Appendix for Animal Use in Research, Development, Testing, Evaluation, and Training

Animal Care and Use Review Office (ACURO)
US Army Medical Research and Development Command

DoD-funded animal work must NOT be initiated until the awardee receives ACURO approval.
Animal work initiated without ACURO approval is noncompliant and may not be funded.
***ACURO will ONLY review protocols that have been approved by your Institutional Animal Care and Use Committee (IACUC). ***

Institutions using United States Department of Defense (DoD) funds to support the use of animals in research, development, testing, and evaluation (RDT&E) or training must adhere to DoD requirements for administrative review and approval by ACURO.

Provide electronic copies (searchable PDF format preferred) of the following documents to ACURO for review and approval:

A. **Appendix**. A completed copy of this **ACURO Animal Use Appendix** (note: if submitting multiple protocols, each IACUC-approved protocol must have a separate ACURO Appendix).

B. **Protocol**. A copy of the **fully approved institutional animal use protocol** that describes all DoD-funded animal use. Note: If the protocol expires within the next 90 days, please wait to submit until your protocol is renewed.

C. **Approval**. Documentation of initial protocol **approval**.

D. **Amendments**. A copy of existing protocol **amendments** applicable to this DoD award and documentation of approval for each.

Questions regarding ACURO protocol review processes should be directed to ACURO at

Phone:301-619-6694
Email: Usarmy.detrick.medcom-usamrmc.other.acuro@mail.mil
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Administrative Data

1. DoD Funding of Protocol

Check one to indicate if all activities described in the submitted protocol are funded by DoD.

☐ **Fully funded by DoD**: All of the experiments described in the accompanying animal use protocol are funded by this DoD award.

☐ **Partially funded by DoD**: This animal use protocol contains some experiments that are NOT funded by this DoD award.

- Note: ACURO review covers only those aspects of the protocol related to work funded by DoD.
- The copy of the protocol submitted **MUST** include highlighting of all information relevant to the DoD-funded portion of the study.

2. Key Personnel & Administrative Data

Complete all sections of the table with the requested information. Consult with your IACUC office as needed.

DoD Award PI Name		
DoD Award PI Email & Telephone		
DoD Funding Source		
DoD Grant/Award/Proposal Number		
Institution where animal studies are conducted		
Protocol PI Name		
Protocol PI Email & Telephone		
IACUC Protocol Title		
IACUC Protocol Number		
☐ If this is a **triennial *de novo* rewrite** submission and the **protocol number has changed** from the original protocol, check this box and enter the original protocol number here: Click here to enter text.		
Attending Veterinarian (AV) Name		
AV Email & Telephone		
IACUC Point of Contact (PoC) Name		
IACUC PoC Email & Telephone		
Institutional Grants Manager Name		
Grants Manager Email & Telephone		

Animal Usage

3. Total Number of Animals to be used by Species and USDA Pain/Distress Category

Required information:

a) Fill in the table for each species used, listing all species of animals used in DoD-funded research and the highest expected USDA Pain/Distress Category for all animals.
b) Note: please list species (by common or scientific name) only. It is NOT necessary to provide a breakdown by strain, substrain, breed, etc.
c) Note: DoD requires this USDA pain category information for all animal species, including mice, rats, and other species not regulated by USDA.
d) See brief definitions below. Full definitions can be found on APHIS form 7023.
    a. Column/Category B: Animals being bred or held for use in research but not yet used for such purposes
    b. Column/Category C: Animals that will experience no more than slight or momentary pain or distress
    c. Column/Category D: Animals that will potentially experience pain or distress for which appropriate anesthetic, analgesic, or tranquilizing drugs WILL be used
    d. Column/Category E: Animals that will potentially experience pain or distress for which appropriate anesthetic, analgesic, or tranquilizing drugs WILL NOT be used

SPECIES	HIGHEST USDA PAIN/DISTRESS CATEGORY (B, C, D, or E)	TOTAL NUMBER

4. Justification for Unalleviated Pain or Distress

Required information (only for USDA Category E studies):

a) Provide in the box below a detailed justification for any unalleviated pain or distress.
b) The justification should provide the rationale for withholding pain relieving medication, including, as applicable:
    a. Procedures or conditions that will result in unalleviated pain or distress
    b. Outcomes or parameters to be measured that can be altered by use of pain relieving medication
    c. Expected effect of pain relieving medications on those outcomes
        i. Note: When applicable, justification should address commonly used pain-relieving medications (e.g., NSAIDs, opioids, local anesthetics, etc).
        ii. When possible, provide references from the scientific literature or data from previous studies that demonstrate the adverse effect on specific study variables to be measured.
    d. Why the effects on these outcomes will compromise the validity of study results
    e. Why you believe the confounding effects of pain-relieving medications will be more severe than the confounding effects of unrelieved pain
c) If this is NOT a category E study, enter "N/A."

