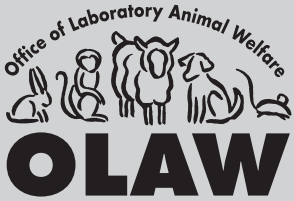
Institutional Animal Care and Use Committee Guidebook

2nd Edition
2002



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This Guidebook is provided for informational purposes only. It neither establishes nor reflects a change in PHS Policy on Humane Care and Use of Laboratory Animals.

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Foreword

The original *OPRR/ARENA IACUC Guidebook* was published in 1992 and has served as a useful resource to the animal research community. This revised edition, the ARENA/OLAW IACUC Guidebook, continues to support the fundamental principle on which the animal care and use program is based: self-regulation with oversight. It clearly demonstrates the increased role of the Institutional Animal Care and Use Committee (IACUC) in ensuring the ethical and sensitive care and use of animals in research, teaching and testing.

This *Guidebook* is the product of an ARENA-established editorial board of knowledgeable individuals who have IACUC experience and are familiar with the evolution of IACUC issues and relevant documents published during the past decade. Sections from the original document have been updated, and new sections added to incorporate state of the art knowledge regarding the functioning of IACUCs and institutional animal care and use programs. This *Guidebook* does not create new or different interpretations of the *PHS Policy on Humane Care and Use of Laboratory Animals*, legislation, or USDA animal welfare regulations.

The most current knowledge and understandings were sought through distinguished authors with experience and expertise. New references, resources and contemporary scientific and "road tested" guidance have been incorporated. For example, the emphasis of the 1996 edition of the *Guide for the Care and Use of Laboratory Animals* on performance goals as opposed to engineering approaches is a theme that resonates throughout. Other new reports, such as the 1997 *Occupational Health and Safety in the Care and Use of Research Animals* and the 1998 *The Psychological Well-Being of Nonhuman Primates*, both published by the National Research Council have offered new insights and approaches that are reflected herein. The AVMA Panel on Euthanasia also published new guidelines in 2001.

Additional knowledge and changing trends in research have mandated broader and deeper coverage of topics in this *Guidebook*. New topic areas include training IACUC members, disaster planning, managing breeding colonies, and the use of transgenic animals. New federal requirements and directives

have been incorporated, and feedback from the field during the past ten years has resulted in emphasis on topics such as the role of the nonaffiliated member, the application of the three R's (reduction, refinement and replacement) of alternatives, and the development of humane endpoints.

It is with a great sense of gratitude and respect for my colleagues who served on the editorial board and to the 30 authors who generously shared their time and expertise that I submit this document to the Office of Laboratory Animal Welfare. I would especially like to express my appreciation to the Project Director, Carol Wigglesworth, and her colleagues in NIH's OLAW who gave untold hours of editing and guidance to make this project not only possible, but also enjoyable. ARENA also gratefully acknowledges the technical review for consistency with the provisions of the USDA animal welfare regulations provided by Dr. Ron DeHaven, Deputy Administrator, Animal Care, APHIS, and his headquarters staff. This has truly been a labor of love by many dedicated individuals in the animal research community and I feel honored to have been a part of this effort.

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Abbreviations and Acronyms

Abbreviations

Guide	ILAR *Guide for the Care and Use of Laboratory Animals*
PHS Policy	*PHS Policy on Humane Care and Use of Laboratory Animals*

Acronyms

A

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
AALAS	American Association for Laboratory Animal Science
AC	Animal Care, APHIS, USDA
ACLAM	American College of Laboratory Animal Medicine
AGRICOLA	National Agricultural Library's Agricultural OnLine Access (USDA)
APHIS	Animal and Plant Health Inspection Service (USDA)
ARENA	Applied Research Ethics National Association
ASLAP	American Society of Laboratory Animal Practitioners
AV	Attending Veterinarian
AVMA	American Veterinary Medical Association
AWA	Animal Welfare Act
AWIC	Animal Welfare Information Center
AWRs	Animal Welfare Regulations (USDA)

C

CAAT	Center for Alternatives to Animal Testing
CCAC	Canadian Council on Animal Care
CDC	Centers for Disease Control and Prevention
CEO	Chief Executive Officer
CFA	Complete Freund's Adjuvant
CFR	Code of Federal Regulations
CIRA	Center for Information on Research with Animals
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora

D

DHHS	Department of Health and Human Services
DOI	Department of the Interior
DVM/VMD	Doctor of Veterinary Medicine or Veterinary Medical Doctor

E

EPA	Environmental Protection Agency
ESA	Endangered Species Act

F

FASEB	Federation of American Societies of Experimental Biology
FBR	Foundation for Biomedical Research
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency

FOIA Freedom of Information Act

FR *Federal Register*

G

GLP Good Laboratory Practices

GPO Government Printing Office

H

HEPA High-Efficiency Particulate Air Filter

HREA Health Research Extension Act, Public Law 99-158

HVAC Heating, Ventilation and Air Conditioning

I

IACUC Institutional Animal Care and Use Committee

IATA International Air Transport Association

IBC Institutional Biosafety Committee

ICLAS International Council for Laboratory Animal Science

IFA Freund's Incomplete Adjuvant

iiFAR Incurably Ill for Animal Research

ILAR Institute for Laboratory Animal Research

IO Institutional Official

IOM Institute of Medicine

IRAC Interagency Research Animal Committee

L

LAMA Laboratory Animal Management Association

LAT Laboratory Animal Technician

LATg Laboratory Animal Technologist

LD Lethal Dose

LD_{50} Lethal Dose 50%

M

mAb Monoclonal Antibody

MRI Magnetic Resonance Imaging

N

NABR National Association for Biomedical Research

NAL National Agricultural Library

NARRC National Advisory Research Resources Council

NAS National Academy of Sciences

NIH National Institutes of Health

NIOSH National Institute of Occupational Safety and Health

NRC National Research Council

NSF National Science Foundation

O

OHSP Occupational Health and Safety Program

OLAW Office of Laboratory Animal Welfare, NIH

OMB Office of Management and Budget

OSHA Occupational Safety and Health Administration

OSTP Office of Science and Technology Policy

P

PHS Public Health Service

PRIM&R Public Responsibility in Medicine and Research

R

RSC Radiation Safety Committee

S

SCAW Scientists Center for Animal Welfare

U

USDA U.S. Department of Agriculture

USFWS U.S. Fish and Wildlife Service, Department of Interior

V

VA Department of Veterans Affairs

W

WHO World Health Organization

WVA World Veterinary Association

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A. The IACUC

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A.1. Timeline, Background and History

Timeline

1950 Formal establishment of Animal Care Panel.

1963 First edition of the *Guide for the Care and Use of Laboratory Animals (Guide)* developed by the Animal Care Panel.

1965 Incorporation of the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

1966 Congress passed the Laboratory Animal Welfare Act (PL 89-544) and the USDA was named the responsible agency.

1967 Animal Care Panel changed its name to the American Association for Laboratory Animal Science (AALAS).

1971 NIH Policy on Humane Care and Use of Laboratory Animals for PHS Supported Institutions.

1971 USDA promulgated standards known as Subpart F, Stolen Animals (AWA).

1973 First Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals.

1974 Public Responsibility in Medicine and Research (PRIM&R) established.

1979 PHS Policy required each animal-using grantee institution to have a PHS Assurance and a committee to maintain oversight of its animal care program.

1979 USDA promulgated standards known as Subpart E, Identification of Animals (AWA).

1982 First PRIM&R Animal Care and Use meeting.

1985 U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training promulgated.

1985 Health Research Extension Act (P.L.99-158) passed by Congress.

1985 Animal Welfare Act Amendments passed by Congress.

1986 Applied Research Ethics National Association (ARENA) established.

1986 PHS Policy revised.

1989 USDA promulgated regulations (known as Parts 1 and 2) implement ing the 1985 AWA amendments.

1990 The structure of the Office for Protection from Research Risks (OPRR) was changed to establish a Division of Animal Welfare.

1990 USDA promulgated standards known as Subpart B, Registration and Subpart C, Research Facilities (AWA).

1991 USDA promulgated standards known as Part 3. In addition, amend ments were made to Part 2: Regulations in Subpart A, Licensing and Subpart D, Attending Veterinarian and Adequate Veterinary Care. (AWA).

1992 First Institutional *Animal Care and Use Committee Guidebook* was developed by a committee under the auspices of the Applied Research Ethics National Association (ARENA) and OPRR.

1996 7th Edition of the *Guide for the Care and Use of Laboratory Animals* revised by an ILAR committee and published by the NRC.

1996 AAALAC became the Association for the Assessment and Accredita tion of Laboratory Animal Care International.

2000 OPRR Division of Animal Welfare was separated from OPRR and became the Office of Laboratory Animal Welfare (OLAW), NIH.

2002 *ARENA/OLAW Institutional Animal Care and Use Committee Guide-book.* Second edition.

Background and History

Prior to the middle of the 20th century the responsibility for animals used in research in the United States was placed directly in the hands of the researchers and the quality of animal care and animal welfare varied tremendously among research institutions. Even within the same school or institution, research laboratories had inconsistent animal care policies and standards of care.

In 1961, a group of veterinarians working for research institutions in the Chicago area formed the Animal Care Panel (ACP). The ACP appointed a committee charged with establishing animal care and use guidelines for research facilities. Their product was the publication of the first edition (1963) of the *Guide for the Care and Use of Laboratory Animals (*referred to in this document as the *Guide)*. Subsequent editions of this publication were supported by the NIH and developed under the auspices of the Institute of Laboratory Animal Resources (ILAR), which was subsequently renamed the Institute for Laboratory Animal Research. The National Acad emy Press, under the auspices of the National Research Council, published the most recent (seventh) edition in 1996. This single document serves as the primary source of laboratory animal care and use standards and guidelines in the United States. The 1996 edition has been translated and published in six languages, and over 400,000 copies have been distributed throughout the world.

In 1963, the ACP saw a need to evaluate the standards of animal care and use practiced in research institutions based on the *Guide,* and appointed an Animal Accreditation Committee. This Committee soon determined that it should function independently of the ACP, and in 1965 incorporated in the state of Illinois as the American Association for the Accreditation of Laboratory Animal Care. This independent accrediting agency changed its name in 1996 to the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Prior to 1966, no U.S. federal law addressed laboratory animal welfare. Local humane societies actively promoted responsible treatment of pets and farm animals. Concurrently, the scientific community was improving the quality of animal care and well-being in the research laboratory. During this time the increasing need for dogs and cats in research was partially fulfilled by animal dealers who obtained these animals in various ways and sold them to research laboratories. A series of articles and news

reports on animal neglect, abuse and pet theft by animal dealers culminated in a 1966 major article and photographs in *Life* magazine. The article suggested a need for regulation and a system of enforcement, especially for dogs and cats used in research. Catalyzed in part by this article, the Laboratory Animal Welfare Act, the first version of what is now known as the Animal Welfare Act (AWA), was passed by Congress in 1966 (Public Law 89-544) establishing legal standards for laboratory animal care and use for the first time in this country. The United States Department of Agriculture (USDA) was named the responsible agency for implementing and enforcing this new law and it promptly began promulgating regulations. Research laboratories and dealers were required to register or license their facilities and undergo inspection by USDA personnel who were authorized to issue citations for non-compliance. These early inspections did not extend into the research laboratory where animal care and use remained under the direction of the research investigator. A number of amendments to the AWA have led to regulations that now include animal transportation, marine mammals, and animals in the research laboratory. However, the USDA regulations currently exclude common laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*), birds, and farm animals used in production agriculture research.

All Public Health Service (PHS) policies on this subject evolved from the 1971 National Institutes of Health (NIH) Policy, "Care and Treatment of Laboratory Animals." That policy referenced several NIH and PHS statements on appropriate care and humane treatment of laboratory animals, among them the *Guide*. It introduced the animal care committee as a means of local assurance of good animal care and use.

The 1971 NIH policy required institutions or organizations using warm-blooded animals in research or teaching supported by NIH grants, awards or contracts to "assure the NIH that they will evaluate their animal facilities in regard to the maintenance of acceptable standards for the care, use and treatment of such animals." The institution could show that it was either accredited by a recognized professional laboratory animal accrediting body (AAALAC) or had established an animal care committee to carry out that assurance function. The minimum number of committee members was not stated, but at least one member had to be a Doctor of Veterinary Medicine. Guidelines for the committee included the *Guide*, all applicable portions of the AWA, and an appended set of Guidelines known as the "Principles for the Use of Laboratory Animals." The committee was required

to inspect the institution's animal facilities at least once a year and report its findings and recommendations to responsible institutional officials. Records of activities and recommendations were required to be available for inspection by NIH representatives.

The first PHS policy regarding animal care and use replaced the NIH policy on July 1, 1973 and continued to accept AAALAC accreditation in lieu of an institutional committee. The January 1, 1979 revision of the PHS policy required each animal-using grantee institution to have "a committee to maintain oversight of its animal care program" and expanded the definition of animal to include all vertebrates. The revised policy also required an institution to submit an Assurance statement to the Office for Protection from Research Risks (OPRR), now the Office of Laboratory Animal Welfare (OLAW), that it is committed to follow the *Guide*, the Principles and the PHS policy requirements, before receiving PHS support for studies in which animals or animal facilities were used.

Institutions were required to include in their Assurance a list of committee members with their position titles and credentials. Committees were composed of at least five members including at least one veterinarian. The members had to be knowledgeable regarding the care and use of animals used in research.

The 1979 PHS policy continued to accept AAALAC accreditation as a means of demonstrating conformance with the *Guide*, but an alternative was annual review of the animal facilities and procedures by the institution's IACUC. Institutions were required to report to NIH (OPRR) any noncon formance with the *Guide* or problems encountered in implementing the PHS policy, and submit annual reports indicating progress toward full conformance. Review of individual proposals or projects by the IACUC was encouraged but not required.

The most recent revision, officially the *PHS Policy on Humane Care and Use of Laboratory Animals* (referred to in this document as the *PHS Policy*), was promulgated in 1986 and reprinted in 1996 and 2000. It further defined and outlined requirements of an animal care and use program. This revised *PHS Policy* includes provisions of the Health Research Extension Act of 1985, enacted on November 20, 1985 as Public Law 99-158. The 1986 *PHS Policy* applies to both extramural and intramural PHS research and requires the Institutional Animal Care and Use Committee (IACUC) mem bers to be appointed by the Chief Executive Officer of the institution. The

IACUC must evaluate and prepare reports on all of the institution's programs and facilities (including satellite facilities) for activities involving animals at least twice each year, and is required to review the care and use of animals in PHS-supported activities. The IACUC, through the Institutional Official (IO), is responsible for compliance with reporting requirements. Minority views filed by members of the IACUC must be included in reports filed under this *PHS Policy*. The *PHS Policy* also requires training or instruction for scientists, animal technicians and other personnel involved in animal care, treatment or use. This training or instruction must include information on the humane practice of animal care and use as well as train ing or instruction in research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress.

The Interagency Research Animal Committee, made up of representatives of federal agencies that use or require the use of experimental animals, promulgated the "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training" in 1985 (see Appendix F). These Principles were subsequently incorporated into the 1986 *PHS Policy*, and remain in effect today as a model for federal agencies that develop specific agency policies for the use of animals.

With the promulgation of the 1986 version of the *PHS Policy*, OPRR (now OLAW) embarked upon an extensive national education program. The program began with the co-sponsorship of one- to two-day workshops in conjunction with Assured institutions at different geographical locations. Many of the early workshops focused on basic provisions set forth in the 1986 *PHS Policy*, such as protocol review and semiannual program evalu ations. That cosponsorship of approximately four to five workshops a year continues today, although the topics are now generally more special ized, covering areas such as performance standards, field studies, and laboratory animal management and technology. Since 1995 OLAW has expanded its educational role to include development of a Web-based tutorial, an extensive Web site with sample documents to assist institu tions in their implementation of the *PHS Policy*, co-sponsorship of ARENA's IACUC 101 program, and this revised ARENA/OLAW Guidebook.

Special interest groups concerned about the acquisition and welfare of animals used in research continue to influence research animal care and use. These groups include local and national humane societies concerned about animal welfare and well-being, and antivivisectionist groups that are opposed to the use of animals in research. The activity of some animal

rights groups escalated and became more vocal in the early 1980s. This activity peaked in a series of illegal break-ins and vandalism and was brought to the forefront of public opinion soon after two incidents involving alleged "animal cruelty" and "insensitivity" in two well-known research institutions. This climate raised public concern and visibility of animals in research and served as a catalyst for amendments and clarifications of guidelines and regulations providing for animal welfare.

New USDA regulations based on the 1985 amendment to the AWA became effective between October 1989 and August 1991. These regula tions require each registered research institution to appoint an IACUC of not less than three members, including a veterinarian, which "serves as the agent of the research facility that assures that the facility is in full com pliance with the Act." The regulations also require a member not affiliated with the institution representing community interests in the proper care and treatment of animals. These USDA Animal Welfare Regulations (AWRs) and the *PHS Policy* contain many common requirements.

The Scientists Center for Animal Welfare (SCAW) was instrumental in pro viding early guidance to institutions on IACUC functions and organization through regional conferences and workshops, culminating in a special 1987 American Association for Laboratory Animal Science (AALAS) publi cation entitled, "Effective Animal Care and Use Committees." Since 1983, training and guidance of this type has also been provided through annual animal care and use conferences sponsored by Public Responsibility in Medicine and Research (PRIM&R) and the Applied Research Ethics National Association (ARENA), regional workshops supported by OLAW, and numerous similar activities. The first *Institutional Animal Care and Use Committee Guidebook* was written by a committee of experts under the auspices of ARENA and published by NIH in 1992. The present edition, published in 2002, is the first revision.

During the 1990s there was an evolution in the ways that IACUCs fulfilled their mandate. This was in part due to increased experience implement ing the *PHS Policy* and AWRs, but may also be attributed to new reports, such as the 1996 *Guide* which emphasizes performance goals as opposed to engineering standards, and the 1997 ILAR report, *Occupational Health and Safety in the Care and Use of Research Animals*, that shifted the focus of occupational health programs to risk based systems. Other factors contributing to this evolution came from the research community, such as the development of transgenic animals and *in vitro* alternatives to the

production of monoclonal antibodies. The IACUC community has also gained a greater understanding of and appreciation for the role of nonaffiliated and nonscientific IACUC members. Humane endpoints in research and innovative ways to address environmental enrichment of primates are other areas that grew in sophistication during the 1990s. Training of IACUC members and animal users has received greater attention and the number of training programs and modules has increased significantly. Finally, OLAW, USDA and AAALAC International have all placed an increased focus on IACUC functions.

While originally borrowed from the human Institutional Review Board structure, the concept of IACUCs to review and ensure animal welfare is now common practice in the animal research community. The goal of each IACUC is to ensure the humane care and use of animals used in research, and compliance with guidelines and regulations, while maintaining flexibility to best meet the unique needs of the institution. Active participation by research scientists allows for the scientific needs of research investigators to be considered; participation by nonaffiliated members incorporates a public conscience; and the involvement of veterinarians ensures appropriate medical care and animal well-being. A program of continuing education is essential to ensure that animal care and use standards and ethical principles continue to be applied at the highest possible level.

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A.2. Authority, Composition and Functions

Each institution that receives PHS support for activities involving vertebrate animals or is subject to the authority of the Animal Welfare Act (AWA) must operate an animal care and use program with clear lines of authority and responsibility. The program must include:

- a properly constituted and functioning Institutional Animal Care and Use Committee (IACUC);
- procedures for self monitoring;
- an adequate veterinary care program;
- an occupational health and safety program (not required under the AWA);
- a personnel training program;
- an environment, housing and management program for animals; and
- appropriately maintained facilities for housing and support.

PHS requires an institutional Animal Welfare Assurance that provides details on the institutional program in order to award funds; USDA requires registration of facilities. Section E.1. and E.1. Table B include additional detail concerning PHS assurances and USDA registration.

Authority

IACUCs derive their authority from the law. They are mandated by the Health Research Extension Act (HREA) of 1985 and the AWA. The laws require the Chief Executive Officer (CEO) of an organization to appoint the IACUC, whose responsibilities are delineated in the law and federal policy and regulations. Office of Laboratory Animal Welfare (OLAW) considers the CEO to be the highest operating official of the organization. The CEO may delegate authority to appoint the IACUC if the delegation is specific and in writing.

Once appointed, IACUCs report to a senior administrator known as the Institutional Official (IO). The IO must have administrative and operational authority to commit institutional resources to ensure compliance with the *PHS Policy* and other requirements. The CEO and IO may be the same

individual, although at large institutions the CEO is typically somewhat removed from operational program involvement. Occasionally IOs are also appointed to serve on IACUCs but this is not advisable because the IACUC reports to the IO, creating potential conflict of interest.

The IACUC's mandate to perform semiannual program evaluations as a means of overseeing the animal care and use program puts the IACUC in an advisory role to the IO. In its semiannual reports the IACUC advises the IO of the status of the institution's compliance, establishes plans and schedules for correcting deficiencies necessary to either maintain or achieve compliance, and makes recommendation to the IO regarding any aspect of the institution's animal program, facilities, or personnel training. This approach of "enforced self-regulation" requires that the IACUC have the full support of the IO responsible for the program.

The IACUC's authority to review and approve protocols is independent of the IO who may not overrule an IACUC decision to withhold approval of a protocol. (The converse is not true, i.e., if an IACUC approves a pro tocol the institution is not required or obligated to conduct the research activity.) An institution may subject protocols to additional institutional review (e.g., department head, biosafety committee, etc.)

Committee Composition

Some IACUC members fulfill specific regulatory requirements (e.g., veterinarian with program responsibility, an individual nonaffiliated with the institution); others have unique roles by virtue of their position (e.g., chairperson).

There are no specific prohibitions regarding individuals filling more than one role on the IACUC, but OLAW strongly recommends against the same person serving multiple roles because the responsibilities and authorities vested in each of the positions are distinct and often require different skills. Appointing one individual to more than one of these roles may circumvent intended checks and balances. Also of importance is the perception of conflict of interest, which can lead to allegations of impropri eties from various sources.

Veterinarian: The *PHS Policy* and AWRs mandate the appointment of a veterinarian with direct or delegated program responsibility to the IACUC. CEOs may appoint more than one veterinarian to the IACUC but the veteri narian with direct or delegated program responsibility must be designated

as such. The veterinarian with program responsibility must have training or experience in laboratory animal science and medicine or in the care of the species being used.

Chair: A knowledgeable and effective leader is crucial to an effective IACUC. This individual needs the full support of the IO. A chair with suffi cient stature (e.g., seniority or tenure) can perform the functions of this position without jeopardy to his/her career. In the case of a large program of animal care and use a co-chair may be desirable.

Nonaffiliated member: The nonaffiliated member is intended to repre sent general community interests. An informed nonaffiliated member can bring significant value to the committee by bringing a non-institutional perspective to the research endeavor. This member has equal status to every other committee member and should be provided the opportunity to participate in all aspects of IACUC functions.

While in the majority of instances effective nonaffiliated members may be willing to serve without reimbursement, in other instances remunera tion for expenses or compensation for time may allow for participation by effective individuals that would not otherwise be possible. OLAW and USDA maintain that nominal compensation is permissible without jeopar dizing a member's non-affiliated status, if it is only in conjunction with service on the IACUC and if the amount of compensation is not so sub stantial that it could be considered to influence voting on the IACUC.

Scientist and nonscientist: *PHS Policy* requires that the IACUC include a practicing scientist experienced in research involving animals, and a member whose primary concerns are in a nonscientific area. Examples of the latter include, but are not limited to, ethicist, lawyer, member of the clergy, and librarian.

Institutions should consider persons with expertise in the disciplines involved in institutional research and teaching programs for service on their IACUCs. In addition to the required categories of membership, it is suggested that individuals with expertise in specific areas pertinent to protocol review and program oversight be considered (e.g., statisticians, occupational health experts, information resource specialists, animal health technicians, and scientific research staff).

There is no requirement that any particular member or category of members be present at all IACUC meetings. However, an institution must have a properly constituted IACUC in order for the IACUC to conduct valid

official business. Many institutions have found that appointing more than the minimum number of members who meet the respective criteria obvi ates problems when an unexpected vacancy occurs**,** and can help the committee meet the quorum requirements necessary for certain official committee actions.

A.2. Table A. Comparison of IACUC Membership Requirements

PHS Policy PHS Policy IV.A. 3. a., b. *Appointed by the CEO* *Minimum of five members:*	**USDA Regulations** 9 CFR, 2.31 (a) (b) *Appointed by the CEO* *Minimum of three members:*
One Doctor of Veterinary Medicine with training or experience in laboratory animal science and medicine who has direct or delegated program authority and respon sibility for activities involving animals at the institution.	At least one Doctor of Veterinary Medicine with training or experience in laboratory animal science and medicine, and who has direct or delegated program respon sibility for activities involving animals at the institution.
One practicing scientist experienced in research involving animals.	
One member whose primary concerns are in a nonscientific area (for example, ethicist, lawyer, clergy).	
One member not affiliated in any way with the institution and not a member of the immediate family of a person who is affiliated with the institution. The *PHS Policy* requires institutions to follow the *Guide*, which states that committee membership should include at least one public member to represent general community interests in proper care and use of animals, and that public members should not be laboratory animal users.	One member not affiliated in any way with the institution and not a member of the immediate family of a person who is affiliated with the institution; person who represents the general community interests in the proper care and treatment of animals; and is not a laboratory animal user (USDA Policy # 15)
	Not more than three members from the same administrative unit of the institution.

Alternate members may be appointed to the IACUC as long as they are appointed by the CEO or other official with authority to appoint members, and there is a specific one-to-one designation of IACUC members and alternates. An IACUC member and his/her alternate may not count toward a quorum at the same time or act in an official member capacity at the same time. Alternates should receive training similar or identical to the training provided to regular IACUC members.

Conflict of Interest

Both the AWRs and *PHS Policy* state that no IACUC member "may par ticipate in the IACUC review or approval of an activity in which that member has a conflicting interest, (e.g., is personally involved in the activity) except to provide information requested by the IACUC."

If the investigator submitting a protocol believes that an IACUC member has a potential conflict, the investigator may request that the member be excluded. When a member has a conflict of interest, the member should notify the IACUC Chair and may not participate in the IACUC review or approval except to provide information. Members who have a conflict of interest may not be counted toward a quorum and may not vote.

Other possible examples of conflict of interest include cases where:

- a member is involved in a potentially competing research program,
- access to funding or intellectual information may provide an unfair competitive advantage, or
- a member's personal biases may interfere with his or her impartial judgment.

Quorum Requirements

Certain official IACUC actions require a quorum: full committee review of a research project (Policy IV.C.2. and AWR §2.31(d)(2)) and suspension of an activity (Policy IV.C.6. and AWR §2.31(d)(6)). "Quorum" is defined as a majority (>50%) of the voting members of the IACUC. Therefore, a protocol is approved only if a quorum is present, and if more than 50% of the quorum votes in favor. *PHS Policy* and AWRs require that in order

to suspend an activity, the IACUC must review the matter at a convened meeting of a quorum of the IACUC and the suspension must be approved by a majority vote of the quorum present.

For reasons other than conflict of interest, abstentions from voting do not alter the quorum or change the number of votes required. For example: If an IACUC has 20 voting members, at least 11 members must be present at a convened meeting to constitute a quorum and approval of a protocol would require a minimum of six votes whether or not there were abstentions.

The requirements of the *PHS Policy* and AWRs take precedence even though they may differ from some commonly used parliamentary proce dures. Institutions may develop their own meeting procedures as long as the procedures do not contradict or are not inconsistent with the require ments of the *PHS Policy* or the AWRs.

A.2. Table B. Federally Mandated Functions of the IACUC

PHS PHS Policy. IV.B.1-8	USDA 9 CFR. 2.31 (c) (1) – (8) and 2.31(d) (5) (6) & (7)
1. Review, at least once every six months, the research facility's program for the humane care and use of animals, using the *Guide* as a basis for evaluation.	1. Review, at least once every six months, the research facility's program for humane care and use of animals, using title 9, chapter 1, subchapter A—Animal Welfare, as a basis for evaluation.
2. Inspect, at least once every six months, all of the institution's animal facilities (including satellite facilities) using the *Guide* as a basis for evaluation. Satellite holding facilities (a facility outside of a core facility or centrally designated area in which animals are housed for more than 24 hours) and areas in which surgical manipulations are performed must always be included.	2. Inspect, at least once every six months, all of the research facility's animal facilities, including animal study areas, using title 9, chapter 1, subchapter A—Animal Welfare as a basis for evaluation. Areas where animals are housed for more than 12 hours are defined as "study areas."
3. Prepare reports of the IACUC evalua tions and submit the reports to the IO. The reports must contain a description of the nature and extent of adherence to the *Guide* and *PHS Policy* and identify specifically any departures from the pro-visions of the *Guide* and *PHS Policy* and state reasons for each departure. The IACUC may determine the best means of conducting an evaluation of its program and facilities. The IACUC may invite *ad hoc* consultants to assist in conducting the evaluation. However, the IACUC remains responsible for the evaluation and report. Reports must distinguish significant deficiencies from minor deficiencies and must contain a reasonable and specific plan and schedule for correcting each deficiency. A significant deficiency is one that is or may be a threat to the health and safety of the animals. Reports must be made available to OLAW upon request.	3. Prepare reports of its evaluations (using the title 9, chapter 1, A – AWR) and submit to the IO. The IACUC may determine the best means of conducting evaluations of the research facility's programs and facilities, provided that no member wishing to participate in any evaluation is excluded. Reports must distinguish significant deficiencies from minor deficiencies and must contain a reasonable and specific plan and schedule with dates for correcting each deficiency. A significant deficiency is one that is or may be a threat to the health and safety of the animals. A significant defi ciency remaining uncorrected beyond the scheduled correction date shall be reported in writing within 15 business days by the IACUC, through the IO, to APHIS and any federal agency funding that activity. Reports must be made available to APHIS and to officials of federal funding agencies for inspection and copying upon request.
4. Review concerns involving the care and use of animals at the institution.	4. Review, and if warranted, investigate concerns involving the care and use of animals resulting from public complaints and from reports of noncompliance received from laboratory or research facility personnel or employees.

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A.2. Table B. Federally Mandated Functions of the IACUC *(continued)*

PHS PHS Policy. IV.B.1-8	USDA 9 CFR. 2.31 (c) (1) – (8) and 2.31(d) (5) (6) & (7)
5. Make recommendations to the IO regarding any aspect of the animal program, facilities or personnel training.	5. Make recommendations to the IO regarding any aspects of the animal program, facilities or personnel training.
6. Review and approve, require modifi cations in, or withhold approval of those components of PHS-conducted or sup-ported animal care and use activities. A complete review is required at least once every three years.	6. Review and approve, require modifi cations in, or withhold approval of those components of proposed activities related to the care and use of animals. Continu ing review of activities required not less than annually.
7. Review and approve, require modifica tions in, or withhold approval of proposed significant changes regarding the use of animals in ongoing activities.	7. Review and approve, require modifica tions in, or withhold approval of proposed significant changes regarding the care and use of animals in ongoing activities.
8. Be authorized to suspend an activity involving animals in accordance with specifications in IV.C.6 of PHS *Policy* (i.e., an activity that is not being conducted in accordance with applicable provision of the AWA, the *Guide*, the institution's Assurance, or *PHS Policy*.) This action may be taken only after review of the matter at a convened meeting of a quorum of the IACUC and a vote for suspension by the majority of the quorum present. The IO in consultation with the IACUC shall review the reasons for suspension, take appro priate corrective action, and report that action with a full explanation to OLAW.	8. Be authorized to suspend an activity involving animals if it determines that the activity is not being conducted in accor dance with the description provided by the investigator and approved by the IACUC. This may be done only after review at a convened meeting of a quorum of the IACUC with the suspen sion vote of a majority of the quorum present. The IO, in consultation with the IACUC, shall review the reasons for the suspension, take appropriate corrective action, and report that action with a full explanation to APHIS and any federal agency funding that activity.

References

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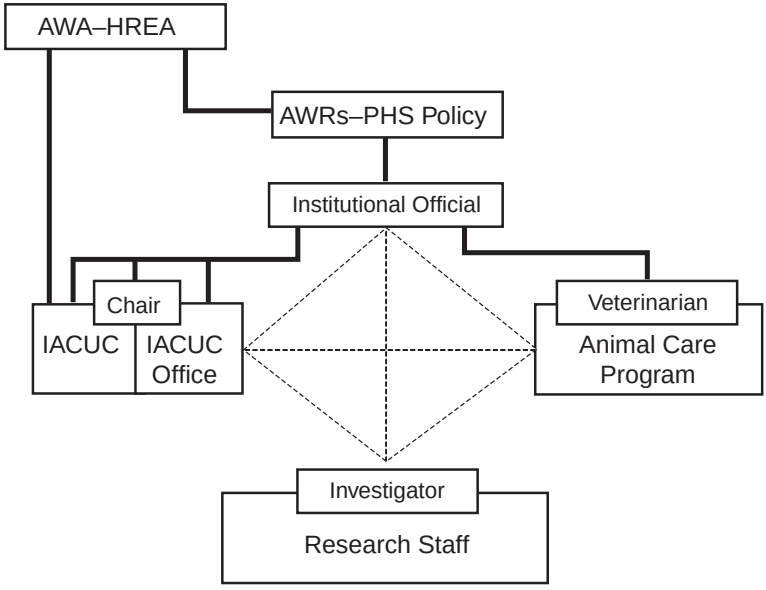
A.3. Operation and Administration

Institutional Responsibility for Animal Welfare

Assuring laboratory animal welfare necessitates a partnership among the Institutional Official (IO), the IACUC, the veterinarian and the investigators. Ultimately, accountability for assuring humane care and use of animals resides with the institution, but this may only be achieved when all of the players, i.e., the investigators and their research staff, the veterinary staff, animal caretakers and technicians, and the IACUC, contribute to a shared goal.

