Institutional Policy

Institutional Animal Care and Use Committee (IACUC) and Animal Care and Use Program (ACUP) of Naresuan University

By
Naresuan University Animal Care and Use Committee)NUACUC)

Institutional Policy

Institutional Animal Care and Use Committee (IACUC) and Animal Care and Use Program (ACUP)

1. GENERAL: At Naresuan University, the policy related to animal use for biomedical research, education, and testing will follow the Act on Animals for Scientific Purposes, B.E. 2558 (2015), the *Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes*, the National Research Council of Thailand, 1999, and the *Guide for the Care and Use of Laboratory Animals (The Guide)*, US National Research Council (2011).

2. PURPOSE: This Policy describes the responsibilities and composition of the Naresuan University IACUC, annual program review and facility inspections, and the components of a protocol using animals, protocol review, post approval monitoring, addressing animal welfare concerns, suspension of animal activities, the responsibilities of the Principal Investigator (PI) and outlines the requirements or policy for animal care and use as well as Whistleblower policy.

The goal of this policy is to ensure that the animal care and use program (ACUP) conducted at Naresuan University Center for Animal Research (NUCAR) complies with applicable international standards, guidelines, and appropriate Thai legislation. It is the goal of Naresuan University that the ACUP will meet international standards and will gain accreditation by the appropriate international inspectorate: the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALACi)

3. CONTENTS

Section Page

4. REFERENCES

1. Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes
2. The Act on Animals for Scientific Purposes, B.E. 2558 (2015)
3. The National Research Council of Thailand, 1999.
4. Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 2011.
5. The American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals: 2020 Edition
6. International Air Transport Association. "Live Animals Regulations." IATA
7. Institutional Administrator's Manual for Laboratory Animal Care and Use. DHHS Publication 88-2959, 1988. Office of Laboratory Animal Welfare (OLAW), National Institutes of Health, Bethesda, MD 20205.
8. Occupational Health and Safety in the Care and Use of Research Animals
9. Institute of Laboratory Animal Resources, National Research Council, National Academy Press, 1997.

5. DEFINITIONS

a. AAALACi – Association for Assessment and Accreditation of Laboratory Animal Care International. This is a private, nonprofit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs.

b. Alternatives – Activities that reduce, refine, or replace the use of animals in biomedical research.

c. Animal – Any vertebrate animal (e.g., traditional laboratory animals, agricultural animals, wildlife, and aquatic species) produced for or used in research, testing, or teaching.

d. Attending Veterinarian (AV) – A veterinarian who has received training or has experience in the care, management, and use of research animals and that has direct program responsibility for activities involving animals at Naresuan University.

e. Facility Inspection and Program Review (FIPR) - The *Guide* requires that the IACUC inspects all animal facilities and study areas annually.

f. Institutional Animal Care and Use Committee (IACUC) – The Committee which is required by the *Ethical Principles* which reviews all proposed animal use protocols, all animal programs, and all Naresuan University animal holding and animal use facilities. This Committee performs review of all proposed use of animals.

g. Institutional Official (IO) – This is the person who is given by the University the authority and responsibility to enforce the *Ethical Principles* and the IACUC policy. In this case, the officer is 'Vice President for Research and Innovation Development.'

h. National Research Council of Thailand (NRCT) — An independent public agency operating under the direct supervision of the Prime Minister. Its main function is to formulate and implement national research policy and strategies.

j. Principal Investigator (PI) – The person responsible for conducting the research or teaching described in the proposed animal use protocol. There is only one PI for each protocol and this person must be on-site. The PI assumes responsibility for the use and care of animals in conformance with procedures set forth in the proposed protocol and in compliance with the *Ethical Principles* and the *Guide*.

k. Naresuan University IACUC (NUACUC) A committee that is responsible for oversight of the animal care and use program of Naresuan university.

l. Protocol – A document prepared by the PI detailing the proposed use of animals.

l. Standard Operating Procedures (SOPs) — A written document or instruction detailing all relevant steps and activities of a process or procedure.

6. APPLICABILITY

a. This policy applies to all investigators receiving funding from Naresuan University, other funding agencies or using the Naresuan University Center for Animal Research (NUCAR).

b. Naresuan University personnel who conduct animal research in facilities outside of Naresuan University will submit a copy of the approved protocol to the Chair, NUACUC, and the facility is considered a satellite facility and may be inspected by NUACUC.

c. Studies involving animal by-products (tissue, blood, cells, antibodies, biochemicals, etc.) obtained entirely from commercial sources (e.g., selected from a company's product catalog) may be conducted at Naresuan University without NUACUC approval. However, transport of animal by-products that have the potential to carry animal pathogens and/or biohazards into the Naresuan University colony (frozen serum and tissue, infected blood products, etc.) must be brought to the attention of the Naresuan University AV and the University Safety Officer. In addition, any biological product that is to be used in an experimental animal (or on a cell line that will be used in an animal) must be listed in the protocol and must be certified free of pathogens and adventitious agents prior to its use. A copy of the certificate must be provided to the IACUC and will be filed with the protocol.

7. AUTHORITY: The NUACUC has the authority to act on behalf of the IO to:

a. Investigate any concerns relating to laboratory animal care and use.

b. Suspend any activity, which violates the *Ethical Principles* and the *Guide,* and/or IACUC policy.

c. Terminate immediately any use of animals that deviates from the approved protocol.

d. Humanely euthanize an animal that in the opinion of the AV is suffering from pain or distress that cannot be alleviated. Certain exceptions may apply when thorough justification is provided in approved, specific protocols.

