Use additional sheets if necessary.)

2. Please provide a description of any unreviewed changes in procedures with respect to animal care and use.

3. Please describe any problems associated with this protocol during the past year.

4. List all changes in personnel involved in your project and indicate: (A) = Add or (D) = Delete.

NOTE: A separate form is needed to add an individual to a protocol. The form can be found on the ACUC homepage or by contacting the ACUC Coordinator.

Signature: _____

Date: _____

Send this completed form to B. Diane Embleton, ORNL Animal Care and Use Coordinator.

Bldg. 4500S, MS-6122, Oak Ridge National Laboratory, Oak Ridge, TN 37831-6122

NOTE: Failure to respond may result in premature expiration of your protocol and possibly suspension of your funding.

Appendix 9

SOP: Bull's Eye Cages

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	Updated July 2000
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S. Bull's Eye Cages

Purpose:

Cages and rooms in which animals are being treated with chemicals in the water, or via other means, will be identified with a Bull's Eye Symbol. This symbol indicates that there are special operating procedures to be followed. This SOP describes the procedures that apply in this situation.

Responsibility:

Research Technician and Animal Care Technician

Procedure/Discussion:

- ? At least one week prior to placing chemically-treated mice into the animal rooms, the Investigator responsible for the experiment shall notify the Laboratory Animal Resource Supervisor. Greater notice is appreciated by the Animal Care Staff. A form for this notification is attached.
- ? A sign will be posted on the outside of the door of the animal room indicating that the room contains some animals treated with test compounds. The sign will include the name of the test compound, the beginning date of the experiment, the type of personal protection equipment required for working with treated mice, the name of the Investigator in charge of the experiment, ACUC protocol number, and telephone numbers for contact persons involved with the experiment. The form for the door is attached.
- ? Each duplex box containing treated mice will be clearly marked with a "Bull's Eye" on the cage card. The Investigator is responsible for identifying the treated cages.
- ? Animal Care Technicians and Research Technicians will wear dust masks and disposable latex, plastic, or rubber gloves while handling mice in Bull's Eye cages.
- ? Bull's Eye cages should be changed/handled last after all unmarked cages in the room are changed/handled.
- ? Feed must be added to Bull's Eye cages with a scoop. DO NOT handle feed on the cages or in the dispenser with your gloved hands.
- ? Carcasses discovered in Bull's Eye cages must be bagged separately and disposed of in the specially marked container in the west freezer on the back dock.
- ? Gloves should be removed and thrown into the trash can in the animal room before you leave the room.
- ? Animal Care and Research Technicians should wash hands after completing work with chemically-treated mice.

<p style="text-align: center;">Oak Ridge National Laboratory Life Sciences Division Laboratory Animal Resources Manual</p>	A-98-LSD-001
	Updated July 2000
IV. Policies and Guidelines	Page 2 of 4

- ? Bull's Eye cages should be stacked separately and washed last. The machine should be acid washed after washing these cages.
- ? The above procedures will be in effect for at least three weeks after the end of treatment and for a minimum of three cage changes. Normal changing procedures will be resumed at that time unless continuation of the special procedure is requested by the Investigator in charge of the experiment. The Investigator will remove the Bull's Eye tags and the sign on the animal room door at the appropriate time.
- ? MSDS sheets for test compounds will be available through the Principal Investigator.
- ? This general procedure will be modified as required by the properties of the test chemical and/or the test procedure.

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TO ANIMAL FACILITY SUPERVISOR:

Notification of chemically-treated animals to be set up for "Bull's Eye" procedure.

CHEMICAL COMPOUND(S) _____

HANDLING PROCEDURE (circle 1 or 2)

- 1. General procedure for handling mice treated with chemicals.
- 2. Alternative or additional procedure (describe).

ROOM NO. _____
 SET UP DATE _____
 FINISH DATE _____
 TOTAL NO. OF CAGES _____

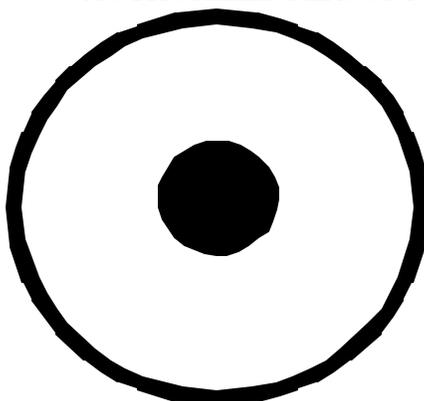
Signed _____
 (Investigator)

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START DATE _____ END DATE _____ ACUC PROTOCOL# _____

