V4

INSTRUCTIONS FOR COMPLETING THE UMD ANIMAL STUDY PROTOCOL-RESEARCH

The Animal Welfare Act (AWA) regulations and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals (PHS Policy) require review and approval of an Animal Study Protocol by the Institutional Animal Care and Use Committee (IACUC) prior to the initiation of any research or teaching activity involving vertebrate animals. Regulatory and guidance documents referred to in these instructions are provided on the UMD IACUC website http://www.umresearch.umd.edu/IACUC/ARAC/

GENERAL INSTRUCTIONS:

When is an Animal Study Protocol Required?

1. An Animal Study Protocol from UMD is required when live animals are either housed or manipulated at UMD or studied in the field.

2. An IACUC-approved protocol from an outside institute is required when UMD-owned animals are either housed or manipulated at the outside institute.

3. If animal work is performed at the outside institute by UMD students or faculty, and the animals are not housed or live animals manipulated at UMD, an IACUC-approved protocol is required from the outside institute; a UMD protocol is not required. A description of the individual's involvement in the study is required by the IACUC.

4. Studies in which tissues are obtained from animals that were used in other IACUC-approved studies AND were euthanized for use in the other studies, do not require a protocol. A protocol is not required to obtain tissues from a slaughterhouse or biological supply house.

5. UMD campus refers to the College Park campus and all facilities under its auspices, including the Central and Western Maryland Research and Education Centers, the Applied Poultry Research Facility, and the Wye Research and Education Center.

Submission of an Animal Study Protocol

Submit the protocol to the IACUC manager located in Lee Building by the first day of the month. The lead time allows for distribution of the protocols and their review by committee members. The IACUC meets on the third Thursday of the month but generally does not meet in August.

Purpose of these Instructions

These instructions are intended to aid you as the investigator in the completion of the protocol form. The goal is to help you to prepare a protocol that can be approved by the IACUC in a single round of review. The purpose of the protocol is to provide a plan for the use of animals in research.

Assistance in Obtaining Protocol Approval

A common mistake made by investigators that leads to delays in getting a protocol approved is the inclusion of more detail in the protocol than is required by the regulations or requested on the form. The IACUC recommends that you seek out the guidance of the University Veterinarian, your facility veterinarian, your animal facility manager, a senior investigator who is familiar with animal research and the IACUC, an IACUC member, and/or the DES representative for help during the preparation of the protocol. The facility manager and veterinarian can advise on facility support for animal housing and procedures. The University Veterinarian is also the facility veterinarian for all facilities except Animal and Avian Sciences and Veterinary Medicine.

There are many opportunities for pre-review during the protocol development stages that help to ensure that your protocol is submitted to the IACUC in near final form. A well constructed protocol will facilitate the review process so that your animal research can proceed rapidly. If invited, attending the IACUC meeting to directly answer questions from IACUC members about your protocol will also speed up approval.

The information that you provide in the protocol should only represent the research that will be conducted that involves animals. As outlined in the *U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training* (http://www.nal.usda.gov/awic/pubs/IACUC/vert.htm), the protocol will be evaluated to assure that the research proposed is an appropriate use of the animals, and that technical assistance and appropriate facilities are available to support the research.

UMD considers all information submitted in the protocol to be privileged and confidential. Once the protocol is approved, however, any portions of the protocol that are not exempt from disclosure under the Freedom of Information Act (FOIA) are subject to release if a request is made under the FOIA.

Section A: ADMINISTRATIVE DATA

A.1. **Title of Project**: Include the common name of the animal used such as fish, mouse, or birds. If applicable, use the same title as the supporting grant/award, but the grant/award must only support this one protocol.

A.2. **Estimated start and completion dates**. The start time is when you want to first order animals or if animals are on hand, when you first wish to manipulate them.

The start date should be no earlier than the first day of the month following submission of the protocol.

A.3. **Type of protocol**. Mark if the protocol is an initial submission, a renewal of an existing or previously expired protocol, or a modification (such as a change in principal investigator or other significant change) of a currently approved protocol. If the protocol is a renewal or a modification, state the protocol number. Protocols expire on the third year anniversary of their approval date and cannot be administratively extended. Full *de novo* review is required to continue work on an expiring protocol.

Field study. Copies of all required permits are to be provided. If permits are not required, then documentation that they are not required must be provided. All applicable information must be provided in each section of the protocol form. The IACUC must be apprised of circumstances in which studies are conducted so they may consider risks to personnel and impact on study subjects.

Location of research. State the location where animals will be housed, live animals manipulated or tissues studied if at a facility outside the UMD campus.

A.4. **Principal Investigator**. There may only be one Principal Investigator (PI) on a protocol. Only UMD faculty and staff can be a PI. The PI assumes all responsibility for the animals, the protocol, and its execution. Other investigators should be listed in "Key Personnel".

Department, UMD Address (Bldg/Room), E-mail, Telephone, FAX. Provide complete and accurate information, as this will be utilized for the life of the protocol.

University Title. Associate, assistant or full professor; post-doctoral student.

A.5. **Key Personnel**. List all personnel who will be conducting procedures involving animals under this protocol. All personnel listed in this section will need to undergo UMD and facility training (see Section M) and, if they handle animals, be enrolled in the Laboratory Animal Handler Medical Surveillance Program (LAHMSP). Investigators not handling animals or conducting procedures on live animals (for example, investigators who use tissues collected by someone listed on the protocol) need not be listed. Medical surveillance program enrollment is also required for investigators receiving non-fixed tissues or fluids from non-human primates whether they are listed on the protocol or not.

A.6. If your protocol will be submitted to ORAA, then provide all appropriate information.

