THE RODENT SEMINAR SERIES

presented by the Research Animal Resources (RAR) and the JHU Animal Care and Use Committee (ACUC) Office

Outline

- Definitions
- Importance & benefits of humane endpoints
  - Regulatory, ethical, and scientific
- Developing appropriate humane endpoints
  - Predetermined, study-specific, precise, objective
- Implementing humane endpoints
  - Assessment of animal pain and distress
  - Criteria for timely intervention
- Case studies

Definitions

- **Experimental endpoint**: a point at which the scientific aims and objectives of the study have been reached
  - Expected timepoint for final data collection
  - Pre-determined prior to the start of the study
  - Every protocol should include experimental endpoints that are both humane and scientifically sound
  - Example:
    - Day 0 — give drug; day 30 — euthanize for tissue collection

Definitions

- **Humane endpoint**: a point at which an experimental animal's pain and/or distress is prevented, terminated, or relieved even if the animal has not reached its defined experimental endpoint
  - Does not always mean euthanasia; can mean terminating painful procedure, giving treatment to alleviate pain or distress, or short-term vs. permanent removal from the study
  - When possible to anticipate, include in protocol and define prior to the study
  - Examples:
    - Animal is in respiratory distress following surgery

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Humane endpoints should be implemented when...

- Scientific results will no longer be valid
- Suffering outweighs experimental benefits of survival
- Suffering has exceeded a humane limit regardless of benefit
- Surrogate endpoints can be employed to prevent pain or death

If applied incorrectly, endpoints could lead to premature decisions and inaccurate data, resulting in waste of animal life.

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Definitions

- **Pain**: complex sensory and emotional experience associated with actual or potential tissue damage\(^1,3\)
  - Result in withdrawal or evasive action towards the stimulus
- **Stress response**: an adaptive biological response to a disturbance in physiological homeostasis or psychological well-being\(^2\)
- **Distress**: an aversive state where the animal has failed to adjust to stressors, and coping mechanisms have failed to re-establish homeostasis\(^2,3\)

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“Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative. Unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals.”

- Guide for the Care and Use of Laboratory Animals (2011)
Importance & Benefits of Humane Endpoints

- Regulatory Imperative
  - Compliance with laws and regulations
- Ethical Imperative
  - Animal welfare
- Scientific Imperative
  - Experimental design and results

Ethical Imperative

- All animal research has ethical costs
- Obligation to treat animals humanely
- Enhance animal welfare and well-being
- Prevent unnecessary pain and distress
- Ensure that animal use is justified
- Potential scientific value must outweigh the ethical costs of research

Regulatory Imperative

“Using animals in research is a privilege granted by society to the research community with the expectation that such use will provide either significant new knowledge or lead to improvement in human and/or animal well-being. It is a trust that mandates responsible and humane care and use of these animals.”
- Guide for the Care and Use of Laboratory Animals (2011)

The Three R’s

- Replacement: methods that avoid using animals
  - Non-animal models or use of a phylogenetically lower animal model
  - Examples:
    - Using a zebrafish instead of a mouse
    - Using cell culture instead of a live animal model
    - Using a computer model instead of an animal
The Three R’s

- **Refinement**: modifications of husbandry or experimental procedures to enhance animal well-being and minimize or eliminate pain and distress
  - Examples:
    - Training a mouse to take a drug delivered on a gel pellet instead of gavaging
    - Holding a mouse on an open hand vs. scruffing

Scientific Imperative

- **Risks of failing to define & implement humane endpoints**:
  - Animals may die, resulting in loss of data (e.g. tissues unusable)
  - Agonal sampling may yield disparate data
  - Confounder for research results

- **Benefits of defining & implementing humane endpoints**:
  - More efficient and effective sample collection
  - More uniform subjects = more uniform data
  - Improved statistics

The Three R’s

- **Reduction**: strategies for obtaining comparable levels of information from the use of fewer animals
  - Examples:
    - Researchers sharing information so that efforts are not duplicated
    - Using imaging to assess the same animal at multiple timepoints rather than using histopathology of multiple animals at various timepoints
    - Does not include animal reuse!

