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2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education

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Guidelines for use of wild mammal species in research are updated from Sikes et al. (2011). These guidelines cover current professional techniques and regulations involving the use of mammals in research and teaching; they also incorporate new resources, procedural summaries, and reporting requirements. Included are details on capturing, marking, housing, and humanely killing wild mammals. It is recommended that Institutional Animal Care and Use Committees (IACUCs), regulatory agencies, and investigators use these guidelines as a resource for protocols involving wild mammals, whether studied in the field or in captivity. These guidelines were prepared and approved by the American Society of Mammalogists (ASM), in consultation with professional veterinarians experienced in wildlife research and IACUCs, whose collective expertise provides a broad and comprehensive understanding of the biology of nondomesticated mammals. The current version of these guidelines and any subsequent modifications are available online on the Animal Care and Use Committee page of the ASM website (http://mammalogy.org/uploads/committee_files/CurrentGuidelines.pdf). Additional resources pertaining to the use of wild animals in research are available at: http://www.mammalsociety.org/committees/animal-care-and-use#tab3.

Resumen

Los lineamientos para el uso de especies de mamíferos de vida silvestre en la investigación con base en Sikes et al. (2011) se actualizaron. Dichos lineamientos cubren técnicas y regulaciones profesionales actuales que involucran el uso de mamíferos en la investigación y enseñanza; también incorporan recursos nuevos, resúmenes de procedimientos y requisitos para reportes. Se incluyen detalles acerca de captura, marcaje, manutención en cautiverio y eutanasia de mamíferos de vida silvestre. Se recomienda que los comités institucionales de uso y cuidado animal (cifras en inglés: IACUCs), las agencias reguladoras y los investigadores se adhieran a dichos lineamientos como fuente base de protocolos que involucren mamíferos de vida silvestre, ya sea investigaciones de campo o en cautiverio. Dichos lineamientos fueron preparados y aprobados por la ASM, en consulta con profesionales veterinarios experimentados en investigaciones de vida silvestre y IACUCS, de quienes cuya experiencia colectiva provee un entendimiento amplio y exhaustivo de la biología de mamíferos no-domesticados. La presente versión de los lineamientos y modificaciones posteriores están disponibles en línea en la página web de la ASM, bajo Cuidado Animal y Comité de Uso: (http://mammalogy.org/uploads/committee_files/CurrentGuidelines.pdf). Recursos adicionales relacionados con el uso de animales de vida silvestre para la investigación se encuentran disponibles en (http://www.mammalsociety.org/committees/animal-care-and-use#tab3).

Key words: animal capture, animal care, animal housing, animal marking, animal use ethics, federal regulation, Institutional Animal Care and Use Committee, trapping

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Overview

Development of the American Society of Mammalogist guidelines

Advances in the study of mammals—from exploring physiological functions to understanding evolutionary relationships and developing management strategies—are predicated on responsible use of animals in research. Founded in April 1919, the American Society of Mammalogists (ASM) is deeply concerned with the welfare of mammals and, in particular, the persistence of natural communities of organisms. In 1928, Joseph Grinnell—one of the founders of the ASM—instructed administrators of Yosemite National Park to maintain the Park as a natural mammalian community without unnecessary or destructive development. Grinnell (1928:76) described various management tactics for Park managers to follow; in particular, he advised that to address an unwanted increase in the bear population, Park officials needed to "devise [some] means whereby troublesome individual bears could be discouraged from raiding food-stores, without doing them serious bodily harm. But I recommend that exceeding care be taken in such procedure, not to rouse, unnecessarily, adverse public opinion, and not to drive away the bears altogether, for they constitute a particularly valuable element in the native animal life of the valley." Thus, Grinnell made informed management recommendations that advocated humane animal care and use with sensitivity toward public opinion. The same is true today as mammalogists work to understand and to protect the sentient organisms they study.

The ASM publication *Guidelines for the Use of Animals in Research* (ad hoc Committee for Animal Care Guidelines 1985) was the 1st effort to codify the expertise and philosophy of experienced, professional mammalogists regarding the use of mammals in research. This single-page statement listed broad considerations, such as concern for numbers of animals used, and highlighted laws that regulated use of animals and available standards. It stated that the investigator should exercise good judgment and prudence when using animals in research. More complete guidance was published by the ASM in 1987 with *Acceptable Field Methods in Mammalogy: Preliminary Guidelines Approved by the American Society of Mammalogists* (ad hoc Committee on Acceptable Field Methods in Mammalogy 1987, http://mammalogy.org/uploads/committee_files/ACUC1987.pdf). The 1987 ASM guidelines, along with those for birds, reptiles and amphibians, and fishes (produced by the other taxon-based professional organizations) were developed at the request of the United States National Science Foundation (NSF) specifically because guidance relevant to wild vertebrates was not available in the 1985 version of the National Research Council's (NRC) *Guide for the Care and Use of Laboratory Animals* (hereinafter *Guide*) (Orlans 1988). Conduct of animal care programs consistent with the *Guide* became required for activities funded by the United States Public Health Services (PHS) under the Health Research Extension Act of 1985, but neither the 1985 version nor subsequent editions of the *Guide* provided specific guidance for wild vertebrates. Even the most recent (2011:32) revision of the *Guide* states that "[t]he Guide does not purport to be a compendium of all information regarding field biology and methods used in wildlife investigations, but the basic principles of humane care and use apply to animals living under natural conditions" and encourages readers to consult qualified wildlife researchers and taxon specialists for additional information.

This and all recent editions of the ASM guidelines include information from the United States and other governments (e.g., Canadian Council on Animal Care 1993) as well as other professional sources where appropriate, such as the Society for the Study of Animal Behaviour (2006), the American Veterinary Medical Association (AVMA 2013a) *Guidelines for the Euthanasia of Animals*, and primary literature. The information contained herein is consistent with existing United States regulations regarding the care and use of vertebrate animals in research and education. Sikes et al. (2012), Sikes and Bryan (2015), and Wingfield (2015) argue that the ethical and appropriate oversight of animal activities requires guidance tailored to the species and conditions involved, and that the appropriate standards for wildlife research are the taxon-specific guidelines prepared by the various taxon-oriented professional societies. The NSF agrees with this conclusion, as evidenced by recent (2013) changes to its Grant Proposal Guide (http://www.nsf.gov/pubs/policydocs/pappguide/nsf15001/gpg_print.pdf) which states that:

> In the case of research involving the study of wildlife in the field or in the lab, for the provision in the PHS Assurance for Institutional Commitment (Section II) that requires the organization to establish and maintain a program for activities involving animals in accordance with the *Guide for the Care and Use of Laboratory Animals* (Guide), the organization has established and will maintain a program for activities involving animals according to the Guide. The organization will follow recommendations specified in the Guide for details involving laboratory animals, and taxon-specific guidelines approved by the American Society of Ichthyologists and Herpetologists, the American Society of Mammalogists, and the Ornithological Council, as is appropriate for the taxon to be studied.

The acceptance of these guidelines is further evidenced by the fact that AAALAC International, an independent organization committed to peer-reviewed assessment and accreditation of animal care programs, adopted the previous (2011) version of the ASM guidelines as a reference document for use by accredited institutions.

The revised guidelines herein are intended to provide investigators and those charged with evaluating animal use in research (e.g., Institutional Animal Care and Use Committees [IACUCs], reviewers and editors of research manuscripts, management agency personnel, graduate committees, and the public) with up-to-date guidance on ethical care and use issues as well as health, safety, and environmental concerns particular to nondomesticated mammals. These guidelines do not provide details concerning how IACUCs are to be constituted or operate, and thus are not intended to replace the *Guide* on these matters. We underscore the fact that fundamental and profound differences exist between activities involving wild mammals, particularly in their natural habitat, and activities with traditional research and domesticated animals in laboratory settings. These differences are detailed elsewhere (Sikes et al. 2012; Sikes and Paul 2013; Sikes and Bryan 2015) and include consideration of appropriate methods for euthanasia or humane killing, the potential for impacts on natural populations, differences in biology of the animals themselves, and differences in handling and husbandry requirements.

Role of the veterinarian

Except in those projects in which investigators are themselves veterinarians or in which veterinarians accompany investigators during all field activities with wild animals, the role of veterinarians in wildlife research will differ substantially from their roles in laboratory research. Because the veterinarian seldom, if ever, accompanies investigators during their field activities, unless the animals can be transported to the veterinarian, the veterinarian could provide medical advice for a specific animal based only upon observations by field personnel. Such actions are not consistent with Section 5 of the model Veterinary Practices Act endorsed by the AVMA (2013b). Section 5 states that:

1. No person may practice veterinary medicine in the State except within the context of a veterinarian–client–patient relationship [VCPR].
2. A veterinarian–client–patient relationship cannot be established solely by telephonic or other electronic means.

Commentary by the AVMA for this section emphasizes "…that because a VCPR requires the veterinarian to examine the patient, it cannot be adequately established by telephonic or other electronic means (i.e., via telemedicine) alone." Thus, for most wildlife research, the veterinarian serves as a valued adviser and consultant during the planning stages or in response to challenges encountered during field activities that stimulate procedural refinements prior to additional activities. It must be stressed that the selection, dosages, and administration routes of pharmaceuticals are best accomplished in consultation with veterinarians having appropriate wildlife experience and expertise, and that this consultation must be prior to use of these compounds. Because the role of the veterinarian in field activities is not necessarily consistent with the expectations of the *Guide* (NRC 2011) and because the *Guide* provides little or no information relevant to issues of primary concern in wildlife investigations (Sikes et al. 2012; Paul and Sikes 2013; Sikes and Paul 2013; Paul et al. 2015; Sikes and Bryan 2015), IACUCs should consider whether these fundamental differences in and of themselves constitute justification for an IACUC-approved departure from the *Guide.* PHS policy (see section below on "Regulation of animal activities") specifies that "…the research project is consistent with the Guide unless acceptable justification for a departure is presented" (National Institutes of Health/Office of Laboratory Animal Welfare [NIH/OLAW] 2015). The Model Wildlife Protocol endorsed by the ASM in 2016 (available at http://mammalogy.org/uploads/committee_files/ModelWildlifeProtocol2016.docx) provides 1 mechanism to facilitate presentation and approval of departures from the *Guide* for wildlife activities.

These guidelines are designed to highlight the concerns one must address within the existing regulatory framework when conducting research and educational activities involving wild mammals. This document is not intended as an exhaustive list of acceptable procedures or issues in all circumstances or with all species. Our goal is to focus attention on the types of issues that should be considered when working with wild mammals and provide resources for addressing those concerns. We emphasize that these guidelines are not intended to constrain ingenuity in meeting research demands, but rather to bring relevant safety, regulatory, and ethical concerns regarding the use of wild mammals to the attention of investigators and oversight bodies. It is the responsibility of the principal investigator (PI) of a project to justify deviations from federal regulations or other applicable guidance during submission of a protocol to the cognizant IACUC.

Tailoring oversight to wildlife research

Oversight of animal use in research and education has almost universally developed from a biomedical perspective in which research was focused on human health and research questions were addressed using domesticated animal models in laboratory environments. Once regulations, guidelines, and expectations were established for oversight of these activities, they were often extended to apply to the study of wild animals in their native environments. This progression is not surprising given the importance and visibility of biomedical research and the number of animals used in such studies but, beyond the basic goals of ensuring humane treatment and minimizing pain or distress, guidelines and regulations designed for biomedical research have little relevance to research on wild animals, particularly in their natural environment. Indeed, using guidance not designed for wild animals will most likely result in ineffective or even inhumane treatment of these taxa. This disconnect occurs because of fundamental differences in the goals of biomedical and wildlife research, the role of animals in these respective research endeavors, and in particular because of fundamental differences between the domesticated animals most often used in biomedical work and the wild subjects used in field research. Consequently, unless guidance documents and expectations are modified extensively before they are applied to wild animals, their utility for biologically appropriate oversight of wild mammals is at best limited and at worst harmful to the animals they are intended to protect.

Sikes and Paul (2013) emphasized many of the obvious differences between biomedical and wildlife research. These include the fact that, rather than using animals as surrogates for humans in studies designed to benefit human health, studies of wildlife are often designed to benefit the study subjects. Individual animals are not so much "used" as they are the objects of study in projects designed to understand various aspects of their biology, including their behavior, ecology, and evolutionary diversification. A 2nd significant difference is that studies of wild animals, particularly those in their natural environments, have the potential for impacts beyond the study subjects because these individuals exist as part of a population and a community. A 3rd difference is that, whereas most biomedical research is conducted with only a few species of purpose-bred mammals, there are more than 5,400 species of wild mammals that are potential subjects of field studies. Closely related species can differ with regard to habitat, handling needs, or husbandry requirements and even wildlife veterinarians are unlikely to have experience with more than a small number of these taxa.

