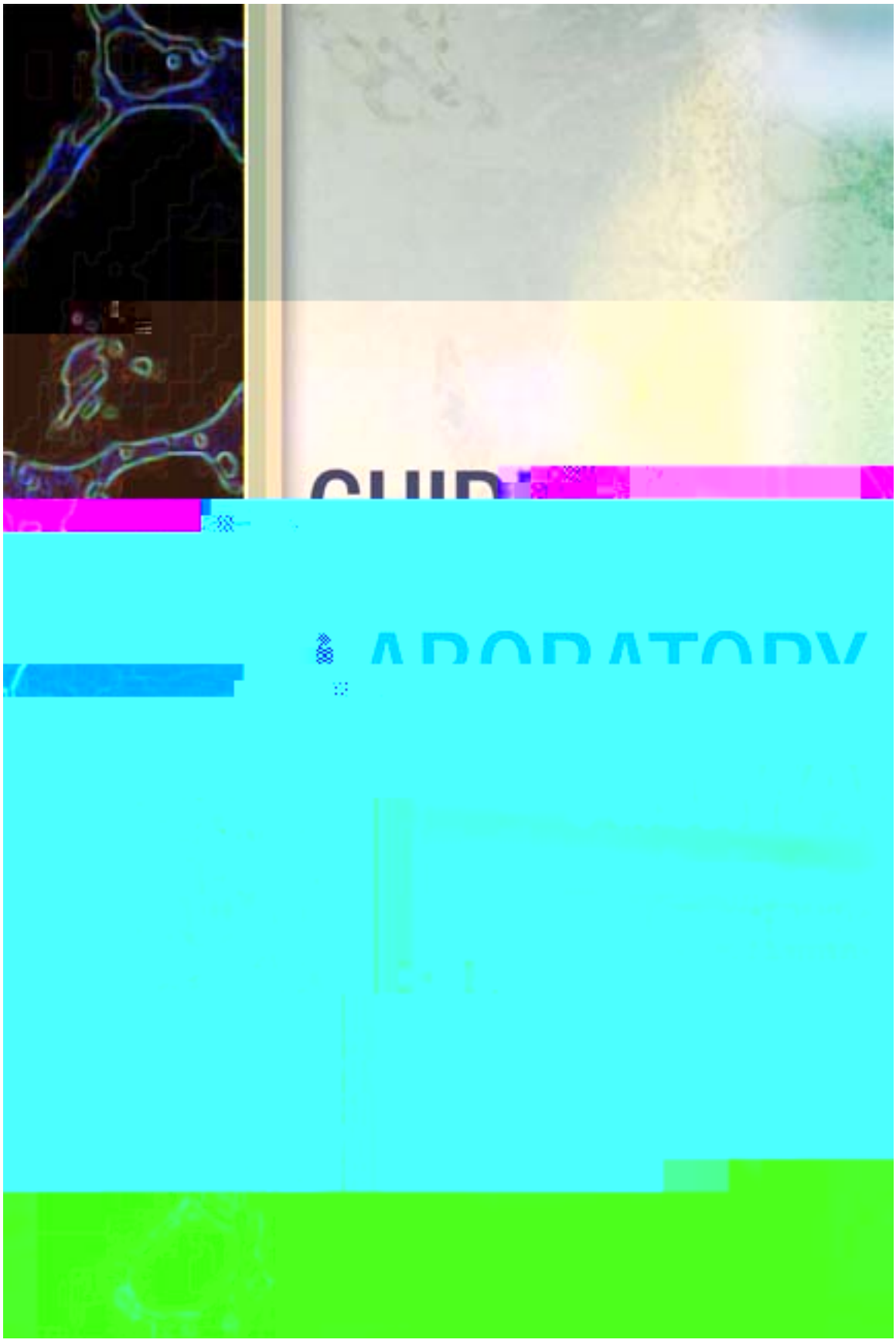

Guide for the Care and Use of Laboratory Animals

PREPUBLICATION DRAFT

Committee for the Update of the
Guide for the Care and Use of Laboratory Animals

Institute for Laboratory Animal Research

Division on Earth and Life Studies

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REVIEWERS

This eighth edition of the Guide for the Care and Use of Laboratory Animals has been reviewed in draft form by individuals chosen for their diverse perspectives and expertise, in accordance with procedures approved by the Report Review Committee of the National Research Council. The purpose of this independent review is to provide candid and critical comments that will assist the Committee in making its published report as sound as possible, and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberation process. The Committee thanks the following individuals for their review of the draft report:

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by John Dowling, Harvard University, and John Vandenbergh, North Carolina State University. Appointed by the National Research Council, they were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered.

Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

PREFACE

The Guide for the Care and Use of Laboratory Animals (the Guide) was first published in 1963 under the title Guide for Laboratory Animal Facilities and Care and was revised in 1965, 1968, 1972, 1978, 1985, and 1996. More than 550,000 copies have been printed since its first publication. The Guide is an internationally accepted primary reference on animal care and use. Use of the Guide is required by the Public Health Service Policy.

The purpose of the Guide, as expressed in the charge to the Committee to Update the Guide for the Care and Use of Laboratory Animals, is to assist institutions in caring for and using animals in ways judged to be scientifically, technically, and humanely appropriate. The Guide is also intended to assist investigators in fulfilling their obligation to plan and conduct animal experiments in accord with the highest scientific, humane, and ethical principles. The recommendations are based on published data, scientific principles, expert opinion, and experience with methods and practices that have proved to be consistent with high-quality humane animal care and use. These recommendations should be used as a foundation for the development of a comprehensive animal care and use program, recognizing that the concept and application of performance standards, in accordance with goals, outcomes and considerations defined in the Guide, is essential to this process.

This Committee has carried forward the balance between ethical and science-bassed practice that has always been the basis of the Guide. In doing so, the Committee has fulfilled its role to provide the research community with an updated tool that allows it to responsibly carry on in a self-tregulatory manner with animal experimentation. Consequently, as professional judgment is exercised, the central notion of performance standards is upheld while the need for more stringent regulations is obviated.

The need for continual updating of the Guide is implicit in its objective "…to provide information that will enhance animal well-being, the quality of research, and the advancement of scientific knowledge that is relevant to both humans and animals" (Chapter 1). The irregular and increasing intervals between updates, reaching a 14-year gap between the seventh edition and this eighth edition, means that important new research findings might wait more

than a decade before being reflected

The Committee acknowledges the contributions of William I. Gay and Bennett J. Cohen in the development of the original Guide. In 1959, Animal Care Panel (ACP) President Cohen appointed the Committee on Ethical Considerations in the Care of Laboratory Animals to evaluate animal care and use. That Committee was chaired by Dr. Gay, who soon recognized that the Committee could not evaluate animal-care programs objectively without appropriate criteria on which to base its evaluations; that is, standards were needed. The ACP executive committee agreed, and the Professional Standards Committee was appointed. NIH later awarded the ACP a contract to "determine and establish a professional standard for laboratory animal care and facilities." Dr. Cohen chaired the ACP Animal Facilities Standards Committee, which prepared the first Guide

OVERVIEW

This eighth edition of the Guide is divided into five chapters and four appendices.

Chapter 1 incorporates some of the material from the Introduction to the last edition and presents key concepts and terminology essential to the premise and utilization of the Guide. The Chapter highlights a commitment to the concepts of the Three Rs (Replacement, Reduction and Refinement) and presents an enhanced discussion of the ethics of animal use and investigator/institutional obligations. The goals and intended audiences of the Guide are also discussed.

Chapter 2 focuses on the overall institutional Animal Care and Use Program (Program), in addition to many of the topics previously covered in Chapter 1. It defines the evolved concept of Program and provides a framework for its intra-institutional integration, including a focus on institutional policies and responsibilities; regulatory considerations; Program and personnel management (including training and occupational health and safety); and Program oversight. The latter includes institutional animal care and use committee (IACUC) functions; protocol and Program review, a new section on post-approval monitoring, and discussion of special considerations, such as humane endpoints and multiple survival surgical procedures. The American College of Laboratory Animal Medicine's "Guidelines for Adequate Veterinary Care" are endorsed.

Chapter 3 focuses on the animals themselves and unlike prior editions, addresses terrestrial and aquatic animals in separate sections reflecting the growing role of aquatic animals in biomedical research. In this chapter, recommendations for housing and environment and enhanced sections on environmental enrichment, animal well-being and scientific validity are presented. The importance of social housing is also emphasized.

In this chapter, space recommendations were minimally expanded based on the Committee's professional and expert opinion and currently applied housing methods. The cage sizes have historically been interpreted as minimum space needs by the users of the Guide, and were labeled as such ("recommended minimum space") in this edition. The use of the word "minimum" does not further restrict users of the Guide because although the space requirements are numbers (i.e., engineering standards), they are utilized within a performance standards framework. In light of many comments submitted to the Committee requesting more information on performance goals and how to achieve them,

rodent breeding recommendations are accompanied by substantial guidance on important considerations, and recommended minimum space for female rodents with litter has been added. Further, the cage height recommendation for rabbits increased to 16".

With respect to NHPs the Committee endorses social housing as the default and has provided some species-specific guidance. Additional NHP groups have been added to include baboons, while the chimpanzees were separated in a new category. These changes were motivated by the Committee's recognition (affirmed by the solicited comments from NHP experts) that these animals need more vertical space, at least in some groups, to exercise their natural habits.

Chapter 4 discusses veterinary care and the responsibilities of the attending veterinarian. It introduces the concept of animal biosecurity to affirm its central role in assuring the health of laboratory animals. It includes recommendations relative to animal procurement, transportation and preventive medicine, and expands the sections on clinical care and management; surgery (introduces intraoperative monitoring); pain and distress; and euthanasia.

Chapter 5 discusses physical plant-related topics and includes updated and new material on such topics as vibration control; physical security and access control; hazardous agent containment; and special facilities for imaging and whole body irradiation, barrier housing, behavioral studies, and aquatic species housing. Detailed discussion of centralized vs. decentralized animal facilities is provided and the concept of variable-volume HVAC systems is introduced with a nod toward energy conservation and efficiency.

Appendix A is the updated bibliography; Appendix B contains the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used

CONTENTS

CHAPTER 1. Key Concepts

This edition of the Guide for the Care and Use of Laboratory Animals (the Guide) strongly affirms the principle that all who care for, use or produce animals for research, testing or teaching must assume responsibility for their well-being. The Guide plays an important role in decision- making regarding the use of vertebrate

Applicability and Goals

In the Guide laboratory animals (also referred to as animals) are generally defined as any vertebrate animal (e.g., traditional laboratory animals, agricultural animals, wildlife and aquatic species) produced for or used in research, testing or teaching. Animal use is defined as the proper care, use and humane treatment of laboratory animals produced for, or used in research testing or teaching.

When appropriate, considerations or specific emphases for agricultural animals and non-traditional species are presented. The Guide does not address in detail agricultural animals used in production agri cultural research or teaching, wildlife and aquatic species studied in natural settings, or invertebrate animals (e.g., cephalopods) used in research. Nevertheless, the Guide establishes general principles and ethical considerations that are also applicable to these species and situations. References in the Guide provide the reader with additional information reg arding statements made in the Guide. Supplemental information on breeding, care, management, and use of selected laboratory animal species is available in other publications prepared by the In

institutions to give careful and deliberate thought to the decision to use animals, taking into consideration the contribution that such use will make to new knowledge, ethical considerations, and the availability of alternatives to animal use (NRC 1992). A practical strategy for decision-making, described as the Three Rs (Replacement, Reduction and Refinement) approach, is discussed in more detail below. Institutions should use the recommendations in the *Guide* as a foundation for the development of a comprehensive animal care and use program and a process for continually improving this program.

Intended Audiences and Uses of the **Guide**

The *Guide* is intended for a wide and diverse audience, including

- The scientific community
- Administrators
- IACUCs
- Veterinarians
- Educators and trainers
- Producers of laboratory animals
- Accreditation bodies
- Regulators
- The public

It is intended that the *Guide* be read by the user in its entirety, as there are many concepts throughout that may be helpful. Individual sections will be particularly relevant to certain users, and it is expected that the reader will explore in more detail the cited references (including those in Appendix A) on topics of interest.

Members of the scientific community (investigators and other animal users) will find Chapters 1 and 2 (and portions of Chapter 4) of the *Guide* useful for assisting in interactions with the IACUC, attending veterinarian and administrators regarding animal care as well as in preparing animal care and use protocols. Scientific review committees and journal editors may choose to refer to multiple sections of the *Guide* to determine if scientists contributing proposals and manuscripts have met the appropriate standards in their planned use of animals. The *Guide* can assist IACUCs and administrators in protocol review, assessment and oversight of an animal care and use program. Veterinarians should find Chapters 3 through 5 valuable for their oversight and support of animal care and use. Educators and trainers can use the *Guide* as a document to assess both the scope and adequacy of training programs supported by the institution. Accreditation bodies will find the *Guide* useful for evaluating many areas of animal care and use programs not subject to strict engineering standards

(see definition below). Finally, members of the public should feel assured that adherence to the *Guide* will ensure humane care and use of laboratory animals.

Readers are reminded that the *Guide* is used by a diverse group of national and international institutions and organizations, many of which are covered by neither the Animal Welfare Act nor the PHS Policy. The *Guide* uses some terminology that is both defined by U.S. statute and denotes a general concept, e.g. "Attending Veterinarian", "Adequate Veterinary Care", and "Institutional Official". Even if these terms are not consistent with terms used by non-U.S. institutions, the underlying principles can still be applied. In all instances where *Guide* recommendations are different from applicable legal or policy requirements, the higher standard should apply.

Ethics and Animal Use

The decision to use animals in research requires critical thought, judgment and analysis. Using animals in research is a privilege granted by society to the research community with the expectation that such use will provide either significant new knowledge or lead to improvement in human and/or animal well-being (McCarthy 1999; Perry 2007). It is a trust that mandates responsible and humane care and use of these animals. The *Guide* endorses the responsibilities of investigators as stated in the U.S. Government Principles for Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (IRAC 1985; see Appendix B). These principles direct the research community to accept responsibility for the care and use of animals during all phases of the research effort. Other government agencies and professional organizations have published similar principles (NASA 2008; NCB 2005; NIH 2002, 2006; for additional references see Appendix A). Ethical considerations discussed here and in other sections of the *Guide* should serve as a starting point and readers are encouraged to go beyond these provisions. In certain situations, special considerations will arise during protocol review and planning. Several of these situations are discussed in more detail in Chapter 2. A practical method for implementation of these concepts is contained in the Three Rs principles.

The Three Rs

In 1959, W. M. S. Russell and R. L. Burch published a practical strategy, referred to as "the Three Rs," – replacement, refinement and reduction– for researchers to apply when considering experimental design in laboratory animal research (Russell and Burch 1959). Over the years, the Three Rs have evolved into an internationally accepted approach for researchers to employ when deciding to

use animals in research, and in designing humane animal research studies.

Replacement refers to methods that avoid using animals. The term includes absolute replacements (i.e., replacing animals with inanimate systems such as computer programs) as well as relative replacements (i.e., replacing animals, such as vertebrates, with animals that are lower on the phylogenic scale).

Refinement refers to modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress. While institutions and investigators should take all reasonable measures to eliminate pain and distress through refinement, IACUCs should understand that with some types of studies, there could be either unforeseen or intended experimental outcomes that produce pain. These outcomes may or may not be eliminated based on the goals of the study.

Reduction includes strategies for obtaining comparable levels of information from the use of fewer animals or for maximizing the information obtained from any given number of animals (without increasing pain or distress) so that in the long run fewer animals are needed to acquire the same scientific information. This approach relies on an analysis of experimental design, applications of newer technologies, the use of appropriate statistical methods, and control of environmentally related variability in animal housing and study areas (see Appendix A).

