Northern Arizona University IACUC Self-Instruction Module for Certification in the Humane Care and Use of Laboratory Animals

How this self-instruction module works. Federal regulations require that individuals who use research animals receive training in Humane Care and Use of Laboratory Animals and in occupational health and safety relevant to using research animals. To allow flexibility for research staff and faculty, the Institutional Animal Care and Use Committee (IACUC) has prepared this self-instruction module to facilitate training. This module addresses humane care and use of animals.

Why is this training required? Federal regulations including the Animal Welfare Act (AWA) and the Health Research Extension Act explicitly require training in the humane care and use of animals for all individuals involved using animals in research or teaching. These regulations are discussed below but require that the following topics must be included in this training:

A. Research methods which don't require animals;
B. Relevant laws regulating humane care and use of animals;
C. Minimizing animal pain and distress;
D. Means to limit the number of animals used;
E. Proper use of anesthetics, analgesics, and tranquilizers;
F. Means to assure research does not unnecessarily duplicate previous research;
G. When and how to report deficiencies in animal care or treatment;
H. Occupational health concerns relevant to the species and study.

Who must complete this training? All persons who use animals for research, teaching, or demonstrations that involves anything other than observing animals in their natural habitat (without materially influencing the animal's behavior) must document NAU-specific training. Although various regulations and policies differ in their definition of an animal (as discussed below), any live vertebrate used for teaching, laboratory demonstration, or research is considered to be an animal at NAU. All such work requires an approved IACUC protocol (defined below) and all involved in this work must complete the Humane Care and Use training. Tissue obtained from dead animals is not regulated but the means by which tissues are obtained is critical. If you obtain tissues from animals killed for non-research/teaching reasons (humane shelters, hunter check stations, etc.) this is not considered animal use and does not require an IACUC protocol. However, if an animal is euthanized for research or teaching purposes or tissues are obtained from a live animal, this is covered by regulations and requires an IACUC protocol. In general, fetal animals are not covered until late-term gestation but the maternal animal is covered.

Humane Care and Use training must be completed once every 3 years. This training may be through training seminars or through reading this module and completing the accompanying quiz. All personnel listed on Institutional Animal Care and Use Committee (IACUC) protocols and all individuals who provide care for animals housed in University sponsored research must complete training before a protocol may be approved.

Individuals working with animals must also complete the occupational health and safety program prior to commencing work. Please contact Annex personnel at 523-1330 if you have any questions about the quiz or to obtain paperwork for the occupational health and safety program.

Historical background. Until the late 1800's, many gave little thought to animal pain and stress. It was accepted philosophy was that animals did not feel pain in the human sense; experiments and dissections were performed with little thought to pain or distress. Public attitudes about this began to change in the 1800's, when individuals began to question the assumption that pain and distress are uniquely human experiences. Anti-vivisection groups then began calling for the end of animal experimentation. While most of society has not subscribed to a wholesale anti-research agenda, public sentiment for animal welfare and

rights continues to grow, as does scientific acceptance of animal cognition. As a result, many are now concerned about research activities that were considered routine until recently. Increasingly complex laws and policies have grown from this concern. Regulations have not necessarily stemmed the tide of public opinion regarding animal research but the trend toward increased regulation will likely continue.

Research methods that don't require animals. Although this module is about humane care and use of research animals, animals must only be used when no other method can suffice. Although many questions cannot be answered without animals, investigators must demonstrate that non-animal methods are insufficient for the proposed study and that the study is of sufficient importance to justify the use of animals. Non-animal systems that must be considered include:

1. Computer models. With sufficient background information, computers modeling can answer many questions that would not otherwise be intuitive. However, given the tremendous complexity of biological systems and limited knowledge about many systems to be examined, computer models are often insufficient to answer biomedical questions,.
2. Mathematical models. Similar to computer modeling, statistical and mathematical models can aid investigators in interpreting existing databut are essentially limited to assisting analysis of what is already known.
3. Plastic models, computer simulations, etc. Plastic models and computer simulations (virtual reality) often supplant the need for live animals in training for surgical or other techniques. For example, plastic models may be excellent for anatomy courses, practice for injection techniques, etc. Computer simulations have replaced live dogs in many physiology courses and can be effective tools in early training for endoscopic surgery, etc.
4. *In vitro* or *ex vivo* systems. Tissue culture (*in vitro*) systems can answer many questions in biomedical science. *In vitro* systems are less complex than *in vivo* systems, which can facilitate data analysis, have lower cost compared to *in vivo* studies, and reduced animal use. However, this decreased complexity also means there are many questions *in vitro* systems will not answer, such as how a toxin that kills cells in culture will behave when diluted in an entire system and interacting with the immune system, etc.
5. Human studies. Studies which may be performed on humans without animal testing, include those that do not involve administration of potentially toxic substances (pharmaceuticals, etc.), implants, etc. In rare cases, products shown safe in *in vitro* systems may be studied in humans without intervening animal studies. However, regulations still require that the vast majority of substances be tested in animals prior to use in humans.

