ANIMAL CARE AND USE PROTOCOL

Mahidol University-Institute Animal Care and Use Committee (MU-IACUC)

COVER SHEET

Protocol number		This section will be completed by the MU-IACUC
Received by IACUC (dd/mm/yy)		
Approved/Request modification (dd/mm/yy)		
Resubmitted (dd/mm/yy)		
Approved/Disapproved by IACUC (dd/mm/yy)		
Approved/Disapproved by IO/Dean (dd/mm/yy)		
Expiration Date (dd/mm/yy)		

Protocol title:

(Thai)……....

.....

(English)……...

……

If this protocol is a part of the main project, please provide the main project title:
(Thai) .....
(English) .....

Funding source(s): .....

Grant proposal: ☐ to be submitted

☐ has been submitted

☐ has been approved. If approved, duration of approval.....

Anticipated protocol period: From ..... **To** .....

Type of animal protocol

[ ] Research: In the Field of .....
[ ] Testing/Monitoring (please specify).....
[ ] Teaching: Course Title/Level.....
[ ] Biological Production: (please specify) .....
[ ] Animal Breeding (please specify).....
[ ] Other (please specify) .....

Principal investigator: Name……..…

(for a student thesis, the principal investigator is the principal adviser, and the student is a co-investigator)

Position: ……**Department** ……...……

Faculty/Institute ……....

…….

Tel. ……**Fax.** ….……

E-mail ……..…..

* **Animal use license no……Expired date…….**

Co- investigator: Name ……...…….

Position: ……**Department** ……...……

Faculty/Institute ……....

…….

Tel. ……**Fax.** ….……

E-mail ……..…..

***Animal use license no……Expired date…….**

Co- investigator: Name ……...……

Position: ……**Department** ……...……

Faculty/Institute ……....

……

Tel. ……**Fax.** ….……

E-mail ……..…..

***Animal use license no……Expired date…….**

Contact person in case of emergency: .....
Office/Affiliation: .....
Phone: .....**E-mail:** .....

**Issued by Institute of Animal for Scientific Purposes Development, NRCT*

Your signature as P.I., Co-investigator on this application verifies that the information herein is true and correct and that you are familiar with and will comply with standard of animal care and use established under the ethical guidelines and policies of the Mahidol University and Office of the National Research Council of Thailand **(**NRCT) and the animal for scientific purpose act., B.E. 2558

Principal investigator: Name……..…

_____ _____
(Signature) (Date)

Co- investigator: Name ……...… ……

_____ _____
(Signature) (Date)

Co- investigator: Name ……...… ……

_____ _____
(Signature) (Date)

****

This section will be completed by the MU-IACUC

Statistical review: Name …… …….……

_____ _____
(Signature) (Date)

Safety review: Name …… ……..……

_____ _____
(Signature) (Date)

Attending veterinarian: Name …… ……..…..…….

* Animal use license no……Expired date……

* * Veterinary practitioner license no……..……Expired date…….

_____ _____
(Signature) (Date)

** Issued by Institute of Animal for Scientific Purposes Development, NRCT*

*** Issued by The Veterinary Council of Thailand*

Head of Faculty/Institute: Name ……...……

_____ _____

(Signature) (Date)

Faculty/Institute: ……

……..……

Approval

MU-IACUC Review:

☐Approved **☐Approval recommended** **☐Disapproved**

……..……

(Chair, MU-IACUC signature, Date)

MAHIDOL UNIVERSITTY
STANDARDIZED RESEARCH PROTOCOL FORMAT FOR PERMISSION OF ANIMAL CARE AND USE

1. Non-technical summary: *(Provide a brief description of the project that is easily understood by non-scientists, expressing its significance and needs for undertaking the study).*

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……

2. Rationale and literature review: *(Include a brief statement of the requirement for the information being sought. Typically, the literature or the experience that led to the proposal will be briefly reviewed, references cited will be provided).*

……

……

……

3. Literature search for duplication: *(This search must be performed to prevent unnecessary duplication of previous experiments).*

3.1 Literature source(s) searched *(database name)*:……..

3.2 Date of search: *(perform the search no earlier than 6 months prior to IACUC meeting, (dd/m/yy)*……

3.3 Period of search *(range of years searched):*……..

3.4 Key words used in search: ……

3.5 Results of search *(provide a narrative description of the results of the literature search)*

……

……. …….