8. RESPONSIBILITIES

8.1. The IO will:

(1) Ensure that research, clinical investigations, diagnostic procedures, and instructional programs are conducted in compliance with *Ethical Principles* and the *Guide*. If there is a conflict or difference between the *Ethical Principles* and the *Guide*, then the stricter of the standards will apply.

(2) Ensure that local animal care, use, procurement, and transportation policies comply with applicable laws, regulations, guidelines, and standards.

(3) Ensure that animals will experience no unnecessary pain, suffering, or distress.

(4) Ensure that efforts will be made to seek and utilize procedures, which minimize pain and distress.

(5) Ensure that alternatives are used if they can fulfill the same scientific objectives.

(6) Ensure that every effort is made to reduce the number of animals used in the research while maintaining statistical validity, that appropriate controls will be in place, and that unnecessarily duplicative research does not occur.

(7) Ensure that an Occupational Health and Safety Program constitutes part of the overall Animal Care and Use Program.

(8) Ensure that the IACUC Chair and the AV have direct access to and report directly to the IO.

(9) Review and approve the minutes, reports, and protocols recommended for approval by the IACUC.

(10) Render reports on the programs for animal care and use as required by the National Research Council of Thailand (NRCT) and/or AAALAC.

(11) Ensure that all scientists, research technicians, animal technicians, and

other personnel involved in the animal care and use at Naresuan University are qualified to perform their duties.

8.2. Department Heads will:

(1) Ensure that all departmental animal research is performed under approved protocols.

(2) Review all protocols submitted by PIs within their department for mission relevance, scientific merit, and the animal care and use program need.

(a) The primary responsibility for scientific review belongs to the department head. His/her signature on the protocol title page certifies that the protocol is scientifically meritorious and relevant to the department's mission. The department head may choose to designate scientific review but retains primary responsibility for this process.

(b) There must be no conflict of interest in the scientific review process (i.e., a co-investigator may not perform the scientific review).

(c) In the event that the department head is also a primary investigator on the protocol, a person designated by the dean must review and sign the protocol cover sheet for scientific merit prior to submission of the protocol to the IACUC.

(3) Nominate member(s) of the department to serve on the IACUC when requested by the IO.

(a) The department IACUC member(s) provide guidance to investigators on correct format and composition of protocols.

(b) The member(s) serve as a liaison between the investigator, their respective departments, and the IACUC.

8.3. The IACUC will:

(1) Review all proposed animal use protocols or proposed changes to ongoing activities to be conducted by PIs for compliance with

applicable laws, regulations, guidelines, and standards.

(2) Ensure that an annual report is submitted for each protocol.

(3) Annually review, inspect, and approve:

(a) The animal care and use program for compliance with all applicable laws, regulations, guidelines, and standards as well as IACUC policies and standard operating procedures (SOPs).

(b) All animal facilities, including laboratories where animal work is performed and/or where animals are housed including laboratories, support and surgical facilities, and field sites.

(4) Prepare a report of deficiencies found and a plan for correction of the deficiencies found.

(5) Review and investigate concerns or complaints involving the use of animals. No person shall be discriminated against or suffer reprisals for reporting violations, and complaints may be made anonymously.

(6) Suspend any activity, which violates the *Ethical Principles*, the *Guide* or applicable laws, regulations, or policies of the University or any use of animals that is conducted without an approved protocol or approved modification to a protocol.

(7) Make recommendations to the IO regarding the animal care and use program.

(8) Ensure that all scientists, technicians, and other personnel involved in the animal care and use program are qualified to perform their duties.

(9) Ensure that scientists, technicians, and other personnel are provided with training opportunities to enhance their knowledge of principles of humane animal care and use.

(10) Ensure that the animal care and use program at Naresuan University is accredited by the AAALAC.

(11) File annual reports as required by the AAALAC and the National

Research Council of Thailand (NRCT).

(12) The IACUC will establish and publicize procedures for reporting conditions or procedures which any observer perceives to violate humane care and use of animals or violate guidelines established by the Institutional Animal Care and Use Program.

8.4. The Chairperson of the IACUC will:

(1) Call and conduct meetings of the IACUC at least every 3 months.

(2) Ensure that all protocols that use animals are reviewed and then approved by the IACUC if they are in accordance with applicable laws, regulations, guidelines, and standards.

(3) Ensure that the composition of the IACUC meets the requirements of all applicable laws, regulations, guidelines, and standards.

(4) Call and conduct annual reviews of the animal care and use program.

(a) Prepare a report to include the adherence of the facility, major and minor deficiencies found, a plan for correction of deficiencies, and a schedule with dates for correction of each deficiency.

(b) Any failure to adhere to the plan and schedule that results in a significant deficiency (one that is or may be a threat to the health or safety of the animals) remaining uncorrected shall be reported in writing within 10 business days to the IO.

(5) Keep a current list of facility deficiencies and update it annually. Prepare a plan and time schedule for correction of facility deficiencies.

(6) Direct recording, preparation and maintenance of the minutes of IACUC meetings and submit them to the Institutional Official.

(7) Direct maintenance of files of approved protocols, inspections, training, and correspondence pertaining to the activities of the IACUC.

(8) Request that the PI, or knowledgeable representative appointed by the PI attend the IACUC meeting where his/her protocol or a related animal care and use issue is to be discussed.

(9) Notify the PI in writing when the protocol is approved and when the PI may commence use of animals as described in the approved protocol.