Each institution should provide a framework with appropriate resources for an animal care and use program that is managed in accordance with the *PHS Policy*, the *Guide*, and the Animal Welfare Regulations (AWRs). Organizations that function effectively have simple, clear and direct lines of responsibility and corresponding authority.



Components of an animal care and use program. Heavy lines represent the mandate from the Animal Welfare Act and Health Research Extension Act that the Secretaries of Agriculture and Health and Human Services develop guidelines for the use of animals in research and for IACUCs, and require established lines of authority from the IO to the IACUC, IACUC staff, and veterinarian. Dotted lines represent the need for cooperation and communication among components.

The *PHS Policy* and AWRs place a strong emphasis on senior management level responsibility and on use of the IACUC as an oversight committee to evaluate the program. The committee needs to work closely with the animal users, the animal care staff, and the responsible veterinarians to ensure a high quality animal care and use program. The IO must support the IACUC by providing appropriate resources.

Responsibilities of the Institutional Official

The IO must have the authority to allocate organizational resources needed to maintain a smoothly functioning animal care and use program based on the recommendations and advice received from:

- the IACUC,
- the veterinarian, and
- the IACUC professional and administrative staff.

The IO should also clearly define and assign responsibilities and reporting channels for other essential program elements such as:

- personnel training,-
- occupational health and safety, and-
- maintenance of facilities.

The IACUC, appointed by the organization's Chief Executive Officer (CEO), must report directly to the IO and be empowered to perform its duties without undue interference. OLAW's experience is that it is usually best for the veterinarian also to report directly to the IO in connection with his or her responsibility for implementing the animal care and use program. In order to provide the intended checks and balances in the system of self-regulation, it is advisable that the veterinarian not serve as Chair of the IACUC or as IO. While it is important that there be a collegial and effective working relationship between the IACUC and the veterinarian, it is impor tant to avoid the potential for real or perceived conflicts of interest.

Role and Responsibilities of the IACUC Staff

The nature of the institution and the volume of animal-based research determine the staffing requirements of an IACUC and the animal care program. Institutions with a high volume of proposals involving animals may require full time IACUC staff. A professional staff with expertise in animal welfare laws, regulations and policies is especially important to provide stability and continuity to animal care and use programs where IACUC chairs and members serve on a rotating basis.

The role of the IACUC staff is to provide administrative support to the IACUC and the IO. It is important however, that neither the IO nor the IACUC Chair over-invest authority or responsibility in the IACUC staff.

The IACUC staff often serve as the gatekeepers of information and com munications for the IO, the IACUC Chair and members, the veterinarian, the animal resource program, the investigators, and other offices within the institution such as public relations and sponsored research. It is important that training and continuing education be provided to program staff so they are knowledgeable of current animal care and use policies and regulations and aware of proposed changes. OLAW workshops, ARENA and PRIM&R annual meetings, ARENA IACUC 101 Training, and SCAW meetings, are examples of useful training and educational opportunities.

IACUC staff responsibilities range from clerical and administrative to pro fessional, depending on the size and complexity of the program.

Some examples of **clerical tasks** are:

- data entry;
- screening protocols for completeness;
- preparing agendas and distributing protocols and other materials to IACUC members;
- sending out reminders of protocol expirations and approval letters;
- maintaining records of protocols and minutes of the meetings, policies and procedures, program reviews and facility inspection reports; and
- coordinating and scheduling the IACUC's meetings, facilities inspec tions and laboratory site visits.

Administrative duties include:

- preparation of minutes and other correspondence and reports, such as the PHS Assurance document, and annual PHS, USDA and AAALAC reports; and
- serving as an information resource for investigators and IACUC members regarding regulatory issues and the status of protocols.

Professional staff duties include:

- providing orientation and training of new IACUC members;
- grant proposal review to ensure consistency in the animal care and use components of the proposal and the protocol submitted to the IACUC;
- pre-review of protocols for federal assurances, scientific and statis tical validity;
- review of literature searches; and
- drafting of institutional policies.

The IACUC staff also maintains federal documents such as the institution's PHS Assurance, USDA registration and reports, and AAALAC accredita tion materials.

Review of Grants and Contracts Submitted to PHS

In order to approve a protocol that involves the use of animals, the IACUC must review the proposed care and use of animals and determine that federal criteria have been met. PHS requires that the project be conducted in accordance with the *PHS Policy*, the AWA, the *Guide*, the institution's Assurance, and all other applicable federal statutes and regulations related to animals. The project should also comply with all institutional policies.

Most IACUCs require use of a standardized protocol application form to assist the investigator in providing the information necessary to ensure compliance. While there is no explicit requirement for the IACUC to do a side-by-side comparison of the information contained in the IACUC proto col review form and the information submitted to PHS, it is imperative that the protocol that the IACUC approves is consistent with the information submitted to PHS. Institutions should devise a mechanism to verify that consistency. If the IACUC requires changes to the protocol that are not

reflected in the grant application, then the PHS funding component must be notified in the follow-up certification of IACUC approval.

Institutions are required to provide PHS with the date of IACUC approval. There is no provision for providing a contingent approval date; the date provided must signify full approval by the IACUC. If an institution has a PHS Assurance, then in most cases the PHS allows a 60-day grace period following the receipt deadline date during which the investigator may secure IACUC approval; otherwise, the application cannot be peer reviewed. If the IACUC review occurs subsequent to the grant submission, then a letter verifying IACUC approval, and stating any modifications required by the IACUC, must be submitted to the funding agency. This grace period is non-existent for some non-federally funded projects and investigators are required to submit evidence of IACUC approval coincident with the grant or contract submission.

If an institution does not have a PHS Assurance, the signature of the official signing the grant application for the organization constitutes a declaration that the institution will submit an Assurance and verification of IACUC approval upon request by OLAW.

Responsibility for Collaborations and Subcontracted Research

Collaborations between institutions can sometimes create ambiguity regard ing responsibility for animal welfare. In cases where an individual investiga tor has appointments at several institutions, or where collaborations occur between institutions, it is advisable to have a formal written agreement, contract or memorandum of understanding between the institutions. This document should originate from the primary collaborative institution (i.e., the institution primarily responsible for directing and/or funding the research) and be signed by the secondary institution.

When an institution receiving PHS funds contracts with a commercial vendor using animals to produce a product, there may need to be IACUC involvement. If a company produces standard antibodies for general sale, that company is not required to file an Assurance with OLAW. However, if a supplier or contractor produces antibodies in animals using an anti gen provided by or at the request of an investigator, the antibodies are considered “custom” and the vendor must have an Assurance on file. The vendor Assurance must be identified on the PHS grant application, and the awardee institution is responsible for verifying that the work is done at an Assured institution.

In addition, while the approaches of funding and regulatory agencies are complementary, they also differ. The *PHS Policy* invests responsibility for animals in the entity that receives PHS funding, known in grant parlance as the "awardee" or "grantee" institution. Accordingly, if there is a concern about a PHS-funded animal activity PHS will likely "follow the money" to determine institutional accountability. Under the AWRs, responsibility generally resides with the institution that houses the animals and with the institution that owns the animals, which may not be the same institution.

PHS may award funds for an activity involving animals only to an entity that has an approved PHS Assurance. When more than one institution is in volved, one of the following four scenarios generally apply:

- An awardee institution and/or a subcontractor or collaborating insti tution can both have PHS Assurances. In this situation, two assured entities are responsible for determining which IACUC will review the research and under which institutional program the research will be covered. While PHS and USDA do not require dual review by both awardee and subcontractor IACUCs (i.e., only one of the assured IACUCs must review and approve the research), OLAW recommends the IACUC of the awardee institution have a mechanism for obtaining a copy of the performance site's IACUC approval. Many times however, both IACUCs will elect to review the research as evidence of shared responsibility and to ensure the research will be conducted in compliance with their own institutional policies and practices in addi tion to meeting the federal laws and regulations.
- If the awardee institution has a PHS Assurance, but the subcontractor or collaborating institution does not, the latter may be required to obtain one. The grant or contract may not be awarded until the Assurance is solicited by OLAW, submitted by the subcontractor, and approved by OLAW. The subcontractor must also submit the date of IACUC review.
- If the awardee institution has a PHS Assurance but the subcontractor or collaborating institution does not, the latter may be brought under the awardee institution's Assurance by an amendment to the Applica bility section of that Assurance. The IO signing the Assurance would then be responsible for the facilities and activities of the subcontrac tor, and the IACUC would be required to include relevant aspects of the subcontractor's facility and program in its semi-annual program review. The subcontractor, in turn, would be required to recognize the authority of the IO and the IACUC of the awardee institution. Most awardee institutions do not elect this option.

- Another possible collaboration, that may or may not involve subcontracting, occurs if an awardee institution does not have an animal program or facility and is therefore not assured, but the investigator will use the facilities of an assured institution. Under these circumstances OLAW requires an "Interinstitutional Agreement Assurance" whereby both IOs agree that the project will be conducted in accordance with the assured institution's Assurance and the investigator will abide by the determinations of the assured institution's IACUC. The effect of such an agreement is to extend the IACUC's oversight to include the particular project, and to meet the *PHS Policy* requirement that the grantee institution be assured.

References

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A.4. Training for Members

For the IACUC to discharge its responsibilities a program of education and training is essential. A well-defined and implemented program, while prima rily directed to the IACUC member, would also be of value to researchers, administrators and others with responsibilities associated with research involving animals.

It is the responsibility of the institution to provide suitable orientation, appropriate materials, adequate resources and training to enable IACUC members to carry out their duties consistent with the *Guide*, the *PHS Policy* and the Animal Welfare Regulations (AWRs). It is important to provide the tools necessary to assist members in understanding and evaluating issues that are brought before them. Appropriate training depends on the size, scope and needs of the research facility, but must incorporate the federal mandates of the IACUC.

Local institutional policies and procedures need to be a part of the training and education program. Frequently, new members find it confusing to understand the differences between the federal policies and requirements and institutional policies and procedures. It is useful to provide an insti tutional policy manual as well as the Web sites for pertinent federal rules and regulations.

Although the plan for training and education can take many different forms, a recommended syllabus with suggested topics for the orientation module and the continuing education module follows.

In addition, ARENA sponsors a basic one-day training course for new IACUC members and persons with IACUC responsibilities – ARENA IACUC 101 – and ARENA IACUC 101 "On the Road." To learn more about this training program, contact the ARENA office at (617) 423-4112 or OLAW at (301) 496-7163, or visit the ARENA or OLAW Web sites (see Appendix A).

Program of Education and Training for New IACUC Members

Orientation Module

(Suggested time – approximately 2 hours)

Suggested Topics

Objectives

1. To introduce members to the role of the IACUC and its evolution-
2. To provide the basic information necessary for IACUC members to-discharge their responsibilities-
3. To provide a forum for response to, and discussion of, members'-concerns and questions-

Conducted by

The IACUC staff, the IACUC Chair or designee, veterinary staff, or consult--ants. Training can be provided by one or more of these individuals.-

Syllabus

1. The IACUC — its evolution and responsibilities-
   - 1.1. Genesis and chronology-
   - 1.2. U.S. Government Principles-
   - 1.3. Benefits and pitfalls of IACUCs-
   - 4.4. Criteria for membership-
   - 4.5. Authority of the IACUC-
   - 4.6. Unique role of the IACUC within the organization-
2. Operation and procedures-
   - 2.1 Proposal (protocol) submission-
   - 2.2 Proposal review-
     - 2.2.1 Process-
     - 2.2.2 IACUC review criteria-
     - 2.2.3 Review by quorum-
     - 2.2.4 Review by designated reviewers-
     - 2.2.5 Post-meeting process-
   - 2.3 Monitoring of approved protocols-
     - 2.3.1 Periodic review (continuing review)-

2.3.2 Protocol changes (amendments)

2.4 Records

2.5 Semiannual reviews

2.5.1 Animal care and use program

2.5.2 Institutional animal facilities

2.6. Handling animal welfare concerns

2.7. Roles, responsibilities, relationships

2.7.1. IACUC

2.7.2. IACUC Program office

2.7.3. Veterinarian

2.7.4. Animal Care Program (e.g., Department of Comparative Medicine or Laboratory Animal Resources)

2.7.5. Institutional Official (IO)

2.7.6. Office of Laboratory Animal Welfare (OLAW), NIH

2.7.7. Animal and Plant Health Inspection Service (APHIS), USDA

2.7.8. Project sponsor/grantor

2.7.9. Community

Suggested Resource Materials

- *Public Health Service Policy on Humane Care and Use of Laboratory Animals.* NIH. Reprinted 2000.
- Health Research Extension Act, P.L.99-158.
- Animal Welfare Act – P.L. 89-544 as amended by P.L. 94-279, P.L. 99-198, P.L. 91-579 and P.L. 101-624.
- Animal Welfare Regulations. 9 CFR.
- *Institutional Administrator's Guide for Animal Care and Use.* NIH. 1988.
- *Guide for the Care and Use of Laboratory Animals.* NRC. 1996.
- *ARENA/OLAW Institutional Animal Care and Use Committee Guidebook.* 2002.
- Institutional IACUC Policies and Procedures Manual.

For additional suggestions see the Core Module in the National Research Council's *Education and Training in the Care and Use of Laboratory Animals – A Guide for Developing Institutional Programs*, pages 11 through 15.

Program of Education and Training for IACUC Members

Recommended Continuing Education Module

(Varying amounts of time – can be incorporated in each IACUC meeting and/or designated or *ad hoc* meetings)

Suggested Topics

Objectives

1. To increase members' knowledge, understanding and awareness
2. To keep members current on:
   - 2.1 Laws (federal, state, local)
   - 2.2 Regulations (proposed, promulgated/issued)
   - 2.3 Directives
   - 2.4 Guidelines
   - 2.5 Developments and trends
   - 2.6 Institutional policies
3. To address issues, concerns and questions raised by IACUC mem bers, institutional staff, and the community.

Conducted by

The IACUC Staff, the Chair or designee, veterinary staff, or consultants.

Syllabus

Agenda based on:

1. Questions and concerns brought to the attention of the IACUC
1. Official directives
3. Publications
4. Notices of, and reports from, conferences, seminars, etc.
5. Animal facility staff and/or veterinarian's observations and recom mendations
6. Facility inspections and program evaluations
7. Problem situations

Suggested Resources: See Appendix A.

A.5. Legal Concerns

The functions and activities of IACUCs are based on two federal laws: the Health Research Extension Act of 1985 (P.L.99-158) (HREA) and the 1985 amendments to the Animal Welfare Act (AWA), the Improved Stan dards for Laboratory Animals Act of 1985 (P.L. 99-198). In addition, other federal rules may pertain to IACUCs, such as the Occupational Safety and Health Administration (OSHA), Food and Drug Administration (FDA), and Good Laboratory Practice (GLP) regulations, and the Endangered Species Act (ESA). Committee members need to be aware of the legal obligations of their institutions, the responsibilities of the IACUC in relation to these institutional commitments, and the regulatory requirements for which they may be personally accountable.

Many states have statutes and regulations in place relevant to laboratory animals as well. Institutional Officials (IOs) and IACUC administrators should ensure that procedures are in place to enable IACUCs to be cognizant of and compliant with state and local laws and regulations that may affect their institution's animal care and use program. A useful reference is the National Association for Biomedical Research (NABR) publication, *State Laws Concerning the Use of Animals in Research.*

Institutions are responsible for informing IACUC members of their re sponsibilities, providing training relative to their role on the IACUC, and ensuring that members have the information necessary to fulfill their duties as IACUC members:

- IACUC members should be provided with documents such as the PHS Assurance with the Office of Laboratory Animal Welfare (OLAW), NIH, the *PHS Policy*, the *Guide* and the Animal Welfare Regulations (AWRs). Committee members should be aware of their institutional registration with the U.S. Department of Agriculture (USDA) and reports of inspec tions and other interactions with Animal and Plant Health Inspection Service (APHIS).
- IACUC members should be free to request through the IACUC Chair or IO, guidance from the institution's legal counsel with regard to Committee actions.
- IACUC members should be provided with information regarding their obligation to treat material as privileged or confidential, especially prior

to final Committee action, or agency funding. In the case of trade secrets or patent applications, such information is protected by law (7USC 2157, Section 27).

- IACUC members should understand that their signatures are legally binding on official IACUC reports such as the six-month program review and facilities inspection report.

Liability

Under *PHS Policy*, the primary responsibility for meeting applicable federal and state rules rests with the research facility or PHS awardee institution. The IO is the individual held responsible on behalf of the research facility for ensuring compliance. Failure to comply with *PHS Policy* could result in OLAW's withdrawal of approval of the institution's Animal Welfare Assurance, thereby making the institution ineligible to receive funds for activities involving animals.

Under applicable statutory provisions (7 U.S.C. Section 2149), the USDA has the authority to order a facility to cease and desist, and to impose a fine for noncompliance with the AWRs and AWA. The AWA provides for penalties of up to $2,500 per count and one year in prison, or both for violations of the AWRs.

Freedom of Information

The Freedom of Information Act (FOIA), 5.U.S.C.552, provides individ uals with a right to access to records in the possession of the federal government. The government may withhold information pursuant to the nine exemptions and three exclusions contained in the Act.

The Electronic FOIA Amendments of 1996 (Public Law 104-231) amended the law in a number of ways that primarily address information systems, use of telecommunications, and electronic reading rooms. Most federal agencies provide guidelines for submitting FOIA requests through their agency Web sites.

Information about federally conducted or supported research projects, PHS Assurance documents, USDA annual reports filed by research facilities, and inspection reports of USDA, Environmental Protection Agency (EPA) and FDA, are generally available to the public under FOIA.

Many states have public records laws and/or open meetings acts, known as “sunshine” laws, which may permit public access to information reviewed and generated by the IACUC, and public attendance at IACUC meetings. However, even in some “sunshine” law states, the IACUC, because it serves in an advisory capacity to the IO, may hold closed sessions. IACUC mem bers need to be aware of specific state laws regarding these issues and should always seek legal counsel if necessary to ensure compliance with applicable laws.

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B. Oversight of the Animal Care and Use Program

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B.1. Program and Facility Review

The *PHS Policy* and Animal Welfare Regulations (AWRs) stipulate that the IACUC must review the program for humane care and use of animals at least once every six months, using the *Guide* as the basis for evaluation for the PHS *Policy* and title 9, chapter I, subchapter A-Animal Welfare for the U.S. Department of Agriculture (USDA). Federal requirements also state that the IACUC must inspect all institutional animal facilities at least once every six months.

Benefits of the Reviews

- Reviews provide an ongoing mechanism for ensuring that the insti tution maintains compliance with applicable animal care and use policies, guidelines and laws.
- Reviews serve as an opportunity for constructive interaction and education for the animal care personnel, research staff and IACUC members.
- Reviews can help an institution prepare for subsequent visits by outside evaluators, such as USDA inspectors, Office of Laboratory Animal Welfare (OLAW) staff and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) site visitors.

A summary of recurring IACUC issues related to semiannual program review and facility inspection identified by AAALAC during site visits is provided in Appendix C.

Resources

OLAW has developed a sample format for the program review and facility inspection that may be modified to meet the institution's needs (see the OLAW Web site). The Table of Contents of the *Guide* or an institution's AAALAC Program Description can also serve as an outline for the semi-annual evaluation.

Conducting Program Evaluations

Key aspects of an animal care and use program that should be emphasized in the semiannual evaluation include:

- IACUC membership, functions and procedures, including protocol review (e.g., using page 10 of the *Guide* as a template, and *PHS Policy* IV.B. and C.);
- facility inspection process;
- provisions for reviewing and investigating concerns regarding animal care and use;
- recordkeeping practices;
- methods employed to meet reporting requirements;
- occupational health and safety program;
- veterinary medical care program; and
- personnel qualifications and training.

Specific procedures to accomplish program evaluation may include pre sentations by appropriate individuals (e.g., the veterinarian, an occupational health and safety representative, etc.) and review of written institutional policies such as standard operating procedures, guidelines on use of anes thetics and analgesics, and euthanasia procedures. Verifying conformance with the USDA Animal Care Policies (1999 *et seq*.) during the semiannual program review will help ensure that current practices are consistent with USDA regulatory interpretations.

Facility Review

All animal housing facilities must be inspected in the semiannual review, including:

- satellite facilities (containment areas outside the central/core animal facility where animals are housed for more than 24 hours *(PHS Policy)*,
- areas in which surgical manipulations are performed *(PHS Policy)*,
- animal study areas (locations where USDA-covered species are held for more than 12 hours) (AWRs), and
- holding facilities (AWRs).

Laboratories in which routine procedures, such as immunization, dosing, and weighing,

are conducted may be evaluated by other means such as random inspec tions. However, the institution, through its IACUC, is still responsible for all animal-related activities regardless of where animals are maintained or the duration of the housing. The IACUC must have reasonable access to these areas for the purpose of verifying that activities involving animals are being conducted in accordance with the proposal approved by the IACUC.

Staffing and Scheduling the Facility Inspections

The IACUC must conduct inspections of facilities at least once every six months. This may be accomplished by assigning specific facilities to sub-committees, which must consist of at least two IACUC members (AWRs). No IACUC member should be excluded should she or he wish to partici pate in an inspection. *Ad hoc* consultants may be used although the IACUC remains responsible for the evaluations and reports. The inspection team should have a working knowledge of the *Guide* and AWRs in order to fully evaluate the facilities that are being inspected. Section B.2. of this Guide-book also provides general guidance in this regard.

Categories to be Inspected

It is helpful for the inspection team to use a list of categories such as:

- sanitation,
- food and water provisions,
- animal identification,
- waste disposal,
- animal health records,
- controlled and/or expired drugs,
- environmental control,-
- occupational health and safety concerns,-
- staff training,
- knowledge of applicable rules and regulations, and
- security.

The IACUC may determine whether the supervisory personnel of various facilities should be notified of the date and time of an inspection. Advance notification allows individuals to be available to answer questions; an unexpected visit may show the facility during usual operations but also may result in a visit having to be rescheduled if key individuals are not available.

Performing Inspections

Adherence to the following recommendations will assist the IACUC in performing inspections:

- An updated list of all facilities to be inspected should be maintained by the IACUC.
- All proposals submitted to the IACUC should specify locations where animal procedures will be performed.
- It is helpful to maintain a list of all facilities including room number, function of the room, species, and deficiencies identified during the previous inspection.
- For satellite areas a contact person is useful.
- For facilities with multiple rooms a floor plan can assist the inspectors.
- If a subcommittee is performing the inspection, a blend of Committee members who last inspected the area with members who did not can bring both continuity and a fresh perspective to the inspection process.
- Notes should be taken throughout the visit to assist in preparation of the final report.
- Apparent deficiencies should be discussed with the person in charge of the facility to ensure that the team's perception of the situation is accurate. In some cases an apparent deviation will be due to the experiment in progress, e.g., withholding of food prior to surgery.
- Use of a checklist provides consistency and helps document that all categories were assessed.

While the inspection of each facility must occur semiannually, there is no regulatory requirement that all facilities at an institution must be inspected at the same time (e.g., during the same month). Therefore, IACUCs at large institutions can stagger these inspections throughout the year, as long as each animal area is inspected at least every six months.

Use of AAALAC Activities as Program Evaluation

Provisions permitting use of *ad hoc* consultants may be invoked by IACUCs to make use of either of the two AAALAC assessment programs (Program Status Evaluation or Accreditation), or pre-assessment preparation activities, to meet the requirements for an IACUC semiannual program evaluation and subsequent report. In order to utilize one of these AAALAC related activities as a semiannual evaluation, the IACUC must ensure that the report complies with IV.B.3. of the *PHS Policy*, and officially endorse the report and submit it to the IO. If an institution is covered by the AWRs, the report must comply with §2.31(c) of the AWRs, at least two IACUC members must participate, no member wishing to participate may be excluded, and the report must be signed by a majority of the IACUC mem bers and include any minority views.

Documentation

A written report of the semiannual program review and facility inspection must be prepared. The AWRs require the report to be signed by a majority of the IACUC. The report must describe the institution's adherence to the AWRs, the *PHS Policy*, and the *Guide*, and identify specifically any devia tions from these documents.

Any deficiencies identified in these reviews must be designated by the IACUC as minor or significant. A significant deficiency is defined as a situation that is or may be a threat to animal health or safety. The IACUC, through the IO, must promptly report to OLAW any serious or continuing noncompliance with the *PHS Policy* or any serious deviation from the provisions of the *Guide*. For both categories of deficiencies, a reasonable and specific plan and schedule with dates for correction must be included in the final report. All individuals to be involved in the corrections should be consulted to ensure that the plan is realistic. If the institution is unable to meet the plan, the IACUC, through the IO, must inform Animal and Plant Health Inspection Service (APHIS) officials within fifteen business days of the lapsed deadline (AWRs). If the activity is federally funded, the relevant funding agency also must be informed.

The report should indicate whether or not any minority views were filed, and minority views must be included in the final document. A copy of the report is sent to the IO and must be kept on file for a minimum of three years. It is often useful for the report to be delivered in person in order to emphasize the findings and plans for action. The institution must notify OLAW of the dates of the semiannual program evaluations and facility inspections in an annual report.

References

OPRR. 1991. The Public Health Service Responds to Commonly Asked Questions. *ILAR News* 33(4): 68-70.

Potkay, S., N. Garnett, J. Miller, C. Pond, and D. Doyle. 1997. Frequently Asked Questions about the Public Health Service Policy on Humane Care and Use of Labo ratory Animals. *Contemporary Topics* 36(2): 47-50.

NIH Guide for Grants and Contracts. December 12, 1999. Notice OD-00-007.

B.2. Animal Environment, Housing and Management

This section provides an overview of the IACUC's role regarding animal environment, housing and management. The *Guide* provides recommendations that are written in general terms and require the application of sound professional judgment (i.e., current best practices). The use of performance standards, or an outcome approach, will direct decisions to optimizing animal well-being while providing a refined animal model for the researcher. Variances from *Guide* recommendations in animal care and husbandry should be based on clear scientific justification, or rationale for an alternative approach to accomplish a performance based *Guide* standard, and must be approved by the IACUC.

B.2.a. General

The *Guide* states:

> Proper housing and management of animal facilities are essential to animal well-being, to the quality of research data and teaching or testing programs in which animals are used, and to the health and safety of personnel. A good management program provides the environment, housing, and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations that can affect research results. Specific operating practices depend on many factors that are peculiar to individual institutions and situations. Well-trained and motivated personnel can often ensure high quality animal care, even in institutions with less than optimal physical plants or equipment.

Animals should be housed in a manner that facilitates the expression of species-typical behavior and minimizes stress-induced behaviors. For social species, housing systems should be designed to accommodate pair or group housing of animals. The *Guide* places responsibility with the IACUC for the review and approval of housing systems; it further recommends follow-up objective evaluations to ensure the housing system is appropriate for the health and well-being of the species and consistent with research objectives.

B.2.b. Animal Environment

Housing

Adequate animal husbandry practices and health maintenance are facili tated by well-constructed and maintained caging or housing systems.

Cages should:

- allow for conspecific social interaction within or between enclosures, adequate ventilation, and observation of animals with minimal dis turbance of them;
- provide a safe and secure environment that permits the normal physi ologic and behavioral needs of the animals to be expressed;
- enable ready access to food and water receptacles and be constructed of materials that balance the needs of the animal with sanitation; and
- be constructed with materials that resist corrosion and withstand chip- ping, cracking or rusting.

Unsealed wood may be acceptable for use as perches or other climbing structures, resting areas, or in the construction of perimeter fences, runs and pens, but wooden items need to be replaced periodically because of wear, damage, and to achieve adequate sanitization.

Cage size requirements/recommendations for most common labora tory animal species are provided by the AWRs and the *Guide*. Cage complexities, vertical height of the cage, and the cage design can influence how an animal uses the cage space provided. The cage must provide sufficient space so that, at a minimum, the animal can turn around and express normal postural adjustments. The animal must have sufficient clean and unobstructed space to move and rest in. Use of wire bottom cages is discouraged for rodents, especially on long-term studies or in larger and older animals, as it may cause foot injury. Use of wire bottom cages should be scientifically justified and approved by the IACUC.

Temperature, Ventilation, Illumination and Noise

Environmental factors can have a profound effect on the health and well-being of animals as well as on the outcome of experimental manipula tion. Temperature, humidity, air pressure differential and air exchange rate, illumination level, and noise levels all may affect animal well-being and research results.

The range of daily temperature fluctuations should be kept to a minimum (e.g., ± 2° F) to avoid large demands on the animals' metabolic and behav ioral processes. Relative humidity should also be controlled (e.g., 30% to 70%). In general, an air exchange rate of 10 to 15 changes per hour is considered an acceptable standard.

Light intensity, duration of exposure, wavelength of light, light history of the animal, pigmentation of the animal and other factors should be considered when establishing an illumination level in the animal room.

Because sound exposure can have variable effects on animals, noise gen erators (e.g., human activities, noisy animals, equipment) should be mini mized in animal areas. Environments should be designed to accommodate animals that make noise, rather than resorting to methods of reducing the noise made by animals.

A review of an animal care and use program should include consideration of environmental standards adopted for the facilities with adequate jus tification for deviations, which are reviewed and approved by the IACUC. While environmental control in outdoor facilities is much less stringent, acceptable ranges in temperature for several species are specified in the AWRs. Reliable methods for monitoring environmental control systems should be in place, including an after-hours monitoring and response program. Back-up heating, ventilation, air conditioning, and lighting sys tems are highly desirable.

B.2.c. Husbandry

Animal Identification

It is imperative that research animals be adequately and appropriately identified and that records pertaining to individuals or groups of animals be maintained. A wide range of acceptable identification methods can be employed, including:

- cage cards,
- subcutaneous transponders,
- ear notches and tags,
- collars,
- colored stains, and
- individual animal tattoos.

The use of toe-clipping to identify individual rodents is discouraged; when necessary, it should be rigorously justified for scientific necessity and done only on very young rodents.

Animal records may consist of a cage card or may involve detailed indi vidual animal information, depending principally on the species and research requirements. Cage cards should include:

- source of the animal,
- strain or stock,
- names and locations of responsible investigators,
- pertinent dates, and
- protocol number.

Feeding

All animals should receive food that is:

- palatable,
- free from contamination, and-
- of sufficient quantity and nutritive value to maintain their good health.-

Specific diets should be selected based on the needs of each species, with special consideration of the requirements for Vitamin C by guinea pigs and some species of nonhuman primates. Animals should be fed at least once a day except under conditions of hibernation, veterinary treatment, pre-procedural fasts, or other justified circumstances. In some species and in some circumstances, varying the diet by providing “treats” can improve animal health and well-being. However, caution should be exercised that animals do not forsake eating their nutritionally balanced diet for treats.

It is known that standard commercial dry bulk foods, when stored properly, retain their nutritional value for six months (generally three months for those containing Vitamin C, unless a stabilized form is used).

To help ensure that fresh, uncontaminated food is provided:

- bags should be stored off the floor,
- the milling date should be known (the date or a code is usually stamped on each bag), and
- the oldest stock should be used first.

Small quantities of food may be kept in animal rooms if stored in tightly covered, leak- and vermin-proof containers; these should not be moved from room to room.

Food should be provided in receptacles that are accessible to all animals in a cage or pen and placed so as to minimize contamination. More than one receptacle may be necessary for some socially housed animals. Food receptacles should be easily cleaned and sanitized, and those functions should be performed on a schedule that meets *Guide* and AWR require ments. With limited exceptions, (e.g., neonatal animals or animals with limited mobility) food should not be placed on the bottom of the cage. Although some species may prefer this presentation, it results in waste and contamination of the food.

Watering

Potable drinking water should be available continuously or provided as often as necessary for the health and well-being of the animal, considering the animal's species, age, condition, and any research requirements. Water may be provided in receptacles (e.g., bowls, bottles or via automatic water ing systems). Whatever method is used, care should be taken to ensure that water does not become contaminated and is actually available. Water may be treated or purified to eliminate contaminants; however, some water treatments may cause physiologic changes, alter microflora, or affect ex perimental results. Sipper tubes and automatic watering devices should be checked daily for patency and cleanliness. Animals occasionally need to be trained to use automatic watering devices. Water bottles generally should be replaced and sanitized rather than refilled.

Bedding

Bedding may be used in the housing of a variety of commonly used laboratory animals. Bedding material should be absorbent and free of any substances that might harm the animals or alter research data. Cedar and untreated softwood products can affect an animal's metabolism (e.g., liver enzymes), which may in turn affect immunologic or other physiologic parameters, and can increase the incidence of cancer. Bedding should be stored off the floor.

Animals may be placed directly on bedding material, a common practice with many rodent species, or bedding may be placed under a slat-bottom cage. Bedding should be used in sufficient amounts and changed as often as necessary to keep the animals clean and dry and the animal room

relatively odor free. Care should be taken to keep bedding from contacting water tubes as this may lead to leakage of water into the cage. The frequency of bedding change depends on several factors, including the number of animals, species, type of caging, and type of bedding.

