

A Unique Application to the IACUC for Studies of Wild Animals in or from Natural Settings

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The format of an application to the Institutional Animal Care and Use Committee (IACUC) frequently is designed to obtain detailed information from medical faculty proposing studies involving a precisely determined number of a few well-characterized species in a controlled laboratory setting. Unfortunately, these application formats typically are less than ideal for marine and field biologists attempting to propose studies of large populations of diverse organisms in a comparatively uncontrolled environment and somewhat unpredictable setting. Traditional IACUC applications rarely address topics of field capture, restraint, marking, animal care in the field, and release of animals back into the environment. Yet the IACUC at institutions that receive federal funds for research involving vertebrate animals is obligated by federal policy to ensure that field studies adhere to the same basic tenets of humane animal care, use, and treatment that guide traditional laboratory animal studies. The University of South Florida IACUC established written policies and developed a specialized application to the IACUC that specifically requests from biologists the relevant details of their proposed field research involving vertebrate animals. These new policies and application format have improved understanding and communication of field biological studies and have helped ensure accountability of vertebrate animal use in the field.

A contemporary Institutional Animal Care and Use Committee (IACUC) often is occupied with the challenges of rodent molecular biotechnology (1), including ensuring that investigators anticipate unique pathologies and phenotypes of genetically engineered mice (2) and identify the earliest possible clinical endpoint in order to avoid or alleviate the pain and discomfort of mice (3). Regardless, an academic institution-based IACUC that oversees an administratively centralized animal care and use program also may be expected to review applications from field and marine biologists that propose studies of wild animals in or derived from natural settings, often referred to as "field studies." Such applications often require the IACUC to consider the use of a considerable array of vertebrate species in diverse environmental settings that frequently cannot be controlled and under circumstances that cannot always be anticipated.

Institutions that receive federal funds for research involving vertebrate animals are obligated by federal policy (4, 5) to ensure that all studies, including field studies, adhere to the same basic tenets of humane animal care, use, and treatment that guide traditional laboratory animal studies (6, 7). Although an IACUC largely comprised of medical faculty is accustomed to reviewing proposals that describe the animal use aspects of grant applications to the National Institutes of Health (NIH), the principle federal agency which funds research involving traditional laboratory species, it also must ensure that biological or medical studies involving wild animals proposed to the National Science Foundation (NSF), the United States Geological Survey (USGS), the United States Department of Agriculture (USDA), or other federal agencies are in accordance with federal law and policy (4-8). Like NIH, these federal agencies require its grantees to comply with the principles of the *Guide for the Care and Use of Laboratory Animals* even though some of the recommendations of the *Guide* clearly are not applicable to field conditions (7).

Background. Federal laboratory animal policy prior to 1986 dealt primarily with the care and maintenance of animals involved in research and not with methods of animal experimentation per se. Prior to 1986, federal policy did not address the observational studies of wild animals in the field, nor did it substantially guide the care of wild animals that were captured and maintained in the laboratory. With implementation of the Health Research Extension Act of 1985 [PL 99-158], federal policies governing humane animal care were extended to include animal use, experimental procedures, administration of anesthesia, euthanasia, and the research techniques used. The Health Research Extension Act of 1985 not only strengthened IACUC oversight of procedures in the conventional laboratory setting to ensure that animal pain and distress were minimized, but this mandate also caused the inclusion of any proposed scientific study that involved free-living wild vertebrate animals, from fish and up the phylogenetic scale to amphibians, reptiles, birds, and mammals.

Biologists of each professional society, including the American Society of Mammalogists, the Ornithological Council, the American Society of Ichthyologists and Herpetologists, the Herpetologists' League, the Society for the Study of Amphibians and Reptiles, the American Fisheries Society, and the American Institutes of Fisheries Research Biologists were asked to promulgate guidelines for the considerable array of taxa of vertebrates that might be encountered in field studies. Biological societies were advised to follow the basic tenets of humane animal care, use, and treatment delineated in the *Guide* and the *Public Health Service Policy (PHS Policy)*. Each society responded with guidelines relevant to their taxa (9-12). These guidelines addressed basic animal needs, how animals should be captured and handled in the field and laboratory, suggested marking techniques, methods of tissue sampling, compliance with permits for collecting animals, transporting animals to the laboratory, and the release of animals back into the environment. As is the case with the *Guide* and *PHS Policy*, guidelines stipulated the responsibility of investigators conducting the studies and endorsed the oversight of the local IACUC. As in the *Guide* and *PHS Policy*, guidelines were deliberately written in general terms to allow applicability to diverse species and settings and left responsibility of achiev-

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ing outcomes with the institution.