5. Animal Procurement

Check the boxes to answer the questions and provide documentation requested, if applicable.

☐ Yes ☐ No	A. Does the protocol involve **USDA regulated animal species**? (Note: most mice & rats bred for use in research are NOT USDA regulated.). If "No" then check "N/A" for question B.
☐ Yes ☐ No ☐ N/A	B. Do you obtain animals from a supplier that holds a current **USDA license**? Note: If supplier claims exemption from USDA licensing, ACURO requires confirmation from your IACUC that the supplier has met the **exemption criteria** outlined in the USDA Animal Welfare Regulations.
☐ Yes ☐ No	C. Will **wildlife** species be used? If "yes" provide ACURO copies of all capture & use **licenses and permits**.
☐ Yes ☐ No	D. Will **privately-owned animals** be used for RDT&E or training? If "yes" provide a copy of the **owner consent form** that will be used. The form must inform the owner that the animal will be used in DoD-funded research.

Animal Care and Use Program Information

6. Institutional Accreditations

a) Check the appropriate boxes for the institution where the animal research will be conducted.
b) **Unaccredited, Unassured Institutions in the United States**: If you check "no" for both 6.i and 6.ii below, submit a statement signed by the Institutional Official stating that the care and use of animals will be conducted in accordance with the standards of the current edition of the National Research Council's Guide for the Care and Use of Laboratory Animals and applicable Federal and DoD regulations.
c) Your institution's IACUC office or attending veterinarian can assist with this information, as needed.

i. **AAALAC International Accreditation**

☐ Yes ☐ No Animal work is being performed at an AAALAC International accredited institution.

ii. **Public Health Service Animal Welfare Assurance Statement**

☐ Yes ☐ No Animal work is being performed at an institution that holds a current assurance with the U.S. Public Health Service Office of Laboratory Animal Welfare (OLAW).

7. Institution's Veterinary Care Program

For institutions that are **NOT** AAALAC accredited, provide in the box below a description of the institution's program of veterinary care. The description should address all of the following, as applicable:
a) Description of routine care of animals by vivarium staff, including daily observation of animals
b) Description of animal care on weekends & holidays and provision of emergency care to animals
c) If the facility uses USDA covered species and your attending veterinarian is part time, confirm that there is a written program of veterinary care
d) For animals on your studies, describe how health and welfare issues are communicated to a veterinarian.
e) If AAALAC accredited, enter "N/A."

****IMPORTANT: read these instructions before filling out sections 8-27****

Details are provided in the grey instructions box for each section below on the required information that must be provided for ACURO review.

Each section below MUST indicate the correct page number in the IACUC-approved protocol that provides the required information. Ensure page numbers are clearly and correctly marked on the protocol document. If your protocol document does not have page numbers, save the document as a PDF and use the page numbering indicated in the PDF reader.

If the protocol provides all the required information for a given item, leave the corresponding "Required information not provided in the protocol" field blank.

If any of the required information is NOT provided in the protocol, add the required information to the "Required information not provided in the protocol" field for each item. Do NOT copy information from the protocol into sections 8-27 of this Appendix form.

If any section is not applicable to your protocol, enter "N/A" for "Page number in protocol."

****Do NOT copy text from the IACUC protocol into sections 8-27.****

Study Design

8. Summary of Study Objectives

Required information: Brief description in language understandable to a nonscientist of how animals will be used to achieve the overall and specific objectives of the protocol

- Page number in protocol: enter page number(s)
- Required information not provided in the protocol:

9. Rationale for Using Animals

Required information: Explanation of why it is necessary to use animals to achieve study objectives

- Page number in protocol: enter page number(s)

● Required information not provided in the protocol:

10. Animal Model Rationale

Required information:
a) Explanation of why this particular animal model is the most appropriate model to answer the scientific question (e.g., what unique physiologic, morphologic, or genetic characteristics does this specific animal species, strain, or breed possess that make it the optimal model?)
b) If multiple species are used, include justification for each species used.

● Page number in protocol: enter page number(s)
● Required information not provided in the protocol:

11. Rationale for Number of Animals to be Used (Statistical Justification).

Required information:
a) Description of the rationale for the number of animals used, including, as applicable:
    a. Methodology used to determine group size and total number of animals
    b. Description of power-based sample size determination, or
    c. Legal mandates that require specific group sizes or total number of animals for certain types of testing

● Page number in protocol: enter page number(s)
● Required information not provided in the protocol:

12. Consideration of Alternatives to Procedures that Cause Pain or Distress (Required for all species of animals used in USDA Category D or E)

Required information:
a) If any animals are listed in USDA Category D or E, the description must include a narrative description of methods and sources used to determine that alternatives were not available for the procedures that may cause pain or distress.
b) Note: DoD regulations require this information for all animal species, including those not subject to USDA regulation.
c) If there are NO animals listed under USDA Category D or E, mark this section "N/A."