Underlying the marked differences in focus and scope between biomedical and wildlife research are profound differences in virtually every aspect of the biology of domesticated animals versus wild taxa. The act of domestication produces animals that interact very differently with humans compared to wild strains. For example, rather than fearing and fleeing from humans, domesticated animals look to us for food, shelter, and often companionship. As Darwin (1868) noted, captive propagation of wild taxa selects for behavioral and morphological traits favored by humans and relaxes selection for traits important for survival in the wild. His observations and our understanding of the genetic basis of these differences led to development of the domesticated strains of animals and plants that form the basis of modern agriculture. Thus, although pet dogs belong to the same species as the wolf, *Canis lupus* (Wilson and Reeder 2005), they clearly differ dramatically from wolves with regard to their interactions with humans. The changes in behavior, morphology, and genome diversity that are part of the domestication process may become evident after remarkably few generations. Lacy et al. (2013) and Willoughby et al. (2015) demonstrated behavioral and genetic changes among captive populations of wild rodents subjected to different breeding regimes after only 6–20 generations, even when breeding protocols were designed to limit evolution in captivity. Importantly, Lacy et al. (2013) also found significant correlations among behavioral and life history parameters such as reproductive success. Other studies of the domestication process have demonstrated associated changes in interactions with humans (Hare et al. 2005), hormonal profiles, and stress responses. Even seemingly subtle selection for "tameability" can have profound influences (Trut et al. 2009), resulting in domestic ferrets that are more "dog-like" than "wild ferret-like" with respect to their social-affiliative behavior and responsiveness to humans (Hernádi et al. 2012), and captive bred-foxes that display dog-like characteristics (e.g., eager to attract human interaction) after only a few generations (Belyaev and Trut 1964). If such changes are evident after only a few generations or decades of captive breeding, how much stronger are the changes associated with extended selection, such as that experienced by traditional laboratory study subjects? Or, perhaps more relevant to work with wild animals, why would one expect wildlife species to respond to stimuli in the same manner as domesticated strains?

Institutions conducting wildlife research should ensure that the IACUC review process includes personnel with appropriate expertise. In many instances, this need is met by having one or more field researchers on the oversight committee. At institutions where field research accounts for a small proportion of protocols, outside consultation can be particularly useful. Even if the oversight body includes wildlife expertise, the diversity of species and research foci encompassed by such research will likely generate occasions when outside consultation is warranted. In these cases, the Animal Care and Use Committee (ACUC) of the ASM can assist in identifying individuals with relevant expertise.

Regulation of animal activities

The use of vertebrate animals, particularly mammals, in research and education is regulated in many countries. In the United States, mammals other than rats of the genus *Rattus* and mice of the genus *Mus* that have been bred for research are regulated by the United States Department of Agriculture Animal and Plant Health Inspection Services (USDA/APHIS). The USDA recognizes exemptions, however, for those studies that meet the definition of a field study as defined by the Animal Welfare Act (AWA, see below). In addition to regulation by the USDA, activities funded by PHS must also comply with relevant provisions of the PHS policies on humane care and use of laboratory animals (NIH/OLAW 2015). Institutions receiving PHS funding involving animal use or assured by the NIH/OLAW must maintain a PHS Assurance with the NIH/OLAW stating that, at a minimum, all PHS-funded activities will be conducted in a manner consistent with the *Guide* and the AVMA *Guidelines for Euthanasia of Animals*. If they choose, institutions may voluntarily extend their PHS Assurance so that it applies to all animal activities rather than only those funded by the PHS. The negative consequences of these latter cases is that all activities, regardless of the source of funding or intellectual focus, must be conducted in a manner consistent with the *Guide* and the AVMA guidelines for euthanasia, both of which are often poorly suited for work with wild animals in the field. It is critical for oversight personnel and investigators to be familiar with the wording of their PHS Assurance to ensure continued compliance with the regulations and policies covering animal use.

Mammalogists conducting virtually any type of research involving wild mammals at an institution subject to federal oversight will be required to consult with their IACUCs to determine if their planned activities are subject to IACUC review and approval; in other words, whether the proposed activities meet the regulatory definition of a "field study" (see below). Investigators are also responsible for procuring all necessary permits from local and federal agencies before conducting any procedure involving live animals. These permit requirements apply whether the PI is working within the United States or elsewhere.

The AWA authorizes the USDA/APHIS to regulate vertebrates used (or intended for use) in research, testing, experimentation, exhibition, or as pets, regardless of whether animals are maintained in a laboratory, wild enclosure, or farm setting. However, the USDA/APHIS does not regulate animals used for food or fiber (or for improving quality of food or fiber), or for improvement of animal nutrition, breeding, management, or production efficiency. As noted above, there also is an exemption for activities that meet the regulatory definition of a field study. It is critical to note that the determination as to whether the proposed activities will meet the definition of a field study should be made by the IACUC rather than the researcher.

The United States Fish and Wildlife Service (USFWS) defines a mammal, in the context of "take," "possession," "transportation," etc., as any member of the class Mammalia, including any part, product, egg, or offspring, or the dead body or parts thereof (excluding fossils), whether or not included in a manufactured product or in a processed food product (50 CFR 10.11). In the context of regulatory requirements, "permit" is any document designated as a "permit," "license," "certificate," or any other document issued by the USFWS to authorize, limit, or describe an activity and signed by an authorized official of the USFWS. Although the focus of this section is on federal and state regulations in the United States, investigators, regardless of their nationality or location of their research, should understand that local, state–provincial, federal–national, or international laws or regulations likely exist that pertain to scientific collecting, transport, possession, sale, purchase, barter, exportation and importation of specimens or parts thereof, or other activities involving native or nonnative species of mammals. Therefore, each investigator must have knowledge of, and comply with, all relevant laws and regulations pertaining to field collection of mammals and the places where fieldwork is being conducted. Federal regulations exist in the United States that pertain to collection, import, export, and transport of scientific specimens of mammals (Endangered Species Act, CITES, and the Lacey Act). These regulations have provisions that allow for civil or criminal penalties and ineligibility for future permits. Researchers living in or conducting research in the United States must obtain permits issued by federal agencies to: import or export specimens of non-endangered species through a nondesignated port of entry; import or export endangered wildlife through any port; import injurious wildlife (those species listed under 50 CFR Part 16); import, export, transport interstate commercially, take, harass or possess endangered species listed under 50 CFR Part 17, or parts thereof, for research or propagation; take, harass, possess, or transport marine mammals (50 CFR Part 18); import or transfer etiological agents or vectors of human disease or living nonhuman primates; collect scientific specimens on national wildlife refuges; import ruminants and swine, including parts, products, and by-products; and import organisms or vectors, tissue cultures, cell lines, blood, and sera. Additional restrictions on importations of mammals, including fluids or tissues, may be imposed by the Centers for Disease Control and Prevention (CDC) or USDA/APHIS, so it is imperative that investigators consult current lists before attempting importation.

Importantly, investigators and IACUCs should be aware that the term "scientific collecting" is typically used by state game agencies in a very different context than by many researchers, who consider "scientific collecting" as removal of individuals from the population. In almost all cases, a state permit will be required for any work with wild vertebrates, whether the animals are removed from the population or not.

When moving specimens of mammals into or out of the United States, researchers are required to file USFWS form 3–177—currently the electronic declaration form (e-Dec) available at www.fws.gov is preferred and may be mandatory at the regional office or port of entry—and any necessary permits required by CITES if species are listed in CITES Appendices I–III, the Endangered Species Act, Lacey Act, or the Marine Mammal Protection Act. Note, however, that CITES-listed materials can move without permit between 2 registered institutions provided that they were first accessioned by the sending institution. Investigators should expect similar regulations in other countries and ensure compliance with all applicable regulations dealing with species of special concern. Investigators also must ascertain whether additional permits are needed when they review state–provincial and federal–national laws and regulations that relate to their planned field investigations. Further, investigators must be familiar with current lists of mammalian species deemed threatened or endangered by appropriate state–provincial or federal–national governments and comply with all laws and regulations pertaining to capture of these and other categories of protected mammals. A list of threatened or endangered species and subspecies under the United States Endangered Species Act is available from the Office of Endangered Species, Fish and Wildlife Service, United States Department of the Interior, Washington, D.C. 20240 (http://www.fws.gov/endangered/). Regulations relevant to these taxa are published in the Code of Federal Regulations, 50 CFR Part 17; amendments to regulations under Title 50 also are published in the Federal Register. Investigators can search for CITES-listed species at http://checklist.cites.org/#/en.

Most states and provinces require scientific collecting permits, and investigators must comply with these requirements and other regulations imposed by agencies in the states or provinces in which they conduct fieldwork in addition to all national and international regulations. States, national and state parks, or other organizations might have additional regulations regarding scientific uses of wildlife on lands under their jurisdiction. All public lands require permits except those lands controlled by the United States Bureau of Land Management, which still requires permission from a District Ranger. Compliance with these regulations and permit requirements is essential. With regard to privately owned land, investigators should obtain permission from the owner, operator, or manager before commencing fieldwork.

Many institutions, and state, provincial, and federal governments, have regulations or recommendations concerning handling and sampling of rodents or other mammals that might be carriers of zoonotic diseases. Investigators must ensure their own safety and that of employees or students by recognizing the disease-carrying potential of the mammals they study. Additionally, as part of their charge of reducing institutional liability, most IACUCs have adopted some form of occupational health screening for all persons involved with animal research. Health screening can range from completion of a simple check-off form inquiring about allergies or other health conditions of investigators, students, and employees, to completion of a much more detailed medical examination and health history.

The variety and number of permits required for work with wild animals is extensive and has no parallel within the biomedical community. Paul and Sikes (2013) review general permitting requirements, but no single document will cover all possible situations. The PI and the oversight body must be well versed regarding required permits. This raises the question of who is responsible for obtaining permits and keeping associated records. Where permits are required, work cannot be conducted legally until the necessary documents have been secured. Although some institutions require permits before an IACUC protocol can be approved or even reviewed, this approach typically increases the workload of both the PI and the IACUC because permit renewal dates rarely coincide with protocol dates. Further, permits are issued to the individual PI rather than to the institution. Thus, a prudent approach is to approve acceptable protocols but specify to the investigator in writing that work cannot commence until all required permits are in place.

Beyond meeting legal requirements, the issuance of permits can provide assurance to the IACUC that any impact of proposed activities on local populations is minimal or is scientifically justified. Whereas IACUCs seldom have the expertise or data necessary to make such judgments, permitting agencies are usually charged with safeguarding these natural resources and have personnel with the appropriate expertise to make informed decisions as part of permit application review and approval. Thus, attention to this major difference between biomedical and wildlife research can be accomplished without additional burden on the IACUC.

Categorization of animal use for USDA compliance

> In 2010, the ASM, in conjunction with the Ornithological Council, reviewed guidance documents available to institutions and developed a joint position regarding categorization of animal use for USDA compliance. This text was first disseminated as a position statement and addendum to the 2007 version of these Guidelines in 2010. The portions of this joint position relevant to work with mammals are included herein.

Two aspects of the terminology used to classify animal usage can cause confusion for activities involving wild animals: classification of the capture of free-ranging animals with regard to the USDA reporting categories for pain and distress and identification of field studies for the purpose of determining when IACUC protocol review, IACUC site inspection, and inclusion on USDA annual reports are required.

USDA reports: pain and distress categories.—The AWA [7 USC 2143(b) (3) (A)] and the implementing regulation (9 CFR 2.36) require that research facilities in the United States subject to these laws file an annual report with the USDA Animal Care Regional Office documenting their research and teaching activities that use live animals covered by the Act and its implementing regulations. A component of this report is classification of animal usage into categories intended to describe the absence, presence, or extent of pain or distress and the use or non-use of drugs to alleviate these conditions.

USDA descriptions for animal reporting categories as defined on the reporting form (APHIS Form 7023) are:

C—Animals upon which teaching, research, experiments or tests were conducted involving no pain, distress, or use of pain-relieving drugs.

D—Animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.

E—Animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, or experiments, surgery, or tests. (An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be attached to the report).

Guidance for classifying animal procedures is provided in Policy 11 of the *Animal Care Policy Manual* (USDA 2015a) published by the Animal Care program of the USDA/APHIS. However, this guidance and the examples therein pertain primarily to procedures conducted in a laboratory setting, usually in the context of biomedical research. Classification becomes especially problematic when institutions are faced with applying regulations intended primarily for laboratory settings and domesticated animals to the very different context of free-ranging wild animals. The 2 critical terms in these descriptions are "pain" and "distress." According to the *Animal Care Policy Manual* Policy 11 (USDA 2015a), a painful procedure is defined as "any procedure that would reasonably be expected to cause more than slight or momentary pain or distress in a human being to which that procedure is applied, that is, pain in excess of that caused by injections or other minor procedures." Distress is not defined in current policy except by example: "Food or water deprivation beyond that necessary for normal presurgical preparation, noxious electrical shock that is not immediately escapable, paralysis or immobility in a conscious animal." Appropriate classification of activities is left to the IACUC.