B.2.d. Facility Maintenance

Cleaning and Sanitation

Cleanliness and sanitation are essential to the operation of an animal facility. The *Guide* and AWRs set forth recommended frequencies and methods for cleaning and sanitation of facilities, equipment and accessories. In general, the frequency and methods should ensure that animals are maintained in a clean, dry environment, free from exposure to harmful contamination and excessive animal odors. Cleaning agents that mask animal odors should not be used as a substitute for good sanitation practices. Cleaning equipment such as mops and buckets should not be moved from room to room due to the potential for cross-contamination.

The most efficient and effective method of cleaning and sanitizing cages and accessories (e.g., feeders, water bottles, sipper tubes) is the use of a mechanical washing machine that provides rinse water temperature of at least 82.2ºC (180ºF) for a time adequate to achieve sanitization. Alternatively, portable high pressure spray washing and disinfection may be used. Least efficient and effective is hand washing and disinfection of such equipment. In general, enclosures and accessories (e.g., cage tops) should be sanitized at least every two weeks. Solid bottom cages, water bottles and sipper tubes should usually be sanitized weekly. The supply lines of automatic watering systems should be flushed and disinfected on a regular basis.

Waste Disposal

A research animal facility generates a significant amount of waste that must be removed and disposed of on a regular, frequent basis. Waste containers should be readily accessible throughout the facility and should be leakproof and equipped with tight-fitting lids. Disposal methods, including incineration and removal to land-fill, must conform to federal, state and local requirements. Some jurisdictions consider all soiled animal bedding from a research facility to be “medical waste,” with consequently more stringent disposal requirements.

If waste must be stored while awaiting disposal, the storage area should be outside the animal holding and clean equipment areas. Animal carcasses and tissues require a separate cold storage area and regularly scheduled removal. Hazardous waste, including carcasses of animals exposed to radioactive or biohazardous agents, must be adequately sterilized and/or contained prior to removal and disposal.

Pest Control

The research animal facility is an active place, with frequent movement of personnel, animals, equipment, containers, and food and bedding, creating ideal conditions for the introduction of pests, including arthropods, birds and wild rodents. Pest control programs are complicated by the potential for harm to animals and personnel, as well as interference with research data by many commonly used pesticides. A regularly scheduled, documented pest control and monitoring program should be implemented, which effectively combines elimination of all entry and harborage sites with good waste disposal and personnel training. If traps are used, methods should be humane.

B.2.e. Emergency, Weekend and Holiday Care

Laboratory animals must be observed by qualified personnel every day, including weekends and holidays to ensure their health and well-being, as well as to promote sound research practices. Skilled assistance, including veterinary care, must be readily available at all times. Names and telephone or pager numbers of those assigned these responsibilities should be prominently displayed in the facility. A disaster plan should be part of the overall facility safety plan which takes into account both personnel and animals (see Section B.6.).

B.2.f. Behavioral Management for Laboratory Animals

There are varying requirements for attention to the behavioral management of laboratory animals, depending on the species of animal and the reference document.

The *Guide* provides recommendations for:

- increasing the complexity of the structural environment,
- addressing the social environment of animals, and

- promoting the expression of species-typical activity in a cohesive behavioral management program for all vertebrate species.

The AWRs require that research facilities develop, document and follow a plan for environment enhancement adequate to promote the psychological well-being of nonhuman primates.

The plan must address:

- the social needs of nonhuman primates;
- environmental enrichment of the primary enclosure through provision of cage complexities, manipulanda, varied food items, foraging or task-oriented feeding methods, and safe personnel interaction; and
- special needs of certain classes of primates (e.g., young animals, animals in psychological distress, some individually housed primates, and some great apes).

Exemptions from some or all of the environment enhancement plan for scientific reasons must be documented in the protocol, approved by the IACUC, and re-reviewed not less than annually. The veterinarian may exempt individual primates from the plan. All exemptions must comply with the AWRs, Part 3, Subpart D, §3.81(e).

The AWRs further require that research facilities develop, document and follow a plan for providing dogs with the opportunity for exercise.

This plan must:

- stipulate specific exercise opportunities for dogs housed individ ually as well as dogs housed in groups based on cage/pen/run floor space; and
- identify the methods, frequency and duration of the opportunity for exercise.

Provisions for exemptions from exercise may be made by the veterinarian in certain instances and the IACUC in others, and must be in accordance with the AWRs, Part 3, Subpart A, §3.8 (d).

Oversight

The IACUC should provide oversight of the behavioral management program in a manner similar to its oversight of other husbandry components of the animal care and use program, and evaluate program outcomes during semiannual reviews.

To adequately discharge this responsibility, the IACUC should have access to training or other orientation materials that will assist the IACUC members in evaluating the adequacy of the program (Bayne 2000). Formal, written plans for nonhuman primate environmental enrichment and canine exercise, established to provide a framework to the behavioral management program, should be approved by the IACUC and reviewed periodically. The committee should identify who is responsible for keeping the plan current and implementing the plan (e.g., an enrichment committee, the AV, etc.). The NRC publication, *The Psychological Well-Being of Nonhuman Primates* (1998), adopted by the Association for Assessment and Accreditation of Laboratory Animal Care International as a Reference Resource for accredited institutions, advises a team approach to development and oversight of the behavioral management program to include investigators, veterinarians and the IACUC.

References

NRC. 1998. *The Psychological Well-Being of Nonhuman Primates*. National Academy Press, Washington, DC.

Bayne, K. 2000. Laboratory animal enrichment. In: *The IACUC Handbook* (J. Silverman, M.A. Suckow and S. Murthy, eds.). CRC Press. New York.

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B.3. Role of the Veterinarian

Adequate veterinary medical care is an essential component of an animal care and use program and is required by the *PHS Policy* and Animal Welfare Regulations (AWRs). Institutions with smaller programs may opt for a part-time consulting veterinarian; the veterinarian's overall responsibilities remain the same in all cases.

It is the institution's responsibility to support ongoing improvements in the animal care and use program through the development and implementation of procedures and policies (e.g., IACUC guidelines) that enhance the health of the animals (ACLAM 1996). Clear provisions should be made to give the veterinarian appropriate authority to execute a program of adequate veterinary care, including access to all animals.

Qualifications

The veterinarian participating in a laboratory animal care and use program must have training or experience in laboratory animal science and medicine, or in care of the species of animals maintained by the institution. Veterinarians can demonstrate the breadth and relevance of their expertise by achieving certification as a Diplomate of the American College of Laboratory Animal Medicine (ACLAM) or through other work experience and career accomplishments. Specialty training programs are available at a number of government, academic and commercial institutions to prepare graduate veterinarians to pursue ACLAM certification. Alternatively, veterinarians may qualify for ACLAM certification by working in a laboratory animal resource program and meeting other specified criteria.

The veterinarian providing support to a laboratory animal care and use program must meet applicable state veterinary practice acts, inclusive of licensure requirements, particularly in the discharging of certain official duties, such as signing interstate health certificates or verifying rabies vaccination or tuberculosis status of animals.

Responsibilities

The chief responsibility of the veterinarian is to provide for the health and welfare of animals. The Report of the American College of Laboratory Animal Medicine on Adequate Veterinary Care in Research, Testing and Teaching provides a detailed description of adequate veterinary care. The details of a veterinary care program will depend on the species of animals employed and the particulars of the laboratory animal use, but in all cases the program and care provided must comply with standard veterinary practice.

The introduction of new animals is an important aspect of the veterinary care program with such considerations as stabilization periods, isolation and quarantine. Animals should be obtained only from licensed dealers or other legitimate sources. One of the prime mechanisms for ensuring high quality laboratory animals is to purchase them from commercial vendors who produce specific pathogen-free stock and maintain rigorous animal health monitoring programs to ensure quality. Generally, most animals are purpose-bred for laboratory use. Certain states have passed legislation requiring that cats and dogs used in research be bred specifically for that purpose.

Random source or wild caught animals are not bred by the supplier (known as Class B dealers), but are obtained from a variety of sources including pounds, shelters or farms that may not conform to the same standards of animal husbandry and health as the research facility. Before their use, clinical evaluation and conditioning of these animals are required to ensure that they are not carrying diseases that can be transmitted to other animals, including humans, or do not introduce uncontrolled variables into research. Research facilities that obtain dogs and cats from sources other than dealers, exhibitors, and exempt persons must hold the animals for five full days, not including the day of acquisition, after acquiring the animal, excluding time in transit, before they may be used by the facility (9 CFR §2.38(j)). Research facilities must comply with the identification of animals requirements set forth in §2.38(g) during this period.

Although selection of high-quality laboratory animals has reduced the prevalence of infectious diseases in research animal colonies, preventive medicine programs, conducted under the guidance of the veterinarian, continue to be important for maintenance of healthy animals.

These programs include:

- immunization against infectious pathogens;
- surveillance of colonies for specific infectious microbial agents;
- disease prophylaxis utilizing pharmaceutical agents;
- isolation and quarantine of incoming animals; and
- separate housing of animals according to species, source or different background microbial floras.

While preventive medicine programs are successful in reducing the incidence of disease, illness and injury may still occur in laboratory animal colonies. The veterinarian is responsible for monitoring animal health, providing adequate diagnostic support through clinical assessments, laboratory diagnosis and necropsy when required, and treating animals when illness or injury necessitates veterinary medical care. Using a documented process, the veterinarian may delegate responsibility for care to trained technical staff but must always be available to provide rapid diagnosis and treatment.

The AWRs stipulate that the veterinarian attend to not only the physical health of animals, but also the psychological well-being of nonhuman primates, and exercise for dogs. The plan for canine exercise must be approved by the Attending Veterinarian (AV) before it can be implemented. Additionally, animals that are exempted from either the canine exercise plan or the nonhuman primate psychological well-being enhancement plan for health, condition or behavioral reasons must be documented by the AV and, unless a permanent condition exists, reviewed by the AV every 30 days.

Specific areas requiring the veterinarian's attention and guidance are:

- the selection and utilization of suitable anesthetic and analgesic agents and methods of euthanasia;
- appropriate selection of species for research projects; and
- proper performance of surgical procedures and adequate pre-operative, surgical, and post-operative care.

The veterinarian should discuss with investigators the design and implementation of study proposals and may provide written guidelines dealing with these and other issues. Collegial exchanges between the investigator and the veterinarian before the submission of a proposal to the IACUC may address many of the Committee's concerns and expedite the review process.

At some institutions, the veterinarian or his/her staff may participate directly as a co-investigator in activities involving animals by providing clinical, surgical or other scientific or technical expertise to the study. Veterinarians sometimes also serve as principal investigators with responsibility for their own research and training programs. In such situations, the IACUC has the same obligation to review and approve the proposed activities as it would for any other investigator. When the veterinarian is personally involved in a research project, he/she must excuse himself/herself from the IACUC review and vote on the project. IACUCs may consider utilizing a consulting veterinarian to assist in review of such projects.

The AWRs require institutions utilizing animals in research and teaching to provide training and instruction to personnel on humane methods of animal maintenance and experimentation. The veterinarian and the animal resource program staff, in conjunction with the IACUC, are usually responsible for providing such training.

The *PHS Policy* requires institutional occupational health and safety programs to ensure that personnel who have laboratory animal contact are included in a risk assessment process and action plan that addresses workplace safety through appropriate educational, industrial hygiene and medical interventions. The veterinarian, in cooperation with appropriate health and safety officials at the institution, is often responsible for the implementation and execution of aspects of the program concerned with animal health and safety issues.

The Veterinarian and the IACUC

The veterinarian occupies an essential position on the IACUC with specific defined functions according to the *PHS Policy* and AWRs. Institutions employing several veterinarians may appoint more than one to the IACUC, but all institutions regardless of size must have at least one veterinarian with direct or delegated program authority and responsibility as a member of the IACUC. A strong veterinary presence on the IACUC has proven beneficial in

many institutions. However, institutions should also be aware that the domination of IACUC activities by the veterinarian(s) may foster or be symptomatic of the disengagement of other members, thereby resulting in a less cohesive and effective IACUC.

The veterinarian should keep abreast of current literature on comparative medicine and laboratory animal science. The knowledge gained often leads to suggestions for alternative techniques, models or species that may enhance animal well-being, augment the study design and help ensure the completion of the proposed study.

Reference

American College of Laboratory Animal Medicine. 1996. Report of the American College of Laboratory Animal Medicine on Adequate Veterinary Care in Research, Testing and Teaching.

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B.4. Occupational Health and Safety

The health and safety of individuals working in animal care and use programs is an area of institutional concern requiring commitment from the senior officials of the institution. The goal of the occupational health and safety program (OHSP) is to prevent occupational injury and illness by avoiding, controlling or eliminating hazards in the workplace. The emphasis of such a program is the prevention of illness and injury, but it also includes provisions for early diagnosis and treatment when necessary.

The IACUC's Responsibility for Occupational Health and Safety

The *PHS Policy* places responsibility for ensuring a safe working environment for personnel involved in the animal care and use program with the institution. An effective OHSP interdigitates with many separate institutional components including animal care and use, research, environmental health and safety, occupational health, and administration and management. A natural point of convergence for these functionally distinct institutional elements at many institutions is the IACUC. Assurance of a safe working environment is one of the topics the IACUC should consider in each animal use proposal and as part of the semiannual program evaluation. It is generally necessary to involve health and safety specialists in the design and implementation of the IACUC review guidelines.

Role of the IACUC in the Occupational Health and Safety Program

Procedures should be developed for conducting a health and safety review of research activities that present hazards. These procedures should be incorporated into the IACUC protocol review process. Procedures to identify and address non-experimental hazards (e.g., during semiannual facility inspections and program reviews) should also be implemented. Communication and other procedural links between the IACUC and the environmental health and safety professional or office should be established, maintained and documented. In some institutions, IACUCs defer review of OHSP to an office of health and safety review.

The IACUC also has a role in ensuring that personnel comply with health and safety requirements (e.g., ensuring personnel have received appropriate training, evaluating compliance with standard operating procedures or institutional policy during semiannual facility inspections, etc.).

Elements of an Occupational Health and Safety Program

An effective program design requires input from health and safety specialists and will include the following elements:

- administrative procedures,
- facility design and operation,
- risk assessment,
- exposure control,
- education and training,
- occupational health-care services,
- personal protective equipment,
- equipment performance,
- information management,
- emergency procedures, and
- program evaluation.

The details of each element will be dictated by the extent and nature of employees' exposure and the type of animal use program.

Personnel Participation in the Occupational Health and Safety Program

A wide range of personnel (e.g., animal care staff, investigators, technical staff, students, volunteers, engineers, housekeepers, security officers, and maintenance personnel who care for or use animals, their tissues or fluids, or who may be exposed to them as a consequence of their job) should be provided the opportunity to participate in the OHSP.

The extent and level of participation of personnel in the OHSP should be based on risk assessment, including:

- hazards posed by the animals and materials used;
- exposure intensity, duration, and frequency;

- susceptibility of personnel; and
- history of occupational illness and injury in the workplace.

Health and safety specialists should be involved in the assessment of risks associated with hazardous activities.

Education and Training

There are ethical and legal requirements to inform individuals of health risks that affect them and appropriate precautions. The objectives of an institution's OHSP can be achieved only if employees are appropriately trained to understand the hazards associated with their work area and job duties, and how those risks are mitigated through institutional policies, engineering controls, work practices, and personal protective equipment.

Training should include information about:

- zoonoses,
- chemical safety,
- microbiologic and physical hazards (e.g., allergens, radiation),
- hazards associated with experimental procedures,
- handling of waste materials, and
- personal hygiene.

Proficiency in work assignments through education and training will also contribute to a safer work environment. Training should be a continuous process, and records of OHSP training of personnel should be maintained.

Preventive Medicine and Provision of Medical Care

The principal means of preventing occupationally acquired illness or injury is by controlling or eliminating hazards. The efficacy of the prevention program will depend on the institution's resource allocation to hazard control and the cooperation or compliance of personnel who are potentially at risk. The quality of the preventive medicine program can also be increased if its development and implementation involves input from trained health professionals.

In addition to established mechanisms for reporting and treating accidents and injuries, the institution should have access to medical expertise in zoonotic diseases and other health risks associated with laboratory animal care. Good communication with medical staff will also facilitate better management of the health of animal care personnel and minimize repeat injuries and infections.

Specific Medical Concerns for Individuals Working in the Animal Research Environment

The complexity of the animal research environment creates numerous classes of hazards.

Physical hazards include:

- animal bites, scratches, and kicks;
- sharps;
- flammable materials;
- high pressure containers and equipment;
- low or single color lighting in animal rooms resulting in poor visibility;
- electric hazards, particularly in areas of water usage;
- ultraviolet and ionizing radiation;
- lasers used in surgical areas;
- inadequate housekeeping practices;
- ergonomic demands;
- machinery; and
- noise.

Chemical hazards result from their flammable, corrosive, reactive, explosive or toxic properties. Burns and irritation of the skin are the most common chemical injuries related to animal care and use.

Allergic reactions to animals, occasionally resulting in the development of occupation-related asthma, are among the most common conditions that adversely affect the health of personnel in the animal research environment. Estimates of the prevalence of allergies in animal care workers range from 10% to 44%. Preplacement screening evaluations, attention to facility design, work practices, and the use of personal protective equipment can

reduce the potential development of laboratory animal allergy and possibly alter its severity.

Infectious diseases also pose a significant risk depending on the species and health status of animals involved and the level of exposure to them by animal care personnel.

Infectious diseases to which animal care personnel may be exposed include:

- viral infections, such as contagious ecthyma, the hepatitides, and *Cercopithecine herpes virus* 1 (Herpes B);
- rickettisal diseases, such as Q fever and cat scratch fever;
- bacterial diseases, such as tuberculosis, salmonellosis, and shigellosis;
- protozoal diseases, such as toxoplasmosis, giardiasis, and cryptosporidiosis; and
- fungal diseases, such as dermatomycosis.

In addition to infections acquired from live animals, animal tissues and excreta can serve as sources of zoonoses. Careful monitoring and quarantine of any animals with potential viral or bacterial infections or parasitic infestations are crucial components of any animal care and use program. It is important to immunize animal care personnel against tetanus. Routine tuberculosis testing is essential and measles vaccination may also be appropriate for workers exposed to nonhuman primates.

Common Occupational Health and Safety Program-wide Pitfalls*

- Instead of being based on hazard identification and risk assessment, the program identifies personnel risk based on animal contact time or frequency.
- There is inadequate training on occupational health and safety topics (e.g., zoonoses, allergies).
- Not all personnel at risk (e.g., students, visiting scientists) are offered inclusion in the program.
- Hazard identification covers experimental hazards, but does not address hazards intrinsic to animal care and use.
- There is inadequate linkage between the IACUC and the institutional safety committee(s).

**From data collected by AAALAC International.*

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B.5. Personnel Training and Education

All staff working with laboratory animals must be qualified to do so in order to ensure the humane treatment of animals. Training is a classic performance standard where the emphasis is on the outcome (i.e., all personnel qualified to do their jobs). Although the *PHS Policy* and Animal Welfare Regulations (AWRs) do not specify a particular program or the frequency with which a program should be offered, the requirement for competence is mandatory.

The AWRs, in Sec. 2.32 (a) and (b), specify:

> It shall be the responsibility of the research facility to ensure that all scientists, research technicians, animal technicians, and other personnel involved in animal care, treatment, and use are qualified to perform their duties. This responsibility shall be fulfilled in part through the provision of training and instruction to those personnel. Training and instruction shall be made available, and the qualifications of personnel reviewed, with sufficient frequency to fulfill the research facility's responsibilities….

The *PHS Policy*, Section IV.C.1.f. places responsibility specifically with the IACUC to ensure that personnel conducting procedures on research animals are appropriately qualified and trained in those procedures.

Personnel training in the care and use of research animals is an important aspect of the alternatives concept (replacement, reduction and refinement) described in Section C.2.a. Training in the recognition and alleviation of animal pain, distress, and abnormalities addresses refinement. Similarly, training in the conduct of animal procedures prepares staff to work without causing unnecessary harm to the animal. Technical proficiency also invokes reduction by avoiding wasted animal lives through failed procedures.

Personnel training should be seen as one of the pillars supporting the animal research program. Training of staff is essential for safeguarding the quality of the animals as a tool of research or testing. A lack of training may

result in inadequate husbandry and poor peri-procedural care, which can undermine the physiological status of the animal thereby potentially impairing the integrity of research results.

Who Should Receive Training?

All staff should receive training if they interact directly with or work in the vicinity of animals. Training made available for each type of staff should be specific to the animal species involved and to the kind of procedures to be performed or animal-related interactions.

For training purposes, staff can be grouped as:

- researchers,
- animal care technicians, and
- other (e.g., maintenance or support staff).

In some institutions, staff may not be clearly divisible into these groups if job responsibilities are more diversified than this classification suggests. For example, facility staff such as animal health technicians may have job functions that include both animal care and research procedures.

Training should also be made available to temporary staff, such as students and visiting scientists. These groups may be difficult to intercept for training unless there is a way to identify them.

Development of a Training Program

A training program should meet the needs of each type of staff, as described above, who work with or around laboratory animals. There are many training resources and methodologies that can be used in the development of a training program: courses, seminars, one-on-one training, conferences, computer-based media and videotapes. When appropriate for the job responsibilities, technicians should be encouraged to pursue certification by professional associations, such as technician certification by the American Association for Laboratory Animal Science and the Academy of Surgical Research.

All staff should have exposure through training to regulatory requirements for animal welfare and occupational health and safety considerations. Staff who work directly with animals should have training that supports the humane care and use of animals in the course of day-to-day procedures.

The AWRs, in Sec. 2.32 (c), require that training and instruction of personnel must include guidance in at least the following areas:

(1) Humane methods of animal maintenance and experimentation, including:
   (i) The basic needs of each species of animal;
   (ii) Proper handling and care for the various species of animals used by the facility;
   (iii) Proper pre-procedural and post-procedural care of animals; and
   (iv) Aseptic surgical methods and procedures;

(2) The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress;

(3) Proper use of anesthetics, analgesics, and tranquilizers for any species of animals used by the facility;

(4) Methods whereby deficiencies in animal care and treatment are reported, including deficiencies in animal care and treatment reported by any employee of the facility.

(5) Utilization of services (e.g., National Agricultural Library, National Library of Medicine) available to provide information:
   (i) On appropriate methods of animal care and use;
   (ii) On alternatives to the use of live animals in research;
   (iii) That could prevent unintended and unnecessary duplication of research involving animals; and
   (iv) Regarding the intent and requirements of the [Animal Welfare] Act.

Training programs should also include information on occupational health and safety. Specific recommendations for general training objectives may be obtained from *Education and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs*. Recommendations for general training objectives are outlined in Table A for each type of staff.

B.5. Table A. General Training Objectives

Topics	Animal Care Personnel	Research Personnel	Other Personnel
Animal welfare laws, regulations, policies, and guidelines	■	■	■
All animals are to be on a protocol	■	■	■
Cage card information	■	■	■
How to report perceived deficiencies in animal care and use	■	■	■
Recognizing pain and distress		■	
Alleviating pain and distress		■	
PI's responsibilities		■	
Protocol requirements	■	■	■
Role of the IACUC	■	■	■
Animal related hazards	■	■	■
Facility hazards	■	■	■
Occupational health and safety concerns	■	■	■
Behavior and appearance of healthy animals	■	■	■
Proper use of cage wash equipment	■		
Assure qualifications of research staff		■	
Humane techniques for animal procedures		■	

Personnel Training Records and Documentation

Although there is no specific requirement to document individual training activities, training records demonstrate that staff have met the training requirements related to their responsibilities in the research animal program, and regulatory or other oversight authorities often request to inspect personnel training records as evidence of an effective program.

Training records have value in tracking the range of topics offered, the frequency of training sessions, and the participation of institutional staff. Such records may include training received in informal settings, e.g., one-on-one instruction, common for teaching animal use methodologies.

Training records may be archived with the IACUC, a training coordinator, research departments or individual laboratories. Whatever the location, training records should be accessible to inspection by any oversight authority, including the IACUC. If training records of research staff are stored in laboratories, a good practice would be to include a brief review of training records among the objectives for the IACUC's semiannual inspection of facilities.

Training Personnel

Many institutions with a large research program have a training coordinator to oversee the training program for all personnel with animal care and use training needs. The training coordinator should be involved in IACUC meetings when institutional training issues are discussed.

Training coordinators should not be the only ones with training responsibilities. The facility staff, (e.g. veterinarians, veterinary technicians, facility managers and animal care technicians), also should be involved in training activities to the greatest extent possible. Their training activities, either with individuals or groups, should be acknowledged as a valuable contribution to the animal research program. In this way, individual expertise is fully utilized and every contact with facility staff offers a training opportunity.

In addition, other staff or outside consultants with specialized expertise can be incorporated into the training program. For example, occupational health professionals may be invited to take part in training on safety related issues. Training in specialized animal methodologies may be best performed by researchers who are accomplished in these techniques. Training program staff, if available, should participate in or oversee the training by outside experts to ensure that the training content is appropriate.

Institutional Support of Training

A high level of staff participation in a training program is essential for achieving the performance standard of staff qualifications necessary for quality research and expected by regulatory authorities. Institutions with mandatory training programs often have the most uniform results.

When training is not mandatory, there is much that an institution can do to encourage participation in the training program. When senior management and IACUC members take part in formal training programs, (e.g., on compliance issues), staff recognize an imperative to attend these sessions. The involvement of outside speakers with recognized expertise is often successful to draw larger groups to a training session. Letters urging staff participation in training programs are effective when sent by senior administrators and the IACUC to department chairpersons and principal investigators.

Methods that increase awareness and availability of information within the institution are valuable to support a training program. A combination of a training manual, newsletters, mailings, posted flyers, brochures and a Web site inform staff about the requirements for training, the institution's animal welfare standards, and the services available in the training program.

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B.6. Emergency Preparedness

B.6.a. Security and Crisis Management

Anti-animal research activities during the past several years against institutions using animals in research, testing and teaching programs have included demonstrations, break-ins, vandalism, life threats and harassment by mail or telephone, arson, and bomb threats. Since the IACUC has responsibility for the welfare of animals at its facility, it shares responsibility for the security of the animals and personnel who care for and use these animals with other units within the institution, such as the units responsible for security, public information, and governmental relations. Institutions receiving federal funds have an obligation to protect the federal investment in research by exercising due diligence in this area. The IACUC can serve a key role in advising the IO and the institution of potential risks and vulnerabilities, and in developing a plan for responding to potential or real threats.

In all cases the IACUC must consider allegations of noncompliance or animal welfare issues as concerns that must be addressed in accordance with relevant *PHS Policy* provisions and Animal Welfare Regulations (AWRs) (see Section D).

There are four key elements to an institution's preparedness:

- an animal care and use program of impeccable integrity;
- a security program based on risk assessment;
- an integrated communication plan with descriptions of research projects in lay terminology, spokespersons and a telephone tree; and
- an internal and external community outreach program that includes legislators and funding agencies.

Crisis Management Team

The establishment of a crisis management team before a crisis occurs is important in order to respond in a timely manner. This team may be comprised of individuals representing the following areas: security, public information, laboratory animal resources, the IACUC, management/research administration (including the IO), legal affairs, and governmental relations. It

is helpful for this team to meet periodically to keep abreast of current issues at the national and local level, and to be apprised of current research activities.

Risk Assessment – Security

The first step in developing a security program is to conduct a risk assessment of the institution's facilities, and evaluation of the existing security system. Organization of a security and communication plan then follow. Some key points include:

1. Determine facility vulnerability.
   a. Look at the research facilities with a "public eye."
   b. Be aware that use of certain animal species can increase vulnerability (e.g., nonhuman primates, cats and dogs).
   c. Be aware that some kinds of research may be perceived to be controversial (e.g., surgical and neuroscience protocols, including drug-addiction studies).
   d. Carefully review protocols that are more likely to generate requests for information under state or federal open records laws, such as items (b) and (c) above.

2. Evaluate the security system.
   a. Review policies regarding access and electronic surveillance systems.
   b. Check location of keys and access to animal rooms; entrances and exit sites such as stairwells and roof access.
   c. Determine who has access to buildings during nights and weekends.
   d. Ensure computer security, network access, etc. with computer administrators.

3. Check storage of research data.
   a. Ensure security of IACUC records and research data, including copies maintained offsite.
   b. Review research protocols for confidential information.
   c. Review protocols for graphic and/or sensitive terminology.

4. Organize a security plan.
   a. Consult with local police to establish procedures.
   b. Establish clear lines of authority and roles in a crisis situation.
   c. Maintain a list of research projects and scientists.

d. Identify ongoing investigations by regulatory agencies.
e. Limit access of delivery persons within animal care facilities.
f. Keep duplicate physical layout plans available off site.
g. Share information with security personnel about activism at other research organizations.
h. Develop a document that will provide pertinent information to the police in the event of an incident such as type of incident, location, animals or property destroyed or stolen, people involved, time, method of entry, and need to check for hazardous materials.

5. Organize a communication plan in the event of an incident during the day, after hours, weekends and holidays.

Communications and Risk Reduction

Institutions using animals need to communicate effectively and on an ongoing basis with the internal and external community and the media. It is important to build these relationships over time and to keep individuals in all of these areas informed about the significance of the work in which animals are used, and the institution's commitment to scientific standards through quality animal care and use. Being proactive by conveying significant advances in research using animals ethically and humanely can reduce the potential for negative public reactions in a crisis situation.

The IACUC Chair and members can interact with institutional public information officers, researchers, veterinarians, technicians and the research administration to identify spokespersons to address animal research issues. These spokespersons should be provided adequate training. Fact sheets should be readily available about the institution's policies and commitment to humane and appropriate animal care and use, the quality of its animal care and use program (including accreditation), and brief summaries of the value and importance of any specific animal use under scrutiny. Written materials need to be written in language understandable to nonscientists. Institutions must be prepared to respond to allegations honestly (i.e., if real noncompliance with relevant policies or regulations is substantiated then the institution must take appropriate action and should be forthcoming about the situation).

In the event of a crisis the facility that is prepared can respond quickly through its spokespersons with accurate and factual information. It is also important for the institution to notify OLAW in such an event so they can confirm

the status of the institution's PHS Assurance and any PHS support, as well as AAALAC, which maintains a crisis communication plan to assist accredited institutions.

Maintaining a high quality animal care and use program, good relationships within the institution and the community, and an effective education program can help to prevent and alleviate many crisis situations and significantly reduce the need for long term damage control.

References

CBRA Crisis and Communications Manual, California Biomedical Research Association. April 2000.

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B.6.b. Disaster Planning

As a fundamental component of the operational plans for most animal facilities, the Disaster Plan is a detailed, site-specific compilation of critical resources that are helpful in a variety of crisis events. The *Guide* recommends that all animal facilities have a Disaster Plan as part of their overall program and that the veterinarian or animal facility manager be part of the official institutional response team. While the *Guide* does not outline the elements of a Disaster Plan, it does suggest that facilities maintain sufficient emergency power necessary to maintain critical services (e.g., heating, ventilation and air conditioning (HVAC) system) and support functions (e.g., freezers, ventilated racks, isolators). Unique components of the facility may require special considerations. The proper institutional authority should approve the final plan so that appropriate resources can be committed during an emergency event. Typically, the IACUC does not have primary responsibility for emergency preparedness, but because emergency events could have significant impact on animals and the animal facility, the committee may choose to assess their site's preparedness during regular semiannual program reviews.

Emergency Management

In addition to the development of a Disaster Plan, an animal facility should consider approaching disaster preparedness from the more encompassing perspective of emergency management. One invaluable resource for emergency management information is the Federal Emergency Management

Agency (FEMA). FEMA is an independent federal agency founded in 1979 that reports directly to the President. FEMA's mission is *to reduce loss of life and property and protect our nation's critical infrastructure from all types of hazards through a comprehensive, risk-based, emergency management program.* FEMA considers an effective emergency management program to consist of four parts:

- *Mitigation* (activities related to preventing future emergencies or minimizing the effects of emergencies that occur);
- *Preparedness* (incorporation of the planning and preparations required to handle an emergency, including the Disaster Plan);
- *Response* (the Disaster Plan put into action when an emergency occurs); and
- *Recovery* (the actions needed to return to normal after an emergency occurs.)

Segments of a Disaster Plan

This section focuses on the Disaster Plan because it is the component of an emergency management program that the IACUC should review as a part of its semiannual program review. The content and scope of the Disaster Plan will be shaped and determined by the individual program and facility. The following approach is one way to create a Disaster Plan and can be useful to the IACUC in evaluating the facility's plan.

A suggested organization method includes:

- developing a planning team,
- defining emergencies,
- identifying critical functions and systems,
- defining resources and contacts,
- developing policies and procedures, and
- training staff and testing emergency equipment.

Developing a Planning Team

The Disaster Plan is best completed by the group of individuals that would respond to an emergency. The emergency response planning team should be comprised of individuals of various backgrounds and expertise, including certain animal facility staff and investigators, as well as representatives

from the facility engineering/maintenance group, security, occupational health services, safety, public relations and risk management. Due to site-specific variables such as the type of facility, hazards, risks and available resources, teams will be as unique as the plan. One of the early actions of the team should be to define its mission, goals and methods of operation. The team will also need to enlist project support from senior management so that resources are allocated for implementation of prescribed action plans. Ultimately, they will also need to integrate the facility Disaster Plan with any site-wide or local Disaster Plans.

Defining Emergencies

FEMA and other emergency management organizations have described various scoring methodologies to help categorize and rank emergencies. They generally divide emergencies (hazards) into three different categories:

- natural emergencies,
- technical emergencies, and
- civil emergencies.