4. Objective(s): *(Provide goal/specific aim of this project)*

……

……

……

5. Experimental design: *(Provide a complete description of what will be done to the animals. Succinctly outline the formal scientific plan and direction for experimentation, sequential description of procedures what will be done to the animals from obtain the animal to the end of study. A diagram or chart may be helpful to explain complex design).*

……

……

……

6. Data analysis and statistical method: *(List the statistical test(s) planned or describe the strategy intended to evaluate the data).*

……

……

…….

7. Animal model and species justification:

7.1 Description of animals

Common name	Genus and species	Strain/ Stock	Age	Weight	Sex	Number
…….	……	……	…	…….	…	…….
…….	……	……	…	…….	…	…….
…….	……	……	…	…….	…	…….

Permanent animal ID method: *(eg. ear tag, ear punch, microchip, tattoo, N/A, other please specify)*

……

……

Special consideration: *(List specialized requirements for the research animals, e.g. certain antibody or virus free, Pasteurella free, etc.)*

……..…….

…….

Source/Vendor**:** ……...

7.2 Scientific justification for animal species and number requested.

7.2.1 Animal model and species justification*: (Provide a scientific justification for the choice of animal model(s). What physiological and morphological characteristics does this animal possess that make it the best possible model?)*

…….

…….

…….

7.2.2 Number of animals required: *(Provide an explanation of how the numbers of animals to be used in each group or total were appropriate. Number of animals used in the experiment should be based on scientific and statistical requirements to achieve objectives).*

……..…….**.....**

…….

…….

8. Animal care:

8.1 Husbandry consideration: *(Briefly describe animal housing and living conditions, routine animal observations, feed and water provisions, etc.)*

8.1.1 Study location: *(Study room where the animals will be housed.)*

…….

……

8.1.2 Housing system:

☐ Clean conventional ☐ Strict hygienic conventional

☐ Isolator maintained ☐ Barrier maintained

☐ Laminar flow

☐ Other, please specify ……...

8.1.3 Caging:

☐ Solid bottom, open top ☐Static filtered top cages

☐ Suspended cages, wire bottom ☐ Metabolic cages

☐ Individual ventilated cage (IVC)

☐ Other, please specify ……....

8.1.4 Cage size: W x L x H, (inch)……..

8.1.5 Caging materials:

☐ Plastic ☐ Stainless steel

☐ Other, please specify …….

8.1.6 Number of animals per cage:…….

8.1.7 Social housing (more than one animal per cage): (*The IACUC requires social housing of all social animals)*

☐ Yes ☐ No

If NO, provide scientific justification for not socially housing the animals. Describe what will be done to replace this social contact with conspecifics.

……

……

8.1.8 Environmental requirements:

Temperature: …….....

Humidity: …….....

Light: ☐ Standard fluorescent

☐ Other, please specify ……..……..…

Light cycle: ☐ Standard 12:12 (ligh:dark)

☐ Other, please specify ……..…

8.1.9 Food:

Type of food: ☐ Standard diet ☐ Other, please specify …….

Feeding schedule:

☐ Routine feeding (Ad libitum)

☐ Other, please specify …….……..…...

8.1.10 Water:

Type of water ☐ Hyperchlorinated …….. ppm.

☐ RO water ……..

☐ Other, please specify ……..…

Provision of water:

☐ Routine feeding (Ad libitum)

☐ Other, please specify ……

8.1.11 Bedding:

☐ No

☐ Yes, please specify ☐ Sterile ☐ Non-sterile

Type of bedding:

☐ Wood shaving ☐ Sawdust

☐ Paper ☐Other, please specify ……

Schedule of bedding changing:

☐ Weekly ☐ At specified interval, every ……day(s)

8.1.12 Environmental Enrichment:

☐ Acceptable

☐ Not acceptable, please justify.

.....

.....

8.2 Is this project intended to conduct the animal experiment in other building?

(This is allowed for conducting experiment(s) only not for housing. In addition, the holding period must be less than 12 hours).

