Chulabhorn Research Institute – Institutional Animal Care and Use Committee (CRI-IACUC)

PROTOCOL COVER SHEET

Protocol Number		This section will be completed by the CRI-IACUC
3-Year Renewal of CRI-IACUC#/ New Protocol		
Received by IACUC		
Approved/Request Revision		
Resubmitted		
Approved/Disapproved by IACUC		
Approved/Disapproved by IO		
Expiration Date		
Notes:		

Animal Protocol Title: .....

.....

If this protocol is a part of the Main Project, please provide the Main Project Title:
(Thai) .....
(English) .....

Anticipated Animal Protocol Period: *(Note: Start date approximately 2 months after protocol submission)*

From **(DD/MMM/YYYY)** ..... **To** **(DD/MMM/YYYY)** .....

Funding Source(s): .....

Grant has been: ☐ Submitted
☐ Approved. From **(DD/MMM/YYYY)**: ..... to **(DD/MMM/YYYY)** .....

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) .....

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 Chulabhorn Research Institute.

Principal Investigator: Name .....

Animal use license number ..... **Expired date** .....

Address: .....

.....

_____

(Signature, Date)

Co-Investigator: Name .....

Animal use license number ..... **Expired date** .....

_____

(Signature, Date)

Co-Investigator: Name .....

Animal use license number ..... **Expired date** .....

_____

(Signature, Date)

Co-Investigator: Name .....

Animal use license number ..... **Expired date** .....

_____

(Signature, Date)

Contact Person in Case of Emergency: .....
Office/Affiliation: .....
Phone: ..... **E-mail:** .....

Director of Laboratory: Name .....

Address: .....

.....

.....

_____

(Signature, Date)

Safety Review: **Name** .....

Address: .....

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.....

_____

(Signature, Date)

AV Review: **Name** .....

Animal use license number ..... **Expired date** .....

Veterinary practitioner license number ..... **Expired date** .....

Address: .....

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_____

(Signature, Date)

Chulabhorn Research Institute
STANDARDIZED RESEARCH PROTOCOL FORMAT FOR PERMISSION OF ANIMAL CARE AND USE

Protocol Title: (Thai) .....

(English) .....

Principal Investigator: .....

Co-Investigator(s): .....

.....

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1. Non-technical Summary: *(Please address the topics below in a way that would be easily understood by a non-scientist, i.e., in narrative style using non-technical terms).*

1.1 Aim(s) of the study

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1.2 Reason for use of laboratory animals

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1.3 Laboratory animal handling, including the key procedures (e.g., treatment) to be used and the durations

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1.4 Expected or potential impacts on the laboratory animals and how these will be managed (i.e., interventions)

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1.5 Benefits of this study

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1.6 Please provide a short (250 words), non-technical summary of the proposed study in Thai.

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2. Background: *(Provide a brief literature review of background information leading to the rationale of the study with a list of references cited.)*

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

3.1 Literature source(s) searched:
In case AI tool(s) has been used in literature review, please specify tool name(s), model(s) and prompt(s)

3.2 Date of search:

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*

3.6 Does the proposed research duplicate any previous work?
[ ] NO [ ] YES

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

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

5. Experimental Design and General Procedures: *(Provide a complete, step-by-step description of the experiment(s). Describe in detail the experimental procedures, especially what will be done from obtaining the animals to the end of animal experiment(s). Diagram(s) or flow chart(s) should accompany complex experimental design)*

6. Data Analysis/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

Animal identification method: *(e.g., tail marking, ear punch, ear tag, microchip, tattoo, or other (please specify)*

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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?).*

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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).*

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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:

[ ] Laboratory Animal Center, Chulabhorn Research Institute (CRI-LAC)
[ ] Other, please specify.....

8.1.2 Housing System:

[ ] Strict hygienic conventional [ ] Laminar flow
[ ] Environmental chamber [ ] Individual Ventilated Cage (IVC)
[ ] Other, please specify.....

8.1.3 Caging:

[ ] Rat
- [ ] Polysulfone shoe box cage:
  - [ ] 42.5 x 26.6 x 18.5 cm
  - [ ] 48 x 26.5 x 21 cm
  - [ ] 50 x 38 x 21.5 cm
- [ ] Individual Ventilated Cage (IVC)
- [ ] Metabolic cage
  - [ ] BW≤300 g
  - [ ] BW>300 g
- [ ] Other, please specify.....

[ ] Mouse
- [ ] Polysulfone shoe box cage
  - [ ] 36.5 x 20.7 x 14 cm
  - [ ] 48 x 26.6 x 15 cm
- [ ] Individual Ventilated Cage (IVC)
- [ ] Metabolic cage
- [ ] Other, please specify.....

8.1.4 Social housing:

[ ] YES, specify number of animal per cage .....
[ ] NO, provide scientific justification .....

8.1.5 Environmental requirements:

Temperature: [ ] 22 ± 1 °C
[ ] Other, please specify.....
Humidity: [ ] 55 ± 10 %
[ ] Other, please specify.....
Light: [ ] Standard fluorescent
[ ] Other, please specify.....
Light cycle [ ] Standard (12:12 hrs.).
[ ] Other, please specify.....