Natural emergencies are the most commonly occurring "disasters" and include weather, seismic or ocean related events. Examples include tornadoes, hurricanes, floods, earthquakes, flood tides, etc. Technical emergencies are mechanical or human failures and include HVAC failures, computer system failures, chemical spills and structural failures. Civil emergencies are deliberate human events such as terrorist attacks, sabotage and labor strikes.

When developing a Disaster Plan, it may be helpful to list each type of emergency and include the primary and secondary effects. Secondary effects can greatly complicate a problem and can affect some critical functions even more than the primary. To help in planning, the list should include the probability of an event occurring (see Table A). The Disaster Plan should be sufficiently general to be responsive to unplanned types of crises.

As a planning exercise in evaluating primary and secondary effects, consider the scenario of a typical, midwinter moderately severe snowstorm, which can present multiple problems. Snow can clog the air intake filters and interfere with the HVAC system, or affect the electrical supply. Snow can contribute to local flooding when it melts. Either snow or flooding may affect employees' ability to get to work or prevent essential deliveries from

being made. If electrical power is lost, and the facility is relying on emergency back-up generators, there may be refueling problems when the fuel reserves are exhausted and delivery trucks can't reach the site. This example shows how the planning exercise can provide valuable modeling information useful in disaster preparation.

Identifying Critical Functions and Systems

Fundamentally, the Disaster Plan should address ways to maintain or cope with the loss of critical functions and systems in the animal facility. To do this, it is important to rigorously identify all critical animal facility specific functions and systems. The critical functions and systems fall into two general categories: mechanical systems and personnel functions (see Table B). It is helpful to compare the list of primary and secondary effects of the different emergencies (Table A) and review their impact on the critical functions and systems. Different scenarios can become the basis for action plans and preparedness activities.

Defining Resources and Contacts

The Disaster Plan can also include lists of available resources and contacts to be used during emergency events. The lists can include various emergency equipment, spare parts, equipment capacities, levels of redundancy built into the mechanical equipment systems and ways to put the equipment into use. Additionally, this section might include critical vendors that can supply services during an emergency, such as a supplier to perform periodic refueling of emergency generator fuel tanks, as well as up to date emergency personnel notification lists, including criteria for contacting specific individuals. More advanced plans stage the level of an emergency and clearly prescribe the type of response for each level. Other pertinent items such as floor layouts, mechanical equipment plans, the names and numbers of national, regional and local emergency response organizations (FEMA, Red Cross, Police, etc.) and local weather information resources, can be included.

Developing Policies and Procedures

The core elements of a Disaster Plan are the policies, guidelines and procedures that are put into action during an emergency. The plan should address very specific emergencies and/or give general outlines for action steps in response to an emergency. Many plans will also focus on coping

with the loss of a critical function or system. This approach is best when it includes evaluation of the reliability of the back-up systems affected during a complex emergency situation. Available resources should be clearly identified and information on how to access the resources included. Clear lines of authority and responsibility should be established and documented.

Training Staff and Testing Emergency Equipment

Personnel are usually familiar with "fire drills" through participation in regular emergency evacuation testing of buildings. Effective disaster planning borrows that concept and conducts the same types of rehearsals for other high-risk emergency situations. Exercising realistic scenarios not only provides practical training but also '"tests" the emergency plans for deficiencies or vulnerabilities. Similarly, emergency equipment should be tested and maintained in working order. Finally, the Disaster Plan should be made readily available to all staff members. Some facilities have the plan available on internal Web sites.

Conclusion

Animal facility management should recognize that emergencies and unexpected problems are inevitable. Adopting the mindset that emergencies are a fact of life and will occur is the first step towards their prevention. Preparedness is critical for emergency avoidance and can reduce, if not eliminate, negative affects. A good Disaster Plan will ensure a quick and effective response and faster recovery. However, the process of emergency management planning is not totally intuitive and a specific effort needs to be made to examine the issues and devise plans. Furthermore, because there are no "formulas" and very few formal requirements for back-up or emergency systems, one facility's plan will not be 100% effective at another facility. The overall process should be dynamic and should be reviewed on a regular basis by the facility. The IACUC may also choose to include it periodically as a part of their semiannual program reviews. The modification or upgrading of functional systems is an ideal time to upgrade the emergency handling potential of the system. Unfortunately, emergency/hazard identification is clearest in retrospect, but the special efforts of prospective disaster planning pay the greatest dividends.

B.6. Table A. Examples of Categories of Emergencies

Natural Emergencies

Primary Emergency	*Secondary Effects*	*Risk of Occurrence*	*Impact*
Earthquake			
Flood			
Coastal flood			
Hurricanes			
Landslides			
Severe storms (thunderstorms)			
Tornadoes			
Tsunamis			
Wildland fires			
Winter storms (snow and ice)			
Drought			
Lightning			

Technical Emergencies

Primary Emergency	*Secondary Effects*	*Risk of Occurrence*	*Impact*
Hazardous chemicals spill			
Radioisotope spill			
Biohazard spill			
Computer system			
HVAC			
Steam heat			
Water supply			
Reverse osmosis or treated drinking water			
Waste water removal			
Solid waste removal			
Security system			
Transportation			
Communication			
Fire safety			
Electricity			

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B.6. Table A. Examples of Categories of Emergencies *(continued)*

Civil Emergencies

Primary Emergency	*Secondary Effects*	*Risk of Occurrence*	*Impact*
Terrorist threat/action			
Strike of personnel			
Demonstrations			
Intrusion			
Computer/network attack			

B.6. Table B. Core Functions of an Animal Facility

Mechanical	**Personnel**
• Ventilation	• Safety
• Cooling	• Communications
• Heating	– Staff
• Cleaning water	– Authorities
• Drinking water for animals	– Public
• Power	• Medical care
• Sewage	• Veterinary care
• Solid waste removal	• Husbandry
• Carcass disposal	– Feeding
• Freezing	– Watering
• Cage sanitation	– Cleaning
• Communication system	– Capturing loose animals
• Transportation	• Medical waste handling
• Shelter	• Carcass disposal
	• Security
	• Supplies
	– Food
	– Bedding
	– Uniforms
	– Personal safety equipment
	– Cleaning

Suggested Reading

Anderson, S. 1998. Hazard Analysis: Preparing for Natural Disasters, *Lab Animal* 27(1):24-29.

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C. Review of Proposals

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C.1. Fundamental Issues

The IACUC is responsible for overseeing and evaluating all aspects of animal care and use, and is charged with reviewing proposals* that involve animals to ensure that the criteria established in the *PHS Policy* and the Animal Welfare Regulations (AWRs) are implemented. In its review of proposals, the Committee's primary goal should be to facilitate compliance with applicable laws, regulations and policies consistent with the performance of appropriate and productive scientific endeavors.

Protocol Review Criteria

Table A lists each review criterion of the *PHS Policy* and AWRs along with the applicable US Government Principles. Since the *PHS Policy* further requires that the provisions of the *Guide* apply, there are many other aspects of research that an IACUC should review, such as food and water deprivation, use of noxious stimuli, and physical restraint. The *Guide* provides useful guidance on these and other practices. Section C.2. Protocol Review Criteria addresses many of the subjects described below in greater detail.

If the IACUC does not have the scientific and technical expertise to evaluate all aspects of a proposal it may bring in outside expert consultants to provide information. Such consultants may not vote. In all cases, the onus should be on the investigator to justify and explain his or her proposed experiments to the satisfaction of the IACUC.

**This Guidebook generally uses the term "proposal" to describe the proposed use of animals. In some cases the term "protocol" is used for ease of readability. For the purposes of this Guidebook "proposal" is interchangeable with the commonly accepted use of the term "protocol."*

C.1. Table A. Regulatory Criteria Applicable to Protocol Review as Defined in PHS Policy and USDA Regulations

U.S. Government Principles Note*: Citations at the end of each Principle refer to other Sections of this Guidebook.*	**PHS Policy on Humane Care and Use of Laboratory Animals**	**USDA AWR 9 CFR Part 2, Subpart C**
Principle I: The transportation, care and use of animals should be in accordance with the AWA (7 U.S.C.2131 et. seq.) and other applicable federal laws, guidelines, and policies*. *For guidance throughout these Principles, the reader is referred to the *Guide*.	C.1.: ...the IACUC shall...determine that the proposed research projects are in accordance with this Policy...the IACUC shall confirm that the research project will be conducted in accordance with the AWA insofar as it applies to the research project, and that the research project is consistent with the *Guide* unless acceptable justification for a departure is presented.	§2.31(d): ...The IACUC shall determine that the proposed activities are in accordance with this subchapter unless acceptable justification for a departure is presented in writing...
Principle II: Procedures involving animals should be designed and performed with due consideration of their scientific relevance to human or animal health, the advancement of knowledge, or the good of society.		§2.31(d)(1)(iii): The PI has provided written Assurance that the activities do not unnecessarily duplicate previous experiments.
Principle III: The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and *in vitro* biological systems should be considered. (See C.2.a. Alternatives)	D.1.: Applications and proposals…that involve the care and use of animals shall contain the following: a.) Identification of the species and the approximate number of animals to be used; b.) rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used…	§2.31(e): A proposal…must contain the following: (1) Identification of the species and approximate number of animals to be used; (2) A rationale for involving animals, and for the appropriateness of the species and numbers of animals to be used…

C.1. Table A. Regulatory Criteria Applicable to Protocol Review as Defined in PHS Policy and USDA Regulations *(continued)*

U.S. Government Principles	PHS Policy on Humane Care and Use of Laboratory Animals	USDA AWR 9 CFR Part 2, Subpart C
Principle IV: Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals. (See C.2.a. Alternatives, and C.2.d. Minimization of Pain and Distress)	IV.C.1.a.: Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design.	§2.31(d)(1)(i): Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals; (ii) the principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, e.g., the Animal Welfare Information Center, used to determine that alternatives were not available. (See also §2.31(e)(4)).
Principle V: Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents. (See C.2.c. Humane Endpoints, and C.2.d. Minimization of Pain and Distress, and C.2.f. Veterinary Review and Consultation)	IV.C.1.b.: Procedures that may cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedation, analgesia, or anesthesia, unless the procedure is justified for scientific reasons in writing by the investigator (and approved by the IACUC).	§2.31(d)(1)(iv): Procedures that may cause more than momentary or slight pain or distress to the animals will: (A) Be performed with appropriate sedatives, analgesics or anesthetics, unless withholding such agents is justified for scientific reasons, in writing, by the principal investigator and will continue for only the necessary period of time; (B) Involve, in their planning, consultation with the attending veterinarian or his or her designee; (C) Not include the use of paralytics without anesthesia.

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C.1. Table A. Regulatory Criteria Applicable to Protocol Review as Defined in PHS Policy and USDA Regulations *(continued)*

U.S. Government Principles	PHS Policy on Humane Care and Use of Laboratory Animals	USDA AWR 9 CFR Part 2, Subpart C
Principle VI: Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure. (See C.2.b. Euthanasia)	IV.C.1.c.: Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly killed at the end of the procedure or, if appropriate, during the procedure. IV.C.1.g.: Methods of euthanasia will be consistent with the recommendations of the AVMA Panel on Euthanasia, unless a deviation is justified for scientific reasons in writing by the investigator [and approved by the IACUC].	§2.31(d)(1)(v): Animals that would otherwise experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized at the end of the procedure or, if appropriate, during the procedure. §2.31(d)(1)(xi): Methods of euthanasia used must be in accordance with the definition of the term set forth in 9 CFR part 1, §1.1 of this subchapter, unless a deviation is justified for scientific reasons, in writing, by the investigator.
Principle VII: The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated. (See B.2.b. Animal Environment, B.2.c. Husbandry, and B.3. Role of the Veterinarian)	IV.C.1.d.: The living conditions of animals will be appropriate for their species and contribute to their health and comfort. The housing, feeding, and non-medical care of the animals will be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. IV.C.1.e.: Medical care for animals will be available and provided as necessary by a qualified veterinarian.	§2.31(d)(1)(vi): The animals' living conditions will be appropriate for their species in accordance with part 3 of this subchapter, and contribute to their health and comfort. The housing, feeding, and nonmedical care of the animals will be directed by the attending veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. §2.31(d)(1)(vii): Medical care for animals will be available and provided as necessary by a qualified veterinarian.
Principle VIII: Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals. (See C.2.e. Personnel Qualifications)	IV.C.1.f.: Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures.	§2.31(d)(1)(viii): Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures.

C.1. Table A. Regulatory Criteria Applicable to Protocol Review as Defined in PHS Policy and USDA Regulations *(continued)*

U.S. Government Principles	PHS Policy on Humane Care and Use of Laboratory Animals	USDA AWR 9 CFR Part 2, Subpart C
Principle IX: Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.	See C.1. above.	See §2.31(d) above.
	D.1.: Applications and proposals… that involve the care and use of animals shall contain the following: …c) a complete description of the proposed use of the animals…	§2.31(e): A proposal…must contain the following: … (3) A complete description of the proposed use of the animals…
	D.1.: Applications and proposals…that involve the care and use of animals shall contain the following: …d) a description of procedures designed to assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research, and that analgesic, anesthetic, and tranquilizing drugs will be used where indicated and appropriate to minimize discomfort and pain to animals.	§2.31(e): A proposal…must contain the following: … (4) A description of procedures designed to assure that discomfort and pain to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research, including provision for the use of analgesic, anesthetic, and tranquilizing drugs where indicated and appropriate to minimize discomfort and pain to animals.

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C.1. Table A. Regulatory Criteria Applicable to Protocol Review as Defined in PHS Policy and USDA Regulations *(continued)*

U.S. Government Principles	PHS Policy on Humane Care and Use of Laboratory Animals	USDA AWR 9 CFR Part 2, Subpart C
		§2.31(d)(1) (ix): Activities that involve surgery include appropriate provision for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. All survival surgery will be performed using aseptic procedures, including surgical gloves, masks, sterile instruments, and aseptic techniques. Major operative procedures on non-rodents will be conducted only in facilities intended for that purpose which shall be operated and maintained under aseptic conditions. Non-major operative procedures and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic procedures. Operative procedures conducted at field sites need not be performed in dedicated facilities, but must be performed using aseptic procedures; §2.31(d) (1) (x): No animal will be used in more than one major operative procedure from which it is allowed to recover, unless: (A) justified for scientific reasons by the principal investigator, in writing; (B) Required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian, or (C) In other special circumstances as determined by the Administrator on an individual basis.

Proposal Review Procedures

The procedural review requirements of the *PHS Policy* or the AWRs take precedence even though they may differ from some commonly used parliamentary procedures. Institutions may develop their own meeting procedures as long as the procedures do not contradict or are not inconsistent with the requirements of the *PHS Policy* or the AWRs.

If a proposal may cause more than momentary or slight pain or distress to animals, the AWRs specifically require investigators to consult with the AV or his or her designee during protocol development. Some committees find it helpful to assign a member a given proposal for in-depth review and liaison with the investigator prior to committee review. Still other committees assign this task to professional IACUC staff. The investigator may choose to consult with these individuals and request a preliminary review before formally submitting a proposal.

The *PHS Policy* and AWRs recognize two methods of review: full committee review and designated member review. The following pertains to review of initial protocols as well as to review of proposed significant changes in previously approved protocols.

- **Full committee review**

  Full committee review of proposals requires a convened meeting of a quorum of the IACUC members. The *PHS Policy* and AWRs are explicit that proposals reviewed by the full committee must receive the approval vote of a majority (>50%) of the quorum present in order receive approval (see A.2. Quorum requirements).

  Some committees designate a specific member or members to serve as primary or primary and secondary reviewers. These individuals, usually chosen for their expertise or familiarity with a given topic, are responsible for an in-depth review of a proposal and sometimes take responsibility for describing the proposal to the full committee and answering questions about the proposal during review by the Committee. Primary and secondary reviewers can also take the initiative to contact the investigator prior to the meeting for clarifications, additional information, or in anticipation of questions the IACUC may raise. The use of primary

reviewers facilitates full committee review by distributing the workload among IACUC members so that each member has responsibility for in-depth review of only a portion of the proposals the IACUC will review. It differs from designated member review (see below), which invests authority to approve a proposal in one or more members.

Review of proposals by the full committee method invokes a deliberative process, and the *PHS Policy* and AWRs require that minutes of IACUC meetings reflect committee deliberations. Minutes should include records of attendance, a summary of the issues discussed and the resolution of issues, and the results of IACUC votes on proposals.

Participation by investigators in meetings in which their proposal is being reviewed is not addressed by either the *PHS Policy* or the AWRs. The participation of the investigator can facilitate the review in a number of ways. Obviously questions can be addressed as they are raised rather than after the meeting, allowing the review process to proceed rather than be interrupted for this exchange of information. Another benefit is the opportunity for the investigator to give a broad overview of how the proposal under review fits into the larger research picture, thus providing additional information regarding the justification and scientific merit. Invariably, both the investigator and the IACUC benefit from such an exchange. The greatest deterrents to participation by investigators in the IACUC meeting are that it may make the meeting last longer, and problems arise if this is an adversarial rather than collegial exchange of information. In any event, the investigator should leave before the final committee deliberations and if he or she is a committee member may not contribute to a quorum or vote.

- **Designated member review**

  To utilize designated member review, each IACUC member must be provided with at least a list of the proposed research protocols or proposed significant changes to previously approved protocols prior to the review. Most committees provide members with an abstract of proposals; in all cases, written descriptions of the research proposals must be made available to IACUC members upon request. All members must

have the opportunity to request full committee review of any proposal. If no member requests full committee review, the Chair designates one or more qualified members to review the proposal (or proposed amendment). These designated members have authority to approve, require modifications in (to secure approval), or request full committee review.

IACUCs with a large volume of proposals to be reviewed find the designated member review option may allow for efficient management of the IACUC workload as well as timely turnaround of requests from investigators for protocol review. Some committees prefer to reserve the designated member review option for certain classes of protocols or amendments; conversely, some IACUCs have devised categories of research activities that must be reviewed by the full committee, e.g., nonhuman primate studies, survival surgeries, etc. If the designated member review method is to be used by PHS-supported institutions then the IACUC's specific procedures for using the method should be described in its PHS Assurance.

Categories of IACUC Actions

As a result of their review of a protocol, an IACUC may take one of several different actions depending upon the findings of the committee: approval, modifications required to secure approval, or withhold approval. An IACUC may also defer or table review if necessary.

The *PHS Policy* and AWRs require the IACUC to notify investigators and the institution in writing of its decision to approve or withhold approval, or of modifications required to secure approval. If approval is withheld the IACUC must provide the reasons for its decision and give the investigator an opportunity to respond.

- **Approval**

  When the IACUC has determined that all review criteria, based on the *PHS Policy* and AWRs, have been adequately addressed by the investigator, the IACUC may approve the proposal, thus providing the investigator permission to perform the experiments or procedures as described.

  An IACUC-approved proposal may be subject to further appropriate review and approval by institutional officials due

to financial, policy, facility, or other institutional or administrative considerations. However, those officials may not approve an activity if it has not been approved by the IACUC.

- **Modifications required to secure approval**

  An IACUC may require modifications to the proposal before granting approval. If the IACUC determines that a proposal is approvable contingent upon receipt of a very specific modification (e.g., receipt of assurance that the procedure will be conducted in a fume hood), or clarification of a specific point, the IACUC may handle these modifications or clarifications as administrative details that an individual, such as the Chair, could verify.

  If a study is unusually complex or involves untried or controversial procedures the IACUC may wish to impose restrictions, (e.g., approval for the use of a limited number of animals as a pilot study with a written report of interim results, or close monitoring by veterinary or other qualified personnel.) If such modifications represent significant departures the IACUC can ask the investigator to revise the protocol to reflect the modifications imposed by the IACUC.

  If the proposal is missing substantive information necessary for the IACUC to make a judgment, or the IACUC requires extensive or multiple modifications, then the IACUC can require that the protocol be revised and resubmitted. If the IACUC wishes to shift to the designated reviewer mode for the approval of the modified protocol, that shift should be explicitly noted in the minutes and the requirements for designated review must be met.

  IACUCs sometimes use terms such as “conditional approval,” “provisional approval” or “approved pending clarification.” Anything less than full IACUC approval via one of the accepted methods described above is not adequate for initiation of animal activities or for submission of an IACUC approval date to PHS in conjunction with a grant application. Therefore, OLAW and USDA recommend that IACUCs either avoid using these terms, or describe them (e.g., in IACUC minutes, Assurance documents, etc.) in sufficient detail to be fully understood.

- **Withhold approval**

  When the IACUC determines that a proposal has not adequately addressed all of the requirements of the *PHS Policy* and AWRs as applicable, the committee may withhold approval. A designated reviewer may not withhold approval; this action may only be taken if the review is conducted using the full committee method of review.

  As indicated above, a higher institutional authority may not administratively overrule an IACUC decision to withhold approval of a proposal.

- **Defer or table review**

  If the proposal requires clarification in order for the IACUC to make a judgment, committee members with certain expertise are not present, the IACUC wishes to seek external consultation, or any of a number of other reasons prevent the IACUC from conducting its review, then the IACUC may wish to defer or table review. Good communication between the IACUC and the investigator can ensure that this action is needed infrequently. However, should it be necessary, the investigator should be informed so that he or she can respond or plan accordingly.

Review of Changes to Approved Protocols

Significant changes to an IACUC-approved protocol must be reviewed and approved by the IACUC before they occur (*PHS Policy* IV.C.1., and AWR §2.31[d][1]). It is prudent for an IACUC to develop a policy on the kinds of changes that are considered significant in order to avoid ambiguity. OLAW has identified the following kinds of significant changes that may serve as examples to guide the IACUC in its determinations:

- change in objectives of a study;
- proposals to switch from nonsurvival to survival surgery;
- change in degree of invasiveness of a procedure or discomfort to an animal;
- change in species or in the approximate number of animals used;
- change in personnel involved in animal procedures;
- change in anesthetic agent(s) or in the use or withholding of analgesics;

- change in methods of euthanasia; or
- change in duration, frequency or number of procedures performed on an animal.

Review of significant changes may be conducted using either the full committee review or the designated member review method described above.

Frequency of Review of Approved Protocols

The *PHS Policy* requires that a complete IACUC review of PHS supported protocols be conducted at least once every three years. This triennial review is interpreted by OLAW as a requirement for *de novo* review, meaning that the criteria and procedures for review specified in IV.C. of the *PHS Policy* must be applied not less than once every three years. The three-year period begins on the actual date of IACUC approval; IACUCs may not administratively extend approval beyond the three years. The triennial review may be conducted using either the full committee review or the designated member review method described above.

AWRs require an annual review, which may be a monitoring mechanism whereby the IACUC requires the investigator to annually report on the status of the protocol, verify that completed activities were conducted in accordance with the approved protocol, describe any proposed departures from the approved protocols, and solicit information about activities projected for the upcoming year. (Proposed significant changes would require IACUC review prior to initiation.) This kind of a monitoring system will satisfy the AWR requirement for annual review, but would not be sufficient for the complete IACUC review required on a triennial basis.

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C.2. Protocol Review Criteria

C.2.a. Alternatives – Replacement, Reduction and Refinement

There is significant interest in the application of alternatives to animals used in research, education and testing. The *PHS Policy* and the AWRs require research institutions to ensure that investigators have appropriately considered alternatives to procedures that can cause more than slight or momentary pain or distress in animals, consistent with sound research design. Through U.S. Government Principle III (Appendix F), the *PHS Policy* further requires that the minimum number of animals be used and that non-animal methods be considered.

The "3 Rs"

Alternatives are framed within the context of the "3 Rs" articulated originally by Russell and Burch in 1959; they include:

1. **Replacement**, or utilizing non-animal models;
2. **Reduction** of numbers of animals used; and
3. **Refinement**, or elimination or reduction of unnecessary pain and distress in animals.

Replacement alternatives utilize:

- living systems,
- non-living systems, or
- computer simulations.

Living systems include *in vitro* methods that utilize organ, tissue or cell culture techniques. Invertebrate animals, such as the fruit fly, have long been used in research and represent another type of living alternative to vertebrate animals. Finally, microorganisms and plants represent living alternatives for some types of research and testing. *If no invertebrate model is appropriate, use of species lower on the phylogenetic scale may be considered a replacement alternative.*

Nonliving systems include physical or mechanical systems and chemical techniques. Mechanical models may be used in the training of specific techniques (cardiopulmonary resuscitation, for example) and have replaced living animals in some cases. Chemical techniques are the most widely used nonliving systems and include such useful systems as the enzyme linked immunosorbent assay (ELISA). Techniques that identify the presence of chemical reactions and enzymes, or simply analyze chemical structure, can all be useful in the prediction of toxicity without the use of animals.

Computer simulations may replace some animal use and can be particularly useful when a question is well defined and there is existing data.

Although opportunities for replacement are numerous in product safety testing and education, they appear more limited in research. If it is demonstrated that there is no *in vitro* alternative to the use of animals, it is important for the IACUC members to focus on the other alternative approaches, reduction and refinement.

Reduction of numbers of animals may be accomplished by a variety of methods described in Table A:

C.2.a. Table A. Methods for Reduction of Numbers of Animals Used

Method	Examples
Rational selection of group size	• Pilot studies to estimate variability and evaluate procedures and effects • Power analysis
Careful experimental design	• Appropriate choice of control groups • Standardizing procedures to minimize variability
Maximizing use of animals	• Performing several terminal procedures per animal • Animals euthanized by one investigator used for tissue needed by another
Correct choice of model	• Use of healthy, genetically similar animals decreases variability
Minimizing loss of animals	• Good post-operative care • Avoid unintended breeding • Plan ahead so the appropriate number of animals needed for studies are ordered or bred
Statistical analysis	• Appropriate use of statistical software can generate maximum information from minimum number of animals

Refinement of technique to reduce or eliminate unnecessary pain and distress in study animals is the most commonly practiced of the 3 Rs, although it is not always recognized as one of the applications.

Investigators are required to consider alternatives to painful procedures, and to avoid or minimize discomfort, distress and pain, consistent with sound scientific practice and the goals of the research. This requires an understanding of the potential of pain or distress in the animals (see Section C.2.d.).

When there is no consensus among IACUC members as to whether a certain procedure actually causes pain or distress in the affected animals, U.S. Government Principle IV should be applied. This Principle states, "Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals."

To assist in this deliberation, the IACUC may need to utilize one or more of the following:

- pilot studies,
- evaluations of clinical signs,
- clinical pathology,
- gross and histological necropsy studies,
- review of comparable literature, and
- consultation with experts.

If there remains any doubt about the presence of pain or distress, the IACUC should err on the side of protecting the animals against the potential of unnecessary pain or distress.

Some refinement opportunities include:

- pain-relieving drugs,
- non-pharmacologic techniques,
- new diagnostic and therapeutic techniques,
- environmental enrichment programs, and
- establishment of more humane endpoints.

Pain-relieving drugs: While it is preferable to design a protocol that prevents pain and distress, when this is not possible the AWRs require that the AV (or designee) be consulted to develop an appropriate plan for the use of anesthetics, analgesics, or other measures, such as anti-inflammatory agents, antibiotics, or sedatives.

New diagnostic and therapeutic techniques: In addition to the use of pain relieving drugs, new diagnostic and therapeutic techniques may have the capability to dramatically reduce the invasiveness of data collection and thereby refine animal research. These include:

- use of sophisticated imaging equipment to replace invasive procedures, and
- blood and tissue sampling techniques that allow for easier collection and the processing of smaller sample sizes.

Environment: The IACUC should consider that environmental factors, such as noises, odors, infrequent or inexperienced handling, or boredom from lack of environmental stimulation can cause unnecessary distress, and that US Government Principle IV should be applied to this area as well. Aside from the AWR requirement to provide environment enhancement for non-human primates, many institutions have implemented environmental modifications for other species with a view to reducing unnecessary distress.

Humane endpoints: The establishment of the earliest possible humane endpoint consistent with the research design may provide an additional opportunity to significantly reduce pain and distress, thereby refining the experiment. For any study that defines death of the experimental animal as the endpoint, the IACUC should ask if there is an earlier point in the study when the necessary data have been collected and the animal could be euthanized without proceeding through more severe illness and death. Or, alternatively, if death is a necessary endpoint, the IACUC could ask for careful ongoing assessment of the animal, so that, when it is determined that death is inevitable, the animal can be euthanized. The Canadian Council on Animal Care Guidelines on Choosing an Appropriate Endpoint in Experiments Using Animals for Research, Teaching and Testing (1998) is an excellent resource for IACUCs. (See also Section C.2.c. Humane Endpoints.)

USDA Requirements for Consideration of Alternatives

USDA AWRs require that investigators consider alternatives to procedures that may cause more than momentary or slight pain or distress and provide a written narrative of the methods used and sources consulted to determine availability of alternatives. Animal Care Policy 12 provides guidance on the requirements for the written narrative, which should include adequate information for the IACUC to assess that a reasonable and good faith effort was made to determine the availability of alternatives or alternative methods. Resources in the area of alternatives include the USDA Animal Welfare Information Center (AWIC); ALTWEB, a Web site maintained under the auspices of the Johns Hopkins University Center for Alternatives to Animal Testing; and the University of California Center for Animal Alternatives (see Appendix A).

C.2.b. Euthanasia

"Euthanasia means the humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death" (AWR). The choice of a method depends on species, age, availability of restraint, skill of the individuals performing euthanasia and other considerations. In a research setting, the method of euthanasia must be consistent with the research goals.

The *PHS Policy* and the AWRs require that an IACUC review and approve methods of euthanasia. The *PHS Policy* specifically states that methods of euthanasia must be consistent with the recommendations of the American Veterinary Medical Association (AVMA) Panel on Euthanasia, unless scientific justifications for alternative methods are presented in writing by the investigator and approved by the IACUC. The most recent Panel convened in 2000, and published its Report in March 2001.

The criteria used as the basis for the Panel's recommendations include:

- minimum pain, distress, anxiety or apprehension;
- minimum delay until unconsciousness;
- reliability and irreversibility;
- safety of personnel; emotional effect on personnel;

- compatibility with requirement and purpose, including subsequent use of tissue;
- compatibility with species, age and health status; and
- drug availability and human abuse potential.

Recommended Methods

The 2000 Report of the AVMA Panel on Euthanasia categorizes methods as acceptable, conditionally acceptable, or unacceptable under specific circumstances.

Acceptable

a. Barbiturates (most species)
b. Carbon dioxide (CO_2)-bottled gas only (most species)
c. Inhalant anesthetics (most species)
d. Microwave irradiation (mice and rats)
e. Tricaine methane sulfate (TMS, MS222) (fish, amphibians)
f. Benzocaine hydrochloride (fish, amphibians)
g. Captive penetrating bolt (horse, ruminant, swine)
h. Ether and carbon monoxide are acceptable for many species, but relatively dangerous to personnel.

Conditionally Acceptable (Requires IACUC Approval of Scientific Justification)

a. Cervical dislocation (birds, small rodents and rabbits)
b. Decapitation (birds, rodents, some other species)
c. Pithing (some ectotherms)
d. Various pharmacological and physical methods

Unacceptable

a. Chloral hydrate, chloroform and cyanide
b. Decompression
c. Neuromuscular blockers
d. Various pharmacological and physical methods
e. Dry ice-generated Co_2

Methods described as conditionally acceptable are considered acceptable when used in deeply anesthetized animals. Some euthanasia methods (e.g., KCl or formalin by intracardiac injection, or exsanguination) are acceptable only under deep general anesthesia.

For more information on methods of euthanasia see Appendix D.

C.2.c. Humane Endpoints

Animals used in research and testing may experience pain from induced diseases, procedures, and toxicity. The *PHS Policy* and AWRs state that procedures that cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. However, research and testing studies sometimes involve pain that cannot be relieved with such agents because they would interfere with the scientific objectives of the study. Accordingly, federal regulations require that IACUCs determine that discomfort to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research, and that unrelieved pain and distress will only continue for the duration necessary to accomplish the scientific objectives.

The *PHS Policy* and AWRs further state that animals that would otherwise suffer severe or chronic pain and distress that cannot be relieved should be painlessly killed at the end of the procedure, or if appropriate, during the procedure.

Developing Humane Endpoints

Criteria used to end experimental studies earlier in order to avoid or terminate unrelieved pain and/or distress are referred to as humane endpoints. An important feature of humane endpoints is that they should ensure that study objectives will still be met even though the study is ended at an earlier point. Ideally, humane endpoints are sought that can be used to end studies before the onset of pain and distress.

It is important to understand that stress may lead to distress when major shifts in biologic function, to which the animal cannot adapt, threaten the animal's well-being. If pain and distress are anticipated, a detailed plan for when and how these will be alleviated should be provided in the protocol.

The plan should include detailed written criteria for the humane endpoints that will be used to determine when animals can be removed from the study, treated, or euthanized. There should be clear directions concerning who can make the decision to euthanize or treat animals, including procedures to follow if a situation arises on weekends, holidays, or in the absence of the responsible study director.

Even if pain or distress are not anticipated, every protocol should contain a contingency plan for dealing with unexpected situations that may arise.

The development and use of humane endpoints can reduce the severity and duration of unrelieved pain and distress. Clinical score sheets can be developed and used to establish humane endpoints for experimental studies. Score sheets are used to record and identify clinical signs and conditions associated with a particular experimental model. Single or multiple clinical signs that are predictive of the current experimental endpoint can then be used to allow for earlier and more humane endpoints.

