General Information:

The Guide: Protocol Review

The animal use protocol is a detailed description of the proposed use of laboratory animals. The following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC:

• a clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee

• availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A, Alternatives)

• justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis; see Appendix A, Experimental Design and Statistics)

• unnecessary duplication of experiments

• nonstandard housing and husbandry requirements

• impact of the proposed procedures on the animals well-being

• appropriate sedation, analgesia, and anesthesia (indices of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A, Anesthesia, Pain, and Surgery)

• conduct of surgical procedures, including multiple operative procedures

• postprocedural care and observation (e.g., inclusion of post-treatment or postsurgical animal assessment forms)

• description and rationale for anticipated or selected endpoints

• criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated

• method of euthanasia or disposition of animals, including planning for care of long-lived species after study completion

• adequacy of training and experience of personnel in the procedures used, and roles and responsibilities of the personnel involved

• use of hazardous materials and provision of a safe working environment.

General Information:

USDA: Protocol Review

The USDA has different requirements for certain animals which are outlined throughout this document. The most common USDA animals that have been used at UTA in the past are hamsters, rabbits, and guinea pigs.
PROTOCOL APPLICATION FORM - SPECIFIC GUIDANCE

1-3. No additional guidance

4. Funding Source

OLAW: Is the IACUC required to review the grant application?

Public Health Service (PHS) Policy and the NIH Grants Policy Statement (Part II, Terms and Conditions) require the institution to verify, before award, that the IACUC has reviewed and approved those components of grant applications and contract proposals related to the care and use of animals. This is not an explicit requirement for the IACUC to do a side-by-side comparison of an application/proposal and the IACUC protocol. However, institutions are responsible for ensuring that the information the IACUC reviews and approves is congruent with what is in the application/proposal. Institutions are free to devise a workable mechanism to accomplish this end. One method to prevent inconsistencies between the information submitted to PHS and that on the IACUC protocol is to implement a procedure for direct comparison. Some institutions have delegated this responsibility to a particular office or position (e.g., sponsored programs or compliance office); others ask departmental chairs to verify consistency. A11

5. Peer Review

OLAW: Is the IACUC responsible for judging the scientific merit of proposals?

Peer review of the scientific and technical merit of an application is considered the purview of the NIH Scientific Review Groups (SRGs), which are composed of scientific experts from the extramural research community in a particular area of expertise. However, SRGs also have authority to raise specific animal welfare concerns that can require resolution prior to a grant award. Although not intended to conduct peer review of research proposals, the IACUC is expected to include consideration of the U.S. Government Principles in its review of protocols. Principle II calls for an evaluation of the relevance of a procedure to human or animal health, the advancement of knowledge, or the good of society. Other PHS Policy review criteria refer to sound research design, rationale for involving animals, and scientifically valuable research. Presumably a study that could not meet these basic criteria is inherently unnecessary and wasteful and, therefore, not justifiable. The primary focus of the SRG is scientific merit and the primary focus of the IACUC is animal welfare. The two bodies have differing constitutions, mandates and functions. However, since it is not entirely possibly to separate scientific value from animal welfare some overlap is inevitable. SRGs may raise concerns about animal welfare and IACUCs may question the scientific rationale or necessity for a procedure. A1

6-8. No additional guidance
9. USDA Classification of Animal Use

*Include total anticipated period of project funding and animal use. Anticipated use of animals more than three years beyond approval date should be included, even though new IACUC approval will be required.

USDA Reporting:

Our institution must make an annual report to the USDA regarding how many USDA classified animals were used in which pain categories. If animals are Category E, justification is required.

11. Special requirements for maintaining Animals

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

VII. The living conditions of animals should be appropriate for their species and contribute to their health and comfort. Normally, the housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied. In any case, veterinary care shall be provided as indicated.

AAALAC: Cage or Pen Space

AAALAC International expects accredited institutions to comply with all national or regional regulations, policies and guidelines, as well as conditions of funding. Additionally, AAALAC International considers performance standards paramount when evaluating the space made available in cages or pens for housing animals used for research, testing or teaching. The performance criteria described in the ILAR Guide, Ag Guide and ETS 123 are used by AAALAC in assessing the adequacy of cage or pen space available to the animal(s).

AAALAC: Social Housing

The Guide states that single housing of social species should be the exception. Social housing will be considered by AAALAC International as the default method of housing unless otherwise justified based on social incompatibility resulting from inappropriate behavior, veterinary concerns regarding animal well-being, or scientific necessity approved by the IACUC (or comparable oversight body). When necessary, single housing of social animals should be limited to the minimum period necessary and, where possible, visual, auditory, olfactory and, depending on the species, protected tactile contact with compatible conspecifics should be provided. In the absence of other animals, additional enrichment should be offered, such as safe and positive interaction with the animal care staff, as appropriate to the species of concern; periodic release into larger enclosures; supplemental enrichment items; and/or the addition of a companion animal in the room or housing area. The institution's policy and exceptions for single housing should be reviewed on a regular basis and approved by the IACUC (or comparable oversight body) and/or veterinarian.
OLAW: May performance standards determine housing issues?

Performance standards are to be applied to housing issues. Outcome-based performance standards are paramount when evaluating cage or pen space for housing animals used for research, research training, and biological testing. While the Guide’s space recommendations are accepted reference points for addressing space needs (Guide pages 50-63), performance standards allow flexibility to improve animal welfare and scientific research. (Guide pages 6-7) An institution’s animal housing practices must be species-specific, appropriate for the animals, and in compliance with all applicable federal and local regulatory requirements. Compliance with the applicable regulations (9 CFR Subchapter A) issued by the U.S. Department of Agriculture under the Animal Welfare Act are an absolute requirement of the PHS Policy (Footnote 2, page 9).

OLAW: May performance standards determine environmental enrichment issues?

An institution’s environmental enrichment practices must be species-specific and appropriate for the animals. Devices that animals climb on or through, perch on, or nest in contribute to, rather than detract from, the animal’s living space and need not be subtracted from the floor dimensions. Some species are upset by the introduction of novel items. Animals should not be subjected to the presence of items that they find distressing. (See Guide pages 52-54). Compliance with the applicable regulations (9 CFR Subchapter A) issued by the U.S. Department of Agriculture under the Animal Welfare Act are an absolute requirement of the PHS Policy (Footnote 2, page 9).

OLAW: Can performance standards be used in determining rabbit housing practices?