USDA classifications as applied to animal capture and noninvasive field procedures.—Mammal capture devices are designed either to hold the animal unharmed (live traps) or to kill the animal outright upon capture. Barring mechanical malfunctions, and with appropriate placement and trap monitoring, animals captured in live traps or nets are simply held without injury until removal. Appropriate training is essential for setting capture devices and for removing animals from those devices. Pain or distress as described in the USDA/APHIS *Animal Care Policy Manual* (USDA 2015a) is unlikely to result from the simple capture of free-ranging mammals in most live traps or capture devices. Thus, Category C is appropriate if these activities occur as part of a project that includes USDA-covered species.

Most tissue sampling and marking techniques conducted in the field are also consistent with USDA Pain or Distress Category C provided that these procedures are not more invasive than peripheral blood sampling. Support for this classification is provided in the *Guidelines for Preparing USDA Annual Reports and Assigning USDA Pain and Distress Categories*. This document is distributed by the NIH Office of Animal Care and Use, which is the oversight office for intramural research. This guidance expressly states that Category C includes most blood collection procedures and tissue collection procedures that involve no or only momentary or slight pain. Based on these same NIH Guidelines, USDA Category C is appropriate also in instances where procedures requiring peripheral tissue sampling or tagging and subsequent release of free-ranging animals necessitate chemical immobilization to conduct the procedures, provided that immobilization is performed only to facilitate the procedure and protect the animal and the researcher from injury rather than to alleviate pain or distress induced by the procedure.

Capture of free-ranging mammals in properly functioning kill traps typically meets the USDA Category C. The same is true of those animals captured in live traps, even if they are subsequently euthanized as part of the research study. The method and quality of death is the criterion for classification. Any method recognized as an approved method of euthanasia by the AVMA is consistent with Category C. Because neither the AWA nor its implementing regulations reference the AVMA's *Guidelines for the Euthanasia of Animals*, for those methods not approved by the AVMA for euthanasia, the IACUC is the deciding body as to whether the method of death meets the regulatory definition of euthanasia as defined by the AWA. Additional information on kill traps and methods of death pertinent to USDA reporting is provided in the section of the AVMA guidelines entitled *Euthanasia and Humane Killing*.

Euthanasia and humane killing.—Even for trapping methods that ordinarily are consistent with Category C, in the event of a problem in the field not associated with experimental manipulations that results in pain or suffering and necessitates pain alleviation, post hoc classification as Category D would be appropriate for that particular animal. For example, if either a live or a kill trap malfunctions, leaving an animal in pain or distress and the animal is either treated with appropriate pharmaceuticals or euthanized in a timely fashion, then Category D is the most appropriate category. In the case of euthanasia, this procedure (rather than treatment) is used to alleviate pain or distress. It is important to note that these cases can occasionally occur with wild animals despite the trapping techniques ordinarily being consistent with Category C. The foregoing is not meant to imply that a higher level of pain or distress should be expected in proposed activities, but simply that PIs and IACUCs should be cognizant of the need to reclassify animals on a post hoc basis should actual events warrant.

Field studies.—Considerable misunderstanding has surrounded the application of the AWA to field research. Regulations promulgated by the USDA under the AWA exempt field studies from IACUC review [9 CFR 2.31(d)] in cases in which field study is defined as "any study conducted on free-living wild animals in their natural habitat that does not harm or materially alter the behavior of the animal under study" (9 CFR 1.1). None of these terms is defined in the regulation or in guidance documents issued by USDA/APHIS, which leaves the IACUC responsible for their interpretation. The same regulation exempts from the inspection requirement of 9 CFR 2.31 "animal areas containing free-living wild animals in their natural habitat." How the definition of field study corresponds to the USDA reporting categories is unclear. In most instances, protocols involving only procedures classified as Category C are consistent with the regulatory definition of a field study, but whether these should be included on annual reports has been uncertain. The USDA provides relevant guidance in their 2015 revision of the *Animal Welfare Inspection Guide* (USDA 2015b) (the guidance document issued to Veterinary Medical Officers charged with inspecting registered institutions) by stating that "animals euthanized, killed, or trapped, and collected, such as for study or museum samples, from their natural habitat via humane euthanasia" should not be included on annual reports to the USDA. Unfortunately, the wording of this document, although not regulatory in the strict sense, could be construed to mean that animals captured, whether euthanized in the field or not, fall under the field studies exclusion and are therefore exempt from IACUC review. *The ASM states emphatically that any activity involving the capture of wild mammals should be subject to review by an IACUC to determine whether the activity meets the regulatory definition of a field study and, if not found to be exempt, to provide appropriate oversight for use of wild mammals in research.*

With regard to IACUC protocol review, the PHS Policy on Humane Care and Use of Laboratory Animals makes no distinction between laboratory and field studies. Guidance from the NIH/OLAW (FAQ A6) states, "[i]f the activities are PHS-supported and involve vertebrate animals, then the IACUC is responsible for oversight in accordance with PHS policy. IACUCs must know where field studies will be located, what procedures will be involved, and be sufficiently familiar with the nature of the habitat to assess the potential impact on the animal subjects. Studies with the potential to impact the health or safety of personnel or the animal's environment may need IACUC oversight, even if described as purely observational or behavioral. When capture, handling, confinement, transportation, anesthesia, euthanasia, or invasive procedures are involved, the IACUC must ensure that proposed studies are in accord with the *Guide*."

Protocols and protocol forms for wildlife studies

Even with standards and guidelines appropriate for wild animals, activities must be described in sufficient detail to permit critical review by oversight bodies. This can best be accomplished by use of protocol forms designed to address concerns relevant to the circumstances and specifics of field work with wild animals. For example, protocols for work with wildlife must describe how animals are captured for study, discuss the potential for capture and the subsequent disposition of nontarget species, and address the uncertainty that often exists regarding the number and sex of animals that might be taken in any trapping or capture event. Other differences include the fact that some wild mammals can be handled only after chemical immobilization and that veterinary care is seldom possible in the field for animals injured during capture or manipulation. These are among the multiple wildlife-specific concerns that must be considered by IACUCs for responsible oversight of activities involving wild animals, but, as noted above, protocol forms designed for biomedical research seldom address these issues. A wildlife-specific protocol form developed collaboratively by the ASM ACUC and the Ornithological Council was featured in a webinar sponsored by NIH/OLAW. A revision of this form is freely available on the ASM website (http://mammalogy.org/uploads/committee_files/ModelWildlifeProtocol2016.docx).

Numbers and species (including endangered taxa)

The *Guide* (NRC 2011) requires that protocols include details concerning the numbers of animals to be used. These details are relevant during IACUC discussions of the "3 Rs" outlined in the *Guide* (Reduction, Refinement, and Replacement—NRC 2011) and direct IACUC members to determine if the fewest animals necessary to accomplish the stated research goals with statistical rigor are being used. Further, oversight agencies such as the USDA focus on appropriate association of animal numbers with procedures or research aims. Frequently, field researchers do not know how many individuals will be needed or sampled; this is particularly true for faunal surveys or other exploratory work common in mammalogy. While animal captures obviously vary with density and environmental conditions, statements in protocols such as, "it is unknown how many animals we will capture" are generally not well received by the IACUC. Although inclusion of statements like, "20 individuals from each species for each locality," is one way to provide numerical limits, a stronger approach is to consider more acceptable generalized statements such as: "In this survey we expect to collect different species of *Oryzomys* and will sample an estimated 25 localities. We will not exceed 20 specimens per species of *Oryzomys* per locality. It is anticipated that the total number of specimens collected during this study will not exceed 500 individuals per year."

The numbers of animals required in field studies will vary greatly depending on study design, species' life history characteristics, and the research questions posed. Behavioral studies might involve capture of only a few animals in which the focus is a specific activity, or capture of an entire population in which all individuals must be marked. In the latter case, the investigator can provide a statement that "all animals in the population will be captured, marked, and released, and it is estimated that this will not exceed 200 individuals per year." Genetic, taxonomic, ecological, and other studies typically require a minimum sample size for statistical analyses. Too few animals might not allow the investigator to address research questions with sufficient scientific rigor and, subsequently, will result in a waste of animals if the results do not provide a robust test of the relevant hypothesis. In these situations, a power analysis might be performed to estimate the number of animals required to obtain statistical significance for a given variance and a minimum expected difference between samples.

IACUCs also are charged with approving the particular species of mammals involved in a project. Biomedical protocols typically use strains of animals that have been domesticated or, if not entirely domesticated, have been maintained in captivity for sufficient time to habituate to interactions with humans. The vast majority of biomedical research is accomplished with only 2 species of mammals—laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*). In addition to the wild counterparts of these 2 domesticated rodents, there exist more than 5,400 species of mammals that field investigators might study scientifically (Wilson and Reeder 2005). For such research, the IACUC will require a protocol in which the investigator provides an adequate description of the study methods, experimental design, and expected results, as well as a summary of related, previous studies. The IACUC might query investigators about planned methods of euthanasia even if the proposed study involves only observation or capture and release of animals. "We are not killing any animals" is a frequent, but unsatisfactory, response to an IACUC because it indicates that the investigator has not considered methods of treatment or euthanasia in the event of an unexpected injury. Importantly, it is usually appropriate for field protocols to list a variety of euthanasia methods to suit the species and conditions encountered.

Because most capture methods for free-ranging mammals are not species specific, it is important for PIs to include, and IACUCs to accept, considerable latitude in the list of target and nontarget species that might be encountered. Species lists that are too narrow can place a PI out of compliance if unexpected captures are made. These unexpected captures often provide particularly important distributional records for species not known to occur in the study area and might even represent previously unidentified species. In some instances, it might be appropriate for species lists to include statements like "all small mammals occurring in the region."

The investigator should provide assurance to the IACUC that all permits necessary for the proposed use of wild mammals have been issued or requested; copies of permits must be available if requested by the IACUC. Although most IACUCs usually do not focus on scientific merit, Principle II of the United States Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training states that "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." IACUCs that deal primarily with biomedical protocols sometimes have difficulty evaluating the merit of field study protocols. Peer review of scientific proposals, approval of project permits by state or federal agencies, and support from academic departmental chairs can provide assurance to the IACUC that the project is sound and the use of animals justified. Although rare, the IACUC might seek an outside assessment or request evidence of peer review to evaluate scientific merit.

In concluding this overview, we emphasize that this document is not intended to be an exhaustive catalog of procedures and requirements; we encourage investigators to consult with their IACUCs during protocol preparation to insure that all oversight concerns are addressed. We also emphasize that final approval of any protocol rests with the IACUC.

General Guidelines

Fieldwork with mammals

Fieldwork is arguably the most challenging form of research for IACUCs and others who typically evaluate use of animals in laboratory-based studies. Fieldwork in mammalogy involves designing and conducting research to address scientific questions while working with mammals in their natural habitats. This process might involve capturing an animal to obtain reproductive and other data and subsequently releasing it to obtain additional information on population dynamics, movements, and habitat relationships. In some cases, the investigator might bring a wild-caught animal into an animal facility for further study. These wild animals might require sedation or anesthesia to facilitate handling, but use of suitable pharmaceuticals is often under federal and state control, so investigators should consult with federal and state drug enforcement agencies and obtain appropriate licenses during the design stage of a study if their use is anticipated. Some drugs (e.g., narcotics) require strict inventory logs and storage in doubly locked areas to prevent unauthorized access, requirements that can be challenging to meet in the context of field work.

Training

Training, especially in the rapidly changing area of research compliance, is extremely important for all individuals handling vertebrate animals. Online training is often required by the IACUC as part of protocol review and approval. Frequently, though, this training is general and oriented toward laboratory environments. The Collaborative Institutional Training Initiative (CITIprogram.org) used by many institutions includes an informative wildlife-specific course in addition to general training in animal use. NIH/OLAW sponsors freely available webinars on animal research topics and at least 1 of these has focused on oversight of wildlife research. These resources are available through the NIH/OLAW education portal at http://grants.nih.gov/grants/olaw/educational_resources.htm. Other wildlife-focused webinars or podcasts have been made available by Public Responsibility in Medicine and Research (PRIM&R) and AAALAC International.

Still other training opportunities are organized by local IACUCs and tailored for their institutions. Procedural training can be provided by veterinarians or technicians experienced in research-oriented procedures. Specialized training can provide the investigator with experience in acceptable methods of restraining, marking, monitoring vital signs, administering injections, taking blood samples, and assessing stress or signs of pain or distress. The investigator is responsible for knowing how to perform procedures in the appropriate setting (field, laboratory, etc.) for which their protocol was approved.

The IACUCs are urged to recognize the investigator as a collaborator who is sometimes well versed in the biology of the taxa studied. Wild vertebrates, particularly mammals, are vastly different in physiology and behavior from the usually highly inbred organisms used in biomedical research (Sikes and Bryan 2015). Wild vertebrates do not inhabit antiseptic, stress-free environments with ad libitum food. With these differences in mind, investigators should serve as resources to their IACUCs and institutional veterinarians and all of these individuals should work together to insure the appropriate use of animals in research.

