

Animal Welfare and Protocol Writing

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NRCT

Ethical Principles and Guidelines for the Use of Animals



Mahanakorn University



Faculty /Institute



Principal Investigator



IACUC Review and Approval



Dean Approval

The Research Proposal

- Before a research program can begin, the scientist must write a detailed outline of the proposed research.
- The document, referred to as a **research proposal**, explains the specific aims and expected results of the research and describes what methods will be used to accomplish these aims.

The Animal Use Protocol

- If the scientist plans to use animals as part of the research, he or she must explain in a separate written document, **called an animal use protocol**, **Why** animals are needed to accomplish the aims, **What** procedures will be performed on the animals and **How** the animals will be housed and cared for throughout the project.

Protocol Review

Benefit MUST outweigh cost

- * Human health
- * Animal health
- * Advancement of knowledge
- * Scientific gain
- * Good of society



- * Animal use
- * Pain
- * Distress
- * Number of animals
- * Species

Protocol Approval

- Protocol approval before any use of animals begins
- Animal protocol – Follow standard form:

“MHKU Animal Care and Use Protocol”

Protocol Review

- Ensures that the animal research is being performed in an **ethical manner**.
- Ensures that the animal research is performed according to the **highest standard**.
- Ensures that animals are not **subjected to unnecessary pain and distress**.

Protocol Review

- Scientifically sound
 - Objective / hypothesis
 - Statistical consultation
 - Study design
- Animals
 - Appropriate species
 - Appropriate number
 - Appropriate housing/care
- Personnel appropriately trained to perform animal manipulations

Protocol Amendment

PI vs IACUC

- Significant change
 - Objective
 - Species or number of animals
 - Degree of invasiveness
 - Switch from nonsurvival to survival surgery
 - Anesthetic or analgesic agents
 - Methods of euthanasia

Protocol Topics

- Non-technical summary
- Rationale and literature review
- Literature search for duplication
- Objective (s)
- Experimental design
- Data analysis and statistical method
- Animal model and species justification
- Animal care
- Animal welfare
- Surgery
- Blood or body fluid withdrawal/tissue collection/injections, tail clip, gavaging
- Restraint with mechanical devices
- Project involving food and water deprivation, or dietary manipulation
- Tumor and disease models, toxicity testing
- Behavioral studies
- Euthanasia / Disposition of animals
- Study endpoint
- Biohazard/safety
- Qualification of personnel

Protocol Writing

Protocol Title

This is an animal protocol, therefore it must have **animal listed in the title**

*** Effect of Antigen A on Vaccine Efficacy.***



*** Effect of Antigen A on Vaccine Efficacy **in Mice**.***



Protocol Writing

Non-technical summary

Provide a brief description of the project expressing its significance and needs for undertaking the study

**** use common language and define all abbreviation which animal will be used and easily understood by non-scientists.****

- Brief narrative description
- Describe simply the reason for the study, which animals will be used, and why.
- State what will be done with animals : Provide brief description of the experimental design
- Tie scope of work into possible human or animal health benefit

Protocol Writing

Rationale and literature review (Background)

Provide a brief description of the project expressing its significance and needs for undertaking the study

- All acronyms must be spelled out first.
- Any statement of discovered fact should be referenced.
- Long, moderate or short to highly technical, aimed at the scientific audience.
- Bring the reader to a jumping off point → The next step

Protocol Writing

Literature search for Duplication

To be performed to prevent unnecessary duplication of previous experiments.

- Literature Source(s) Search: Generally performed by Librarian
Date of Search: Perform no earlier than six months prior to the IACUC meeting.
- Period of Search: Provided by librarian
- Key Words of Search: hit, animals species used, agent type.

Protocol Writing

Literature searches for Duplication

- BRD (Biomedical Research Database)
<http://www.scitechweb.com/acau/brd/>
- FEDRIP (Federal Research in Progress)
<http://grc.ntis.gov/index.html>
- CRISP (Computer Retrieval of Information of Scientific Projects)
<http://crisp.cit.nih.gov/>

Protocol Writing

Literature search for Duplication

Result of Search

Provide a narrative description of the results of the literature search.