The three "Rs" of research. The underlying theme of Federal humane care and use regulations is the reduction of pain and distress of research animals. These regulations are based on the 3 "Rs" of animal research, which are:

1. Reduce--use of the fewest animals necessary to obtain statistical significance. Usually this means using a small number of animals but sometimes investigators propose using too few animals. If too few animals are used to obtain statistical significance, the animals used were wasted. Statistical power analysis is often useful to ensure neither too few nor too many animals are used.
2. Refine--using less painful or invasive techniques. For example, a subcutaneous Alzet pump may eliminate frequent injections. Refinement also includes use of persons experienced in the planned techniques, since post-operative pain is significantly reduced with increased surgeon skill.
3. Replace--replacing animals with non-animal systems (computer, tissue culture, etc.).

RELEVANT LAWS AND POLICIES REGULATING HUMANE CARE AND USE OF ANIMALS IN RESEARCH AND TEACHING

1. ANIMAL WELFARE ACT: The Animal Welfare Act (AWA) was first passed in 1966 (Code of Federal Regulations Title 9, Subchapter A, Parts 1-4). The AWA was written to prevent theft of pet dogs

and cats and ensure humane treatment of dogs, cats, and monkeys. It has been revised several times and now addresses humane treatment of most warm-blooded vertebrates used in research, teaching, large scale breeding, or exhibits (including zoos). The AWA does not cover mice (*Mus*) and rats (*Rattus*) raised specifically for research, any birds, or farm animals (including horses) used for agricultural (food and fiber) research. Farm animals used for biomedical studies are covered by the AWA.

The US Department of Agriculture (USDA) administers the AWA. USDA veterinarians are the primary AWA enforcement officers and perform unannounced inspections of research institutions on a regular basis to check for AWA compliance. AWA regulations must be followed regardless of how the research or teaching is funded. The regulations are "performance standards," meaning they provide strict definitions of how things must be done with little room for professional judgment.

The AWA and Guide (see below) both require that the Institutional Animal Care and Use Committee (IACUC) approve all animal-based research or teaching. This approval must be obtained before the activity begins. This approval process is described in detail below. IACUC review ensures all studies meet all requirements for humane use of animals.

The AWA is very specific in prohibiting certain activities. For example, multiple major survival surgeries may not be performed without strong scientific justification (unless required as veterinary care for the animal's well being). **Major surgeries** are defined as those which may seriously debilitate the animal, such as orthopedic surgery, or which enter a body cavity (thorax or abdomen). Requests for multiple major survival surgeries require strong scientific justification and IACUC approval. In addition, requests for multiple major survival surgery on one animal in different studies requires approval from the regional USDA offices, if the animal is of a regulated species.

Since the AWA is Federal law, criminal penalties may be imposed for non-compliance. Penalties range from warning citations to fines (up to hundreds of thousands of dollars). Inspectors can immediately stop any research with regulated species that they feel is grossly non-compliant. They may also ask funding agencies to revoke project funding.

USDA policy statements. Due to complexities of the AWA, the USDA has issued several policy statements to clarify specific items. These statements address subjects such as required veterinary care, pain relief for painful procedures, and requirements to prove there are no alternatives to potentially painful procedures. These policy statements may be viewed at the website: http://www.aphis.usda.gov/ac; click on "publications" and scroll down to "APHIS policy manual."

2. PUBLIC HEALTH SERVICE (PHS) POLICY: The PHS oversees the National Institutes of Health (NIH), Center for Disease Control (CDC), and other human health and research institutions. The strict PHS policies on research animal use are outlined in the "Guide for the Care and Use of Laboratory Animals" (the Guide) and in "Principles for the Utilization and Care of Vertebrate Animals in Testing, Research, and Training." The definition of an animal in the Guide is broader than in the AWA; PHS considers all vertebrates to be animals.

Unlike the AWA, the Guide is a Federal policy rather than law. Those who receive Federal research funds must follow the Guide for animals used in research or teaching. Most research foundations also require compliance with the Guide. The NAU Assurance Statement to NIH states that all research with vertebrate animals here will comply with the Guide, regardless of funding source. Non-compliance is punished by revocation of Federal research funds rather than criminal penalty. Recently, many prominent universities have lost Federal funding for extended periods of time while alleged non-compliance was investigated. Some laboratories have essentially lost their ability to compete for Federal funds after such investigations. In addition, publicity associated with revoked funding has the potential to significantly impact an

institution's credibility. At NAU, failure to comply with the Guide may result in loss of animal-research privileges, regardless of funding source.

Many policies in the Guide are similar to the AWA, although the Guide is often more stringent than the AWA. Universities must guarantee compliance with the Guide through an "Assurance Statement" filed with the Federal government before research may be funded. At NAU, our Assurance Statement states the university applies the principals of the Guide to all animals, regardless of how the research or teaching efforts are funded. Similar to the AWA, the Guide does not cover farm animals (including horses) used for agricultural (food and fiber) research. Farm animals used for biomedical studies are covered by the AWA. Unlike the AWA, the Guide does cover laboratory mice, rats, and birds.

Several of the major topics of the Guide are addressed below, in the section discussing the IACUC.