● Page number in protocol: enter page number(s) or n/a (category B or C)
● Required information not provided in the protocol:

13. Experimental Design

Required information:
a) Explanation of experimental design that will allow reviewers to understand and follow the progress of animals through the proposed procedures
b) Clearly understandable account of numbers of animals and their distribution into experimental groups
    a. Inclusion of flow charts illustrating experimental design and summary tables of the experimental groups is encouraged.

c) Scientific justification for the use of only one sex of animal, when applicable
d) Description of the projected experimental endpoints, including maximum time on study for animals
e) Note: Animal numbers described in this section must be consistent with animal numbers described elsewhere in the protocol and in this Appendix.

- Page number in protocol: enter page number(s)
- Required information not provided in the protocol:

Surgery and Other Potentially Painful Procedures

14. Description of Surgical and Other Potentially Painful Procedures

Required information:
a) Description of each type of surgical or other potentially painful procedure planned in sufficient detail that someone with appropriate training in surgery and animal use could repeat it with minimal variation
b) Statement regarding whether or not aseptic technique will be used for surgical procedures. If aseptic technique is not practiced, provide detailed justification.
c) For the purposes of this question, a potentially painful procedure is one that may produce a level or duration of pain that would normally require the use of anesthetics or analgesics.
d) If surgery or other potentially painful procedures will NOT be conducted, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

15. Immediate and Long-Term Post-procedural Monitoring/Observations/Treatment

Required information:
a) For any surgical or other potentially-painful procedures, provide a description of immediate (during anesthetic recovery), intermediate (1-3 days post-procedural), and long-term post-procedural monitoring, including frequency of monitoring and how you will monitor for and address any potential pain/distress.
b) Typical signs of pain or distress specific to the animal species and procedures being performed and plans to mitigate the pain or distress, including timing of administration of pain relieving medications
c) If used, provide a copy of any pain scoring sheets.
d) If surgery or other potentially painful procedures will NOT be conducted, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

16. Multiple Major Survival Surgeries

Required information:
a) Scientific justification for multiple major survival surgeries performed on the same animal
b) If multiple major survival surgeries will NOT be performed on the same animal, write "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

Other Procedures / Technical Methods

17. Paralytic Agents

Required information:
a) Description of how paralytic agents will be used
b) Justification for use
c) Monitoring methods used to ensure animals are adequately anesthetized while under the influence of paralytics
d) Note: the use of paralytic agents without anesthesia is prohibited.
e) Note: paralytic agents include neuromuscular blocking agents (such as pancuronium, rocuronium, gallamine, succinylcholine, and others) that produce generalized muscle paralysis without loss of consciousness. General anesthetics (e.g., isoflurane, ketamine, etc.) are NOT considered paralytic agents.
f) If paralytics will NOT be used, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

18. Genotyping

Required information:
a) Description of any methods used for sample collection for genotyping or other DNA analysis, including:
    a. Age of animals at sampling
    b. Amount and type of tissue obtained for sampling
    c. Anesthetic and/or analgesic use and criteria for use
b) If NO tissue samples are collected for genotyping, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

19. Adjuvants

Required information:
a) Adjuvants to be used and the plan for their use
b) Expected adverse effects
c) Frequency and method of injection site monitoring
d) Response plan (e.g., endpoints, treatment, etc.) in the event of an adverse reaction
e) Any use of adjuvants that have significant potential to induce pain (e.g., Complete Freund's Adjuvant) must be addressed in Section 12 of this appendix (Consideration of Alternatives to Procedures that Cause Pain or Distress).
f) If adjuvants will NOT be used, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

20. Use of Non-pharmaceutical Grade (NPG) Agents in Animals

Required information:
a) NPG agents to be used in animals are identified as such in the protocol
b) If NPGs are to be used but are not identified as NPG in the protocol, provide communication from IACUC that NPG usage is approved.
c) If NPG agents will NOT be used, enter "N/A"

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

21. Prolonged Restraint

Required information:
a) Note: this section is for prolonged or long-term physical restraint only; it is NOT intended for short-term actions such as brief restraint for routine blood collection, etc.
b) Justification and detailed description of any prolonged restraint to be used during the study (e.g., primate chairs, restraint boards, etc.)
c) Duration of restraint and frequency of animal observations
d) Procedures for habituation or training of animals to the device prior to the restraint, including the plan for management of animals that fail to adapt to restraint
e) If prolonged restraint will NOT be used, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