The application format used by an academic-based IACUC often is designed to draw detailed information from medical faculty proposing the use of precisely determined numbers of a single, well-characterized species in a conventional laboratory setting. Such application formats frequently prove absurd at best to marine and field biologists accustomed to studying large populations of diverse organisms, discovering organisms previously unknown to science, and using substantially different experimental approaches.

In response to this need to improve how information was requested from biologists regarding their proposed field investigations, the University of South Florida's IACUC established written policies based on the guidelines promulgated by each of the professional biological societies (9-12), in accordance with federal law (8) and policy (4, 5), and after considering other reviews (13-18). We developed a specialized application to the IACUC titled *Application for the Study of Wild Animals in or from Natural Settings*, which better communicates the proposed field study to reviewers on the IACUC and is viewable in its entirety at <http://www.research.usf.edu/cm/applications.htm>.

As recommended by the NSF (7), local policy was revised so that IACUC membership included a biologist. This new member provided the IACUC with an understanding of the nature and impact of proposed field investigations, the housing and care of the species to be studied, and the risks associated with maintaining wild vertebrates in captivity. The IACUC learned from its biologist that field studies contribute to the conservation and well-being of wild animals, because efforts to protect indigenous animal species often are dependent on an understanding of the species present, the nature of their habitat, and their ecology, genetics, anatomy, physiology, and distribution.

With the assistance of faculty biologists, this new application design accommodates a range of proposals to the IACUC from the unobtrusive observation of wild animals in natural settings; through their capture, restraint, marking and release; to their transportation to and confinement in the laboratory. The application also requests a description of the planned methods of wild-animal maintenance and care, specimen collection, test substance administration, surgery, and other experimental procedures. Appropriately, its format follows the continuum of field research activities that involve wild animals, beginning with a declaration of anticipated study locations and an acknowledged understanding of the required permits and then proceeds through methods of animal capture, restraint, marking, care, and release. The format provides multiple opportunities for early completion of the application when the proposed study involves only less obtrusive procedures.

Wildlife permits. While drafting the application, both the IACUC and assisting biologists developed a greater appreciation for the challenges that confront the IACUC and the unique regulatory and logistical features of field studies. The IACUC was reminded that state and federal agencies already review applications for permits that authorize the use of wild animals. The United States Fish and Wildlife Service (USFWS) issues many of the necessary permits, which are found in 50 CFR, Section 1-100. In so doing, the USFWS considers the proposed study's scientific merit, its impact on native populations, the number and taxa of animals to be encountered, the methods and period of study, and any restrictions deemed necessary to minimize deleterious effects. Threatened and endangered species status is reviewed by the USFWS, except in the case of marine mammals and fishes reviewed by the National Marine Fisheries Service, prior to issuing permits (19). Regulations governing scientific permits for endangered species are found in 50 CFR 17.22 and, for threatened species, in 50 CFR 17.62. During the conduct of the study, agents of the USFWS may inspect the location, records,

permits, and any wildlife kept under the authority of the permit. The USFWS Service-wide Permits Issuance and Tracking System assesses the impact of permitted activities on wild animal populations.

A research activity described in a single protocol submitted to the IACUC may be subject to multiple laws, and multiple permits may be required. Studies conducted outside of the United States must be in accordance with the Convention on International Trade in Endangered Species (CITES) codified as part of the Endangered Species Act (19), and all wildlife regulations of the country in which the research will be performed. The Wild Bird Conservation Act, which supplements CITES, requires permits for the scientific research and import of many species of birds, found at 50 CFR 15.22. Permits for the taking of any of the nearly 830 species of birds protected by the Migratory Bird Treaty Act are found at 50 CFR 21. A Bald and Golden Eagle Protection Act permit is required for research involving the bald eagle *Haliaeetus leucocephalus* or the golden eagle *Aquila chrysaetos*. Bird banding and marking activities require a permit under 50 CFR 21.22 issued by the United States Geological Survey-Biological Resources Division's Bird Banding Laboratory and using bands issued by the USFWS. Wildlife or their parts or products must be imported, exported, or transported through designated ports in order to allow for inspection by Customs and USFWS officers in accordance with the Lacey Act and Amendments and its regulations found at 50 CFR 14. Permits issued to conduct research involving marine mammals in accordance with the 1972 Marine Mammal Protection Act are provided in 50 CFR 18. In addition, the site where the research will be performed may also be regulated, for example by either the Bureau of Land Management, the National Park Service, the Forest Service, or another academic institution, zoological park, oceanarium, or aquarium.