[ ] No [ ] Yes

If YES, please provide information below:

1. Where the experiment is expected to be conducted? Please indicate the building name and room number.

……

……

2. Please provide the animal experimental procedures in detail.

……

……

3. Estimated total time period that live animals will be kept in the laboratory is……hours

4. How will the animal sample or carcass be disposed?

……..

……..

9. Veterinary medical care: *(Describe the routine veterinary care. List the criteria used for health evaluation while the animals are on study).*

……

……

……

10. Animal welfare:

10.1 Does the proposed research duplicate any previous work?

☐ Yes ☐ No

If YES, explain why it is scientifically necessary to duplicate the experiment.

……

……

10.2 Replacement, Reduction and Refinement. (*Briefly describe how you have considered each of the following alternatives (the 3Rs) or why they are not applicable).*

10.2.1 <u>Replacement of animals</u> *(e.g., with in vitro models, computer models or less sentient animals)*:

…….…… ……

……

……

10.2.2 <u>Reduction in the number of animals</u> *(e.g., using appropriate statistical methods in the design and analysis of the study; reduction in experimental variability by using animals of defined genetic or microbiological status):*

……

……..

……

10.2.3 <u>Refinement of experimental procedures to minimize pain or distress</u>
(e.g., early endpoints; use of analgesics, anesthetics or sedatives; techniques that reduce stress in the animal.):

……..……..

……..…..

……..….

10.3 Potential animal pain and distress assessment:

10.3.1 Please indicate pain category according to USDA Pain and Distress. (Appendix A)

1) Number of animals: - Category B .....
- Category C.....
- Category D.....
- Category E.....

2) Pain relief/Prevention.....

10.3.2 During the study:

1) How often will the clinical condition of animals be monitored?

…….

2) Who will monitor the clinical condition of the animals?

…….

10.3.3 Are the animals expected to experience any specific study-induced or related problems (i.e. health problems, pain, distress, complications, etc.) **or any health problems as a result of the phenotype of the animal?**

☐ Yes ☐ No If YES, please answer the following questions:

1) Describe the expected problems. …….

……..

2) What criteria(s) will be used to assess pain, distress, or discomfort?

Check all that apply:

☐ Inactivity

☐ Loss of appetite

☐ Loss of weight ☐ 5% ☐ 10 % ☐ 15% ☐ 20% weight loss

☐ Restlessness

☐ Abnormal resting postures, somnolence or hunched posture

☐ Licking, biting, scratching, or shaking a particular area

☐ Failure to show normal patterns of inquisitiveness

☐ Failure to groom, causing and unkempt appearance

☐ Guarding (protecting the painful area)

☐ Loss of mobility

☐ Red stain around the eyes of rats

☐ Self-mutilation

☐ Labored breathing

☐ Tumor

☐ Unresponsiveness

☐ Other (please list) …….

10.3.4 Literature search for alternative to procedure that cause pain & distress

10.3.4.1 Literature source(s) searched: *(database name)* .....
10.3.4.2 Date of search: *(perform the search no earlier than 6 months prior to IACUC meeting, (dd/m/yy)* .....
10.3.4.3 Period of search *(range of years searched)*: .....
10.3.4.4 Key words of search: .....
10.3.4.5 Results of search: (*provide a narrative description of the results of the literature search)*

……..……..

……

10.4 Anesthesia

☐ Yes ☐ No

If YES, please answer the following questions:

1) Preanesthetic preparation: …….…….
2) Anesthetic agent(s) used: …….
3) Dosage: …….
4) Volume: ……
5) Route of administration: ……..…….
6) Frequency of anesthesia: …….…….
7) Length of anesthesia: ……
8) Who is responsible for monitoring anesthesia?……..…
9) If an inhalation anesthetic is used, describe scavenging of the waste anesthetic gas.

…….....

10) What criteria(s) will be used to assess level of anesthesia?

……..

Check all that apply:

☐ Respiration rate ☐ Body temperature ☐ Heart rate
☐ ECG ☐ Toe pinch ☐ Tail pinch
☐ Corneal reflex ☐ Pedal reflex ☐ Muscular relaxation
☐ Color of mucous membrane
☐ Other (pulse oximeter, respirometer) please list …….…….

11) How animals are kept warm? …….…….

.