8.1.6 Food:

Type of food: [ ] Standard diet [ ] Other,.....
Feeding schedule: [ ] Ad libitum [ ] Other,.....

8.1.7 Water:

Type of water: [ ] RO water contains 2-4 ppm chlorine
[ ] Other, please specify.....
Provision of water: [ ] Ad libitum [ ] Other,.....

8.1.8 Bedding:

Type of bedding: [ ] Corn cob [ ] Other,.....
Schedule of changing: [ ] Twice a week [ ] Other,.....

8.1.9 Environmental enrichment: It is CRI policy to provide environmental enrichment through nesting material and shelter object for all laboratory animals.

[ ] Acceptable

[ ] Not acceptable. Please justify.

[ ] Other. Please justify.

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9. Transportation of animals

9.1 In this animal protocol, are any animal experiments planned to be conducted in other buildings?

[ ] NO

[ ] YES, specify room number, building and mean of transport .....

.....

9.2 Estimated durations (e.g. hours) that live animals will be kept in the other buildings (e.g. laboratories).

.....

9.3 How will the animal carcasses be disposed of after the experiments are concluded?

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

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11. Animal Welfare:

11.1 Briefly describe how you have considered each of the following alternatives (the 3Rs) or why they are not applicable.

11.1.1 Replacement of animals *(e.g., with in vitro models, computer models or less sentient animals):*

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.....

.....

11.1.2 Reduction in the number of animals *(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; sharing tissue among investigators):*

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

.....

.....

.....

11.2 Potential animal pain and distress assessment:

11.2.1 Please indicate pain category according to USDA (see Appendix A).

1) Number of animals: - Category B (*no pain*) .....
- Category C (*momentary/slight pain*) .....
- Category D (*pain with alleviation*) .....

** For Category D, which interventions, methods, or measures are used to prevent or minimize pain, distress, or discomfort? (Check all that apply)*

[ ] *Anesthetics,* [ ] *Analgesics,* [ ] *Tranquilizing drugs,* [ ] *Humane endpoints, and/or*

[ ] *Other, specify* .....

- Category E (*pain with no alleviation*) .....

*** For Category E, provide strong scientific justification as to why pain-relieving drugs or other methods for relieving pain cannot be used on animals*

.....

.....

.....

11.2.2 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?

[ ] NO [ ] YES

If yes, please answer the following questions:

1) Describe the expected problems.

.....

.....

.....

2) What criteria 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
[ ] Unresponsiveness

[ ] Self-mutilation
[ ] Labored breathing
[ ] Other (please list)

3) How often will the animals be monitored for these signs of pain and distress?

.....

4) Who will monitor the animals?

.....

11.2.3 Early endpoint is used *(The animals are humanely euthanized prior to the expected date of study termination)*

[ ] NO [ ] YES

Early endpoint criteria used are .....

.....

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

1) Literature source(s) searched: .....
2) Date of search: .....
3) Period of search **(range of years searched)**: .....
4) Key words of search: .....
5) Results of search: .....

11.3 Anesthesia

[ ] NO [ ] YES, *specify the following:*

1) Pre-anesthetic preparation .....

2) Type, dosage and route of anesthesia used

[ ] Isoflurane (inhalation): 4-5% for induction and 1–3% for maintenance

[ ] Other, specify .....

3) Frequency of anesthesia .....
4) Length of anesthesia .....
5) Who is responsible for maintaining anesthesia .....
6) Methods used to monitor anesthesia, frequency of monitoring

.....

.....

7) If inhalation anesthetics are used, describe the system for scavenging waste anesthetics gas.

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.....

8) What criteria will be used to assess level of anesthesia? Check all that apply (✓):

[ ] Respiration rate
[ ] Heart rate
[ ] Toe pinch
[ ] Tail pinch
[ ] Corneal reflex

[ ] Color of mucous membrane
[ ] Muscular relaxation
[ ] Other (pulse oximeter, respirometer) please list.....

9) How are animals kept warm?

.....

.....

11.4 Analgesics and/or tranquilizers:

[ ] NO [ ] YES

If yes, please answer the following:

1) Type of analgesics used, if applicable.....
2) Dose .....
3) Route of administration .....

11.5 Describe post-anesthetic/ analgesics / tranquilizers treatment or intervention:

.....

.....

12. Surgery:

[ ] NO [ ] YES

If yes, please answer the following:

12.1 Surgical procedure is:

[ ] Non-survival	[ ] Survival
[ ] Major	[ ] Minor
[ ] One time	[ ] Multiple

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

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.....

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

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.....

12.4 Procedure: *Describe in detail any surgical procedures planned. (may add a reference)*

.....

.....

12.5 Pre- and post-operative provisions: *Detail the provisions for both pre-and post-operative care, including provisions for post-surgical observations.*

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12.6 Describe long-term care of any chronic survival procedures.

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12.7 Multiple survival surgery procedures: *Multiple major operative procedures on the same animal must be adequately justified for scientific reasons by the P.I. in writing.*

12.7.1 Procedures:

.....

