**A close up of a logo

Description automatically generatedAnimal Care and Use Protocol for Aquatic Animal Study**

**Faculty of Science, Mahidol University–Institutional Animal Care and Use Committee (MUSC–IACUC)**

**COVER SHEET**

**1. Overview**: *This section will be completed by MUSC–IACUC*

**A picture containing text, receipt, font, screenshot

Description automatically generated**

**2. Protocol title:** *Including type of animal to be used in the protocol title*

(Thai)

(English)

If applicable,

2.1 This protocol is a part of the **main research project** entitled

(Thai)

(English)

2.2 Principal investigator of the **main research project**

Name

Degree Position

Affiliation

**3. Principal investigator of the submitted protocol:** *For a student thesis, principal investigator is the principal adviser, and student is a co-investigator*

Name

Degree Position

Affiliation

Telephone Email

Animal use license number (*issued by Institute of Animal for Scientific Purposes Development, NRCT*) Expired date

**4. Co-investigators of the submitted protocol**

**4.1 Co-investigators directly involved with animals**

4.1.1 Name

Degree Position

Affiliation

Telephone Email

Animal use license number Expired date

4.1.2 Name

Degree Position

Affiliation

Telephone Email

Animal use license number Expired date

**4.2 Co-investigators NOT directly involved with animals**

4.2.1 Name

Degree Position

Affiliation

Telephone Email

4.2.2 Name

Degree Position

Affiliation

Telephone Email

**5. Designated contact person in case of an emergency**

Name

Affiliation

Mobile phone Email

**6. Designated contact person for protocol writing inquiries**

Name

Affiliation

Mobile phone Email

Have you previously attended the annual protocol writing training organized by MUSC-IACUC?

□ No, □ Yes, *specify the most recent year of attendance*

**7. Type of animal protocol**

□ Research in the field of

□ Testing or monitoring, specify

□ Teaching, specify (course, class)

□ Animal breeding, specify (species, strain, genotype)

□ Other, specify

**8. Anticipated protocol period:** *Approximate date at least 2 months after protocol submission*

from to

**9. Funding**

□ Received from

Funding period from to

□ To be requested from

Funding period from to

□ Other, specify

**10. Payment method**

□ Payment from Department of

□ Payment from Research grant, specify details of invoice

□ Other, specify

**11. Signatures:** *Include all investigators*

*Your signature as Principal investigator/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 Date

( )

Co-investigator Date

( )

Co-investigator Date

( )

Head of Department Date

( )

Faculty/Institute

**This section will be completed by MUSC–IACUC**

Statistical review Date

(Suntaree Unhapipat, Ph.D.)

Safety review Date

(Nattapon Panupinthu, M.D., Ph.D.)

Veterinary review Date

(Napawan Hirunwiroj, D.V.M., M.M.)

Animal use license no. U1-08176-2562 Expired date 6/10/2027

Veterinary practitioner license no. 01-12817/2561 Expired date 18/07/2028

**12. Approval**

MUSC–IACUC review: □ Approved

□ Approval recommended

□ Disapproved

MUSC–IACUC Chair Date

(Professor Emeritus Dr. Nateetip Krishnamra)

**BODY OF PROTOCOL**

**1. Non-technical summary:** *Provide a brief, only one A4 page, and* ***simplified description*** *of the project expressing rationale,* *methods (****especially when involving animals*** *e.g., type and age of animals, treatment, anesthesia, euthanasia etc.), project significance, needs for the use of animals and potential benefits of the study*.

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

**3. Objectives:** *Provide goals or specific aims of this protocol*

**4. Potential benefits of the study:** *Explain how the study is important to human or animal health and the advancement of knowledge*

**5. Experimental design and animal 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 and statistical methods:** *Describe statistical methods to be used for analysis of the results and for testing the hypothesis*

**7. Animal model and justification**

7.1 Provide description of animals in Table below

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Common name** | **Species** | **Strain** | **Age** | **Weight** | **Sex** | **Number** |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |

7.2 Describe specialized requirements for the research animals

7.3 Provide list of sources or vendors

□ Nature: *Perform without contravention to law and careful execution. Recognizing the health of animals, endangered species and ecosystem*

□ Laboratory animals: specify the source with genetic quality and health certificates

□ Commercial source, specify

□ Other, specify

Describe mode of transportation

7.4 Describe the method(s) to prevent injury and/or infection during transportation

7.5 Is the quarantine required?

□ No

□ Yes, specify the method, location and duration

7.6 Provide a scientific justification for the choice of animal model used: *Which is/are appropriate characteristic(s) of this animal model?*

7.7 Provide an explanation and statistical justification of how the proposed numbers for animals in each group and in total are appropriate for this study

**8. Animal care:** *Provide all husbandry consideration*

8.1. Study location (specify room number, name of building or facility)

8.2 Housing system

□ Open system □ Closed system □ Semi system

□ Other (e.g., sheltered, outdoor or naturalistic system), specify

8.3 Macroenvironment (i.e., animal holding space)

Temperature □ Ambient □ Other, specify (ºC)