Oversight of field studies

Although field studies as defined by the AWA (those conducted on free-living animals in their natural habitat that do not involve invasive procedures, harm, or materially alter behavior of an animal) are exempt from IACUC review [9 CFR 2.31 (d)], many institutions interpret the AWA in a broader sense and require IACUC review of all laboratory, classroom, and fieldwork involving vertebrates. For those studies that require review and approval by the IACUC, many references for common field procedures for mammals are available (e.g., Kunz and Parsons 2009; Martin et al. 2011; Ryan 2011); these sources should be consulted by the investigator during protocol preparation and referenced as needed. Further, some institutions may have standard procedure descriptions available for use by all investigators preparing protocols.

Trapping Techniques

Considerations for capturing mammals

Although capture of wild mammals is a common element of field studies, physical capture is not always necessary and investigators can sometimes use other procedures to monitor free-living animals. These include obtaining acoustic signatures (ultrasonic detectors), visual data (still or video cameras), or nondestructive tissue samples (sticky hair snares to remove hair) from free-ranging mammals without substantially altering the animals' behavior. In general, these techniques are not of concern to IACUCs unless they involve capture (e.g., capturing bats in mist nets to identify species before animals are released and their vocalizations recorded as they retreat), harassment, or visiting nest sites during critical times in a species' life cycle (e.g., bat nursery roost or seal pup nursery). Concerns may also exist that the investigator's presence can alter animal behavior or place animals or personnel at risk for exposure to pathogens or other harm (Klailova et al. 2010). Individual IACUCs and institutional policies vary widely regarding exemptions for observational studies and thus investigators should become familiar with their institutional policies before beginning any work with mammals.

Common reasons to capture mammals include livetrapping to tag (with radiotransmitters, necklaces, ear tags, or passive integrated transponder [PIT] tags), mark (number, band, hair color, freeze brand, ear tag, or toe clip), or collect tissue. Regardless of approach, the potential for pain, distress, or suffering must be considered. A variety of methods and devices are available for trapping wild mammals. Techniques for capture of specific species of mammals are detailed in summary sources (Wilson et al. 1996), internet sites devoted to specific subsets of mammals (e.g., http://www.fishwildlife.org/index.

php?section=furbearer_management&activator=27 for furbearers), and especially in articles from the primary literature. Possibilities include live traps (e.g., Sherman, box, mist nets, snares, Tomahawk, Havahart, pitfall, nest box, and artificial burrow), kill traps (e.g., Museum Special, Conibear, and pitfalls), and other specialty traps for particular species or purposes. Shooting with a firearm might be necessary to obtain specimens of some species. We address each of these general categories of traps in greater detail below.

Live capture

Investigators conducting research requiring live capture of mammals assume the responsibility for using humane methods that respect target and nontarget species. Methods for live capture include those designed for small mammals (Sherman, Tomahawk, and Havahart traps, pitfalls, artificial burrows, and nest boxes), medium-sized to large mammals (Tomahawk, Havahart, and foot-hold traps, snares, corrals, cannon nets, culvert traps, and darting), bats (mist nets, harp traps, and bags), and fossorial mammals (Baker and Williams 1972; Hart 1973). Methods of live capture should not injure or cause excessive stress to the animal. Adequate measures should be taken to ensure that captured animals are protected from predation and temperature extremes and have food and water available, as needed, while restrained in traps. For permanent trapping grids or webs, the investigator might provide shelters over traps to protect captured animals from environmental conditions (Kaufman and Kaufman 1989; Parmenter et al. 2003).

Use of steel foot-hold traps for live capture of animals must be employed with caution due to the potential for injury or capture of nontarget species (Kuehn et al. 1986). For some taxa, foot-hold traps, including leg snares, might present the only available or the most effective means of capture (Schmintz 2005; see also http://www.fishwildlife.org/index.php?section=furbearer_management&activator=27 for specific techniques and trap recommendations). When their use is approved, investigators have an ethical obligation to use steel foot-hold traps of a sufficient size and strength to hold the animal firmly. Foot-hold traps, other than snares, with rubber-padded or offset jaws should be used to minimize potential damage to bone and soft tissue and associated discomfort for those cases in which animals will remain alive in these traps. Snares or spring foot-hold traps must be checked at suitable frequencies. These observations should be at least daily, but might be more frequent depending upon target species, the potential for capture of nontarget species, and environmental conditions. Captured animals must be assessed carefully for injury and euthanized when necessary. Nontarget species, if uninjured, should be released immediately although their release, as with target species, might require chemical immobilization to facilitate handling and prevent injury to the animal or researcher.

The number of traps set at a particular time and location should not exceed the ability of the investigator(s) to monitor them at reasonable intervals. Because frequent checking of traps is the most effective means of minimizing mortality or injury to animals in live traps, the investigator should consider staking or visibly flagging a trap line (or otherwise devising some effective system) to ensure that all traps are recovered and removed reliably and efficiently. Regular monitoring ensures that target animals remain in good condition while in traps and allows prompt release of nontarget species with no ill effects due to capture. Monitoring intervals vary and are dependent on target species, type of trap, weather, season, terrain, and number and experience of investigators. Generally, live traps for nocturnal species are set before dusk and checked shortly after sunrise. Traps are then retrieved or closed during the day, where warranted, to prevent capture of diurnal, nontarget taxa. Live traps for very small mammals, particularly shrews, should be checked more frequently (e.g., every 1.5 h—Hawes 1977) to minimize mortality due to the higher metabolism of these taxa. Similarly, taxa of larger size but with particularly high metabolic rates (e.g., *Mustela*) might also require shorter intervals between observations. Live traps for diurnal species should be set in shaded areas or under trap shelters (Kaufman and Kaufman 1989) and checked every few hours in warm weather. Traps should then be retrieved or closed at dusk, when appropriate, to prevent unintended capture of nocturnal taxa.

Thermoregulatory demands, especially for small mammals, can induce stress even if the duration of captivity is short. Thermoregulatory stress can be minimized by providing food sources (Do et al. 2013) and nesting material in the live trap. Because most live traps for small mammals are constructed of metal and conduct heat readily, it might be necessary to insulate traps to minimize hypo- or hyperthermia in captive animals. Insulation can be accomplished by using items such as cotton or synthetic fiber batting, leaves, or twigs to provide dead air space between the animal and conducting surface and to provide escape from the temperature extremes. Critical temperature tolerance limits vary with species and environmental conditions. Investigators must be responsive to changing weather conditions and modify trapping procedures as necessary to minimize thermal stress to trapped animals.

If disturbance of live traps (removal of captured individual, trap damage) by larger carnivores, birds, or other animals is problematic, trap enclosures (Getz and Batzli 1974; Layne 1987) or other methods to secure traps might be required. Pitfall traps can be fitted with raised covers to minimize capture of nontarget species, to provide cover from rain and sun, and to prevent predation by larger animals. Pitfall traps used for live capture may require small holes at the bottom of the containers to allow drainage in rainy weather, or enhancements such as small sections of polyvinyl chloride pipe to provide shelter from other captured animals.

Captured small- and medium-sized mammals should be handled by methods that control body movements without restricting breathing. Covering an animal's eyes may reduce struggle to escape. Restraint by a mesh or cloth bag allows the investigator to mark, measure, or otherwise sample an individual through mesh or the partially opened end of the bag (e.g., *Cynomys gunnisoni*—Davidson et al. 1999). Some small mammals can be transferred directly from a trap to a heavy-duty plastic bag for handling or to a heavy cloth bag or cage for transport. The design of some traps (e.g., box-type traps such as Sherman or Tomahawk live traps) also allows them to be used as a temporary cage for easy and safe transport.

Traps used for live capture of larger mammals include box traps, clover traps, and culvert traps. Some large mammals (e.g., ungulates and kangaroos) can be herded along fences into corrals or captured with cannon nets or drop nets projected from helicopters using net guns. These methods require extensive training of personnel and immediate attention to the animals to prevent injury while minimizing stress and distress in particularly sensitive species (e.g., *Antilocapra americana*). For such large-scale capture efforts, it may be useful to contract a veterinarian to assist with any injured or stressed animals. Depending on the nature of the activity, individuals captured using these techniques might need to be sedated, otherwise restrained, or have their eyes covered until the investigator's work is completed (Braun 2005).

Large mammals also can be captured by delivering a sedative into the hip or shoulder musculature using a dart gun. Chemical immobilization, whether for capture or sedation, requires training by a wildlife veterinarian and thorough knowledge of proper dosage, antidote, and sedative effect. In many instances, having a reversal agent on hand is advisable. The location selected for dart placement can vary seasonally and with animal condition. Individuals familiar with the species should be consulted prior to employing this procedure. An excellent reference for chemical immobilization of mammals is Kreeger and Arnemo (2012). Location of the animal within the habitat should be considered in light of time necessary for sedation and recovery to avoid injury or mortality during recovery; sedated mammals must be monitored closely during procedures. Baits laced with tranquilizers have been described (Braun 2005), but these should be used with caution to prevent sedating nontarget species. Finally, local, state, or federal regulations might restrict use of certain drugs (e.g., narcotics), which may impact their use in field settings.

In procedures with domesticated species, sedated animals are always monitored until they recover. Similar monitoring of wild mammals might not be possible or appropriate. The presence of humans is a stressor for most wild animals and they will often attempt to flee before they are fully recovered. Depending upon the species and habitat, it may be advisable to place sedated animals in a safe location and then retreat, rather than remaining in close proximity to individuals as they recover (Sikes and Bryan 2015). In no instance should sedated animals be left in close proximity to water or exposed to potential predators or aggressive conspecifics while under the influence of immobilizing drugs.

Net-gunning from helicopters is a method frequently used for capture of ungulates and at times for capture of other types of mammals. Depending upon the species and activities involved, animals captured in nets may be sedated or they may be handled without sedation.

Bats can be captured effectively and humanely with mist nets, harp traps, bag traps, or by hand (Kunz and Parsons 2009). Mist nets should not be left unattended for > 15 min when bats are active. Captured bats should be removed from nets immediately to minimize injury, drowning, strangulation, or stress. Proper and thorough training of assistants beforehand is essential so that bats are removed without injury to delicate wing bones and patagia. If a bat is badly tangled, it can be removed by cutting strands of the net; although costly, mist nets can be repaired or replaced. Nets should not be operated in high winds because these conditions can place undue stress on bats and further entangle them in nets. Mist nets should generally be operated only at night or during crepuscular periods and closed during the daytime to prevent capture of nontarget taxa (e.g., birds). The number of mist nets operated simultaneously should not exceed the ability of investigators to check and clear nets of bats. For example, mist nets should not be used where large numbers of bats might be captured simultaneously (e.g., at cave entrances) because numbers can quickly overwhelm the ability of investigators to remove individuals efficiently and safely. In these situations, harp traps or sweep nets are preferable (Wilson et al. 1996). Although harp traps do not require constant attention, they should be checked regularly, especially when a large number of captures is expected within a short period of time. Investigators using harp traps should guard against predators entering the trap bag or biting captured bats, predation of 1 bat species on another, rabies transfer, or suffocation due to large numbers of bats caught in a short time (Kunz and Parsons 2009).

Roosting bats can sometimes be captured by hand. The use of suitable gloves should be considered as they provide protection from bites while allowing the investigator to feel the body and movements of the bat, thereby preventing injury to both the investigator and the study subject. Long, padded tissue forceps can be used to extract bats from crevices, but extreme care should be taken to avoid injury to delicate wing bones and membranes (Kunz and Parsons 2009). Investigators should consider how the time of year when bats are studied might impact their survival. For example, large or repeated disturbance of maternity colonies can cause mortality of offspring and colony abandonment (O'Shea and Bogan 2003). Repeated arousal of hibernating bats can lead to mortality because of depletion of critical fat stores (Thomas 1995). Individuals working with bats should also follow the most current precautions to prevent spread of the fungus causing White Nose Syndrome (https://www.whitenosesyndrome.org/resource/universal-precautions-management-bat-white-nose-syndrome-wns). Reeder et al. (2015) is a particularly useful reference regarding the potential for spreading diseases while conducting research on at-risk populations.

Capture myopathy and injuries

Wild mammals typically will experience some level of stress during capture regardless of the capture technique employed. For those methods that involve pursuit of the target animal, personnel must be especially cognizant of the potential for exertional rhabdomyolysis (ER or capture myopathy). The potential for ER can be minimized by limiting pursuit times, restricting captures to periods when environmental conditions minimize the chance that an animal will overheat, carefully selecting drugs used for immobilization, and ensuring the expertise of the capture team. When pursuit is used for capture, the research team should monitor animal temperature and have materials on hand to quickly reduce core temperature if necessary.