A.7. Provide the source of your funding for animal care and procurement.

Section B: ANIMAL REQUIREMENTS

B.1. **Species, Age/Weight, Sex, Stock, or Strain, Common or Scientific name**. Provide specific information if known regarding these characteristics of the

animals. If you are using transgenic or knock-out strain(s) of animals, provide the background strain(s) and the designation of the genetic mutation. Consult the nomenclature rules (http://www.informatics.jax.org/mgihome/nomen/index.shtml) as proper nomenclature will be essential for ordering and tracking animals.

Source. State the vendor of each species. If procured in the wild, state "wild-caught". All animal orders must comply with the Central Animal Procurement System (CAPS). See http://www.umresearch.umd.edu/IACUC/ARAC/. Rodents are generally ordered from approved vendors through CAPS. If rodents or rodent products are obtained from a source other than an approved source, contact the University Veterinarian.

Holding Location(s). Indicate where animals are proposed to be housed (building). The room number is not necessary. You must indicate if it is necessary to hold animals anywhere outside of a core animal facility for more than 12 hours. In addition if you need to hold the animals in this space for more than 24 hours, a satellite facility must be established and approved by the IACUC. Contact the University Veterinarian if you think your animals will need housing outside of a designated animal facility.

Number of Animals. This column should reflect the total number of animals estimated and justified under Section E3. If multiple species are used, provide the numbers separately for each species. If the study is a renewal, include the numbers of animals to be carried over from the previous protocol. The number of animals used on the protocol must be tracked and an amendment with justification submitted to the IACUC if the approved number is surpassed. It is acceptable to inflate the number of animals needed if unexpected deaths are possible or to perfect surgical or other techniques prior to initiating planned experiments.

B.2. **Description of Animal Usage**. Providing the approximate number of animals used each year, the maximum number of animals on hand at any given time and the maximum number of weeks any single animal may be housed is helpful to the facility manager and veterinarian to determine caretaker workloads and resource allocations.

B.3. **Number of Animals On Hand**. State the number of animals on hand or carried over from the previous protocol. Again, this number is included in the total number of animals required in the study under Section B1.

B.4. **Animal Accountability**. The IACUC has established general guidelines for what is considered an animal for the purpose of accountability. Unless specifically used in the study, nursing rodent pups and embryonated eggs are not considered animals and do not count against approved animal numbers. However, the IACUC wishes to have estimates on the number of these animals.

Section C: TRANSPORTATION

Animals may need to be transported to different animal procedure or holding facilities in or outside of the primary animal building. If animals are to be transported, state that all UMD and facility guidelines and policies will be followed. The guidelines can be found at http://www.umresearch.umd.edu/IACUC/ARAC/. If different or beyond the scope of the guidelines, then describe the transportation procedures. Consult as needed with your facility veterinarian about routes and the type of cages required. Include destinations, procedures, vehicle designation (i.e. State van), and containment to be utilized.

Section D: LAY SUMMARY

Briefly, limiting to 300 words, state the study objectives and goals. Minimize abbreviations, technical terms and jargon. When they are necessary, technical terms and abbreviations should be defined. Your response should be written so that the general public would readily understand, i.e. use language that a high school student would comprehend.

This is a summary and not a detailed description of the experiment or animal use. It should explain why the work is important and how the results of the study might benefit humans and/or animals.

Section E: RATIONALE FOR ANIMAL USE

You should use language that a high school student would understand. Explain technical terms, and define abbreviations the first time they are used.

E.1. **Justify why animals are required.** You should include why non-animal alternatives cannot be used. This is a very important issue because you are asking for the privilege of using animals for procedures that rarely will benefit them and will most likely result in their death. There must be a compelling potential for benefit to human or animal health to warrant the use of animals. Explain why an animal model is necessary for this study as opposed to cell cultures, computer simulations or other non-animal models.

Examples:

- This study requires a functioning animals with a highly developed nervous system and a complex multicellular structure. Cell culture systems are usually composed of a single type of cell that cannot be used to demonstrate nervous system function.

- The investigation of the effects of lesions to the parts of the brain that help to control vision such as the hypothalamus can only be performed on living organisms with both a well-developed and intact nervous system and an immune system.

- Living animal cells are necessary to study the production of proteins. Female germ cells have been used very successfully for these types of studies and are superior to extracts of cultured cells.

E.2. **Justify appropriateness of species selected**. Describe why the animal model selected is essential for the proposed study as opposed to a lower order vertebrate or an invertebrate. Justification should be based on sound scientific reasoning. Cost savings alone is not an adequate justification for using a particular species. Assuming that animals are indeed necessary, the least sentient species capable of providing the needed data should be used. The hierarchy of sentient species can be a subject of disagreement, but generally it goes as follows:

- Nonhuman primates
- Larger animals commonly kept as pets, such as dogs and cat
- Larger animals commonly used as farm animals, such as pigs and goat
- Rabbits
- Rodents (guinea pigs, hamsters, rats, mice
- Non-mammalian vertebrates (poultry, amphibians, reptiles, fish)
- Invertebrates (crustaceans, slugs
- Smaller life forms (insects, arachnids, worms)
- Single cell organisms (yeast, bacteria, etc)

Justification for using a particular species may include:

- The presence of previous work in the biomedical literature validates the use of a particular species in an animal model of a human disease.
- The existence of a large body of previous laboratory data would have to be repeated if another species was used instead.
- Characteristics of the species render it uniquely suited to the proposed research.
- Size, availability, and cost.
- Availability of reagents or research tools unique to that species.