Refinement and reduction goals should be balanced on a case-by-case basis. In other words, reduction should not serve as a rationale for reusing an animal or animals that have already undergone experimental procedures especially if the well-being of the animals would be compromised. Principal Investigators are strongly discouraged from advocating animal reuse as a reduction strategy. Studies that may result in severe or chronic pain or significant alterations in the animals' ability to maintain normal physiology, or adequately respond to stressors, should contain descriptions of appropriate humane endpoints or provide science-based justification as to why a particular, commonly accepted humane endpoint cannot be employed. Veterinary consultation must occur when pain or distress is beyond the level anticipated in the protocol description or when interventional control is not possible.

Key Terms Used in the **Guide**

The Committee to Update the Guide believes that the terms set out below are important for a full understanding of the Guide. Accordingly, we have defined these terms and concepts to provide the users of the Guide with additional assistance in implementing their responsibilities.

Humane Care

Humane care means those actions taken to assure that laboratory animals are treated according to high ethical and scientific standards. Implementing a humane care program, and creating a laboratory environment in which humane care and respect for animals is valued and encouraged, underlie the core requirements of the Guide and the system of self-regulation it supports (Klein and Bayne 2007).

Animal Care and Use Program

Animal care and use program ("Program") means the policies, procedures, standards, organizational structure, staffing and practices put into place by an institution to achieve humane care of animals in the laboratory and throughout the institution. This includes the establishment and support of an institution's animal care and use committee (IACUC) or equivalent ethical oversight committee and the maintenance of an environment in which the IACUC can function successfully to carry out its responsibilities under the Guide, U.S. laws and U.S. policies. Chapter 2 contains a more expansive discussion of the applicability to, and importance of, the Guide to animal care and use programs.

Engineering and Performance Standards

Engineering standard means a standard or guideline that specifies in detail a method, technology or technique for achieving a desired outcome, and does not provide for modification in the event that acceptable alternative methods are available or unusual circumstances arise. Engineering standards are prescriptive and provide limited flexibility for implementation. However, an engineering standard can be useful to establish a baseline, and are easier to use in evaluating compliance.

Performance standard means a standard or guideline that, while describing a desired outcome, provides flexibility in achieving this outcome by granting discretion to those with responsibility for managing the animal care and use program, the researcher, and the IACUC. The performance approach requires professional input, sound judgment and a team approach to achieve specific goals. It is essential that the desired outcomes and/or goals are clearly defined, and that appropriate performance measures are regularly monitored, in order to verify success of the process. This approach can be advantageous because many variables (such as the species and previous history of the animals, facilities, expertise of the people, and research goals) can be taken into consideration so that the implementation of the standard can be best tailored to meet the recommendations in the Guide.

Ideally, engineering and performance standards are balanced, setting a target for optimal practices, management and operations while encouraging flexibility and judgment, if appropriate, based on individual situations (Gonder et al. 2001). Scientists, veterinarians, technicians and others have extensive experience and information covering many of the topics discussed in the Guide. For those topics in which information is insufficient or incomplete, sustained research into improved methods of laboratory animal management, care and use is needed for the continued evaluation and improvement of performance and engineering standards.

Practice Standards

Practice standard means the application of professional judgment by qualified, experienced individuals to a task or process over time, which has been demonstrated to benefit or enhance animal care and use. Much of the basis for professional judgment comes from information obtained from the peer-reviewed scientific literature and textbooks. However, similar to many other disciplines, the application of professional judgment also relies on time-proven experiences in the field (for additional information see chapter 2). Therefore, in the absence of published scientific literature or other definitive sources, where experience has demonstrated that a particular practice improves animal care and use, such standards have been utilized in determining appropriate recommendations in the Guide. In most situations, the Guide is intended to provide flexibility so that institutions can modify practices and procedures with changing conditions and new information.

Policies, Principles and Procedures

Policies commonly derive from a public ag ency or private entity. Policies are generally practical statements of collect

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Program Management

An effective Program requires clearly defined roles that align responsibility with regulatory and management authority. U.S. federal law creates a statutory basis for the Institutional Official (IO), the Attending Veterinarian (AV), and the Institutional Animal Care and Use Committee (IACUC). The Guide endorses these concepts as important operating principles for all U.S. and non-U.S. animal care and use programs. Effective leadership within and collaboration among these three components, which not only oversee but also support animal users, are necessary (Lowman 2008; Van Sluyters 2008). In addition, interactions with regulatory and funding agencies and accreditation organizations are an integral part of the Program.

As summarized here and discussed throughout the Guide, the primary oversight responsibilities within the Program rest with the IO, the AV and the IACUC. The roles fit within a defined organizational structure where the reporting relationships, authorities and responsibilities of each are clearly defined and transparent. Taken together they establish policies and procedures, ensure regulatory compliance, monitor Program performance and support high-quality science and humane animal use. A program that includes these elements, and establishes a balance among them, has the best chance of efficiently utilizing resources while attaining the highest standards of animal well-being and scientific quality (Bayne and Garnett 2008; Van Sluyters 2008).

Program Management Responsibility

The Institutional Official

Institutional Official – The individual who, as a representative of senior administration, bears ultimate responsibility for the Program, and is responsible for resource planning and ensuring alignment of Program goals with the institution's mission.

The Institutional Official (IO) bears ultimate responsibility for the Program, although overall direction of the Program should be a shared responsibility among the IO, AV and IACUC. The IO has the authority to allocate the needed resources and ensure the Program's overall effectiveness. Program needs should be clearly and regularly communicated to the IO by the AV, the IACUC and others associated with the Program

(facilities management, occupational health and safety, scientists, etc.). As a representative of senior administration, the IO is responsible for resource planning and ensuring alignment of Program goals of quality animal care and use, with the institution's mission.

The Attending Veterinarian

The Attending Veterinarian(AV) is responsible for the health and well-being of all laboratory animals used at the institution. The institution must provide the AV with sufficient authority, including access to all animals, and resources to manage the program of veterinary care. The AV should oversee other aspects of animal care and use(e.g., husbandry, housing) to ensure that the Program complies with the Guide.

Institutional mission, programmatic goals, including the nature of animal use at the institution, and Program size will determine whether full-time, part-time, or consultative veterinary services are needed. If a full-time veterinarian is not available on site, visits by a consulting or part-time veterinarian should be at intervals appropriate to programmatic needs. In such instances, there must be an individual with assigned respon sibility for daily animal care and use and facility management. While institutions with large animal care and use programs may employ multiple veterinarians, management of veterinary medicine, animal care, and facility operations by a single administrative unit is often an efficient mechanism to administer all aspects of the Program.

The Guideendorses the American College of Laboratory Animal Medicine's (ACLAM) "Guidelines for Adequate Veterinary Care" (ACLAM 1996). These guidelines include veterinary access to all animals and their medical records, regular veterinary visits to facilities where animals may be housed or used, provisions s tofals maysas and comvetent celidicad, preentative, andgemargncyf veterinary care, andah l anima, prcturement and rianportratiod.

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to work effectively, there should be clear and regular communication between the AV and the IACUC.

The Institutional Animal Care and Use Committee

The IACUC (or institutional equivalent) is responsible for assessment and oversight of the institution's Program components and facilities. The IACUC should have sufficient authority and be provided with resources (e.g., staff, training, computer resources) to fulfill this responsibility. Detailed information on the role and function of the IACUC is provided later in this chapter.

Collaborations

Inter-institutional collaboration has the potential to create ambiguities regarding responsibility for animal care and use. In cases of collaboration between institutions that involves animal use (beyond merely transporting animals), institutions should have a formal written understanding (e.g., contract, memorandum of understanding or inter-institutional agreement) between the institutions. The written agreement should address the responsibility for offsite animal care and use, animal ownership and IACUC review and oversight (AAALAC 2003). In addition, IACUC's from both participating institutions may choose to review protocols for the work being conducted.

Personnel Management

Training and Education

All personnel involved with the care and use of animals must be adequately educated, trained and/or qualified in basic principles of laboratory animal science to help assure high quality science and animal well-being. The number and qualifications of personnel required to conduct and support a Program depend on several factors, including the type and size of institution, the administrative structure for providing adequate animal care, the .;14sdfalgcrals

institution-sponsored discussion and trai ning programs and reference materials applicable to their jobs and the species being cared for, should be provided to each employee responsible for animal care (Kreger 1995). Coordinators of institutional training programs can seek assistance from the Animal Welfare Information Center (AWIC), the Laborat ory Animal Welfare and Training Exchange (LAWTE), AALAS and ILAR (NRC 1991). The Guide to the Care and Use of Experimental Animals by the Canadian Council on Animal Care (CCAC 1993) and guidelines from other countries are valuable additions to the libraries of laboratory animal scientists (Appendix A).

Occupational Health and Safety of Personnel

Each institution must establish and maintain an Occupational Health and Safety Program (OHSP) as an essential part of the overall program of animal care and use (CFR 1984a, b, c; DHHS 2007; PHS 2002). The OHSP must be consistent with federal, state, and local regulations and should focus on maintaining a safe and healthy workplace (Gonder 2002; Newcomer 2002; OSHA 1998a). The nature of the OHSP will depend on the facility, research activities, hazards, and animal species involved. The National Research Council's publication Occupational Health and Safety in the Care and Use of Research Animals (NRC 1997) contains guidelines and references for establishing and maintaining an effective, comprehensive OHSP (also see Appendix A). An effective OHSP requires coordination between the research program (as represented by the investigator), the animal care and use program (as represented by the AV, IO and the IACUC), the environmental health and safety program, occupational-health services, and administration (e.g., human resources, finance, and facility-maintenance personnel). Establishment of a safety committee may facilitate communication and promote ongoing evaluation of health and safety in the workplace. In some cases there is a regulatory requirement for such a committee. Operational and day-to-day responsibility for safety in the workplace resides with the laboratory or facility supervisor (e.g., principal investigator, facility director, or veterinarian) and depends on performance of safe work practices by all employees.

Control and Prevention Strategies

In developing a comprehensive OHSP a hierarchy of control and prevention strategies should be followed that begins with the identification of hazards and the assessment of risk associated with those hazards. Managing risk involves the following steps: first, the appropriate design and operation of facilities and use of appropriate safety equipment (engineering controls); second, the development of processes and standard operating procedures (SOPs; administrative controls); and finally, the provision of appropriate personal protective equipment (PPE) for employees. Managing risk, utilizing these strategies, requires that personnel be trained, adhere to good personal hygiene, be knowledgeable about the hazards in their work environment, understand the proper selection and use of equipment, follow established procedures, and utilize PPE provided.

Hazard Identification and Risk Assessment

The institutional OHSP should identify potential hazards in the work environment and conduct a critical assessment of the associated risks. An

to protect the animal-care and investigative staff, other occupants of the facility, the public, animals, and the environment from exposure to hazardous biologic, chemical, and physical agents used in animal experimentation (Frasier 2005; NIH 2002; DHHS 2007). When necessary, these facilities should be separated from other animal housing and support areas, research and clinical laboratories, and patient-care facilities. They should be appropriately identified, and access to them should be limited to authorized personnel.

Facilities, equipment, and procedures should also be designed, selected, and developed to reduce the potential of physical injury or health risk to personnel, (NIOSH 1997a, b). Engineering controls to address the potential for ergonomic injury should be considered in situations such as lifting of heavy equipment or animals (AVMA 2008). The potential for repetitive motion injuries in animal facilities (e.g., maintenance of large rodent populations and other husbandry activities) should be assessed. Engineering controls and equipment are frequently utilized to limit or control personnel exposure to animal allergens (Harrison 2001; Huerkamp et al. 2008).

The selection of appropriate animal-housing systems requires professional knowledge and judgment and depends on the nature of the hazards in question, the types of animals used, the limitations or capabilities of the facilities, and the design of the experiments. Experimental animals should be housed so that potentially contaminated food and bedding, feces, and urine can be handled in a controlled manner. Facilities, equipment, and procedures should be utilized for appropriate bedding disposal. Safety equipment should be properly maintained and its function periodically validated. Appropriate methods should be used for assessing and monitoring exposure to potentially hazardous biologic, chemical, and physical agents where required (e.g., ionizing radiation), or where the possibility of exceeding permissible exposure limits (PELs) exists (CFR 1984b).

Personnel Training

Personnel at risk should be provided with clearly defined procedures, and in specific situations, personal protective equipment (PPE) to safely conduct their duties, understand the hazards involved, and be proficient in implementing the required safeguards. They should be trained regarding zoonoses, chemical, biologic and physical hazards (e.g., radiation and allergies), unusual conditions or agents that might be part of experimental procedures (e.g., the use of human tissue in immunocompromised animals), handling of waste materials, personal

attending veterinarian, animal care technician and occupational health and safety professionals may enhance compliance.

The BMBL (DHHS 2007) and the National Research Council (NRC 1997) recommend practices and procedures, safety equipment, and facility requirements for working with hazardous biologic agents and materials. Facilities that handle agents of unknown risk should consult with appropriate CDC personnel about hazard control and medical surveillance. The use of highly pathogenic "select agents and toxins" in research requires that institutions develop a program and procedures for procuring, maintaining and disposing of

Medical Evaluation and Preventive Medicine for Personnel

Development and implementation of a program of medical evaluation and preventive medicine should involve input from trained health professionals, such as occupational-health physicians and nurses. Confidentiality and other medical and legal factors must be considered in the context of appropriate federal, state, and local regulations (e.g., PL 104-191).

A pre-employment health evaluation and/or a health-history evaluation before work assignment is advisable to assess potential risks for individual employees. Continuing periodic medical evaluations are advisable for personnel in specific risk categories. For example, personnel required to use respiratory protection may also require medical evaluation to ensure that they are physically and psychologically able to use the respirator properly (Sargent and Gallo 2003). An appropriate immunization schedule should be adopted. It is important to immunize animal-care personnel against tetanus (NRC 1997). In addition, pre-exposure immunization should be offered to people at risk of infection or exposure to specific agents such as rabies virus (e.g., if working with species at risk for infection) or hepatitis B virus (e.g., if working with human blood or human tissues, cell lines or stocks). Vaccination is recommended if research is to be conducted on infectious diseases for which effective vaccines are available. More specific recommendations can be found in the BMBL (DHHS 2007). Pre-employment or pre-exposure serum collection is advisable only in specific circumstances as determined by an occupational health and safety professional (NRC 1997). In such cases, identification, traceability, retention, and storage conditions of samples should be considered, and the purpose for which the serum samples will be used must be consistent with applicable federal and state laws.

Zoonosis surveillance should be a part of an OHSP (DHHS 2007; NRC 1997). Personnel should be instructed to notify their supervisors of potential or known exposures and of suspected health hazards and illnesses. Clear procedures should be established for reporting all accidents, bites, scratches, and allergic reactions (NRC 1997).

Laboratory animal allergy has become a significant issue for individuals in contact with laboratory animals (Bush and Stave 2003; Gordon 2001; Wolfle and Bush 2001; Wood 2001). The medical surveillance program should promote the early diagnosis of allergies (Bush 2001; Bush and Stave 2003; Seward 2001) and include evaluation of an individual's medical history for preexisting allergies. Personnel training should include information on laboratory animal allergies,

preventive control measures and proper techniques for working with animals (Gordon et at. 1997; Schweitzer et al. 2003; Thulin et al. 2002). PPE should be used to supplement, not replace, engineering or process controls (Harrison 2001; Reeb-Whitaker et al. 1999). If PPE for respiratory protection is necessary, appropriate fit testing and training should be provided.

Nonhuman primate diseases that are transmissible to humans can be serious hazards (NRC 2003a). Animal technicians, veterinarians, investigators, students, research technicians, maintenance workers and others who have contact with nonhuman primates, or their tissues and body fluids, or who have duties in nonhuman primate housing areas should be routinely screened for tuberculosis. Because of the potential for Macacine herpesvirus 1 (formerly Cercopithecine herpesvirus 1 or Herpes B virus) exposure, personnel who work with or handle biological samples (blood and tissues) from macaques should have access to and be instructed in the use of bite and scratch emergency-care stations (Cohen et al. 2002). Injuries associated with macaques, their tissues or body fluids, or caging and equipment with which the animals have had direct contact, should be carefully evaluated and appropriate post-exposure treatment and follow-up implemented (ibid; NRC 2003a). Medical care for bites, scratches, and puncture wounds from other animal species should also be available (Cohen et al. 2002; DHHS 2007).