OLAW concurs with the Guide that animals "should be housed under conditions that provide sufficient space...to meet physical, physiologic, and behavioral needs." (Guide pages 50-51). The height of an enclosure can be important to allow for expression of species-specific behaviors and postural adjustments. Cage height should take into account the animals typical posture and provide adequate clearance for the animal from cage structures, such as feeders and water devices (Guide page 56). Space allocations should be assessed, reviewed, and modified as necessary by the IACUC considering the performance indices and special needs determined by the characteristics of the animal (Guide page 56). IACUCs may consider the use of a rabbit cage that is 14 inches in height, if appropriate for specific animals. The IACUC should establish, through performance indices related to animal well-being, that the cage provides sufficient space to meet the physical, physiologic and behavioral needs of the animal. For example, the rabbit must be able to hold its ears in an upright position (if this is natural for the breed) and ears must not be forced to fold over by contact with the cage ceiling.

12. Animal Transportation

OLAW: What are the institution’s responsibilities in ensuring that animals are shipped safely and in reporting adverse events that occur in shipment of animals to or from the institution?

Animals should be transported according to international, federal, state and local regulations summarized in the Guide (page 107). Needs of the animals and protection of personnel should be considered in advance of transportation and met during loading, transportation and unloading, as described in the Guide (pages 107-109). OLAW expects all parties involved in the transportation of
animals to apply due diligence in assuring that animals are shipped under appropriate conditions to prevent morbidity or mortality due to temperature extremes or other adverse events. When animals are shipped from an institution, that institution should consider and address all relevant factors to ensure safe transport of the animals. OLAW expects shipping institutions to report adverse events that occur to animals in transit. Receiving institutions should notify the shipping institution when animals are received in extremis or dead.

The Guide: Animal Transportation

For noncommercial sources of animals, in particular, it is important for the veterinarian or the veterinarian's designee to review the health status and other housing and husbandry requirements before authorizing shipment of animals. This action will ensure that effective quarantine practices are implemented for incoming animals and address any special requirements needed to ensure animal well-being (Otto and Tolwani 2002). Special considerations may be necessary for transporting animals during certain phases of their life or in certain conditions, such as pregnant, perinatal, and geriatric animals; animals with preexisting mellitus; and animals surgically prepared by the supplier (FASS 2010). Although ensuring animal biosecurity during transportation is always important, it is of particular importance for immunocompromised, genetically modified, and specific pathogen-free rodents (Jacoby and Lindsey 1998). For these animals, reinforced disposable shipping containers with filter-protected ventilation openings and internal food and water sources help ensure that microbial contamination does not occur during transit. Transportation of animals in private vehicles is discouraged because of potential animal biosecurity, safety, health, and liability risks for the animals, personnel, and institution. For aquatic species and amphibians, special considerations are required for transportation in an aqueous or sufficiently moist environment, and special attention should be given to avoiding temperature extremes for poikilotherms.

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training: I. The transportation, care, and use of animals should be in accordance with the Animal Welfare Act (7 U.S.C. 2131 et. seq.) and other applicable Federal laws, guidelines, and policies.

UTA IACUC Protocol Requirement:

Transportation of animals must conform to all institutional guidelines/policies and federal regulations. If transporting animals, please complete and submit alongside your application the Request for Transportation of Animals form.

16. Invasive Procedures (other than blood collection, catheterization, intubations, etc.)

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

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.
V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

16(a-c). Anesthesia

The Guide: Anesthesia

The selection of appropriate analgesics and anesthetics should reflect professional veterinary judgment as to which best meets clinical and humane requirements as well as the needs of the research protocol. The selection depends on many factors, such as the species, age, and strain or stock of the animal, the type and degree of pain, the likely effects of particular agents on specific organ systems, the nature and length of the surgical or pain-inducing procedure, and the safety of the agent, particularly if a physiologic deficit is induced by a surgical or other procedure (Kona-Boun et al. 2005). Preemptive analgesia (the administration of preoperative and intraoperative analgesia) enhances intraoperative patient stability and optimizes postoperative care and well-being by reducing postoperative pain (Coderre et al. 1993; Hedenqvist et al. 2000). Analgesia may be achieved through timely enteral or parenteral administration of analgesic agents as well as by blocking nociceptive signaling via local anesthetics (e.g., bupivacaine). Most anesthetics cause a dose-dependent depression of physiologic homeostasis and the changes can vary considerably with different agents. The level of consciousness, degree of antinociception (lack of response to noxious stimuli), and status of the cardiovascular, respiratory, musculoskeletal, and thermoregulatory systems should all be used to assess the adequacy of the anesthetic regimen. Interpretation and appropriate response to the various parameters measured require training and experience with the anesthetic regimen and the species.

OLAW Frequently Asked Questions - PHS Policy on Humane Care and Use of Laboratory Animals, Use of Non-Pharmaceutical-Grade Compounds in Animals:

In compliance with Federal Animal Welfare Regulations and guidance and standard veterinary medical practice the IACUC expects that investigators will use pharmaceutical grade medications whenever they are available, even in acute/terminal procedures. Non-pharmaceutical grade compounds should only be used after specific review and approval by the IACUC for reasons such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost savings is not a justification for using non-pharmaceutical grade compounds in research animals.

UTA Guidelines:

Anesthesia in Laboratory Animals (includes Formulary)

Analgesia in Laboratory Animals (includes Formulary)

UTA IACUC Suggested References:

The following sites contain information regarding different types of anesthesia and analgesia and their common applications for different species:
18. **Restraint** (chairs, slings, tethers, stanchions, metabolism cages or other devices)

The Guide: Physical Restraint:

Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation. Animals are restrained for brief periods, usually minutes, in many research applications. Restraint devices should be suitable in size, design, and operation to minimize discomfort, pain, distress, and the potential for injury to the animal and the research staff. Prolonged restraint, including chairing of nonhuman primates, should be avoided unless it is essential for achieving research objectives and is specifically approved by the IACUC. When restraint devices are used, they should be specifically designed to accomplish research goals that are impossible or impractical to accomplish by other means or to prevent injury to animals or personnel. The following are important guidelines for restraint:

- Restraint devices should not be considered a normal method of housing, and must be justified in the animal use protocol.

- Restraint devices should not be used simply as a convenience in handling or managing animals.

- Alternatives to physical restraint should be considered.

- Personnel should be properly trained on the appropriate selection and use of restraint devices.

- The period of restraint should be the minimum required to accomplish the research objectives.

- Animals to be placed in restraint devices should be given training (with positive reinforcement) to adapt to the equipment and personnel.

- Animals that fail to adapt should be removed from the study.

- Provision should be made for observation of the animal at appropriate intervals, as determined by the IACUC.