Example: This research will examine the genetics of mammary tumors, which occur only in female mammals. Zebrafish strains with mutations in some of the genes of interest have been identified, but cannot be used for the study of mammary tumors. In addition, few human or mouse mammary tumors can be grown in tissue culture, making the mouse the ideal organism for generating primary mammary tumors.

E.3. **Justify number of animals to be used**. The number of animals selected for a procedure should be the minimum number required to obtain statistically valid results. When possible, a statistical analysis should be used to justify group sizes or experimental repetitions. Commonly, a power analysis is the most appropriate tool. However, applying statistical principles to justify numbers can be difficult due to the lack of knowledge regarding the potential outcomes and variance.

The goal is not to minimize the number of animals used but to determine the right number of animals for obtaining valid results. Therefore, you might even find out that you need to ask for more animals per group than you thought would be necessary. It is acceptable to ask for animals that will be used to perfect surgical or other techniques prior to initiating planned experiments. This is preferable to beginning a large experiment that will develop technical problems which might cause pain or distress to the animals. Consider using cadavers from other approved protocols in advance of performing any procedure on a live animal.

It is also acceptable to ask for animals that will be used in pilot experiments in addition to animals requested for more robust experiments. Pilot experiments can be used to demonstrate feasibility or provide a justification for proceeding with larger, more tightly controlled experiments.

Use a flow chart or table. It is understood that the size of the groups and the number of experiments proposed represents the good faith judgment of the PI and is based on the PI's scientific expertise. Estimates cannot predict the number of animals with precision, so the protocol should state that analysis of the initial experiments will be performed and the group size adjusted if the results indicate that changes in the size of the experimental groups or the number of experiments will be needed.

> Example: We estimate that we will need to purchase 200 animals for this study because we will be using five animals per group, and examining the effects of five compounds (including vehicle) at four doses of drug per compound with two replications (to assure reproducibility) per determination. Past experience and review of referenced or refereed publications have shown that a group size of 5 provides reasonable assurance of statistical power for this type of study. However, if our initial experiments justify a larger or smaller sample size we will amend our protocol. The total numbers requested will be: 5 animals/group x 5 compounds/group x 4 doses per compound x 2 trials = 200.

If the study represents a new animal model, drug or procedure, a statistical analysis may not be appropriate. If the study is a continuation of ongoing work or parallels current or previous experiments where the magnitude of the effects and, the degree of variance is known, then a short summary of statistical principles that were applied to arrive at the group size(s) may be appropriate.

When the use of animals is for the harvesting of normal tissues, organs or fluids for *in vitro* use or *in vivo* transfer, briefly cite expected usage levels to provide the quantity of tissues or fluids needed for the study. If no prior experience is available, state an anticipated tissue/fluid harvest per animal with a description of the process.

> Example: In our experience, 10 rats are required to generate enough cells for one experiment. Since we plan to conduct one experiment per week, we need 520 rats per year. This number of animals will enable us to test 10 drugs at the desired dosage.

If rodents are to be used for breeding, then the following categories can be used in justifying and estimating your animal numbers:

- An estimate of the numbers of progeny intended for experimental use or export
- An estimate of the progeny expected
- The numbers used for breeding (including founders and initial mates)
- The numbers of progeny needed for continuation of the experimental line
- The numbers that will be euthanized due to undesirable genotype

An outline, a table, or a flow chart for each strain listed can be very helpful in presenting this information. Excessive detail in this section is frustrating to both the PI and the IACUC and can only lead to delays in ASP approval.

> Example: We estimate that 100 homozygous mutant animals will be needed to obtain valid results. These animals will be divided into three groups of 15 receiving different doses of the drug, along with 5 untreated controls and parallel groups of wild type animals given the same dosages in two experiments. From our initial founder animals (~3) we will obtain ~10 heterozygous animals along with 10 wild type animals which we will use as controls. We will cross heterozygotes to generate ~50 litters of 8 animals each, which should contain 100 homozygous animals if they are born in the expected ratio. If we find that this breeding scheme does not generate the required number of animals we will submit an amendment describing the new breeding scheme. Estimated number of animals: 3 founders + 3 wild type mates to generate 20 F1 progeny, including 10 heterozygotes for breeding. These will be mated and expanded to generate 400 mice of which we estimate that ~100 will be homozygous mutants for our studies. The remainder will be used as controls or for continued breeding or euthanized. Total: 6+20+400 = 426.

For research involving chemical mutagenesis, a statistical justification is appropriate.

> Example. Drug X at the dose we have proposed causes mutations in about 1 locus out of 1000 loci. Therefore, 1 in every 1000 gametes from a mutagenized male might be expected to carry a mutation in a gene of interest. Based on the frequency of mutations, the following number of animals per year are anticipated:
>
> 60 BALB/c males to be mutagenized in 3 sets of 20. Based on previous experience, we expect that 6/20 mutagenized males will

regain fertility after drug X treatment. The fertile males will be mated to 3 C57BL/6J females each to generate 3 litters averaging 8 pups per litter. 6 males x 2 females (one female is used twice) x 8 pups/litter x 3 litters = 192 G1 x 3 drug x treatments/year = 571 G1 progeny, 50% male and 50% female. ~288 G1 females will be discarded. ~ 250 G1 males will be crossed to 3 C57BL/6 females = 750 litters at 8 pups/ litter = 6,000 pups to be screened for dominant mutations. Sperm and tissues from all G1 males will be cryopreserved and archived for sequencing and later mutation retrieval.
60 treated males +36 C57BL/6 females + 576 G1 progeny = 672 mice
250 G1 matings generates 6000 mice for screening. Total 6672

If the protocol calls for post-harvest use of embryos or fetal tissues, only the dams need be counted, rather than the number of embryos/feti. Only if the feti are individually used for data collection, such as a fetal surgery experiment, would the feti be separately accounted for. However, the IACUC requests approximate numbers of these animals. Exceeding these numbers is not a requirement for a modification to the protocol.