Establishing and implementing humane endpoints is best achieved by a collaborative effort on the part of investigators, veterinarians, and animal care staff.

Moribund Condition as a Humane Endpoint

Moribund has been defined as "in the state of dying," or "at the point of death." A moribund condition may be an appropriate humane experimental endpoint for some studies where there is the induction of severe disease states and high rates of mortality. Pre-emptive euthanasia of moribund animals can prevent further pain and distress.

Objective data-based criteria that are predictive of impending death can be used to implement timely euthanasia to avoid spontaneous deaths. FDA regulatory testing guidelines allow for humane killing of animals that are moribund. However, it is important to recognize that euthanasia of a moribund animal does not eliminate pain and distress that may be experienced during progression to a moribund condition. It should also be noted that while death is not a required endpoint for routine toxicity testing, animals are often found dead during studies. Establishing procedures to detect and humanely euthanize moribund or pre-moribund animals can reduce the number of animals that die spontaneously. In addition to reducing animal pain and distress, euthanasia of moribund animals allows for the collection of tissues and other biologic specimens that may otherwise be lost or rendered unusable when an animal is found dead.

Various clinical signs are indicative of a moribund condition in laboratory animals. These typically include one or more of the following:

- impaired ambulation which prevents animals from reaching food or water,
- excessive weight loss and emaciation,
- lack of physical or mental alertness,
- difficult labored breathing, and
- inability to remain upright.

Animals should be observed frequently enough to detect signs of impending death so they can be euthanized in a timely manner. When increased morbidity or mortality is expected, a minimum of twice daily observation is recommended. Animals not likely to survive until the next scheduled observation should normally be euthanized. In situations where animals are often found dead, closer and more frequent observation for moribund animals should be considered to reduce spontaneous deaths. Euthanasia of animals that are moribund or experiencing severe pain and distress should always be done in a manner that produces the least possible amount of additional pain and distress.

Other Humane Endpoints in Research

Animals used to study tumor biology, to develop new cancer therapies, and to evaluate the carcinogenic potential of substances may experience pain and distress. Frequent and appropriate monitoring of animals during tumor development is necessary to allow for appropriate intervention before significant deterioration or death. Effective monitoring systems and endpoints should include limits on tumor size and severity of tumor-associated disease. Altered physiologic, biochemical, and other biomarkers may be potentially more objective and reproducible endpoints than clinical signs for such studies.

Genetically engineered animal models are sometimes accompanied by unintended and unpredicted alterations that adversely affect animal well-being. Investigators need to establish a plan for addressing unanticipated adverse outcomes for genetically altered animals. There should be a plan for systematic characterization of phenotypes to facilitate assessment of their possible utility and timely decisions on disposition or retention. IACUCs should provide oversight of such studies to ensure that animal welfare problems are handled in an effective and prompt manner.

Animals with induced infections may experience significant pain and/or distress during progression of the disease. Early physiologic and biochemical changes during infection have been found to be useful humane endpoints rather than death or moribund condition. Specific decreases in body temperature have been found to be effective early predictors of eventual death for some infections in rodents. Vaccine potency testing typically involves challenging immunized animals with infectious agents. While such testing has commonly used lethality as the endpoint indicative of insufficient protection, some regulatory authorities now allow euthanasia of moribund animals.

Toxicity Testing

Animals used in toxicity testing can experience pain and distress when toxic effects are produced. Toxicity testing regulations allow treatment of pain and distress in test animals only if there is no interference with the study. As a result, animals are rarely treated in toxicology studies because of the potential confounding effects of analgesics. Consequently, management of pain and distress in toxicity studies is accomplished largely by euthanizing animals that are experiencing significant pain and distress.

Current regulatory guidelines state that animals in toxicology studies obviously in pain or showing signs of severe and enduring distress should be euthanized, rather than allowing them to survive to the end of the scheduled study. Humane endpoints should be established and used for toxicology studies in order to further minimize pain and distress.

Death as an Endpoint

Since it provides an objective and unequivocal data point, death has historically been used as an endpoint in cancer, infectious disease and other animal studies, especially for regulatory purposes (e.g., drug safety/efficacy studies). Increased public interest and regulation have led to a re-evaluation of this practice. Much of the concern arose from the use of traditional LD_{50} tests for chemicals and drugs to determine acute toxicity. However, regulatory testing requirements for acute toxicity now allow for animals that are moribund or exhibiting clinical signs of severe pain and distress to be euthanized rather than to die spontaneously. Euthanasia also provides tissues more appropriate for subsequent study and alleviates potential suffering by the animal. Hence, euthanasia is often preferable to death for both scientific and ethical reasons.

The use of death as an endpoint is discouraged and must always be justified. Endpoints other than death must always be considered and should be used whenever the research objective can be attained with non-lethal endpoints. Use of death as an endpoint must be justified in writing in proposals and its use must be approved by the IACUC prior to beginning a study.

C.2.c. Table A. Examples of Humane Endpoints for Studies with Potential Lethality

Endpoint	Characteristics	Applications
Tumor growth or effects	Tumor exceeds 10% of normal body weight; necrosis, infection, ulceration, interference with ambulation or eating/drinking	Subcutaneous or intraperitoneal tumors and hybridomas
Prolonged inappetence/ Cachexia	Rapid loss of weight (>20% of normal body weight) and/ or condition	Metastatic disease, chronic infectious disease
Inability to ambulate	Prolonged recumbency	Many
Signs of severe organ or system involvement	Respiratory: rapid or labored breathing, coughing, rales	Toxicity testing; systemic disease
	Cardiovascular: shock, hemorrhage, anaphylaxis	
	Gastrointestinal: severe diarrhea or vomiting	
	Peripheral Nervous System: flaccid or spastic paralysis	
	CNS Signs: circling, blindness, dementia, convulsion	
Progressive hypothermia	Decrease of 4-6ºC in body temperature in rodents	Infectious disease studies; vaccine potency studies
Moribund or pre-moribund state	Define with specific clinical signs and euthanize when reached	Many

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C.2.d. Minimization of Pain and Distress

It is the responsibility of the IACUC to critically evaluate all research protocols for the potential to cause pain or distress and assess the steps that are to be taken to enhance animal well-being.

As required by the *PHS Policy* and the AWRs, and reiterated in the *Guide*, the IACUC is mandated to review protocols to ensure that pain and distress are minimized in laboratory animals. The AWRs stipulate that the IACUC determine that the principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animal and has provided a written narrative description of the methods and sources used to determine that alternatives were not available. Additional guidance from the USDA on this subject is provided in their policies. The *Guide* states that the IACUC should ensure the protocol addresses:

- appropriate sedation, analgesia, and anesthesia;
- criteria for timely intervention, removal of animals from study, or euthanasia if painful or stressful outcomes are anticipated; and
- details of postprocedural care.

The protocol must provide adequate information for the IACUC to assess the potential animal pain and/or distress resulting from the study and the effectiveness of the pain- and distress-relieving agents proposed for use. Criteria for re-dosing the animal should also be established. The AV must be consulted for any procedure that has the potential to cause more than momentary pain or distress.

Examples of procedures which the *Guide* suggests may have the potential to cause pain or distress, include:

- physical restraint,
- survival surgeries,
- food or water restriction,
- death as an endpoint,
- noxious stimuli,
- skin or corneal irritancy testing,
- tumor burdens,
- intracardiac or orbital sinus blood sampling, and
- abnormal environmental conditions.

Assessing Pain and Distress

Numerous references indicate that both laboratory animals and humans receive and process noxious stimuli using similar mechanisms and with similar thresholds of awareness. The pain tolerance, or maximum stimulus intensity voluntarily accepted, varies between species and between individuals of the same species, including humans. Pain typically results from stimuli that damage tissue or have the potential to damage tissue. An animal's response to pain is often adaptive to reduce movement to minimize reinjury and aid recuperation. However, this response may lead to physiological and behavioral changes which impact negatively on both the animal's well-being and the research results.

Fundamental to the relief of pain is the ability to recognize its clinical signs in various species of animals. Due to the inability of animals to verbalize, it is essential that animal care staff and researchers receive adequate training on how to recognize clinical signs of pain and distress. Tables A, B, and C at the end of this section identify some of the indicators.

According to the NRC report on pain and distress, while there are no generally accepted criteria for distress, there are a number of metabolic, physiologic and behavioral parameters that are altered in distressed animals. These include changes in reproductive performance, elevation in glucocorticoid levels and elevation in catecholamine levels. It is necessary to use objective assessments, which means choosing appropriate parameters and quantifying observations. Numerous models for scoring pain and distress have been published and involve assigning a numeric score to

observations with the aid of descriptors. It is often useful to start with a general set of observations for assessing pain and distress such as change in body weight, physical appearance/posture or changes in unprovoked and provoked behavior. The assessment system should then be modified on a case-by-case basis using specific changes that may be anticipated in a particular study.

Alleviation of Pain and Distress

Accepted best practices for dealing with the possibility of unrelieved pain and distress should be considered and incorporated into protocols unless there is a sound scientific rationale for deviation from those practices. The investigator must also provide an assurance that unrelieved pain will continue for only the minimum period of time necessary to attain the study objectives.

Protocol methodology should be considered which decreases the potential for pain or distress. In addition to thorough searches of the literature, this can be done through the careful use of pilot studies to determine earlier endpoints or less invasive alternatives.

Pharmacologic treatment of pain or distress should be given as consistent with the type of pain/distress and the needs of the research question. The veterinarian must be consulted for all such protocols and should provide guidance to investigators and the IACUC. The responses of different species to different anesthetics, analgesics or tranquilizers vary and are not fully defined. Often the effects of a given drug have only been examined in a single species and definitive information, for example, on cardiovascular and respiratory function or on the ability to relieve the perception of noxious stimuli, is missing. As a result, dosages have been developed on the basis of the amount required to produce cessation of movement when the animal is confronted by what is assumed to be a painful manipulation, in conjunction with an adequate recovery. Because of the imprecise nature of the studies, dosage ranges are often quite wide, requiring a very conservative approach to their use. The use of drug mixtures further complicates the choice of an adequate dose. Numerous reference texts exist and IACUCs may request that the veterinarian prepare current charts of recommended doses as an institutional resource for investigators.

Non-pharmacologic treatments should also be employed. This may include special housing considerations, dietary and other environmental enrichments, adjustments and careful supportive care.

Summary

It is the responsibility of the investigator to show she or he has considered all the options for minimizing pain and distress that do not compromise the scientific validity of the experiment. The committee's deliberations regarding the management of potential pain and distress in a protocol should be documented. Personnel should be trained in pain and distress management. The IACUC should ensure that there is a mechanism in place for prompt reporting of sick animals to the veterinary staff.

C.2.d. Table A. Definitions of Terminology Related to Pain and Distress

Analgesia	A complete loss of sensitivity to pain.
Anesthesia	A total loss of sensation in a part of or in the entire body.
Distress	An aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and shows maladaptive behavior.
Pain	An unpleasant sensory or emotional experience associated with actual or potential tissue damage.
Sedation	A state characterized by decreased awareness of surroundings, relaxation, and sleepiness. Analgesia is not present.
Tranquilization	A state of mental calming, decreased response to environmental stimuli, and muscle relaxation. No sleep, analgesia or anesthesia is present, even at increased dosage.

From: *American College of Laboratory Animal Medicine. 1997.* Anesthesia and Analgesia in Laboratory Animals. *D.F. Kohn, S.K. Wixson, W.J. White and G.J. Benson, eds. Academic Press.*

C.2.d. Table B. Signs of Acute Pain

Sign	Explanation
Guarding	Attempting to protect, move away, or bite
Vocalization	Crying out when palpated or forced to use affected area
Mutilation	Licking, biting, scratching, shaking, or rubbing
Restlessness	Pacing, lying down and getting up, or shifting weight
Sweating	In species that sweat (horses)
Recumbency	Unusual length of time
Depression	Reluctance to move or difficulty in rising
Abnormal appearance	Head down, tucked abdomen, hunched, facial distortion, or pallor

Reprinted with permission from SOMA, 1987. Soma, L.R. 1987. Assessment of animal pain in experimental animals. Lab Anim Sci *37:71-74. Reprinted with permission from Recognition and Alleviation of Pain and Distress in Laboratory Animals. Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC. 1992.*

C.2.d. Table C. Signs, Degree and Length of Surgically Produced Pain*

Surgical Site	Signs of Pain	Degree of Pain	Length of Pain
Head, eye, ear, mouth	Attempts to rub or scratch, self-mutilation, shaking, reluctance to eat, drink, or swallow, reluctance to move	Moderate to high	Intermittent to continual
Rectal area	Rubbing, licking, biting, abnormal bowel movement or excretory behavior	Moderate to high	Intermittent to continual
Bones	Reluctance to move, lameness, abnormal posture, guarding, licking, self-mutilation	Moderate to high: upper part of axial skeleton (humerus, femur) especially painful	Intermittent
Abdomen	Abnormal posture (hunched), anorexia, guarding	Not obvious to moderate	Short
Thorax	Reluctance to move, respiratory changes (rapid, shallow), depression	Sternal approach, high; lateral approach, slight to moderate	Continual
Spine, cervical	Abnormal posture of head and neck, reluctance to move, abnormal gait—"walking on eggs"	Moderate to severe	Continual
Spine, thoracic or lumbar	Few signs, often moving immediately	Slight	Short

**Based on observations of dogs.*

Reprinted with permission from Recognition and Alleviation of Pain and Distress in Laboratory Animals. Committee on Pain and Distress in Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, Washington, DC. 1992.

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C.2.e. Personnel Qualifications

In evaluating proposed research projects, the *PHS Policy* and the AWRs require the IACUC to assess whether personnel conducting procedures are appropriately qualified and trained in those procedures (IV.C.1.f and 2.31(d)(1)(viii)). A similar requirement can be found on page 10 of the *Guide* and in U.S. Government Principle VIII (see Appendix F).

Developing Guidelines

To facilitate evaluation of personnel qualifications and training during protocol review, each IACUC should develop a list of items to be assessed as well as a list of classifications of personnel required to participate in such training. This could be a list of qualifications and training items specific to protocols according to procedures and or manipulations proposed or the list could be broad enough to cover all aspects of the institution's training requirements (see Section A.4).

A procedure specific checklist might include:

- proficiency in handling specific specie(s),
- proficiency in pain-relieving methods,
- proficiency in surgical manipulations,
- proficiency in aseptic techniques,
- proficiency in pain management,
- proficiency in euthanasia,
- proficiency in pre- and post-operative care,
- Drug Enforcement Administration (DEA) license, and
- approval by safety office.

A checklist of institutional requirements that need to be satisfied as a component of protocol review might include the following in addition to those above:

- completion of occupational health and safety risk assessment,
- demonstrated knowledge of relevant rules and regulations,
- enrollment in occupational health and safety program,
- attendance at compliance training session, and
- viewing of safety training video.

Classifications of employees whose qualifications and training may require assessment include:

- investigators,
- research technicians,
- animal husbandry personnel, and
- veterinarian and veterinary technicians.

An important decision to be made by the IACUC is the level of training required of an investigator not actually involved in the day-to-day manipulation and care of the animals. If the investigator is responsible for the research activity and the animals involved, should she or he demonstrate proficiency in the areas indicated above? Is the investigator responsible for training personnel in the lab? If yes, should she or he demonstrate proficiency in those areas? An IACUC policy on this issue will prevent conflict later.

Evaluating Qualifications and Training

To prevent problems related to assessment of qualifications and training during protocol review, it is helpful if the IACUC determines any training needs during the protocol development and veterinary consultation. Discussion of new techniques, procedures, or manipulations at this time can provide the impetus for a training opportunity for both the veterinary staff and the research staff with demonstrated proficiency completed prior to protocol review. This training experience should be so noted in the protocol or otherwise documented.

Maintaining a database of all participants in the facility's training program who use laboratory animals will facilitate assessment of qualifications and training. With such a database, preliminary evaluation of an individual's

expertise can be an administrative task performed by the IACUC or staff assigned to assist with managing the animal care program. If a deficiency is noted, a follow-up memo can be sent to the investigator stating that protocol review is pending until training requirements have been completed.

IACUCs should note that high morbidity or mortality rates or requests for more animals than originally planned may indicate a training opportunity and should be followed up in the context of the relevant protocol, either immediately or during the semiannual review.

Evaluating the qualifications and training of new personnel or those proposing to use new techniques, procedures, or manipulations will necessitate another approach by the IACUC.

New Personnel

One way to manage the training of new personnel is to initiate an IACUC policy that no protocol will be reviewed until training requirements have been satisfied. Such training would need to incorporate all institutional requirements as well as those specific to the work expectations of the individual, and might include those listed above.

New Techniques, Procedures or Manipulations

When an investigator proposes new techniques, procedures, or manipulations, the IACUC must assure itself that the personnel are qualified to perform the work. If no training module on a particular technique, procedure, or manipulation exists, it is possible that the most closely aligned existing module can be used. If the personnel have not demonstrated proficiency through one of the training modules (see Section A.4), the IACUC can consider the following options:

- The IACUC may mandate that the individual(s) complete pertinent training before the protocol can be reviewed. This assumes the IACUC has a policy that stipulates adequate qualifications and training as a condition of protocol review.
- If no relevant training module exists, a possible course of action would be to stipulate that the veterinarian supervise the new technique, procedure, or manipulation pending certification of training or demonstration of proficiency. If there are no in-house personnel with the necessary expertise, the IACUC can seek a consultant for advice

> and training. This should not be viewed as a confrontational event, but rather one with educational value for both the veterinarian and the research staff. Documentation of this training experience should be made in the IACUC files or database.

In summary, evaluation of personnel qualifications and training is an essential component of the review of animal use protocols to ensure the humane care and use of laboratory animals. The challenge to IACUCs is to perform this evaluation in an efficient, consistent and uniform manner.

C.2.f. Veterinary Review and Consultation

Each IACUC is required by the AWRs and the *PHS Policy* to have as one of its members a Doctor of Veterinary Medicine with direct or delegated program authority and responsibility for animal activities at the institution.

The AWRs and the *PHS Policy* require that the veterinarian be trained or experienced in laboratory animal science and medicine for the species used at the institution; the *Guide* recommends the IACUC veterinarian be American College of Laboratory Animal Medicine (ACLAM) certified or have equivalent experience.

The Report of the American College of Laboratory Animal Medicine on Adequate Veterinary Care in Research, Testing and Teaching (1996) states:

> The veterinarian must be involved in the review and approval of all animal care and use in the institutional program. This includes advising on the design and performance of experiments using animals as related to model selection, collection and analysis of samples and data from animals, and methods andtechniques proposed or in use. This responsibility is usually shared with investigators, the IACUC, and external peer reviewers.

The veterinarian plays a key role in IACUC protocol review, as described below.

Reviewing Animal Use Protocols

The veterinarian can integrate his or her experience and training with that of the investigator and advise the investigator on selection of species, their sex, age and/or size. The veterinarian can assess the ability of the animal facility and its staff to support the proposed species and associated procedures.

When the selection criteria have been established, the veterinarian can assist the IACUC in reviewing the proposed procedures and techniques appropriate to the goals of the study.

Reviewing Protocols for Potential Pain and Distress

The AWRs require that investigators proposing procedures that may cause more than momentary or slight pain or distress to the animals will consult with the AV or his or her designee. Similarly, the veterinarian has implicit responsibilities outlined in the AWRs to assess the potential for pain and distress that might be associated with the proposed animal activities, and to recommend the use of pain alleviating drugs, whenever possible, to counteract those conditions.

Reviewing Protocols Involving Surgery

The veterinarian can ensure that appropriate provision is made for pre-operative and post-operative care of the animals in accordance with established veterinary medical and nursing practices. As noted in the AWRs and the *Guide*, all survival surgery should be performed using aseptic procedures, including the use of surgical gloves, masks, sterile instruments, and aseptic techniques.

The veterinarian may provide the IACUC with assessment of the following:

- preparation of the animal for the surgical intervention, to include the use of pre-anesthetic drugs where indicated, and appropriate anesthetic agents;
- that the individual(s) performing the surgery has adequate experience or training for the specific procedures outlined in the study;

- that aseptic techniques are appropriate for the procedure; and
- that adequate post-operative care, to include post-operative analgesics where indicated, is provided.

Reviewing Protocols To Ensure Humane Euthanasia of Animals

The American Veterinary Medical Association (AVMA) provides guidance on the most humane methods to be used for euthanasia of animals, to include those used in research, testing and training. Their most recent recommendations are contained in the "2000 Report of the AVMA Panel on Euthanasia" (*JAVMA* Vol. 218, No. 5, pages 669-696). The veterinarian on the IACUC, using that publication or subsequent editions as the principal reference, can assess the investigator's proposed method of euthanasia.

After Protocol Review and Approval

Following IACUC approval of protocols, the veterinarian is in a position, through periodic visits to the animal facility and animal activity areas, to observe and evaluate animal well-being and decide whether the animal activities are being conducted in accordance with the conditions described or referenced in the protocol. The veterinarian, by virtue of training and experience, is able to serve in advocacy, oversight, and intervention roles that are distinct and unique among the IACUC members and research staff.

Checklist

*Some Examples of the Veterinarian's Responsibilities During Protocol Development and Review**

- Choice and use of appropriate analgesics/anesthetics
- Verification of appropriate drug dosages, route of administration and choice of agent
- Assistance in selection of appropriate animal model
- Identification of refinement initiatives to ensure that manipulations have a minimal impact on animal welfare
- Oversight of aseptic surgery and peri-operative care
- Oversight of animal health and husbandry pertinent to the protocol and the entire colony

- Identification of possible iatrogenic complications of model and procedures selected
- Ensuring there are appropriate remediation efforts for iatrogenic complications
- Serving as an occupational health and safety (including zoonoses) resource
- Serving as a regulatory compliance resource
- Assistance in identifying appropriate endpoints and in ensuring humane euthanasia.

**This checklist is not all-inclusive; rather it provides examples of the veterinarian's responsi bilities, which may vary with each proposal.*

C.3. Other Protocol Review Considerations

C.3.a. Agricultural Research

Farm animals are used in a variety of research contexts, including:

- vaccine trials,
- studies of basic biological processes,
- studies of pharmacokinetics and organ transplantation, and
- studies of nutritional, breeding and management methods to increase the supply and quality of food and fiber.

Unlike typical laboratory animals, farm animals used for research and teaching may be housed in many different kinds of environments, ranging from traditional laboratory environments to enclosed or extensive farm settings. Because of these factors, as well as the regulatory complexity surrounding farm animal oversight, determining standards for the evaluation of research, teaching, and testing using farm animals is more complicated than for other laboratory animals.

Applicability of PHS Policy and the AWRs

Farm animals used for improving animal nutrition, breeding, management, production efficiency, or the quality of food and fiber are specifically excluded from the definition of "animal" in the AWA. The *PHS Policy* applies to vertebrates used in research, research training and biological testing, funded by the PHS. Some Assurances extend coverage of the *PHS Policy* to all animal activities at an institution. Hence, farm animals used in research, teaching or testing may be covered by the *PHS Policy* and the AWRs. Farm animals used in agricultural research may not be covered by either.

OLAW advises institutions that uniform and consistent standards are an essential ingredient in a quality animal care and use program. Public perception of a potential double standard should also be considered.

Standards for Evaluation of Agricultural Animal Research and Teaching

In 1988, a consortium of organizations and agencies developed guidelines for the care and use of farm animals, the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (known as the *Ag Guide*). The *Ag Guide*, revised in 1999, was written to aid IACUCs in the evaluation of projects involving farm animal research or teaching "for which the scientific objectives are to improve understanding of the animal's use in production agriculture and that may require a simulated or actual production setting." The *Ag Guide* is comprised of overview chapters covering institutional policies, veterinary care, husbandry, and physical plant, as well as specific species chapters for horses, cattle, poultry, and sheep and goats. Adoption of the *Ag Guide* by an institution is voluntary, although the USDA endorses it as a basis for animal care review of USDA competitive grant submissions and projects receiving experiment station funding.

This dual system of oversight for research and agricultural animals can pose challenges for IACUCs. In order to be relevant to commercial production, agricultural research must often be conducted under conditions similar to those found on commercial farms. However, there are practices that are common in commercial agriculture that would not ordinarily be permitted under the regulations governing research; for example, castrating young animals without anesthesia or closely confining animals in cages or stalls throughout the production cycle. But determining whether a particular protocol is agricultural or biomedical research, and which standards should be applied, is not always straightforward. For example, studies of basic biological processes in farm animals may benefit food and fiber production, but may also have human health implications. USDA Policy 26 provides some clarification, stating that farm animals used to manufacture and test biologicals for nonagricultural or nonproduction animals, or for humans, are considered research animals and thus are regulated under the AWA. But gray areas remain, and IACUCs need to consider animal welfare, protocol requirements, and research or teaching goals when setting standards.

Recently, there has been recognition that some melding of these different guidelines and standards may be necessary and appropriate. For example, the *Guide*, while intended to apply only to farm animals used for research purposes, recognizes that such animals may sometimes be housed in farm settings, and recommends the *Ag Guide* as a useful resource in such situations. And although USDA-APHIS decided to regulate farm animals used in research in 1991, they did not develop specific standards; instead, they adopted the *Ag Guide* and the *Guide* as guidance documents (Policy 29).

AAALAC also uses both the *Guide* and *Ag Guide* as reference documents for the accreditation of farm animal facilities and programs. Thus, the use of a performance-based approach is desirable.

Review of Protocols and Facilities

Institutions employ a number of different approaches to reviewing activities involving animals used for agricultural research and teaching. Some have a single committee that reviews all protocols, while others have a subcommittee or even a separate committee that reviews agricultural animal research protocols. (As applicable, committees must comply with the membership and review procedures required by the *PHS Policy* and the AWRs.) There are benefits and limitations associated with each of these approaches. However, what is most important is that the institution ensures uniform and high-quality oversight of all research, teaching, and testing activities involving animals, regardless of the species or the type of research being conducted.

For thorough oversight of agricultural animal care and use, it is particularly important that there be agricultural expertise on the IACUC. The *Ag Guide* suggests that the IACUC include, among other members:

- a scientist from the institution with experience in agricultural research or teaching involving agricultural animals;
- an animal, dairy or poultry scientist who has training and experience in the management of agricultural animals; and
- a veterinarian who has training and experience in agricultural animal medicine and who is licensed or eligible to be licensed to practice veterinary medicine.

There are unusual aspects of agricultural research that deserve careful consideration by IACUCs. As mentioned previously, there are certain husbandry practices common on commercial farms that have the potential to cause pain or distress that would not ordinarily be permitted under the regulations governing research. The *Ag Guide* recommends that IACUCs review these procedures, as well as husbandry conditions that do not meet the standards outlined in the *Ag Guide*, even if they are considered normal practice. Another unusual aspect of agricultural research is that the animals may be killed and marketed for human food at the end of studies, which means that there are special considerations with respect to avoiding residues from therapeutics and other drugs.

The extent of oversight is another issue that IACUCs need to address. At institutions with agricultural colleges, there may be multiple lines of authority for animal facilities and animal ownership. In addition, animals may be housed at off-site facilities at some distance from the main unit. The IACUC needs to ensure that there is adequate oversight of all animals under approved protocols. Agricultural and veterinary extension faculty may also conduct research or teach using privately owned animals on private farms, and the IACUC should consider whether or not these activities need to be covered by protocols.

Finally, the facilities in which agricultural animals are housed are often older than typical laboratory animal facilities. Because many of these facilities are semi-enclosed or open, there may be problems with rodent control and some other aspects of maintenance. Recordkeeping in agricultural animal facilities may be less complete than that required in conventional lab animal facilities. The IACUC should be aware that there can and should be a high standard of animal care even in modest facilities. The development and implementation of standard operating procedures for these facilities can help to ensure a consistent standard of animal care.

Conclusion

Although not always required by law, the monitoring of food and fiber animal research and teaching activities can significantly benefit an institution by improving the overall quality of the animal care program. Because agricultural research often has the improvement of food or fiber production as an endpoint, standards may differ from those for research animals. This does not mean, however, that different ethical standards should be used by an IACUC in considering the use and care of farm animals used for food and fiber research. Experimental goals and animal welfare should both be considered when evaluating the use and treatment of these animals.

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C.3.b. Antibody Production

Antibodies are important tools for research. Depending on research needs antibodies may be produced by polyclonal or monoclonal technique. Each technique requires that specific issues be addressed in animal protocols. IACUCs should ensure adequate training of personnel in the use of proper technique when any method of immunization is proposed. The advantages of a centralized service utilizing skilled technicians to meet multiple research groups' needs for polyclonal and monoclonal antibodies is another refinement which may enhance animal welfare in larger research programs. There are also many commercial sources of antibodies made to order.

A good resource is "Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies." AWIC Resource Series, No. 3. August 1997. Call Number: aHV4701.A94no.3. ISBN 090076791X. The document includes over 500 bibliographic citations regarding adjuvants and antibody production methods compiled from scientific journals, proceedings and newsletters. A company/institute listing of suppliers of antibodies and antibody production products is included. Emphasis is placed on citing comparative studies and research into alternative methods.

Polyclonal Antibody Production

Injection of an immunogen (e.g., protein, virus, bacterium) into an animal produces a humoral response, which induces the production of a population of heterogeneous antibodies, with varying specificities toward different molecular regions (epitopes) of the immunogen. Two types of lymphocytes (T cells, derived from the thymus, and B cells, derived from marrow) are responsible for the production of polyclonal antibodies. Polyclonal antibodies produced in response to infection can be effective in recognizing and eliminating foreign material, but the heterogenicity of the product limits its use in research and industry.

Adjuvants

To increase the immune response, the immunogen may be combined with an adjuvant. Adjuvants stimulate the rapid and sustained production of high titers of antibodies with high avidity. Adjuvants may facilitate the immune response through three basic mechanisms:

- Adjuvants may serve as a depot for the antigen, which should increase the duration of antigen exposure and the antibody response.
- Adjuvants may stimulate immune cells.
- Adjuvants may enhance macrophage phagocytosis after binding the antigen as a particulate (a carrier/vehicle function).

The use of adjuvants is required for many antigens which by themselves are weakly immunogenic. Adjuvant selection remains largely empirical. Antigens that are easily purified or available in large quantities may be good choices for starting with the least inflammatory adjuvants for immunization. Should antibody response not be suitable, a gradual increase in the inflammatory level of the adjuvant would then be warranted.

The choice of the appropriate adjuvant is important from both the aspect of the end result (high antibody response) and the welfare of the immunized animal. Many of the adjuvants have the capacity to cause inflammation, tissue necrosis and pain in animals. A major charge to investigators is to minimize animal use and discomfort.

Freund's incomplete adjuvant (IFA) is a water/oil emulsion containing immunogen, paraffin oil and an emulsifying agent. Addition of killed mycobacteria to the oil phase (Freund's complete adjuvant, CFA) enhances the immune response. Multiple exposures to CFA will cause severe hypersensitivity reactions. The use of CFA can be painful and alternative adjuvants should be considered. Abscesses, granulomas and tissue sloughs may occur at injection sites. However, a recent report (Halliday) suggests that when the NIH intramural guidelines are meticulously followed, assuring aseptic technique and adding the judicious use of chemical sedation, the use of CFA for immunization is a humane procedure. Undesirable and painful side effects must be minimized or eliminated by careful preparation of inoculum, the use of appropriate routes of administration, adequate separation of injection sites, and the use of a small amount of inoculum per site.

Because of the severity of the secondary immune response to mycobacterium in CFA, IFA must be used with booster antigen administrations in cases where CFA has been used in the initial injection.

For many years CFA was the only effective adjuvant, but this is no longer true. Other adjuvants are available as alternatives and may be suitable for use in an investigator's experiments.

Route of Injection

The range of recommendations for routes and sites of administration of antigen-adjuvants preparations, volumes per site and number of sites per animal for different species vary in the literature and institutional guidelines. Particularly with the use of CFA, it is important to note that the severity of potentially painful inflammatory reactions may be minimized by injection of a small volume of inoculum per site and the use of multiple injection sites when appropriate. Injection sites must be sufficiently separated to prohibit coalescing of the inflammatory lesions.

Using multiple sites for immunization also provides more foci for antigen presentation and the involvement of more lymph nodes. Intradermal and subcutaneous routes are commonly used to take advantage of antigen-processing dendritic cells present within the dermis. Hair should be clipped from intradermal and subcutaneous injection sites, and the site should be aseptically prepared with betadine or nolvasan scrub followed by alcohol or other appropriate antiseptics. The following recommendations apply primarily to antigen solutions in CFA or IFA. Volumes ranging from 0.05 ml to 0.10 ml per site have been recommended for intradermal injections in rabbits. A total of five intradermal sites has been recommended. Because intradermal sites ulcerate with FCA, sterile inocula must be used and the site must be properly disinfected to prevent secondary bacterial infection. Subcutaneous injection volumes in the rabbit vary from recommendations of 0.10 ml to 0.25 ml to 0.40 ml per site. Number of sites recommended varies from 4 to 10.

Footpad injections in rabbits are prohibited. Where scientific justification is provided, footpad injections may be permitted in rodents, but only in one hind foot, and with the animals housed on soft bedding. Suggested maximum injection volumes can range from 0.01 to 0.05 for mice and 0.10 ml for rats. The need for footpad injections must be critically evaluated by the IACUC before approval.

Sometimes direct inoculation into lymph nodes, such as the popliteal lymph node, is used. With practice these nodes often can be palpated and the injection performed percutaneously.