(10) Investigate or appoint an appropriate subcommittee to investigate any report of inhumane or inappropriate use of research animals or any violation of policy.

(11) Notify the PI in writing at least 60 days before the annual review is required and provide the PI with an annual checklist to describe the protocol and the anticipated use of animals for the upcoming year.

(12) Ensure that all correspondence involving the IACUC is reviewed at each IACUC meeting.

(13) Approve minor modifications to protocols.

(14) Delegate administrative functions as necessary to the IACUC Administrator.

8.5. The Principal Investigator (PI) will:

(1) Prepare, submit, and have an approved protocol for the use of animals before any research using animals is performed.

(2) Ensure that protocols submitted by PIs of other institutions identify an affiliation of the principal investigator.

(3) Obtain signed approvals from each of the department heads of Naresuan University prior to submission of protocols to the IACUC. The review and signature of the department head serve to validate scientific mission relevance.

(4) Sign an ‘Assurance Statement’ which affirms responsibility for the performance of the research conducted under the protocol and assures the continued humane care and use of animals used in the research for which he/she is PI. Furthermore:

(a) The research must be performed in accordance with the description provided in the IACUC-approved protocol and any approved major and minor modifications.

(b) Ensure adherence to the approved protocols, attention to animal health and to unexpected changes in animal facilities, safety procedures, and strict oversight of all staff, coworkers, etc. working under the protocol are the responsibility of each PI.

(c) The PI must ensure that he/she and all personnel working on the protocol will be properly trained in basic animal care and principles of use, and have received occupational health clearance to work with animals. The PI will ensure that all training for relevant personnel is documented fully.

(d) Each PI is required to apply any new techniques that could reduce the number of animals needed, could reduce pain or distress, or could replace animals with non-animal systems.

(e) The PI must use pharmaceutical or veterinary-grade medications and materials whenever they are available, even in acute procedures. Non-pharmaceutical-grade chemical compounds may only be used to make anesthetic drugs or any other type of medication for use in animals after specific review and approval by the IACUC for reasons, such as scientific necessity or non-availability of an acceptable veterinary or human pharmaceutical-grade product. Cost saving alone is not an adequate justification for using non-pharmaceutical-grade compounds.

(5) Obtain approval for modifications to an approved protocol before modifications are implemented.

(6) Ensure that all individuals and all activities associated with any protocol involving biohazards are following current guidelines and regulations issued by the Center for Disease Control and Prevention. (CDC)/National Institutes of Health (NIH, USA) as well as Naresuan University.

(7) Assume responsibility for the disposal of animals following their completion of the protocol to determine whether euthanasia or transfer of the animal to another protocol is the best course of action.

(8) Submit annual Protocol Reports to the IACUC as requested, describing progress, conclusions, and publications as well as any developments in the field that might alter the number of animals needed or the procedures to be used. Protocols are generally reviewed and approved for a 3-year period, but annual reports to the IACUC by the PI are reviewed to determine whether each protocol should continue. Modifications made in the protocol during the year should be detailed in the annual report.

(9) Submit, if required, information requested by the IACUC as a result of their Facility Inspection and Program Review and address any deficiencies in the PI's area of responsibility.

(10) Wait until official written approval is received from the IACUC Chair authorizing that the protocol may be initiated before beginning research.

(11) Submit an official memorandum to the IACUC Chair when the protocol work has been completed and no additional animal support is planned. Request closure of the protocol and include a brief summary of the findings and disposition of the animals.

(12) Ensure that all the data is objectively and honestly obtained.

8.6 The Attending Veterinarian (AV) will:

(1) Procure, maintain, and provide health care to animals housed in institute facilities and maintain required records of those activities. Provision of animal husbandry and health care responsibilities shall be conducted in accordance with applicable laws, regulations, guidelines, and standards including all standard operating procedures of the Center for Animal Research, Naresuan University. Any procurement of animals other than under an approved protocol and at the direction of the AV is strictly prohibited.

(2) Provide protocol support as needed to research personnel to ensure that all animal use procedures are performed in accordance with applicable laws, regulations, standards, and guidelines.

(3) Provide training to investigators and technicians with regard to animal care and use and maintain training records.

(4) Provide veterinary consultation to PIs during drafting of each protocol.

(5) Maintain the Institute's AAALAC Accreditation standard of quality animal care and related documentation.

(6) Serve as a voting member on the IACUC.

8.7 . IACUC Administrator will provide administrative support for the IACUC

(1) Schedule, coordinate, record, attend, and document Committee meetings and inspections

(2) Prepare correspondence

(3) Maintain protocol files, Committee records, and other relevant documents

(4) Prepare reports as required by the AAALAC and the Institute.

9. IACUC MEMBERSHIP

a. The IACUC membership may vary with the needs of the Institute, but must be in accordance with the *Ethical Principles* and the *Guide*. The IACUC voting membership will include, but may not be limited to:

(1) A scientist experienced in animal research and having a track record of high-quality research.

(2) A veterinarian trained in laboratory animal science, preferably with experience in research animal care and use that has direct program responsibility for activities involving animals at Naresuan University (Attending Veterinarian).

(3) At least one person representing community interests. (Non-Affiliated) This individual cannot have any liaison with the Institute other than as a member of the IACUC and shall not be an immediate family member of any person affiliated with the Institute.

(4) At least one person whose primary background is outside of biomedical science (Non-scientist).

(5) At least 1 representative from each department within Naresuan University who is a major user of animals.