E.4. **Justify duplicative research**. In addition to considering animal alternatives, you are also required to avoid performing unnecessary duplications of research studies. If duplicative studies are necessary, provide justification. The critical concept is that unnecessary duplication is not allowed. Acceptance of new ideas in science is often dependent upon the ability of other scientists to duplicate previous work, if scientifically important to do so.

Section F: EXPERIMENTAL DESIGN AND ANIMAL PROCEDURES

F.1. **Briefly describe the experimental design and animal procedures**. Describe all procedures that will be performed on individual animals. Include enough information in the description of the procedures to enable the IACUC members to visualize each procedure as it will be performed. This section is divided into sub-sections asking for information about different classes of procedures, i.e. administered substances, bleeding, restraint, survival surgery, other experimental procedures, anticipated resultant effects and endpoints.

You should not provide any more details than is specified on the protocol form. Details regarding anesthesia and euthanasia are requested elsewhere. For more complex experiments, it is helpful to provide a flow chart to make the experimental design clear. The description of the animal procedures should stand by itself. If, however, general guidelines or SOPs are referenced, they must have been previously approved by the IACUC and approval dates included. If in doubt, include the body of the SOP in this section. Approval dates of ARAC-approved guidelines are not required. The IACUC should not have to read another document such as a grant application or journal article to understand what you propose.

Define all abbreviations the first time they are used to facilitate comprehension. Do not use technical language that only specialists in your field would understand. Make sure it is clear which procedures will be performed on which species.

F.2. **Administered Substances**. Include the substance, dose or concentration, route, volume frequency, injection site if applicable and needle size if applicable. Include all administered substances even if from multiple experiments. Administered substances may be mentioned in sub-section F.1., but details are provided in this sub-section. The use of non-pharmaceutical grade compounds is not allowed unless a) there is a scientific necessity; b) there are no acceptable veterinary or human pharmaceutical-grade compound available; and c) there is specific review and approval by the IACUC.

F.3. **Blood Collection**. Provide the method, site, volume and frequency of blood withdrawal. Also state if the blood draw is terminal. Caution should be observed not to withdraw excessive amounts during survival bleeds. The acceptable quantity and frequency of blood sampling is dependent on the circulating blood volume of the animal and the red blood cell (RBC) turnover rate. The approximate circulating blood volume of rodents is 55 to 70 ml/kg of body weight. Of the circulating blood volume, approximately 10% of the total volume can be safely removed every 2 to 4 weeks, and 1% every 24 hours. Refer to the ARAC guidelines on bleeding animals for more information (http://www.umresearch.umd.edu/IACUC/ARAC/).

F.4. **Methods of Restraint**. Note that restraint methods can also include chemical restraint, and if used as such, list the name of the drug(s) and dose(s) here as well as in Section I.

F.5. **Survival Surgery**. Surgical methods including aseptic technique, the surgical procedure, suturing, intraoperative care and monitoring, and postoperative analgesia and monitoring.

F.5.a. **Describe Surgical Procedure**. Only survival surgery is to be described in this section. Non-survival surgery is defined as a surgical procedure in which the animal is euthanized prior to recovery from general anesthesia. In preparing a protocol that includes survival surgery, consultation with the facility or University Veterinarian to ensure that the proper techniques are described and that the facility can support the proposed surgery will facilitate approval.

Describe intraoperative support procedures for the animal, i.e. methods for maintaining body temperature and methods for assessing depth of anesthesia (heart rate, respiration rate, etc.). Describe surgical preparations including preoperative medications and withholding food.

F.5.b. **Aseptic Methods**. Sterile instruments and aseptic technique are required for ALL species (rodents, birds, ferrets, etc.). Describe surgical preparations including hair clipping and skin disinfection procedures. Describe methods of instrument

sterilization between surgeries, i.e. cold sterilant, hot beads, etc. Survival surgery on rodents, birds, and bats should be performed in accordance with the ARAC Guideline: "Guidelines for Survival Surgery" http://www.umresearch.umd.edu/IACUC/ARAC/.

F.5.c. **Who will perform the surgery?** Provide the names of all individuals performing animal surgery and describe their qualifications to perform the specific procedures listed in terms of related training and experience.

F.5.d. **Postoperative Care**. Describe supportive therapy that is required (e.g. supplemental heat source, fluids, etc.) and state the observations the designated person will use to evaluate the animal's health status in the immediate postoperative period until the animal has recovered from anesthesia. In addition, describe longer term postoperative care needs such as analgesia, suture/staple removal, catheter flushing, etc., that accompanies the surgical procedure(s). A written record should be kept of observations during the postoperative period for each animal.

F.5.e. **Has major survival surgery been performed on any animal prior to being placed in this study**? Major Survival Surgery is defined as a major operative procedure from which an animal is allowed to recover from anesthesia. Major operative procedures are defined as procedures that penetrate a body cavity or any procedure that has the potential to cause permanent impairment of physical or physiological function. More than one major survival surgery on a single animal must be justified.

F.5.f. **Will more than one major survival surgery be performed on any animal while on this study?** Major survival surgery is defined in section F.5.e. If more then one major operative procedure is to be performed on a single animal, the need for multiple procedures usually constitute parts of a single experimental paradigm which must be justified in this section.

F.6. **Anticipated Resultant Effects**. Include anticipated effects on the animals during the study. Provide specific details for each procedure that can affect the pain or distress potential for the animals.