3. Laws regulating use of wildlife. Numerous laws regulate research and educational use of wildlife. If you will work with any wildlife, you must check with federal, state, and, if applicable, tribal officials to ensure compliance. Major regulations include:

a. **The Committee on the International Trade of Endangered Species (CITES)**—although the title is International Trade, this act also covers interstate activities involving endangered species;

b. **The Migratory Bird Act**—this act covers any work done with or that may affect migratory birds. Thus, you will likely need a permit if you plan to net migratory birds or spread nets which may inadvertently trap migratory birds.

c. **State Game and Fish** agencies require permits to trap or hold most wildlife species, unless the species is considered vermin. National forests and tribal governments often have additional permit requirements. Due to the diversity of species used at NAU, it is difficult to provide a blanket description of permits which may be required. Check with the IACUC chair if you have any question regarding permits. *A copy of all relevant permits must be attached to the original IACUC protocol and maintained for the duration of project activity.*

4. Miscellaneous Arizona state laws which relate to research.

a. **Arizona animal cruelty law.** This law criminalizes specific acts of animal cruelty. Violations range from misdemeanors to felonies, depending on the species involved and the type of cruelty. This law does not exempt research animals. Adherence to an IACUC approved protocol should protect an investigator and their students. Definitions in the law (directly quoted from the statute) are:

1. "Animal" means a mammal, bird, reptile or amphibian.
2. "Cruel mistreatment" means torture or inflict unnecessary serious physical injury...or to kill an animal in a manner that causes protracted suffering....
3. "Cruel neglect" means to fail to provide an animal with...food, water or shelter.

Misdemeanor offenses include the following occurrence to any animal under a person's custody or control:

1. Knowingly...or recklessly subjects any animal...to cruel neglect or abandonment;
2. Knowingly...or recklessly fails to provide medical attention...to prevent protracted suffering;
3. Knowingly...or recklessly inflicts unnecessary physical injury to any animal;
4. Recklessly subjects any animal to cruel mistreatment;
5. Knowingly...or recklessly kills any animal...of another person without...consent of the owner.

Felonious offenses include the following occurrence to any animal under a person's custody or control:

1. Knowingly subjects any animal...to cruel neglect or abandonment that results in serious physical injury to the animal.
2. Knowingly subjects any animal to cruel mistreatment.

This law does not prohibit release of unclaimed shelter animals to research facilities. In fact, Arizona has a law stating such release is permissible. However, most animal shelters have policies prohibiting such release due to concerns over public opinion.

b. Arizona research facility protection act. It is a class 6 felony for a person to "intentionally release an animal that is lawfully confined for scientific, research, commercial, or educational purposes without the consent of the owner." Animal rights groups periodically break into research facilities to release animals. Not only does this usually have dire consequences for the animals (domestic white mice don't do well in a field of predators), it is a felony under Arizona law.

5. Controlled substances act. Drugs that may be addictive or have the potential for social abuse are classified as controlled substances. The Department of Justice, Drug Enforcement Administration (DEA) oversees use of these agents. A principal investigator must obtain a DEA license to order and use scheduled drugs, such as ketamine, pentobarbitol, buprenorphine, morphine, and several others. These must be kept behind double locks (a 2-lock safe or a locked safe in a locked room) and records must be kept of all quantities used. For information contact the U.S. Dept. of Justice, DEA, 3010 N. 2d Ave, Suite 301, Phoenix, AZ 85012.

6. Good laboratory practice acts (GLPs). The Food and Drug Administration requires Standard Operating Procedures, strict (and audited) quality control, and rigorous record keeping for FDA safety assessment studies. These studies include preclinical and clinical studies of drug efficacy and toxicity, new medical devices, and food additives. GLP regulations do not apply to most university studies but certain contract studies with pharmaceutical, biomedical, or device companies may have to be performed as per GLP regulations. It is imperative that you prepare for this in advance; the FDA will not accept data not performed as per GLPs.

THE IACUC

Both the AWA and PHS require the IACUC to oversee use of animals in research and teaching. The primary mission of the IACUC is to ensure regulatory compliance and humane care and use of animals through enforcement of AWA and PHS policies. Most IACUCs also compose additional policies and operating procedures to address welfare concerns specific to their institution's research program. The IACUC reports directly to the Institutional Official (IO), who is responsible to the federal government for institutional regulatory compliance. At NAU, the IO is the Vice Provost for Research and Graduate Studies.

IACUC composition is clearly stipulated by Federal regulation. At least one scientist, one non-scientist, one non-university employee (outside member), and one veterinarian trained in laboratory animal medicine must be on the committee. The outside member represents the community to assure concerns of general public are heard. The veterinarian is to assure that choice of animal species is suitable to the study and that all aspects of humane care and use of animals are addressed.

The IACUC protocol. The first step an investigator must take before beginning any use of animals at NAU is completion of an IACUC protocol form. No animals may be obtained or used before the IACUC formally

approves the protocol. The protocol form is available on the webpage of the NAU Office of the Vice Provost for Research and Graduate Studies.

Protocols with potentially painful procedures must be prepared in consultation with the Institutional Veterinarian, who reviews procedures to ensure less invasive techniques are not available, and that pain and distress alleviation are sufficient. The investigator then sends the completed protocol form to the IACUC chair, who distributes the protocol to the committee for review.