Personnel Security

While contingency plans normally address natural disasters, institutions should also consider the threat that criminal activity, such as personnel harassment and assault, and facility trespassing, arson, and vandalism pose to laboratory animals, research personnel, equipment and facilities, and biomedical research within the institution. Preventive measures should be considered, including pre-employment screening, and physical and information technology security (Miller 2007).

Investigating and Reporting Animal Welfare Concerns

Safeguarding animal welfare is the responsibility of every individual associated with the Program. The institution must develop methods for reporting and investigating animal welfare concerns. In the U.S., responsibility for review and

are reported anonymously; taking corrective actions if deemed necessary; and providing a report of the issue, findings and actions taken to the IO. Reported concerns and any corrective actions taken should be documented.

Mechanisms for reporting concerns should be posted in prominent locations within the facility and on applicable institutional web site(s) with instructions on how to report the concern and to whom. Multiple points of contact, including senior management, IO, IACUC Chair and the AV are recommended. The process should include a mechanism for anonymity, compliance with applicable whistleblower policies, non-discrimination against the concerned/reporting party and protection from reprisals.

Training and regular communication with employees regarding the Institution's animal use activities may reduce potential concerns. Personnel, such as custodial, maintenance and administrative staff, who are farther removed from the animal use, should also be included.

Program Oversight

The Role of the IACUC

IACUC Constitution and Function

The responsibility of the IACUC is to oversee and routinely evaluate the Program. It is the institution's responsibility to provide suitable orientation, background materials, access to appropriate resources, and, if necessary, specific training to assist IACUC members in understanding their roles and responsibilities, and evaluating issues brought before the committee.

Committee membership includes the following:

- A Doctor of Veterinary Medicine certified (e.g., ACLAM, ECLAM, JCLAM, KCLAM) or with training and experience in laboratory animal science and medicine or in the use of the species at the institution.
- At least one practicing scientist experienced in research involving animals.
- At least one member from a nonscientific background, drawn from inside or outside the institution.
- At least one public member to represent general community interests in the proper care and use of animals.

Public members should not be laboratory animal users, be affiliated in any way with the institution, or be members of the immediate family of a person who is affiliated with the institution. While the public member may receive compensation for their participation and ancillary expenses (meals, parking, travel, etc.), the amount should be sufficiently modest that it does not become a substantial source of income, thus potentially compromising the association with the community and public at large.

For large institutions with many administrative units or departments, no more than three voting members should be associated with a single administrative unit of the institution (CFR 1985). The size of the institution and the nature and extent of the Program will determine the number of members of the committee and their terms of appointment. Institutions with broad research programs may need to choose scientists from a number of disciplines and experience to properly evaluate animal use protocols.

The committee is responsible for oversight and evaluation of the entire Program and its components as described in the Guide. Its oversight functions include review and approval of proposed animal use (protocol review); proposed significant changes to animal use; regular inspection of facilities and animal use areas; regular review of the Program; on-going assessment of animal care and use; and establishment of a mechanism for receipt and review of concerns involving the care and use of animals at the institution. The committee must meet as often as necessary to fulfill its responsibilities. Records of committee meetings and results of deliberations should be maintained. Program review and facilities inspections should occur at least annually or more often as required (e.g., Animal Welfare Act and PHS Policy). After review and inspection, a written report (including any minority views) should be made to the IO on the status of the Program.

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avoiding unnecessary animal use or duplication of experiments. For some IACUC questions, input from outside experts may be advisable or necessary. In the absence of evidence of a formal scientific merit review, the IACUC may

invasive experiments, the experimental and humane endpoints are closely linked (Wallace 2000) and should be carefully considered during IACUC protocol review. While all studies should employ endpoints that are humane, studies that commonly require special consideration include those that involve tumor models, infectious diseases, vaccine challenge, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, organ or system failure, and models of cardiovascular shock.

The Principal Investigator, with precise knowledge of both the objectives of the study and the proposed model, should identify, explain and include in his or her animal use protocol a study endpoint that is both humane and scientifically sound. The identification of humane endpoints is often challenging because multiple factors must be weighed, including the model, species (and sometimes strain or stock), animal health status, study objectives, institutional policy, regulatory requirements, and occasionally conflicting scientific literature. Determination of humane endpoints should involve the Principal Investigator, the veterinarian, and the IACUC, and should be defined when possible prior to the start of the study (Olfert and Godson 2000; Stokes 2000). Information that is critical to the IACUC's assessment of appropriate endpoint consideration within a protocol includes precisely defining the humane endpoint (including assessment criteria); the frequency of animal observation; training of personnel responsible for assessment and recognition of the humane endpoint; and the response required upon reaching the humane endpoint. An understanding of pre-emptive euthanasia (Toth 2000), behavioral or physiologic definitions of the moribund state (ibid), and the use of study-specific animal assessment records (Morton 2000; Paster et al. 2009) can aid the Principal Investigator and IACUC when considering or developing proposed endpoints. When novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the Principal Investigator, IACUC and veterinarian. A system for communication with the IACUC should be in place both during and after such studies. Numerous publications address specific proposals for the application and use of humane endpoints (e.g., CCAC 1998; ILAR 2000; OECD 1999; Toth 1997; UKCCCR 1997).

Unexpected Outcomes

Fundamental to scientific inquiry is the investigation of novel experimental variables. Because of the potential for unexpected outcomes that may affect animal well-being when highly novel variables are introduced, more frequent monitoring of animals may be required. With their inherent potential for unanticipated phenotypes, GMAs are an example of when increased monitoring

for unexpected outcomes could be implemented (Dennis 1999).

GMAs, particularly mice and fish, are important animal models, and new methods and combinations of genetic manipulation are constantly being developed (Gondo 2008). Regardless of whether genetic manipulation is targeted or random, the phenotype that initially results is often unpredictable and may lead to expected or unexpected outcomes that impact the animal's well-being or survival at any stage of life. For example, instances have occurred in which genetic modification has lead to unexpected immunodeficiency, requiring the GMA offspring to be held under specialized bio-exclusion conditions (Mumphrey et al. 2007). The promoter sequences used to direct expression of transgenes to specific tissues have varying degrees of specificity ("leakiness") that can lead to unexpected phenotypes (Moorehead et al. 2003). These examples illustrate the diversity of unanticipated outcomes and emphasize the need for diligent monitoring and professional judgment, to ensure the animals' well-being (Dennis 2000). The first offspring of a newly generated GMA line should be carefully observed from birth into early adulthood for signs of disease, pain or distress. Investigators may find that the phenotype precludes breeding of particular genotypes or that unexpected infertility occurs. Such situations could lead to increases in the numbers of animals used and revision of the animal use protocol. When the initial characterization of a GMA reveals a condition that negatively impacts animal well-being, this should be reported to the IACUC, and more extensive analysis may be required to better define the phenotype (Brown et al. 2000; Crawley 1999; Dennis 2000). This may help to determine whether proactive measures can be taken to circumvent or alleviate the impact of the genetic modification on the animal's well-being, and to establish humane endpoints specific to the GMA line.

Physical U-.001.0002 Tc79Rd

Prolonged restraint, including chairing of nonhuman primates, should be avoided unless it is essential for achieving research objectives and is specifically approved by the IACUC (NRC 2003b). Systems that do not limit an animal's ability to make normal postural adjustments, such as subcutaneous implantation of osmotic minipumps in rodents, back pack-fitted infusion pumps in dogs and nonhuman primates, and free-stall housing for farm animals, should be used when compatible with protocol objectives. Animals that do not adapt to necessary restraint systems should be removed from the study. When restraint devices are used, they should be specifically designed to accomplish research goals that are impossible or impractical to accomplish by other means or to prevent injury to animals or personnel.

The following are important guidelines for restraint:

- Restraint devices should not be considered a normal method of housing, and must be justified in the animal use protocol.
- Restraint devices should not be used simply as a convenience in handling or managing animals.
- Alternatives to physical restraint should be considered.
- The period of restraint should be the minimum required to accomplish the research objectives.
- Animals to be placed in restraint devices should be given training (with positive reinforcement) to adapt to the equipment and personnel.
- Animals that fail to adapt should be removed from the study.
- Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.
- Veterinary care must be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates temporary or permanent removal of the animal from restraint.
- Clear explanation of the purpose of the restraint and its duration

animal training for a specific protocol-related task. Close monitoring of the animals should occur to ensure that food and fluid intake meets the animal's nutritional needs (Toth and Gardiner 2000). Body weights should be recorded at least weekly and more often for animals requiring greater restrictions (NRC 2003b). Written records should be maintained for each animal to document daily food and fluid consumption, hydration st atus, and any behavioral and clinical changes used as criteria for temporary or permanent removal of an animal from

Housing systems for agricultural animals used in biomedical research may or may not differ from those used in agricultural research. Animals used in either can be housed in cages, stalls, paddocks or pastures (ibid). Some agricultural studies need uniform conditions to minimize environmental variability, and some biomedical studies are conducted in farm settings. Thus, the protocol, rather than the category of research, should determine the setting (farm or laboratory). Decisions on categorizing research uses of agricultural animals and defining standards for their care and use should be based on the researcher's goals and concern for animal well-being, and should be made by the IACUC. Regardless of the category of research, institutions are expected to provide oversight of all research animals and ensure that pain and distress are minimized.

The *Guide* applies to agricultural animals used in biomedical research, including those maintained in typical farm settings. For animals maintained in a farm setting, the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS 2010) is a useful resource. In particular, information dealing with environmental enrichment, transport and handling may be helpful in both agricultural and biomedical research settings. Additional information regarding facilities and management of farm animals in an agricultural setting can be obtained from the Midwest Plan Service (1987) and from agricultural engineers or animal-science experts.

Post-Approval Monitoring

Continuing IACUC oversight of animal activities is required by federal laws, regulations and policies. A variety of mechanisms can be used to facilitate ongoing protocol assessment and regulatory compliance. Post-approval monitoring (PAM) is considered here in the broadest sense, consisting of all potential types of protocol monitoring following initial protocol approval by the IACUC. PAM helps ensure the well-being of the animals and may also provide opportunities to refine research procedures. Methods include continuing protocol review; laboratory inspections (as part of regular facilities' inspections or conducted separately); veterinary or IACUC observation of select procedures; observation of animals by animal care, veterinary, and IACUC staff and members; and external regulatory inspections and assessments. The IACUC, veterinary, animal care and compliance staff may all conduct PAM, which may also be used as an educational tool.

Disaster Planning and Emergency Preparedness

Animal facilities may be subjected to unexpected conditions that result in the catastrophic failure of critical systems or significant personnel absenteeism, or other unexpected events that severely compromise on-going animal care and well-being (ILAR 2010). Facilities must have a disaster plan. The plan should define the actions necessary to prevent animal pain, distress, and deaths due to loss of systems such as those controlling ventilation, cooling, heating, or provision of potable water. If possible the plan should describe how the facility will preserve animals necessary for critical research activities or that are irreplaceable. The geographic locale may provide guidance as to the probability of a particular type of disaster.

Disaster plans should be established in conjunction with the responsible investigator(s) taking into consideration both the priorities for triaging animal populations and institutional needs and resources. Animals that cannot be protected from the consequences of the disaster or relocated must be humanely euthanized. The disaster plan should identify essential personnel who should be trained in advance in its implementation. Efforts should be taken to assure personnel safety and provide access to essential personnel during or immediately following disasters. Such plans should be approved by the institution and be part of the overall institutional disaster response plan that is coordinated by the IO or another senior level administrator. Law enforcement and emergency personnel should be provided with a copy of the plan for comment and integration into broader, area-wide planning (Vogelweid 1998).

Chapter 2 References

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CHAPTER 3. Environment, Housing, and Management

This chapter provides guidelines for th e environment, housing, and management

Terrestrial Animals

Terrestrial Environment

Microenvironment and Macroenvironment

The microenvironment of an animal is the physical environment immediately surrounding it; that is, the primary enclosure such as the cage, pen, or stall. It contains all the resources with which the animals directly contact and also provides the limits of the animals' immediate environment. The microenvironment is characterized by many factors, including illumination, noise, vibration, temperature, humidity, and gaseous and particulate composition of the air. The physical environment of the secondary enclosure, such as a room, a

Temperature and Humidity

Maintenance of body temperature within normal circadian variation is necessary for animal well-being and animals should be housed within temperature and humidity ranges appropriate for the species, to which they can adapt with minimal stress and physiologic alteration. The ambient temperature range in which thermoregulation occurs without the need to increase metabolic heat production is called thermoneutral zone (TNZ) and is bounded by the upper (UCT) and lower critical temperatures (L CT). To maintain body temperature under a given environmental temperat ure animals adjust physiologically (including metabolism) and behaviorally (including their activity level and resource use). For example, the thermoneutral zone of mice ranges between 26°C and 34°C (Gordon 1993). At lower temperatures, building nests and huddling for resting and sleeping allows them to thermoregulate by behaviorally controlling their microclimate. Although mice choose temperatures below their LCT of 26°C during activity periods, they strongly prefer temperatures above LCT for maintenance and resting beha

The dry-bulb temperatures listed in Table 3.1 are broad and generally

macroenvironment, e.g., static filter-top (isolator) cages. Some species may require conditions with high relative humidity (e.g., selected species of nonhuman primates, tropical reptiles, and amphibians; Olson and Palotay 1983). In mice, both abnormally high and low humidity may increase pre-weaning mortality (Clough 1982). In rats, low relative humidity, especially in combination with temperature extremes, may lead to ringtail, a condition involving ischemic necrosis of the tail and sometimes toes (Crippa et al. 2000; Njaa 1957; Totten 1958). For some species, elevated relative humidity may impact an animal's ability to cope with thermal extremes. Elevated microenvironmental relative humidity may also lead to high intra-cage ammonia concentrations in rodent isolator cages (Corning and Lipman 1991; Hasenau et al. 1993), which can be irritating to the nasal passages and alter some biological responses (Gordon et al. 1980; Manninen et al. 1998). In climates where it is difficult to provide a sufficient level of environmental relative humidity, animals should be closely monitored for negative effects such as excessively flaky skin in

in optimizing ventilation of micro- and macroenvironments (Hughes and Reynolds 1995).

Direct exposure of animals to air moving at high velocity (drafts) should be avoided as the speed of air to which animals are exposed affects the rate at which heat and moisture are removed from an animal. For example, air at 20°C moving at 60 linear feet per minute (18.3 m/min) has a cooling effect of approximately 7°C (Weihe 1971). Drafts can be particularly problematic for neonatal homeotherms, which may be hairless and have poorly developed mechanisms for thermoregulatory control, for mutants lacking fur, and for semi-aquatic amphibians that can desiccate.

Provision of 10-15 fresh-air changes per hour in animal housing rooms is an acceptable guideline to maintain macroenvironmental air quality by constant volume systems and may also ensure microenvironmental air quality. Although this range is effective in many animal-housing settings, it does not take into account the range of possible heat loads; the species, size, and number of animals involved; the type of primary enclosure and bedding utilized; the frequency of cage-changing; the room dimensions; or the efficiency of air distribution within the macroenvironment and between it and the microenvironment. In some situations, the use of such a broad guideline might overventilate a macroenvironment containing few animals, thereby wasting energy, or underventilate a microenvironment containing many animals, allowing heat, moisture, and pollutants to accumulate.

Modern heating, ventilation, and air conditioning (HVAC) systems, e.g., variable air volume (VAV) systems, allow ventilation rates to be set in accordance with heat load and other variables (see Chapter 5). These systems offer considerable advantages with respect to flexibility and energy conservation, but should always provide a minimum amount of air exchange, as recommended for general use laboratories (Bell 2008; DiBerardinis et al. 2008).