Intramuscular injections, usually made in the biceps femoris or quadriceps muscle mass, generally are lower volumes of 0.25 ml to 0.20 –0.40 ml. Care must be exercised to avoid adjacent nerves and blood vessels as well as fascial planes when injecting into a muscle bundle. Disagreement exists as to the appropriateness of intramuscular injection of CFA. The intramuscular route of injection is recommended in some institutional guidelines and specifically discouraged in other guidelines. Intramuscular injection is generally not recommended in rodents because of limited muscle mass.

For TiterMax®, intradermal, subcutaneous, and intramuscular routes are recommended with volumes per injection site ranging from 0.01 to 0.25 ml in small and large animals. For Ribi®, intradermal, subcutaneous and intramuscular routes are recommended with volumes per injection site ranging from 0.05 to 0.50 in small and large animals.

Monoclonal Antibody Production

Monoclonal antibodies (mAbs) are homogeneous because they are produced by hybrid cells derived from a single antigen-stimulated B cell. The production of mAbs involves two phases. In the first phase an animal (usually a mouse) is immunized with the antigen of interest. Immunization of the antigen is often performed with an adjuvant, as discussed above. Splenocytes are harvested from the responding animal, and are fused with a myeloma cell line for *in vitro* propagation.

Before the immunization protocol begins, the methodology for detecting the specific antibody of interest in the mouse sera and tissue culture supernatants is developed. Otherwise, significant time and animal resources may be wasted later in the mAb-developing phase. Test bleeds should be performed in order to determine if the mice are responding to the immunizations. Most immunologically based assays for determining if the desired antibodies are being produced require less than 10 microliters of mouse serum. Once an appropriate response has been confirmed the mice should be boosted again and typically after three days from the boost the mice should be euthanized and spleens harvested.

The second phase is production of adequate quantities of mAb for a project or analysis. There are two major methods: *in vitro* and the ascites method.

The ascites method has been one of the most popular means for producing large quantities of highly concentrated monoclonal antibodies since its inception in 1972. However, improved techniques and culture media have demonstrated that mAbs can be produced by *in vitro* techniques at a quality and concentration that are similar to that of ascites. The National Research Council's report on Monoclonal Antibody Production specifically states "*in vitro* methods for the production of monoclonal antibodies should be adopted as a routine method unless there is a clear reason why they cannot be used…". In accordance with the *PHS Policy* and the *Guide*, alternatives to the use of animals (*in vitro* techniques) for the production of mAbs must be considered in place of the ascites method. (See the Office of Extramural Research Guidance concerning the Production of Monoclonal Antibodies in Animals, *NIH Guide for Grants and Contracts*, Notice OD-00-019, 2/3/2000, and the 11/17/97 OPRR Dear Colleague letter on Production of mABs Using Mouse Ascites Method).

The ascites method should only be used after *in vitro* failure of each cell line has been demonstrated, or other adequate justification is provided. Analysis of individual cell lines is necessary because the production performance of each hybridoma cell line grown *in vitro* is highly variable. Despite this variability, work performed by Petrie indicates that at least 90% of all hybridomas that are placed on *in vitro* production protocols will yield adequate amounts of high quality mAbs.

Several resources for the *in vitro* production of mAb are available. Some institutions have core facilities that may provide an *in vitro* mAb production service. The NIH also sponsors a national cell culture core facility (National Cell Culture Center, Minneapolis, MN; http://www.nccc.com).

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C.3.c. Breeding Colonies

Investigators maintain breeding colonies for a variety of reasons. A breeding colony may be required for an established animal model because:

- the animal model is not commercially available,
- young animals have very specific age or weight requirements that cannot be fulfilled by a commercial breeding colony, or
- physiological status of the mutant animal is too severely affected for it to survive shipment.

Investigators developing a new spontaneous or induced mutant animal model need to maintain their own breeding colony because there is no alternative source for this mutant. While trying to establish a breeding colony for a new mutant model, the investigator is also simultaneously working to determine phenotype, to identify affected physiological system(s), and define inheritance pattern.

To review standard operating procedures for breeding colonies, the IACUC will need information about colony management. Examples of necessary information include:

- number of breeders and number of young per cage,
- breeding system including number of females per male or continuous versus interrupted mating,
- weaning age,
- separation of animals at weaning, and
- methods for identification of individual animals.

Large numbers of animals may be required to maintain a breeding colony. The exact number of animals can only be approximated because it is impossible to predict in advance the exact number and sex of offspring. The estimated number of animals should clearly distinguish between:

- breeders,
- young that cannot be used in experiments because they are of the wrong genotype or sex, and
- animals that will be subject to experimental manipulations.

Colony management practices should be briefly described in the investigator's animal protocol, and justification provided for departure from standard institutional practices.

Determining which animals to include in the estimated number of animals on an animal protocol can be challenging to the investigator and the IACUC in the absence of IACUC-developed guidelines. The estimated number of animals that are kept for breeding purposes and not subject to any experimental manipulations should be part of the animal protocol.

Studies involving genetic analysis are animal intensive. Genetic analysis can involve determining if a single gene has dominant or recessive inheritance, identifying different genes involved in a quantitative (polygenic) trait,

or fine mapping to determine chromosomal location of a mutant gene. It is possible for the investigator to estimate the number of animals required, but difficult for the IACUC to evaluate this estimate in the absence of experience.

Up to 1200 mice are required to map a single gene with recessive inheritance and full penetrance, and have adequate numbers of progeny for developmental studies, phenotyping and linkage analysis. This number assumes a breeding colony of 10 to 12 pair matings with a 6- to 8-month reproductive lifespan, around 90% productive matings, replacement of breeders, and no unusual mutant infertility or mortality.

Up to 1100 mice are required for quantitative trait loci analysis using analysis of F2 progeny. The number assumes small breeding colonies of two inbred parental strains (4 to 6 pairs) and two reciprocal F1 hybrids (2 to 4 pairs), no unusual infertility, replacement of breeders at 6- to 8-month intervals, and generation of between 500 and 1000 F2 mice for genotyping.

Up to 750 mice are required to construct a congenic strain using "speed'" congenic genotyping methods. This number assumes a breeding colony of 10 to 12 breeding pairs, replacement of breeders, and progeny for phenotyping and genetic linkage. If the homozygous mutant does not breed and the congenic strain must be developed using intercross matings, the estimated number of mice increases to 1,200.

After founder transgenic or 'knock-out' mice have been identified, between 80 and 100 mice may be needed to maintain and characterize a line. The number assumes up to five breeder pairs per line, breeder replacement, no unusual infertility and adequate numbers of weanlings for genotyping and phenotyping characterization.

If a study requires fertilized one-cell eggs, embryos or fetuses, the protocol should indicate the number of eggs, embryos or fetuses that are required for proposed studies. The estimated number of experimental animals may be limited to the number of female animals that are mated and euthanized or surgically manipulated to collect the required eggs, embryos or fetuses. In this situation, males might be listed as breeders if they are not subject to any experimental manipulation.

If a suckling animal will be subject to any manipulation, such as thymectomy, toe clip or ear notch for identification, tail tip excision for genotyping, or behavioral tests, the estimated number of manipulated sucklings must

be included in the number of animals used. If suckling animals will be euthanized at or prior to weaning because they are the wrong genotype or sex for the experiment, then they may be included as animals held or euthanized but not subject to experimental manipulations.

One option is for the IACUC to request estimated animal numbers as follows:

Estimated number of weaned and adult animals to be subject to experimental manipulations	_____*
Estimated number of suckling animals to be subject to experimental manipulations	_____*
TOTAL	_____

*Estimated numbers should be further subdivided based on invasiveness of procedures using institutional criteria:

Estimated number of breeders held but not subject to experimental manipulations	_____
Estimated number of suckling animals to be euthanized at or prior to weaning, and not subject to experimental manipulation	_____

In summary, the IACUC's role for oversight regarding breeding colonies includes ensuring that the need for a breeding colony has been established based on scientific or animal welfare concerns, that the procedures used in the breeding colony are evaluated and approved by the IACUC on a regular basis (e.g., as part of the semiannual program review), that there is a mechanism for tracking animals, and that the standards of care and animal well-being for the animals in the breeding colony are consistent with the *Guide*.

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C.3.d. Field Studies

Federal requirements and the *Guide* focus primarily on the care and use of laboratory animals in research facilities. The same guiding principles, however, apply to the use of vertebrate species in field studies.

Application of the requirements and guidelines often pose unique challenges to the investigator and the IACUC because of the nature of field research. For example, field sites are often at a distance and may be remote, making it impractical for IACUC inspections. One solution is to require the investigator to provide photos, videotapes or other information that can help the committee evaluate the use of animals. For some projects the committee can find a consultant near the field site to perform an inspection and report to the IACUC. If the studies fall under the AWRs, at least two members of the IACUC must conduct the site inspections. Other difficulties relate to the nature of the research and the populations to be studied, which may be unfamiliar to the IACUC.

Professional field biologists in organizations devoted to the study of fish, amphibians, reptiles, birds, and mammals have prepared guidelines for field work with these populations; these guidelines form a useful reference and can assist the investigator in planning, and the IACUC in reviewing, field research using vertebrate animals. The references at the end of this section cite such guidelines. Appendix E includes a list of professional societies and contact information. These organizations can assist by referring the IACUC to appropriate individuals and authorities.

There is a comprehensive set of laws intended to protect wild animal populations. Appendix E describes these laws and the manner in which they are implemented and enforced. Virtually all activities involving birds, for example, require permitting under the Migratory Bird Treaty Act, the Endangered Species Act, the Bald and Golden Eagle Protection Act, or other permitting requirements. The investigator must be able to assure the IACUC that all necessary federal and state permits have been or will be obtained before research begins.

The proposed study can be assessed by the IACUC in a manner similar to laboratory studies if the protocol prepared by the PI addresses the following relevant items:

- species selection,
- site selection, and
- methodologies employed.

Species Selection

The investigator should provide information on the population to be studied and a rationale for choosing that particular population. The U.S. Fish and Wildlife Service (USFWS) issues many of the necessary permits. In issuing permits the USFWS assesses the risk to the animal population and the IACUC may rely on that assessment rather than attempt to determine the potential impact on the population.

With regard to small or declining populations, many state wildlife or natural resource agencies also issue research permits. In the event that a state research permit is required and has been issued, the IACUC may assume that the state agency has assessed the risk to the population and found it to be acceptable.

An IACUC that has additional questions about the selection of species or the impact on the population to be studied may require the investigator to provide additional information or the Committee may consult with biologists with relevant expertise.

Site Selection

The selection of the study site for the research should maximize the opportunity for data collection and minimize the disruption caused by the investigator. The selection process should also take into consideration other activities in the area, such as agricultural practices, tourism, and hunting, which may interfere with the research protocol.

Permission to utilize the site may be necessary and the investigator must be able to assure the IACUC that necessary permits or permission have or will be obtained. Appendix E describes various site-specific permits required for field investigations.

Methodologies Employed

The potential short- and long-term effects of procedures on individual animals should be evaluated in all protocols. If animals are to be captured, the methods used and the numbers involved should be detailed in the protocol submitted to the IACUC. There should be a description of measures taken to prevent potential injuries and alleviate potential distress, and of the possible impact of capture on subsequent behavior and survival of the animals.

If animals are to be monitored individually, the investigator must indicate whether they will be identified by natural markings or will be artificially

marked. If the animals are to be artificially marked, there must be a description of methods to be used and potential trauma (e.g., paint markings may increase visibility to predators). Capture and marking methods are often a matter of practicality and usually have been developed and evaluated over a period of time. There is a substantial body of literature regarding the effect of mark-and-recapture studies and other study techniques on wild animals. The IACUC or investigator may rely on consultation with experts in the relevant discipline for this information. In issuing permits the USFWS also assesses capture and marking activities, and the IACUC may rely on that assessment in considering the appropriateness of a particular technique.

Field experimental procedures are commonly used to test hypotheses. In all instances, any potential pain or distress to an individual animal must be assessed and the investigator's justification evaluated in the context of the potential value of the data to be obtained.

Techniques for remotely recording behavioral or physiological data in the field are valuable and often minimally invasive. When possible, the least invasive procedures should be chosen (e.g., use of hormone assays of urine or feces rather than blood samples). When removal of individuals is necessary to take measurements or tissue samples, the IACUC should take into account the degree of invasiveness of the procedure and potential problems associated with return of the animal to the field. For example, animals should be released in a condition that enables them to avoid predators, seek shelter, and survive inclement weather.

Individual animals may also be treated experimentally to alter their behavior or physiology by surgery or drugs. Any invasive surgery, such as organ removal or implanting transmitters, should be done using aseptic technique. The use and choice of anesthesia will be affected by field conditions because some agents are difficult to transport or use in field conditions. Anesthetics that do not clear from the system quickly may require holding the animal longer as they may compromise the animal's ability to survive when released. The potential for human consumption of contaminated game species should also be considered.

Procedures involving site manipulation should be adequately justified by the investigator. For example, investigators may remove or, in rare and well-justified cases, add a predator; however, state law may prohibit releases of non-native invasive species. If fences are erected to limit movement of individuals or populations, the impact on other species should be considered.

Euthanasia of wildlife in the field can raise unique and challenging issues. The Report of the AVMA Panel on Euthanasia includes considerations and techniques for euthanasia of wildlife and should be used by the IACUC as a resource.

Conclusion

Many of these issues are difficult to address definitively, but their consideration will help the IACUC judge the potential impact and value of the study proposed, and can be expected to assist the investigator in obtaining maximum information from the study with minimum negative impact on the animals studied or their environment. The IACUC should ensure that the investigator complies with applicable regulations and policies and obtains any required permits; the IACUC may wish to obtain copies. Many of the issues arising from proposals to conduct field research on vertebrate animals will require the judgment of experienced professionals in the field and the IACUC should feel free to seek advice or consultation if necessary.

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C.3.e. Hazardous Materials

The IACUC must pay particular attention to proposals employing potentially hazardous materials, including:

- radioactive substances,
- infectious microorganisms,
- biological toxins,
- hazardous chemicals, and
- recombinant DNA.

These all have the potential of causing harm to animals in the facility and the personnel caring for and using them.

Some hazardous materials are strictly controlled by federal, state and local regulations. Radiation Safety Committees (RSCs) and Institutional Biosafety Committees (IBCs) have been mandated by the federal government to ensure that certain radioactive materials and recombinant DNA materials are handled safely. The role of these committees may be extended to consider research involving human and animal pathogens. The IACUC should be generally familiar with the responsibilities of the various safety committees and organizations at their institution and the institution should ensure that the functions of the committees are coordinated. Animal research proposals should be consistent with the procedures required by the IBC.

In addition to the various safety committees, institutions should have professional staff or resources available to handle chemical, biological and radiological agents. The National Research Council publication, *Occupational Health and Safety in the Care and Use of Research Animals*, is a valuable resource for IACUC members. This publication covers a wide variety of occupational health and safety issues, including information on working with hazardous materials in research animals.

Radioactive Materials

The U.S. Nuclear Regulatory Commission (USNRC) directly, or by its state designee, issues licenses permitting institutions to procure, use and dispose of specified radioactive materials. These licenses do not cover:

- X-ray machines;
- high voltage accelerators;
- electron microscopes; and
- radioactive materials from sources other than reactor by-products, although these are all sources of ionizing radiation.

RSCs have oversight for the procurement, use and disposal of radioactive materials; therefore, their approval should be coordinated with IACUC review of any proposal that involves radioactivity. General information on potential health risks from exposure to ionizing radiation can be found in the USNRC Regulatory Guide.

Biohazardous Materials

Infectious diseases may be a factor in many animal studies due to natural infections as well as those specifically induced as part of research. Consensus biosafety guidelines have been established for the use of animals in research involving infectious agents (*Biosafety in Microbiological and Biomedical Laboratories*). These guidelines provide a concept for assessing risks and selecting appropriate safeguards. Four biosafety levels, which consist of combinations of practices, safety equipment and facilities, are described in this CDC/NIH document.

Certain human pathogens as listed in the Select Agent List (Appendix A, 42 CFR 72.6) must be registered and approved by the Centers for Disease Control (CDC) prior to transfer from one registered facility to another. Similar requirements are in place with the USDA for the transfer of foreign animal disease agents.

The NIH publication, *Guidelines for Research Involving Recombinant DNA Molecules*, promulgated by the NIH Office of Biotechnology Activities, also includes four biosafety levels and represents a key reference for work involving recombinant microorganisms. Recombinant DNA experiments involving animals also require approval from the IBC.

Hazardous Chemicals

In addition to animal care concerns, activities involving hazardous chemicals require procedures for:

- chemical storage and disbursement,
- dosage preparation and challenge procedures, and
- waste management and disposal practices.

It is also necessary to determine whether the chemicals will be present in feed, feces or urine. A rigorous review to ensure appropriate safety practices, containment equipment and facility safeguards is essential for animal experiments involving chemical inhalation.

Proposals submitted to the IACUC must include sufficient documentation to assess the adequacy of precautions to control exposure of personnel to the hazardous agents involved in animal experiments. The identification by the IACUC of protocols involving hazardous chemicals (e.g., the use of known carcinogens to induce tumors in animal models, determinations of carcinogenicity, mutagenicity, or teratogenicity, or acute toxicity studies) is essential for institutional compliance with health and safety standards. The Occupational Safety and Health Administration (OSHA) laboratory standard "Occupational Exposure to Hazardous Chemicals in the Laboratory" is of particular importance. The IACUC should be familiar with the requirement in this standard for a chemical hygiene plan for controlling exposures to hazardous chemicals. Written standard operating procedures may be required describing appropriate safety precautions and specific "designated areas" where hazardous chemicals will be used or stored.

One health and safety issue common to most IACUCs concerns the use of the inhalation agent ether for anesthesia and euthanasia. Ether forms explosive peroxide when stored in metal containers and must be used with special precautions because of its volatility and flammability. Ether must be used with special ventilation and kept away from flames or electrical ignition sources. Carcasses of animals euthanized with ether should be stored in explosion proof well-ventilated areas and not incinerated until the ether is volatilized. Other inhalation anesthetics, such as halothane, methoxyflurane and nitrous oxide, although not without some degree of toxicity in an occupational setting, are less hazardous when used with proper precautions and a waste gas scavenging system. Methoxyflurane is the most toxic of these inhalation agents to humans, and safe practices should be closely scrutinized by the IACUC.

Another class of hazardous chemical routinely encountered in the laboratory environment is aldehydes. Specific OSHA guidelines are available for handling aldehydes and other chemicals. *Material Safety Data Sheets*, which provide useful information on specific hazardous chemicals, must be accessible on site for each hazardous agent present.

Hazardous Waste

Animal wastes contaminated with radioactive materials, recombinant organisms, infectious agents or other hazardous chemical agents must be carefully managed to avoid human exposure or damage to the environment. Special efforts should be made in experimental design to minimize the generation of wastes containing hazardous chemicals. Those containing radioactivity in addition to hazardous chemicals are particularly difficult to deal with. Wastes containing infectious agents should be decontaminated, preferably in a steam autoclave, before disposal. Incineration is the recommended treatment for contaminated feed and bedding. The professional health and safety staff, who have responsibility for hazardous waste management at the institution, should review institutional policies when animal care proposals involving hazardous materials are received.

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C.3.f. Instructional Use of Animals

Any instructional use of live, vertebrate animals that is supported by the PHS is governed by the *PHS Policy*. The applicability of the AWRs depends upon the species used. Most institutions have chosen to require that all instructional use of animals, regardless of funding source or species, be reviewed by the IACUC.

It may be appropriate for students, at both undergraduate and graduate levels, to participate in the conduct of experiments involving laboratory animals for the purpose of education. All instructional proposals should clearly identify the learning objectives and justify the particular value of animal use as part of the course, whether it is demonstration of a known phenomenon, acquisition of practical skills, or exposure to research. In all cases, consideration must be given to alternative approaches to attaining the desired educational objectives, in accordance with the U.S. Government Principles.

Adequate supervision and training are especially important as the techniques learned by students may be carried into subsequent research careers. It is recommended that students receive instruction in the ethics of animal research and applicable rules and regulations prior to undertaking any experimentation. When students work in an investigator's laboratory, the IACUC must ensure that the students receive appropriate supervision and training in animal care and use. The *PHS Policy* and AWRs have specific training requirements that apply to all animal users, including students. Student projects involving protocols different from those approved for the instructor's laboratory must be reviewed and approved on their own merits by the IACUC.

Experiments sometimes entail behavioral observation with no intervention, or minor painless interventions, such as choices of food or living accommodations. Such projects teach the rigors of conducting a research project and the variability inherent to biological or biobehavioral systems. These exercises generally involve little or no distress to the animals, but still require IACUC approval.

Some procedures present additional concerns. Selected examples are listed below:

- Behavioral studies that involve conditioning procedures in which animals are trained to perform tasks using mildly aversive stimuli, such as the noise of a buzzer, may be potentially stressful to the animals.

For other behavioral studies using non-aversive stimuli, such as running mazes, it may be necessary to maintain animals at a reduced body weight to enable food treats to be used as an effective reward. Experiments involving food and water restriction for teaching purposes must be rigorously justified and carefully monitored.

- Some behavioral studies produce potentially high levels of distress, including those using aversive stimuli, such as unavoidable noxious electric shock and surgical ablations or drug-induced lesions designed to affect the animal's behavior or performance. The educational benefits of such procedures should be carefully reviewed and clearly justified, bearing in mind that studies involving unrelieved pain or distress are generally inappropriate when employed solely for instructional purposes (U.S. Government Principle IX).
- Laboratory studies in physiology, neurophysiology, biology, and pharmacology often involve observations and experiments using animals. For all procedures, including those in which animals are euthanized to obtain tissues (e.g., in the teaching of anatomy or tissue harvest for *in vitro* procedures), the procedures and method of euthanasia, if any, must be reviewed by the IACUC. The number of animals used should always be the minimum necessary to accomplish the objectives of the proposed educational activity.

Animal Use in Veterinary Teaching

Many North American veterinary schools use live animals to teach anesthesia, animal handling, surgical procedures, recovery from anesthesia, post-operative management and postmortem examinations following terminal procedures. Animals designated for teaching may be kept long term and participate in many classes over the course of a year or more.

All instructional use of animals in non-survival as well as survival instructional procedures should be reviewed by the IACUC. Repeated procedures on designated teaching animals should be limited and reviewed by the IACUC. Federal limitations on multiple survival surgeries must be observed. Cost savings alone is not an adequate reason for performing multiple survival surgical procedures.

Some schools make alternatives available for those students who do not wish to participate in animal laboratories. Alternatives to the use of animals acquired specifically for instruction include the use of client-owned animals, or dogs and cats from animal control facilities that are made

available for surgical neutering. Plastic models and other model systems are increasingly being used to teach manual skills.

Animals that develop unique and/or terminal conditions may be donated to a veterinary school for research and/or teaching purposes. The use of these animals needs full IACUC review.

Animal Use in Agricultural Instruction

Flocks and herds of agricultural animals are often maintained by agricultural schools to teach husbandry, production, and showmanship. Animals used for these practices are not covered by the *PHS Policy* (unless supported by PHS) or the AWRs. However, research procedures (e.g., *in vitro* fertilization), should have committee review. IACUCs charged with reviewing the use of animals in activities with agricultural applications will find *A Guide for the Care and Use of Agricultural Animals in Agriculture Research and Teaching* useful in conducting their evaluation.

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C.3.g. Surgery

Surgical procedures are a common component of animal research activities, and IACUCs are often called upon to assess the details of these procedures. Further, the IACUC is responsible for determining that personnel are qualified and trained in the procedures to be performed.

Definitions

Major surgery: Penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions.

Minor surgery: Does not expose a body cavity and causes little or no physical impairment.

Survival surgery: The animal awakes from surgical anesthesia.

Non-survival surgery: The animal is euthanized before recovery from anesthesia.

Reviewing Protocols for Surgical Procedures

Some of the aspects of a surgical procedure that the IACUC reviews are:

- details of the procedure (e.g., the actual procedure itself, pre- and post-operative care, aseptic technique, sequence of multiple procedures);
- appropriateness of the species for the procedure proposed;
- qualifications of the personnel performing the surgical procedures;
- species-specific and procedure-specific facility requirements;
- patient monitoring practices in the surgical and post-surgical periods; and
- personnel occupational health and safety issues.

The veterinarian should always be one of the IACUC's primary sources of information on surgery and post-operative issues. Other sources include the AWRs (9 CFR 2.31(d)(1) (ix) and (x)), the *PHS Policy,* the *Guide*, and other publications referenced at the end of this section. While the numerous references available provide background and a basis for reviewing surgical protocols, the IACUC relies on professional judgment to review the unique situations surrounding surgery in an experimental setting. Surgical procedures performed in a research setting have review requirements that may be different from those in a routine veterinary clinical setting.

Some of the surgical procedures proposed in research are experimental and may require ongoing review by the IACUC as the procedure is developed. Model development protocols, and close collaboration with the veterinarian and other experienced individuals, can be helpful in these circumstances.

To perform a meaningful review, the IACUC must be provided with details of proposed surgical procedures. Such details give the IACUC the opportunity to assess the level of the investigator's knowledge and need for additional training.

Multiple Major Survival Surgery (MMSS)

The *Guide* discourages multiple major survival surgery. The AWRs state that animals may not be used in these procedures unless:

- there is a scientific justification (e.g., related components of the same study) provided by the principal investigator in writing;
- the MMSS are required as a routine veterinary procedure or to protect the health and well-being of the animal, as determined by the attending veterinarian; or
- under other special circumstances which have been approved by the Administrator of APHIS.

The *Guide* suggests that reasons for MMSS may include procedures that are related components of a research project, procedures that will conserve scarce animal resources, or procedures conducted for clinical reasons. The *Guide* precludes cost savings as the sole justification for MMSS. Subsequent to approval of MMSS, the IACUC should ensure that there is sufficient ongoing oversight of the project.

Special Considerations

Some procedures are difficult or impossible to perform in some species of animals due to the nature of the animal (e.g., anatomical variation such as lack of a gall bladder, size of the animal, or size of a particular organ; sensitivity to antibiotics; or tolerance to a particular procedure). This can be an issue when a protocol involves an established procedure in a new animal model. Such protocols require particular attention and guidance from the IACUC.

If a procedure may cause more than momentary or slight pain or distress, the AWRs prohibit the use of paralytics without concurrent anesthesia.

Some procedures may require specialized facilities to ensure their success. For example, major survival surgery in non-rodents requires dedicated surgical facilities. Details of such physical requirements can be found in the *Guide*. The IACUC should assess the availability of necessary facilities during the protocol review process.

Patient Monitoring

The sophistication of patient monitoring required varies with the species and the procedure, but during protocol review, the IACUC should expect evidence of the following:

- a pre-surgical assessment;
- adequate monitoring of depth of anesthesia and animal homeostasis during the surgical procedure;
- support such as fluid supplementation, external heat or ventilation;
- monitoring and support during anesthetic recovery; and
- post-surgical monitoring details, (e.g., what will be done and how often, who will be responsible, and the name and phone number of the individual to contact in the case of post-surgical complications).

Recordkeeping

Recordkeeping is an essential component of peri-operative care. For major surgical procedures on non-rodent mammals, an intra-operative anesthetic monitoring record should be kept and included with the surgeon's report as part of the animal's records. This record should be available to the personnel providing post-operative care. Post-operative records, at a minimum, should reflect that the animal was observed until it was extubated and had recovered the ability to stand. These should be supplemented by records evaluating the animal's recovery, administration of analgesics and antibiotics, basic vital signs, monitoring for infection, wound care, and other medical observations.

Occupational Health and Safety

Surgical situations can present certain occupational health and safety risks related to:

- use of inhalation anesthetics,
- use of certain species or a species under certain circumstances (e.g., pregnant sheep), or
- use of certain devices (e.g., lasers).

If the circumstances warrant it, the IACUC should consult with the applicable biosafety personnel.

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C.3.h. Transgenic Animals

A *spontaneous mutation* is a naturally occurring heritable alteration in the genetic code. Spontaneous mutations have been observed in virtually all species. An *induced mutation* is a man-made alteration in the genetic code. Induced mutant is a generic term including transgenic and targeted mutations that are created to study over-expression or under-expression of a specific gene. The altered gene must be predictably transmitted to offspring for a spontaneous or an induced mutation to be useful in research. To date, the majority of induced mutations have been made in laboratory mice of the genus *Mus* or laboratory rats of genus *Rattus*. Although mice are used as examples in the following discussion, the general considerations are applicable to induced mutants of any species.

Transgenic refers to insertion of exogenous DNA (deoxyribonucleic acid) into cells. Typically, cDNA (complimentary deoxyribonucleic acid) made from specific mRNA (messenger ribonucleic acid) is inserted into cells using microinjection, electroporation or certain nonpathogenic viruses. (Electroporation is the brief application of an electric field to a cell to increase permeability of the cell membrane for purposes of introducing drugs or genes into the cell.) Each of these methods has been used to insert new DNA into the pronucleus of a fertilized mouse egg and to create transgenic mice. The manipulated fertilized eggs may or may not be cultured *in vitro* for one to three days before they are surgically implanted into the oviducts or uterus of pseudopregnant female mice. The inserted DNA incorporates in chromosomes of a percentage of embryos developing from the micro-injected eggs. The DNA incorporates at different genetic locations and a different number of copies of the DNA may incorporate in different embryos. Thus, each embryo has the potential to become a unique transgenic mouse even though the same quantity and type of DNA was injected into genetically identical fertilized eggs. All manipulated, fertilized eggs do not become live born transgenic mice. Losses occur at every step from injection through gestation and delivery.

Mice can carry transgenes, but unless the cDNA is incorporated into germ cells, the mouse is unable to transmit the transgene to its offspring. A mouse that passes the transgene to the descendants is called a ‘founder. Thus, many fertilized eggs have to be injected to obtain a few transgenic mice, and only a few of these transgenic mice will be ‘founders’ of this transgenic line.

Targeted mutation refers to a process whereby a specific gene is made nonfunctional (‘knocked-out’) or less frequently made functional (‘knocked-in’). Creation of a targeted mutation requires several steps in the laboratory. The specific gene is identified, cloned and manipulated to make it nonfunctional (‘knocked-out’). The manipulated gene is attached to another DNA sequence called a promoter and introduced into embryonic stem (ES) cells by electrical or chemical methods. These ES cells are cultured in special media that permits identification of ES cells incorporating the manipulated gene. ES cells incorporating the manipulated gene are injected into an early embryo (blastocyst). The ES cell injected blastocysts are surgically implanted into the uterus of pseudopregnant female mice. Some injected blastocysts develop into viable embryos and gene deficient ‘knock-out’ mice are born.

Many blastocysts have to be injected to obtain a few new 'knock-out' mice, and only a few of the new 'knock-out' mice will incorporate the 'knocked-out' gene in their germ cells and become 'founders'.

If a project uses a spontaneous or induced mutant model and the mutant animal can be purchased from a resource or commercial colony, review of this project is similar to review of any other project. If a project uses an induced mutant model and only breeders are available from the source, review of this project is similar to review of any other breeding colony**.** In either case, the IACUC should determine if the mutant gene will result in a severely debilitating phenotype, if anything can or will be done to ameliorate such phenotype, and what endpoints will be used to determine when a mutant animal will be euthanized. Simple husbandry measures can modify the severity of some mutant phenotypes. For example, ground feed or moist feed can extend life and improve growth of mutants with missing or malformed teeth. Food and water on the bottom of the cage may be easier for mutant rodents with neuromuscular abnormalities to access than food in a traditional feeder built into a cage lid. Extra bedding helps dwarf mice reach food and water. Extra bedding helps absorb urine produced by diabetic mice or other mice that excrete large quantities of urine. A normal cage mate, a solid bottom cage with extra bedding, or a slight increase in room temperature can benefit mutant rodents that have problems maintaining body temperature (Beamer, 1986).

When an investigator prepares a proposal that includes development of a new mutant model, information about clinical abnormalities associated with the phenotype, special husbandry requirements, etc. will not be available. However, the investigator should include general criteria for euthanasia if a severe debilitating phenotype develops, and provide the IACUC with this information when the new mutant has been developed or at the next annual review.

The standard of 'normal' for a mutant animal may or may not be the same as for a non-mutant animal. If the mutant phenotype does not impact clinical well-being of the animal, the same standard of 'normal' can be used for mutant and non-mutant animal. In the mouse, brown (gene symbol Tyr<b>) and short ear (Bmp5<se>) are examples of spontaneous mutations

that produce no observable, clinical impact on the well-being of the mouse. If the mutant phenotype has minimal impact on the well-being of the animal, the standard of 'normal' can be similar for mutant and non-mutant animal. Hypogondal (Gnhr<hpg>) and 'little' (Ghrhr<lit>) are examples of spontaneous mutations with minimal impact on well being of the mouse. Homozygous hypogondal mice are normal in all ways except for small, non-functional gonads. Homozygous 'little' mice are smaller than non-mutant littermates. Growth hormone transgenic mice tend to have larger body size than normal, but are otherwise clinically normal with the exception of reduced fertility.