For the IACUC approval, specific items must be addressed in the protocol, including:

1. All who will work with vertebrate animals must be listed and have completed training in humane use of animals as well as all techniques in the protocol. They must also participate in the University occupational health program before working with animals.
2. A brief abstract must explain why animals must be used in this work. The abstract must be written in lay terms understandable to the non-scientist committee members.
3. In addition to the lay abstract, the investigator must provide a detailed description of experimental design, data collection and analysis. Regulations stipulate animal research is defensible only if it advances human or animal health, scientific knowledge, or benefits society.
4. Housing must be appropriate for the species and allow for animals' health and comfort.
5. The investigator must explain why the study must be performed on vertebrate animals.
6. Investigators must explain why the species chosen is appropriate and justify the number of animals used (statistically if possible). The investigator must document that animals will be obtained legally and transported according to Federal regulations.
7. A description of steps to minimize pain and distress is required, as discussed below.
8. Surgical techniques must be described in detail, as discussed below.
9. Post-procedural analgesia must be appropriate to the procedures, as discussed below.
10. *A literature search or similar documentation must verify this work does not unnecessarily duplicate previous work and that no less invasive, painful, or stressful procedures are available. Alternatives to animal use (computer models, in vitro systems) must be considered in this search, as discussed below.*
11. The investigator must explain euthanasia methods and assure they are humane and comply with the American Veterinary Medical Association (AVMA) Panel on Euthanasia. Animals suffering severe, unrelenting pain must be euthanized unless the IACUC previously approved an exemption based on scientific justification.
12. The investigator must submit current copies of all relevant permits.

Any exceptions to these procedures must be approved by the IACUC prior to their occurrence.

The IACUC does not usually judge scientific merit of protocols but may evaluate merit of non-peer reviewed studies (i.e. no NSF, NIH, etc. review) to ensure animal use is warranted. Use of animals may not be warranted if a study has limited scientific value.

Protocol Review. When a protocol is submitted, the IACUC chair assigns a primary reviewer to it. This reviewer is responsible for thoroughly evaluating the protocol but all committee members are given the opportunity to examine it. IACUC members have 2 weeks to review protocols after distribution. If any committee member has significant concerns about the animal welfare, justification for use of animals, occupational risks, etc., they may call for full committee review during this two-week period. Further consideration of the protocol must then be suspended until it is discussed at the next committee meeting. If no members call for full committee review, the primary reviewer compiles comments from all members and

their own review. They forward these comments to the committee chair, with one of the following recommendations:

1. Approve as proposed—procedures may begin as outlined and the project.
2. Approved with conditions—the project may not begin until all conditions are met.
3. Returned for clarification— key information necessary to determine if animal use is appropriate is missing; the protocol must be resubmitted with the additional information.
4. Approval denied—the project is not approved and cannot be performed.

Modifications to an approved protocol. Any changes in animal use must be approved as modifications to the original protocol. Thus, if your protocol states you will use 25 mice and ketamine anesthetic, you must submit a modification request to use more than 25 mice or to use pentobarbital. In most cases, a simple explanation and justification will suffice. Modifications must be submitted when new personnel (not previously listed on the protocol) will handle animals.

Annual reports of approved protocols. IACUC protocols may legally be valid for 3 years but annual reports must be submitted each year for protocols to continue. Failure to submit an annual report must result in protocol inactivation on the anniversary of approval. The investigator cannot then legally perform animal studies until annual reports allow reactivation. Failure to submit reports will also prevent IACUC approval of other activities for the investigator. The third annual report is a final report, as protocols may not extend beyond 3 years. If a study continues for more than 3 years, investigators must submit a new full protocol. Forms for modifications, annual, and final reports may be downloaded from the web at http://www.nau.edu/%7Egrants/ac_fms.htm. The renewal protocol will not be approved until a final report is filed for the previous protocol.

Protocol suspension. The IACUC must suspend ANY activities that deviate from the approved protocol and must report such suspensions to the USDA (for regulated species) and applicable funding agencies. Funding agencies often stop financial support to entire institutions while investigating alleged non-compliance. If non-compliance is confirmed, the agency often permanently withdraws financial support from the study.

Several topics create challenges for those composing protocols. Some of the more common of these topics are:

Potentially painful or distressful procedures. Studies must be designed to minimize animal discomfort, distress, and pain. Anything that may be painful to a human (including a child) must be considered painful or distressful to animals. Investigators must describe steps taken to minimize pain and distress including: analgesics, acclimating animals to the study system to reduce stress, anxiolytics, specialized surgical training, etc. If pharmaceuticals or acclimatization will interfere with the study, the investigator must provide strong scientific evidence of this and receive IACUC approval before the study. Analgesics must be given after potentially painful procedures unless the IACUC previously approves withholding analgesia based on strong scientific justification. Investigators must also document that no alternatives exist to potentially painful procedures. The most common way to document no alternatives for painful or distressing procedures is a computer data base literature search. The protocol must contain the following information about such searches: the names of the databases searched (i.e. Medline, Biosys, etc.), dates of the database searched (i.e. Medline 1966-present), key words used, the date the search was performed, and who performed the search. The search should document there are no alternatives to potentially invasive or painful procedures and that the research is not duplicative. Occasionally, alternative information sources may substitute for the database search. Check with the IACUC chair or Institutional Veterinarian if you have questions about this requirement.