Humidity, specify (%)

Ventilation system, specify

Light source □ Natural □ Fluorescent or LED, specify intensity (lux) □ Other, specify source and intensity (lux)

Light cycle □ Ambient □ 12:12 hours □ Other, specify

Requirement of the noise and vibration control

□ Not applicable □ Yes, specify

8.4 Microenvironment (i.e., water that directly contacts with the animals)

Water system

□ Recirculation □ Flow-through or single-pass

□ Static □ Other, specify

Type of water □ Freshwater □ Seawater □ Brackish water

Source of water

Water quality control

Parameter, specify

Salinity (ppt), specify

Frequency of testing, specify

Changing schedule, specify the interval (days) and the percentages of new water

Requirement of the pretreatment and chemical removal

□ Not applicable □ Yes, *specify*

Life support system

□ Not applicable □ Yes, *specify*

Behavioral management

□ Not applicable □ Yes, *specify*

Social management

□ Social housing, *provide number of animals per tank*

□ Single housing, *provide scientific justification*

8.5 Sanitation, *describe the materials and methods used at the animal housing facility*

8.6 Food

□ Commercial feed □ Other, *specify*

Feeding schedule, *specify*

8.7 Aquatic animal tank/pool, *provide size, volume and material used*

8.8 Requirement of substrate

□ Not applicable □ Yes, *specify*

**9. Health monitoring:** *Describe the criteria used for health evaluation of the animals*

**10. Animal welfare**

10.1 Provide information on literature search for duplication: *This search must be performed to prevent unnecessary duplication of previous experiments*

10.1.1 Database(s) searched

10.1.2 Date of literature search (must be within six months prior to submission date)

10.1.3 Range of years searched

10.1.4 Key words used

10.1.5 Results of literature search: *Does the study duplicate any previous work?*

□ No

* Yes, *explain why it is scientifically necessary to duplicate previous experiment*

10.2 Does the study comply with the 3R principle? *Provide adequate explanations*

10.2.1 Replacement of animals, *e.g., with in vitro models, computer models or less sentient animals*

10.2.2 Reduction in the number of animals, *e.g., using appropriate statistical methods in the design and analysis of the study, reduction in experimental variability using animals of defined genetic or microbiological status*

10.2.3 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*

10.3 Will anesthesia be performed?

□ No—*proceed to 11*

□ Yes, *select and describe or specify the followings*

Type of anesthesia

□ Non-chemical method, *describe*

□ Chemical method, *specify the followings*

a) Name of anesthesia used

b) Dosage

c) Route of administration

d) Stage of anesthesia

**11. Surgery**

11.1 Will surgery be performed?

□ No—*proceed to 12*

□ Yes, *answer all that apply in 11.2 to 11.7*

11.2 Type of surgical procedures, *check all that apply*

Procedure: □ Underwater, □ Out of water

Technique: □ Re-circulating, □ Non-re-circulating

11.3 Location: *Give room number for conducting the proposed procedures*

11.4 Name the person who will perform the surgery and indicate qualification, training, or experience

11.5 Describe surgical procedures

11.6 Describe provision for both pre- and post-operative cares including provisions for post-surgical observation including pain management

11.7 Describe long-term care for chronic survival

**12. Blood or body fluid withdrawal, tissue or organ collection:** *Describe in the Table below*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Procedures** | **Anatomical location** | **Needle size or catheter size and length** | **Biopsy size** | **Volume collected (ml)** | **Frequency** |
| Blood withdrawal |  |  |  |  |  |
| Body fluid withdrawal |  |  |  |  |  |
| Tissue collection |  |  |  |  |  |
| Other, *describe* |  | | | | |

**13. Use of non-pharmaceutical grade compounds**

13.1 Will animals be treated with non-pharmaceutical grade compounds?

□ No—*proceed to 14*

□ Yes, *answer all that apply in 13.2 and 13.3*

13.2 Give information on name, source, formulation, concentration, site and route of administration and potential side effects

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

**14. Animal restraint**

14.1 Will animals be restrained with any mechanical devices?

□ No—*proceed to 15*

□ Yes, *answer all that apply in 14.2 and 14.3*

14.2 Describe the device, duration of restraint, frequency of observation, and steps to assure comfort and well-being

14.3 Provide scientific justification for prolonged complete restraint

**15. Food deprivation or dietary manipulation**

15.1 Does this protocol involve food deprivation or dietary manipulation?

□ No—*proceed to 16*

□ Yes, *describe methods for assessing physical conditions, discomfort stress and distress during the course of study. Include clinical signs and symptoms expected.*

15.2 Provide detail of these procedures in Table below

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Procedures** | **Amount restricted or added** | **Duration** | **Compound supplemented** | **Compound excluded** | **Frequency** |
| Food deprivation |  |  |  |  |  |
| Nutrient alteration |  |  |  |  |  |

**16. Tumor study, use of disease models and toxicity testing**

16.1 Does this protocol involve tumor study, use of disease models or toxicity testing?

□ No—*proceed to 17*

□ Yes, *answer all that apply in 16.2 and 16.3*

16.2 Describe methods for assessing physical conditions, stress, pain and discomfort during the course of study. Include clinical signs and symptoms expected.