10.5 Analgesics and/or tranquilizers:

☐ Yes ☐ No

If "YES", please specify

1) 1.1. Type of analgesics used ……
   1.2. Agent(s)…….
2) Dosage…….
3) Route of administration ……
4) Schedule……..

10.6 Describe post-anesthetic treatment or intervention:

…….

…….

11. Surgery:

☐ Yes ☐ No

If YES, please answer the followings:

11.1 Surgical procedure is: ☐ Non-survival ☐ Survival
☐ Major ☐ Minor
☐ One time ☐ Multiple

11.2 Location: *Give the location/room number for the proposed surgical procedure.*

…….

…….

11.3 Surgeon/qualification*: Indicate who will perform the surgery, and his/her qualifications, training, or experience in the proposed procedure*.

…….

…….

11.4 Procedure: *Describe in detail the surgical procedure*. ……..…

…….

……..

11.5 Pre- and post-operative provision: *Detail the provision for both pre-and post-operative care, including provisions for post-surgical observation.*

…….

……

11.6 Describe long-term care of chronic survival procedure.

…….

…….

11.7 Multiple survival surgery procedures: *Multiple major operative procedures on the same animal must be adequately justified for scientific reasons by the principal investigator in writing.*

11.7.1 Procedure: …….

……..…

……..

11.7.2 Scientific justification:

…….

……...

……

11.7.3 Who will be the responsible for post-surgical care and treatment?

…….

……

……

12. Blood or body fluid withdrawal/tissue collection/injections, tail clip, gavaging

Describe in detail: method(s), needle size(s), volume(s) collected or administered, and frequency of collection or injection.

	Method/Anatomic location	Needle size/ catheter size and length	Biopsy size	Volume collected (ml)	Volume administered (ml)	Frequency
Blood withdrawal	…….	…….	……	…….	…….	…….
Body Fluid withdrawal	…….	…….	…….	…….	…….	…….
Tissue collection	…….	…….	…….	…….	…….	…….
Injection/ infusion	…….	…….	…….	…….	…….	…….
Tail clip	…….	…….	…….	…….	…….	…….
Gavaging	…….	…….	…….	…….	…….	…….
Other *(specify)*	…….	…….	…….	…….	…….	…….

Total blood volume ……. ml. in total ……. study days or ……. months

13. Restraint with mechanical devices:

☐ Yes ☐ No

If YES, describe device, duration of restraint, frequency of observation, conditioning procedures and steps to assure comfort and well-being.

…….

…….

If prolonged restraint is used, must provide justification:

…….

…….

14. Project involving food and water deprivation, or dietary manipulation:

☐ Yes ☐ No

If YES, describe methodology. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study. Include clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

☐ Individual animal's weight is monitored every …… days.

☐ Individual animal's weight is not monitored.

	Amount restricted/added	Duration	Compound supplemented	Compound deleted	Frequency
Food restriction	…….	…….	…….	…….	……
Fluid restriction	…….	…….	…….	…….	……
Nutrient alterations	…….	…….	…….	…….	……

15. Tumor and disease models, toxicity testing:

☐ Yes ☐ No

If YES, describe methodology used for tumor/disease and/or toxicity testing. State objective criteria used to assess physical condition and pain, discomfort, stress, and distress during the course of study, including clinical signs or manifestations expected from the procedure. What criteria will be used to determine a humane endpoint before severe morbidity and death?

…….

…….

16. Behavioral studies:

☐ Yes ☐ No

If YES, describe in detail types of behavioral manipulations, including placement in testing chambers or apparatus, use of adverse stimuli, duration of test periods, and frequency of test periods.

…….

…….

……..

17. Study and Humane endpoint:

17.1 State the project study endpoint for the animals. *Indicate whether recovery, euthanasia, or death is/are expected; specific plan for determining when the animal experimentation phase will be stopped.*

……..

……..

……..

17.2 Early endpoint is used *(the animals are humanely euthanized prior to the expected terminate study day):*

☐ Yes ☐ No

Early endpoint criteria used are ……..

…….

17.3 Death or moribundity as an endpoint is used

17.3.1 Criteria that establish when the endpoint has been reached.

……

……

17.3.2 A plan for monitoring the animals both before and after a change in any of the above aspects, providing care if appropriate, and increasing the level of monitoring must be described.