Surgical Procedures. The IACUC must have very specific information about surgical procedures before it can approve them. Required information includes: location where surgeries will be performed, steps taken to ensure asepsis, anesthetic monitoring, recovery methods, and post-operative analgesia. Surgery cannot be performed without adequate anesthesia; paralytics are rarely approved and cannot be used without anesthetics. Survival surgeries must be conducted aseptically, with sterile gloves, sterile instruments, sterile skin preparation, and use of a sterile surgery field. Routine surgical skin preparation is not recommended for amphibians or fish, as it damages the slime layer and fosters post-operative infections. As per the Guide, surgeries on non-rodent species must be performed in the designated surgical suite in the Annex. The Guide states that surgery on rodents may be performed in laboratories but only in an area designated exclusively for surgery. The NAU IACUC also allows surgeries on some ectotherms to occur in similar surgical areas in investigator laboratories. Field surgeries must also utilize aseptic technique.

Exogenous heat sources are required for most species during surgery and recovery. Hypothermia is a significant cause of anesthetic death in many species. Failure to provide exogenous heat for reptiles may extend the anesthetic recovery to several days!

Post-anesthetic care. Any time an animal is anesthetized, post-anesthetic care must continue until animals have regained righting and swallowing reflexes, and spontaneous breathing. Such care must include sufficient monitoring, exogenous heat sources, and supplemental oxygen as needed.

Death as an endpoint. The term "Death as an endpoint" is potentially confusing. Many studies require euthanasia for tissue analysis, etc. This is not "death as an endpoint." Rather, this refers to not euthanizing moribund animals but leaving them to die on their own. This practice is rarely justifiable scientifically and regulations prohibit use of death as an endpoint without strong scientific justification. For studies in which animals will likely die, investigators must usually choose a point at which animals will be euthanized. In almost every case, such points provide reliable data while decreasing animal distress.

Adoption. Under certain circumstances research animals may be transferred to other investigators or adopted to private homes at the end of studies.

Euthanasia. All forms of euthanasia must comply with the AVMA Panel on Euthanasia unless the investigator can provide scientific justification of alternative methods.

Where animals may be housed. Usually animals must be housed in the Research Annex vivarium and may not be held for more than 12 consecutive hours in other locations without scientific justification and previous IACUC approval. Occasionally the IACUC approves alternate housing in investigator laboratories. All housing must comply with regulations and university policies and is subject to IACUC semi-annual inspection. All animals must be cared for daily; the University Veterinarian must examined unexpectedly ill or dying animals. Animals in field studies are rarely held for more than brief periods of time. You must provide scientific justification to hold animals in field situations. USDA inspectors may also demand they inspect any field facilities in which you hold regulated species for more than 12 hours.

Animal procurement. No live vertebrates may be obtained or used without prior IACUC approval and notification of Annex personnel before animals are ordered. Annex personnel must monitor procurement to ensure animals are obtained legally and that the number of animals does not exceed that approved by the IACUC. Most vertebrates may only be obtained from approved vendors to assure that only pathogen free animals enter the facility. The IACUC and University Veterinarian must previously approve exemptions to this policy.

Other IACUC responsibilities. The IACUC must ensure animal use is humane and justified but their responsibilities and oversight of animal use does not end with protocol approval. The committee ensures

ongoing regulatory compliance of all studies, continued humane care and use of vertebrate animals, and adequacy of training and occupational health programs for those working with animals. Thus, Federal regulations require that the IACUC:

1. Review and approve all changes in animal activities via protocol modifications;
2. Inspect animal housing areas and laboratories every 6 months;
3. Ensure adequate veterinary care and oversight;
4. Maintain records to verify compliance with all regulations;
5. Over see investigator and student training required by law (such as this module).

Mechanisms for reporting problems (whistleblower policy). The AWA and Guide require institutions to investigate suspected animal abuse and non-compliance with regulations or IACUC protocols. Serious complaints (as determined by the IACUC) must be reported to Federal authorities. Both State and Federal law protect any person who reports suspected violations from retaliation. To report suspected violations or problems in animal care, contact any of the following:
1. Dr. Roger Van Andel, the University Veterinarian (523-6714);
2. IACUC chairperson ;
3. or Dr. Carl A. Fox, Vice Provost for Research and Graduate Studies (523-4340).

Visitors. Visitors are prohibited from entering animal rooms or engaging in animal-related activities (including field studies) unless informed of hazards unique to the area and pre-authorised by the Institutional Veterinarian. Persons involved in one time animal contact laboratories, such as educational laboratories, will be assessed on a case-by case basis but all participants must be advised of allergy or zoonotic disease risks. Gloves and lab coats should be available for all participants and surgical masks should be available for those who request them. Individuals with more than casual, one time animal exposure must be added to the protocol via a modification and must complete humane care and use and occupational health questionnaire.