- Veterinary care must be provided if lesions or illnesses associated with restraint are observed. The presence of lesions, illness, or severe behavioral change often necessitates the temporary or permanent removal of the animal from restraint.

- The purpose of the restraint and its duration should be clearly explained to personnel involved with the study.
19. Food/Fluid Restriction

OLAW: May investigators restrict animals' food and fluid?

Ingestion of food and fluid are requirements for proper nutrition. When food or fluid is restricted, the amount of the regulated item earned during testing and the amount of the regulated item freely given should be recorded to ensure each animal receives its minimum daily requirements. (NRC 2003, Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research, Washington: National Academies Press) The IACUC must evaluate the level of restriction and potential adverse consequences in regulating food or fluid. The IACUC must also evaluate the methods for assessing the health and well-being of animals involved in activities that regulate food or fluid consumption. The IACUC has the authority to approve scientific justifications for departures from the recommendations in the Guide. For instance, using scheduled access to food or fluid sources may be justified by describing procedures based on performance standards that assure adequate maintenance of hydration, body weight, and behavioral and clinical health. It may be necessary to monitor both food and fluid intake if regulation of one influences consumption of the other. The Guide discusses food and fluid restriction on pages 30-31.

17. Surgery (Survival, Multiple, Terminal)

OLAW: What are the requirements for conducting rodent survival surgery?

The Guide requires that aseptic technique be followed for all survival surgical procedures. The manner in which asepsis is achieved varies by species. Modification of standard techniques may be desirable or even required, but should not compromise the well-being of the animals. The design of a surgical facility should accommodate the species to be operated on and the complexity of the procedure to be performed.

(Guides page 144). For most rodent survival surgery, “an animal procedure laboratory is recommended; the space should be dedicated to surgery...and appropriately managed to minimize contamination from other activities conducted in the room at other times.” (Guides page 144).

Surgical procedures can be categorized as major or minor. (See Guides page 117.) Major survival surgery penetrates and exposes a body cavity, produces substantial impairment of physical or physiologic functions, or involves extensive tissue dissection or transection (e.g., laparotomy, thoracotomy, joint replacement, and limb amputation). Minor survival surgery does not expose a body cavity and causes little or no physical impairment (e.g., wound suturing, peripheral vessel cannulation, percutaneous biopsy, and most procedures routinely done on an outpatient basis in veterinary clinical practice). Animals undergoing a minor survival surgical procedure typically do not show significant signs of postoperative pain, have minimal complications, and quickly return to normal function. OLAW recognizes the authority of the IACUC to determine whether specific manipulations used in research are major operative procedures. The IACUC’s determination must be based on a detailed description of the procedure and the anticipated or actual consequences, as characterized by the investigator. In some cases, the classification by the IACUC of a procedure as major or minor may be readjusted post-procedurally depending on clinical outcome. If the IACUC, after thorough review, determines that the surgical procedure only penetrates but does not
expose a body cavity and that the procedure does not produce substantial impairment, the IACUC may conclude that it is not a major operative procedure.

U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training: Procedures with animals that may cause more than momentary or slight pain or in distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

USDA Animal Care Policy #3: "Non-survival surgeries require neither aseptic techniques nor dedicated facilities if the subjects are not anesthetized long enough to show evidence of infection." However, in addition to the duration of the procedure, other factors such as the degree of tissue trauma or the surgical site (e.g., gastrointestinal surgery) may influence the risk of intraoperative infection and/or sepsis for which the use of aseptic technique may be indicated. Non-survival surgeries of this type will be evaluated on a case-by-case basis to determine whether aseptic technique must be used. If non-survival surgery is used as a method of collecting tissues for subsequent implantation into another animal, the use of aseptic technique is a critical requirement in minimizing infection in the recipient animal and therefore must be used when operating on the donor animal.

UTA IACUC Requirement: Surgeries Not Requiring Use of Aseptic Technique

For non-survival procedures not requiring the use of aseptic technique, the following guidelines must be used. At minimum, a clean lab coat or gown, a mask, and clean gloves should be worn. However, please note that procedures conducted on animals exposed to hazardous agents may require additional appropriate personal protective equipment. The use of clean, non-sterile instruments and supplies is acceptable. Non-survival surgeries not performed aseptically or in a dedicated facility must be performed in a clean area, free of clutter, and using acceptable veterinary sanitation practices analogous to those used in a standard examination/treatment room. The location where surgery is conducted must not be used for other purposes during the time of surgery. Personnel present in the area must observe strict cleanliness practices for both themselves and the animals.

17(b). If performing Multiple Survival Surgeries on a single animal, please provide scientific justification, citing the number of surgeries to be performed.

The Guide: Multiple Survival Surgeries

Multiple Survival Surgical Procedures Surgical procedures in the laboratory setting may be categorized as major or minor (USDA 1985). Whether a procedure is major or minor should be evaluated on a case-by-case basis, as determined by the veterinarian and IACUC (NRC 2003b; Silverman et al. 2007; for additional discussion see Chapter 4, Surgical Procedures).

Regardless of classification, multiple surgical procedures on a single animal should be evaluated to determine their impact on the animal’s wellbeing. Multiple major surgical procedures on a single animal are acceptable only if they are (1) included in and essential components of a single research project or protocol, (2) scientifically justified by the investigator, or (3) necessary for clinical reasons.
Conservation of scarce animal resources may justify the conduct of multiple major surgeries on a single animal, but the application of such a practice on a single animal used in separate protocols is discouraged and should be reviewed critically by the IACUC. When applicable, the IO must submit a request to the USDA/APHIS and receive approval in order to allow a regulated animal to undergo multiple major survival surgical procedures in separate unrelated research protocols (USDA 1985, 1997a). Justifications for allowing animals not regulated by the USDA to undergo multiple survival procedures that meet the above criteria should conform to those required for regulated species. If multiple survival surgery is approved, the IACUC should pay particular attention to animal well-being through continuing evaluation of outcomes. Cost savings alone is not an adequate reason for performing multiple major survival surgical procedures. Some procedures characterized as minor may induce substantial postprocedural pain or impairment and should similarly be scientifically justified if performed more than once in a single animal.

OLAW: Are multiple major survival surgical procedures permitted on a single animal? Surgical procedures should be defined as major or minor on a case-by-case basis and evaluated by the veterinarian and IACUC to determine their impact on the animal’s well-being. (Guide pages 30, 117) Multiple procedures that may induce substantial post-procedural pain or impairment may be conducted on a single animal only if justified by the PI, and reviewed and approved by the IACUC. Multiple major surgical procedures on a single animal are acceptable only if they are:

- included in and essential components of a single research project or proposal;
- scientifically justified by investigator;
- or necessary for clinical reasons. (Note, clinically necessary procedures do not necessarily require review and approval by the IACUC in advance of the procedure.)