Wild mammals usually struggle or attempt to flee when approached by humans and can injure themselves as a result. Excessive rates of injury warrant review of the animal handling procedures. Whereas minor injuries can often be treated by the field personnel, severely injured animals typically are humanely killed.

Kill traps and shooting

Some studies require that free-living mammals intentionally be killed (e.g., collection of specimens for museum collections). In all cases, methods must provide an efficient and quick death that minimizes pain. In some cases, individuals may be livetrapped and then humanely killed. Where live traps are used, animals should be humanely killed as quickly as possible (see methods below) without damaging materials needed for research. The AVMA notes that "kill traps do not consistently meet the POE's [Panel of Euthanasia] criteria for euthanasia, and may be best characterized as humane killing under some circumstances. At the same time, it is recognized they can be practical and effective for scientific animal collection or pest control when used in a manner that ensures selectivity, a swift kill, and no damage to body parts needed for field research" (AVMA 2013a:40).

Traps suitable for kill trapping include snap traps (e.g., Victor and Museum Special) for rat- and mouse-sized mammals, kill traps (e.g., Macabee) designed for subterranean species, harpoon traps for moles, snares for carnivores and furbearers, and Conibear or similar body-grip traps for medium-sized mammals. Trapping techniques that use drowning as a means of killing have been described as inhumane or unethical by some (e.g., Powell and Proulx 2003; AVMA 2013a). However, submersion trapping systems can be effective and appropriate for furbearers found in or near waterways. Such systems rely on equipment (e.g., steel foot-hold traps with 1-way cable slides and locks) or techniques that cause the furbearer, upon capture, to quickly and irreversibly submerge until death (http://www.fishwildlife.org/index.php?section=furbearer_management&activator=27).

Pitfall kill traps can provide the best trapping option for some small mammals (e.g., rodents and shrews), many of which are much more effectively captured with pitfalls than by other means. These traps are particularly efficient where trapping must be continuous or cannot be accomplished using live traps or snap traps that need resetting between captures. Pitfalls used with drowning fluids add a measure of preservation that can be useful for scientific collections and detailed study of internal organs. Additional circumstances in which pitfalls are effective are outlined in Beacham and Krebs (1980) and Garsd and Howard (1981).

Ethical use of pitfall kill traps requires the minimization of pain or distress. The pitfall designed by Howard and Brock (1961) does this by using 70% ethanol (or similar alcohol) as the main ingredient of the drowning fluid. Evaporation of alcohol is retarded by a thin layer of light mineral oil and hexane (2:1) added to the solution. Small mammals that fall into the trap (and hence the drowning fluid) lose buoyancy almost immediately due to the surfactant action of hexane and mineral oil and thus submergence and death occur rapidly. Alcohol then infuses the body and acts as a preservative. As long as the solution is deeper than the head-body length of the target animals, individuals cannot struggle or escape by standing on the bottom of the trap. Use of formalin or ethylene glycol in pit traps, however, is not approved by the ASM. Pitfalls used as kill traps should have covers or other means of excluding nontarget species. If the traps will not be operational for extended periods, they should be constructed such that the kill jar and its fluid can be removed to prevent unwanted captures.

Drowning in conjunction with other trapping methods

Several of the methods described above include a primary capture device (trap) that induces death by drowning. While the AVMA (2013a:102) does not consider drowning an acceptable form of euthanasia, it acknowledges that "the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia (i.e., distinguishes between euthanasia and methods that are more accurately characterized as humane killing)" (AVMA 2013a, Section S7.6). Further, in a 2011 "Literature Review" regarding thoracic compression, the AVMA states that "when scientifically justified, the IACUC has and should employ the authority to approve killing techniques not listed as recognized forms of euthanasia" (AVMA 2011). The ASM urges the use of other trapping and killing techniques whenever possible. However, wild mammals held in traps are unable to avoid predators or to forage, so in situations where other trapping options are not feasible and capture limits an animal's ability to maintain itself (e.g., through continued foraging or defense against predators), the ASM considers death by drowning to be more humane than alternatives that cause prolonged distress or discomfort.

In all cases, investigators should strive to use the trap that will inflict the least trauma and result in a clean, effective kill. Most traps should be checked at least once a day; in the event that a captured animal is still alive, it should be immediately dispatched according to methods approved by the IACUC. The AVMA offers these recommendations regarding kill traps: "Mechanical kill traps are used for the collection and killing of small, free-ranging mammals for commercial purposes (fur, skin, or meat), scientific purposes, to stop property damage, and to protect human safety. Their use remains controversial and kill traps do not always render a rapid or stress-free death consistent with the criteria established for euthanasia by the POE. For this reason, use of live traps followed by other methods of euthanasia is preferred. There are a few situations when that is not possible (e.g., pest control) or when it may actually be more stressful for the animals or dangerous for humans to use live traps" (AVMA 2013a:40).

An effective way (sometimes the only way) to collect certain species of mammals is by use of a firearm. Investigators using this method must be experienced in safe handling of firearms and must adhere to laws and regulations related to their possession and use. The firearm and ammunition should be appropriate for the species of interest so that the animal is killed swiftly without excessive damage to the body (including the skull). A .17 or .22-caliber rifle chambered for an appropriate cartridge (.17 HMR, .22 Long Rifle, .22 Short, .223, etc.) and loaded with bullets or shotguns loaded with appropriate shot sizes are suitable for medium-sized mammals. Generally, small mammals (chipmunk size or smaller) can be taken with .22-caliber rifle or handgun loaded with #11 or #12 (dust) shot, whereas animals the size of rabbits can be taken with shotguns loaded with #4–#6 shot. Large mammals should be taken with a high-velocity rifle of a suitable caliber, where legal, or shotguns using appropriate ammunition (e.g., rifled slugs or larger shot). After the animal has been shot, it should be retrieved and processed promptly for the purpose for which it was collected. Additional information regarding killing via gunshot is provided in the section below on "Euthanasia and Humane Killing."

Marine mammals

All marine mammals in United States territorial waters are covered by the Marine Mammal Protection Act of 1972. Many species also are covered by the Endangered Species Act of 1973. The latest versions of both Acts can be found at the United States Marine Mammal Commission web site (http://www.mmc.gov/about-the-commission/our-mission/endangered-species-act-and-other-legislation-and-agreements/). These Acts prohibit any form of "take," including terminal capture, live capture, or tagging of marine mammals without appropriate federal permits. Exceptions are made for certain aboriginal or traditional harvests of marine mammals and for commercial fisheries that might incidentally take marine mammals as part of normal fishing operations. Permit application forms and instructions can be found on the National Marine Fisheries Service web site (http://www.nmfs.noaa.gov/prot_res/overview/permits.html) and at the USFWS web site (http://permits.fws.gov/).

Methods of live capture for marine mammals include nets (ranging from purse seines to small, handheld hoop nets) and mechanical clamps with lines that are placed over an animal's peduncle while it rides the bow pressure wave of a vessel. Many live capture techniques for smaller cetaceans are reviewed by Asper (1975). Some dolphins or small whales (e.g., *Phocoena*, *Delphinapterus*) can be captured by hand in shallow water (Walker 1975). Although polar bears (*Ursus maritimus*) and some species of pinnipeds (e.g., northern elephant seal, *Mirounga angustirostris*) can be captured using remotely injected chemicals, chemical immobilization of marine mammals is risky due to the possibility of losing animals by drowning or overdose (Dierauf and Gulland 2001). The Society for Marine Mammalogy has developed detailed guidelines for the treatment of marine mammals in field research, which the ASM endorses. The most current version of these guidelines is available at. www.marinemammalscience.org/about-us/ethics/marine-mammal-treatment-guidelines. Euthanasia for marine mammals was reviewed by Greer et al. (2001).

Holding of marine mammals in captivity is regulated by the Marine Mammal Protection Act, the Endangered Species Act, and the AWA; the latter administered by USDA/APHIS. The AWA regulations include species-specific criteria for pool and pen sizes, construction methods, water quality, food storage and handling, and routine healthcare. The most current AWA regulations can be found on the USDA/APHIS web site.

Use of domestic dogs in research

Highly trained domestic dogs are increasingly being used in research to locate study animals or specimens. Because of their olfactory abilities, these service animals can make many types of collecting efforts more efficient and can allow types of field work that are otherwise not feasible. Dogs used in this manner should be considered part of the research team rather than study animals and should be accorded care as such. In cases in which dogs are owned by the institution, their veterinary care is equivalent to occupational health considerations for personnel.

When dogs are used to locate live animals, the potential stress or harm they might cause to target individuals should be considered. For animals that alert handlers to the presence of target animals, the stress caused by the dog may be equivalent to that caused by native predators or humans. When dogs are used to find samples (e.g., scats), there is less potential impact on target animals as they may no longer be in the vicinity, but nontarget animals may still be affected. Additionally, investigators and oversight personnel should take precautions to minimize the potential for exchange of pathogens between native populations and service dogs. As the use of dogs in field studies becomes more commonplace, more specific guidelines are sure to follow.

Tissue Sampling and Identification

Tissue sampling

The collection of small amounts of tissue from small wild mammals is routine and often required for studies involving DNA, proteins (e.g., hemoglobins, albumins, enzymes), or physiological assays (e.g., hormonal levels, antibody titers). Tissue samples can be obtained in conjunction with some marking procedures (e.g., toe clips, patagial, or ear biopsies). These techniques are treated below under "External Marks." Even when these techniques are not required for identification, small external tissue samples are frequently taken from unsedated animals with little difficulty and are generally consistent with USDA Pain or Distress Category C (see above). When blood is required, many procedures do not require anesthesia and can be conducted in the field by appropriately trained personnel. After tissue collection and prior to release, individuals should be observed to ensure that no trauma or adverse reaction has occurred as a consequence of capture, handling, or tissue or blood collection.

Multiple factors must be considered when determining the most appropriate method for obtaining blood samples. Various morphological attributes of a species (e.g., size of the orbit, absence of tail or external pinnae, presence of cheek pouches) can limit potential sites of blood or tissue collection. The size of the animal also might restrict collection sites and limit the quantity of blood (≤ 1.5% of body mass) that can be removed. The training and experience of the individuals performing the procedure is important as unskilled personnel can cause significant trauma with some techniques. The procedures for blood collection and the qualifications of study personnel must be reviewed by the IACUC. More specialized procedures for blood collection from small mammals are as follows:

Obtaining blood from the facial vein.—This technique, which has been used on laboratory mice for many years, allows collection of 4–10 drops of blood with minimal discomfort to the animal (see USDA news release at www.ars.usda.gov/is/pr/2005/050921.htm). The procedure is described (including text, photos, and video) at http://www.medipoint.com/html/rat_video_demonstraton.html (*note:* this citation is not intended to be an endorsement of this commercial product by the ASM).

Obtaining blood from the caudal vein.—Extracting blood from the caudal vein is a relatively simple procedure that involves the use of a needle (more difficult in small rodents) or lancet to puncture the caudal vein. Alternatively, excising the distal 1–2 mm of the tail can yield a small amount of blood; the tail tip can be used for DNA extraction.

Obtaining blood from the retro–orbital sinus.—Retro–orbital bleeding should be used when less invasive blood collection methods have been determined to be unsuitable. To minimize the chances of damage to the eye, this technique should be performed by trained and experienced individuals. The use of very short-acting anesthesia (e.g., isoflurane or sevoflurane) in a plastic bag will immobilize rodents in 15–20 s, thereby making the procedure safer for the rodent and the handler.

External marks

Individual identification of mammals is necessary for many types of studies, both in the laboratory and the field. Identification marks can be natural (stripe pattern, color, or mane patterns) or can be applied by the investigator. Of primary concern is the distance from which the animal must be identified and the time period over which the mark must endure. It is worth noting that marking techniques that do not permit identification at a distance or are not permanent might require repeat trapping and handling of animals for reapplication of marks. On large bodied species, natural variation in fur or whisker patterns (West and Packer 2002) or marks left by previously sustained injuries (e.g., scars on wings, ears, or flukes) often suffice for permanent identification at a distance.

When appropriate naturally occurring marks are not available, external dyes, freeze brands, or paint marks might provide the visibility and degree of longevity required. Identification marks can be made with nontoxic hair dyes or paint. Care should be taken to ensure that substances used for external marks are nontoxic and do not alter the behavior of animals or subject them to increased predation (e.g., by adversely affecting the animal's natural camouflage or increasing its visual or olfactory detectability). Dye marks on juveniles or subadults are of more limited duration because of rapid molting and thus may require more frequent renewal. Freeze branding is an effective means of marking bats and other species, and marks might last several years (Sherwin et al. 2002). Tattooing and ear punches provide a more persistent means of identification but require handling of animals for individual recognition and may not be permanent in some species.