Occupational health and safety. A wide variety of laboratory animals are used in research and teaching. They generally fall into two categories: Specific Pathogen Free (SPF) and conventional. SPF animals include most laboratory-bred rodents. Conventional animals include farm animals, wild animals, etc., although some pigs are SPF. Although all animals are potential sources of zoonoses (infections spread from animals to humans), SPF rodents pose more of an allergy risk than a risk for spreading zoonotic disease. Non-SPF animals spread several zoonotic diseases if appropriate precautions are not taken.

The IACUC and University Veterinarian are responsible for ensuring that all faculty, students, and laboratory participants have proper occupational health and safety training and risk assessment prior to animal exposure. Individuals must fill out health risk questionnaire before beginning animal work. Health care providers at Fronske Student Health Center then review the questionnaires and schedule examinations for those with more than casual animal contact. If potential problems such as allergies, etc. are discovered, individuals are advised of preventive measures that may avoid medical complications. Any exposures to potentially infectious animal body fluids or tissues or any development of allergies to animals should be reported to the IACUC or University Veterinarian and the appropriate health care provider. Current tetanus vaccination is required for all who work with animals. Other vaccinations may be required for specific studies. For projects where animals are exposed to test substances that pose a threat to human health, separate programs will be initiated and specific standard operating procedures (SOPs) created based on situation.

Health risks by species. The following is a partial list of zoonoses that may be encountered at NAU. The following is a general but not comprehensive overview of common zoonoses in laboratory and field settings. Many diseases not usually zoonotic may present problems for immunocompromised individuals. Please contact the University Veterinarian if you require more specific information.

Domestic or Laboratory Rodents/Rabbits: Most rodents used in research are specific pathogen free (SPF). The major zoonotic risk with these animals is allergies. Rats are one of the most allergenic animals, up to 30% of people working with rodents for prolonged periods develop allergies. Many people also develop allergies to rabbits. These allergies are often serious and may prevent the individual from continuing work with rodents. In the worst cases, allergies and asthma may be life threatening. The most allergenic proteins are in the urine and saliva. Lymphocytic choriomeningitis virus has been reported (rarely) in some laboratory-bred rodents. The disease causes swelling of the brain in humans and is potentially fatal. As such, it is the most serious zoonotic disease of these animals, although it is extremely rare.

Wild Rodents/Rabbits:

Allergens--Rodent and rabbit salivary and urinary proteins and dander are highly allergenic.

Dermatomycosis (ringworm)--This fungus causes scaly skin lesions that are easily treated.

Hantavirus--Hantavirus is the most serious zoonoses of wild rodents. It is endemic in Arizona deer mice (*Peromyscus maniculatus*), which are the most common carrier. Many other rodent species also carry the virus. Zoonotic disease often occurs via fecal aerosol; direct contact with infected rodents is not required. Care should be exercised when entering areas or facilities with high concentrations of rodent feces. Hantavirus causes pneumonia in people with a 70% case fatality rate. There are no effective treatments. Precautions to take when working with wild rodents, including double gloving, use of respirators (surgical masks are insufficient), and protective clothing like long-sleeve laboratory coats.

Lymphocytic Choriomeningitis Virus--As mentioned above, this flu-like disease may progress to serious inflammation of the brain but is rare.

Plague (*Yersinia pestis*)--Spread by the rat flea, this flu-like disease is most commonly associated with prairie dogs. The organism may be spread via rat flea or, occasionally, by respiratory droplets. May progress to fatal septicemia; respiratory exposures are often fatal. This is a very serious disease but is curable if treatment begins early.

***Staphylococcus* and *Streptococcus* spp**.--These bacteria are usually spread by bites. SPF rodents may have these bacteria.

Rabies--Rabies is discussed more completely below. It is rarely seen in this group of animals. They are susceptible to the virus but quickly die when demonstrating abnormal behavior.

Rat-bite fever (*Streptobacillus moniliformis*)--This flu-like disease spread by bite wounds is usually not serious.

Tularemia (Francisella tularensis)--A non-specific flu-like disease with an incubation period of 1-10 days, tularemia often progresses to fatal septicemia. Spread is by aerosol, ingestion, direct contact, or by arthropods (ticks). This is commonly seen when people necropsy animals but don't wear gloves. Lesions in infected animals include white spots on the liver and occasionally in the spleen or lymph nodes. The disease is most common in wild rabbits but may be seen in some rodents as well.

Amphibians, Reptiles, and Fish: Zoonotic diseases of amphibians, reptiles, and fish are common; wear gloves if possible and wash hands after handling animals.

***Campylobacter, Yersinia enterocolitica,* and other enterics (*Klebsiella*, *Aeromonas*, *Citrobacter*, *Serratio*, etc.)**--These are common in most of these animals; symptoms are similar to Salmonella.

Equine encephalitis-- Reptiles and amphibians may carry the virus but it is unknown whether they are a source for human infection

Erysipelothrix rhusiopathiae--Crocodilians are known to carry this bacterium that may cause septicemia in people.

Fungal infections seen include Aygomycosis, Aspergillus, etc.

Mycobacteriosis--Several amphibians and reptiles carry *Mycobacteria* (mariner's itch [*M. marinum*], rarely TB). Human are infected through scratches, bites, or during cage cleaning.