Individually ventilated cages and other types of specialized primary enclosures, that either directly ventilate the enclosure using filtered room air or are ventilated independently of the room, can effectively address animals' ventilation requirements without the need to increase macroenvironmental ventilation. However, cautions mentioned above regarding high velocity air should be considered (Baumans et al. 2002; Krohn et al. 2003). Nevertheless, the macroenvironment should be ventilated sufficiently to address heat loads, particulates, odors, and waste gases released from primary enclosures (Lipman 1993).

If ventilated primary enclosures contain adequate filtration to address contamination risks, air exhausted from the microenvironment may be returned to the room in which animals are housed, although it is generally preferable to exhaust these systems directly into the building's exhaust system to reduce heat load and macroenvironmental contamination.

Static isolation caging (without forced ventilation), such as that used in some types of rodent housing, restricts ventilation (Keller et al. 1989). To compensate, it might be necessary to adjust husbandry practices, including sanitation and cage change frequency; selection of contact bedding; placement of cages in a secondary enclosure; animal densities within cages; and/or decrease of macroenvironmental relative humidity to improve the microenvironment and heat dissipation.

The use of recycled air to ventilate animal rooms may save energy but entails risks. Because many animal pathogens can be airborne or travel on fomites (e.g., dust), exhaust air recycled into heating, ventilation, and air conditioning (HVAC) systems that serve multiple rooms presents a risk of cross contamination. Recycling air from non-animal use areas (e.g., some human occupancy areas and food, bedding, and supply storage areas) may require less intensive filtration or conditioning and pose less risk of infection. The risks in some situations, however, might be too great to consider recycling (e.g., in the case of nonhuman primates and biohazard areas). The exhaust air to be recycled should be filtered, at minimum, with 85- 95% ASHRAE efficient filters to remove airborne particles before it is recycled (NAFA 1996). Depending on the air source, composition and proportion of recycled air utilized (e.g., ammonia and other gases emitted from excrement in recirculating air from animal rooms), consideration should also be given to filtering volatile substances. In areas that require filtration to ensure personnel and/or animal safety (e.g., hazardous containment holding), filter efficiency, loading, and integrity should be assessed. The successful operation of any HVAC system requires regular preventive maintenance and evaluation, including measurement of its function at the level of the secondary enclosure. Such measurements should include supply- and exhaust-air volumes, fluctuation in temperature and relative humidity, air pressure differentials between spaces, as well as critical mechanical operating parameters.

Illumination

Light can affect the physiology, morphology, and behavior of various animals (Azar et al. 2008, Brainard et al. 1986; Erkert and Grober 1986; Newbold et al. 1991; Tucker et al. 1984). Potential photostressors include inappropriate

photoperiod, photointensity, and spectral quality of the light (Stoskopf 1983). Numerous factors can affect animals' needs for light and should be considered when an appropriate illumination level is being established for an animal holding room. These include light intensity and wavelength, duration of the animal's current and prior exposure to light; pigmentation; circadian rhythm; body temperature; hormonal status; age; species; sex; and stock or strain of animal (Brainard 1989; Duncan and O'Steen 1985; O'Steen 1980; Saltarelli and Coppola 1979; Semple-Rowland and Dawson 1987; Wax 1977). More recent studies in rodents and primates have shown the importance of intrinsically photosensitive retinal ganglion cells, distinct from rods and cones, for neuroendocrine, circadian and neurobehavioral regulation (Berson et al. 2002; Hanifin and Brainard 2007). These cells can respond to light wavelengths that may differ from other photoreceptors and may influence the type of lighting, light intensity and wavelength selected for certain types of research.

In general, lighting should be diffused throughout an animal holding area and provide sufficient illumination for the animals' well-being while permitting good housekeeping practices, adequate animal inspection including the bottom-most cages in racks, and safe working conditions for personnel. Light in animal holding rooms should provide for adequate vision and for neuroendocrine regulation of diurnal and circadian cycles (Brainard 1989).

Photoperiod is a critical regulator of reproductive behavior in many animal species (Brainard et al. 1986; Cherry 1987), therefore inadvertent light

light intensity under which it was raised has been reported to be near the threshold of retinal damage in some individual albino rats according to histologic, morphometric, and electrophysiologic evidence (Semple-Rowland and Dawson 1987). Some guidelines recommend a light intensity as low as 40 lux at the position of the animal in midcage (NASA 1988). Rats and mice generally prefer cages with low light intensity (Blom et al. 1993), and albino rats prefer areas with a light intensity of less than 25 lux (Schlingmann et al. 1993a). Young mice prefer much lower illumination than adults (Wax 1977). Thus, for animals that have been shown to be susceptible to phototoxic retinopathy, light should be between 130 and 325 lux in the room at cage level.

Light intensity decreases with the square of the distance from its source. Therefore, the location of a cage on a rack impacts the intensity of light to which animals contained within are exposed. Light intensity may differ by as much as 80-fold in transparent cages from the top to the bottom of a rack, while differences up to 20-fold have been recorded within a cage (Schlingman et al. 1993a, b). Management practices, such as rotating cage position relative to the light source (Greenman et al. 1982) or providing animals with ways to control their own light exposure by behavioral means (e.g., nesting or bedding material adequate for tunneling), can be used to reduce inappropriate light stimulation. Variable-intensity lights are often used to accommodate the needs of research protocols, certain animal species, and energy conservation. However, such a system should also provide for the observation and care of the animals. Caution should be exercised since increasing daytime room illumination for maintenance purposes has been shown to change photoreceptor physiology and can alter circadian regulation (NRC 1996; Remé et al. 1991; Terman et al. 1991).

Noise and Vibration

Noise produced by animals and animal-care activities is inherent in the operation of an animal facility (Pfaff and Stecker 1976) and noise control should be considered in facility design and operation (Pekrul 1991). Assessment of the potential effects of noise on an animal warrants consideration of the intensity, frequency, rapidity of onset, duration, and vibration potential of the sound and the hearing range, noise-exposure history, and sound-effect susceptibility of the species, stock, or strain. Similarly, occupational exposure to animal or animal care practices generating noise may also be of concern for personnel and if of sufficient intensity may warrant hearing protection.

Separation of human and animal areas minimizes disturbances to both human and animal occupants of the facility. Noisy animals such as dogs, swine, goats, nonhuman primates, and some birds (e.g., zebra finches), should be

housed away from quieter animals, such as rodents, rabbits, and cats. Environments should be designed to accommodate animals that make noise, rather than resorting to methods of noise reduction. Exposure to sound louder than 85 dB can have both auditory

Terrestrial Housing

Microenvironment (Primary Enclosure)

All animals should be housed under conditions that provide sufficient space as well as supplementary structures and resources required to meet physical, physiologic, and behavioral needs. Environments that fail to meet the animals' needs may result in abnormal brain development, physiologic dysfunction and behavioral disorders (Garner 2005; van Praag et al. 2000; Würbel 2001) that potentially compromise both animal well-being and scientific validity. The primary enclosure or space may need to be enriched to prevent such effects and improve animal well-being (see also section on Environmental Enrichment, page 107).

An appropriate housing space or enclosure should also account for the animals' social needs. Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility (see also section on Behavioral and Social Management, page 126). Structural adjustments are frequently required for social housing (e.g., perches, visual barriers, refuges), and important resources (e.g., food, water, and shelter) should be provided in such a way that they cannot be monopolized by dominant animals (see also section on Environmental Enrichment, page 107).

The primary enclosure should provide a secure environment that does not permit animal escape and should be made of durable materials that resist corrosion, withstand the rigors of cleaning and regular handling, and are not detrimental to the health and research use of the animals. The enclosure should be designed and manufactured to prevent accidental entrapment of animals or their appendages and should be free of sharp edges or projections that could cause injury to the animals or personnel. It should have smooth, impervious surfaces with minimal ledges, angles, corners, and overlapping surfaces so that accumulation of dirt, debris, and moisture is minimized and cleaning and disinfecting are not impaired. All enclosures should be kept in good repair to prevent escape of or injury to animals, promote physical comfort, and facilitate sanitation and servicing. Rusting or oxidized equipment that threaten the health or safety of animals need to be repaired or replaced. Less durable materials, such as wood, may be appropriate in select situations, e.g., outdoor corrals, perches, climbing structures, resting areas, and perimeter fences for primary enclosures. Wooden items may need to be replaced periodically because of damage or difficulties with sanitation. Painting or sealing wood surfaces with non-toxic materials may improve durability in many instances.

Flooring should be solid, perforated or sl atted with a slip-resistant surface. In the case of perforated or slatted floors, the holes and slats should have smooth edges. Their size and spacing need tobe commensurate with the size of the housed animal to minimize injury and the development of foot lesions. If wire-mesh flooring is used, a solid resting area

1994). Novelty of enrichment through rotation or replacement of items should also be a consideration; however, changing the animal environment too frequently may be stressful.

Enrichment programs should be reviewed by the IACUC, researchers and veterinarian on a regular basis to ensure that they are beneficial to animal well-being and consistent with the goals of animal use. They should be updated as needed to ensure that they reflect current knowledge. Personnel responsible for animal care and husbandry should receive training in the behavioral biology of the species they work with to appropriately monitor the effects of enrichment, as well as identify the development of adverse or abnormal behaviors.

Like other environmental factors (such as space, light, noise, temperature, and animal care procedures), enrichment affects animal phenotype and may impact the experimental outcome. Thus, it should be considered an independent variable and appropriately controlled.

Some scientists have raised concerns that environmental enrichment may compromise experimental standardization by introducing environmental variability, adding not only diversity to the animals' behavioral repertoire but also variation to the animals' responses to experimental treatments (e.g., Bayne 2005; Eskola et al. 1999; Gärtner 1999; Tsai et al. 2003). A systematic study in mice did not find evidence to support this viewpoint (Wolfer et al. 2004), indicating that housing conditions can be enriched without compromising the precision or reproducibility of experimental results. However, further research in other species may be needed to confirm this conclusion. Moreover, it has been

such as an indoor portion of a run. Shelters should be large enough to accommodate all animals housed in the enclosure, should be accessible at all times to all animals, have sufficient ventilation, and be designed to prevent build-up of waste materials and excessive moisture. Houses, dens, boxes, shelves, perches, and other furnishings should be constructed in a manner and made of materials that allow cleaning or replacement in accord with generally accepted husbandry practices.

Floors or ground-level surfaces of outdoor housing facilities can be covered with dirt, absorbent bedding, sand, gravel, grass, or similar material that can be removed or replaced when needed to ensure appropriate sanitation. Excessive build-up of animal waste and stagnant water should be avoided by, for example, using contoured or drained surfaces. Other surfaces should be able to withstand the elements and be easily maintained.

Successful management of outdoor housing relies on stable social groups of compatible animals; sufficient and species-adequate feeding and resting places; an adequate acclimation period in advance of seasonal changes when animals are first introduced to outdoor housing; training of animals to cooperate with veterinary and investigative personnel; (e.g., to enter chutes or cages for restraint or transport); and adequate security via a perimeter fence or other means.

Naturalistic Environments

Areas such as pastures and islands afford opportunities to provide a suitable environment for maintaining or producing animals and for some types of research. Their use results in the loss of some control over nutrition, health care and surveillance, and pedigree management. These limitations should be balanced against the benefits of having the animals live in more natural conditions. Animals should be added to, removed from, and returned to social groups in this setting with appropriate consideration of the effects on the individual animals and on the group. Adequate supplies of food, fresh water, and natural or constructed shelter should be ensured.

Space

General Considerations for all Animals

An animal's space needs are complex, and consideration of only the animal's body weight or surface area may be inadequate. Important considerations for determining space needs include the age and sex of the animals; the number of

enrichment devices (e.g., novel objects, toys, foraging devices) should not be considered part of the floor space.

The space recommendations presented here are based on professional judgment and experience. They should be considered the minimum for animals housed under conditions commonly found in laboratory animal housing facilities. Adjustments to the amount and arrangement of space recommended in the following tables should be reviewed and approved by the IACUC. They should be based on performance indices related to animal well-being and research quality as described in the preceding paragraphs with due consideration of the AWRs and PHS Policy and other applicable regulations and standards.

It is not within the scope of the Guide to discuss the housing requirements of all species used in research. For species not specifically indicated, advice should be sought from the scientific literature and from species-relevant experts.

Laboratory Rodents

Table 3.2 lists recommended minimum space for commonly used laboratory rodents housed in groups. If they are housed singly or in small groups, or exceed the weights in the table more space per animal may be required, while larger groups may be housed at slightly higher densities.

Space needs and the effects of social housing, group size and density

TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups

Animals	Weight (g)	Floor Area/Animal [a]		Height [b]		Comments
		in^2	cm^2	in	cm	
Mice in Groups[c]	<10					

[d] Other considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects to the mother or the litter.

Other Common Laboratory Animals

Tables 3.3 and 3.4 list recommended minimum space for other common laboratory animals and for avian species. These allocations are based, in general, on the needs of pair- or group-housed animals. Space allocations should be re-evaluated to provide for enrichment or to accommodate animals that exceed the weights in the tables, and should be based on species characteristics, behavior, compatibility of the animals, number of animals, and goals of the housing situation (Held et al. 1995; Lupo et al. 2000; Raje 1997; Turner et al. 1997). Singly housed animals may require more space per animal than that recommended for group-housed animals, while larger groups may be housed at slightly higher densities. For cats, dogs and some rabbits, housing enclosures that allow greater freedom of movement and less restricted vertical space are preferred (e.g., kennels, runs, or pens instead of cages). Dogs and cats, especially when housed individually or in smaller enclosures (Bayne 2002), should be allowed to exercise and provided with positive human interaction. Species-specific plans for housing and management should be developed. Such plans should also include strategies for environmental enrichment.

TABLE 3.3 Recommended Minimum Space for Rabbits, Cats and Dogs Housed in Pairs or Groups

Animals	Weight Kg[a]	Floor Area/Animal[b] ft^2	m^2	Height[c] in	cm	Comments
Rabbits	<2	1.5	0.14	16	40.5	Larger rabbits may require more cage height to allow animals to sit up.
	Up to 4	3.0	0.28	16	40.5	
	Up to 5.4	4.0	0.37	16	40.5	
	>5.4[c]	≥5.0	≥0.46	16	40.5	
Cats	≤4	3.0	0.28	24	60.8	For cats vertical space with perches is preferred and may require additional cage height.
	>4[d]	≥4.0	≥0.37	24	60.8	
Dogs[e]	<15	8.0	0.74		-[f]	Cage height should be sufficient for the animals to comfortably stand erect with their feet on the floor.
	Up to 30	12.0	1.2		-[f]	
	>30[d]	≥24.0	≥2.4		-[f]	

[a] To convert kilograms to pounds, multiply by 2.2.

[b] Singly housed animals may require more space per animal than recommended for pair- or group-housed animals.

[c] From cage floor to cage top.

[d] Larger animals might require more space to meet performance standards (see text).

[e]

If it is necessary to house animals singly (for example, when justified for experimental purposes; for provision of veterinary care; or for incompatible animals), it should be for the shortest duration possible. If single animals are housed in small enclosures an opportunity for periodic release into larger enclosures with additional enrichment items should be considered, particularly for animals housed singly for extended periods of time. Singly housed animals may require more space per animal than recommended for pair- or group-housed housed animals, while larger groups may be housed at slightly higher densities. Because of the many physical and behavioral characteristics of nonhuman primate species and the many factors to consider when using these animals in a biomedical research setting, species-specific plans for housing and management should be developed. Such plans should include strategies for environmental and psychological enrichment.