(6) There shall be no more than 3 voting members from the same department.

(7) The person recording the minutes shall be a non-voting member.

(8) The IACUC Chair to be appointed by the IO.

b. Whenever possible, all members shall have an alternate member who will be trained in IACUC procedures and responsibilities, who will receive the same general information as IACUC members to stay abreast of current issues, and who may serve on various IACUC subcommittees.

c. Nonvoting, Ad Hoc members may include the IACUC Administrator, other consulting veterinarians, animal care personnel, technicians, subject matter experts, and other consultants.

d. Should expertise be unavailable from within the IACUC, a subject matter expert may be contacted in person or in writing to advise the Committee.

e. All members must obtain an initial eight hours of training in laboratory animal care issues and ethics as directed by the *Ethical Principles* and the *Guide*.

10. GENERAL IACUC PROCEDURES

10.1. Institutional Animal Care and Use Committee Meetings:

(1) The IACUC will meet at least every 3 months or more frequently as necessary to review and discuss protocols, reports, the results of

program, facility reviews, and other animal care and use issues brought before the Committee.

(a) A tentative monthly IACUC meeting schedule with a deadline date for protocol submission will be made available to PIs and IACUC members.

(b) IACUC meetings will be held on the day scheduled if:

- There is new or pending IACUC business OR

- It has been 3 months since the last meeting

(2) A quorum consisting of a majority (more than 50 %) of the IACUC voting membership is required for each official meeting. No member may vote on a protocol for which that member has a conflict of interest (i.e., is a PI or co-investigator) and that member may not be counted toward a quorum for that vote. (Note: An alternate may only vote in place of the primary member specified in the appointment memorandum). If a quorum cannot be attained on the scheduled date, a new date, which is to be as close to the scheduled date as possible, will be selected by the IACUC Chair.

(3) At least 3 working days prior to the IACUC meeting, primary and alternate members receive packets or via electronic mail containing the upcoming meeting agenda and supporting documentation. The packets may include copies of protocols to be reviewed, minutes of previous meetings, a list of protocols approved by the IACUC during the interim since the last meeting, inspection reports, and general information pertaining to facilities and the general health and welfare of research animals. Packets may be sent electronically. PIs need to be assured that the information is confidential.

(4) IACUC members may contact the Chair or the IACUC Administrator 7 days prior to a regularly scheduled meeting to place issues on the agenda or to request that a consultant, investigator, or other personnel be invited to the meeting.

10.2. Facility Inspection and Program Review (FIPR).

A review and inspection of the Animal Care and Use Program and facilities will be conducted by the IACUC or IACUC representatives every six months. Reports of the IACUC on evaluations and inspections will be reviewed and signed by a majority of IACUC members. Minority views, if expressed, must be included.

10.3. Protocol Review Process

(1) PIs will submit animal use protocols using the standard protocol format to the IACUC Administrator for IACUC review after the protocol has received review and signature from the department head and any additional support personnel as indicated.

(2) All new protocols will receive full Committee review at the next appropriate IACUC meeting. All protocols received prior to 7 working days before the scheduled monthly IACUC meeting will be discussed at that month's IACUC meeting. Any protocol received within 7 working days of the meeting may be held until the following meeting.

(3) Each protocol to be reviewed will be submitted to all IACUC members. They will have at least 3 working days to review the protocol prior to the IACUC meeting.

(4) If prior to the meeting an IACUC member raises issues or makes suggestions regarding the protocol which the PI needs to address, the member should:

(a) Indicate his/her remarks in writing.

(b) Forward these remarks in writing to the IACUC administrator at least 2 working days before the scheduled IACUC meeting.

(c) The IACUC Chair or administrator will forward these to the PI 1 working day before the meeting inviting the PI to attend and discuss these issues. No written response is required of the PI at this time.

(d) New issues regarding the protocol may also be brought up to the PI during the meeting without prior notification.

(e) At any time, each member of the IACUC may also contact the PI directly, or ask the IACUC administrator to do so to ask any questions about the proposed protocol.

(5) At the IACUC meeting:

(a) In some cases, the PI, or the PI's representative, will give a brief overview of the protocol and answer all IACUC questions. After all issues have been satisfactorily discussed, the PI will be asked to leave.

(b) The Committee will then discuss the protocol and vote.

(c) By a majority vote the IACUC may decide to:

- Approve the protocol as is.

- Request modifications or clarifications to secure approval. When this occurs, the Committee generally authorizes designated members to approve the revised protocol on behalf of the IACUC when resubmitted. However, the IACUC reserves the right to require that the revised protocol be resubmitted at the next convened meeting for full Committee review or be distributed via e-mail for Committee review. When distributed via e-mail, members have 5 working days to comment or request that the resubmitted protocol be reviewed at the next full meeting.

- Disapprove the protocol. The PI will be made aware of the reasons for this decision.

- Table or defer review.

(6) The IACUC Chair communicates the IACUC's decision, comments, and requests to the PI. It is the responsibility of the PI to assure that all revisions requested by IACUC members are addressed and incorporated into the protocol before the protocol is forwarded to the IACUC Chair for approval. The PI must respond to this communication in writing within 3 months of the initial review.

(7) In reviewing the protocol, the IACUC will:

(a) Consider whether the protocol contains all required management and regulatory elements as defined by this Policy and the Protocol Template, and whether the body of the protocol is understandable and complete. The IACUC will determine if the proposal meets the current standards and practices for pain management, adjuvant use, humane end-point determination, etc. as defined in applicable laws, regulations, guidelines, and standards.