Show that

1. There are no applicable non-animal alternative
2. There are not unnecessary duplicating previous experiments.

It is up to the IACUC to see that adequate information is provided.

Protocol Writing

Objectives/Hypothesis

State the objective of this protocol to be accepted or rejected.

- non-technical terms
- Type a full sentence:
“ The objective of this protocol is to determine or develop.....”
- More than one objective is acceptable.
The objective is to:
 - 1) determine.....
 - 2) test the vaccine.....etc
- Number the objectives for clarity.

Protocol Writing

Materials and Methods/Experimental design

What will happen to the animals?

- complete description of the proposed use of animals, all necessary information needs to be included here
- clearly description of the numbers of animals and their distribution
- Identify all groups in the design
 - * Include control and experimental groups
 - * Number of animals per group
 - * Number of iterations of testing/sampling/ injections
- outline the formal scientific plan and direction for experimentation
- describe the experiment design of sequential studies
- for more than one species used, make sure it is clear which procedures will be performed on which species

With Humane Standards

Protocol Writing

Materials and Methods/Experimental design

- Flow charts, time lines and tables are very useful
- Describe any non-surgical manipulations: Injections, scans (x-rays), sample collection, Route, volumes, frequency included
- Outline All Surgical manipulations
 - * Describe surgical manipulations including site preparation, surgical approach, and unique techniques for each surgical procedure
 - * Provide information on Pre- and post operative care
 - * Anesthesia and Analgesia
 - * Intra-operative monitoring
 - * Wound Closure and suture removal
- Conclude with the study endpoint and the total number of animals per species required for this particular experiment.
- The universal study endpoint is always “euthanasia when moribund or survival to X days following exposure”.

Protocol Writing

Justification for the Use of Animals

How will the proposed use of animals benefit human or animal health?

- Good representative of the human disease; physiological and/or morphological.
- There is a wide body of knowledge regarding the model.
- Used in the previous protocol and need to complete.
- Look for how sensitive this model is to the agent.
- Alternative to another animal

Protocol Writing

Justification for the Use of Animals

How will the proposed use of animals benefit human or animal health?

- Disease
 - cause
 - currently therapy
 - how proposed animal experiments might better
- Simple language
- Explain the medical terms

Protocol Writing

Justification for animal species

- Describe the characteristic of the animal that make appropriate for the study.
- Use the least sentient species
 - Apes (chimps, orangutans, gorillas)
 - Monkeys (baboons, rhesus monkeys, marmosets)
 - Large animals (dogs, cats, pigs, goats)
 - Rabbits
 - Rodents (guinea-pigs, hamsters, rats, mice)
 - Non-mammalian vertebrates (poultry, amphibians, reptiles, fish)
 - Invertebrates (crustaceans, slugs)
 - Smaller life forms (insects, arachnids, worms)
 - Single cell organisms (yeast, bacteria etc.)

Protocol Writing

Justification for animal species

- Describe the characteristic of the animal that make appropriate for the study.
- Use scientific name “Genus and Species
- Age, application for some species like rodents.
- Weight, especially application to primate, large rodents and rabbits
- Sex, male or female – may break out the number for each

Protocol Writing

Justification for the number of animals

Determine the right number of animals for obtaining valid results

- Justify group size or experimental repetitions:
 - match exactly those described in experimental design.
 - use statistical analysis
 - consult a biostatistician
 - be include animals for controls or technique development
- For new surgical or other techniques
 - studies on cadavers from other approved protocols
- Pilot experiments to demonstrate feasibility or provide a justification for proceeding with subsequent studies.

Protocol Writing

Animal Care Consideration

Determine whether the living conditions are appropriate for the animals

Routine housing and care conditions.

- study room
- floor space
- cage changing
- feeding
- cage flooring
- group housing
- environment
- watering

Specialized living conditions

- care for surgically altered animals
- special light cycle
- special diets
- using metabolic cage

Protocol Writing

Animal Care Consideration

Determine whether the living conditions are appropriate for the animals

Examples of non-standard...