CAUTION - HAZARDOUS CHEMICALS IN USE

AUTHORIZED PERSONNEL ONLY



CHEMICAL(S) BEING TESTED:

WEAR GLOVES WHEN HANDLING MICE IN CAGES MARKED WITH A BULL'S EYE
(Additional Information/MSDS sheets in Rm _____ Bldg. _____)

	INVESTIGATOR	DIVISION SAFETY OFFICER
Name		David Edds
Work Phone		574-1221
Pager		873-6410
Home Phone		938-5208

Appendix 10

HVAC Summary

HEATING, VENTILATION & AIR CONDITIONING (HVAC) SYSTEM

BUILDING 9210

Room No.	Use	Fresh Air	Treatment	Air Changes	Pressure In water	Humidity Control	Date Assessed
105	Animal	100%	95%	13	+ 0.02	40-60%	06/25/02
108	Animal	100%	95%	13	+ 0.02	40-60%	06/25/02
109	Animal	100%	95%	12	+ 0.04	40-60%	06/25/02
110	Animal	100%	95%	12	+ 0.05	40-60%	06/25/02
111	Animal	100%	95%	13	+ 0.05	40-60%	06/25/02
112	Animal	100%	95%	13	+ 0.04	40-60%	06/25/02
113	Animal	100%	95%	13	+ 0.04	40-60%	06/25/02
114	Animal	100%	95%	12	+ 0.03	40-60%	06/25/02
115	Animal	100%	95%	12	+ 0.04	40-60%	06/25/02
116	Animal	100%	95%	12	+ 0.02	40-60%	06/25/02
117	Animal	100%	95%	13	+ 0.04	40-60%	06/25/02
118	Animal	100%	95%	13	+ 0.04	40-60%	06/25/02
120	Animal	100%	95%	12	+ 0.03	40-60%	06/25/02
121	Animal	100%	95%	13	+ 0.04	40-60%	06/25/02
201	Animal	100%	95%	12	+ 0.04	40-60%	06/25/02
202	Animal	100%	95%	13	+ 0.04	40-60%	06/25/02
203	Animal	100%	95%	12	+ 0.03	40-60%	06/25/02
204	Animal	100%	95%	12	+ 0.04	40-60%	06/25/02
205	Animal	100%	95%	12	+ 0.05	40-60%	06/25/02
206	Animal	100%	95%	11	+ 0.04	40-60%	06/25/02
208	Animal	100%	95%	10	+ 0.07	40-60%	06/25/02
209	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
210	Animal	100%	95%	11	+ 0.05	40-60%	06/25/02
211	Animal	100%	95%	11	+ 0.06	40-60%	06/25/02
212	Animal	100%	95%	11	+ 0.06	40-60%	06/25/02
213	Animal	100%	95%	12	+ 0.07	40-60%	06/25/02
215	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
216	Animal	100%	95%	10	+ 0.07	40-60%	06/25/02
217	Animal	100%	95%	11	+ 0.04	40-60%	06/25/02
218	Animal	100%	95%	10	+ 0.07	40-60%	06/25/02
219	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
220	Animal	100%	95%	11	+ 0.05	40-60%	06/25/02
301	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
302	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
303	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
304	Animal	100%	95%	11	+ 0.05	40-60%	06/25/02
305	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
306	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
308	Animal	100%	95%	11	+ 0.05	40-60%	06/25/02

309	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
310	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
311	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
312	Animal	100%	95%	10	+ 0.07	40-60%	06/25/02
313	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
314	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
315	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
316	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
317	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
318	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
319	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
320	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
321	Animal	100%	95%	11	+ 0.07	40-60%	06/25/02
323	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02
324	Animal	100%	95%	10	+ 0.60	40-60%	06/25/02
325	Animal	100%	95%	10	+ 0.06	40-60%	06/25/02

BUILDING 4500S

Room No.	Use	Fresh Air	Treatment	Air Changes	Pressure In water	Humidity Control	Date Assessed
147	Animal	100%	95%	13	+0.02	50-60%	06/27/02
Rack	Animal	100%	HEPA	85	+0.08	50-60%	06/27/02

BUILDING 4501

Room No.	Use	Fresh Air	Treatment	Air Changes	Pressure In water	Humidity Control	Date Assessed
317	Animal	100%	HEPA	14	+0.02	40-60%	06/27/02

BUILDING 3500

Room No.	Use	Fresh Air	Treatment	Air Changes	Pressure In water	Humidity Control	Date Assessed
MIR Lab.	Lab. & Animal	100%	95%	10	+ 0.01	40-60%	06/27/02