F.7. **Provide Experimental and Humane Endpoints**. Endpoint criteria are usually timelines or a medical state used to intervene to prevent unnecessary pain and distress. They describe when it is time to either euthanize an animal to prevent suffering, discontinue a painful procedure or provide treatment to relieve pain or distress. Experimental endpoints are based on experimental design such as a timeline to bleed or euthanaize an animal. Humane endpoints determine when you must intervene due to adverse outcomes or complications.

Endpoint criteria should apply to animals before, during and after experimental procedures. For example, the pain experienced by an arthritic transgenic mouse

may be alleviated with analgesics, but the lack of mobility may ultimately cause body weight loss and a need for euthanasia prior to reaching the experimental endpoint. In this case, setting a body weight loss standard of 15-20% would constitute a humane endpoint and would be followed even before the start of the study.

For all studies, death as an experimental endpoint should be minimized and must be scientifically justified. Refer to the ARAC Guideline, "Guidelines for Endpoints in Animal Study Protocols" (http://www.umresearch.umd.edu/IACUC/ARAC/) for further details on setting appropriate endpoints. Examples of endpoint criteria include a limit on weight loss as a percentage of body weight (often 20-25%); anorexia for an extended time (e.g., three days); sudden pain or distress that cannot be controlled with analgesics, sedatives, or tranquilizers; or severe medical conditions that cannot be controlled with appropriate therapy (e.g., severe systemic infections, kidney or liver failure, or heart disease).

Score sheets and plans for monitoring animals and subsequent intervention are also required.

F.8. **Animal Procedure Location(s).** Provide the building and room number for all laboratory or special animal activity spaces, or animal facility spaces that will be used for all live animal procedures to include surgeries, euthanasia, and tissue harvesting. Specialized surgical facilities are required for major operative procedures proposed in higher species such as rabbits, cats and ferrets. Surgery on birds, bats and rodents must be performed in suitably prepared areas in accordance with the ARAC Guideline: "Guidelines for Survival Surgery of Rodents, Bats and Birds" (http://www.umresearch.umd.edu/IACUC/ARAC/).

Section G: PAIN AND DISTRESS

G.1. **Categories**. Under most conditions, a procedure that would cause pain or distress in a human causes the same in animals. The USDA Animal Welfare Regulations stipulate that the number of research animals used by an institution must be reported annually to the USDA. It is important for information on Category III procedures to be complete and accurate – once submitted to USDA, the public can obtain this information under the Freedom of Information Act.

If multiple procedures will be performed on an animal, the animal is placed in the category appropriate for the most painful/distressful procedure. One animal cannot be placed in multiple categories. The animal numbers displayed in this section should match the total numbers in Sections B and E3. If multiple species are used, list each species separately.

Category I - Minimal, transient, or no pain or distress. (USDA Column C) These procedures are considered to produce minimal, transient, or no pain or distress when performed by competent individuals using recognized methods.

Examples:
- Administration of anesthetics, analgesics, fluids, and oral medications
- Blood collection (except intracardiac, and periorbital in some species)
- Euthanasia as performed in accordance with recommendations of the AVMA Guidelines on Euthanasia
- Intracerebral inoculations in neonatal rodents

Category II - Pain or distress relieved by appropriate measures. (USDA Column D) These procedures may produce pain or distress, but are performed using appropriate and adequate anesthetics, analgesics, or tranquilizers and followed with appropriate measures to alleviate pain or distress.

Examples:
- Surgery
- Electrical shocks including shock reinforcement or using voltage that is accepted as generally causing pain in humans
- Use of any agent that induces excessive inflammation or necrosis
- Drug or radiation toxicity testing, including survivability determinations, producing pain and distress
- Infectious disease work depending on the point of intervention
- Intracardiac blood collection
- Periorbital collection of blood from any species except mice and hamsters

Category III - Unrelieved pain or distress (USDA Column E) These procedures include those listed above for Category II which are performed without appropriate and adequate anesthesia or analgesia; or which are not followed with appropriate measures to alleviate pain or distress; or which are not amenable to relief by therapeutic measures. Withholding of anesthetics, analgesics, or tranquilizers can only be allowed if it is scientifically justified.

Examples:
- Lethal dose studies (e.g., LD50 studies) that allow animals to die without intervention
- Pain studies that would not be possible if pain-relieving agents were administered; and
- Psychological conditioning experiments that involve painful stimuli such as a noxious electrical shock that cannot immediately be avoided by an animal.

G.2. **Literature Search**. For protocols describing animal research categorized as Category II or III, a literature search of at least two different databases must be performed to identify any alternative procedures that are less painful. The key words used must be directed at minimizing pain and distress and determining if alternatives to the animal model or procedure are possible. Key words that limit the search only to the proposed animal model are not acceptable. For example, if you

are using ferrets, you should determine if the same procedures can be done in other animals such as rodents.

Suggested databases are MEDLINE, AGRICOLA, ALTWEB, PUBMED and BIOSIS. State the date of the search, the years covered by the search, and the keywords and/or search strategy used. The results of the search should be BRIEFLY summarized. In some highly specialized fields of study, conference proceedings or subject-expert consultants, etc., may provide more relevant and current information.

For animals in Category II or III, you must also consult with your facility veterinarian or the University Veterinarian when planning these procedures.

Section H: ANESTHESIA AND ANALGESIA

List the agent, procedure requiring the agent, dose, route, frequency, and if not already mentioned elsewhere, any requirement for and duration of pre-anesthetic fasting, methods of anesthetic monitoring, and care during anesthetic recovery. Completing this section should involve consultation with your facility veterinarian or the University Veterinarian.