Metallic or plastic tags and bands or collars may be suitable for identification at appreciable distances on large terrestrial species. Tags typically are applied to the ears of terrestrial mammals and to flippers of seals and sea lions. Use of individually numbered tags on small mammals necessitates handling the animal each time an individual is to be identified. Although they frequently are used with a high degree of success, ear tags might inhibit grooming of ears and promote infection by parasites in some rodents (Ostfeld et al. 1996), although potential for infection likely varies with species and environment. Further, unless carefully sized, tags might snag, either during grooming or when animals move through vegetation, resulting in loss of the tag (Wood and Slade 1990). Eartags might also affect typical movements of the pinnae (e.g., Preyer reflex) in free-ranging animals. Many of the problems associated with ear tags are reduced in laboratory settings where such tags may be especially useful for long-term identification. Ear tags may not be an option for species with greatly reduced pinnae (e.g., many shrews). Wing bands for bats should be applied so that they slide freely along the forelimb, which may necessitate cutting a slit in the wing membrane in some cases. Another external marking option for bats is an appropriately sized bead-chain necklace (Barclay and Bell 1988).

Individuals in some taxa can be identified by unique patterns of ear punches (where a small amount of tissue is removed from external pinnae using a surgical hole punch). Ear punches, however, may become unidentifiable over time in free-ranging individuals because of healing, subsequent injuries sustained in the field, or obscuration by hair. In some species, toe clips may be used. Toe clipping involves removal of 1 or more digits or terminal phalanges (generally only 1 per foot) and provides a permanent identifying mark. Both ear punches and toe clipping typically require recapture as neither is generally suitable for identification at a distance. Because both of these methods involve removal of a small amount of tissue, they might be especially appropriate in studies where tissue samples also are required.

According to the *Guide* (NRC 2011:75), toe clipping "should be used only when no other individual identification method is feasible." Justification for toe clipping of wild mammals should include consideration of the natural history of the species, how the feet are used in the animal's environment, and the size of the toe. Digits generally should not be removed from the forefeet of subterranean or fossorial taxa because they are used for digging, nor should primary digits be removed from arboreal or scansorial taxa because they are used for climbing. Toe clipping in species with fleshy digits should be avoided. Toe clipping might be especially suitable for permanent identification in small species (e.g., *Chaetodipus*, *Perognathus*, *Peromyscus*, *Reithrodontomys*, and *Sorex*) and in neonates of larger taxa. Toe clipping and ear punches should not be used for marking bats, which instead can be effectively marked with wing punches or freeze brands. Toe clips and ear punches should be performed with sharp, sterilized instruments. Anesthetics and analgesics generally are not recommended as prolonged restraint of small mammals to administer these substances and consumption of the analgesic substances (e.g., creams) via licking likely cause more stress and harm than conducting the procedure without their use. A recent study in laboratory mice showed that toe clipping as late as 17 days of age caused no microscopic reaction as late as 10 weeks of age and that the use of vapocoolant anesthetics caused more harmful effects than toe removal without anesthetics (Paluch et al. 2014; but see Braden et al. 2015 regarding the use of vapocoolant anesthetics in general). The adverse effects of additional handling for application of anesthetics or analgesics with wild taxa are likely to be substantially greater than was observed by Paluch et al. (2014) with laboratory mice.

Radiotransmitters provide a mechanism to monitor movements and survival of animals and, therefore, also serve to identify individuals. Transmitters can be attached externally with surgical or skin glue or a collar, or implanted into the body cavity. External attachment often can be accomplished in the field (Rothmeyer et al. 2002; Munro et al. 2006), whereas more invasive implantation might require transport to a laboratory where sterile conditions can be maintained during surgery. Investigators using collars should take into account the possibility that an animal will grow or undergo seasonal changes in neck circumference (e.g., male cervids); the devices used should be designed to accommodate such changes (Strathearn et al. 1984). If external transmitters are attached using glue, members of some species will groom each other excessively to remove adhesive from their fur (Wilkinson and Bradbury 1988). Surgical implantation and explantation and more invasive procedures, which should be performed by a veterinarian or individuals who have received specialized training, usually require a suitable recovery period before the animal can be released. Before using radiotransmitters, an investigator should consider the weight of the transmitter relative to the body mass of the target species or individual. Generally, the transmitter should represent < 5–10% of the individual's body weight (Wilson et al. 1996). As an alternative to radiotransmitters, light-emitting diodes or similar markers might be fastened externally to some species for nocturnal observation.

Internal tags

PIT tags are electronic devices encased in glass or resin capsules. They do not emit constant signals but can be interpreted with a remote reader in much the same way that barcodes are scanned. PIT tags are becoming progressively smaller but can still be too large for some species or individuals; their use in very small individuals should be approached cautiously. Tags are injected subcutaneously using a modified large-bore hypodermic syringe and are suitable for many field and laboratory identification needs. Tags should be massaged away from the point of insertion subdermally to prevent loss and allow healing at the point of entry. Currently available PIT tag readers must be held in reasonably close proximity to the tag (~ 10 cm) to function and thus their use with large, aggressive taxa (e.g., *Procyon* and *Lynx*) will usually require anesthesia both for application and for subsequent reading of the tag to prevent injury to the animal and investigators. Because of the resultant stress for both subject and investigator, other methods of tagging large mammals, such as using radiotransmitters or naturally occurring markings, may be preferable. Ingestion of colored plastic particles or radioactive isotopes (such as P^{32}) in bait can be used to mark feces for studies of movements of individuals or groups of individuals, although this method is of limited use for uniquely marking a large number of individuals.

Chemical immobilization for application of marks and tissue sampling

Depending on the biology of the target species, its body size, and the goals of the study, captured animals might require chemical immobilization for safe and effective handling. Investigators should consider that stress and restraint associated with immobilization might be greater than applying or reading a particular mark or taking noninvasive tissue samples without immobilization. Whether immobilization is required must be considered on a case-by-case basis. If pain is slight or momentary and the animal can be handled without unreasonable risk of self-injury or injury to personnel, anesthesia might be contraindicated so that the animal can be released immediately. Procedures that can cause more than momentary or slight pain or distress or species in which the struggling animal is a danger to itself or personnel should be performed with appropriate sedation, analgesia, or anesthesia (Interagency Research Animal Committee [IRAC] 1985:V). In these instances, field-portable anesthetic machines allow use of isoflurane and similar inhalants to provide a reliable anesthetic and rapid recovery after the animal is no longer exposed to the gas. Use of anesthesia for blood sampling will depend on collection procedure and species-specific considerations. Some anesthetics (e.g., ketamine) depress blood pressure and make blood collection lengthier and potentially dangerous. Anesthesia also might alter the blood component (e.g., cortisols) under investigation. Use of anesthesia should be weighed against risk of mortality because some species are very sensitive to anesthetics (e.g., felids—Bush 1995; Kreeger and Arnemo 2012). Selection of anesthetics and analgesics should be conducted in consultation with a specialist—such as a wildlife veterinarian—knowledgeable regarding the use of these substances in species of mammals other than standard laboratory or companion taxa. The investigator should conduct a literature review to search for alternatives as well as for anesthetics and analgesics used in closely related species (Kreeger and Arnemo 2012). Physiological measurements required for experimental purposes also can affect the choice of anesthesia. Sedatives, anxiolytics, and neuromuscular blocking agents are not analgesic or anesthetic and hence do not relieve pain; these substances must be used in combination with a suitable anesthetic or analgesic (NRC 2011).

Maintenance of Wild-Caught Mammals in Captivity

Procurement and holding conditions

When wild-caught individuals are to be held for an extended period or transported, the investigator must provide a secure means of containment, sufficient food and moisture, and appropriate ambient conditions and must consider the potential for transfer of parasites or pathogens, and the safety of the investigator(s) (see section on "Human Safety"). Cages must be constructed to minimize possibility of injury or escape, to provide adequate ventilation, to allow for protection from wastes; cages generally should be of sufficient size to permit the captive individual to make appropriate postural adjustments (NRC 2011, 9 CFR 3.125). Some types of live traps (e.g., Sherman traps and Tomahawk traps) can be used as holding or transport cages for short periods of time for appropriate species.

Captive mammals held for an extended period (> 12 h for USDA-regulated species, which includes all wild mammals in the United States) must be provided with suitable sources of food and moisture. Food can be provided at the time of capture. For many small mammals, especially rodents, fruits or vegetables (e.g., grapes, celery, cabbage, lettuce, or slices of apple or potato) with high moisture content will suffice during transport or short periods of captivity until more permanent housing, food, and water can be provided. Water bottles should generally be avoided during transport because they will leak and dampen bedding.

Care must be taken when transporting captive animals to prevent their exposure to temperature extremes or precipitation, to provide adequate ventilation, and to minimize stress. Regardless of cage construction, minimizing disturbance is best. This may include placing transport cages in cool, darkened settings and minimizing noise and movement. In some instances, these conditions can be achieved simply by placing a drape over the cage, provided air flow is sufficient and temperatures are not extreme.

Free-ranging mammals may carry diseases and almost certainly harbor ecto- and endoparasites. Some IACUCs require treatment for ectoparasites before transport, and most will require quarantine of newly captured individuals before entering an animal care facility. Even if these precautions are not required, the investigator should take appropriate steps to minimize potential impacts to other captive animals and to humans. Most ectoparasites can be controlled by dusting with commercial flea and tick powder. Treatments for endoparasites are more involved and generally should be pursued after consultation with a veterinarian. Investigators should contact the local institutional occupational health office for information on risks to humans from species of mammals under consideration before transport, housing, or maintenance.

Maintenance environments

When individuals of wild species are to be maintained in captivity for > 12 h, the caging or holding environment must be selected carefully to accommodate species-specific requirements and to minimize stress. Cages or pens of an appropriate size and construction must adequately contain animals for their health and safety and that of investigators and animal care personnel. Because of the great variety of mammalian species that might be maintained, no specific guidelines for cage materials or size are possible, but considerations should be given to all aspects of the ecology, physiology, and behavior of target species. Guidelines developed for husbandry of domesticated species, including the *Guide* (NRC 2011), are seldom appropriate for wild-caught individuals and may constitute inappropriate or even inhumane treatment. Because of their capture as free-ranging individuals, nondomesticated species may perform better in larger cages or pens than those used for similarly sized domesticated species (Fowler 1995). Temperature, humidity, lighting, and noise levels also must be within appropriate limits. An excellent source of information on the specific needs of wild-caught species is the ASM's *Mammalian Species* series (https://mspecies.oxfordjournals.org). Additional valuable information usually can be obtained directly from investigators or animal care staff familiar with a particular species. Investigators proposing to maintain wild-caught mammals in captivity are encouraged to contact other researchers or institutions experienced with the taxa in question and to consult with the IACUC's attending veterinarian before submitting a protocol. Investigators should realize that housing requirements often will represent departures from the *Guide* (NRC 2011) or the PHS policy on use of laboratory animals (NIH/OLAW 2015), even if optimum for the proper maintenance of nondomesticated taxa; as a result, investigators should be prepared to justify proposed maintenance requirements to the IACUC and the IACUC approve departures.

Careful selection of bedding materials and substrate is critical to meeting the needs of nondomesticated species. Materials used should simulate as closely as possible the animal's natural environment and structure. Such materials might include sand or fine woodchips for desert species, soil and leaf litter for shrews and fossorial forms, and hay or straw for other species of rodents. The quantity of bedding also might be important if a dense covering (e.g., straw) allows establishment of refuges or runways that are components of the natural environment of the target species. More generally, some form of refuge should be provided in which captive individuals can remain concealed when possible because their availability influences behavior (Rusak and Zucker 1975).

Olfactory cues are a fundamental component of the natural environment of most mammals, and the design of husbandry practices should incorporate the maintenance of familiar scents to maximize animal comfort. Individuals frequently scent mark to establish territory boundaries and ownership. Frequent bedding changes and cage washing eliminates normal scent cues and places captive individuals in a novel and potentially stressful environment. Investigators can reduce stress that accompanies cleaning by changing bedding and cage equipment on a less frequent cycle than typically used for domesticated species (often 1 or 2 times weekly for laboratory rodents). Additionally, investigators can mix a small amount of old bedding with fresh bedding. Species adapted to arid conditions (e.g., *Onychomys*) will likely perform best when bedding changes occur every 10–14 days (or even less frequently), while others (e.g., *Sigmodon*) may require more frequent (e.g., weekly) changes. Because scent marks often are deposited on watering devices or cage lids, disturbance associated with being placed into a novel environment can be reduced by changing these devices on a different schedule from that used for caging and bedding so that individuals are not placed in an environment completely devoid of familiar scents. The importance of establishing and maintaining familiar surroundings, especially as identified by olfactory cues, cannot be overemphasized.