(b) The IACUC will determine whether proposed protocols involving cats, dogs, or nonhuman primates can only be performed in those species or whether some other species or test system could produce comparable data. The IACUC will evaluate whether the species of nonhuman primate proposed for use is the most suitable and PIs will be required to contact other animal facilities for nonhuman primates use.

(8) After the IACUC approval, the IACUC administrator will assign a protocol identification code and prepare for the IACUC approval document signed by the IACUC Chair.

(9) The written notification from the IACUC Chair and the IACUC approval document will be sent to the PIs. The PIs must receive IACUC approval prior to the start of the research.

(10) Protocols are approved for a three-year period.

10.4. Protocol Format and Content

1. Protocols must use the Naresuan University Protocol Template. The current template can be found on NUCAR website or obtained from the IACUC Administrator. The protocol format includes requirements of the Act on Animals for Scientific Purposes, B.E. 2558 (2015), Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes and the Guide (2011).

2. Protocols must include much specific information. The data required are detailed in the protocol template. In general, the IACUC is primarily interested in issues of animal care and use, while the Department Heads are primarily responsible for scientific merit and mission relevance. However, since the approval of animal use is tied to the scientific value of the information gained, the IACUC may decide on the basis of scientific merit whether the use of the animals is justified. The IACUC may also require additional scientific review.

   The outline format consists of the following information: the cover page [Protocol Title, Principal Investigator, Co-Investigator(s)], 1. Non-technical summary, 2. Rationale, 3. Literature review, 4. Literature Search for Duplication, 5. Experimental design and general procedures, 6. Animal model, 7. Animal care, 8. Veterinary medical care, 9. Animal welfare, 10. Surgery, 11. Blood/ Body Fluid Collection, 12. Sample collection, 13. Restraint with mechanical devices, 14. The project involves food and fluid restriction, or dietary manipulation, 15. Tumor and disease models, toxicity testing, 16. Endpoints, 17. Euthanasia/ disposition of animals after completion of study, 18. Necropsy, 19. Animal tissue and carcasses disposal, 20. Biohazard/Safety, 21. List of References, 22. Qualification, 23. Assurances. However, some information may be added to the format to meet local IACUC needs.

3. Protocols involving potential pain or distress.

   (a) A painful procedure is any procedure which would be reasonably expected to cause more than slight or momentary pain or distress when applied to humans, (i.e., pain in excess of that caused by injections or other minor procedures).

   (b) PIs must check with the AV at the time their protocol is prepared with regard to classification of certain procedures and approved methods of euthanasia.

4. Protocols must contain a detailed description for monitoring the animals and include provisions for supportive care (e.g., placing food in the cage within easy access, supplemental fluids, extra bedding etc.). A protocol not providing supportive or palliative care for clinically ill animals has to be fully justified.

5. Protocols should be scientifically rigorous, yet be written in a clear and logical style. An intelligent reader should be able to understand, in general, why and how the PI is going to use the animals.

6. The protocol must include a section on safety or hazards if infectious (human or animal), chemical or radioisotope hazards exist. This section should explain in detail how these elements will be managed by the PI.

7. The protocol must include a section on training of the PI and associated personnel who will perform the procedures described in the protocol. Training includes educational background, experience, and knowledge with the procedures and animal species to be used. Specific formal training should be cited. IACUC members and/or attending veterinarian may observe and document a technique performed by an investigator as part of their animal use and training oversight responsibilities.

8. The protocol must also include the Assurance Statement detailed in the Protocol Template, followed by the typed name and signature of the PI.

10.5 Protocol Modifications

(1) There are two categories of modifications:

(a) *Major modifications* require full IACUC approval and include but are not limited to the following:

- Changing the scientific direction of a protocol.

- Increasing the number of non-human primates, dogs or cats, or increasing the number of other animals by more than 10%.

- Changing to a species other than that approved in the original protocol.

- Change to more severe painful procedures.

- Adding surgery to the protocol.

- Changing the Principal Investigator. The new PI must submit a training statement of competency with his/her request and a signed statement agreeing with all the conditions imposed on the original PI.

(b) *Minor modifications* to the original protocol do not require full IACUC approval. Typical minor modifications might include the following:

- Changing doses or routes of administration of a drug or using a non-pharmaceutical-grade medication.

- Recording or measuring additional variables (e.g. locomotor activity, heart rate) in the whole animal that are no more invasive than procedures currently approved under the protocol,

- Using additional investigative compounds or veterinary health drugs that are functionally similar to those in the original protocol.

- Changing personnel supporting the protocol. All new personnel must submit a statement outlining training credentials with the request.

- Changing the number of small animals used, 10% or less of the original number. All requests exceeding the 10% is a major amendment.

- The IACUC may direct a PI to provide additional information for individual experiments (for example drugs to be tested in a drug screening model) in a minor modification prior to the start of each experiment.

(c) Guidance from the IACUC Chair or the AV should be sought when the PI is unsure whether proposed changes constitute a major or minor modification.

(d) The final decision on modification classification will be made by the Chair in consultation with the AV.

(2) All modifications must be documented in a provided format to the IACUC Chair for filing with the protocol record. All modifications should be noted in the annual report of the protocol.

(3) Major modification submission and review:

(a) Major modifications are submitted and approved as a protocol amendment. The protocol amendment template is available on the NUCAR website and from the IACUC administrator.