Cost savings alone are not an adequate justification for performing multiple major survival surgical procedures. (Guide page 30).

Note that under USDA regulations (AWR 2.31 (x) A-C), “No animal will be used in more than one major operative procedure from which it is allowed to recover, unless: (A) Justified for scientific reasons by the principal investigator, in writing; (B) Required as routine veterinary procedure or to protect the health or well-being of the animal as determined by the attending veterinarian; or (C) In other special circumstances as determined by the Animal Care Administrator on an individual basis. Written requests and supporting data should be sent to the Animal and Plant Health Inspection Service, Animal Care, 4700 River Road, Unit 84, Riverdale MD 20737-1234.”

OLAW: Are laparoscopic procedures considered major surgery?

Any laparoscopic surgery that produces substantial impairment of physical or physiological function must be considered a major operative procedure. Whether the laparoscopic procedure is classified as major or minor, the IACUC must ensure that the appropriate analgesia, sterile technique, and perioperative monitoring is employed.

AAALAC: What level of monitoring and record-keeping are expected for rodent surgery?

There is clear general consensus in relevant resources (e.g., the Guide, NRC 2011; Medical Records for Animals Used in Research, Teaching, and Testing: Public Statement from the American
College of Laboratory Animal Medicine, ILAR 2007; Rodents: Laboratory Animal Management, NRC 1996; Research Animal Anesthesia, Analgesia and Surgery, SCAW 2007) that monitoring of rodents during surgery is critical so that animals are maintained under a surgical plane of anesthesia and that therapeutic intervention can be provided should unexpected physiological responses occur. Perioperative assessment of the physiological status (especially body temperature, but depending on other factors, also respiratory rate, heart rate, blood pressure, blood gases, ECG, etc.) and anesthetic depth are valuable metrics for this purpose. Monitoring is also key to ensuring that sound research data will ultimately be collected from the animals. The level of detail contained in the records should accurately reflect the monitoring being performed.

Therefore, while AAALAC does not have a policy that stipulates the level of documentation for surgical procedures, the Guide does recommend that pre-surgical planning include consideration of record-keeping, and AAALAC would expect that this would occur and that the level of monitoring and record-keeping would be adjusted to the type of procedure, health of the animal, etc. Good record-keeping is also important so the Institutional Animal Care and Use or Oversight Body (IACUC/OB) can track whether or not a specific animal had undergone more than one survival surgical procedure, as multiple survival surgical procedures need to be handled in a specific manner by the IACUC/OB. To summarize, then, there is no “cookie-cutter” approach to monitoring and documentation associated with surgical procedures, but AAALAC site visitors would expect all the factors described to be evaluated by the IACUC/OB for all surgical procedures when making these determinations.

Note: Each investigator may develop his/her own surgical log. Template available from ACF Manager upon request.

20(b) and 21(b). For the drugs, biologics, or reagents listed in D above, if these agents are not human or veterinary pharmaceutical-grade substances, provide a scientific justification for their use and describe methods that will be used to ensure appropriate preparation and administration.

OLAW: May investigators use non-pharmaceutical-grade compounds in animals?

OLAW and USDA agree that pharmaceutical-grade chemicals and other substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and/or interfere with the interpretation of research results. However, it is frequently necessary to use investigational compounds, veterinarian- or pharmacy-compounded drugs, and/or Schedule I controlled substances to meet scientific and research goals. The IACUC is responsible for evaluating the potential adverse consequences of such agents when used for research. In making its evaluation, the IACUC may consider factors including, for example:

- grade,
- purity,
- sterility,
• acid-base balance,
• pyrogenicity,
• osmolality,
• stability,
• site and route of administration,
• compatibility of components,
• side effects and adverse reactions,
• storage, and
• pharmacokinetics.

The IACUC may use a variety of administrative methods to review and approve the use of such non-pharmaceutical-grade agents. For example, the IACUC may establish acceptable scientific criteria for use of these agents within the institution, rather than on a case-by-case basis. Investigators and IACUCs should consider relevant animal welfare and scientific issues including safety, efficacy, availability of pharmaceutical-grade compounds, and the inadvertent introduction of new variables. Cost savings alone are not an adequate justification for the use of non-pharmaceutical-grade or compounded drugs in animals. Although the potential animal welfare consequences of complications are less evident in non-survival studies, the scientific issues remain the same. The principles and need for professional judgment outlined above apply to non-survival studies.

Procedures that may cause more than momentary or slight pain or distress to animals must be relieved by sedation, analgesia, or anesthesia using veterinary or human pharmaceutical-grade compounds, unless the use of an investigational chemical or formulation is scientifically necessary, appropriately justified, and approved by the IACUC. The use of a non-pharmaceutical-grade euthanasia agent must meet the same criteria.

On March 1, 2012, OLAW, with USDA and AAALAC, offered additional information through a webinar on the Use of Non-Pharmaceutical-Grade Chemicals and Other Compounds in Research with Animals Use of Non-Pharmaceutical-Grade Chemicals and Other Compounds in Research with Animals. Here you will find a recording of the webinar, a transcript that includes answers to numerous questions, plus examples of situations for the use of non-pharmaceutical-grade substances.

• A pharmaceutical grade compound is a drug, biologic, or reagent that is approved by the Food and Drug Administration (FDA) or for which a chemical purity standard has been established by the United States Pharmacopeia-National Formulary (USP-NF), or British Pharmacopeia (BP). According to guidance from the FDA, "pharmaceutical secondary standards" are acceptable for use in clinical animal studies if obtained from a reputable source and comply with compendia standards.
• A listing of pharmaceutical-grade drugs and biologics is available through the FDA database. The Orange Book is the reference for FDA-approved human drugs. The Green Book is the reference for FDA-approved veterinary drugs.

• Veterinary compounding is the customized manipulation of an approved drug by a veterinarian, or by a pharmacist upon the prescription of a veterinarian, to meet the needs of a research study. IACUCs considering the use of veterinary compounding for research purposes are advised to consult: https://www.avma.org/KB/Resources/Reference/Pages/Compounding.aspx for more information about federal regulations.

• United States Department of Justice Drug Enforcement Agency controlled substances Schedule I and II-IV drugs may be used in biomedical research according to the standards of the Code of Federal Regulations 1301.13. (Source: http://grants.nih.gov/grants/olaw/faqs.htm#useandmgmt_4)

OLAW: May investigators use expired pharmaceuticals, biologics, and supplies in animals?