All species of mammals require some form of moisture in captivity, although water sources and requirements vary widely among species. Most mammals are best maintained with liquid water provided in various containers or via lickable watering systems. However, some taxa, such as kangaroo rats (*Dipodomys*) and pocket gophers (*Thomomys*), do not consume free water in the wild because they obtain moisture directly from their food and/or retain metabolic water (Boice 1972). These taxa can be maintained in captivity by periodically feeding small amounts of moisture-containing produce such as cabbage, lettuce, celery, or apple. The frequency of these supplemental feedings is dependent upon the ambient humidity in the environment and the water physiology of the species in question. If provided with ad libitum access to free water, xeric-adapted species can become dependent upon these sources (Boice 1972), which can result in changes in physiological functions that may confound some studies.

Environmental enrichment

Because the lack of stimulation in a captive environment can result in development of stereotypic behaviors that confound research interests, environmental enrichment can be a critical component of husbandry for nondomesticated mammals. Enrichment might be as simple as increasing structural complexity in the cage or providing additional materials for manipulation. For example, the captive environment of woodrats (*Neotoma*) kept in false-bottom cages can be improved by providing rodent chow directly in the cage rather than in a feeder attached to the cage front. This allows these natural hoarders to regularly rearrange food within their cage. Their environment can be further enhanced by providing strips of cardboard that will simulate the woody debris these animals use to construct nests in the wild. Other species of rodents also can benefit from inclusion of fibrous materials from which to construct nests. Chipmunks (*Neotamias*) and red squirrels (*Tamiasciurus*) are very active and can be difficult to maintain in captivity, but they can be housed successfully by using cages that incorporate 3-dimensional structures (e.g., hanging branches and perches) along with a cage floor sufficient for digging and caching food. For some species, hiding food in cardboard boxes allows the animal to "forage," thereby providing an important form of enrichment.

The social structure of the target species also must be taken into account when housing captive mammals. Captive situations that permit an approximation of the natural social structure of the target species are likely to be most successful and minimize stress. Individuals of species that are social or gregarious should be housed with other individuals when possible, but care must be taken to ensure compatibility. Of course, investigators must be aware of seasonal changes in social structure and modify housing environments accordingly to minimize stress and control reproduction.

Captive housing of bats

Housing and caring for bats in captivity presents several particular challenges for investigators and IACUCs. Animal care programs should be designed in consultation with rehabilitation or zoo experts or other researchers experienced with the care of the type of bats to be held (e.g., husbandry of fruit bats versus insectivorous bats will differ substantially). To the extent possible, housing conditions should imitate wild conditions. For nonhibernating species or for hibernating species housed during the active season, a flight cage that allows for and encourages free-flight should be provided. Obstacles such has hanging chains or hanging dividers require maneuverability during flight (the appropriateness of which varies by bat species), which should maintain natural behaviors and help prevent obesity, a frequent occurrence in captive conditions. Multiple roosting pouches, as well as numerous food and water dishes are preferable, so as to minimize competition when individuals are housed together. A flight cage should have double-wired walls to prevent escape. The external wire should be sturdy, such as hardware cloth. The internal wire will vary by species (depending upon body size, including size of the claws on hind feet), but window screen is often selected. Excellent guidelines for the housing and care of bats under captive conditions, including dietary considerations, can be found in Barnard (2011) and Lollar and Schmidt-French (1988).

For temperate, hibernating species, housing animals under hibernation conditions is often required by the study design or is otherwise desirable. Bats housed in modified refrigerators or in hibernation chambers require significantly less room than active-season bats (bats can be placed in small cages; e.g., wire mesh (reptile) cages in which bats will roost on the sides). Hibernating animals must be maintained at temperatures appropriate for the study species (determined by field data from natural hibernacula, which exist for many species) and require very high relative humidity (> 95%). These conditions (housing of flying animals in small cages, housing at very cold temperatures, and housing at very high relative humidity) deviate from the "normal" limits for laboratory animals and may necessitate special approval from the IACUC. Animals captured in the late fall or early winter and having sufficient body fat will enter hibernation in response to low ambient temperatures. Hibernating bats must be provided with water using either small trays or other waterers (e.g., chick waterers, which may be filled from attached jars, with marbles placed into the bottom to prevent accidental drowning of torpid bats). Unless one is studying a species that routinely feeds during the winter, the provision of food is neither required nor desired. Hibernating bats can be housed successfully under these conditions for

5–6 months (their normal period of hibernation). We strongly recommend that researchers consult with other bat specialists who routinely house hibernating species, as the necessary environmental chambers are typically specially designed and/or modified for bats. When housing hibernating animals, including bats, disturbances must be kept to a minimum to prevent repeated arousal and the resultant exhaustion of energy reserves. Hibernating animals should be checked remotely (using equipment that does not cause disturbance, including sound at frequencies the animals can receive) or observed only infrequently, often at greater than 2-week intervals.

Observation intervals for captive wild mammals

Animals held in captivity will require routine care, which includes food, water, and bedding changes as appropriate for the species. Animal care guidelines and regulations also specify periodic observation to monitor the health and well-being of individual animals. The *Guide*, for example, states (p. 112) that "[a]ll animals should be observed for signs of illness, injury, or abnormal behavior by a person trained to recognize such signs. As a rule, such observations 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." As with the remainder of the *Guide*, these statements reflect standard husbandry practices for domesticated species and, in many instances, will not be appropriate for wild animals held in captivity. There are no domesticated hibernators, for example, and a daily disturbance of hibernating mammals would quickly lead to death as energy reserves are depleted by repeated arousal. Similarly, wild animals, particularly those recently brought into captivity, will be stressed by regular close contact with humans. In such instances, PIs and IACUCs must balance the disturbance associated with regular observations of nondomesticated animals against the information acquired from such monitoring. For animals hibernated in captivity or animals housed in naturalistic outdoor enclosures that permit foraging, observations may be conducted less frequently or may be accomplished using indirect signs such as disappearance of food and deposition of feces.

Separation of taxa and minimizing stress

The Animal Welfare Regulations (9 CFR 3.133) state that animals housed in the same primary enclosure must be compatible. That is, prey species should not be maintained near carnivores and diverse taxa of carnivores generally should not be housed together. Closely related species of some rodents frequently co-occur in nature and often can be housed in the same room without difficulty.

The general principles for identifying captive mammals in pain or distress are abnormal appearance or behavior. Normal appearances and behavior are determined by species-specific characteristics and personal experience of the handlers. Because behavioral changes are the primary means for identifying pain or distress, all personnel working with animals should understand the normal behavioral patterns of the species they are housing. Thus, all animals should be monitored by trained staff.

Pain is generally easy to identify if it is associated with an injury or physical abnormality, but stress or distress might not be due to pain and thus not immediately recognizable. IACUCs generally consider that procedures that cause pain or distress in humans likely also will cause pain or distress in other animals. Symptoms of pain in animals are species specific but may include anorexia, rapid or labored respiration, immobility, increased aggression, lack of grooming, periocular and nasal porphyrin discharge, and abnormal appearance or posture (NRC 2008, 2009). An extensive list of indicators of pain for a variety of laboratory animals is available from Cornell University at https://www.iacuc.cornell.edu/documents/IACUC009.01.pdf, but animal care personnel should be aware that wild mammals often will provide little or no sign of pain or distress until the condition is acute because overt signs of pain or distress would be strongly selected against in nature, where predators or competitors may cue in to such signs.

Release of captive mammals

Release of wild-caught mammals that have been held in captivity might be justified in the case of 1) endangered or threatened species, 2) species of special concern due to population dynamics, management needs, or conservation initiatives, or 3) individuals held for only short periods of time. Research designs that require release of captive animals as part of an experimental manipulation must be planned to minimize both potential impacts on local population and stress to the released individuals.

Concerns regarding release of individuals held in captivity for more than short periods include:

- Introduction of individuals into an area without available refuge and resources (especially problematic with highly territorial species)
- Alteration of population genetics
- Introduction of individuals not acclimated to the local environment
- Introduction of pathogens acquired in captivity to wild populations
- Stress on local populations and released individuals
- Excessive exposure to predation of released individuals due to inappropriate foraging cycles (entrained by captive light cycles or environments), extensive foraging due to not having caches built up for winter months, or lack of familiarity with local resources
- Disruption of social systems
- Lack of appropriate foraging skills (forgotten or never acquired)
- Legality of reintroduction of captive animals (varies with state and country)

Decisions regarding release and permissible durations of captivity prior to release are often species- or project specific and must be made on a case-by-case basis. Holding members of a species for 1 or a few days to recover from surgical implantation of a transmitter or data logger is usually appropriate. In contrast, release of highly territorial animals held for even short periods into the same environment from which they were captured can be problematic because vacant territories can be usurped such that reintroduction of a former resident virtually guarantees a conflict that would not have occurred had the resident not been removed. For additional information regarding the potential release of marine mammals, investigators are referred to the best practices for these taxa developed by the National Marine Fisheries Service (http://www.nmfs.noaa.gov/pr/pdfs/health/release_guidelines.pdf). Final disposition of captive animals is of concern, but the integrity of natural populations and humane treatment of released individuals must be the highest priorities in project design and IACUC deliberations.

Euthanasia and Humane Killing

The *Guide* defines euthanasia as "the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress" (NRC 2011:123). Euthanasia is often accomplished as a 2-step process that involves use of an agent to depress or eliminate central nervous function and a 2nd step to stop the heart. The 1st action causes the animal to become unconscious and insensitive to pain while the second is the actual cause of mortality. Although both of these goals can be accomplished with a single agent, the primary concern is alleviating pain immediately. Whatever the method, the objective is that the animal feels no or only momentary pain, distress, or anxiety.

Euthanasia techniques are evaluated and approved by the IACUC during review of the animal protocol. Investigators should be aware that the *Guide* (NRC 2011) requires that methods of euthanasia be consistent with the AVMA Guidelines on Euthanasia (AVMA 2013a) unless an exception is justified for scientific or medical reasons. The AVMA distinguishes between euthanasia and humane killing and notes that methods of killing other than those deemed "acceptable methods of euthanasia" might be justified in situations with free-ranging wild animals. In the section on wildlife, the AVMA writes "that the quickest and most humane means of terminating the life of free-ranging wildlife in a given situation may not always meet all criteria established for euthanasia (i.e., distinguishes between euthanasia and methods that are more accurately characterized as humane killing)" (AVMA 2013a, Section S7.6).

Investigators and oversight personnel should realize that the AVMA guidelines for euthanasia are a poor fit for many, if not most, field settings, and thus often warrant IACUC-approved departures from the *Guide* and the AVMA guidelines. The treatment of gunshot as a form of euthanasia or humane killing in the AVMA guidelines is one such example. The AVMA contends that the minimum energy recommended for euthanasia by gunshot of animals smaller than 180 kg is 407 J (AVMA 2013a:37). Satisfying these conditions would require use of a handgun on the order of a .357 Magnum to achieve these energy levels with most readily available ammunition. This is clearly an inappropriate level of energy for most smaller species of mammals. It is also the position of the AVMA that gunshot cannot be considered euthanasia in wildlife unless "bullet placement is to the head (targeted to destroy the brain)" (AVMA 2013a:82). Such a procedure, particularly with cartridges of the muzzle energy specified by the AVMA, renders the skull and perhaps large parts of the animal carcass unusable and is thus inconsistent with most scientific collecting efforts that rely on obtaining high-quality specimens for research. Gunshot through the brain also risks aerosolizing brain tissue and thus may contribute to the spread of rabies viruses or prions if present in the target individual. Justification for deviations from approved AVMA euthanasia methods for studies of wildlife can include citation of published literature (such as these ASM guidelines) and incompatibility with study requirements.

Field methods for killing should be as quick and painless as possible, compatible with study design, and the size, behavior, and biology of the focal species. In case of injury resulting from capture or handling, and when nothing can be done to alleviate pain or distress or when recovery is not expected, euthanasia or humane killing is indicated. Except when specifically excluded by permit or law (e.g., with endangered/threatened species), protocols involving fieldwork should explicitly indicate the circumstances for and method of euthanasia for voucher and distressed or injured animals to accommodate unplanned injuries even when animal mortality is not an anticipated outcome. In the case of Endangered Species, investigators should request conditions on their permit that will allow them to terminate the lives of injured animals unlikely to survive in the wild unless treated, particularly where the animal is in pain or distress and veterinary care is not available. Importantly, investigators are advised to list, and oversight bodies to accept, a range of methods to achieve a humane death for target and nontarget species under the variable environmental conditions that may be encountered.