BUILDING 1505

Room No.	Use	Fresh Air	Treatment	Air Changes	Pressure In water	Humidity Control	Date Assessed
153	Fish	85%	95%	9	N/A	40-60%	06/27/02
263 lab.	Fish	85%	95%	20	N/A	40-60%	06/27/02

BUILDING 1504

Room No.	Use	Fresh Air	Treatment	Air Changes	Pressure In water	Humidity Control	Date Assessed
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1 Tank Room	Fish	85%	95%	9	N/A	40-60%	06/27/02
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Appendix 11

Cage Inventory

Cage Inventory

Building	Cage Type/Size	Number of Cages
9210	Open duplex/5" X 10.75" = 53.75 sq. in. (each side = 53.75 sq. in.) Vent. duplex/12/25" X 12.25" = 150 sq. in. (each side = 75 sq. in.) Vent. Single/8.75" X 12" = 105 sq. in.	11,613 (23,613) 605 (1210) 294
4500S	Ventilated/6.5" X 11.25" = 73 sq. in.	199
4501	Open rat/19' X 10" = 190 sq. in. Metabolic rat cages Open mouse/13" X 7.5I = 97.5 sq. in. Open mouse/19' X 10" = 190 sq. in.	39 7 75 17
3500	Open duplex/5" X 10.75" = 53.75 sq. in. (each side = 53.75 sq. in.)	1 (2)
9224 storage	Open duplex/5" X 10.75" = 53.75 sq/ in. (each side = 53.75 sq. in.)	1100 (2200)
Storage Units (720 sq. ft. at ORNL)	New Thoren single and duplex ventilated cages for the new planned SPF rodent facility	6,620

Environmental Sciences Division Aquatic Animal-Holding Materials Inventory¹

Type	Total	In Use	Stored	Type of Use:
Bldg. 1505, Rm 263				
1 gal. Aquarium	11	0	11	General purpose aquatic holding tanks
2 gal. Aquarium	36	2	34	General purpose aquatic holding tanks
10 gal. Aquarium	14	11	3	General purpose aquatic holding tanks
15 gal. Aquarium	12	10	2	General purpose aquatic holding tanks
50 gal. Aquarium	1	0	1	General purpose aquatic holding tanks
2 m (150 gal.) Living Stream	8	8	0	Medaka culture; general purpose aquatic holding tanks
Bldg. 1505, Rm 153				
2 m (150 gal.) Living Stream	4	1	3 ²	Temperature-controlled water-bath for medaka embryo tests; general purpose aquatic holding tanks
150 gal. Circular Tank	2	0	2	General purpose aquatic holding tanks
Bldg. 1504				
2 gal. Aquarium	51	2	49	Invertebrate culture; general purpose aquatic holding tanks
3 gal. Aquarium	3	0	3	General purpose aquatic holding tanks
20 gal. Aquarium	3	1	2	Invertebrate culture; general purpose aquatic holding tanks
40 gal. Aquarium	1	0	1	General purpose aquatic holding tanks
50 gal. Aquarium	7	0	7	Fish display tanks; general purpose aquatic holding tanks
80 gal. Aquarium	2 ³	1	1	General purpose aquatic holding tanks
150 gal. Aquarium	1	1	0	Fish display tanks; general purpose aquatic holding tanks
180 gal. Aquarium	1	1	0	General purpose aquatic holding tanks
2 m (150 gal.) Living Stream	10	4	6 ⁴	Temperature-controlled water-bath for toxicity test; general purpose aquatic holding tanks
250 gal. Circular Tank	16	0	16 ⁵	General purpose aquatic holding tanks
500 gal. Circular Tank	4	1	3 ⁶	Fathead minnow holding; general purpose aquatic holding tanks
25 m spiraling stream, approx. 500 gal.	8 ⁷	5	3	Stream microcosm experiments; no vertebrates currently being used

¹ Also have numerous outdoor ponds of various sizes, none of which are currently in experimental use.

² Only one of the stored tanks remain in Rm 153; two more have been dismantled and are stored in an outdoor storage area.

³ One additional tank, not included in this total, is stored in Bldg. 1506

⁴ Five of these stored tanks remain in Bldg. 1504; one tank has been dismantled and is stored in an outdoor storage area.

⁵ Seven of these stored tanks remain in Bldg. 1504; nine tanks have been dismantled and are stored in an outdoor storage area.

⁶ None of these stored tanks remain in Bldg. 1504; the three tanks have been dismantled; one tank is stored in an outdoor storage area and two tanks are loaned to the University of Tennessee Knoxville.

⁷ Sections to construct approximately 6 additional streams are stored in Bldg. 1503 greenhouse.