The use of expired pharmaceuticals, biologics, and supplies is not consistent with acceptable veterinary practice or adequate veterinary care. Euthanasia, anesthesia and analgesia agents should not be used beyond their expiration date, even if a procedure is terminal. Other expired materials should not be used unless the manufacturer verifies efficacy beyond the expiration date, or the investigator is able to document to the satisfaction of the IACUC that such use would not negatively impact animal welfare or compromise the validity of the study. The veterinarian and IACUC must maintain control over the use of expired medical materials in order to meet their responsibilities to avoid or minimize discomfort, pain or distress to animals.

AAALAC: Non-Pharmaceutical-Grade Compounds

A pharmaceutical-grade compound is defined as any active or inactive drug, biologic or reagent, for which a chemical purity standard has been established by a recognized national or regional pharmacopeia (e.g., the U.S. Pharmacopeia (USP), British Pharmacopeia (BP), National Formulary (NF), European Pharmacopoeia (EP), Japanese Pharmacopeia (JP), etc.). These standards are used by manufacturers to help ensure the products are of the appropriate chemical purity and quality, in the appropriate solution or compound, to ensure stability, safety, and efficacy. AAALAC International acknowledges that in an animal care and use program non-pharmaceutical-grade compounds often are necessary for scientific research. Where the use of non-pharmaceutical-grade substances may be essential for the conduct of science, the goal of the IACUC (or comparable oversight body) should be to consider the health and well-being of the animals while aiding the researcher in minimizing potentially confounding experimental variables and maximizing reproducibility of the research. AAALAC distinguishes between two scenarios when considering the use of non-pharmaceutical-grade compounds:

Clinical Use - compounds used for the clinical treatment of animals and to prevent or reduce/eliminate animal pain or distress. Whenever possible, pharmaceutical-grade compounds must be used.

Research Use - compounds used to accomplish the scientific aims of the study. If available, and suitable, pharmaceutical-grade compounds are preferred; but when non-pharmaceutical-grade
preparations are used, AAALAC International will expect investigators and the IACUC (or comparable oversight body) to consider the following factors:

• Use must be compliant with applicable national or regional regulatory guidelines and requirements and the requirements of relevant funding agencies;

• A scientific justification is provided;

• The pharmaceutical-grade compound is not available in the appropriate concentration or formulation or the appropriate vehicle control is unavailable

• The compound is required to generate data that are part of an ongoing study or that are comparable to previous work;

• The chemical properties of the compound are appropriate for the study and the route of administration (e.g., the purity, grade, stability in and out of solution, solution vehicle properties, pH, osmolality, and compatibility of the solvent and other components of final preparation). In some cases the reagent-grade of the compound may be as or more pure than the pharmaceutical-grade; and

• The method of preparation, labeling (i.e., preparation and use-by dates), administration and storage of formulations should be appropriately considered with the aim of maintaining their stability and quality (i.e., to prevent inadvertent co-administration of infectious agents or contaminants).

The Guide: Non-Pharmaceutical-Grade Compounds

Use of Non-Pharmaceutical-Grade Chemicals and Other Substances The use of pharmaceutical-grade chemicals and other substances ensures that toxic or unwanted side effects are not introduced into studies conducted with experimental animals. They should therefore be used, when available, for all animal-related procedures (USDA 1997b). The use of non-pharmaceutical-grade chemicals or substances should be described and justified in the animal use protocol and be approved by the IACUC (Wolff et al. 2003); for example, the use of a non-pharmaceutical-grade chemical or substance may be necessary to meet the scientific goals of a project or when a veterinary or human pharmaceutical-grade product is unavailable. In such instances, consideration should be given to the grade, purity, sterility, pH, pyrogenicity, osmolality, stability, site and route of administration, formulation, compatibility, and pharmacokinetics of the chemical or substance to be administered, as well as animal welfare and scientific issues relating to its use (NIH 2008).

24. If applicable, describe in sufficient detail the Experimental Endpoints of the study. (The experimental endpoint of a study occurs when the scientific aims and objectives have been reached.)

The Guide: Experimental and Humane Endpoints

The experimental endpoint of a study occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is
prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental end-points that result in unrelied or severe animal pain and distress, including death. The humane endpoint should be relevant and reliable (Hendriksen and Steen 2000; Olfert and Godson 2000; Sass 2000; Stokes 2002). For many invasive experiments, the experimental and humane endpoints are closely linked (Wallace 2000) and should be carefully considered during IACUC protocol review. The PI, who has precise knowledge of both the objectives of the study and the proposed model, should identify, explain, and include in the animal use protocol a study endpoint that is both humane and scientifically sound. Information that is critical to the IACUC’s assessment of appropriate endpoint consideration in a protocol includes precise definition of the humane endpoint (including assessment criteria), the frequency of animal observation, training of personnel responsible for assessment and recognition of the humane endpoint, and the response required upon reaching the humane endpoint. An understanding of preemptive euthanasia (Toth 2000), behavioral or physiologic definitions of the moribund state (ibid.), and the use of study-specific animal assessment records (Morton 2000; Paster et al. 2009) can aid the PI and IACUC when considering or developing proposed endpoints. When novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is an effective method for identifying and defining humane endpoints and reaching consensus among the PI, IACUC, and veterinarian.

Also see UT ARLINGTON’S IACUC POLICY & PROCEDURES FOR RESEARCH INVOLVING ANIMALS - IV.A.3. Suggested Signs and Symptoms for Judging the Moribund Condition (state of dying) in Rodents.

25. Intervention for Pain or Distress

The Guide: Experimental and Humane Endpoints

The experimental endpoint of a study occurs when the scientific aims and objectives have been reached. The humane endpoint is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved. The use of humane endpoints contributes to refinement by providing an alternative to experimental endpoints that result in unrelied or severe animal pain and distress, including death. The humane endpoint should be relevant and reliable (Hendriksen and Steen 2000; Olfert and Godson 2000; Sass 2000; Stokes 2002). For many invasive experiments, the experimental and humane endpoints are closely linked (Wallace 2000) and should be carefully considered during IACUC protocol review. The PI, who has precise knowledge of both the objectives of the study and the proposed model, should identify, explain, and include in the animal use protocol a study endpoint that is both humane and scientifically sound. Information that is critical to the IACUC’s assessment of appropriate endpoint consideration in a protocol includes precise definition of the humane endpoint (including assessment criteria), the frequency of animal observation, training of personnel responsible for assessment and recognition of the humane endpoint, and the response required upon reaching the humane endpoint. An understanding of preemptive euthanasia (Toth 2000), behavioral or physiologic definitions of the moribund state (ibid.), and the use of study-specific animal assessment records (Morton 2000; Paster et al. 2009) can aid the PI and IACUC when considering or developing proposed endpoints. When novel studies are proposed or information for an alternative endpoint is lacking, the use of pilot studies is
an effective method for identifying and defining humane endpoints and reaching consensus among
the PI, IACUC, and veterinarian.