Parasites-- Worms are common in reptiles and amphibians; most are not zoonotic. Zoonotic infections with reptile pentastomids are rare but are the most serious. Pentastomid eggs can contaminate cages and spread during cleaning. In humans they may encyst in lymph nodes, liver, lungs, etc., calcify, and induce inflammation. Reptile ticks are not zoonotic but can spread zoonoses, including relapsing fever, etc.
Q-fever--This disease has been spread by pythons. It is described under "Farm Animals."
Salmonellosis--This bacterium is very common in these animals and causes severe gastroenteritis (vomiting, diarrhea), occasionally septicemia, disseminated abscesses and rarely death. As such, it is usually considered the most serious zoonotic disease of these animals. Many animals are asymptomatic carriers; up to 90% of various populations shed *Salmonella* in feces. *Salmonella* cannot usually be eliminated but antibiotics can cause increased resistance. Animals can be infected before or at birth through contact with other animals or by eating feces (typical for hatchlings).
Ticks--Reptile ticks are not zoonotic but can spread equine encephalitis virus, etc.

Birds:

Campylobacter spp.--The zoonotic potential of avian *Campylobacter* has not been fully investigated. These bacteria may cause diarrhea in children.
Equine Encephalitis Viruses-- Birds may act as reservoirs for Equine Encephalitis, and a few other zoonotic viruses, depending on the avian species.
***E. coli*--**Severe forms are rarely spread from birds but can be found in feces.
Giardia--birds may serve as a reservoir of *Giardia spp.*
Listeria--Avian sources of this bacterium reportedly cause human conjunctivitis.
Psittacosis--Caused by *Chlamydiophilia(Chlamydia) psittaci*, psittacosis is usually contracted from inhaled organisms from psittacine (parrots, etc.), duck, and turkey feces. Transmission occurs by fecal aerosolization, which may be facilitated by feather dust.Strains from other bird species appear to be less virulent for humans but cases from pigeons have been reported. Clinical signs vary but include acute fever, headache, rash, muscle pain, chills, and shortness of breath. Occasionally disease may become serious, with symptoms of debilitation, pneumonia, meningitis, kidney disease, and liver lesions if untreated.
Salmonella spp.--Birds commonly carry this bacterium but human infection from birds is rare. Signs in people are as described above.
Tularemia--as described below. Avian transmission is unlikely but birds carry the bacterium.
Tuberculosis--Many birds carry *M. avium intracellulare*. Not traditionally a common cause of human tuberculosis, these species have become a major concern in immunosupressed humans, especially HIV patients.
Yersinia pseudotuberculosis--Birds are a major reservoir but the bacterium is more common in Europe than in the US. Symptoms include severe intestinal inflammation, headaches, anorexia, vomiting, arthritis, skin sores, and occasionally death. Treatment is challenging.

Cats and Dogs:

Allergens--Cat salivary proteins and dander are highly allergenic.
Cat-scratch fever (*Bartonella henesleii*)--This organism usually causes self-limiting lymphadenopathy.
Cryptosporidiosis, *Giardia*--These enteric organisms may be zoonotic from dogs and cats, the evidence is unclear.
Dermatomycosis (ringworm)--Skin infection caused by these fungi is usually easy to treat.
Parasites-- A few roundworms of dogs and cats are zoonotic. The major parasites of concern are of the genus *Ancylostoma* and *Toxocara*, causing cutaneous or visceral larval migrans, respectively (parasites migrate through subcutaneous tissues or major organs).
Rabies-- Rabies is rare but devastating, causing severe neurological symptoms and death. All bites by wild mammals and other potentially infected animals should be reported immediately to one's

supervisor. Care should be exercised when handling wild animals or tissue, as infected animals may shed the virus in the saliva for days before signs of illness appear. Rabies vaccination is available for those working with high-risk species. Those who refuse vaccination will not be allowed to work with high-risk species.

Staphylococcus* and *Streptococcus* spp., *Capnophagia spp., etc. are often spread by bites. They cause mild to moderate subcutaneous infections.

Toxoplasmosis--Toxoplasmosis is caused by *Toxoplasma gondii*. This is common in cats, although most people are infected from eating undercooked beef. Cats spread the organism via feces. The organism must sporulate for 48 hrs post-defecation before it is infective, so daily litter box changes prevent infection. Disease is usually mild, with swollen lymph nodes, mild fever, fatigue, and headaches. Rarely, more serious illness involves the lungs, heart, brain or liver. The biggest risk for severe toxoplasmosis is for immunocompromised individuals and fetuses. Severe damage may occur in developing fetuses, with miscarriage, stillbirth, or congenital defects resulting. *T. gondii* titer evaluation is recommended for females of childbearing age exposed to cat feces or *T. gondii*. Women may wish to request their supervisor t for temporary job reassignment during pregnancy.

Wild Non-Rodent Mammals (skunks, bats, foxes, racoons, opossums, etc.):

Dermatomycosis (ringworm)--As described above.

Leptospirosis--the bacterium is spread primarily through urine. It can infect numerous organs in humans, causing fever, headache, rashes, and occasionally death.