TABLE 3.5 Reccomended Minimum Space for Nonhuman Primates Housed in Pairs or Groups

Animals	Weight Kg[a]	Floor Area/Animal[b] ft^2	m^2	Height[c] in	cm	Comments

Animals/Enclosure	Weight, kg [a]	Floor Area/Animal [b]	
		ft^2	m^2
	>50[c]	≥15.0	1.35
Swine			
1	<15	8.0	0.72
	Up to 25	12.0	1.08
	Up to 50	15.0	1.35
	Up to 100	24.0	2.16
	Up to 200	48.0	4.32
	>200[c]	≥60.0	5.4
2-5	<25	6.0	0.54
	Up to 50	10.0	0.9
	Up to 100	20.0	1.8
	Up to 200	40.0	3.6
	>200[c]	≥52.0	4.68
>5	<25	6.0	0.54
	Up to 50	9.0	0.81
	Up to 100	18.0	1.62
	Up to 200	36.0	3.24
	>200[c]	≥48.0	4.32
Cattle			
1	<75	24.0	2.16
	Up to 200	48.0	4.32
	Up to 350	72.0	6.48
	Up to 500	96.0	8.64
	Up to 650	124.0	11.16
	>650[c]	≥144.0	12.96
2-5	<75	20.0	1.8
	Up to 200	40.0	3.6
	Up to 350	60.0	5.4
	Up to 500	80.0	7.2
	Up to 650	105.0	9.45
	>650[c]	≥120.0	10.8
>5	<75	18.0	1.62
	Up to 200	36.0	3.24
	Up to 350	54.0	4.86
	Up to 500	72.0	6.48
	Up to 650	93.0	8.37
	>650[c]	≥108.0	9.72
Horses	--	144.0	12.96
Ponies			
1-4	--	72.0	6.48
>4/Pen	≤200	60.0	5.4
	>200[c]	≥72.0	6.48

[a] To convert kilograms to pounds, multiply by 2.2.

Terrestrial Management

Behavioral and Social Management

Activity

Animal activity typically implies motor activity but also includes cognitive activity and social interaction. Animals maintained in a laboratory environment are generally restricted in their activities compared to free-ranging animals. The animals' natural behavior and activity profile should be considered during evaluation of suitable housing or behavioral assessment. Forced activity for reasons other than attempts to meet therapeutic or approved protocol objectives should be avoided. High levels of repetitive, unvarying behavior (stereotypies, compulsive behaviors) may reflect disruptions of normal behavioral control mechanisms due to housing conditions or management practices (Garner 2005; NRC 1998a).

Dogs, cats, rabbits, and many other animals benefit from positive human interaction (Augustsson et al. 2002; Bayne et al. 1993; McCune 1997; Poole 1998; Rennie and Buchanan-Smith 2006; Rollin 1990). Dogs can be given additional opportunities for activity by being walked on a leash, having access to a run, or being moved into areas for social contact, play, or exploration (Wolff and Rupert 1991). Loafing areas, exercise lots, and pastures are suitable for large farm animals, such as sheep, horses, and cattle.

Social Environment

Appropriate social interactions among members of the same species (conspecifics) are essential to normal development and well-being (Bayne et al. 1995; Hall 1998; Novak et al. 2006). When selecting a suitable social environment, attention should be given to whether the animals are naturally territorial or communal and whether they should be housed singly, in pairs, or in groups. An understanding of species-typical natural social behavior (e.g., natural social composition, population density, ability to disperse, familiarity and social ranking) is key to successful social housing.

Not all members of a social species are necessarily socially compatible. Social housing of incompatible animals can induce chronic stress, injury and even death. In some species, social incompatibility may be sex biased; for example, male mice are generally more prone to aggression than female mice, and female hamsters are generally more aggressive than male hamsters. These risks of social incompatibility are greatly reduced if the animals to be grouped are raised together from a young age, if group composition remains stable, and if

design of the animals' enclosure and environmental enrichment facilitate the avoidance of social conflicts. Social stability should be carefully monitored and in cases of severe or prolonged aggression, incompatible individuals need to be separated.

For some species, developing a stable social hierarchy will entail agonistic interactions between pair or group members, particularly for animals introduced as adults. Animals may have to be introduced to each other over a period of time and they should be monitored closely during this introductory period and thereafter to ensure compatibility.

Single housing of social species should be the exception and should be justified based on experimental requirements or veterinary-related concerns regarding animal well-being. In these cases, single housing of social animals should be limited to the minimum period necessary, and where possible, visual, auditory, olfactory and tactile contact with compatible conspecifics should be provided. In the absence of other animals, enrichment should be offered such as positive interaction with the animal care staff and additional enrichment items or addition of a companion animal in the room or housing area. The need for single housing should be reviewed on a regular basis by the IACUC and veterinarian.

Procedural Habituation and Training of Animals

Habituating animals to routine husbandry or experimental procedures should be encouraged, whenever possible, as it may assist the animal to better cope with a captive environment by reducing stress associated with novel procedures or people. The type and duration of habituation needed will be determined by the complexity of the procedure being performed. In most cases, principles of operant conditioning may be employed during training sessions, using progressive behavioral shaping, to induce voluntary cooperation with procedures (Bloomsmith et al. 1998; Laule et al. 2003; NRC 2006a; Reinhardt 1997).

Husbandry

Food

Animals should be fed palatable, uncontaminated diets that meet their nutritional and behavioral needs at least daily, or according to their particular requirements, unless the protocol in which they are being used requires otherwise. Subcommittees of the National Research Council Committee on Animal Nutrition have prepared comprehensive reports of the nutrient

requirements of laboratory animals (NRC 1977, 1982, 1993, 1994, 1995a, 1998b, 2000, 2001, 2003a, 2006b, 2006c, 2007). These publications consider issues of quality assurance, freedom from chemical or microbial contaminants and presence of natural toxicants in feedstuffs, bioavailability of nutrients in feeds, and palatability. There are three types of diets classified by the degree of refinement of their ingredients. Natural-ingredient diets are formulated with

stored off the floor on pallets, racks, or carts in a manner that facilitates sanitation. Opened bags of food should be stored in vermin-proof containers to minimize contamination and to avoid potential spread of pathogens. Exposure to elevated storage room temperatures, extremes in relative humidity, unsanitary conditions, insects and other vermin hastens food deterioration. Storage of natural ingredient diets at less than 21°C (70° F) and below 50% relative humidity is recommended. Precautions should be taken if perishable items—such as meats, fruits, and vegetables and some specialty diets (for example, select medicated or high fat diets)—are fed, because storage conditions may lead to variation in food quality.

Most natural-ingredient, dry laboratory-animal diets stored properly can be used up to 6 months after manufacture. Non-stabilized vitamin C in manufactured feeds generally has a shelf-life of only 3 months, but commonly employed stabilized forms can extend the shelf-life of feed. Refrigeration preserves nutritional quality and lengthens shelf-life, but food-storage time should be reduced to the lowest practical period and the manufacturers' recommendations should be considered. Purified and chemically defined diets are often less stable than natural-ingredient diets, and their shelf-life is usually less than 6 months (Fullerton et al. 1982); these diets should be stored at 4°C (39°F) or lower.

Irradiated and fortified autoclavable diets are commercially available and are commonly used for axenic, microbiolo Th.0d01 f .0s-.00002ife ofl

more discussion on food and fluid regu lation as an experimental tool see Chapter 2 and NRC 2003b). Benefits acured by moderate caloric restriction in

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Softwood beddings have been used, but the use of untreated softwood shavings and chips is contraindicated for some protocols because they can affect metabolism (Vesell 1967; Vessell et al. 1973, 1976). Cedar shavings are not recommended, because they emit aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity (Torronen et al. 1989; Weichbrod et al. 1986, 1988). Prior treatment with high heat (kiln-drying or autoclaving) may, depending on the material used and the concentration of aromatic hydrocarbon constituents present, reduce the concentration of volatile organic compounds; however, the amounts remaining may be sufficient to affect specific protocols (Cunliffe-Beamer et al. 1981; Nevalainen and Vartiainen 1996).

Manufacturing, monitoring, and storage methods used by vendors should be considered when purchasing bedding products. Bedding may be contaminated with toxins and other substances, bacteria, fungi, and vermin. Bedding should be transported and stored off the floor on pallets, racks, or carts in a fashion consistent with maintenance of quality and avoidance of contamination. Bags should be stored sufficiently away from walls to facilitate cleaning. During autoclaving, bedding can absorb moisture and as a result lose absorbency and support the growth of microorganisms. Therefore, appropriate drying times and storage conditions should be used or alternatively, gamma-irradiated materials utilized if sterile bedding is indicated.

Bedding should be used in amounts sufficient to keep animals dry between cage changes, and, in the case of small laboratory animals, care should be taken to keep the bedding from coming into contact with the sipper tubes, because such contact could cause leakage of water into the cage.

Sanitation

Sanitation—the maintenance of environmental conditions conducive to health and well-being—involves bedding change (as appropriate), cleaning, and disinfection. Cleaning removes excessive amounts of excrement, dirt and debris, and disinfection reduces or eliminates unacceptable concentrations of microorganisms.

The frequency and intensity of cleaning and disinfection should depend on what is needed to provide a healthy environment for an animal. Methods and frequencies of sanitation will vary with many factors, including the normal physiologic and behavioral characteristics of the animals; the type, physical characteristics, and size of the enclosure; the type, number, size, age, and reproductive status of the animals; the use and type of bedding materials;

caging and husbandry practices used, including the use of regularly changed contact or noncontact bedding, regular flushing of suspended catch pans, and the use of wire-bottom or perforated-bottom cages. In general, enclosures and accessories, such as tops, should be sanitized at least once every 2 weeks. Solid-bottom caging, bottles, and sipper tubes usually require sanitation at least once a week. Some types of cages and housing systems might require less-frequent cleaning or disinfection; these might include large cages with very low animal density and frequent bedding changes; cages that house animals in gnotobiotic conditions with frequent bedding changes; individually ventilated cages; and cages used for special situations. Other circumstances, such as filter-topped cages without forced air ventilation, animals that urinate excessively (e.g., diabetic or renal patients), or densely populated enclosures, might require more frequent sanitation.

The increased use of individually ventilated cages (IVCs) for rodents has led to investigations regarding the maintenance of a suitable microenvironment with extended cage sanitation intervals and/or increased housing densities

rooms) should be regularly cleaned and disinfected as appropriate to the circumstances and at a frequency based on the use of the area and the nature of likely contamination. Vaporized hydrogen peroxide or chlorine dioxide are effective compounds for room decontamination, particularly following completion of studies with highly infectious agents (Krause et al. 2001) or contamination with adventitious microbial agents.

Cleaning implements should be constructed of materials that resist corrosion and withstand regular sanitation. They should be assigned to specific areas and should not be transported between areas with different risks of contamination without prior disinfection. Worn items should be replaced regularly. The implements should be stored in a neat, organized fashion that facilitates drying and minimizes contamination or harborage of vermin.

Assessing the Effectiveness of Sanitation

Monitoring of sanitation practices should fit the process and materials being cleaned and may include visual inspection and microbiological and water temperature monitoring (Compton et al. 2004 a, b; Ednie et al. 1998; Parker et al. 2003). The intensity of animal odors, particularly that of ammonia, should not be used as the sole means of assessing the effectiveness of the sanitation program. A decision to alter the frequency of cage-bedding changes or cage-washing should be based on such factors as ammonia concentration; bedding condition; appearance of the cage and animals; and the number and size of animals housed in the cage.

Mechanical washer function should be evaluated regularly and include examination of mechanical components such as spray arms and moving headers as well as spray nozzles to ensure they are functioning appropriately. If sanitation is temperature dependent, the use of temperature sensing devices (e.g., thermometers, probes, or temperature sensitive indicator strips), is recommended to ensure that the equipment being sanitized is exposed to the desired conditions.

Whether the sanitation process is automated or manual, regular evaluation of sanitation effectiveness is recommended. This can be performed by evaluating processed materials by microbiologic culture or the use of soil detection systems (e.g., ATP bioluminescence) and/or by confirming the removal of artificial soil applied to equipment surfaces before washing.

Pest Control

Programs designed to prevent, control, or eliminate the presence of or infestation by pests, are essential in an animal environment. A regularly scheduled and documented program of control and monitoring should be implemented. The ideal program prevents the entry of vermin into and eliminates harborage within the facility (Anadon et al. 2009; Easterbrook et al. 2008). For animals in outdoor facilities, consideration should also be given to eliminating or minimizing the potential risk associated with pests and predators. Pesticides can induce toxic effects on research animals and interfere with experimental procedures

Population Management

Identification

Animal records are useful and variable, ranging from limited information on identification cards to detailed computerized records for individual animals (Field et al. 2007). Means of animal identification include room, rack, pen, stall, and cage cards with written or bar-coded or radio frequency identification (RFID) information. Identification cards should include the source of the animal, the strain or stock, names and contact information for the responsible investigators, pertinent dates, and protocol number, when applicable. Where applicable, genotype information should be included in the identification, and consistent, unambiguous abbreviations should be used where the full genotype nomenclature (see below) is too lengthy.

Further, the animals may wear collars, bands, plates, or tabs; be marked by colored stains; ear notches/punches and tags; tattoos; subcutaneous transponders; and freeze brands. As a method of identification of small rodents, toe-clipping should be used only when no other individual identification method is feasible. It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age (Castelhano-Carlos et al. 2010; Schaefer et al. 2010), especially if toe-clipping and genotyping can be combined. Under all circumstances aseptic practices should be followed. Use of anesthesia or analgesia should be commensurate with the age of the animals (Hankenson et al. 2008).

on experimental use, and necropsy findings where applicable. Basic demographic information and clinical hist ories enhance the value of individual animals for both breeding and research and should be readily accessible to investigators, veterinary staff, and animal-care staff.

Cryopreservation of fertilized embryos, ova, ovaries, or spermatozoa should also be considered as a safeguard against alterations in transgenes over time or accidental loss of GMA lines (Conner 2002; Liu 2009). When animals with multiple genetic alterations are required, this often involves crossing different GMA lines and can lead to the production of offspring with genotypes that are not of interest to the researcher, either as experimental or control animals as well as unexpected phenotypes. Carefully designed breeding strategies and accurate genotype assessment can help to minimize the generation of animals with unwanted genotypes (Linder 2003). Newly generated genotypes should be carefully monitored and new phenotypes that negatively impact well-being should be reported to the IACUC and managed in a manner to ensure the animals' health and well being.

Accurate recording, with standardized nomenclature h si

Aquatic Environment

Microenvironment and Macroenvironment

As with terrestrial systems, the microenvironment of an animal is the physical environment immediately surrounding it; that is, the primary enclosure such as the tank, raceway or pond. It contains all the resources with which the animals are in direct contact and also provides the limits of the animals' immediate environment. The microenvironment is characterized by many factors, including

The specific parameters and frequency

advanced containing biological filters (biofilters) that promote conversion of ammonia to nitrite and nitrate via nitrifying bacteria, protein skimmers (foam fractionators) and particulate filters to remove undissolved and dissolved proteins and particulate matter, carbon filters to remove dissolved chemicals, and ultraviolet or ozone units to disinfect the water. The systems generally contain components to aerate and degas the water to prevent gas over-saturation, heat or cool the water, and automated dosing systems to maintain appropriate pH and conductivity. Not all elements are found on all systems and some components may accomplish multiple functions. Re-circulating systems may be designed so that multiple individual tanks are supplied with treated water from a single source, as is the case with "rack" systems utilized for zebrafish (Danio rerio) and Xenopus laevis and tropicalis, as examples (Fisher 2000; Koerber 2009; Schultz 2003).

The development and maintenance of the biofilter is critical for limiting ammonia and nitrite accumulation in re-circulating systems. The biofilter must be of sufficient size (i.e., contain a sufficient quantity of bacteria) to be capable of processing the bioload (level of nitrogenous waste) entering the system. The microorganisms supported by the biofilter require certain water quality parameters. Alterations in the aquatic environment, such as rapid changes in salinity, temperature, and pH, as well as the addition of chemicals or anti-microbials, may significantly impact the microbial ecology of the system, and therefore water quality and animal well-being. If damaged, biofilter recovery may take weeks (Fisher 2000). Changes in water quality parameters (such as pH, ammonia, and nitrite) may negatively impact animal health and the efficiency of the biofilter, therefore species sensitive to change in water quality outside of a narrow range require more frequent monitoring.