In the case of mutants where phenotype involves clinical abnormalities, the standard for 'normal' may have to be modified to encompass the expected phenotype. For example, 4 to 5 week old homozygous dystrophic mice (Lama<dy-2J>) have difficulty abducting hindlegs and have an abnormal gait. As these mice age, muscular weakness progresses in hindlegs and eventually extends to involve all skeletal muscles. The standard for 'normal' for homozygous dystrophic mice must include difficulty abducting hindlegs and an abnormal gait. Adenopolyposis coli 'knock-out' mutant mice (Apc<Min>) are clinically normal until the intestinal polyps develop, after which time the mice become anemic and lose weight. Experimental endpoints for these latter and similar mutant models should focus on (1) ability of the mutant to access feed and water, (2) response of the mutant to stimuli, and (3) general condition of the mutant (i.e., is the mutant excessively thin, showing progressive weight loss or hunched posture?).

Many institutions have a centralized induced mutant facility that receives the genetic material from investigators and performs the manipulations to develop 'founder' transgenic or 'knock-out' mice. The 'founder' mice are returned to the investigator who undertakes breeding to expand the line. Review of the centralized induced mutant facility should focus on personnel qualifications, animal related practices such as aseptic surgery, and average number of mice required to produce 'founders' for a single DNA construct, recognizing, however, that the number of mice required is a very rough estimate because of differences in responses of different strains or stocks of mice, variations in success rate for different DNA constructs, and subtle or less subtle uncontrollable environmental changes.

In many non-mutant model experiments, an investigator can accurately estimate the exact number of animals required to test a hypothesis. However, when creating an induced mutant, there are major variables that make it difficult to accurately estimate the number of required animals, including:

- differences in percent successful microinjections of pronuclei or successful incorporations of altered gene into ES cells,
- differences in percent successful surgical transfers of fertilized eggs or blastocysts, and
- differences in percent successful incorporation of exogenous DNA or altered gene into germ cells of induced mutant mice.

Different strains of mice vary in their responses to each of these manipulations. Different genes ('constructs') vary in the ease with which they insert as a transgene or are 'knocked-out'. These variables remain even when the same skilled people perform each manipulation.

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C.4. Monitoring of Approved Protocols

After the IACUC has approved a protocol, it has a responsibility to ensure that procedures are carried out in the laboratory or classroom as described in the protocol. This section will briefly review ways that the IACUC can monitor the conduct of approved protocols.

Acquisition and Tracking

Animals should be obtained only from licensed dealers or other legal sources, and it is incumbent upon an institution to establish mechanisms to monitor and document the number of animals acquired and used in approved activities. This is best accomplished if animal purchases may be made only through the institution's animal resource facility or other appropriately designated office. Once animals have been acquired, they should be included in a tracking system. Many institutions have automated systems that will alert an appropriate individual when an investigator has reached a preset percentage (e.g., 80 to 90%) of the number of animals approved for a specific project, and can prevent ordering animals in excess of the number approved. Institutions with small programs using limited numbers of animals may choose to maintain a manual log of IACUC approved activities and numbers of animals acquired.

Tracking animal use becomes more complicated when investigators maintain breeding colonies. Keeping track of animal usage may be accomplished by requiring that investigators with breeding colonies maintain accurate records. Investigators can be required to report to the designated office, at regular intervals, the number of animals born, weaned, or used in studies. This report can be tallied against the numbers in the approved protocol.

Compliance Specialist

Some IACUCs have a full or part-time compliance specialist who monitors procedures in vivaria, laboratories, and classrooms, and reports his or her observations to the IACUC. This individual should have laboratory animal training and experience, and be authorized to conduct announced or unannounced laboratory inspections on behalf of the IACUC. In addition to this role, the compliance specialist may periodically survey individual

laboratories to ensure that actual procedures used are consistent with protocols. The survey may include meeting with investigators and staff to review concerns, answer questions, and identify procedures that may deviate from those originally approved by the IACUC. In cases of deviation, the specialist should notify the IACUC.

Eyes and Ears

Research, veterinary, and husbandry staff should be aware of approved procedures for use on animals when they have responsibility for those animals. This may be accomplished by informing these individuals in staff meetings or by making standard operating procedures and animal use protocols readily accessible in the laboratory or vivarium. These practices help to ensure that procedures being used are, in fact, those that were approved by the IACUC. Maintaining an open environment in which staff can discuss apparent departures from approved procedures with the investigator often facilitates compliance and the rapid correction of deviations. Staff must also be free to report perceived deviations to the IACUC, which must then consider such concerns (see Section D).

Semiannual Inspection

During the semiannual facility inspections, IACUC members should note the use of animals and may verify that the observed procedures are consistent with the protocol on file.

Retrospective Reporting of Adverse Events

The USDA requires that the number of covered animals used in each pain/distress category be reported annually, but there are currently no explicit federal requirements for reporting of unexpected or unintentional changes in pain category, morbidity, or mortality after the event. The USDA does expect any significant deviations from the expected pain/distress category to be reported correctly. Institutions may choose to require an accounting of unexpected, unintentional, or adverse events as a means of identifying deficiencies in procedures, faults in study design, or need for additional personnel training.

Review of Publications

In academic institutions and many companies, much research is eventually published. Some IACUCs choose to review some published descriptions of animal use to verify that work was done according to the approved protocol.

Conclusion

Although no IACUC has the staff or time to observe all animal use in an institution, the IACUC can help establish a climate of compliance. To ensure that animal use conforms to local policy and federal regulations, it is prudent for the IACUC to confirm that animals are used according to protocol.

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D. Evaluation of Animal Care and Use Concerns

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Evaluation of Animal Care and Use Concerns

To help ensure that laboratory animals receive humane care and use or treatment in accordance with the highest ethical standards, laws, regula tions and policies governing animal research, the IACUC must review and, if warranted, address any animal-related concerns raised by the public or institutional employees. Procedures must be established to ensure that concerns are communicated to the IACUC. The Committee must review each concern in a timely and systematic manner and, when necessary, take prompt, appropriate corrective actions.

PHS Policy, Animal Welfare Act, and USDA AWR requirements

The *PHS Policy* requires the IACUC to "review concerns involving the care and use of animals at the institution", and the *Guide* states that the IACUC is responsible for "establishment of a mechanism for receipt and review of concerns involving the care and use of animals." The Animal Welfare Act (AWA) (7 U.S.C. 2142; Section 13) requires training of personnel who are involved in animal care or treatment, including "methods whereby defi ciencies in animal care and treatment should be reported." The AWRs (9 CFR Part 2, Subpart C, 2.32 (c)(4)) require each research facility to provide the methods whereby any employee of the facility can report defi ciencies in animal care and treatment. In addition, the AWRs, Section 2.31(c)(4)) require the IACUC to "review and, if warranted, investigate con cerns involving the care and use of animals at the facility resulting from public complaints received and from reports of noncompliance received from laboratory or research facility personnel or employees."

In addition, the AWRs (9 CFR Part 2, Subpart C, Section 2.32(c)(4)) state that "no facility employee, Committee member, or laboratory personnel shall be discriminated against or be subject to any reprisal for reporting viola tions of any regulation or standards under the [Animal Welfare] Act."

Compliance

To ensure compliance with federal law, regulations, and policies, it is strongly recommended that each IACUC develop and implement policies and pro cedures to ensure that all animal care and use concerns are brought to its attention for consideration. Some of the elements that should be included

in these procedures are described below (see IACUC Responses to Complaints). Institutional policy should contain provisions to protect the confidentiality of those who report concerns as well as anyone against whom allegations are directed, while allegations are under investigation. The policy should also address mechanisms for protecting complainants from reprisals.

Origins of Concerns or Complaints

Some common sources include:

- *animal care and use personnel* – these individuals should receive instruction in institutional training programs to report perceived deficiencies in animal care or use to the IACUC.
- *other personnel* – these persons (e.g., secretarial, maintenance, security staff) are likely to direct concerns to a member of the research, animal care or veterinary staff, but they should be instructed to report concerns to the IACUC.
- *employee "hotlines" or ombudsmen* – personnel responsible for these functions should be sensitive to animal-related concerns and notify the IACUC Chair of any that may arise.
- *the public* – they are most likely to direct complaints to senior institutional representatives who should promptly forward them to the IACUC Chair.
- *anonymous* – these complainants may or may not be institutional employees.
- *the media* – stories appearing in newspapers, and on television or radio, etc. may contain or evoke concerns about animal care and use; such reports should be evaluated by the IACUC, and, when appropriate, the institution should proactively address them.

Methods for Reporting Concerns

To facilitate communication, the names and phone numbers of contact persons, including IACUC members, the veterinarian, security office, and ombudsman/hotline, if one exists, should be posted in or near the entrance to animal facilities or listed on a Web site that is readily available to institutional employees. This information should also be provided during training sessions as described above.

Although written concerns are more convenient to deal with, complainants may not be willing to submit them in this manner. In such cases, the individuals who receive concerns should document them fully to ensure that the issues are clear and to prevent misunderstandings. Requests for anonymity should be honored to the extent possible.

IACUC Responses to Complaints

While specific methods for evaluating concerns about animal care and use may vary from institution to institution, all methods should contain these elements:

- There should be a procedure for verifying stated concerns.
- Verified concerns should be related to the AWRs, the *PHS Policy* or institutional policies.
- There should be guidelines for effecting appropriate corrective measures, when necessary.

One of the roles of the IACUC is to review all concerns about the animal care and use program, regardless of origin, and investigate them if warranted. The IACUC Chair is normally responsible for ensuring that concerns are addressed, but may delegate investigation to a subcommittee. If the Chair has, or is perceived to have, a conflict of interest, the Institutional Official (IO) should delegate the responsibility for assuring that the concern is addressed to another non-conflicted member of the IACUC.

Concerns may include situations or activities ranging from those in which animals are reported to be in immediate, actual or perceived jeopardy to those in which violations of the AWRs or institutional Animal Welfare Assur ances are alleged to be occurring but animals are not in apparent danger. They may focus on allegations of past policy and procedure violations.

The course of action taken by the IACUC should be driven by the potential significance of the alleged situation. For example, conditions that report edly jeopardize the health or well-being of animals should be evaluated immediately. To cope promptly with such situations, some institutions have policies whereby a veterinarian or other designated person is authorized to halt procedures which they believe do not comply with institutional poli cies until the IACUC can be convened and consider the matter formally. Similarly, situations that may involve potential criminal activity or human safety should be reported promptly to the institution's law enforcement or

occupational health and safety officials. Allegations of other ongoing policy or procedural matters may not require such same-day attention, but should not be deferred merely as a matter of convenience. Emergency meetings may be necessary in these cases to ensure prompt consideration of concerns.

IACUC procedures for handling complaints may involve reviewing them with the veterinarian. Depending on the nature of the concern, the IO, legal counsel, and the person who submitted or fielded the complaint may also be invited to participate. Based on the results of its initial evaluation, a course of action—which may include further investigation—will then be determined and implemented. The IACUC should acknowledge receipt of concerns when the complainant is known. Details concerning the complaint, complainant, persons against whom allegations may have been directed, and the investigations in progress are usually considered confidential. However, when the Committee releases the report of its findings (including corrective actions, if applicable), those reports may become accessible to the public under state "sunshine" laws, and if provided to Federal regulatory agencies, under the Freedom of Information Act.

The AWRs and the *PHS Policy* authorize the IACUC to suspend an activity after review of the matter at a convened meeting of a quorum of the IACUC and with the suspension vote of a majority of the quorum present. Suspensions must also be reviewed by the IO in consultation with the Committee; appropriate corrective action taken by the IO is reported to OLAW.

Most institutions have developed self-regulatory policies and procedures that supplement formal suspensions by the IACUC and are intended to ensure adherence to institutional and regulatory requirements. Depending on the severity of noncompliance or deviation from accepted practices, these range from counseling and mandatory remedial training to specific monitoring of animal use, temporary revocation of animal use privileges, or termination of employment.

Model

One model for considering concerns about animal care and use is outlined on the following pages. This example may not apply to all institutions, and may be adapted, as needed, in designing guidelines that are appropriate for individual institutions.

Suggessted IACUC Procedures for the Investigation of Animal Care and Use Concerns*

Initial Evaluation and Actions

Upon receipt of a concern the IACUC Chair should convene a meeting of the IACUC. After initial review of the complaint the IACUC should determine whether it requires further investigation and immediate action, further investigation but no immediate action, or no action. Once this decision has been made, the IACUC should determine which individuals or other institu tional or noninstitutional offices may require notification at this time.

If immediate action appears warranted because animal or human welfare may be compromised, the IACUC should notify the IO and proceed accord ingly. Veterinary medical intervention, suspension of a research activity, and/ or notification of appropriate safety, occupational health, or other officials, are examples of actions that may be taken immediately to protect animal or human welfare. In accordance with the AWRs (9 CFR Part 2, Subpart C, Section 2.31[d][7]), if an activity is suspended, the IO shall report that action to APHIS and any federal agency funding that activity. If the activity is supported in any way by the PHS, the IACUC, through the IO, must promptly notify OLAW (PHS Policy, IV.F.3.) (OPRR Reports 94-02, 1/12/94).

Investigation

Should the IACUC determine that further investigation is required, the Chair, or another individual or subcommittee appointed by the Chair, should conduct the investigation and report back to the IACUC. It is important to avoid actual or perceived conflicts of interest in this process.

The IACUC should charge the designated person or group with its require ments for information gathering and impose a completion date. The assigned completion date will depend on the IACUC's determination of whether immediate remedial action may be required.

***DISCLAIMER**

Neither the AWRs nor the PHS Policy provide specific guidance regarding the consideration of concerns or the institutional conduct of investigations. Owing to the considerable diversity of concerns that may arise and the contexts in which they may be voiced, no one set of procedures will be suitable for investigating all potential situations that involve violations of or deviations from animal care and use practices required by the PHS Policy, AWRs, the Guide and other federal statutes and regulations regarding animals. Consequently, the following suggestions are broad, intended for general use, and not intended for application in all situations.

The nature of the information required will vary depending on the circum stances, but often involves:

- interviewing complainants (if known), any persons against whom allegations were directed, and pertinent program officials;
- observing the animals and their environment; and
- reviewing any pertinent records, (e.g., animal health records, protocol, and other documents).

The designated investigator(s) should provide a report to the IACUC which summarizes:

- the concern(s),
- the results of interviews,
- the condition of animals and their environment, and
- the results of records and other document reviews.

The report should also contain:

- any supporting documentation such as correspondence, reports, and animal records;
- conclusions regarding the substance of the concerns *vis-à-vis* re quirements of the AWRs, the *PHS Policy*, the *Guide*, and institutional policies and procedures; and
- recommended actions, if appropriate.

Outcomes and Final Actions

Upon receipt and evaluation of the report, the IACUC may request further information or find that:

- there was no evidence to support the concern or complaint,
- the concern or complaint was not sustained, but a) related aspects of the animal care and use program require further review or b) other institutional programs may require review, or
- the concern or complaint was valid.

Subsequent actions of the IACUC may include:

- implementing measures to prevent recurrence (such measures often include changes in administrative, management or IACUC policies and procedures, and may include sanctions*);
- notifying the IO and the AV of its actions;
- notifying funding or regulatory agencies, as required; and
- notifying the complainant, any persons against whom allegations were directed, and pertinent program officials (appropriate supervisory and management staff, the public affairs office, institutional attorneys, etc.).

**Note on Sanctions:* Aside from empowering the IACUC to suspend a pre viously approved activity, the AWRs and the *PHS* Policy are silent regarding IACUC- or institutionally imposed sanctions.

Some institutions, as part of their programs, have developed policies and procedures that authorize the IACUC to impose sanctions on behalf of the institution. In other institutions, IACUCs recommend actions to the IO for implementation, and in still others, there exists a combination of these approaches. Some of the institutional sanctions that have been devised include:

- counseling;
- issuing letters of reprimand;
- mandating specific training aimed at preventing future incidents;
- monitoring by the IACUC or IACUC-appointed individuals of research, testing, or training involving animals;
- temporary revocation of privileges to provide animal care or to conduct research, testing, or training that involves animals, pending compliance with specific, IACUC-mandated conditions;
- permanent revocation of privileges to provide animal care or to conduct research, testing, or training that involves animals; and
- recommending to the IO that institutional (e.g., reassignment, termi nation of employment) sanctions be imposed.

Concerns Unrelated to Animal Care and Use

The IACUC may determine, either in its initial evaluation of a concern or as a result of investigation, that violations of non-animal-related institutional policies and procedures, local, state or federal statutes, regulations, or laws may have occurred (e.g., scientific misconduct, misuse of monies, fraud, theft, etc). In such cases, those findings should be reported to appropriate institutional officials or committees for their consideration.

E. Recordkeeping and Communications

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E.1. Recordkeeping and Reporting

Introduction

The *PHS Policy* and AWRs include recordkeeping and reporting requirements. The responsibility for these functions should be clearly delegated. Usually the IACUC office is assigned this task. The individuals responsible should understand federal animal use requirements and the institution's program, and should also be aware of the Freedom of Information Act (FOIA) and any state open records laws. Many of the reports written may be accessible under such laws, and care should be taken to use language that is clear and precise to ensure accurate interpretation.

Recordkeeping

Minutes

The *PHS Policy* and the AWRs require that the institution maintain "minutes of IACUC meetings, including records of attendance, activities of the Committee, and Committee deliberations" (PHS Policy IV. E; 9 CFR Part 2 Subpart C 2.35 (a)(1)). The IACUC has some latitude in the degree of detail in these minutes.

Records of attendance: Although members may arrive late or leave during a meeting, generally a member is marked as either present or absent. An exception would be when the IACUC member leaves the meeting room during discussion of a protocol on which that member is a participant. If the temporary absence of a member drops the number of members present below the quorum, this should be noted in the minutes. Certain official IACUC actions require a quorum (see Section A.2. Quorum Requirements).

Activities of the Committee include corrections or approval of previous minutes; presentation of program, policy, facility and compliance reports; and decisions on policies, protocols, and amendments.

Deliberations refers to the discussion and reasons leading to particular IACUC decisions. Although some IACUCs maintain a verbatim record (e.g., audio or videotapes), minutes should include as a minimum a summary of the key points discussed prior to a committee decision.

Protocols

The *PHS Policy* and the AWRs require that animal applications and proposed significant changes be retained for the duration of the animal activity and for an additional three years after the end of the activity. Proposals submitted to the IACUC must be kept for three years even if approval was not granted or animals were not used. The records must show whether or not IACUC approval was given.

Other records

Both the *PHS Policy* and the AWRs require that semiannual IACUC reports and recommendations be retained by the institution. PHS also requires that the OLAW Assurance and reports of accrediting agencies (e.g., AAALAC) be kept on file. USDA requires additional records on dogs and cats acquired, transported, sold, or euthanized by the research facility. Animal health records are not usually maintained by the IACUC but are kept in the animal facility. All these records must be kept for at least three years; and must be accessible to PHS, APHIS, and funding agencies for inspection or copying (see Table A).

Reporting Requirements

PHS Assurance

In order to qualify for support from the PHS for activities involving animals, institutions must provide an Assurance of Compliance with the *PHS Policy*. The Assurance is a written agreement that fully describes the institution's program and commits the organization to comply with the PHS Policy, and in which the institution outlines in detail its policies and procedures. A sample Assurance is available at the OLAW Web site. Institutions that are not accredited by AAALAC must submit, with their Assurance, the most recent IACUC semiannual program evaluation. The completed Assurance, signed by the IO with appropriate authority, is submitted to and evaluated by OLAW. Upon final approval by OLAW an Assurance number (in the format A####-01 where # is a digit) is assigned to the institution. Assurances are approved for a period of up to five years, after which time the institution must submit a new Assurance. A list of institutions with approved Assurances is available on the OLAW Web site.

It is important that the approved Assurance document is distributed appropriately within the institution and that members of the IACUC are familiar with this document, as compliance with the Assurance is required to be eligible for PHS funding.

USDA Registration

Institutions that use species of animals covered by the AWRs for research, testing, experiments, or teaching on its premises as specified in the AWA are required to be registered with the Animal Care division of the Animal and Plant Health Inspection Service (APHIS), using APHIS form 7011. The form is submitted to APHIS via the Regional Director of Animal Care (AC) for the state in which the facility has its principal place of business. At academic institutions, the submission is usually made by the institution, not the individual departments or schools, and signed by the IO. An approved USDA registration is given a number in the format ##-X-####, where X is a letter (R for research institution) and # is usually a digit. The registration may be renewed every three years. The institution is required to notify the AC Regional Director within ten (10) days of any change in the name, address, ownership or operations affecting its status as a research facility. The Regional Director may place a facility that has not housed animals for two years in inactive status. The registration can be cancelled by written request if a facility no longer uses, or intends to use, animals (see Table B).

Semiannual Facility Inspections and Program Evaluations

The *PHS Policy* and the AWRs require that the IACUC evaluate the institution's animal program at least once every six months, including an inspection of facilities, and submit a report to the IO. The *PHS Policy* allows the IACUC discretion in how it evaluates its facilities and program. The report format is not mandated, but OLAW offers models for both facility inspections and program reviews on its Web site.

The report must contain a description of the nature and extent of the institution's compliance with the *PHS Policy* and *Guide*; any departures must be identified and modifications proposed, with a plan and timetable for correction. Any minority views of IACUC members must be included.

Minor and significant deficiencies must be distinguished. A significant deficiency is defined as one that "is or may be a threat to the health or safety of animals." Program or facility deficiencies, including accidents or natural disasters, which cause injury, death, or severe distress in animals, are, by definition, 'significant.' Examples of minor deficiencies include chipped paint and burnt-out light bulbs. The report must also identify any facilities that are AAALAC accredited.

The IACUC may utilize AAALAC program status evaluations, accreditation, or pre-assessment preparation activities as a semiannual evaluation. To be used as the semiannual report, the report must include all the information required in Section IV.B.3 of the *PHS Policy* (see Table C), and be approved by vote of the IACUC.

Semiannual reports are only submitted to OLAW under two circumstances:

1) If an institution is not accredited by AAALAC, a copy of the most recent semiannual report must be submitted to OLAW with a new or renewal Assurance.
2) Upon request by OLAW or other PHS representatives.

USDA requirements are essentially the same as those for PHS with three exceptions:

1) The AWRs include additional reporting requirements if the schedule and plan for correcting a deficiency is not followed. Failure to correct a significant deficiency in accordance with the specified schedule and plan must be reported in writing within fifteen business days by the IACUC, through the IO, to APHIS and any federal agency funding the activity.
2) USDA requires that reports be reviewed and signed by a majority of IACUC members.
3) USDA does not require the identification of facilities accredited by AAALAC.

As with the PHS semiannual review, AAALAC processes may also fulfill the requirements for the USDA semiannual report provided Section 2.31 (c) requirements are met, as listed in Table C.

Annual Report

The IACUC at an institution with an approved PHS Assurance must submit an annual report to OLAW through the IO. This report details changes in the animal care and use program, IACUC membership, and AAALAC accreditation status. Minority reports from IACUC members must be included. It also includes the dates of semiannual review and reports submitted to the IO. The PHS and AAALAC annual reporting dates may be synchronized with the USDA reports.

A sample annual report format is provided on OLAW's Web site and may be utilized, but is not required.

On or before December 1, each facility registered with the USDA must submit an annual report to the APHIS, AC Regional Director, for the state in which the facility is registered. Form 7023 is usually prepared by the IACUC and signed by the CEO or IO. It lists the number of each covered species used, by pain categories. The report includes assurances that animal care and use are at professionally accepted standards, that alternatives to painful procedures have been considered (see Section C.2.a. Alternatives) and that AWRs are followed.

When an IACUC-approved deviation from USDA standards and regulations is required for scientific or other reasons, the report must address the reasons for the deviation, and the number and species of animals affected.

Suspension and Noncompliance

At an institution with an approved PHS Assurance, the IACUC must report promptly, through the IO, the circumstances and actions taken in the following instances:

- any serious or continuing non-compliance with the *PHS Policy,*
- any serious deviation from the provisions of the *Guide,* and
- any suspension of any activity by the IACUC.

It is recommended that the institution contact OLAW immediately following the event, and send a formal report, describing the circumstances and any actions taken, to OLAW after IACUC and IO review. Similarly, accredited institutions must report promptly to AAALAC serious issues relating to the animal care and use program, such as investigations by the USDA or OLAW, or other serious incidents or concerns that negatively affect animal well-being.

If the IACUC suspends any activities involving USDA-covered animals, the IO files a report with the AC Regional Director, in consultation with the IACUC. After reviewing the reasons for the suspension and taking appropriate corrective actions, the IO is responsible for submitting a full explanation to APHIS and any federal agency funding the activity (see Tables E and A.2.B.).

E.1. Table A. Federal Requirements: Recordkeeping

Records	PHS Policy	AWRs
IACUC Minutes	• Records of attendance, activities and deliberations	• Records of attendance, activities and deliberations
Protocols	• Records of proposed activities using animals • Record of proposed significant changes • Outcome of IACUC review	• Records of proposed activities using animals • Record of proposed significant changes • Outcome of IACUC review
Basic Documents	• Semiannual IACUC reports and recommendations • Assurance Document • Records of accrediting body determinations	• Semiannual IACUC reports and recommendations
Animal	• Refer to Guide regarding clinical records, pedigree information, standardized nomenclature, etc.	• Records on acquired live dogs/cats or offspring including seven types of information • Records on dogs/cats transported/sold/euthanized including three types of information • "Random Source" Certificates (See 2.133 (f) and (g))
Other Requirements	• All records must be kept for three years; records that relate to applications, proposals, and proposed significant changes must be maintained for the duration of the activity plus three years. • Accessible for inspection or copying by OLAW or other PHS officials	• All records must be kept for three years; records that relate to applications, proposals, and proposed significant changes must be maintained for the duration of the activity plus three years. • Accessible to APHIS and Federal agency officials • USDA may extend the records retention requirements pending completion of an investigation.
Reference	Policy IV.E.	9 CFR Part 2, Subpart C 2.35

E.1. Table B. Federal Requirements: Assurance and Registration

	PHS Institutional Assurance	USDA Research Facility Registration
When required	• To receive PHS support for animal activities • Approved for up to five years	• Animals covered by USDA Regulations held or used for regulated purposes • Updated every three years
Submit to	• Office of Laboratory Animal Welfare (OLAW)	• APHIS, AC Regional Director
Submitted by	• IO	• Signed by person with legal authority to bind organization
Forms	• Institutional letterhead	• APHIS Form 7011
Contents	• List all components of institution to be included, including sq. ft. of each facility, species housed and average daily inventory by species • Describe lines of authority and responsibility • List qualifications, responsibility, authority and percent of time contribution of each veterinarian • IACUC membership list and description of IACUC procedures • Describe occupational health program • Synopsis of training/instruction offered to personnel involved with animals	• Location of research facilities • Number of covered species used annually • Sources of Federal funds
After submission	• Indicate AAALAC accreditation and include semiannual IACUC report if not accredited • OLAW may negotiate changes before approval	• Notify APHIS via AC Regional Director within ten (10) days of change of operation
Reference	PHS Policy IV.A.	9 CFR Part 2, Subpart C 2.30

E.1. Table C. Federal Requirements: Report of Semiannual Evaluations

	PHS Semiannual Report	USDA Semiannual Report
Timetable	• Every six months; an AAALAC report may fulfill these requirements	• Every six months; an AAALAC report may fulfill these requirements
Submit to	• IO	• IO
Submitted by	• IACUC, as Committee action	• IACUC; signed by a majority of members
Form used	• Not specified	• Not specified
Contents	• Describe adherence to *Guide* and *PHS Policy* and departures from *Guide* and *PHS Policy* • State reasons for departures; identify significant and minor deficiencies; include plan/schedule to correct deficiencies; include minority views • Approved by IACUC • Maintained by institution • Available to OLAW upon request • Other: Identify facilities accredited by AAALAC	• Describe adherence to AWRs and departures from AWRs; • State reasons for departures; identify significant and minor deficiencies; include plan/schedule to correct deficiencies; include minority views • Reviewed and signed by majority of IACUC members • Maintained by Research Facility Available to APHIS and funding agency upon request • Other: Report failure to adhere to plan/schedule through IO to APHIS and funding agency within 15 working days
Reference	PHS Policy IV.B.3. IV.E.1.d & IV.F.4.	9 CFR Part 2, Subpart C 2.31 (c) (3)

E.1. Table D. Federal Requirements: Annual Report

	PHS Annual Report	USDA Annual Report
Timetable	• At least once every 12 months; may be synchronized with USDA or AAALAC reporting period	• On or before December 1, describing activity during fiscal year (October 1–September 30)
Submit to	• OLAW	• APHIS, AC Regional Director
Submitted by	• IACUC, through the IO	• Signed or certified by CEO or IO
Form used	• Not specified; may use OLAW sample format provided	• APHIS Form 7023
Contents	Report changes in: • AAALAC accreditation status • Animal care/use program (as described in Assurance) • IACUC membership • IO • Report date(s) IACUC conducted semiannual evaluations and submitted reports to IO. Include any minority views of IACUC members.	• Location of all facilities • Endorse assurance statements • Common name and number of animals being bred, conditioned or in holding not being used (col. B) • Common name and number of animals used where: – Animals experience no pain or distress (col. C) – Drugs were used to alleviate pain or distress (col. D) – Drugs were not used to alleviate pain or distress because drugs would have interfered with the results or interpretation of the procedure (col. E) • An explanation of column E procedures, as justified by the investigator and approved by the IACUC, must be summarized in an attachment. It must include species and number of animals affected.
Reference	PHS Policy IV.F.1, 2 & 4.	9 CFR Part 2, Subpart C 2.36

E.1. Table E. Federal Requirements: Suspensions and Noncompliance

	PHS Suspension/ Noncompliance Report	USDA Suspension Report
Submitted by	• IACUC through IO	• IO with IACUC consultation
Submit to	• OLAW	• APHIS and federal agency funding the activity
When required	• Suspension of an activity by the IACUC • Serious deviation from the *Guide* (unless previously approved by the IACUC) • Serious or continuing noncompliance with the *PHS Policy*	• Suspension of an activity by the IACUC
Contents	• Full explanation of circumstances • Description of corrective action taken • Minority views filed by IACUC	• Full explanation of circumstances • Description of corrective action taken
Reference	PHS Policy IV.C.6. & 7. and IV.F.3. & 4	9 CFR Part 2, Subpart C 2.31(d)(7)

References

AAALAC International. *Connection Newsletter* Summer 2000, pages 1-4.

Potkay, S., et al. Frequently Asked Questions about the Public Health Service Policy on Humane Care and Use of Laboratory Animals. *Contemporary Topics* 36(2)47-50, March, 1997.

NIH Guide to Grants and Contracts. Notice OD-00-007, 12/21/99.

E.2. Communications

It has never been easier to communicate with others, and at the beginning of the 21st century the use of nontraditional means of communication such as electronic mail (email), Web sites, and Internet chat rooms provide new opportunities for rapid communication.

Electronic communication offers advantages and disadvantages. Modes of communication available to the IACUC vary in speed and ease of use, clarity, and security. Some permit easy communication with an entire committee or an entire institution; and some include a permanent record that can be retained for later reference.

Regulations and Policies

Most of the regulations governing the IACUC were written before the Internet became pervasive, but OLAW has presented some guidelines for the IACUC regarding the use of email and similar modes of communication (Garnett and Potkay, *ILAR Journal* 37:190-192, 1995).

The guidelines state that email is an appropriate medium for transmitting animal protocols, IACUC meeting agenda and minutes, institutional policies, and other matters related to the animal care and use program. However, OLAW states that the conduct of IACUC meetings should allow greater opportunity for members to interact than that permitted by email. Sequential, one-on-one communication (polling) by email, telephone, or fax should not take the place of a convened IACUC meeting or voting, although it is an appropriate mechanism for providing all IACUC members with the opportunity to call for full committee review of a protocol prior to initiating the designated reviewer method of protocol review. OLAW recommends that traditional meetings, in which a quorum of IACUC members is in the same room, should be the standard method for conducting IACUC business such as protocol review, review of annual and semiannual reports, and suspensions.

Under “exceptional circumstances” an IACUC may be permitted to conduct a meeting using electronic conferencing such as telephone or audio-visual conferences. To be considered a valid convened meeting,

the alternate approach must include a high degree of interactivity and allow for careful consideration of issues. Each member must be in direct communication with every other member in attendance, and a quorum of actively participating members must be maintained. Minutes of an electronic conference would be written and retained as for any other convened IACUC meeting.

If the IACUC wishes to use electronic methods for IACUC meetings or other activities, the proposed procedures should be described in the institutional Assurance and approved by OLAW.

Efficiency and Security

Email and the Internet have dramatically increased the speed and volume of information conveyed. Many institutions publish animal use policies and forms on a public Web site, and some include information such as IACUC membership and meeting times. Some have also developed mechanisms for submitting animal use protocols or modifications electronically, which can potentially eliminate tedious data entry and facilitate review and approval, and recordkeeping.

The increasing use of email for communication with investigators and the IACUC also has the potential for speeding up the review process, provided that messages do not get lost in a barrage of email from other sources. Undoubtedly, more institutions will automate protocol submission and move toward more efficient review processes during the next decade.

The continued development of electronic or digital signatures and password-only access to certain information is important. There is a widespread concern that electronic systems are not secure. IACUC databases should be maintained on institutional intranets, as opposed to the Internet, to minimize the vulnerability of systems. As Internet security improves, these issues should become less of an obstacle to the use of electronic communication for carrying out work of the IACUC.

Reference

Garnett, N. and S. Potkay. Use of Electronic Communications for IACUC Functions. *ILAR Journal* 37(4)190-192, 1995

Appendices

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Appendix A: Resources

ALTWEB

Web: *http://altweb.jhsph.edu*

ALTWEB is a Website created under the auspices of the Johns Hopkins Center for Alternatives to Animal Testing that is dedicated to providing information about and fostering the development of scientifically acceptable alternatives to the use of animals in testing and research. Alternatives are defined as methods that reduce animal use, replace whole animal tests, or refine existing tests by minimizing animal distress.