(b) The amendment is reviewed initially by the IACUC administrator and the AV or any other coordinating personnel before submitted to the IACUC.

(c) Upon receipt and administrative acceptance by the IACUC administrator, the major amendment is sent to all Committee members via e-mail. Members are given at least 3 working days to review the amendment, make comments, and request a full Committee review. Comments by designated members may be made without requesting full Committee review.

(d) If any member (primary or alternate) requests full Committee review then the amendment is held until the next IACUC meeting and reviewed in the same manner as a new protocol.

(e) If no member requests a full Committee review, the chair assigns at least three IACUC members as primary designated-reviewers. The primary reviewers act on behalf of the IACUC and communicates any IACUC comments and concerns to the PI and judges whether responses are adequate. This process should be completed within 3 months of receipt of the original modification. Once all concerns are adequately addressed, the protocol is recommended for approval in the IACUC meeting.

(f) The amendment is officially approved when the PI receives official notification from the IACUC Chair.

(4) Memorandums for both major and minor modifications must be completed as per the protocol amendment template which requires the

PI state the proposed modification, justify the modification, provide personnel training documentation (when applicable), and sign an assurance statement.

10.6. Investigating Animal Use Related Concerns

Procedures for investigating alleged abuses of animal use are as follow:

(1) All allegations reported to the IACUC will be evaluated by the IACUC Chair, AV, and the IACUC administrator. This subcommittee will decide if there is any basis for the allegation. If an animal is in imminent danger, corrective action will be taken immediately. All written allegations and allegations determined to have basis will be investigated fully.

(2) The IACUC Chair will bring the issue to the IACUC. If additional information is required, the Chair will designate a subcommittee of at least 2 members to investigate the complaint. The results of all completed investigations will be reviewed by the IACUC. At any time, the IACUC Chair or subcommittee can request the full IACUC meeting be convened to discuss the complaint.

(3) All persons involved will be informed of the purpose of the investigation and the manner in which it will be conducted.

(4) Those against whom the complaint is addressed will have an opportunity to explain their side of the issue.

(5) The results of the IACUC investigation will be available to all involved.

(6) The IACUC may suspend a previously approved protocol. In brief, the IACUC may suspend a protocol only after review of the matter at a convened meeting of a quorum of the IACUC by a majority vote of the quorum present.

11. Animal care and Use Program

11.1. Animal Husbandry

Naresuan University has established “NUCAR” as the centralized animal facility designed to serve all the university researchers for all studies using

laboratory animals such as mice, rats, guinea pig and rabbits. All of the rodents and rabbits used in NUCAR are procured from reliable sources. Only animals authorized in the NUACUC approved protocol are ordered, and the animal acquisition is processed solely by NUCAR. The quality assurance (QA) procedure randomly evaluates the health status of the animals received from reliable sources upon arrival and at the end of the acclimatization period. The rodent QA and quality surveillance (QS) procedures include abnormal physical signs or behaviors such as changes in body weight, amount of water and food intake, autonomic signs, stress, and general appearance.

All animals are observed daily by an animal caretaker assigned to each room. Each animal is observed for well-being, feed, water, and housing conditions. Animal caretakers are trained as part of their job responsibilities to recognize abnormal physical signs or behaviors, such as changes in body weight, amount of water and food intake, autonomic signs, stress, and general appearance. The mice, rats, and Guinea pig's cages either shoe box cages and individually ventilated cages (IVC) are made of durable, nontoxic, and heat resistant materials: polycarbonate or polysulfone free of sharp edges. An appropriate housing space is a major account for the animal's social need. Population density in each cage/group is judged by the AV based on the recommendation from the Guide during protocol review. The standard transparent cage system with an interlinking system for social interaction are used for rabbits (6 cages/rack) and this can promote their natural behavior pattern. Autoclaved corncob bedding is used as bedding material for all animals.

11.2. Social housing

Social housing of all social research animal species is the default housing environment at NUCAR. Single housing of social species (other than short term recovery from experimental manipulation) must be justified based on experimental requirements and described in the protocol. The justification can be submitted for review and approval by the IACUC.

When necessary, single housing of social animals should be limited to the minimum period necessary and, where possible, visual, auditory, olfactory and, depending on the species should be provided. In the absence of other animals, additional enrichment should be offered, such as safe and positive interaction with the animal care staff, as appropriate to the species or supplemental enrichment items in the cage.

11.3. Environmental Enrichment

Enrichment program has been implemented to improve the welfare of the animals such as polycarbonate huts, PVC tubes and nesting material to encourage the animals to express their natural behaviors like digging, and nest building. For rabbits, elevated shelve is available in their cages to retreat themselves in case of disturbances. All nude mice must be provided with nesting material or a plastic/paper hut, or shelter. Sterilized rice straw or good quality hay is put in the rabbit cages as an enrichment item.

Requests for exceptions to this enrichment program must be reviewed and approved by the IACUC.

11.4. Disease surveillance program

The procedures used in health surveillance at NUCAR generally include serologic tests, bacterial cultures, parasitological examinations. Each of these may include very few or many procedures to detect different infectious agents or disease processes. Surveillance with rodent sentinels is required for all long-term rodent colonies that house more than 6 months. Sentinel program for rodent colonies is used to define the microbial status of rodent colonies, surveillance is conducted for subclinical and clinical diseases that may interfere with research results.