Also see **UT ARLINGTON’S IACUC POLICY & PROCEDURES FOR RESEARCH INVOLVING ANIMALS** - IV.A.3. Suggested Signs and Symptoms for Judging the Moribund Condition (state of
dying) in Rodents.

26. Disposition of Animals

(euthanized, release to former habitat, adoption, other)

The Guide: Euthanasia

Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness
and death without pain or distress. Unless a deviation is justified for scientific or medical reasons,
methods should be consistent with the AVMA Guidelines on Euthanasia (AVMA 2007 or later
editions). In evaluating the appropriateness of methods, some of the criteria that should be
considered are ability to induce loss of consciousness and death with no or only momentary pain,
distress, or anxiety; reliability; irreversibility; time required to induce unconsciousness;
appropriateness for the species and age of the animal; compatibility with research objectives; and
the safety of and emotional effect on personnel. Euthanasia may be planned and necessary at the
end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics,
sedatives, or other treatments. Criteria for euthanasia include protocol-specific endpoints (such as
degree of a physical or behavioral deficit or tumor size) that will enable a prompt decision by the
veterinarian and the investigator to ensure that the endpoint is humane and, whenever possible, the
scientific objective of the protocol is achieved (see Chapter 2).

Generally, chemical agents (e.g., barbiturates, nonexplosive inhalant anesthetics) are preferable to
physical methods (e.g., cervical dislocation, decapitation, use of a penetrating captive bolt);
however, scientific considerations may preclude the use of chemical agents for some protocols. It
is essential that euthanasia be performed by personnel skilled in methods for the species in
question and in a professional and compassionate manner. Special attention is required to ensure
proficiency when a physical method of euthanasia is used. Death must be confirmed by personnel
trained to recognize cessation of vital signs in the species being euthanized. A secondary method
of euthanasia (e.g., thoracotomy or exsanguination) can be also used to ensure death. All methods
of euthanasia should be reviewed and approved by the veterinarian and IACUC.

OLAW: Is the use of carbon dioxide an acceptable euthanasia agent?

Although CO2 is generally considered an acceptable euthanasia agent for small animals when
properly administered, its acceptability is predicated on a number of critical factors that are
because neonatal rodents are resistant to the hypoxia-inducing effects of CO2 and require longer
exposure times to the agent, alternative methods should be considered such as injection with
chemical agents, cervical dislocation, or decapitation.
UTA IACUC Requirement:

Death of the animal must be assured by a physical method. Justification is required for method of euthanasia, especially if using guillotine, cervical dislocation, or other physical means. See also:

UTA Euthanasia and Humane Endpoints SOP

UTA Guillotine Maintenance SOP

27. Hazards to Personnel

OLAW: What is required for an occupational health and safety program?

The PHS Policy requires a "health program for personnel who work in laboratory animal facilities or have frequent contact with animals" (IV.A.1.f). The Guide states that, "Each institution must establish and maintain an occupational health and safety program as an essential part of the overall Program of animal care and use. The nature of the OHSP will depend on the facility, research activities, hazards, and animal species involved." (Guide pages 17-23)

"A comprehensive OHSP should include a hierarchy of control and prevention strategies that begins with the identification of hazards and the assessment of risk associated with those hazards." (Guide page 18) An effective occupational health and safety program must encompass all personnel that have contact with animals. Depending on the species of animal or the amount of animal exposure, the program may not affect all personnel equally. Minimally, the program should include:

• pre-placement medical evaluation;
• identification of hazards to personnel and safeguards appropriate to the risks associated with the hazards;
• appropriate testing and vaccinations;
• training of personnel regarding their duties, any hazards, and necessary safeguards;
• policies and facilities that promote cleanliness;
• provisions for treating and documenting job-related injuries and illnesses;
• facilities, equipment, and procedures should be designed, selected, and developed to reduce the possibility of physical injury or health risk to personnel;
• good personal hygiene practices, prohibiting eating and drinking, use of tobacco products, and application of cosmetics and/or contact lenses in animal rooms and laboratories; and
• personal protective equipment (PPE).

AAALAC: Occupational Health & Safety Program

An occupational health and safety program must be part of the overall animal care and use program. The basic elements of a program include hazard identification and risk assessment,
personnel training and protection, written procedures and policies regarding hazard use and monitoring, and medical evaluation and preventive medicine.

The extent and level of participation of personnel in the program should be based on the hazards posed by the animals and materials used; on the exposure intensity, duration, and frequency; on the susceptibility of the personnel; and on the history of occupational illness and injury in the particular workplace. A health history evaluation is advisable before work assignment to assess potential risks for individual employees. Periodic medical evaluations and appropriate immunization schedules are advisable for some risk categories. Immunization of animal care personnel against tetanus is important.

UTA's Occupational Health Program Requirements:

All personnel performing surgery are required to wear a mask, gloves, and gown. All personnel entering a room where animals are present must wear a gown.

http://www.uta.edu/research/administration/departments/rs/animals-subjects-iacuc/occupational-health-program.php

UTA's Environmental Health and Safety Program:

You must contact Environmental Health and Safety, 817-272-2185; for approval if you plan to use radioisotopes or any other material that poses a biological safety concern. For more information, see http://www.uta.edu/campus-ops/ehs/biological/.

28. Personnel


- adequacy of training and experience of personnel in the procedures used, and roles and responsibilities of the personnel involved.

UTA IACUC Requirement:

The IACUC at UTA requires a list of the people who will be working on your protocol and what their role will be, i.e., surgery, invasive procedures, euthanasia, etc. Any personnel performing restraint, anesthesia, or euthanasia MUST be trained by either the veterinarian or the PI. A description of how personnel will be trained on specific procedures must be included. Training on injections and invasive procedures must first be done on cadavers before live animals are used. The number of live animals requested in your application must incorporate, through description and justification, your needs for both training and experimental procedures. The ACF Manager is available for assistance. See contact information on Page 1.

31.(1) Objective and Significance of the Project benefits, advancement of knowledge, etc.
U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training:

II. Procedures involving animals should be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society.

OLAW: Is the IACUC responsible for judging the scientific merit of proposals?