The University of South Florida IACUC supplements its assessments of wild animal proposals and on-going studies in part by relying on the permitting process of the USFWS and other agencies. Knowledge of the regulations pertaining to the specific animals proposed for a study was entrusted to the applicant biologist, who must obtain permits necessary for carrying out the study prior to its initiation. The biologist then provides copies of permits when reporting episodes of wild animal use to the animal care service when requested but at least annually.

Characteristics and number of animals. The IACUC had grown accustomed to reviewing comparatively narrow-focused applications that justify the characteristics of a few genotypes. It soon learned that studies of free-living vertebrates often involve many species of a considerable range of diverse creatures of the more than 20,000 species of fishes, 8000 species of amphibians and reptiles, and 9000 species of birds, each with varied and often poorly known behavioral, physiological, and ecological characteristics. The IACUC learned that some species encountered during the conduct of the study might not have been anticipated or even known to science before the onset of the study.

The number of animals required for a field study varies but is typically much larger than that of conventional laboratory studies, in part because the intent of field studies is to define the prevalence and movement of species and because biologists have substantially less control over biotic and abiotic conditions in the field that produce experimental variation. Although field studies might propose the need for a relatively large number of animals from the conventional laboratory perspective, the number of animals needed might in fact be a very small percentage of the resident wild-animal population. The IACUC also learned that it is not always possible to predict at the initiation of a field study all of the observation or collection opportunities or the number of animals or species to be encountered in the field.

Further, the IACUC was introduced to the concept that pain perception by many species of vertebrates may not be uniform

over various portions of their bodies, and a broad extrapolation of pain perception across taxonomic lines may not be appropriate (11, 12). It also learned that no concise or specific compendium of approved methods for field research encompassing all species, settings, and methods is available. These were challenging realizations for the IACUC overseeing a diverse, centralized animal care and use program that had grown accustomed to a rather well defined process in a principally medical setting. New policies and assurances were established in order to memorialize a new and better understanding between biologists and the IACUC, viewable at <http://www.research.usf.edu/cm/policies.htm>.

Assurances. When biologists sign the new application they assure the IACUC that they have adequate experience, training, and knowledge regarding the housing, feeding, and care requirements of the animals to be studied "to the extent that these factors are known or applicable to the study." This last phrase from the biological societies' original guidelines (9-12) became a favorite reminder to IACUC members that a substantial contribution of field investigations is an increased understanding of characteristics of the subject animals. With their signature, applicant biologists also assure the IACUC that the taxa chosen was well suited to answer the question posed. This assurance often is given somewhat reluctantly, because as the biologists were fond of reminding the IACUC, the question posed in the study typically is "what are these animals like?". The applicants also assure that the number of animals proposed would be the minimum necessary for accomplishing the goals of the study. Biologists assure that they will direct the research activity in the field, and assist with tracking it by reporting episodes of wild animal use, the range of taxa, and the number of animals encountered or used, at intervals appropriate to the study, at least once each year. Reports of wild-animal use are made to the Division of Comparative Medicine animal purchasing agent, who maintains an inventory of the number of animals used in each IACUC-approved protocol.

Capture and restraint. Field studies in their simplest form consist of the direct observation of free-ranging animals under natural conditions, but the objectives of most field studies mandate that individual animals be captured one or more times. Prior to the capture or removal of animals, investigators should make an effort to understand the population status of the taxa involved. Capture techniques that have minimal impact on the animal and are environmentally benevolent are used whenever possible. Return to the natural environment is incorporated into the sampling design whenever feasible.

Unaccustomed to capture techniques, the IACUC was taught that acceptable capture techniques that have more than a minimal impact on fish include gill netting, electrofishing, the use of ichthyocides, and the use of hooks or spears (12), on amphibians and reptiles include trapping and netting (11), on birds include netting and trapping (10), and on mammals include trapping, netting, and capture darts which deliver an immobilizing drug (9). Capture devices such as nets and traps should be checked frequently to prevent animal injuries or mortality.

Biologists are typically authorities on the species under study and familiar with the subject animals' response to disturbance, sensitivity to capture and restraint, and requirements for captive maintenance "to the extent that these factors are known or applicable to the study" (9-12). As in the laboratory setting, procedures should be selected that avoid or minimize distress to the animals consistent with sound research design. Procedures that cause more than momentary or slight distress to the animals must be performed with appropriate sedation, analgesia, or anesthesia, except when scientifically justified and approved by the IACUC. Living conditions of animals held at field sites should be appropriate for the involved animals and contribute to their health

and well-being.