11.5. Prolonged Restraint

Prolonged restraint (greater than 12 hours) should be avoided. Strong scientific justification must be given if prolonged restraint is necessary to achieve research objectives. Physical restraint is not considered a normal method of housing and should not be used simply as a convenience in handling or managing animals in accordance with the Guide. Conditioning is necessary to accustom nonhuman primates, dogs, cats and rabbits to restraint devices. Animals will not be placed in the devices that are not properly set up. Animals may be monitored by unannounced visits by veterinary personnel or IACUC members.

11.6. Food and fluid regulation

Animals housed at NUCAR receive food and water ad libitum. Food and/or fluid Regulation must be approved by the IACUC and justified based on the scientific objectives of the study. The least amount of Restriction that will achieve the objectives must be used. Studies that typically require food/fluid Regulation involve a) use of food/fluid consumption to motivate animals to perform novel or learned tasks, b) research of the motivated behaviors and

physiologic mediators of hunger and thirst, and c) homeostatic regulation of energy metabolism or food balance.

Food restriction studies must ensure that the diet is nutritionally adequate so that the animal's metabolic requirements are met and the animal receives the minimum daily requirement of protein, fats, and carbohydrates plus vitamins and minerals to stay healthy. This principle applies unless the study is intended to investigate the minimum nutritional requirement of a certain food component or if the intent of the study is to test hypotheses related to the effects of nutrient deficiency or weight loss. Animals must be closely monitored to ensure that food and fluid intake meets nutritional needs. Body weight should be measured at predetermined intervals and written records must be maintained for each animal to document daily food and fluid consumption, hydration status, and clinical or behavioral changes. These changes may be used as criteria for temporary or permanent removal from the study.

Food and/or fluid Regulation is not recommended in rodents under eight weeks of age and no rodent can be completely deprived of fluids for more than 24 hours. Pre-anesthetic fasting or water restriction is also not regulation.

11.7. Surgery Procedures

Procedures that require surgery must be fully and explicitly described in procedure.

(1) All survival surgery (that from which an animal recovers) must be performed in accordance with applicable laws, regulations, standards, and guidelines.

(2) When major operative procedures are conducted on non-rodents, this and any recovery process must be performed in a facility dedicated for that purpose (i.e., a surgery room) during which time it must be constantly monitored. Major operative surgery is any surgical intervention that penetrates and exposes a body cavity or any procedure that produces permanent impairment of physical or physiological functions.

(3) Minor operative surgery, non-survival surgery, and all surgery on rodents may be performed in a non-surgery room (e.g., a portion of the procedure room) but surgery must be performed using aseptic techniques.

(4) No animal will be used for more than one major surgical procedure and allowed to recover unless the multiple procedures are explicitly justified by the PI in the protocol. Nevertheless, the animal must be allowed to fully recover from the effects of the previous surgical anesthesia and all vital signs must be within normal limits unless variations are the result of an approved surgical manipulation before the next experimental surgical procedure is performed. However, where a subsequent veterinary procedure is needed to protect the health of the animal, this can be determined and permitted by the attending veterinarian.

(5) A plan for appropriate post-operative care and monitoring must be detailed in the protocol.

(6) According to the *Guide*, "The AV must provide guidance or oversight to surgery programs and oversight of post-surgical care," and "The investigator and veterinarian share responsibility for ensuring that post-surgical care is adequate."

11.8. Freund's Adjuvant

The use of Freund's adjuvant must be completely described and explicitly justified, since this agent can cause pain and distress and alternative methods are sometimes suitable. Complete Freund's Adjuvant (CFA) is inappropriate as a priming agent for the ascites method of monoclonal antibody production and will not be permitted.

11.9. Blood Collection

Collection of blood from the retro-orbital venous sinus or plexus

(1) Collection of blood from the retro orbital venous sinus or plexus is considered by the IACUC to be a painful procedure. This procedure must be done under full surgical anesthesia unless scientifically justified.

(2) For mice drawing blood from the mandibular vein is recommended as an alternative to retro-orbital bleeding. Obtaining blood from the mandibular vein in mice is considered a non-painful procedure and may also be undertaken without anesthesia.

11.10. Euthanasia

The means of euthanasia must be stated and must adhere to the most current recommendations of the American Veterinary Medicine Association (AVMA). Both acceptable methods of euthanasia and methods that are acceptable with conditions are accepted by the IACUC. If the use of a euthanasia method that is acceptable with conditions is proposed in the protocol, the PI must review the conditions that must be met and assure the IACUC that they will be followed. If animals are given CO_2 or lethal drug injection, death must be assured by follow-up heart auscultation. The use of decapitation (small animals) or cervical dislocation without anesthesia requires a justification.

Laboratory rodents should be euthanized in their home cage and must not be placed in unfamiliar groups. Activities that contribute to distress in rodents include transport, handling (in animals not accustomed to it), disruption of compatible groups, and elimination of established scent marks. While eliminating all sources of distress may not be practical or possible, the selected method of euthanizing rodents must minimize these sources of potential distress. Methods of euthanasia likely to elicit distress vocalizations or pheromones that other animals could hear, or smell should be performed in another location, if transportation distress can be minimized.

Specific humane endpoints must be clearly defined in all animal protocols, particularly for all USDA Category D and E.

11.11. Tissue Sharing

(1) Sharing of animal tissues is encouraged because it decreases the overall numbers of animals needed. Tissues may be collected at the time of euthanasia.

(2) A full protocol review is not required to use tissue from dead or dying (during euthanasia) animals.

When an animal is euthanized by the original PI as part of the research procedure, another PI wishing to use tissue from the dead animal should provide the description to the protocol PI directly, who should then include it in his/her annual protocol update.