- High/Low salt diet; high/low fat diet, etc
- Drugs / Compounds (Sprite, alcohol) added to drinking water
- Single housing, metabolic caging

For each non-standard situation...

- Include description of what is added
- Provide reason
- Identify an individual responsible for overseeing the process
- Develop lab book
- Label appropriately

Protocol Writing

Veterinary Care Provision

- Routine
 - daily observation or more frequently and by whom
 - indicate what will happen if the animal becomes ill during the study and requires supportive therapy
 - if the animal will be euthanized and by whom
 - justification for not providing supportive care for clinically ill animals
- Emergency
 - during recovery after surgical
 - fighting
 - etc.

Protocol Writing

Animal Welfare

Replacement, Reduction and Refinement.

Replacement with non-animal techniques

Not common but should be considered

- organ, tissue or cell culture > incubators for cell lines
- immunologic bench assays > bioassays involving animals
- computer simulations > model the pharmacokinetics
- *invitro* techniques > growing monoclonal antibodies

Protocol Writing

Animal Welfare

Replacement, Reduction and Refinement.

Reduction

- use the correct model
- minimum group size as needed to obtain statistically significant data
- shared control groups/ share tissue
- use newer instrument that improves precision
- preliminary screening in non-animal systems
- well trained staff and good animal care