……

……

17.3.3 Identification of personnel responsible for evaluation, record keeping, notification of the investigator and/or veterinarian and persons responsible for euthanasia must be described.

……

……

18. Euthanasia / Disposition of animals

18.1 Disposal of animals after completion of activity, the animals will be:

☐ Euthanized ☐ Returned to production/breeding unit/facility inventory

☐ Transferred to another research project:

– Protocol No. ……..…… and investigator …….

☐ Other (Please describe) ……

18.2 Euthanasia method

☐ CO2-compressed carbon dioxide gas in cylinders

☐ Anesthetic/Sedative(s)

Agent(s) …….…….

Dosage …….....

Route of administration ……

☐ Cervical dislocation

☐ performed with anesthesia

☐ performed with no anesthesia, provide scientific justification……

☐ Decapitation, provide scientific justification…….

☐ Other (Please describe) ……..

18.3 State how death will be verified before disposal: ……….

……..

……

19. Necropsy/ Selected tissue and sample collection

[ ] No

[ ] Yes, please describe.

– Location.....

– Who will do it, and what is their experience in the technique used? ....

……

– Personnel protective equipment (PPE) .....

…….

20. Animal tissue and carcasses disposal: *Describe method used to dispose animal tissue and carcasses.*

……

……

21. Biohazard/safety:

☐ Infectious agent (s) is/are used: specify ……

☐ Biohazardous chemical or carcinogen or radioactive material is/are used specify ……

☐ Recombination agent(s) is/are used: specify ……...…

☐ None

21.1 Provide a list of any potential biohazards associated with this protocol.

Specify biosafety level ☐ ABSL 1 ☐ ABSL 2 ☐ ABSL 3 ☐ ABSL 4

21.2 Explain any safety precaution or program designed to protect personnel From biohazard and any surveillance procedure in place to monitor potential exposure.

……..……..

…….

…….

21.3 Explain how the waste is decontaminated and disposed.

…….

…….….

21.4 List primary safety equipment and personnel protective equipment requirements.

…….....

…….……

21.5 List procedures if any accident, injury or illness occurs.

……..

…….

21.6 List specific treatment provision for accidental exposure.

……

…….

21.7 List relevant occupational medical health provision.

……

……

22. Qualification of personnel:

List all individuals who will be involved in this protocol. If personnel do not have experience in working with animals, state how they will be trained

Name	Responsibilities	Description of relevant experience or training
……	…….	……...
……	…….	……...
……	…….	……...
……	…….	……...
……	…….	……...
……	…….	……...

As Principal investigator on this protocol, I verifies that the information herein is true and correct and that I am familiar with and will comply with standard of animal care and use established under the ethical guidelines and policies of Mahidol University, and Office of the National Research Council of Thailand (NRCT). Additionally, I acknowledge my responsibilities and provide assurances for the followings:

A. Animal use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the MU-ACUC.

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

C. Statistical assurance: I assure that I have consulted with qualified statistician to evaluate the statistical design or strategy of this proposal, and that the minimum number of animals needed for scientific validity are used.

D. Biohazard/safety: I have taken into consideration, and I have made the proper coordinations

regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unneccessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the responsibility for implementing animal use alternatives where feasible, and conducting 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. Research studies: This protocol **IS** or **IS NOT** (circle one) associated with a grant application. If yes, I certify that this protocol is essentially the same as the study found in the grant application or program/project. The MU-ACUC and the funding agency will be notified of any changes in the proposed project, or personnel, relative to this application. I will not proceed with animal experiment until approval by the MU-ACUC is granted.

…… …….