Investigators who need to collect animal tissues (including blood) regularly from live animals should prepare protocols for this purpose.

Transfer of live animals among approved protocols (with veterinary review, IACUC approval and proper documentation) is acceptable. The sharing of animals is one way of meeting the alternatives concept of reduction of animal numbers and is encouraged by the IACUC, provided such sharing does not violate the restriction against multiple, major survival surgeries on a single animal.

11.12. Training

All personnel using animals are required to be properly trained in the species and procedures they are using before they start any experiments. IACUC and OCHSC offer several topics of training including Protocol writing, Statistics used for sample size calculation, Basic technique in laboratory animals, Introduction to Occupational Health and Safety etc. PI and co-investigators are encouraged to attend these trainings in order to perform the research in laboratory animals properly ethically, and correctly.

All IACUC, OHSC and NUCAR staff are encouraged to participate in the National Meeting/Seminars/Conference involving animal care and use organized by National Research Council of Thailand (NRCT) and Thai Association for Laboratory Animal Science (TALAS) or other related organizations. Researchers and NUCAR staff who work with animals in the animal biosafety laboratory level 2 (ABSL2) are required to do fit test at least one a year and attend the “Biosafety and Biosecurity Training Course”. to increase their ability to recognize and reduce hazard in a containment and comply with Pathogen and Animal Toxin Act. B.E.2558 (2015), polices, and regulations.

PIs and AV or an animal technical specialist are required to present during initial phase of new study to make sure the co-investigator can properly perform techniques in animals especially oral gavage.

11.13. Death-as-Endpoint

Death-as-Endpoint procedures must be scientifically justified. A plan to provide enhanced care to moribund animals must be included. The plan may include a clinical score sheet developed specifically by the PI for the study. Endpoints are a critical component of every study which must be reviewed by the IACUC. It is ideal when the scientific aims and the study objectives can be accomplished without adverse effects, pain, or distress to the animal. However, this is not always possible and careful consideration must be given to the:

- Scientific requirements of the study;

- Expected and/or possible adverse effects that the research animals may experience (pain, distress, illness, etc.);
- Probable time course and progression of those adverse effects;
- Earliest most predictive indicators of present or impending adverse effects

Where pain or distress is a necessary part of the study, a humane endpoint must be used and approved by the IACUC.

11.14. Studies Involving Wildlife

Detailed descriptions of all capture, handling and chemical restraint procedures, and explanations of their appropriateness are essential with studies involving wildlife. The necessity for these procedures must be clearly established. Criteria used to assess suitability for release must be clearly stated. Provisions for recovery, treatment or euthanasia of injured animals and disposal of carcasses must be specified. If traps are used, the type of trap, its injury potential, and the monitoring frequency of the traps are important considerations. Protocols for field studies involving euthanasia must include justification for the method used.

11.15. Records and Reports

a. The IACUC administrator will maintain all records of IACUC activities.

b. The IACUC administrator will maintain records of approved protocols including approved amendments for a minimum of three years after completion of the protocol.

c. The IACUC administrator will maintain records of the minutes of IACUC meetings. These minutes will include a record of attendance, the title and PI for each protocol voted upon, and a brief summary of all business discussed at the meetings. The IACUC Administrator will maintain the file of the minutes.

d. The IACUC administrator will maintain records of the IACUC's semiannual Facility Inspection and Program Review (FIPR) and recommendations (including minority views). The inspection reports contain a description of the nature and extent of the facility and must distinguish between significant versus minor deficiencies. A significant deficiency is one that in the judgment of the IACUC and the IO is or may be a threat to the health or safety of the animals.

If program or facility deficiencies are noted, the reports must contain a reasonable and specific plan and schedule for correcting each deficiency. Any failure to adhere to the plan and schedule resulting in a significant continuation of the deficiency shall be reported in writing by the IACUC to the IO within 10 days.

e. Reporting suspension of animal care and use activity. The IACUC will report any suspended activity to the IO as soon as possible.

f. The IACUC Administrator maintains IACUC training records on primary investigators and IACUC members.

g. Annual reports

(1) The Center for Animal Research, Naresuan University through the IO submits annual reports to the following:

(a) Fiscal year report on or around 15 December of the current calendar year. AAALAC will send a memorandum requesting the Annual Report covering animal and facility use for previous fiscal year.

(2) The reports assure:

(a) That professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by the institute.

(b) That each PI has considered alternatives to painful procedures.

(c) That the facility is adhering to the *Ethical Principles* and the *Guide*.

11.16. Whistleblower policy

Naresuan university encourages the reporting of concerns related to animal welfare by issuing the Whistleblower policy. This policy provides the mechanisms to report concerns regarding animal welfare or concerns about the

specific use of animals at or in association with Naresuan University. Any individual who has concerns related to the use of animals in teaching, research, or outreach in the University either at NUCAR or the laboratory outside NUCAR may express those concerns without fear of reprisal. Any individual may report concerns directly by calling the number or emailing to the IACUC email addresses provided in the Whistleblower poster. Reports can be handled confidentially and may be submitted anonymously. All reports will be reviewed by the committee and the conclusion will be submitted to the IO. No employee or student of the University will be subject to reprisal for reporting suspected violations or animal welfare concerns.

Kornkanok Ingkaninan

Associate Professor Dr.Kornkanok Ingkaninan

Vice President for Research and Innovation Development

Institutional Official (IO)

25/02/2023