Continuous or timed flow-through systems can be utilized where suitable water is available to support the species to be housed (e.g., aquaculture facilities). These systems may utilize extremely large volumes of water, as water is not reused. The water may be used "as is" or processed before use, for example by removing sediments, excessive dissolved gases, chlorine or chloramines, and by disinfecting with UV or ozone (Fisher 2000; Overstreet 2000). Static systems vary in size from small tanks to large in-ground ponds. These systems may utilize mechanical devices to move and aerate water.

Temperature, Humidity and Ventilation

The general concepts discussed in the Terrestrial Animals section also apply to the aquatic setting. The majority of aquatic or semi aquatic species (fish, amphibians and reptiles) used in research are poikilotherms. Poikilotherms

depend, for the most part, on the temperature of their environment to sustain physiologic processes, such as metabolism, reproduction, and feeding behavior (Browne and Edwards 2003a; Fraile 1989; Maniero 1997; Pough 1991). Temperature requirements are based on the natural history of the species and can vary across life stage (Green 2002; Pough 1991; Schultz 2003). Water temperature may be controlled at its source, within the life support system, or by controlling the macroenvironment. Some semi-open systems depend on source water temperature and thus enclosure water temperature will vary with that of the source water (e.g., raceways by a river).

The volume of water contained within a room can impact room temperature, temperature stability and relative humidity. Likewise the thermal load produced by chiller/heater systems can affect the stability of the macroenvironmental temperature. Air handling systems need to be designed to compensate for these thermal and moisture loads. Macroenvironmental relative humidity levels are generally defined by safety issues and staff comfort, since room humidity is not critical for aquatic species; however, excessive moisture may result in condensation on walls, ceilings, and tank lids, which may support microbial growth and serve as a source of contamination or create a conducive environment for metal corrosion. In a dry environment (e.g., indoor heating during cold weather or outdoor housing in some climates/seasons), evaporation rates may be increased, potentially requiring the addition of large quantities of water to the system and monitoring for increases in salinity/conductivity, contaminants or other water quality aberrations. Semi-aquatic species (e.g., some amphibians and reptiles) may need elevated microenvironmental humidity (in excess of 50%-70% relative humidity), which may require maintaining elevated macroenvironmental humidity levels (Pough 1991; St. Claire 2005).

Room air exchange rates are typically governed by thermal and moisture loads. For fish and some aquatic amphibians, the microenvironmental air quality may impact water quality (i.e., gas exchange), however, appropriate life support system design may reduce its importance. Air borne particulates and compounds (e.g., volatile organic compounds and ammonia) may dissolve in tank water and impact animal health (Koerber 2009). As the aerosolization of water can lead to spreading of aquatic animal pathogens (e.g., protozoa, bacteria) within or throughout an aquatic animal facility, this process should be minimized as much as feasibly possible (Roberts-Thomson et al. 2006; Wooster and Bowser 2007; Yanong 2003)

- Allow access to adequate food and removal of food waste.
- Restrict escape or accidental entrapment of animals or their appendages.
- Are free of sharp edges and/or projections that could cause injury.
- Allow for observation of the animals with minimal disturbance.
- Are constructed of non-toxic materials that do not leach toxicants or chemicals into the aquatic environment
- Do not present electrical hazards directly or indirectly

Environmental Enrichment and Social Housing

Environmental enrichment strategies for many aquatic species are not well established. The implications of a barren versus an enriched environment on well-being, general research, growth and development are unknown or poorly defined. This also applies to individual vs. group (social) housing for many species. When utilized, enrichment should elicit species appropriate behaviors and be evaluated for safety and utility.

Generally, schooling fish species are housed with conspecifics, and many amphibians, especially anuran speciesmay be group-housed. Aggression in aquatic animals does occur (van de Nieuwegiessen et al. 2008; Speedie and Gerlai 2007) and, as for terrestrial animals, appropriate monitoring and intervention may be necessary (Matthews 2002; Torreilles 2007). Some species need appropriate substrate (e.g., gravel) to reproduce or need substrate variety to express basic behaviors and maintain health (Overstreet 2000). Improved breeding success in enriched environments has been reported but further research in this area is needed (Carfagnini 2009). For many species, visual barriers, hides and shading are appropriate as, for example, for Xenopus laevis (Alworth 2009; Torreilles 2007). Most semi-aquatic reptiles spend some time on land (e.g., basking, feeding, digesting, and ovipositing) and terrestrial areas should be provided as appropriate for the species.

Sheltered, Outdoor, and Naturalistic Housing

Animals utilized in aquaculture are often housed in situations that mimic agricultural rearing and may be in outdoor and/or sheltered raceways, ponds, or pens with high population densities. In these settings, where natural predation and mortalities occur, it may be appropriate to measure animal “numbers” by utilizing standard aquaculture techniques such as final production biomass (Borski 2003).

Space

Space recommendations and housing density varies extensively with the species, age/size of the species, life support system, and type of research (Browne et al.

Husbandry

Food

The general principles relating to feeding of terrestrial animals are applicable to aquatic animals. Food should be stored in a type-appropriate manner to preserve nutritional content, minimize contamination and prevent entry of pests.

Pest Control

Terrestrial animal pest control principl

animals are maintained utilizing group (vs. individual) identification, detailed animal records still need to be maintained. General animal information that may routinely be captured, particularly in biomedical research with fish, includes: species; genetic information (parental stock identification, genetic composition); stock source; stock numbers in system; tank identification; system life support information; breeding; deaths; illnesses; animal transfers within and out of the facility; and fertilization/hatching information (ibid; Matthews 2002). Feeding records should also be maintained for aquatic animals (e.g., food offered, acceptance) as well as an accurate tracking of non-expired food supplies to ensure sustainance of nutritional profile. Records should also be maintained for any live cultures (e.g., hatch rates and information to ensure suppliers' recommendations are being met; ibid).

Records of water quality testing for system and source water and maintenance activities of the life support system components are important in tracking and maintaining water quality. The exact water quality parameters tested and testing frequency should be clearly established and will vary with such factors as the type of life support system, animals, and research as discussed in the Water Quality section (page 160). Detailed tracking of animal numbers in aquatic systems is often possible if accurate records of transfers, breeding and mortalities are maintained (ibid). In some cases where animals are housed in large groups (e.g., some *Xenopus* colonies) periodic censuses may be undertaken to obtain an exact count. In large-scale aquaculture research it may be more appropriate to measure biomass of the system vs. actual numbers of animals (Borski 2003).

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immobilization, sedation, analgesia, anesthesia, and euthanasia. In addition, the veterinarian should provide guidance and oversight to surgery programs and perioperative care involving animals.

Animal Procurement and Transportation

Animal Procurement

All animals must be acquired lawfully , and the receiving institution should ensure that all procedures involving animal procurement are conducted in a lawful manner. Prior to procuring anim als, the Principal Investigator should confirm that there are sufficient facilities and expertise to house and manage the species being acquired. Procurement of animals should be linked to the prior

does not pose risks to animal well-being or personnel safety. During times of extreme temperatures animal transport may not be possible if an appropriately heated or cooled means of

inadvertently introduced; a comprehensive ongoing program for evaluating animals' health status, including access to all animals; and containment and eradication –if desired- of introduced infectious agents. Related program components include procedures for evaluating and selecting appropriate animal suppliers (these may include quarantine and confirmation of animal health status if unknown); treatment of animals or their products at entry to minimize disease risks (e.g., surface disinfection of fish eggs); establishment of a comprehensive pest control program that may include evaluation of the health status of feral animals; procedures to ensure that all biologicals administered to animals are free of contamination; and development of appropriate procedures for intra- and inter-facility animal transport. For example, transport of animals to laboratory and other facilities outside of the animal facility can present challenges to animal biosecurity (Balaban and Hampshire 2001). Additional details pertaining to these topics can be found in other sections of the Guide.

Quarantine and Stabilization

Quarantine is the separation of newly received animals from those already in the facility, in a way that prevents potential spread of contaminants, until the health and possibly the microbial status of the newly received animals have been determined. Transportation of animals can be stressful and may induce recrudescence of subclinical infections harbored by an animal. An effective quarantine program minimizes the risk of introduction of pathogens into an established colony. The veterinary medical staff should implement procedures for evaluating the health and, if appropriate, the pathogen status of newly received animals, and the procedures should reflect acceptable veterinary medical practice and federal and state regulations applicable to zoonoses (Butler et al. 1995). Effective quarantine procedures are particularly helpful in limiting human exposure to zoonotic infections from nonhuman primates, such as mycobacterial infections which necessitate specific guidelines for handling of these animals (Lerche et al. 2008; Robertst and Andrews 2008). Information from suppliers on animal quality should be sufficient to enable a veterinarian to determine the length of quarantine, to define the potential risks to personnel and animals within the colony, to determine whether therapy is required before animals are released from quarantine, and,

animals from other shipments to preclude transfer of infectious agents between groups.

Depending on the health status of the colony animals and consistent with the animal biosecurity program in place, rodents or other animals being moved outside of an animal facility for procedures, such as imaging or behavioral testing, may need to be held separately from their colony of origin until their health status is evaluated.

Regardless of whether the animals are quarantined, newly received animals should be given a period for physiologic, behavioral, and nutritional acclimation before their use (Obernier and Baldwin 2006). The length of time for acclimation will depend on the type and duration of animal transportation, the species involved, and the intended use of the animals. For animals not typically housed in research settings, considerations should be given to providing means to assist with animal acclimation, for example, shearing sheep before they are brought indoors. The need for an acclimation period has been demonstrated in mice, rats, guinea pigs, nonhuman primates, and goats; it is likely important for other species as well (Conour et al. 2006; Capitanio et al. 2006; Kagira et al. 2007; Landi et al. 1982; Prasad et al. 1978; Sanhouri et al. 1989; Tuli et al. 1995).

Separation by Health Status and Species

Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate the potential for anxiety and physiologic and behavioral changes due to interspecies conflict (Arndt et al. 2010). Such separation is usually accomplished by housing different species in separate rooms. In some instances, however, this might be accomplished with cubicles, laminar-flow units, cages that

- As a rule, New World (South and Central American), Old World African, and Old World Asian species of nonhuman primates should be housed in separate rooms. Simian hemorrhagic fever (Renquist 1990) and simian immunodeficiency virus (Hirsch et al. 1991; Murphy-Corb et al. 1986), for example, cause only subclinical infections in African species but induce clinical disease in Asian species.
- Some species should be housed in separate rooms even though they are from the same geographic region. For example, squirrel monkeys (*Saimiri sciureus*) and tamarins (*Saguinus oedipus*) might be latently infected with herpesviruses (*Herpesvirus saimiri* and *Herpesvirus tamarinus*, respectively), which could be transmitted to and cause a fatal epizootic disease in owl monkeys (*Aotus trivirgatus*) (Barahona et al. 1975; Hunt and Melendez 1966; Murphy et al. 1971).

Intraspecies separation might be essential when animals obtained from multiple sites or sources, either commercial or institutional, differ in pathogen status, e.g., rat theilovirus in rats, mouse hepatitis virus in mice, bacterial gill disease in rainbow trout, *Pasteurella multocida* in rabbits, Macacine herpesvirus (B virus) in macaque species, and *Mycoplasma hyopneumoniae* in swine.

Surveillance, Diagnosis, Treatment, and Control of Disease

All animals should be observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs. As a rule, this should occur at least daily, but more frequent observations may be required, such as during postoperative recovery, when animals are ill or have a physical deficit, or when animals are approaching a study endpoint. Professional judgment should be used to ensure that the frequency and character of observations minimize risks to individual animals and does not compro

Procedures for disease prevention, diagnosis, and therapy should be those currently accepted in veterinary and laboratory animal practice. Health monitoring programs also include veterinary herd/flock health programs for livestock, and colony health monitoring programs for aquatic and rodent species. Access to diagnostic laboratory services facilitate veterinary medical care and can include gross and microscopic pathology, hematology, microbiology, parasitology, clinical chemistry, molecular diagnostics, and serology. If a disease or infectious agent is identified within a facility or colony, the choice of therapy should be made by the veterinarian in consultation with the investigator. If the animal is to continue on study, the selected treatment plan should be therapeutically sound and, when possible, should interfere minimally with the research process.

Subclinical microbial infections (see Appendix A, Pathology, Clinical Pathology, and Parasitology) occur frequently in conventionally maintained rodents but also can occur in facilities designed and maintained for production and use of pathogen-free rodents if the microbial barrier is breached. Examples of infectious agents that can be subclinical but which may induce immunologic changes or alter physiologic, pharmacologic, or toxicologic responses are noroviruses, parvoviruses, mouse hepatitis virus, lymphocytic choriomeningitis virus, and *Helicobacter* spp (Besselsen et al. 2008; Clifford and Watson 2008; NRC 1991 a, b, c). Scientific objectives of a particular protocol, the consequences of infection within a specific strain of rodent, the potential for zoonotic disease, and the adverse effects that infectious agents might have on other animals or protocols in a facility should determine the characteristics of rodent health-surveillance programs and strategies for keeping rodents free of specific pathogens.

The principal method for detecting microbial infections in animal populations is serologic testing, flow cytometric bead immunoassays or immunofluorescent assays. Other methods of detecting infections, such as DNA analysis using a polymerase chain reaction (PCR), microbial culture, clinical chemistry (e.g., LDH virus), histopathology, and other validated emergent technologies can also be used to make or confirm a diagnosis. Transplantable tumors, hybridomas, cell lines, blood products, and other biologic materials can be sources of murine viruses that can contaminate rodents as well as human viruses that may pose risks to laboratory personnel (Nicklas et al. 1993). Rapid and effective assays are available to monitor microbiologic contamination and should be considered prior to introducing such material into animals (Peterson 2008). Because health monitoring programs are dependent on the size and complexity of the Program, the species involved, and the institutional research focus, it is beyond the scope of the *Guide* to go into details about health monitoring programs for all species. Additional references can be found in

Appendix A (Disease Surveillance, Diagnosis and Treatment; Pathology, Clinical Pathology and Parasitology; and under Species-Specific References).

Clinical Care and Management

Healthy, well cared for animals are a prerequisite for good quality animal-based science. The structure of the veterinary care program, including the number of qualified veterinarians should be appropriate to fulfill the program's requirements. This will vary by institution, species used, and the nature of the animal use. To be effective in providing clinical care, the veterinarian should be familiar with the species, the various uses of animals in the institutional research, teaching, testing, or production programs and have access to medical and experimental treatment records.

Medical Management

There should be a timely and accurate method for communication of any abnormalities or concerns regarding animal health, behavior, and well-being to the veterinarian or the veterinarian's designee. The responsibility for bringing these concerns forward rests with all those involved with as2gshu7(exso7 0 TDc)3 Tc .0003 Tw (ab

Emergency Care

Procedures must be in place to provide for emergency veterinary care both during and outside of regularly scheduled hours. Such procedures must enable timely reporting of animal injury, illness, or death by animal care and research staff. A veterinarian or the veterinarian's designee must be available to expeditiously assess the animal's condition, treat the animal, investigate an unexpected death, or advise on euthanasia. In the case of a pressing health problem, if the responsible person (e.g., investigator) is not available or if consensus between the investigator and veterinary staff cannot be reached concerning treatment, the veterinarian

the procedures will be conducted; and perioperative animal health assessment and care (Brown and Schofield 1994). Aveterinarian should be involved in discussions surrounding the selection of anesthetic agents and doses to be used as well as the plan for perioperative analgesic use. If a nonsterile part of an animal, such as the gastrointestinal tract, is to be surgically exposed or if a se. immunosuppes ions,proeperative anatib-1628(i)1(oic s maybe s]TJ -0-1.24 TD -0001 Tc -.0001

Surgical Procedures

In general, surgical procedures are categorized as major or minor and, in the laboratory setting, can be further divided into survival and nonsurvival. As a general guideline, major survival surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic functions (such as laparotomy, thoracotomy, joint replacement, and limb amputation), or involves extensive tissue dissection or transection(Brown et al. 1993). Minor survival surgery does not expose a body cavity and causes little or no physical impairment (such as wound suturi ng; peripheral vessel cannulation; percutaneous biopsy; routine agricultural animal procedures such as castration, and most procedures routinely done on an “outpatient” basis in veterinary clinical practice). Typically, animals re covering from these minor procedures do not show significant signs of postope rative pain, have minimal complications,

In non-survival surgery, an animal is euthanatized before recovery from anesthesia. It might not be necessary to follow all the techniques outlined in this section if nonsurvival surgery is performed; however, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean (Slattum et al. 1991). For non-survival procedures of extended duration, attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome.