Protocol Writing

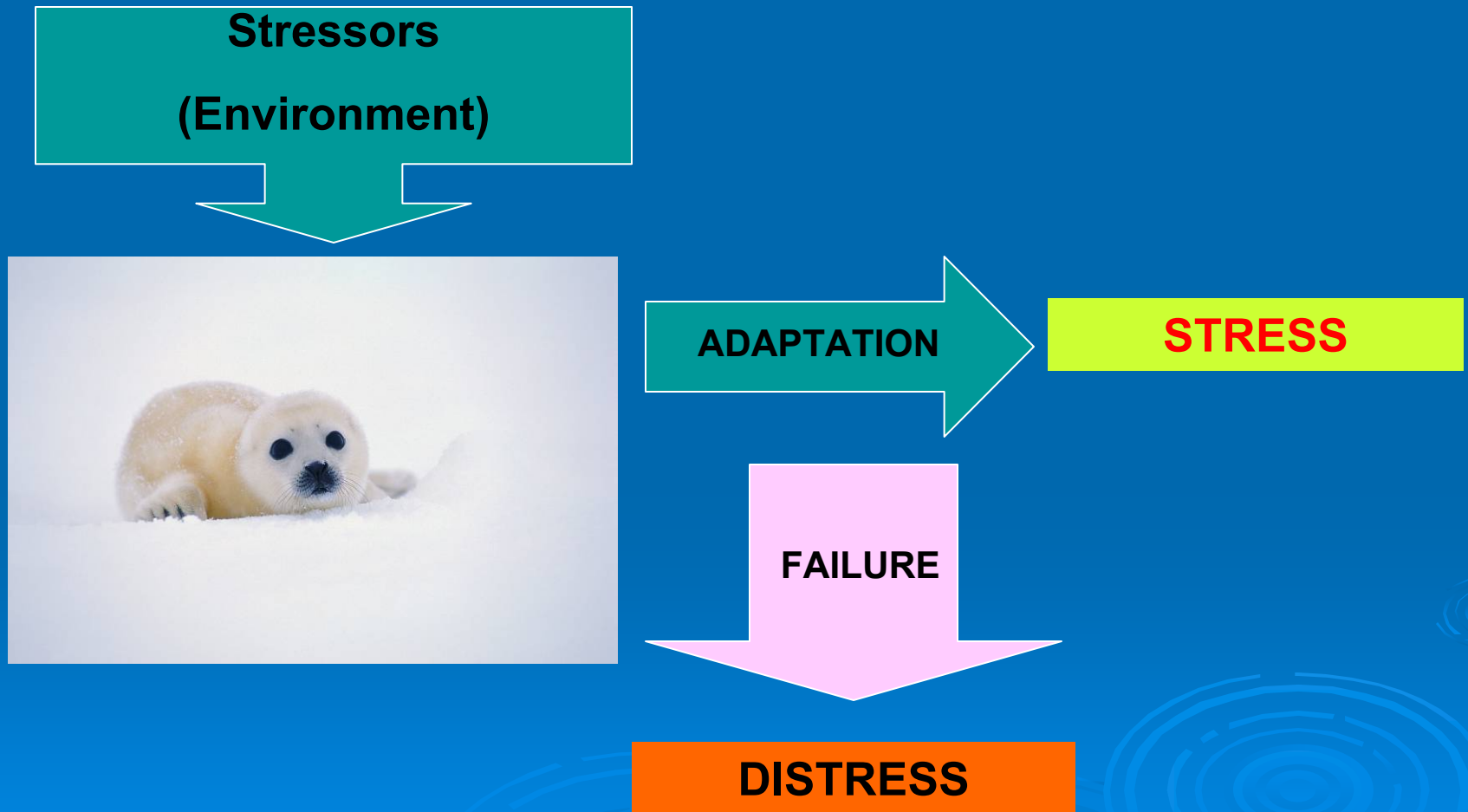
Animal Welfare

Replacement, Reduction and Refinement.

Refinement: changing experiments or procedures to reduce pain or distress

- pilot experiment
- adjust techniques
- skilled technicians
- training
- anesthesia/analgesia
- close observation
- early endpoint

Pain and Distress



Protocol Writing

Pain and Distress assessment

No pain : routine procedures e.g. injection, deep palpation, blood collection from vein, observation studies of animal behavior, tissue collection after euthanasia

Alleviated pain : appropriate anesthetics or analgesics will be administered to avoid or alleviate pain e.g. surgery, tattooing, small tumor removal

Unalleviated pain : animals are subjected to painful procedures without the use of anesthetics, analgesics, or tranquilizers e.g. lethal dose studies, pain studies

Protocol Writing

Observe signs of pain

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 unkempt appearance

Guarding (protecting the painful area)

Loss of mobility

Red stain around the eyes of rats

etc.

Who will observe the animals? How often? Criteria?

Protocol Writing

Anesthesia and Analgesia

- Pre-anesthetic fasting
- Duration of anesthesia
- Agents, dose, routes, and site
- Indicate needle size
- Methods of monitoring and care during anesthetic recovery
- Analgesic (pre-emptive and post procedure provision)

Who will be administering the analgesics, anesthetics during the study ?

Protocol Writing

Surgery

Survival surgery: the animal regains consciousness after anesthesia.

- aseptic techniques
- properly prepare the incision site
- clip the hair and disinfect the skin

Non-survival surgery: the animal is euthanized while under anesthesia and does not regain consciousness

- minimum the surgeon should wear gloves
- clip the surgical site
- instruments and work area should be cleaned

Protocol Writing

Surgery

- Major surgery:**
- Surgery that penetrates and expose a body cavity, such as the chest or abdomen after anesthesia.
 - Surgery that produces substantial physical or physiological impairment

Minor surgery: the less invasive surgery

Multiple major survival surgeries:

- Scientific justification
- Conservation justification
- Medical justification

Protocol Writing

Facilities for Aseptic Surgery

Major survival operative procedures on non-rodent species

- conducted only in dedicated facilities intended for that purpose and under aseptic conditions
- separate areas for surgical support, animal preparation, surgeon preparation, operating room, and animal recovery.

Non-major operative procedures and all rodents surgery

- do not require a dedicated facility but must be using aseptic technique

Protocol Writing

Surgical methods

- Aseptic techniques
- Surgical approach
- Suturing
- Peri-operative care and monitoring
- Analgesia
(pre-emptive and postoperative)

Protocol Writing

Post-Operative Procedures

- Post operative care
 - *By whom? How frequency?
 - * Weekends, holidays, after hours care and monitoring
- How will pain and distress be monitored?
- How will pain and distress be evaluated?