Parasites-- The raccoon ascarid worm, genus *Baylisascaris* (and occasionally the skunk ascarid), can be an agent of larval migrans, preferrentially migrating through Central Nervous System tissues or the eye. There is no effective treatment.

Rabies--Rabies is endemic in Arizona skunks, foxes, and bats; the disease has never been documented in North American opossums. Occasionally the disease is seen in other species of wild and domestic animals. Although rodents are theoretically susceptible to rabies, cases are very rare and zoonotic risk has not been confirmed. Occasionally rabies is spread through saliva or bat feces (guano) without bite or scratch wounds. The virus can remain viable in frozen tissues for an extended period. Anyone who works with wild carnivores, some other species of wild mammals, or bats must receive rabies prophylaxis (vaccination) before beginning work.

Salmonellosis--As described above.

Farm Animals: Zoonotic diseases of horses, cattle, sheep, goats, and pigs include:

Dermatomycosis--As described above.

Erysipelothrix rhusiopathiae--Pigs often carry this bacterium.

Leptospirosis--As described above.

Rabies--Although rabies is often thought of as a disease of carnivores or bats, cattle and horses have been a source of human exposure.

Orf--This poxvirus causes self-limiting skin vesicles in sheep and people.

Q fever-- The spirochete *Coxiella burnetii* causes this disease, which is spread in ruminants uterine fluids. Human disease is rare but has been seen in researchers and support staff. The disease is flu-like but can progress to serious endocarditis, particularly in those with other cardiac disease. Illness can be life threatening.

Salmonellosis--Salmonelloisis relatively rare in these species, except horses.

Non-human primates:

Dermatomycosis--As described above.

Enteric bacterial infection--The most common zoonotic diseases of NHPs are enteric organisms that can cause serious diarrhea in humans. Common diseases include: shigellosis, campylobacteriosis, yersiniosis, and entamoebiasis. These enteric organisms are shed when primates develop stress-related diarrhea. Human infections can result in serious diarrhea.

Herpes B virus--This virus is related to the human cold sore virus. More than 90% of macaques carry the virus. It is present in saliva, feces, and other fluids of viremic monkeys. Viremia is usually induced by stresses such as transport, anaesthesia, etc. Non-viremic animals are not contagious. Human cases are very rare (less than 70 in the 1900's) but the case fatality rate exceeds 70%. Infection is spread to humans by bites, scratches, or infected bodily fluids. Antiviral agents (acyclovir, etc.) may prevent death but must be given lifelong.
Poxviruses--Several pox viruses infect macaques and are zoonotic. These organisms are rare but not usually fatal to humans.
Tuberculosis-- Tuberculosis is often caused by *Mycobacterium tuberculosis* or *M. bovis*. As discussed above, tuberculosis associated with birds may be caused by *M. avium* or *M. intracellularae*. Multi-drug resistant TB is an "emerging disease" in the US but in a research facility tuberculosis is more often anthroponotic rather than zoonotic. This means humans transmit the disease to research animals, particularly old-world primates, rather than vise-versa. Individuals who work with non-human primates must document they are tuberculosis-free via negative skin test every 6 months. Those with false positive skin tests must obtain a chest radiograph every 5 years to document no pulmonary lesions.

Protection. The following steps help prevent spread of zoonotic disease or development of allergies:

Personal protective equipment: All personnel should wear dedicated lab coats and gloves to work with animals. Hypoallergenic gloves are available to decrease risk of latex allergy development. Hands should be washed after completing procedures involving animals. Persons at high risk of developing allergies should also consider wearing NIOSH-approved N95 respirators. A respiratory function test may be recommended prior to use of these respirators. Additional personal protective equipment (PPE) may be appropriate for particular species or studies. Those who work with other high-risk studies/species, such as hantavirus studies, must contact occupational health personnel to discuss appropriate personal protective equipment.

Suspected allergies or infectious agent exposure. Individuals who suspect allergic or infectious disease exposure should immediately vacate the animal room or laboratory. Serious or life-threatening exposures are medical emergencies and appropriate care must be pursued immediately. For less serious situations, the person should immediately remove contaminated PPE and wash hands, face, or affected areas with soap and water, and report to their supervisor. The University Veterinarian should also be contacted to formulate plans for future work.

Vaccinations. Vaccinations are not available or recommended for most laboratory animal zoonoses. Rabies vaccination may be required for those working with high-risk species (bats, skunks, etc.).

Coxiella burnetii (Q-Fever) titer may be considered for those working with pregnant ruminants.

Toxoplasma antibody titer may be considered for those working with cats and other species harbouring parasites. Toxoplasma is of minimal risk for immunocompetent non-pregnant workers.

Tuberculin testing. Although tuberculosis is a zoonotic disease of primates, the risk of spread from humans to primates is much greater than risk of zoonotic spread. Employees working with non-human primate contact must contact the University Veterinarian to establish a regular testing regime.

Alternative employment. Employees with medical conditions that preclude continued animal work are offered different positions. This includes pregnant or immunocompromised personnel.

Illness. It is recommended that a physician, in consultation with the University Veterinarian, evaluate all personnel with direct laboratory animal contact who develop unexpected illnesses.