The type and dose of anesthetic, analgesic or sedative must be appropriate for both the species being used and the type of pain or distress being prevented/relieved. Doses and routes of administration should be clearly appropriate and effective, i.e., commonly accepted or published doses, or a description of your experience with the agent and dose described which demonstrates its effectiveness. The frequency and/or indications for drug administration should be provided, e.g. every 12 hours, as needed, etc.

If agents are to be given "as needed", a brief description of the indications for its administration should be provided, e.g., "at the first indication of discomfort as evidenced by lethargy, anorexia, hunched posture, eye squinting, or vocalization".

If anesthetics or analgesics must be withheld when a procedure will cause more than slight or momentary pain or distress, then it must be scientifically justified, in Section G.

Section I: BIOLOGICAL MATERIAL FOR USE IN ANIMALS

Biological material and animal products such as cell lines, tissues, and tumors that are introduced into research animals can harbor human or animal pathogens (e.g. ectromelia, lymphocytic choriomeningitis and mouse hepatitis) which can subsequently infect animal colonies.

You are responsible for ensuring that the biologic materials used will not endanger the health of the live animals used in your study or other animals housed in the

animal facility. The University Veterinarian can provide you with information about the tests required. In this section, describe the material and tests performed. The documentation of the testing should be submitted as an attachment.

Section J: ANIMAL DISPOSITION / EUTHANASIA

J.1. A description of euthanasia for ALL animals on the protocol must be clearly described. Methods of euthanasia should be consistent with the recommendations of the AVMA Guidelines on Euthanasia unless the IACUC approves deviations for scientific reasons. Methods which are not consistent with the recommendations in the AVMA Guidelines must be scientifically justified.

Improper technique can cause pain and suffering to animals, so you must be trained to properly and humanely perform euthanasia, or any other procedure on an animal, until an experienced person has trained you and you feel confident performing the technique. Euthanasia agents should not be used beyond their expiration date.

J.2. **Common Methods of Euthanasia.**

- Rodents. Carbon dioxide (CO_2) or anesthetic gas asphyxiation; over-crowding should be avoided; dry ice is not allowed as a source of carbon dioxide - only compressed gas tanks may be used. Barbiturate overdose given intraperitoneal .
- Rabbits. Barbiturate overdose via the lateral ear vein.
- Ferrets, cats, horses, other large animals. Barbiturate overdose by injection in cephalic, jugular, or other vein.
- Neonates are in general resistant to low oxygen levels; thus carbon dioxide is not very effective as a euthanasia agent unless there is prolonged exposure. Neonates of larger species (cats, pigs) can be given intravenous euthanasia agents. Rodent neonates offer special challenges. Due to the difficulties of performing intravenous injections and the difficulty of properly anesthetizing rodent pups, quick decapitation with scissors, with or without carbon dioxide or anesthetic gas asphyxiation prior to decapitation is allowed.

J.3. **Method to Ensure Death**. It is very important to ensure an animal is really dead before placing it in a bag for disposal. It is easy to mistake a deeply anesthetized animal for a dead animal. Because of the risk for recovery from CO_2 asphyxiation and the difficulty of verifying death in small rodents, thoracotomy, decapitation or cervical dislocation after apparent death from CO_2 are ways to ensure the irreversibility of the procedure in rodents.

J.4. **Conditionally Acceptable Methods**. The AVMA Guidelines on Euthanasia classify methods of euthanasia as acceptable, conditionally acceptable and unacceptable. Acceptable methods are those that consistently produce a humane death when used as the sole means of euthanasia.

Conditionally acceptable methods are those techniques that by the nature of the technique or because of great potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature.

Unacceptable techniques are those methods deemed in-humane under any condition or pose a substantial risk to the human applying the technique. Justification is required for methods that are conditionally acceptable or unacceptable.

The following methods of euthanasia are not considered humane when used alone, but may be acceptable if the animal is unconscious (anesthetized). These methods require special justification.

- Air embolism. Injection of air intravenously can be accompanied by convulsions, other neurological signs, and vocalization in a conscious animal. The animal should be under general anesthesia.
- Cervical Dislocation. Cervical dislocation is used to euthanize small birds, mice, immature rats weighing less than 200 grams and rabbits weighing less than 1 kg. You should anesthetize or sedate the animals first, unless there are scientific reasons for not doing so approved by the IACUC. Advantages of cervical dislocation include 1) it may induce rapid unconsciousness, 2) it does not chemically contaminate tissue, and 3) it is rapidly accomplished. However, there is some experimental evidence that brain activity and sensory capabilities do not end immediately after dislocation. In addition, it may be aesthetically displeasing to personnel.
- Decapitation. Decapitation without sedation or anesthesia is an important issue, especially for rodents and small rabbits. The primary justification for this method is the need to recover tissues and body fluids that are chemically uncontaminated sedatives or anesthetic agents. Special commercial guillotines designed to accomplish decapitation in a uniformly instantaneous manner should be used. However, the handling and restraint required to perform this technique may be distressful to animals, the guillotine blade is a hazard to personnel performing the technique, the technique may be aesthetically displeasing to personnel, and there is some experimental evidence that brain activity and sensory capabilities do not end immediately. Consequently, the use of decapitation without prior anesthesia or sedation should be used in research settings only when scientifically justified by the user and approved by the IACUC.
- Exsanguination. This method involves the near-complete withdrawal of blood from an animal. Because anxiety is associated with very low blood pressure, exsanguinations should not be used as a sole means of euthanasia. It can be used to ensure death in unconscious animals.
- Intracardiac injection. Administration of injectable euthanasia agents into the heart provides rapid loss of consciousness and death. However, intracardiac injections should only be performed if heavily sedated, anesthetized, or comatose animals unless the IACUC approves it after considering a justification. The same holds true for blood collection from the heart.