22. Behavioral Testing or Behavioral Modification Techniques

Required information:
a) Description of any behavioral tests or behavioral modification methods, including use of aversive stimuli (such as foot shocks), food or water regulation, etc.
b) Description of how animal welfare will be ensured, including acclimation if applicable
c) For food or water regulation, provide the following information:
    a. Describe any plans for temporary removal of animals from the food/water regulation regimen and criteria for reinstating food/water regulation.
    b. Include endpoint criteria related to food/fluid regulation (e.g., % weight loss, hydration status, etc.)
d) If behavioral tests or behavioral modification will NOT be used, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

23. Exceptions/exemptions/departures from the *Guide* or Animal Welfare Regulations

Required information: Description of and appropriate justification for any proposed exceptions to the Animal Welfare Regulations or to "must" standards of the *Guide* (e.g., enrichment restrictions in primates, cage space restrictions, etc.)

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

24. Other Procedures (e.g., antemortem sample collection, electrocardiograms, imaging procedures, stress induction, etc.)

Required information:
a) Description of all other procedures that will be performed while conducting this research but have not been addressed elsewhere in this Appendix
b) If the protocol involves induction of stress for experimental purposes, describe duration, severity, and endpoint criteria for the procedures.
c) If no other procedures will be conducted, enter "N/A."

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

Endpoints & Euthanasia

25. Humane Early Endpoints

Required information:
a) Description of potential adverse outcomes, including adverse effects due to experimental manipulations, agents administered, or any other potential adverse effects not already addressed elsewhere in this Appendix
b) Description of specific, objective criteria that will be used to determine early removal from the study for humane reasons prior to the planned study endpoint (e.g., percentage of weight loss, tumor size that inhibits movement, number of abdominal taps, abdominal distension, loss of locomotion, hypothermia, decreased food or water consumption, decreased activity, pain nonresponsive to analgesics, etc.)

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

26. Death as an Endpoint

Required information:
a) Scientific justification for any proposal in which animals are allowed to die as a result of the experimental procedures without the benefits of early euthanasia or treatment to alleviate pain
b) Explanation of why endpoints prior to death are not adequate to achieve the study objectives

- Page number in protocol: enter page number(s) or n/a
- Required information not provided in the protocol:

27. Euthanasia Methods

Required information:
a) Description of all methods of euthanasia to be used in this study, including methods used to confirm death
b) Scientific justification for use of any euthanasia method not consistent with the most recent edition of the AVMA Guidelines for the Euthanasia of Animals

- Page number in protocol: enter page number(s) or n/a

- Required information not provided in the protocol:

Animal Use Assurances

28. Protocol PI Assurances:

U.S. DoD and Federal regulations specifically require several written assurances from the PI. Carefully read each item and sign below.

As the Principal Investigator on this protocol, my signature below confirms the following:

A. **Painful Procedures**: Pain and distress in animals will be limited to what is unavoidable in the conduct of scientifically valuable research; analgesic, anesthetic, or tranquilizing drugs will be used when appropriate to minimize pain or distress; and a veterinarian was consulted regarding any painful procedures in this protocol.

B. **Animal Use**: The animals authorized for use in this protocol will be used only in the activities and manner described herein unless a modification is approved by the IACUC, or equivalent animal welfare body, and ACURO prior to implementation.

C. **Duplication of Effort**: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

D. **Statistical Assurance**: I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis and determined that the most appropriate number of animals needed for scientific validity will be used.

E. **Training**: I verify that the personnel performing the animal procedures, manipulations, or observations described in this protocol are technically competent and qualified and have been properly trained to ensure that animals will experience no unnecessary pain or distress as a result of the procedures/manipulations.

F. **Responsibility**: I assure that all individuals associated with this project will demonstrate the appropriate regard for the health, comfort, welfare, and well-being of the research animals. I pledge to implement animal use alternatives where feasible, and conduct humane and lawful research.

G. **Scientific Review**: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

H. **Wildlife studies** (if applicable): All research involving wildlife will be performed in compliance with all applicable laws and regulations and with all required permits or licenses.

I. **Animal procurement**: All animals will be procured in compliance with all applicable laws and regulations. Furthermore, USDA regulated animals will be obtained only from USDA licensed suppliers unless exempted under the provisions of the Animal Welfare Regulations.

J. **Disposition**. Animals used in RDT&E or training will have a nonterminal disposition (e.g., adoption, retirement, inter-institutional transfer) whenever possible.

K. **Privately-owned animals** (if applicable). If privately-owned animals are used for research, owner consent will be obtained, and owners will be informed that the study is supported by the Department of Defense.

L. **Annual review of protocols**: Our IACUC conducts annual continuing review of animal use protocols when required by the Animal Welfare Regulations or other applicable regulations.

SUBMISSION IS NOT COMPLETE WITHOUT PROTOCOL PI's SIGNATURE ON THIS PAGE

Protocol PI Printed Name	Protocol PI Signature	Date