Aseptic Technique

Aseptic technique is used to reduce microbial contamination to the lowest possible practical level (Mangram et al. 1999). No procedure, piece of equipment, or germicide alone can achieve that objective (Schonholtz 1976). Aseptic technique requires the input and cooperation of everyone who enters the surgery area (Belkin 1992; McWilliams 1976). The contribution and importance of each practice varies with the procedure. Aseptic technique, regardless of the species, includes preparation of the patient, such as hair or feather removal and disinfection of the operative site (Hofmann 1979); preparation of the surgeon, such as the provision of appropriate surgical attire, face masks, and sterile surgical gloves (Chamberlain and Houang 1984; Pereira et al. 1990; Schonholtz 1976); sterilization of instruments, supplies, and implanted materials (Bernal et al. 2009; Kagan 1992b); and the use of operative techniques to reduce the likelihood of infection (Ayliffe 1991; Kagan 1992a; Ritter and Marmion 1987; Schofield 1994; Whyte 1988).

While the species of animal may influence the manner in which principles of aseptic technique are achieved (Brown 1994; Cunliffe-Beamer 1983), inadequate or improper technique may lead to subclinical infections that can cause adverse physiologic and behavioral responses (Beamer 1972; Bradfield et al. 1992; Cunliffe-Beamer 1990; Waynforth 1980, 1987) affecting surgical success, animal well-being, and research results (Cooper et al 2000). General principles of aseptic technique should be adhered to for all survival surgical procedures (ACLAM 2001).

Specific sterilization methods should be selected on the basis of physical characteristics of materials to be sterilized (Callahan et al. 1995; Schofield 1994). Autoclaving and plasma and gas sterilization are effective methods most commonly used to sterilize instruments and materials. Sterilization indicators should be used to validate that materials have been properly sterilized (Berg 1993). Alternative methods, used primarily for rodent surgery, include liquid chemical sterilants and dry heat sterilization. Liquid chemical sterilants should

care of surgical incisions. Appropriate medical records should also be maintained. After recovery from anesthesia, monitoring is often less intense but should include attention to basic biologic functions of intake and elimination, behavioral signs of postoperative pain, monitoring for postsurgical infections, monitoring of the surgical incision site for dehiscence, bandaging as appropriate, and timely removal of skin sutures, clips, or staples (UFAW 1989).

Pain and Distress

potential to be exhibited (UFAW 1989). 0 Tc 0 T01 T7 (ar

An integral component of veterinary medical care is prevention or alleviation of pain associated with procedural and surgical protocols. Pain is a complex experience that typically results from stimuli that damage tissue or have the potential to damage tissue. The ability to experience and respond to pain is

physiologic, and biochemical indicato rs of well-being (Dubner 1987; Karas 2002;

procedures. Neuromuscular blocking agents (e.g., pancuronium) are sometimes used to paralyze skeletal muscles during surgery in which general anesthetics have been administered (Klein 1987). When these agents are used during surgery or in any other painful procedure, many signs and reflexes used to assess anesthetic depth are eliminated because of the paralysis. However, autonomic nervous system changes (e.g., sudden changes in heart rate and blood pressure) can be indicators of pain related to an inadequate depth of anesthesia. It is imperative that any proposed use of neuromuscular blocking drugs be carefully evaluated by the veterinarian and the

The selection of specific agents and methods for euthanasia will depend on the species involved, the animal's age, and the objectives of the protocol. Generally, chemical agents (such as barbiturates and non-explosive inhalant anesthetics) are preferable to physical methods (such as cervical dislocation, decapitation, and use of a penetrating captive bolt); however, scientific considerations might preclude the use of chemical agents for some protocols.

Although carbon dioxide (CO_2) is a commonly used method for rodent euthanasia, there is ongoing controversy regarding its aversive characteristics as an inhalant euthanasia agent. This is an area of active research (Conlee et al. 2005; Danneman et al. 1997; Hackbarth et al. 2000; Kirkden et al. 2008; Leach et al. 2002; Niel et al. 2008) and further study is needed to optimize the methods for CO_2 euthanasia in rodents (Hawkins et al. 2006). Its acceptability as a euthanasia agent for small rodents should be evaluated as new data becomes available. Because neonatal rodents are resistant to the hypoxia-inducing effects of CO_2 and require longer exposure times to the agent (Artwohl et al. 2006), alternative methods should be considered (e.g., injection with chemical agents, cervical dislocation, or decapitation; Klaunberg et al. 2004; Pritchett-Corning 2009).

It is essential that euthanasia be

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CHAPTER 5. Physical Plant

A well-planned, well-designed, well-constructed, properly maintained and managed facility is an important element of humane animal care and use, as it facilitates efficient, economical, and safe operation (see Appendix A, Design and Construction of Animal Facilities). The design and size of an animal facility depend on the scope of institutional research activities, the animals to be housed, the physical relationship to the rest of the institution, and the geographic location. Effective planning and design should include input from personnel experienced with animal-facility design, engineering and operation, as well as from representative users of the proposed facility. Use of computational fluid dynamics (CFD), building information modeling and literature on post-occupancy analysis of space use may provide benefits when designing facilities and caging (Eastman et al. 2008; Reynolds 2008; Ross et al. 2009). An animal facility should be designed and constructed in accord with all applicable building codes. In areas with substantial seismic activity the recommendations of the Building Seismic Safety Council should be incorporated into building planning and design (BSSC 2001; Vogelweid et al. 2005). As animal model development and use would be expected to change during the life cycle of an animal facility, facilities should be designed to accommodate changes in utilization. Modular units (such as custom-designed trailers or prefabricated structures) should comply with construction guidelines described in this chapter.

Building materials should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture- and vermin-proof, fire-resistant, seamless materials are most desirable for interior surfaces. Surfaces should be highly resistant to the effects of cleaning agents, scrubbing, high-pressure sprays, and impact. Paints and glazes should be nontoxic if used on surfaces with which animals will have direct contact. In the construction of outdoor facilities, consideration should be given to surfaces that withstand the elements and can be easily maintained.

Location

Quality animal management and human comfort and health protection require separation of animal facilities from personnel areas, such as offices and conference rooms. Separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should

make it possible to place animal-housing areas next to or near research laboratories but separated from them by barriers, such as entry locks, corridors, or floors. Additional considerations include the impact of noise and vibration generated from within the facility and from surrounding areas of the building, as well as security of the facility. Animals should be housed in facilities dedicated to or assigned for that purpose and should not be housed in laboratories merely for convenience. If animals must be maintained in a laboratory to satisfy the scientific aims of a protocol, then that space should be appropriate to house and care for the animals and its use limited to the period during which it is required. If needed, measures should be taken to minimize occupational hazards related to exposure to animals both while in the research area and during transport to and from the area.

Centralization versus Decentralization

In a physically centralized animal facility, support, care, and use areas are adjacent to the animal-housing space. When decentralized, animal housing and use occurs in space that is not solely dedicated to animal care or support, or is physically separated from the support areas and animal care personnel. Centralization often reduces operating costs, providing a more efficient flow of animal care supplies, equipment and personnel; more efficient use of environmental controls; and, less duplication of support services. Centralization generally reduces the needs for transporting animals between housing and study sites, thereby minimizing the risks of transport stress and exposure to disease agents, and generally provides greater security by providing the opportunity to control facility access and increases the ease of monitoring staff and animals.

Decentralized animal facilities generally cost more to construct because of the requirement for specialized environmental systems and controls in multiple sites. Duplicate equipment (for example, cage washers) may be needed, or soiled materials may need to be moved distances for processing. Decentralization may be preferred for certain specialized research services such as imaging, quarantine, and proximity to research facilities, or for biosecurity reasons. Decentralization may be needed to accommodate large or complex equipment, such as magnetic resonance imaging, or to permit space sharing by users from multiple facilities or institutions. The opportunity for exposure to disease agents is much greater in these situations and special consideration should be given to biosecurity including transportation to and from the site, quarantine before or after utilizing the specialized research area, and environmental and equipment decontamination. In any event, the decisions leading to a selection of physically

centralized vs. decentralized animal facilities should be made early and carefully,

- Space for administrative and supervisory personnel, including space for training and education of staff
- Showers, sinks, lockers, toilets, and break areas for personnel
- Security features, such as card-key systems, electronic surveillance, and alarms
- Areas for maintenance and repair of specialized animal housing systems and equipment

Construction Guidelines

Corridors

Corridors should be wide enough to facilitate the movement of personnel and equipment. Corridors 6-8 ft wide can accommodate the needs of most facilities. Floor-wall junctions should be designed to facilitate cleaning. Protective rails or bumpers are recommended and, if provided, should be sealed or manufactured to prevent access to vermin. In corridors leading to dog or swine housing facilities, cage-washing facilities and other high-noise areas, double-door entry vestibules or other noise traps should be considered. Similar entries are advisable for areas leading to non-human primate housing as a means to reduce the potential for escape. Double-door entry vestibules also permit air-locks in these and other areas where directional airflow is critical for containment or protection. Wherever possible, water lines, drainpipes, reheat coils and valves, electric-service connections, and other utilities should be accessible via interstitial space or through access panels or chases in corridors outside the animal rooms. Fire alarms, fire extinguishers, and telephones should be recessed, installed high enough, or be protected by protective guards to prevent damage from the movement of large equipment.

Animal-Room Doors

For safety, doors should open into animal rooms; however, if it is necessary that they open toward a corridor, there should be recessed vestibules. Doors with viewing windows may be needed for safety and other reasons. However, the ability to cover viewing windows might be considered in situations where exposure to light or hallway activities would be undesirable, e.g., to avoid disturbing the circadian rhythm. Red-tinted windows, which do not transmit specific wavelengths of visible light between corridors and animal rooms, have proved useful for mouse and rat holding rooms as both species have a limited ability to detect light in the red portions of the spectra (Jacobs et al. 2001;

Drainage

Where floor drains are used, the floors should be sloped and drain traps kept filled with liquid. To minimize prol onged increases in humidity, drainage should allow rapid removal of water and drying of surfaces (Gorton and Besch 1974). Drainpipes should be at least 4 in(10.2 cm) in diameter. In some areas, such as dog kennels and agricultural-animal facilities, larger drainpipes (>_ 6 in) are recommended. A rim- and/or trap-fl ushing drain or an in-line comminutor may be useful for the disposal of solid waste. When drains are not in use for long periods, they should be capped and sealed to prevent backflow of sewer gases, vermin, and other contaminants; lockable drain covers might be advisable for this purpose in some circumstances.

Floor drains are not essential in all animal rooms, particularly those housing rodents. Floors in such rooms can be sanitized satisfactorily by wet vacuuming or mopping with appropriate cl eaning compounds or disinfectants. However, floor drains capped when not in use may provide flexibility for future housing of non-rodent species.

Walls and Ceilings

Walls and ceilings should be smooth, moisture-resistant, nonabsorbent, and resistant to damage from impact. They should be free of cracks, of unsealed utility penetrations, and of imperfect junc tions with doors, ceilings, floors, walls, and corners. Surface materials should becapable of withstanding cleaning with

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Heating, Ventilation and Air-Conditioning (HVAC)

A properly designed and functioning HV AC system is essential to provide environmental and space pressurization control. Temperature and humidity control minimizes variations due either to changing climatic conditions or to differences in the number and kind of animals and equipment in animal holding space, i.e., room or cubicle. Pressurization assists in controlling airborne contamination and odors by providing directional airflow between spaces. Areas for quarantine, housing and use of animals exposed to hazardous materials, and housing of non-human primates should be kept under relative negative pressure, whereas areas for surgery or clean-equipment storage should be kept under relative positive pressure with clean air. HVAC systems should be designed for reliability (including re

Temperature is best regulated by having thermostatic control for each holding space. Use of zonal control for multiple spaces can result in temperature variations between spaces in the zone because of differences in animal densities within the spaces and heat gain or loss in ventilation ducts and other surfaces within the zone. Individual space control is generally accomplished by providing each space with a dedicated reheat coil. Valves controlling reheat coils should fail closed and steam coils should be avoided or be equipped with a high temperature cut-off system to prevent space overheating and animal loss with valve failure. Humidification is generally controlled and supplemented on a system or zone basis. Control of humidification in individual holding spaces may be desirable for select species with reduced tolerance for low relative (e.g, non-human primates) or high humidity (e.g ., rabbits). Holding spaces should be designed to minimize drafts and temperature gradients.

Most HVAC systems are designed for average high and low temperatures and humidities experienced in a geographic area within ±5% variation (ASHRAE 2009). Moderate fluctuations in temperature and relative humidity outside suggested ranges are generally well tolerated by most species commonly used in research as long as they are brief and infrequent. Consideration should be given to measures that minimize fluctuations in temperature and relative humidity outside the recommended ranges due to extremes in the external ambient environment. Such measures can include partial redundancy, partial air recirculation, altered ventilation rates, or the use of auxiliary equipment. In the event of an HVAC system or component failure, systems should at the minimum supply facility needs at a reduced level, address the adverse effects of loss of temperature control, and, where necessary, maintain critical pressurization gradients. It is essential that life-threatening heat accumulation or loss be prevented during mechanical failure. Temporary needs for ventilation of sheltered or outdoor facilities can usually be met with auxiliary equipment.

Air handling system intake locations should avoid entrainment of fumes from vehicles, equipment, and system exhaust. While 100% outside air is typically provided, when recirculated air is used, its quality and quantity should be in accord with recommendations in Chapter 3. The type and efficiency of supply and exhaust air treatment should be matched to the quantity and types of contaminants and to the risks that they pose. Supply air is usually filtered with 85 – 95% dust spot efficient filters (ASHRAE 2008). In select instances, higher efficiency filters, for example, HEPA, may be beneficial for recirculated supply air, and air supplied to or exhausted from specialized areas such as surgical and containment facilities (Kowalsky et al. 2002).

Power and Lighting

The electrical system should be safe and provide appropriate lighting, a sufficient number of power outlets, and suitable amperage for specialized equipment. In the event of power failure, an alternative or emergency power supply should be available to maintain critical services (e.g, the HVAC system, power to ventilated caging systems (Huerkamp et al. 2003), or life support systems for aquatic species) or support functions (e.g., freezers and isolators) in animal rooms, operating suites, and other essential areas. Consideration should be given to outfitting moveable equipment for which an uninterrupted power is essential, for example, ventilated racks, with twist-lock plugs to prevent accidental removal from the power supply.