- Pithing. Pithing is the destruction of the central nervous system by mechanical means. Either the brain or the spinal cord, or both may be destroyed, depending on the species and additional methods of euthanasia used. Pithing is a physical means of euthanasia, and thus should only be used if nonphysical methods are not appropriate. Accordingly, pithing is generally used as an adjunctive procedure to ensure death in an animal that has been rendered unconscious by other means. For some species with anatomic features which facilitate easy access to the central nervous system, such as frogs, pithing may be used as a sole means of euthanasia; but anesthetic overdose is a more suitable method.
- Rapid freezing. Formation of ice crystals on the skin and in tissues of an animal may cause pain or distress. Quick freezing of deeply anesthetized animal is acceptable.

J.5. **Disposal of Euthanized Animals.** Animal carcasses which have NOT been contaminated with hazardous agents are to be disposed of as biological waste in accordance with facility and DES guidelines. DES should be consulted on proper disposal methods for carcasses contaminated with hazardous agents. The proper disposal of contaminated carcasses should be described in Section K and not here.

Section K: HAZARDOUS AGENTS

K.1. Identify any agent (radionuclides, biological agents, hazardous chemicals, recombinant DNA) that will be administered to the animals as part of this study. Refer to DES for assistance in describing the use of hazardous agents in animal research. DES will assist you in obtaining all of the appropriate safety documents specified in this section (e.g., radiation safety protocols, Recombinant DNA documents, etc.), which should be submitted as attachments. The use of hazardous agents requires the signature of the DES representative(s) under Section O before a protocol can be approved. The description of the agents on the form defines the level of detail required for this section. The disposal of carcasses contaminated with hazardous agents should be described here as well.

Radionuclides. Identify radioactive isotopes used *in vivo* and their activity. The Radiation Safety Committee may need to review and approve the use of radioactive materials in animals.

Biological Agents. List human pathogens (viruses, bacteria, parasites) and any human tissue, blood, cells, etc. to be used *in vivo*. A Biological Materials Research Registration Document must be filed with DES and approved by the UMD Institutional Biosafety Committee (IBC) for the use of these biological agents. Identify the Registration number. See Appendix B of the NIH Guidelines for a list of infectious agents at:
http://www4.od.nih.gov/oba/rac/guidelines_02/APPENDIX_B.htm .

Hazardous Chemical or Drugs. List any hazardous chemicals or drugs (carcinogens, mutagens, formaldehyde, inhalant anesthetics, etc.) that will be

administered to animals. All laboratories that use hazardous chemicals must submit a written Chemical Hygiene Plan with written Standard Operating Procedures (SOPs) to the Division of Environmental Safety. The Chemical Hygiene Plan and SOPs should identify the appropriate health and safety precautions including protective equipment, designated areas, and any engineering controls. If hazardous chemicals are a component of your protocol, omission from your Chemical Hygiene Plan will delay the review process.

Recombinant DNA, including recombinant microorganisms and transgenic animals. Identify any nonexempt Recombinant DNA (rDNA) used *in vivo* e.g. genetically modified microorganisms and constructs used for development of transgenic or knock-out animal models. A recombinant DNA registration document must be filed with and approved by the UMD Institutional Biosafety Committee prior to initiation of the study; unless the rDNA qualifies for registration simultaneous with initiation, e.g. ABSL-1 constructs. See NIH's rDNA Guidelines at: http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html .

The use of hazardous agents requires the signature of the DES representative(s) under Section O before a protocol can be approved.

K.2. Indicate the Animal Biosafety Level (1, 2 or 3) at which the animal study will be conducted. Check the "NA" box if no biosafety precautions are required.

K.3. Describe the precautions and procedures that will be implemented for work involving identified hazardous agents. Check the "NA" box of no hazardous agents will be used for this animal study.

K.4. Identify agents (with concentrations/activities) that are administered to the animals, method(s) of administration, and identify the concentrations/activities that are expected to be expelled in the animal waste products. Specify procedures for handling wastes if contaminated. Check the "NA" box if no hazardous agents will be administered to animals during this study.

K.5. Identify agents that are administered that are expected to remain in tissues or the carcass when the materials are ultimately disposed and the expected concentrations/activities. Specify procedures for handling carcasses/tissues if considered contaminated. Check the "NA" box if no hazardous agents will be administered to animals during this study.

Section L: SPECIAL CONCERNS OR REQUIREMENTS

List any items or procedures that may require special care or attention by either the PI or the animal facility during the performance of the study. Include procedures that may adversely affect the animals, how those effects will be detected and the actions that will be taken to support the animals and to minimize pain or distress. Information regarding and identification of animals that may require special care

due to surgical alterations (e.g. splenectomy, adrenalectomy, etc.) or genetic manipulations should be recorded.

List any unusual requirements that the animal facility management may need to consider to support the study, including importation, specialized housing (e.g. sterile cages for immunocompromised animals), lighting, feed, water or a need for other than routine veterinary care. Also include the use and storage of specialized pieces of equipment and special off-hour or weekend/holiday requirements.

Any deviations from the *Guide to the Care and use of Laboratory* Animals (http://www.nap.edu/html/labrats/) must be justified.