American Association for Laboratory Animal Science (AALAS)

9190 Crestwyn Hills Drive
Memphis, TN 38125
Tel: 901-754-8620
Fax: 901-753-0046
Web: *http://www.aalas.org/*

AALAS is an association of over 9,300 individuals dedicated to the humane care and treatment of laboratory animals and to quality research. It serves as a forum for the exchange of information and expertise in the care and use of laboratory animals.

American College of Laboratory Animal Medicine (ACLAM)

Web: *http://www.aclam.org/index.html*

The ACLAM is an organization of board certified veterinary medical specialists who are experts in the humane, proper and safe care and use of laboratory animals. ACLAM establishes standards of education, training, experience and expertise necessary to become qualified as a specialist and recognizes that achievement through board certification. ACLAM promotes the advancement of knowledge in this field through professional continuing education activities, and the development of educational materials.

American Society of Laboratory Animal Practitioners (ASLAP)

11300 Rockville Pike
Suite 1211
Rockville, MD 20852
Tel: 301-231-6349
Fax: 301-231-6071
Email: aslap@aaalac.org
Web: *http://www.aslap.org/*

The ASLAP is an organization of veterinarians and veterinary students that promotes the acquisition and dissemination of education and training in the practice of laboratory animal medicine.

American Veterinary Medical Association (AVMA)

1931 North Meacham Road
Suite 100
Schaumburg, IL 60173
Tel: 847-925-8070
Fax: 847-925-1329
Email: avmainfo@avma.org
Web: *http://www.avma.org*

The AVMA, a not-for-profit national association of veterinarians, was established in 1863 and has a current membership representing approximately 85% of the veterinary medical profession. The Association aims to advance the science and art of veterinary medicine, including its relationship to public health, biological science, and agriculture. It provides a forum for the discussion of issues of importance to the veterinary profession, and for the development of official positions. The Association is the authorized voice for the profession in presenting its views to government, academia, pet owners, the media, and other concerned publics.

Animal Welfare Information Center (AWIC)

National Agricultural Library, USDA
10301 Baltimore Avenue, 5th Floor
Beltsville, MD 20705-2351
Tel: 301-504-6212
Fax: 301-504-7125
Email: awic@nal.usda.gov
Web: *http://www.nal.usda.gov/awic/*

AWIC, a component of the USDA National Agricultural Library, is dedicated to providing information for improved animal care and use in research, teaching, and testing. AWIC also offers educational activities that are geared towards meeting the information requirements of the Animal Welfare Act, and publishes bibliographies, information resource guides, and other publications.

Applied Research Ethics National Association (ARENA)

132 Boylston Street
Fourth Floor
Boston, MA 02116
Tel: 617-423-4112
Fax: 617-423-1185
Email: PRMR@aol.com
Web: *http://www.arena.org/*

ARENA is a membership organization for those involved in the day-to-day application of ethical principles, governmental regulations, and other policies regarding research and clinical practice. ARENA services include sponsorship of national and regional meetings, the dissemination of current information on research ethics, and the provision of opportunities for networking among members through a quarterly newsletter.

Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International)

11300 Rockville Pike
Suite 1211
Rockville, MD 20852-3035
Tel: 301-231-5353
Fax: 301-231-8282
Email: accredit@aaalac.org
Web: *http://www.aaalac.org/*

AAALAC International is a private, non-profit organization that promotes the humane treatment of animals in science through a voluntary accreditation program. The rigorous, peer review of the animal care and use program promotes scientific validity and demonstrates accountability. AAALAC also offers independent program status evaluations to assist institutions in determining their preparedness for accreditation and to help institutions improve their animal care and use program.

Canadian Council on Animal Care (CCAC)

315-350 Albert Street
Ottawa ON K1R 1B1
Tel: 613-238-4031
Fax: 613-238-2837
Email: lroach@bart.ccac.ca
Web: *http://www.ccac.ca/english/welcome.htm*

CCAC is the national peer review agency responsible for setting and maintaining standards for the care and use of animals used in research, teaching and testing throughout Canada. CCAC guidelines and publications provide useful information for animal care and use committees concerning optimal physical and psychological care of animals according to acceptable scientific standards.

Center for Alternatives to Animal Testing

Johns Hopkins University School of Hygiene and Public Health
111 Market Place, Suite 840
Baltimore, MD 21202-6709
Tel: 410-223-1612
Fax: 410-223-1603
Email: caat@jhsph.edu
Web: *http://caat.jhsph.edu*

Foundation for Biomedical Research (FBR)

818 Connecticut Avenue, NW
Suite 303
Washington, DC 20006
Tel: 202-457-0654
Fax: 202-457-0659
Email: info@fbresearch.org
Web: *http://www.fbresearch.org/index.html*

The FBR was established in 1981 to improve the quality of human and animal health by promoting public understanding and support of the ethical use of animals in scientific and medical research. FBR produces a wide variety of educational resources to help the general public understand why animals are so important in the search for new and better ways to treat the diseases that afflict both people and animals.

IACUC.ORG

Web: *http://www.iacuc.org/*

IACUC.ORG is an information resource developed by AALAS for members and staff of institutional animal care and use committees. It is a link archive where online resources are organized by menus and submenus, which serve as organizing tools enabling users to quickly point to a topic of interest, such as example protocol forms or disaster plans used by other institutions.

Institute for Laboratory Animal Resources (ILAR)

2101 Constitution Avenue, NW
Washington, DC 20418
Tel: 202-334-2590
Fax: 202-334-1687
Email: ILAR@nas.edu
Web: *http://www4.nas.edu/cls/ilarhome.nsf*

A component of the National Academy of Sciences, ILAR is responsible for authoritative reports on subjects of importance to the animal care and use community, and for serving as a clearinghouse for information about animal resources. Its mission is to develop and make available scientific and technical information on laboratory animals and other biological research resources to the scientific community, the federal government, and the public.

NETVET Veterinary Resources

Web: *http://netvet.wustl.edu/vet.htm*

NETVET is a comprehensive website that categorizes and organizes veterinary medical and animal-related information on the Internet in a relevant, user friendly format. Much of the information is of interest to IACUCs.

Office of Laboratory Animal Welfare (OLAW)

National Institutes of Health
RKL1, MSC 7982
6705 Rockledge Drive
Bethesda, MD 20892-7982
Tel: 301-496-7163
Fax: 301-402-2803
Email: olaw@od.nih.gov
Web: *http://grants.nih.gov/grants/olaw/olaw.htm*

OLAW is responsible for the administration and implementation of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals. Located at the National Institutes of Health, OLAW administers an educational program for PHS-supported institutions and investigators, negotiates Animal Welfare Assurances, and evaluates compliance with the *PHS Policy*.

Public Responsibility in Medicine and Research (PRIM&R)

132 Boylston Street
Fourth Floor
Boston, MA 02116
Tel: 617-423-4112
Fax: 617-423-1185
Email: PRMR@aol.com
Web: *http://www.primr.org/*

PRIM&R is a national nonprofit organization dedicated to educating the medical and legal professions, industry and the public about the ethical, legal, and policy dimensions of appropriate and ethical research. Through PRIM&R conferences a broad range of issues regarding research, clinical practice, ethics, and the law are addressed, including the operation of Institutional Animal Care and Use Committees.

Scientists Center for Animal Welfare (SCAW)

7833 Walker Drive, Suite 410
Greenbelt, MD 20770
Tel: 301-345-3500
Fax: 301-345-3503
Email: info@scaw.com
Web: *http://www.scaw.com/*

The SCAW is a non-profit educational association of individuals and institutions whose mission is to promote humane care, use, and management of animals involved in research, testing or education in laboratory, agricultural, wildlife or other settings. It offers an ongoing forum for the exchange and evaluation of scientific information about the care, treatment, well-being and ethical use of animals.

United States Department of Agriculture (USDA), Animal Care (AC)

4700 River Road, Unit 84
Riverdale, MD 20737-1234
Tel: 301-734-7833
Fax: 301-734-4978
Email: ace@aphis.usda.gov
Web: *http://www.aphis.usda.gov/ac/*

The Animal Care (AC) component of the USDA's Animal and Plant Health Inspection Service (APHIS) is responsible for the enforcement of the Animal Welfare Act (AWA). The AWA sets minimum standards of care and treatment for most warm-blooded animals used in research. Three regional offices employ field veterinary medical officers (VMOs) who regularly conduct unannounced inspections of research facilities for compliance with the USDA animal welfare regulations.

University of California Center for Animal Alternatives (UCCAA)

One Shields Avenue
Davis, CA 95616-8684
Tel: 530-752-1800
Fax: 530-754-8606
Email: animalalternatives@ucdavis.edu
Web: *http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm*

The UCCAA collects, disseminates, and facilitates access to information concerning animal alternatives, serving primarily the scientists and staff on the nine University of California campuses. The purpose is to improve the well-being and quality of life of research animals, but also to optimize their contribution to education and research.

ResearchTraining.org

Web: *http://www.researchtraining.org*

ResearchTraining.org is a Website developed by the Medical Research Service in the VA Office of Research and Development. Its purpose is to help VA and non-VA institutions meet research training mandates. The site includes free web-based courses and exams for research staff and IACUC members, and an IACUC Administrator's site where administrators can review the records of staff members who pass exams.

Appendix B: Office of Laboratory Animal Welfare

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Appendix C: Mandatory IACUC Issues Identified During AAALAC International Site Visits

(See Section B.1. Program and Facility Review)

- Inadequate review and follow-up of the animal care and use program
- Need for more rigorous protocol review
- Inadequate records of IACUC activities
- Assurance of participation in and adequacy of training programs
- Inadequately addressing issues pertaining to pain and distress
- Need for IACUC to review and approve deviations from the *Guide*
- IACUC assurance of adequate veterinary care
- Inadequate IACUC oversight of animals in satellite/contract facilities
- Committee composition and participation
- Changes in protocol without IACUC review and approval
- No three year complete review of protocols/annual review of PHS-funded research
- Allowing ordering of animals without assignment to an animal use protocol
- Not all animals covered by a protocol (e.g., breeding animals)
- Absence of exercise and psychological well-being plans for dogs and nonhuman primates
- Committee not appointed by the CEO
- Inadequate facility inspections (e.g., laboratories)
- Inadequate training of IACUC
- Inadequate intensity of oversight of program

Presented in order of most common citation to least frequent citation.

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Appendix D: Recommendations of the 2000 AVMA Panel on Euthanasia

(See C.2.b. Euthanasia)

The 2000 AVMA Panel on Euthanasia Report characterizes euthanasia methods by type:

1) Inhalant agents,
2) Noninhalant pharmaceutical agents, and
3) Physical methods.

The Panel further classifies the methods into those that are considered acceptable, conditionally acceptable and unacceptable when used as the sole means of euthanasia.

Euthanasia of Homeothermic (Warm-blooded) Animals

Inhalant Agents

Inhalant Anesthetics: The Panel recommends the use of halothane, enflurane, sevoflurane, methoxyflurane, isoflurane and desflurane (in order of preference) for animals under 7 kg. Although acceptable for use in larger animals these agents are not often used due to cost and difficulty in administration. Induction with methoxyflurane (metofane) is unacceptably slow in some species. Ether was formerly used extensively, but is now only conditionally acceptable due to irritation of mucous membranes and risk of fire and explosion. Nitrous oxide (N_2O) does not produce anesthesia, and may produce hypoxemia and cardiac or respiratory arrest. It may be used in combination with other anesthetics to speed anesthesia onset. It is important to minimize exposure of personnel to these potentially toxic agents; therefore fume hoods must be used.

Carbon Dioxide (CO_2): Carbon dioxide is an effective and widely used agent to euthanize rodents. This method causes hypoxia attributable to depression of vital centers. Use of carbon dioxide generated by other methods (e.g., dry ice, fire extinguishers) is not acceptable. Compressed CO_2 gas in cylinders is the only recommended source of carbon dioxide, since the inflow to the euthanasia chamber can be regulated. An optimal flow rate will displace at least 20% of the chamber volume per minute. In some species (e.g., rats) prefilling the chamber to 70% or more will produce rapid unconsciousness with minimal distress. Young animals, and some burrowing and diving animals, are relatively resistant to the hypoxemic effect of CO_2. Since the effects of carbon dioxide are reversible, it is important to ensure that the animals are dead.

Other agents: Nitrogen and argon are listed as conditionally acceptable methods for death by hypoxemia, and are relatively safe. Although effective, they may cause distress and other methods are preferred. Carbon monoxide induces unconsciousness without significant discomfort, and is considered acceptable for euthanasia for dogs, cats, and other small mammals. However, it is dangerous to use, and the Panel recommends it only if proper precautions are observed.

Non-inhalant Agents

Barbiturates: Injection of barbiturates, particularly sodium pentobarbital, is the most rapid and reliable method of euthanasia for most research animals. In non-rodent species, barbiturates are given intravenously to be most effective. A sedative or tranquilizer may be given prior to the barbiturate in animals that are difficult to restrain. Intraperitoneal injection is also acceptable when necessary if restraint or intravenous administration would be more stressful. In rodents, intravenous barbiturate for euthanasia is not common, since equally humane and less time-consuming methods are available. Intraperitoneal injection of barbiturate is acceptable for euthanasia in small mammals.

Sodium pentobarbital is listed as a Schedule II drug by the U.S. Drug Enforcement Administration (DEA). Current federal drug regulations require strict accounting for barbiturates, and they must be used under the supervision of personnel registered with the DEA. Some effective euthanasia solutions contain barbiturates in combination with other agents, and are listed Schedule III and are less restricted in use.

Potassium Chloride (KCl): KCl induces immediate cardiac arrest without any significant depression of the central nervous system. Hence, it must only be used after the animal is deeply anesthetized.

Neuromuscular Blocking Agents (Succinycholine, Curare, etc.): These drugs induce muscular paralysis and death by suffocation. They are not acceptable for euthanasia.

Physical Methods

Physical methods are sometimes necessary to obtain scientifically valid data and, while aesthetically displeasing to some individuals, are humane when properly performed by skilled and experienced personnel with appropriate, well-maintained equipment. The Panel considers most physical methods to be conditionally acceptable.

Cervical Dislocation: This is frequently used for mice, poultry and other small birds, immature rats weighing less than 200 grams and rabbits weighing less than one kilogram. Cervical dislocation is described in the 2000 AVMA Report as a humane technique for euthanasia of rodents and small rabbits in research, which induces rapid loss of consciousness without chemically contaminating tissue. Its use must be scientifically justified and approved by the IACUC on a case-by-case basis. As part of the approval process the IACUC must be assured that the personnel are appropriately qualified in the use of this method for the specific species involved. It is critical that personnel performing these procedures are thoroughly trained, usually by practicing the procedure on anesthetized animals.

Decapitation: Decapitation may be used to euthanize rodents and small rabbits. Except in neonatal animals, a guillotine is generally used. The section should be through the atlanto-occipital joint. The 2000 AVMA Report recommends that decapitation be done only when scientifically justified and approved by the IACUC on a case-by-case basis. As part of the approval process the IACUC must be assured that the personnel are appropriately skilled in the use of this method for the specific species involved.

Microwave Irradiation: This method is used when a project requires fixation of mouse or rat brain metabolites *in vivo* without losing anatomic integrity of the brain. Commercial microwave chambers will render a rodent unconscious in less 100 msec. and dead in under one second. These instruments differ from household units in that they direct most of the microwave energy at the head of the animal. Only instruments designed for this purpose and having the appropriate power and microwave distribution may be used.

Penetrating Captive Bolt: This method is conditionally acceptable for ruminants, horses, and swine when chemical agents are scientifically contraindicated. Use of a non-penetrating captive bolt only stuns and should not be attempted as the sole means of euthanasia.

Euthanasia of Poikilothermic (Cold-blooded) Animals

The 2000 Report of the AVMA Panel on Euthanasia addressed the euthanasia of poikilothermic animals and in doing so pointed out that the available objective information on these species in the literature limits the guidelines that can be developed. The Panel also pointed out the differences in the metabolism, respiration and tolerance to cerebral hypoxia between these species and homeothermic animals must be considered when selecting a method of euthanasia.

Chemical Agents: Intraperitoneal administration of pentobarbital is an effective method of euthanasia in amphibians, turtles and snakes. Tricaine methane sulfonate (MS222) or benzocaine hydrochloride may be placed in the water of amphibians and fish to produce anesthesia and prolonged contact will produce death. Inhalant anesthetics may be used for amphibians and reptiles. Due to the low oxygen requirements for reptiles, the onset of unconsciousness and death will be significantly lengthened.

Physical Methods: Poikilotherms may be euthanized by stunning followed by decapitation, pithing, or some other method to ensure death. In frogs and toads, pithing the brain and spinal cord (double pithing) is an effective and acceptable method.

Additional and Adjunctive Methods

The 2000 Report of the AVMA Panel on Euthanasia included additional methods that, under appropriate circumstances, would produce a humane death. For specifics, consult the Panel report published in *JAVMA* Vol. 218, No. 5, March 1, 2001.

Appendix E: Federal and State Permits Required for Field Studies

(See Section C.3.d. Field Studies)

One research protocol may be subject to multiple laws and therefore multiple permits might be required. It is most commonly the case that both state and federal permits are needed in addition to site-specific permits for research conducted on federal- or state-owned property.

U.S. Fish and Wildlife Service

The permits administered by the U.S. Fish and Wildlife Service (USFWS) are found in 50 CFR, Sections 1 - 100. The general permit conditions found in 50 CFR 13 state that any person accepting and holding a permit acknowledges the necessity for close regulation and monitoring of the permitted activity by the Government. By accepting such permit, the permittee consents to and must allow entry by agents or employees of the USFWS upon premises where the permitted activity is conducted at any reasonable hour. Service agents or employees may enter such premises to inspect the location; any books, records, or permits required to be kept by this subchapter; and any wildlife or plants kept under authority of the permit. The regulations also provide for permit suspension and revocation if permit terms and conditions are violated.

USFWS has developed a system to assess the impact of permitted activities on populations. Known as the Service-wide Permits Issuance and Tracking system, this tool allows permit biologists to determine the cumulative impact of permitted activities on wildlife populations with a high degree of precision.

To take, possess, or transport any bald eagle (*Haliaeetus leucocephalus*) or any golden eagle (*Aquila chrysaetos)*, or the parts, nests, or eggs of such birds, a Bald and Golden Eagle Protection Act permit is required, although banding and marking may be authorized under a Migratory Bird Treaty Act permit. The USFWS will accept a single application for both permits provided that it includes all of the information required for an application under each applicable part.

CITES

The Convention on International Trade in Endangered Species, commonly referred to by the acronym CITES, is an international treaty codified in U.S. law as part of the Endangered Species Act. It regulates import and export of wildlife and plants listed on its three appendices. Appendix I species require both an export permit from the country of origin and an import permit from the importing country. Commercial trade is prohibited but import and export for scientific research is allowed, subject to very strict permitting requirements. Permit issuance criteria require capture and import methods that minimize the risk of injury, damage to health or cruel treatment. Permits are not issued if the proposed import or export would be detrimental to the survival of the species.

Endangered Species Act

The Endangered Species Act prohibits the taking of any species listed as endangered or threatened. The endangered species list is found in 50 CFR 17.11. The term "take" means to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct. Both civil and criminal penalties can be imposed on a violator. Exceptions are made for scientific research and for activities that will enhance the survival of the species. Permits are required for such activities and are issued by the USFWS (except in the case of marine mammals and fishes, which are issued by the National Marine Fisheries Service). The regu• lations governing scientific permits for endangered species are found at 50 CFR 17.22; regulations for permits for threatened species are found at 50 CFR 17.62.

In considering whether to grant a permit, the permitting official will consider the purpose for which the permit is required, the probable direct and indirect effect on the population of issuing the permit, whether the permit would conflict with programs intended to enhance the survival probabilities of the population, whether the permit would reduce the threat of extinction, opinions or views of experts in matters germane to the application, and whether the expertise, facilities, or other resources available to the appli• cant appear adequate to successfully accomplish the objectives stated in the application.

An issued permit may contain conditions that the permitting authority chooses to impose, including requirements for humane conditions (50 CFR 13.41). For instance, the permit may limit the time a researcher may spend in a colony of seabirds, limit capture methods, or otherwise dictate limits on research methodology. Applications for endangered species permits are published in the Federal Register and afford the public an opportunity to comment or object.

Lacey Act

The original Lacey Act dates back to 1900; what is currently referred to as the Lacey Act is actually the Lacey Act Amendments of 1981. It is not specific to research, but pertains to research involving the import and export of wildlife. Most commonly, the import and export of wildlife is conducted by museums acquiring or trading specimens but there is also importation and exportation of live animals for research purposes. The law covers all fish and wildlife and their parts or products. Under this law, it is unlawful to import, export, sell, acquire, or purchase fish, wildlife or plants taken, possessed, transported, or sold: 1) in violation of U.S. or Indian law, or 2) in interstate or foreign commerce involving any fish, wildlife, or plants taken, possessed or sold in violation of State or foreign law.

Regulations pertaining to the import, export, and transportation of wildlife are found at 50 CFR 14. Generally, wildlife must be imported through designated ports in order to allow for inspection by Customs officers and/or USFWS law enforcement officers. Permits can be obtained to import wildlife for scientific purposes through nondesignated ports. Otherwise, for import of species not listed on CITES or the Endangered Species List, importers or their agents must file with the Service a completed Declaration for Importation or Exportation of Fish or Wildlife (Form 3-177), signed by the importer or the importer's agent, upon the importation of any wildlife at the place where Service clearance (permission for release from the port) is requested.

Marine Mammal Protection Act

The 1972 Marine Mammal Protection Act established a Federal responsibility to conserve marine mammals with management vested in the Department of Interior for sea otter, walrus, polar bear, dugong, and manatee. The Department of Commerce is responsible for cetaceans and pinnipeds, other than the walrus. With certain specified exceptions, the Act establishes a

moratorium on the taking and importation of marine mammals as well as products taken from them, and establishes procedures for waiving the moratorium and transferring management responsibility to the States. The 1988 amendments include the listing of conditions under which permits may be issued to take marine mammals for the protection and welfare of the animals, including importation, public display, scientific research, and enhancing the survival or recovery of a species.

Scientific permits are provided for by 50 CFR 18. If the application is for a scientific research permit, it must include a detailed description of the scientific research project or program in which the marine mammal or marine mammal product is to be used including a copy of the research proposal relating to such program or project and the names and addresses of the sponsor or cooperating institution and the scientists involved. Where the species is listed as endangered or threatened or has been designated as depleted, the applicant must also provide a detailed justification of the need for such a marine mammal, including a discussion of possible alterna• tives, whether or not under the control of the applicant.

Migratory Bird Treaty Act

The original 1918 statute implemented the 1916 Convention between the U.S. and Great Britain (for Canada) for the protection of migratory birds. Specific provisions in the statute include the establishment of a federal prohibition, unless permitted by regulations, to "pursue, hunt, take, cap• ture, kill, attempt to take, capture or kill, possess, offer for sale, sell, offer to purchase, purchase, deliver for shipment, ship, cause to be shipped, deliver for transportation, transport, cause to be transported, carry, or cause to be carried by any means whatever, receive for shipment, transporta• tion or carriage, or export, at any time, or in any manner, any migratory bird, included in the terms of this Convention . . . for the protection of migratory birds . . . or any part, nest, or egg of any such bird." (16 U.S.C. 703)

The Secretary of the Interior is authorized to determine, periodically, when, consistent with the Conventions, "hunting, taking, capture, killing, posses• sion, sale, purchase, shipment, transportation, carriage, or export of any . . . bird, or any part, nest or egg" could be undertaken and to adopt regulations for this purpose.

The title "Migratory Bird Treaty Act" (MBTA) is a misnomer because the Act does not apply only to birds that migrate long distances or across international borders, but to nearly 830 species of birds. Permits for the taking of birds protected by the MBTA are found at 50 CFR 21.

Banding and marking activities require a permit under 50 CFR 21.22. These permits are issued by the U.S. Geological Survey–Biological Resources Division's Bird Banding Laboratory. A banding permit authorizes the placement of USFWS-issued bands on birds. Additional authorization is required for the use of auxiliary markers (such as colored leg bands, paint marks, wing tagging, radio transmitters), mist nets, cannon or rocket nets, or chemical means of capturing birds. Permits are specific to taxa or even species. Special authorization is required for endangered species, eagles, waterfowl, and hummingbirds. The Bird Banding Laboratory may also authorize the taking of blood and feather samples. Requests to band in more than one state must be justified.

Other MBTA permits are obtained from the USFWS. These include permits for scientific collecting (50 CFR 21.23). The regulation does not limit the number of individuals that may be collected, but the USFWS by practice and policy does.

Wild Bird Conservation Act

The Wild Bird Conservation Act (WBCA) prohibits the import of any bird into the United States other than those specifically listed in the regulations as permissible. For any other species, a permit is required. This law supplements CITES, as many species of birds are also listed on CITES Appendices I and II.

Permits may be issued for scientific research, and are in addition to any other permits that might be required. So, for instance, if the species is also a CITES-listed species, both a CITES and a WBCA permit are required. The regulation for scientific research permits is found at 50 CFR 15.22. Applications must detail (among other things) why the applicant is justified in obtaining a permit, and a complete description of the scientific research to be conducted on the exotic bird requested.

Site-specific Permit Requirements

Site-specific permits are in addition to the permits described above. A permit to conduct research on federal property confers no right to conduct research without other legally required permits.

Bureau of Land Management

The Bureau of Land Management (BLM) has no specific requirements or permits for scientific research activities. General use regulations under 43 CFR 2920 govern all non-federal use of the lands managed by the BLM. The local BLM office is to consider the duration of the anticipated use and its impact on the public lands and resources. Permission will be given only for those uses that conform with BLM plans, policy, objectives and resource management programs. For some research activities, a permit may not be required as the regulations provide that no land use authorization is required under the regulations in this part for casual use of the public lands. An application must include a description of the proposed land use in sufficient detail to enable the authorized officer to evaluate the feasibility of the proposed land use, the impacts if any, on the environment, the public or other benefits from the proposed land use, the approximate cost of the proposal, any threat to the public health and safety posed by the proposal and whether the proposal is, in the proponent's opinion, in conformance with BLM plans, programs and policies.

National Parks

National Park Service (NPS) regulations prohibit possessing, destroying, injuring, defacing, removing, digging, or disturbing from its natural state living or dead wildlife or fish, or the parts or products thereof, such as antlers or nests (36 CFR 2.1.). Section 2.2 prohibits the taking of wildlife, except by authorized hunting and trapping activities conducted in accordance with paragraph (b) of this section and the feeding, touching, teasing, frightening or intentional disturbing of wildlife nesting, breeding or other activities. Possession of a weapon, net, or trap without a permit is prohibited.

There is no specific regulation pertaining to scientific research other than the collecting regulation discussed below. Currently, the NPS policy regarding research is found in its Administrative Guide, which pertains to all scientific research, Application Procedures and Requirements for Research and Collecting Permits, and the Guidelines for Study Proposals. In 1999, NPS began an effort to develop a research and collecting permit and reporting

system. Researchers are required to submit research proposals, which are reviewed by the NPS for scientific validity and actual or potential impact to park resources, among other things. The NPS may impose any conditions it deems appropriate. In reviewing applications, the NPS considers, among other things, whether the proposed research contributes information useful to an increased understanding of park resources or addresses problems or questions of importance to science or society and shows promise of mak• ing an important contribution to humankind's knowledge of the subject matter. The qualifications of the applicant are also reviewed.

Scientific collecting, including the taking of plants, fish, wildlife, rocks or minerals is regulated by 36 CFR 2.5. A specimen collection permit may be issued only to an official representative of a reputable scientific or educa• tional institution or a State or Federal agency for specific purposes described in the regulations. A permit to take an endangered or threatened species listed pursuant to the Endangered Species Act, or similarly identified by the States, may not be issued unless the species cannot be obtained outside of the park area and the primary purpose of the collection is to enhance the protection or management of the species. In park areas where the enabling legislation authorizes the killing of wildlife, a permit that authorizes the kill• ing of plants, fish or wildlife may be issued only when the superintendent approves a written research proposal and determines that the collection will benefit science or has the potential for improving the management and protection of park resources. In park areas where enabling legislation does not expressly prohibit the killing of wildlife, a permit authorizing the killing of plants, fish or wildlife may be issued only when the superintendent approves a written research proposal and determines that the collection will not result in the derogation of the values or purposes for which the park area was established and has the potential for conserving and perpetuat• ing the species subject to collection. In park areas where the enabling legislation prohibits the killing of wildlife, issuance of a collecting permit for wildlife or fish or plants, is prohibited.

National Forests

Forest Service laws and regulations prohibit all activities that are not expressly allowed by regulation or permit under 36 CFR 251, and the regu• lations do not address scientific research specifically. The guidelines for special use permits are found in 36 CFR 251.54. The two-tier screening process entails, among other things, determinations that authorization of the proposed activity is consistent or can be made consistent with the standards and guidelines in the applicable forest land and resource

management plan required under the National Forest Management Act and 36 CFR part 219, and that the proposed activity does not materially impact the characteristics or functions of the environmentally sensitive resources or lands identified in Forest Service Handbook 1909.15, chapter 30.

National Wildlife Refuges

When a national wildlife refuge is created, it is considered closed to the public until it is expressly opened by its manager. The refuge managers are, under the National Wildlife Refuge System Administration Act of 1966, to allow "compatible" wildlife dependent recreation. In 1997, Congress enacted the National Wildlife Refuge System Improvement Act (NWRSIA), which retained the compatibility standards, but required FWS to define what it is and establish a process for compatibility determinations. On October 18, 2000, the USFWS issued its Final Compatibility Regulations (65 FR 62457-62483). The regulation defines compatibility as, "a proposed or existing wildlife-dependent recreational use or any other use of a national wildlife refuge that, based on sound professional judgment, will not materially interfere with or detract from the fulfillment of the National Wildlife Refuge System mission or the purpose(s) of the national wildlife refuge." This determination is to be made by the refuge manager.

The primary concern of refuge managers under the statutes and regulations is to "administer a national network of lands and waters for the conservation, management, and, where appropriate, restoration of the fish, wildlife, and plant resources and their habitats within the United States for the benefit of present and future generations of Americans." The NWRSIA specifically defines the terms 'conserving,' 'conservation,' 'manage,' 'managing' and 'management' to mean, "to sustain, and where appropriate, restore and enhance, healthy populations of fish, wildlife, and plants, utilizing, in accordance with applicable Federal and State laws, methods, and procedures associated with modern scientific resource programs. Such methods and procedures include, consistent with the provisions of this Act, protection, research, live trapping and transplantation, and regulated taking."

State Laws and Regulations

Virtually all States regulate activities involving wildlife, including scientific research. The Center for Wildlife Law has published a handbook entitled *State Wildlife Laws Handbook*, which covers all State wildlife statutes, although it does not include permitting regulations. State regulations would be found in the States' analogues to the Code of Federal Regulations. Most State regulations also require permits for research on State-owned lands.

Professional Societies

Animal Behavior Society

Website: *http://www.animalbehavior.org/*

Contact: Animal Behavior Society
Indiana University
2611 East 10th Street, #170
Bloomington, IN 47408-2603
(812) 856-5541

American Fisheries Society

Website: *http://www.fisheries.org*

Contact: American Fisheries Society
5410 Grosvenor Lane, Suite 110
Bethesda, MD 20814-2199
(301) 897-8616

American Society of Ichthyologists and Herpetologists

Website: *http://199.245.200.110/*

Contact: ASIH has no staffed office. Leadership and committee members, including the Animal Care and Use Committee, are listed on the ASIH website, which also includes an on-line directory of mem• bers' e-mail addresses.

American Society of Mammalogists

Website: *http://www.mammalsociety.org/*

Contact: ASM has no staffed office. Leadership and committee members are listed on the ASM website.

The Ornithological Council

Website: *http://www.nmnh.si.edu/BIRDNET*

Contact: The Ornithological Council
3713 Chevy Chase Lake Drive, Apt 3
Chevy Chase, MD 20815
(301) 986-8568

The Wildlife Society

Website: *www.wildlife.org*

Contact: The Wildlife Society
5410 Grosvenor Lane
Bethesda, MD 20814
(301) 897-9770
(301) 530-2471 Fax

Appendix F: U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training

The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species. Whenever U.S. Gov• ernment agencies develop requirements for testing, research, or training procedures involving the use of vertebrate animals, the following principles shall be considered; and whenever these agencies actually perform or spon• sor such procedures, the responsible Institutional Official shall ensure that these principles are adhered to:

I.- The transportation, care, and use of animals should be in accor• dance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.*

II.- Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

III.- The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simu• lation, and in vitro biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investi• gators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate seda• tion, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI.- Animals that would otherwise suffer severe or chronic pain or dis• tress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

VIII. Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals.

IX.- Where exceptions are required in relation to the provisions of these Principles, the decisions should not rest with the investigators directly concerned but should be made, with due regard to Principle II, by an appropriate review group such as an institutional animal care and use committee. Such exceptions should not be made solely for the purposes of teaching or demonstration.

** For guidance throughout these Principles, the reader is referred to the* Guide for the Care and Use of Laboratory Animals *prepared by the Institute of Laboratory Animal Resources, National Academy of Sciences.*

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