Light fixtures, timers, switches, and outlets should be properly sealed to prevent access to vermin. Recessed energy-efficient fluorescent lights are commonly used in animal facilities. Spectral quality of lights may be important for some species when maintained in the laboratory. In these cases full spectrum lamps may be appropriate. A time-controlled lighting system should be used to ensure a uniform diurnal lighting cycle. Override systems should be equipped with automatic timeout or a warning light to indicate the system is in override mode. System performance and override functions should be regularly evaluated to ensure proper cycling. Dual-level lighting may be considered when housing species that are sensitive to high light intensity, such as albino rodents. Low intensity lighting is provided during the light phase of the diurnal cycle, whereas higher intensity lighting is provided, as needed (e.g., when personnel require enhanced visibility). Light bulbs or fixtures should be equipped with protective covers to ensure the safety of the animals and personnel. Moisture-resistant switches and outlets and ground-fault interrupters should be used in areas with high water use, such as cage-washing areas and aquarium-maintenance areas.

Storage Areas

Adequate space should be provided for storage of equipment, supplies, food, bedding, and refuse. Corridors are not appropriate storage areas. Storage space can be decreased when delivery of materials and supplies is reliable and frequent; however, it should be ample enough to accommodate storage of essential commodities to ensure the animals' uninterrupted husbandry and care (e.g., should delivery be delayed). Bedding and food should be stored in a separate area free from vermin and protected from the risk of contamination

from toxic or hazardous substances. Areas used for food storage should not be subject to elevated temperatures or relative humidity for prolonged periods. Refuse-storage areas should be separated from other storage areas. Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal-tissue waste; this storage area should be kept below 7°C (44.6°F) to reduce putrefaction of wastes and animal carcasses. These areas should be constructed in a manner that facilitates cleaning.

Noise Control

Noise control is an important consideration in an animal facility and should be addressed during the planning stages of new facility design or renovation (see Chapter 3). Noise-producing support functions, such as cage-washing, are commonly separated from housing and experimental functions. Masonry walls, due to their density, generally have excellent sound attenuating properties; however, similar sound attenuation can be achieved using many different materials and partition designs. Generally, acoustic materials applied directly to the ceiling or as part of a suspended ceiling of an animal room present problems for sanitation and vermin control and are not recommended. However, sanitizable sound-attenuating materials bonded to walls or ceilings might be appropriate for noise control in some situations. Experience has shown that well-constructed corridor doors, sound-attenuating doors, or double-door entry vestibules can help to control the transmission of sound along corridors. An excellent resource on partition design for sound control may be found in the publication, Noise Control in Buildings: A Practical Guide for Architects and Engineers (Warnock and Quirt 1994).

Attention should be paid to attenuating noise generated by equipment (ASHRAE 2007b). Fire and environmental-monitoring alarm systems and public-address systems should be selected and positioned to minimize potential animal disturbance. The location of equipment capable of generating sound at ultrasonic frequencies is important as some species can hear such high frequencies. Selecting equipment for rodent facilities that does not generate noise in the ultrasonic range should be considered.

Vibration Control

Vibration may arise from mechanical equipment, electrical switches and other building components, or from remote sources with ground-borne transmission to

the inside. Regarding the latter, special consideration should be given to the building structure type especially if th e animal facility will be located over, under, or adjacent to subways, trains, or automobile and truck traffic. Like noise, different species can detect and be affected by vibrations of different frequencies and wavelengths. Therefore, attempts should be made to identify all vibration sources and isolate or dampen them with vibration suppression systems (ASHRAE 2007b).

Facilities for Sanitizing Materials

A dedicated, central area for sanitizing cages and ancillary equipment should be provided. Mechanical cage-washing equipment is generally needed and should be selected to match the types of caging and equipment used. Consideration should be given to such factors as:

- Location with respect to animal rooms and waste-disposal and storage areas
- Ease of access, including doors of sufficient width to facilitate movement of equipment
- Sufficient space for staging and maneuvering of equipment
- Provision for soiled waste disposal and prewashing activities
- Ease of cleaning and disinfection of the area
- Traffic flow that separates animals and equipment moving between clean and soiled areas
- Air pressurization between partitioned spaces to reduce the potential of cross contamination between soiled and clean equipment
- Insulation of Ef <0e
-

Environmental Monitoring

Monitoring of environmental conditions within animal holding spaces and other environmentally sensitive areas within the facility should be considered. Automated monitoring systems, which notify personnel of excursions in environmental conditions, including temperature and photoperiod, are advisable to prevent animal loss or physiologic changes which may occur as a result of system malfunction. If provided, system function and accuracy should be regularly verified.

Special Facilities

Surgery

The design of a surgical facility should accommodate the species to be operated on and the complexity of the procedures to be performed (Hessler 1991; see also Appendix A, Design and Construction of Animal Facilities). For most survival surgery performed on rodents and other small species such as aquatics and birds, an animal procedure laboratory, dedicated to surgery and related activities when used for this purpose and managed to minimize contamination from other activities conducted within the room at other times, is recommended. The surgical facility, including that used for rodents, by necessity becomes larger and more complex as the number and size of animals or the complexity of procedures increase. For instance, a larger facility may be required for procedures on agricultural species, to accommodate large surgical teams, imaging devices, robotic surgical systems, and/or laparoscopic equipment towers. Surgical facilities for agricultural species may additionally require floor drains, special restraint devices and hydraulic operating tables. The association of surgical facilities with diagnostic laboratories, imaging facilities, animal housing, staff offices, and so on should be considered in the overall context of the complexity of the surgical program. Surgical facilities should be sufficiently separate from other areas to minimize unnecessary traffic and decrease the potential for contamination (Humphreys 1993). Centralized surgical facilities are cost-effective in equipment, conservation of space and personnel resources, and reduced transit of animals. They also sustain enhanced personnel safety and enhanced professional oversight of both facilities and procedures.

For most surgical programs, functional components of aseptic surgery include surgical support, animal preparation, surgeon's scrub, operating room,

and postoperative recovery. The areas that support those functions should be designed to minimize traffic flow and separate the related, nonsurgical activities from the surgical procedure in the operating room. The separation is best achieved by physical barriers (AORN 1993) but might also be achieved by distance between areas or by the timing of appropriate cleaning and disinfection between activities. The number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of postoperative wound infection (Fitzgerald 1979). Traffic in the operating room itself can be reduced by the installation of an observation window, a communication system (such as an intercom system), and judicious location of doors.

Control of contamination and ease of cleaning should be key considerations in the design of a surgical facility. The interior surfaces should be constructed of materials that are monolithic and impervious to moisture. Ventilation systems supplying filtered air at positive pressure can reduce the risk of postoperative infection (Ayscue 1986; Bartley 1993; Schonholtz 1976). Careful location of air supply and exhaust ducts and appropriate room-ventilation rates are also recommended to minimize contamination (Ayliffe 1991; Bartley 1993; Holton and Ridgway 1993; Humphreys 1993). To facilitate cleaning, the operating rooms should have as little fixed equipment as possible (Schonholtz 1976; UFAW 1989). Other features of the operating room to consider include surgical lights to provide adequate illumination (Ayscue 1986), sufficient electric outlets for support equipment, gases to support anesthesia, surgical procedures, and gas-powered equipment, vacuum, and gas-scavenging capability.

The surgical-support area should be designed for washing and sterilizing instruments and for storing instruments and supplies. Autoclaves are commonly placed in this area. It is often desirable to have a large sink in the animal-preparation area to facilitate cleaning of the animal and the operative site. A dressing area should be provided for personnel to change into surgical attire; a multipurpose locker room can serve this function. There should be a scrub area for surgeons, equipped with foot, knee, or electric-eye surgical sinks (Knecht et al. 1981). To minimize the potential for contamination of the surgical site by aerosols generated during scrubbing, the scrub area should usually be outside the operating room and animal preparation area.

A postoperative-recovery area should provide the physical environment to support the needs of the animal during the period of anesthetic and immediate postsurgical recovery and should be so placed as to allow adequate observation of the animal during this period. The electric and mechanical requirements of monitoring and support equipment should be considered. The type of caging and support equipment will depend on the species and types of procedures but

Imaging

In vivo imaging offers non-invasive methodology for evaluating structure and function at the level of the whole animal, tissue, or cell, and allows for the sequential study of temporal events (Chatham and Blackband 2001; Cherry and Gambhir 2001). Imaging devices vary in the technology used to generate an image, body targets imaged, resolution, hazard exposure and requirements for use. Imaging devices may be self shielded and require no modifications of the surrounding structure to operate safely, or they may require concrete, solid core masonry, lead-, steel-, or copper-lined walls, or other construction features to operate safely or minimize interference with devices and activities in adjacent areas. As these devices are often expensive to acquire and maintain, and may require specialized support space and highly trained personnel to operate, shared animal imaging resources may be preferable. Consideration should be given to the location of the imaging resource. Whether located within the animal facility or in a separate location, cross contamination between groups of animals, different animal species, or between animals and humans (if the device is used for both animal and human subjects) is possible because these devices may be difficult to sanitize (Klaunberg and Davis 2008; Lipman 2006). If the imaging resource is located outside of the animal facility, appropriate transportation methods and routes should be developed to avoid inappropriate exposure of humans to animals in transit. If possible, animals should not be moved past offices, lunch rooms, or public areas where people are likely to be present.

As imaging may require the subject to be immobile, often for extended time periods during image acquisition, provision should be made for delivery of anesthetics and carrier gas and to scavenge waste anesthetic gas and provision for adequate animal monitoring (Balaban and Hampshire 2001). Remote storage of gas tanks is generally required in facilities using magnetic resonance (MR) scanners as the magnetic field requires ferrous materials to be maintained a safe distance away from the magnet. Site selection of MR scanners requires special attention because of their weight, the fringe field generated (especially from

emit ionizing or magnetic radiation. Imaging devices with difficult to sanitize components should be covered with a disposable or sanitizable material when not in use.

Whole Body Irradiation

Total body irradiation of small laboratory animals may be accomplished using devices that emit either gamma- or X-rays. Devices are usually self shielded and, as a result of the shielding material weight, may require special site considerations. Devices containing gamma-emitting sources are subject to regulations, which require adherence to specific security, monitoring, and personnel clearance requirements (NRC 2008). The site selected for irradiators should also take into consideration whether they are to be used for animals and biologics, as well as the source and microbial status of the animals to be irradiated. Locating them within the animal facility may require access be provided to personnel who would normally not require it or necessitate bringing animals into a facility where they are not normally housed.

Hazardous Agent Containment

The goal of containment is to "reduce or eliminate exposure of laboratory workers, other persons, and the outside environment to potential hazardous agents" (DHHS 2007). This is accomplished by employing appropriate practices and equipment, vaccinating personnel if a vaccine is available, and through the proper design and operation of the physical plant. Animal facilities used to study biological agents that are infectious to humans are categorized into different biosafety levels of escalating

consulted for specific design and engin

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APPENDIX A: Additional Selected References

USE OF LABORATORY ANIMALS
- x Alternatives
- x Ethics and Welfare
- x Experimental Design and Statistics
- x Research and Testing Methodology

PROGRAM MANAGEMENT
- x General References
- x Laws, Regulations and Policies
- x Education
- x Monitoring the Care and Use of Animals
- x Occupational Health and Safety

ENVIRONMENT, HOUSING and MANAGEMENT
- x General References
- x Environmental Enrichment
- x Genetics and Genetically Modified Animals
- x Species-Specific References – Environment, Housing and Management
    - o Agricultural Animals
    - o Amphibians, Reptiles and Fish
    - o Birds
    - o Cats and Dogs
    - o Exotic, Wild and Zoo Animals
    - o Nonhuman Primates
    - o Rodents and Rabbits
    - o Other Animals

VETERINARY CARE
- x Transportation
- x Anesthesia, Pain and Surgery
- x Disease Surveillance, Diagnosis and Treatment
- x Pathology, Clinical Pathology, and Parasitology
- x Species-Specific References – Veterinary Care
    - o Agricultural Animals
    - o Amphibians, Reptiles and Fish
    - o Birds
    - o Cats and Dogs

- Exotic, Wild and Zoo Animals
- Nonhuman Primates
- Rodents and Rabbits

DESIGN and CONSTRUCTION of ANIMAL FACILITIES

USE of LABORATORY ANIMALS

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APPENDIX B: 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. Government 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 sponsor 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 accordance 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 simulation, 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, investigators 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 sedation, 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 se vere 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.

VII. The living conditions of animals shou ld 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 in vestigators 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 comm ittee. 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 for Laboratory Animal Research, The National Academies.

APPENDIX D: About the Authors

Janet C. Garber (Chair), DVM, PhD, received her Doctor of Veterinary Medicine degree from Iowa State University and her PhD in patho-physiology from the University of Wisconsin. Her experiences have included infectious disease research at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID), primate medicine and research, GLP device and materials evaluation, and transplantation immunology. Her current interests are in the areas of laboratory animal facility management, infectious diseases, occupational health and safety and research program management. She most recently was Vice President, Safety Assessment, at Baxter Healthcare Corporation and is now a consultant with Garber Consulting, LLC in North Carolina. Dr. Garber is currently a member of the Council on Accreditation, AAALAC, International, and previously served as Chair of the Council. She served on the ILAR committee to revise the Guide for the Care and Use of Laboratory Animals and the committee on Occupational Health and Safety in the Care and Use of Research Animals.

R. Wayne Barbee, PhD, is Associate Professor and Associate Director of Research at the Department of Emergency Medicine, School of Medicine, Senior VCURES (Virginia Commonwealth University Reanimation Engineering Science Center) Fellow and chair of the IACUC at the Virginia Commonwealth University. Dr. Barbee holds a masters degree and doctorate in Physiology with three decades of research involving a wide variety of animals (bats, cats, crabs, dogs, rodents and swine) in a number of experimental settings. His research has focused on circulatory shock andresuscitation, acute and chronic rodent surgery, and analysis of rodent hemodynamics. He has been associated with IACUCs at small, medium and large institutions for over two decades, and is familiar with the oversight of animal care and use programs. He has served on multiple study sections for both the NIH and DOD. Dr. Barbee also served as an Oxford, UK 2006 fellow (recipient, VCU Harris-Manchester Award) where he examined policies, training, and security issues related to animal care and use within the UK.

Joseph T. Bielitzki, MS, DVM, is Research Manager, University of Central Florida. Dr. Bielitzki has worked with non human primates in the laboratory

environment for 20 years. Over this period he has worked with macaques (pig-tail, long tail, Japanese, rhesus, stump-tail), baboons (yellow, green and hybrids) squirrel monkeys, capuchin monkeys, mangabeys, gibbons, chimpanzees, orangutan, bonobos and gorillas. In the area of non human primates his area of expertise is in enteric diseases, nursery rearing, and colony management. He has also worked with mice and rats in a variety of international facilities. He was instrumental in the writing and acceptance of the NASA Bioethical Principles for the Use of Animals in Research (NPD 8910.1). He speaks frequently on IACUC function and the importance of ethics in the use of animals. His background includes experience in academia, industry and government in the role of attending veterinarian, program manager, and researcher.

Leigh Ann Clayton, DVM, is Director of Animal Health at the National Aquarium in Baltimore where she also chairs the Animal Welfare Committee. Dr. Leigh Clayton has worked in the zoo/aquarium field or the exotic pet medicine field exclusively since 2000. As she has worked with animals held in aquatics systems both in re-circulating fresh and salt water, she is experienced in managing disease and accomplishing preventive health programs for fishes, amphibians, and reptiles as well as birds and mammals. She is Diplomate of the American Board of Veterinary Practitioners (Avian). Dr. Clayton has routinely used her knowledge of nitrogen cycling and the basics of a variety of life support system designs to solve health issues in

Dennis F. Kohn , DVM, PhD, is Professor Emeritus of Clinical Comparative Pathology at Columbia University. He received his DVM from Ohio State University and a doctorate in Medical Microbiology from West Virginia University. He is board certified by th e American College of Laboratory Animal Medicine. He has directed laboratory animal resource/comparative medicine programs at West Virginia University Medical Center, University of Texas Medical School at Houston, and Health Sciences Division, Columbia University. His research interests have dealt primarily with the pathogenicity of Mycoplasma pulmonisin the respiratory tract of laboratory rats, and the experimental M. pulmonis. He is a past president of the American College of Laboratory Animal Medicine

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