Protocol Writing

Prolong Restraint

Prolong restraint should be avoided unless it is essential for achieving research objectives

- Restraint devices are not normal methods.
- Restraint devices should not be used for convenience.
- Period of restraint should be minimum.
- Training the animals
- Provide veterinary care

Protocol Writing

Restrictions of Food and Water

- The reason for restricting food or water
- The period and frequency of food or water deprivation
- The procedure for monitoring the animals

In prolonged deprivation, be included

- Physiological or behavioral parameters used in monitoring the animals.
- Criteria for temporary or permanent removal of an animal from the study.

Protocol Writing

Endpoint Criteria

The criteria used to intervene in research studies to prevent unnecessary pain and distress

For early removal from study...

- A limit on weight loss 20-25 %
- Extend anorexia over 3 days
- Sudden pain or distress that cannot be controlled with analgesics, sedatives or tranquilizers
- Severe medical conditions that cannot be controlled with appropriate therapy
- Maximum tumor volumes or tumor weight

Protocol Writing

Euthanasia and Disposition of Animals

Gentle or Easy death

To kill an animal with the minimum of physical
and mental suffering

- painless
- rapid and complete
 - peaceful
 - safe for the operator, observer
 - simple to perform

2000 Report of the AVMA Panel on Euthanasia

Protocol Writing

Euthanasia and Disposition of Animals

- State the method of euthanasia (according to SOP).
- Giving needle size and syringe size range, if applicable.
- State how death will be ensure when euthanizing rodents with CO₂.
- State who will perform the euthanasia DVM personnel, PI and staff (by name)

AVMA Panel on Euthanasia

Animal Manipulations

Injections

- List all types of injection and the procedure
- Provide detail on needle and syringe size.

Biosamples

- All blood, urine, feces, tissue etc sampling from living animals should be annotated.
- For blood, list the volume to be taken, the frequency and how.

Table: Site of injection, maximum normally accepted volume and needle size

Species	Oral	Subcutaneous	Intramuscular	Intraperitoneal	Intravenous
Mouse (20 g.)	0.5 ml	Scruff, 0.5 ml/site 25 G	Quadriceps/posterior thigh, 0.01 ml/site, 25G	1.0 ml, 25G	Lateral tail vein, 0.2 ml, 25G
Rat (200-250g.)	2.0 ml	Scruff, back, 0.5ml/site <23G	Quadriceps/posterior thigh, 0.1 ml/site, <23G	5.0 ml, <23G	Lateral tail vein, Sublingual vein , penile vein
Guinea pig (300-400g.)	5.0 ml	Scruff, back, 1.0 ml/site <21G	Quadriceps/posterior thigh, 0.1 ml/site, <21G	10-15 ml, <21 G	Ear vein, saphenous vein, dorsal penile vein, 0.5 ml, <23G
Rabbit (2-2.5 kg.)	7.5 ml	Scruff, flank, 1.5 ml./site <21G	Quadriceps/posterior thigh, lumbar muscles, 0.2ml/site <21G	20ml, < 21G	Marginal ear vein, 10 ml, (slowly), <21G

Practicable volumes of blood to take from various animals.

Blood volume (ml)	MOUSE	RAT	GUINEA PIG	RABBIT
Total blood Volume (ml/kg.)	80	50	75	70
Available volume When bled out (ml/kg.)	25	20	35	35
Maximum safe Volume at one bleeding (ml/kg.)	7	5	7	7

BIOHAZARD/SAFETY

- Potential occupational health and safety issues?
- Any hazardous agents?
- How they will be handled?



Training

- To understand the potential hazards.
 - From animals
 - The use of specific agents
- Safety practices to minimize the risk of exposure.
- The Available Health Care Service.



Personnel Qualification and Training

Name	Qualification and Training	Procedures
A	BSc Trained... on...	Animal handling. IV Injections. Euthanasia.
B	PhD Trained... on...	Catheter Placement. Splenectomy.