16.3 What are the criteria for humane endpoint in this protocol?

**17. Behavioral study**

17.1 Does this protocol involve behavioral study?

□ No—*proceed to 18*

□ Yes, *answer the followings*

17.2 Describe type of behavioral manipulation

17.3 Describe the protocol involving the use of testing apparatus or aversive stimulus and detail of duration and frequency of the testing period

**18. Study endpoints**

18.1 Describe the endpoint for the animals in this protocol. *Indicate whether recovery, euthanasia, or death is/are expected, and when the animal experimentation phase will be stopped.*

18.2 Humane (early) endpoint is used *(i.e., animals are humanely euthanized prior to the expected day of termination)*

□ No

□ Yes, *provide criteria for humane endpoint*

18.3 Death or moribund as an endpoint is used

□ No

□ Yes, *answer the followings*

18.3.1 Provide criteria that establish when this endpoint has been reached, and describe how animals will be monitored and cared for

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

**19. Animal euthanasia and disposition**

19.1 After completion of the study, animals will be:

□ Euthanized

□ Returned to housing facility

□ Transferred to another animal project: *Provide protocol number and name of principal investigator*

□ Other, specify

19.2 Describe the two-step euthanasia method according to AVMA guidelines

This protocol uses:

□ A chemical method followed by a mechanical method

□ A mechanical method followed by a chemical method

Describe chemical method:

□ Immersion:

□ Pharmaceutical-grade clove oil at ml/L for minutes

□ MS-222 at mg/L for minutes

□ Magnesium salts at mg/L for minutes

□ Ethanol at % ml/L for minutes

□ 2-phenoxyethanol at ml/L for minutes

□ Benzocaine at mg/L for minutes

□ Lidocaine at mg/L for minutes

□ Isoflurane at mg/L for minutes

□ Sevoflurane at mg/L for minutes

□ Quinaldine sulfate at mg/L for minutes

□ Other, specify

□ Injection:

□ Pentobarbital at mg/kg

□ Ketamine at mg/kg

□ Ketamine-medetomidine at mg/kg

□ Other, specify at mg/kg

Route of administration

□ Intramuscular, □ Intravenous, □ Intraperitoneal, □ Intracoelomic, □ Intracadiac

Describe mechanical method:

□ Cervical transection

□ Chilling using: □ Ice, □ Cold water for minutes

□ Cranial concussion

□ Decapitation

□ Exsanguination

□ Maceration

□ Penetrating captive bolt

□ Pithing

□ Other, specify

□ Use another method besides the two-step euthanasia method, *describe and provide strong scientific justification*

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

**20. Necropsy and tissue collection:** *Will this project involve necropsy and/or tissue collection?*

□ No—*proceed to 21*

□ Yes, *provide room number, personnel with qualification*

**21. Animal tissue and carcass disposal:** *Describe method used to dispose animal tissues and carcasses*

**22. Occupational health and safety**

22.1 Select types of hazards associated with this protocol, also provide name, source and amount to be used in each category

□ Hazardous chemicals (e.g., carcinogen, mutagen and teratogen)

□ Infectious agents, *provide the certificate of biosafety approval*

□ Radiation equipment and radioactive elements

□ Recombination agents

□ Other, specify

□ None

22.2 Specify biosafety level: □ ABSL-1 □ ABSL-2

22.3 Explain how the wastes associated with these hazards are decontaminated and disposed

22.4 Explain how the carcasses associated with these hazards are disposed

22.5 Explain any safety precautions and protective measures (e.g., biosafety cabinet and proper PPE) to protect personnel from those hazards and list any surveillance procedures in place to monitor any potential exposure

22.6 In the event of an accident, provide immediate procedures and early treatment to minimize potential injury or illness, as well as details of available medical support in the case of serious exposure

**23. Qualification of personnel:** *List PI and all co-investigators who will be* ***directly*** *involved with animals in this submitted protocol*

|  |  |  |
| --- | --- | --- |
| **Name** | **Responsibility** | **Relevant experience and qualification** |
|  |  |  |
|  |  |  |
|  |  |  |

As the 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 the standard of animal care and use established under the ethical guidelines and policies of Mahidol University, and the 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 MUSC–IACUC.

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

**C. Statistical assurance:** I assure that I have consulted with a 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. Occupational health and safety:** I have taken into consideration, and I have made the proper co-ordinations regarding all applicable rules and regulations concerning hazard identification, prevention and protection 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 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 study:**

□ This protocol is associated with a grant application. I certify that this protocol is essentially the same as the study found in the grant application or program/project. The MUSC–IACUC 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 MUSC–IACUC is granted.

□ This protocol is not associated with a grant application.

Principal investigator Date

( )