Euthanasia or humane killing should be conducted by personnel properly trained in the procedure used. Proper technique includes a follow-up examination to confirm death. Standard evidence of death include dilated pupils and absence of heartbeat as well as failure to respond to a toe pinch or touch of the eye; cessation of breathing is not a sufficient criterion. Decapitation, cervical dislocation, or thoracotomy (i.e., open biopsy of lung, pleura, hilum, and mediastinum) may be conducted after administration of euthanizing drugs, as consistent with study requirements, to insure that animals do not revive (AVMA 2013a). The AVMA considers decapitation and cervical dislocation, when properly performed by experienced and trained personnel, acceptable methods of euthanasia for some study designs and research needs, although justification for use of these methods should include details concerning intended use of the animals and the reasons why other euthanasia methods are unsuitable. Decapitation or cervical dislocation can be used as a method of euthanasia on animals that are first anesthetized or sedated, but investigators should be aware that administering sedatives or anesthesia before euthanasia might add distress or impose additional pain on the animal. For many small-bodied species, cervical dislocation can be accomplished efficiently in the field without sedation by experienced personnel. Investigators should be aware, however, that this procedure can alter body measurements and damage skulls of smaller species, so its use on individuals that will be archived as museum specimens should be carefully considered.

Although euthanasia of small mammals in field settings can be accomplished using any of the techniques approved by the AVMA, use of injectable controlled substances or inhalants can be challenging due to risks to investigators and stress to the animals. Investigators and IACUCs are also reminded to verify the legal requirements for using controlled substances away from the premises listed on DEA registration and for administration by individuals other than licensed veterinarians if such will not be present at the site of administration. If the method of killing includes controlled substances, toxins, or lead projectiles, the PI and IACUC must consider the possibility of secondary toxicity if the carcass is left in the field and consumed by other animals. These concerns might be alleviated by removing or burying the carcass, but removal of carcasses, particularly of large animals, also deprives the biotic community of these valuable resources (Sikes and Bryan 2015). Further, the use of controlled substances requires DEA registration and availability of the compounds themselves. Availability might be further compromised if the study involves foreign travel.

PIs and IACUCs should be aware that the Veterinary Mobility Act of 2014 permits veterinarians, but not other DEA registrants, to transport and use controlled substances at sites other than their registered address. It is also important to note that most states require a VCPR that may or may not extend to wildlife and the use of controlled substances by someone other than the veterinarian. As noted previously in the section on the "Role of the veterinarian," the language of Section 5 of the model Veterinary Practices Act endorsed by the AVMA in 2013 (AVMA 2013b) precludes establishment of a VCPR exclusively by telephonic or other electronic means. Thus, to provide medical advice, including whether or not to euthanize an animal on the basis of its condition, it would presumably be necessary for a veterinarian to accompany the investigator on all field excursions. To insure compliance, investigators and IACUCs should consult the language of the regulations for the practice of veterinary medicine and the use of controlled substances in the state in which research activities are conducted.

Thoracic compression offers an acceptable method of field euthanasia for some mammals and has been used effectively for decades by practicing mammalogists. Thoracic compression is not listed as an acceptable form of euthanasia in the 2013 edition of the AVMA *Guidelines for the Euthanasia of Animals*, but, as noted above, procedures other than those considered acceptable as euthanasia can be used for humane killing and can be authorized by the IACUC. The AVMA's (2011) "Literature Review" on thoracic compression states that "when scientifically justified, the IACUC has and should employ the authority to approve killing techniques not listed as recognized forms of euthanasia. This might include approving thoracic compression where it represents the most humane option available or practicable, or approving the use of drugs with analgesic properties that may not be scheduled drugs." The AVMA further states that "thoracic compression should not be prohibited where its use is necessary to minimize animal suffering or is scientifically justified (such as under the oversight of an Institutional Animal Care and Use Committee)" (AVMA 2011). AAALAC International similarly considers thoracic compression acceptable in these situations. In their adoption of the AVMA *Guidelines* as reference materials, AAALAC International made a single exception, which focused on the use of thoracic compression in wildlife studies (AAALAC International 2015):

> Exception: Thoracic (cardiopulmonary, cardiac) compression is a method used to euthanize wild small mammals and birds, mainly under field conditions. According to the 2013 Guidelines, thoracic compression is an unacceptable means of euthanizing animals that are not deeply anesthetized or insentient due to other reasons P.41, M3.12 and P.83, S7.6.3.3. The Council on Accreditation recognizes the need for the use of thoracic compression in conscious wild small birds and mammals in situations where alternate techniques are not feasible or objectives of the protocol are such that the Institutional Animal Care and Use Committee (IACUC), and/or competent authority, grants approval for this method, training for the technique is provided, and its continued approval is re-evaluated as more scientifically-based data regarding its use becomes available.

Thoracic compression has the advantage of requiring no additional equipment and thus can be used in any situation where appropriately sized animals can be brought to hand. It does not distort important body measurements, destroy needed tissues and skeletal elements, or alter hormonal profiles via the introduction of foreign substances. The ASM considers thoracic compression a humane method of killing when the investigator is skilled in this procedure and when the individuals to be killed are sufficiently small that the thoracic cavity can be collapsed to prevent inspiration.

Methods of euthanasia acceptable to the AVMA—their advantages, disadvantages, and effectiveness—are reviewed in the AVMA *Guidelines for the Euthanasia of Animals* (AVMA 2013a). This report also provides information on inhalant agents, noninhalant pharmaceutical agents, and physical methods of euthanasia. The American College of Laboratory Animal Medicine (ACLAM) has also evaluated rodent euthanasia and identified 3 issues of concern: euthanasia of fetal and neonatal rodents, use of carbon dioxide for euthanasia, and impact of euthanasia techniques on data collection. Publications by ACLAM (https://www.aclam.org/Content/files/files/Public/Active/report_rodent_euth.pdf) provide appropriate directives on these topics.

For collecting methods using kill traps, it is important to recall the AVMA position that, although kill traps do not always render a rapid or stress-free death consistent with their criteria for euthanasia, situations exist when live traps and subsequent euthanasia are not possible or when it might be more stressful to animals or dangerous to humans to use live traps as opposed to kill traps (AVMA 2013a). Finally, whether the termination of life is considered euthanasia or humane killing, these acts must be performed with a conscious respect for their effect on other animals (including human observers). Fear in other animals (including conspecifics and in some cases heterospecifics) can be triggered by distress vocalizations, fearful behavior, and release of odors and pheromones by a frightened animal (AVMA 2013a). Thus, killing should be done outside the perceptive range of other individuals whenever possible. Judgment should be exercised with regard to killing animals in view of the general public without providing them training or education about its necessity to avoid negative reactions to the procedures by observers.

Vouchering of Specimens and Ancillary Materials

Investigators must always plan for the disposition of animals from wild populations when a study is completed or when animals are procured unexpectedly during the study. The latter might result from incidental deaths when animals are found dead in traps, nets, or on roadways. All specimens and ancillary material generated from field studies should be deposited with relevant data into an accredited research collection when possible. The ASM Systematic Collection Committee has compiled a list of accredited collections in the Western Hemisphere (Hafner et al. 1997). The information is available online at http://www.mammalsociety.org/committees/systematic-collections. Deposition of specimens and ancillary materials in permanent collections maximizes benefits from each specimen generated, ensures access to valuable data by future investigators, and provides vouchers for individuals or species used in published research. Further, in some instances, archived specimens may be used in lieu of sacrificing additional individuals as part of future studies. The ASM recommends that when IACUCs approve field studies that include collection or otherwise might result in mortalities or the collection of potential surplus biological material from either target or nontarget taxa, PIs designate an ASM-approved systematic collection in which to deposit their specimens. The submission of materials, of course, should be commensurate with the needs and capacities of the systematic collection. Exceptions to this practice should be justified in the approved protocols. ASM recommends that investigators coordinate with these institutions at the earliest possible stages of project development in order to adequately budget for the expenses involved in voucher deposition and curation.

Human Safety

Working with wild mammals, particularly in field situations, involves inherent risks, both biotic (e.g., bites, pathogens, parasites, and venomous plants and animals) and abiotic (e.g., lightning and temperature extremes). Fortunately, most of these risks can be minimized with basic training, planning, mentoring, and experience. Investigators have the responsibility to ensure that personnel handling, transporting, or maintaining wild-caught mammals are qualified and familiar with the associated hazards (e.g., bites and exposure to body fluids) as well as the requirements of the target species (e.g., bats—Constantine 1988). With appropriate preparation and training, investigators can adequately protect themselves and collaborators while conducting fieldwork with mammals (Kunz et al. 1997).

Many universities and other institutions offer field courses, workshops, and online programs designed to provide investigators and students with proper training in fieldwork and activities with wild-caught mammals. Occupational health programs also provide guidance for avoiding biological, chemical, and other hazards. Sources such as the CDC (1998, 1999; http://www.cdc.gov/) or state health departments offer current information and precautions for personnel conducting epidemiological studies or working with populations suspected of posing specific health risks. Additionally, the ASM provides updated guidelines relative to hantavirus pulmonary syndrome for mammalogists and wildlife researchers working with rodents; these recommendations should be broadly applicable to field studies of other mammal taxa (Kelt et al. 2010). The document by Kelt et al. (2010) also makes the important clarification that earlier published guidelines by the CDC (1998, 1999) were never intended to apply to field investigators conducting nonviral-focused research on rodents. Special precautions (e.g., vaccinations) to ensure human safety might be necessary when transporting individuals known or suspected of carrying potentially lethal pathogens such as hantavirus or the rabies virus. In areas where zoonotics are known to occur, bagging traps with a gloved hand and bringing them to a central processing area that follows institutional biosafety recommendations might be sufficient, although additional precautions might be required at the time of final processing of the captured animal, depending on the data required. Although chloroform is considered highly hazardous to personnel due to attendant risks of cancer and liver toxicity (https://www.osha.gov/dts/chemicalsampling/data/CH_227600.html), use of this substance under open-air field conditions might be appropriate because it kills ectoparasites that can pose risks to the researcher through transmission of disease.

Many IACUCs will require the investigator to document in their protocols the potential risks to human health and safety while working with target species of wild-caught mammals. However, investigators and IACUC members should remain cognizant that risks from zoonoses vary depending on study species, local environmental conditions, personnel attributes, and the potential pathogens. Accordingly, the safety precautions employed should match potential risks.

Summary

These updated guidelines on the use of mammals, including wild species, emphasize that investigators are responsible for compliance with federal and state guidelines regulating care and use of animals in research, exhibition, and instruction. Investigators should work with IACUCs to develop research protocols that allow scientific research objectives to be completed successfully while complying with animal welfare regulations. A rational, well-justified protocol, written succinctly and completely, will facilitate a positive and productive dialog with the IACUC. The task of the IACUC is to provide assurance to federal regulatory agencies and the public that animal research is being accomplished in accordance with the regulations and intent of the AWA and to work with researchers and educators to develop appropriate protocols. IACUCs must be strong advocates for animal welfare and humane animal use in research and education, especially when investigators provide clear justification for animal use and expertise upon which the IACUC can rely. These interactions foster strong, positive, and professional relationships between the IACUC and the investigator.

From initial design to completion of a study, investigators should exercise good judgment and prudence when using animals in research. IACUCs appreciate working with investigators who provide details of their research designs and goals. The "3 Rs" of Reducing the number of individuals without compromising statistical validity or biological significance, Replacing "higher" animals with "lower" ones, and Refinements of techniques and care to minimize pain or distress to animals (NRC 2011) are important goals for field mammalogy. A cap on the number of animals collected is usually imposed by state and sometimes federal permitting agencies and, likewise, is expected by the IACUC. Underestimates of the number of animals needed for a study might invalidate results. Therefore, a sufficient number of animals (i.e., the number needed to meet research goals) must be clearly requested and justified. "Replacement" in mammals might be achieved by using cell lines, voucher materials from previous studies, or computer simulations where possible. Further, larger mammals are not usually collected in surveys or for genetic work. Rather, they can be subsampled by ear punches or hair combs, or tissues might be requested from mammalian research collections where this material is already be archived as specimens. Other alternatives include using carcasses of species of interest (especially larger carnivores or ungulates) that have been trapped or hunted for other purposes. However, investigators are reminded that such sources may introduce undesirable biases associated with age, sex, or size. Finally, an example of "Refinement" might include using behavioral responses as indicators of social dominance rather than outcomes of physical combat.

Most field investigators already embrace the ethical treatment of animals because of their respect for nature and their dedication to wild species. The guidelines provided here were developed to assist investigators in maintaining compliance and understanding the evolving suite of animal welfare regulations. How we view use of mammals in research does not differ much from that of Joseph Grinnell when he visited Yosemite Valley nearly 100 years ago. Knowledge of most aspects of mammalian biology has advanced, but we still struggle with a basic understanding of our place in nature. Mammalogists continue to explore the farthest reaches of the Earth. In contrast, the public and even some scientists in other fields have become sufficiently removed from what is wild that we must be prepared to answer the question "what good is it?" That is, we must be able to communicate to a broad audience the applied and theoretical values of research on wild mammals. Proactive consideration of humane treatment of our study animals will help prevent retroactive criticism of our ethics and our research. With this in mind, the ultimate design of research programs, including the methods and techniques to address research objectives, are the responsibility of the investigator. Guidelines can provide current information on ethical and regulatory standards, but they cannot replace individual judgment or the drive, ingenuity, and curiosity that lead investigators to generate new and insightful advances in science.

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