(Principal investigator) Date

Appendix A

USDA Pain Levels:

USDA Category B	USDA Category C	USDA Category D	USDA Category E
Breeding or Holding Colony Protocols	No more than momentary or slight pain or distress and no use of pain-relieving drugs, or no pain or distress. For example: euthanatized for tissues; just observed under normal conditions;	Pain or distress appropriately relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.	Pain or distress or potential pain or distress that is **not** relieved with anesthetics, analgesics and/or tranquilizer drugs or other methods for relieving pain or distress.
	Examples	**Examples**	**Examples**
	1. Holding or weighing animals in teaching or research activities. 2. Injections, blood collection or catheter implantation via superficial vessels. 3. Tattooing animals. 4. Ear punching of rodents. 5. Routine physical examinations. 6. Observation of animal behavior. 7. Feeding studies, which do not result in clinical health problems. 8. AVMA approved humane euthanasia procedures. 9. Routine agricultural husbandry procedures. 10. Live trapping. 11. Positive reward projects.	1. Diagnostic procedures such as laparoscopy or needle biopsies. 2. Non-survival surgical procedures. 3. Survival surgical procedures. 4. Post operative pain or distress. 5. Ocular blood collection in mice. 6. Terminal cardiac blood collection. 7. Any post procedural outcome resulting in evident pain, discomfort or distress such as that associated with decreased appetite/ activity level, adverse reactions, to touch, open skin lesions, abscesses, lameness, conjunctivitis, corneal edema and photophobia. 8. Exposure of blood vessels for catheter implantation. 9. Exsanguination under anesthesia. 10. Induced infections or antibody production with appropriate anesthesia and post-op/post-procedure analgesia when necessary.	1. Toxicological or microbiological testing, cancer research or infectious disease research that requires continuation until clinical symptoms are evident or death occurs. 2. Ocular or skin irritancy testing. 3. Food or water deprivation beyond that necessary for ordinary pre-surgical preparation. 4. Application of noxious stimuli such as electrical shock if the animal cannot avoid/escape the stimuli and/or it is severe enough to cause injury or more than momentary pain or distress. 5. Infliction of burns or trauma. 6. Prolonged restraint. 7. Any procedures for which needed analgesics, tranquilizers, sedatives, or anesthetics must be withheld for justifiable study purposes. 8. Use of paralyzing or immobilizing drugs for restraint. 9. Exposure to abnormal or extreme environmental conditions. 10. Psychotic-like behavior suggesting a painful or distressful status. 11. Euthanasia by procedures not approved by the AVMA.

(**Note:** there is no USDA Category A.)

Guidelines for determining USDA classification in protocols involving tissue collection before/after euthanasia and/or animal perfusion:

If an animal will be euthanatized by an approved physical or chemical method of euthanasia solely for the collection of tissues (after the animal's death), the procedure should be classified as USDA C.

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy), and the animal will then be allowed to recover, the procedure should be classified as USDA D (survival surgery).

If an animal will be anesthetized so that non-vital tissues can be collected (liver or skin biopsy, etc.); and the animal will then be euthanatized, the procedure should be classified as USDA D (non-survival surgery). In this scenario, it is necessary to justify why the animal couldn't be euthanatized (USDA category C) rather than anesthetized.

If an animal will be anesthetized so that vital tissues can be collected (heart, both kidneys or lungs, whole liver, etc.), the animal will obviously succumb to the procedure. To determine whether this will be euthanasia or non-survival surgery, we must consider the definition of euthanasia. A critical component of this definition is "rapid unconsciousness followed by loss of cardiac, respiratory and brain function". Based on this definition, procedures which require tissue manipulation or other prolonged techniques prior to the animals death (more than a few minutes) should be classified as non-survival surgery (USDA D). Similarly, if an animal will be anesthetized so that the tissue can be collected in the "freshest" possible state (i.e. heart) and the tissues will be rapidly excised, the procedure should be classified as euthanasia (USDA C). (Note: In this scenario, it is difficult to justify why the animal couldn't be euthanatized rather than anesthetized.)

If an animal will be anesthetized so that it can be chemically perfused, the same "test of time" applies (i.e.: long, technical manipulations should be classified as USDA D; while rapid intravascular injection of the perfusate without other manipulations should be classified as USDA C).

NOTE: Because the USDA classification system is based on the "potential for pain, distress or discomfort," the anesthetic/euthanasia drug dose becomes a critical concern. For example, if a known "euthanasia dose" of pentobarbital will be administered, drug irreversibility is assumed. Thus, once the animal is confirmed to be in an anesthetic plane (toe pinch response, etc.), tissues can be collected/ procedures can be performed without the concern about what the animal will be perceiving. This procedure would then be classified as USDA C. The Committee recommends using a euthanizing dose whenever possible. Other methods may be appropriate with proper scientific justification.