Although not intended to conduct peer review of research proposals, the IACUC is expected to include consideration of the U.S. Government Principles in its review of protocols. Principle II calls for an evaluation of the relevance of a procedure to human or animal health, the advancement of knowledge, or the good of society. Other PHS Policy review criteria refer to sound research design, rationale for involving animals, and scientifically valuable research. Presumably a study that could not meet these basic criteria is inherently unnecessary and wasteful and, therefore, not justifiable.

31.(2) Detailed Description of Procedures

OLAW: What criteria should the IACUC consider when reviewing protocols?

IACUCs must confirm that:

- the protocol is consistent with the Guide unless a scientific justification for a departure is presented and is acceptable to the IACUC;
- the protocol conforms with the institution’s Assurance;
- the protocol will be conducted in accordance with the USDA Animal Welfare Regulations if applicable; and
- the protocol meets the requirements of the PHS Policy at IV.C.1.a.-g.

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

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress established, is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia. Surgical or other painful procedures should not be performed on unanesthetized animals paralyzed by chemical agents.

VI. Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.

The animal use protocol is a detailed description of the proposed use of laboratory animals. The following topics should be considered in the preparation of the protocol by the researcher and its review by the IACUC:

- rationale and purpose of the proposed use of animals
- a clear and concise sequential description of the procedures involving the use of animals that is easily understood by all members of the committee
- availability or appropriateness of the use of less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation (see Appendix A, Alternatives)
- justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis; see Appendix A, Experimental Design and Statistics)
- unnecessary duplication of experiments
- nonstandard housing and husbandry requirements
- impact of the proposed procedures on the animals' well-being
- appropriate sedation, analgesia, and anesthesia (indices of pain or invasiveness might aid in the preparation and review of protocols; see Appendix A, Anesthesia, Pain, and Surgery)
- conduct of surgical procedures, including multiple operative procedures
- postprocedural care and observation (e.g., inclusion of post-treatment or postsurgical animal assessment forms)
- description and rationale for anticipated or selected endpoints
- criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated
- method of euthanasia or disposition of animals, including planning for care of long-lived species after study completion, adequacy of training and experience of personnel in the procedures used, and roles and responsibilities of the personnel involved
- use of hazardous materials and provision of a safe working environment.

31.(3) Species and Justification for number of animals

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

III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer stimulation, and in vitro biological systems should be considered.

Justification of the species and number of animals proposed; whenever possible, the number of animals and experimental group sizes should be statistically justified (e.g., provision of a power analysis; see Appendix A, Experimental Design and Statistics).

OLAW: Is the IACUC responsible for tracking animal usage?

Although the PHS Policy does not explicitly require a mechanism to track animal usage by investigators, it does require that proposals specify a rationale for the approximate number of animals to be used and be limited to the appropriate number necessary to obtain valid results. This implicitly requires that institutions establish mechanisms to document and monitor numbers of animals acquired and used, including any animals that are euthanatized because they are not needed. Monitoring should not exclude the disposition of animals inadvertently or necessarily produced in excess of the number needed or which do not meet criteria (e.g., genetic) established for the specific study proposal. Institutions have adopted a variety of administrative, electronic, and manual mechanisms to meet institutional needs and PHS Policy requirements. A7

OLAW: Should the IACUC consider the three "Rs" of alternatives when reviewing protocols? (Refinements to research, Reduction of animal numbers, and Replacement with non-animal models)

The federal mandate in U.S. Government Principle IV to avoid or minimize discomfort, distress, and pain in experimental animals consistent with sound scientific practices, is synonymous with a requirement to implement refinements (e.g., less invasive procedures or use of analgesia). Similarly, the mandate in U.S. Government Principle III to use the minimum number of animals necessary to obtain valid results is synonymous with a requirement to reduce animal numbers. U.S. Government Principle III further states that mathematical models, computer simulation, and in vitro biological systems should be considered, and is synonymous with a requirement to replace non-animal models wherever possible. Thus, consideration of the three "Rs" should be incorporated into IACUC review, as well as other aspects of the institution’s program (e.g., investigator training). D6

31.(4) Experience with Proposed Animal Model Manipulation

The Guide: Training and Education

All personnel involved with the care and use of animals must be adequately educated, trained, and/or qualified in basic principles of laboratory animal science to help ensure high-quality science and animal well-being. The number and qualifications of personnel required to conduct and support a Program depend on several factors, including the type and size of the institution, the administrative structure for providing adequate animal care, the characteristics of the physical plant, the number and species of animals maintained, and the nature of the research, testing, teaching, and production activities. Institutions are responsible for providing appropriate resources to support personnel training (Anderson 2007), and the IACUC is responsible for providing oversight and for evaluating the effectiveness of the training program (Foshay and Tinkey 2007). All Program personnel training should be documented.
OLAW: What kind of training is necessary to comply with PHS Policy, and how frequently should it be provided?

The U.S. Government Principles, Health Research Extension Act of 1985 and the PHS Policy repeatedly refer to appropriately trained, qualified, and experienced personnel, and availability of instruction and training. The institution is responsible for the training of its staff. The size and nature of institutional research programs varies significantly and accounts for the corresponding variation in the scope and depth of instructional programs and the frequency at which they are offered.

Discussion of appropriate training is found throughout the five chapters of the Guide. At a minimum, the PHS Policy and the Guide (page 15) require institutions to:

- ensure that individuals who use or provide care for animals are trained and qualified in the appropriate species-specific housing methods, husbandry procedures, and handling techniques;
- ensure that research staff members performing experimental manipulation, including anesthesia and surgery, are qualified through training or experience to accomplish such procedures humanely and in a scientifically acceptable fashion;
- provide training or instruction in research and testing methods that minimize the number of animals required to obtain valid results and minimize animal distress;
- ensure that professional staff whose work involves hazardous biological, chemical, or physical agents have training or experience to assess potential dangers and select and oversee the implementation of appropriate safeguards; and
- ensure compliance with any initial and continuing education regarding State requirements for the licensing of veterinary or animal health technicians.

1 The U.S. Government Principles direct that, "...housing, care, and feeding must be directed by a veterinarian or other scientist trained and experienced in the proper care, handling, and use of the species being maintained or studied (VII) and that, Investigators and other personnel shall be appropriately qualified and experienced for conducting procedures on living animals. Adequate arrangements shall be made for their in-service training, including the proper and humane care and use of laboratory animals" (VIII).

2 The Health Research Extension Act of 1985 requires that, "scientists, animal technicians, and other personnel involved with animal care, treatment, and use have available to them instruction or training in the humane practice of animal maintenance and experimentation..." (Sec. 495.(c) (1) (B)).