Restraint procedures, including confinement, physical restrictions, and drug-induced immobilization, should be those that cause the least amount of restraint necessary, that can be accomplished in the shortest period of time, that reduce or eliminate contact between the handler and the animal, and that minimize hazards to personnel and the animal, whenever possible within the constraints of study design.

Marking. Marking of wild animals is a basic method of many field studies and provides a way of determining the movements, abundance, and population dynamics of those animals. Investigators and the IACUC should carefully consider the nature and duration of restraint required by the marking technique, the amount of tissue affected, whether distress is momentary or prolonged, whether the animal after marking will be at greater-than-normal risk, whether the animal's desirability as a mate is reduced, and whether the risk of infection or abscess formation is minimal.

Acceptable marking techniques of fish include fin-clipping, freeze-branding, electrocauterization, tagging, radiotelemetry, and radioisotopes (12); of amphibians and reptiles include scale clipping, banding, tagging, shell marking, radiotelemetry, tattooing, electrocauterization, branding, and radioisotopes (11); of birds include banding, dyes, collars, tagging, and radiotelemetry (10); and of mammals include tagging, banding, radiotelemetry, tattooing, spot-shaving, radioisotopes, and freeze-branding (9). The investigator and the IACUC should consider the potential for pain and discomfort associated with each of these techniques and whether they should be preceded by a general or local anesthetic and/or followed by a topical antiseptic.

Wild-animal care. Maintenance of wild animals in their natural setting must incorporate, as far as possible, those aspects of the natural habitat deemed important to the survival and well-being of the animal. Adequacy of maintenance should be judged by monitoring factors such as appearance, activity level, general behavior, rate of growth, change in body weight, breeding success, and rate of survival. Nutritionally balanced diets must be provided, or natural foods should be duplicated as closely as possible. Natural light, ventilation, temperature, and humidity conditions should be provided, unless these are factors under investigation.

Whenever wild-caught animals are brought into a laboratory, they must be maintained under conditions that comply with the *Guide*, unless the purpose of the study requires the simulation of the natural setting or when the wild animals housed in the laboratory require conditions other than those prescribed by the *Guide*. In such instances, the design of enclosures and methods of care must accommodate salient features of the animal's ecology, morphology, physiology, and behavior. Investigators and the IACUC should consider whether newly captured animals that are brought to the laboratory should be quarantined from resident animals for a period of at least 30 days.

Specimen collection. Methods used for sampling tissues or specimens from wild animals should be designed to obtain the maximum amount of scientific data with the least amount of animal handling, restraint, and distress and involve the minimum number of animals. Methods that cause more than slight or momentary pain or discomfort require the use of appropriate anesthetics and/or analgesics. Aseptic sampling techniques and surgical procedures should be used. Biologists and the IACUC should consider whether antimicrobial drugs should be administered after sampling procedures.

Euthanasia. Methods of euthanasia should be consistent with the methods recommended by the biological societies (9-12) and the 2000 Report of the American Veterinary Medical Association Panel on Euthanasia (20). The collection of animals and their preparation as museum specimens contributes to research

and teaching of systematic zoology. Each animal collected should serve as a source of information on many levels (e.g., anatomy, physiology, and genetics) to ensure the maximum utility of each animal and to minimize the need for duplicate collecting. Formalin fixation of dead specimens is acceptable, but euthanizing unanesthetized specimens by immersion in a formalin solution is unacceptable.

Occupational health and safety. Many wild animals are potentially hazardous to research staff, either from traumatic injury, infectious disease, venoms, or poisons. Staff working in the field should maintain current tetanus immunization status, and those working with carnivores or bats should maintain current rabies immunization status. Biologists should ensure that the design of the field study does not compromise the health and safety of the staff working in the field or other animals in the area.

Release. Whenever practical and ecologically appropriate, as soon as possible after capture and upon completion of the study, wild-caught animals should be released at the site of the original capture. Release should be contingent upon ensuring that the animal's ability to survive has not been impaired and that the animal can be expected to function normally. Wild animals should be released when conditions are conducive to the animal's survival and when their release is not likely to spread pathogens. Release should not occur if laws or regulations so prohibit or when it may be detrimental to the well being of the existing native animals.

Although the IACUC is introduced to new challenges by proposals to study wild animals in or from natural settings, the application and reporting process is assisted by adopting an application format tailored specifically for such studies and the unique policies that guide them. It is also helpful to recognize that respect for all forms of life is an inherent characteristic of biologists who are well aware that respectful treatment, care, and use of wild animals involved in research are both ethical and scientific necessities.

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