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12.7.2 Scientific justification:

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.....

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

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13. Blood or Body Fluid Withdrawal/ Tissue Collection/Injections, Tail Clip, Gavage

Describe in detail the method(s), needle sizes, volume(s) collected or administered, and frequency of collection or injections:

	Anatomic Location	Needle Size/ Catheter Size and length	Biopsy Size	Volume Collected (ml)	Volume Administered (ml)	Frequency (if the frequency is not regular please specify)
Blood Withdrawal						
Body Fluid Withdrawal						
Tissue Collection						
Injection/ Infusion						
Tail Clip/ Puncture						
Gavage						
Other						

- **The total number of blood collections per animal is ..... time(s) (not including blood collection at euthanasia)**
- **The total blood volume per animal collected for this study is ..... ml (not including blood collection at euthanasia)**
- **Period of experiment ..... (days/ weeks/ months - please circle one)**

14. Use of Non-Pharmaceutical Grade Substances or Compounds

[ ] NO [ ] YES

If yes, provide the following information:

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.....

.....

.....

Name of substances or compounds, site/route of administration and method of preparation

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.....

Provide scientific justification for the use of non-pharmaceutical grade compounds

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Information submitted for consideration (*if available, certificate of analysis, test report of contaminants and potential side effect*s)

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15. Restraint with Mechanical Devices:

[ ] NO [ ] YES

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

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.....

.....

If prolonged restraint is used, must provide justification:

.....

.....

.....

16. Projects Involving Food and Water Restriction / Deprivation, or Dietary Manipulation:

[ ] NO [ ] YES

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					

17. Tumor and Disease Models, Toxicity Testing:

[ ] NO [ ] YES

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?

.....

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18. Behavioral Studies:

[ ] NO [ ] YES

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

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19. Euthanasia / Disposition of Animals

19.1 After completion of activity, animals will be:

[ ] Euthanized
[ ] Returned to production/breeding unit/facility inventory
[ ] Transferred to another research project:
- please list protocol # ..... and Investigator.....

[ ] Other (please describe).....

19.2 Method of euthanasia .....

[ ] CO_2 overdose

[ ] Anesthetic overdose

[ ] Isoflurane (inhalation): 5% with exsanguination

[ ] Other, specify .....

[ ] Cervical dislocation (only for mice and rats not more than 200g; provide scientific justification)

.....

[ ] Decapitation, provide scientific justification

.....

[ ] Other, describe and provide scientific justification

.....

19.3 State how death of the animals will be verified before disposal

.....

19.4 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. Study Endpoint: *(State the projected study endpoint for the animals. Indicate whether recovery, euthanasia, or death is expected; and the specific plan for determining when the animal experimentation phase will be stopped).*

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IF death or moribundity is used as an endpoint, please answer all that applies:

20.1 Provide criteria that establishes when this endpoint has been reached, and describe how animals will be monitored and cared for .....

.....
.....
.....

20.2 List persons responsible for evaluating animal condition, record keeping, and notifying the PI and/or veterinarians to perform euthanasia .....

.....
.....
.....

21. Hazard/Safety:

21.1 Chemical hazards

[ ] None
[ ] Hazardous chemicals are used: specify
[ ] Unknown

Chemical Name	Hazard Category (✓all that apply)			
	Carcinogen	Radioactive material	Mutagen	Other

*please provide references

21.2 Biological hazards

[ ] None
[ ] Hazardous biological agents are used: specify
[ ] Unknown

Name of Biological Agent	Hazard Category		BSL level
	Non-infectious	Infectious	

*please provide references

21.3. Safety management (please provide SOP where applicable)

List primary safety equipment and personnel protective equipment requirements.

List procedures if accident, injury or illness occurs.

List specific treatment provision for accidental exposure.

List relevant occupational medical health provision.

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Explain any safety precautions or programs designed to protect personnel from chemical/ biological hazards and any surveillance procedures in place to monitor potential exposures.

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21.4 Waste management (please provide SOP where applicable)

Explain how the waste is decontaminated and disposed of.

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22. Study Personnel Qualifications and Training:

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

Name/Degree(s)	Responsibility/ Procedures	Qualification, Relevant Experience and Training

23. Assurances: As Principal investigator on this protocol, I verify 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 Chulabhorn Research Institute, 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 modification is specifically approved by the CRI IACUC prior to its implementation.

B. Duplication of Effort: I have made every effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with a qualified individual who evaluated the experimental design with respect to the statistical analysis, and that the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken into consideration and made the proper coordination regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, 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 unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral, ethical 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. Painful Procedures: (A signature for this assurance is required by the Principal Investigator if the research being conducted has the potential to cause more than momentary or slight pain or distress even if an anesthetic or analgesic is used to relieve the pain and/or distress.)

I am NOT conducting biomedical experiments, which may potentially cause more than momentary or slight pain or distress to animals.

I. Research studies: The CRI IACUC 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 CRI IACUC is granted.

Signature.....

(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; positive reward projects; routine procedures; injections; and blood sampling.	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.)