3 The PHS Policy requires that institutions seeking an Assurance provide "a synopsis of training or instruction in the humane practice of animal care and use, as well as training or instruction in research or testing methods that minimize the number or animals required to obtain valid results and minimize animal distress, offered to scientists, animal technicians, and other personnel involved in animal care, treatment or use" (IV.A.1.g.); that medical care for animals will be provided by "qualified" veterinarians (IV.C.1.e.) and that, Personnel conducting procedures on the species being maintained or studied will be appropriately qualified and trained in those procedures (IV.C.1.f.).
UTA IACUC Requirement:

If a student lacks experience with the procedures they will be carrying out, please include a statement certifying that you will train them on all necessary procedures.

Appendix B-Literature Search


- unnecessary duplication of experiments

USDA: Where does the legislation say that I have to do a literature search?

The Animal Welfare Act (Title 7, U.S. Code), as written and approved by Congress, emphasizes minimizing pain and distress, but does not mention how alternatives consideration should be documented. It states in Section 13(a)(3)(B): “that the principal investigator consider alternatives to any procedure likely to produce pain or distress in an experimental animal;” Title 9 of the Code of Federal Regulations (9CFR, Part 2, Sec. 2.31 (d)(1)(ii)) gives the USDA regulation on how consideration of alternatives should be accomplished. It mentions AWIC, which relies on multiple database literature searching, as a resource: “The IACUC shall determine that... The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources, e.g., the Animal Welfare Information Center, used to determine that alternatives were not available;” The Federal Register (Vol. 54, No. 168, Thursday, August 31, 1989) gives the USDA rationale for making the alternatives consideration a written requirement and suggests a series of databases that can be searched to document whether or not alternatives are available: “The principal investigator must provide a written narrative of the sources, such as biological abstracts, MEDLINE, the Current Research Information Service (CRIS), and the Animal Welfare Information Center that is operated by the National Agricultural Library. We believe that in fulfilling this requirement Committee members will discuss these efforts with the principal investigator in reviewing the proposed activity. We also believe that considerations of alternatives will be discussed during Committee meetings where proposed activities are presented for approval, and made part of the meeting minutes...” The legislation indicates that the investigator must provide a written narrative which demonstrates to the IACUC that alternatives, useful or not, were at least considered in the experimental design. The literature search is suggested as the best way to demonstrate this. IACUC members, including the nonaffiliated member, a visiting AC inspector, or a member of the public can follow a printed search strategy, view the list of databases and keywords, and verify that the investigator has made a good faith effort to demonstrate whether or not alternatives exist and why he/she will or will not adopt them. The literature search if far less questionable than a check-off box or a sentence or two saying there are no alternatives written on the protocol form. "Policy #12: Consideration of Alternatives to Painful/Distressful Procedures" is posted on the Animal Care website (APHIS/AC Policy Manual) and clarifies what USDA inspectors have been saying for a long time. Policy 12 defines alternatives and goes on to say: "Alternatives should be considered in the planning phase of the animal use proposal. As indicated when these regulations were finalized in 1989, APHIS continues to recommend a database search as the most effective and efficient method for demonstrating compliance with the requirement to consider
alternatives to painful/distressful procedures. However, in some circumstances (as in highly specialized fields of study), conferences, colloquia, subject expert consultants, or other sources may provide relevant and up-to-date information regarding alternatives in lieu of, or in addition to, a database search. Sufficient documentation, such as the consultant’s name and qualifications and the date and content of the consult, should be provided to the IACUC to demonstrate the expert’s knowledge of the availability of alternatives in the specific field of study. For example, an immunologist cited as a subject expert may or may not possess expertise concerning alternatives to in vivo antibody production.

When a database search is the primary means of meeting this requirement, the narrative should include:

• the name(s) of the databases searched (due to the variation in subject coverage and sources used, one database is seldom adequate);

• the date the search was performed;

• the time period covered by the search; and

• the search strategy (including scientifically relevant terminology) used.

What is the minimum number of databases that must be searched?

The literature search is a performance, not an engineering, standard. Although there is no minimum number, no one database reviews all the literature in all research fields. Databases do overlap somewhat in the journals they index and the subject areas they cover, but they also complement each other. Testing a new medical device, for example, might involve searching biomedical, engineering, and even computer sciences databases. The objectives of the search are to demonstrate whether or not alternatives are available and, if so, why or why can they not be used. A thorough search usually requires more than one database.

How do I run an alternatives search if I am doing toxicology testing and don’t know what compounds are being tested?

If the type of compound is known, the search is easily run. If not, it is important to remember that alternatives means more than simply searching for a replacement technique. The investigator can search for a method which uses fewer animals, where mortality is not the endpoint, or techniques that minimize pain or distress. Even environmental enrichment can be considered an alternative.

The legislation specifically mentions alternatives to the painful procedure. Why do I need to search for any more than that specific part of the study?

The painful procedure must be examined in the context of the entire study. This information is often buried within the paper. Databases generally keyword search for words or phrases in the title, abstract, or descriptor. The painful procedures are sometimes, but not often, mentioned in those categories. Therefore, a broader view is needed to see if the study’s ultimate objectives can be met with alternative methods. Beside the legal aspect, there are many other benefits of the alternatives search. Many researchers run literature searches when designing a study. This helps determine if the research is original or "unnecessary duplication" (which must be documented). Such searches can easily be tailored to address alternatives. The search shows the investigator will stand behind
his/her work and that it is as humane as possible. Sometimes, the adoption of an alternative method is more economical (ie using an in vitro model vs. housing a colony of animals). It may provide more meaningful data by avoiding confounding factors in the experimental design such as distress in the animal model. Hopefully, with a little patience and understanding, researchers will see the alternatives search as a valuable tool for improving the quality of research and not a dreaded Federal mandate.

UTA IACUC Suggested Reference:

3R Guide: a new database of 3R resources

Norecopa (the Norwegian 3Rs centre) and the Animal Welfare Information Center, Beltsville (AWIC) have launched a database called 3R Guide (https://norecopa.no/3r-guide-database). Their aim is to offer investigators and facility staff a "one-stop shop" where they can locate key resources.

3R Guide is located on the same website as two other databases: TextBase (which lists textbooks and other written material within laboratory animal science) and NORINA (a database of products that may be used to replace or supplement animal use in teaching and training). Search results from one database are automatically displayed for the other databases, as well as search results from the website itself.

3R Guide, TextBase and NORINA are all available on https://norecopa.no/3r-guide, which in itself is one large database.