Section M: TRAINING

Research personnel using animals must be trained so that they are qualified to perform research procedures on animals. The IACUC must ensure that personnel are qualified to perform the proposed procedures on animals as part of the review process. You as the PI are responsible for the training of your laboratory personnel. Training and proficiency must be documented for all personnel who will be working with animals on your study. You will be responsible to ensure that all involved will comply with the procedures described in the protocol.

Specific topics required by the USDA AWA Regulations and PHS Policy are 1) humane methods of animal maintenance and experimentation, including basic needs of each species of animal; proper handling and care of animals; 2) proper pre-procedural and post-procedural care; 3) aseptic surgical methods and procedures; 4) the concept, availability and use of research or testing methods that limit the use of animals or minimize animal distress; 5) proper use of anesthetics, analgesics and tranquilizers for any species used; and 6) methods whereby deficiencies in animal care and treatment are reported.

Although academic degrees are indicators of educational experience, they are not often useful in evaluating an individuals experience in animal research.

The IACUC must assure itself that you and the key personnel listed in Section A are qualified to perform the work. If you or your key personnel do not have the necessary training or experience to perform the proposed procedure, contact the University Veterinarian to arrange for training. You must complete the "Principal Investigator and Animal Users" course prior to your protocol being approved. All of your key personnel must also complete the course prior to working with animals, but completion can be after protocol approval. It would be helpful to complete all training prior to protocol review. If you or your key personnel are unable to obtain all training before submitting your protocol, you can admit this on your animal protocol form. However, make sure that all training is completed before animal procedures begin. For more information on UMD training requirements for animal use, see http://www.umresearch.umd.edu/IACUC/education.htm

Training and experience should only address the animal user's training and experience for the procedures listed in the protocol that they will actually be performing and should address both experimental procedures, i.e. injections, blood withdrawals, etc., as well as surgical procedures. Short, bulleted statements are sufficient.

Section N: CERTIFICATIONS AND ACKNOWLEDGMENTS

All protocol forms must be signed by the Principal Investigator. Your signature as the Principal Investigator in this section certifies the following:

1. You are responsible for the conduct of the procedures and humane care of your research animals. This extends to all individuals listed on the protocol.

2. You will conduct all animal work in accordance with the protocol and report all significant changes in writing to the IACUC for review and approval prior to the initiation of the study change. Significant changes are defined as those that have the potential to impact substantially and directly on the health and well being of the experimental animals. Examples of significant changes include additions or deletions of the PI, addition of surgery, change in animal species, change in pain level, change in overall study objectives, addition of hazardous agents, etc. For minor protocol changes, contact the University Veterinarian, IACUC Chair or IACUC manager.

3. You will certify that the proposed research is not duplicative of previous research unless a justification is approved.

4. You and all personnel listed in Section A are enrolled in the Animal Handler Medical Surveillance Program Exposure Program or equivalent. If information or assistance is needed for enrolling in the Program, see the Health Services web site at http://www.health.umd.edu/services/occupationalhealth.html, 301-405-8172.

5. You will maintain complete animal records such as census sheets, animal health, surgery etc.

6. You have considered alternatives to procedures that may cause more than momentary or slight pain and distress and has provided a written narrative description of the methods and sources used to determine that alternatives were not available as specified by the AWA regulations. This narrative appears in Section H.

7. That all personnel listed in Section A have completed the "Guidelines for Principal Investigator and Animal User" course and have been trained in all procedures described in the protocol prior to initiating animal procedures.

Section O: CONCURRENCES

It is your responsibility as the Principal Investigator to obtain the signature of your Department Chair. Protocols originating from a Department Chair do not require a concurring signature from your Dean. This signature certifies the availability of facilities and appropriateness of the budget and research effort. The signature is required prior to the protocol's submission.

Signature(s) of representative(s) of the Division of Environmental Safety are required for studies utilizing hazardous agents. The signature is required prior to protocol approval, but not submission. You are not responsible for obtaining this signature.

You are responsible for obtaining the signature of the facility manger or facility veterinarian where your animals will be housed. This signature is not required for field studies unless captured animals will be housed in UMD facilities. The University Veterinarian serves as the facility veterinarian for all facilities except Animal and Avian Sciences and Veterinary Medicine. The signatory only certifies that he/she has reviewed the protocol and that the facility has the infrastructure and veterinary care resources/information to support the protocol. Approval is not withheld or delayed for other reasons. If the signatory has questions outside of the areas of infrastructure and veterinary care, those questions must be presented to the IACUC Chair. The signature is required prior to protocol approval but not submission.

Section P: APPROVAL

The Chairperson of the IACUC has authority for final approval of the protocol.

"Instructions for Emergency Animal Treatment & Care Form"

- Complete and submit along with the completed protocol for IACUC review.
- Multiple forms may be used if care is different for multiple species listed on the protocol or if care is different for individual experimental groups.
- All information listed on this form should be the same as listed in the protocol without contradiction regarding animal care issues or endpoints.

List of procedures – emphasize procedures that may result in serious animal health complications.

Points of contact (POC) – does not have to be the PI, but should be the person working directly with the animals who has an intimate knowledge of their experimental status and overall health.

Potential or expected complications – directly tied to the "list of procedures" and therefore if there are multiple procedures listed, there will likely be multiple complications listed.

Circumstances requiring contact - directly tied to the "list of procedures" and therefore if there are multiple procedures listed, there will likely be multiple circumstances listed.

Treatment – if the treatment cannot be prescribed by the veterinarian, then please be diligent in listing restrictions and specific treatments for all listed complications.

Euthanasia – if euthanasia is not at veterinary discretion, then be diligent in listing restrictions and specific criteria for all listed complications. If the POC must be notified prior to euthanasia, then ensure accurate contact information and POC availability at critical stages of the experiment.