Importance of the ACUC Protocol

- Affects IACUC’s ability to assess whether proposed endpoints are appropriate
- Conveys important information about:
  - Animal model and anticipated phenotype
  - Study goals
  - Anticipated complications, based on prior literature, if any
  - Precise definitions of humane endpoint
  - Criteria by which animal will be assessed
Developing Humane Endpoints

- “What is happening to the animal?”
  - Utilize prior literature about your model
  - What response do we expect?
    - What part of that response is necessary for the model?
  - What specific complications can we anticipate?
  - How do we assess for abnormalities?
  - Example: if we are placing a cranial implant, we have to consider what would happen if the implant becomes dislodged – does the animal have a revision surgery, or will the animal be euthanized?

Examples of Humane Endpoints

- “What do we know about the animal?”
  - Biology of the species, breed, strain/stock, genotype
  - Individual health status
  - Scientific literature

Developing Humane Endpoints

- Defined ahead of the study, if possible
  - Described clearly in the protocol
  - Reviewed and approved by the ACUC
- Can include unforeseen complications
  - Update the ACUC if new endpoints are developed – may require amendment
  - Communication of established endpoints to all individuals involved in animal work
  - Periodic re-evaluation and refinement
  - Collaboration between PI, ACUC, and veterinarian

Diagrams:
- ACUC
- PI & lab members
- Vets, vet techs, & care staff

- Head tilt
- Tumor exceeding 1 cm and impairing mobility
- SQ abscess refractory to treatment
- Fight wounds
Defining Humane Endpoints in the ACUC Protocol

- Terms should be well-defined, precise, study-specific
  - Provides the ACUC with a clear idea of the expected clinical presentation of the animals
  - Ensures consistency between observers assessing the animals (lab members, care staff, veterinarians)

“Humane endpoints should be well-defined, precise, study-specific, measurable, and objective.”

How could the description of this endpoint be improved?

“This study involves nerve injury to the limb. Neuropathic pain can result in self-mutilation. Because this study evaluates pain, analgesics are contraindicated. Rat will be assessed daily. Any small (<3 mm) and superficial skin wounds to the affected limb will be closely monitored; if no other clinical signs of pain (disuse of the limb, hunching, lethargy, decreased BCS) are observed, no treatment will be implemented. Rats will be euthanized if they engage in self-mutilation that results in large (>3 mm) or deep (muscle or bone exposure) wounds, if wounds become necrotic or infected (purulent discharge), or if signs of systemic illness or pain (described above) are observed. RAR veterinarians will be consulted on wound management if indicated.”

ACUC protocol scenario

What are potential shortcomings of the way this humane endpoint is written?

“This study involves nerve injury to the limb. Rats will be euthanized if they engage in self-mutilation.”

- Wide spectrum of clinical signs and severity that would qualify as self-mutilation.
- Does not state how often animals will be observed.
- Could result in early implementation of endpoint (euthanasia) and loss of study data.
- Does not specify why there are no alternatives to euthanasia for this animal (such as pain medication).

ACUC protocol scenario

What are potential shortcomings of the way this humane endpoint is written?

“Chinchillas treated with the drug may exhibit some neurologic signs; chinchillas with neurologic signs will not be euthanized unless they exhibit failure to thrive.”

- Wide variety of clinical signs and severity that qualify as neurologic signs.
- Some neurologic signs may impact ability to perform basic functions like eat or drink, so the need to keep an animal in that condition would need to be justified.
- Non-specific definition of “failure to thrive”.
- Could negatively impact animal welfare.
How could the description of this endpoint be improved?

“Chinchillas treated with the drug may exhibit some neurologic signs expected as a side effect of the drug (head tilt, mild nystagmus, or mild ataxia). Chinchillas will be assessed daily; if they are able to eat, drink, and move about the cage without impaired mobility, no treatment will be implemented. Chinchillas that exhibit more severe neurologic signs (incoordination that prevents walking; severe head tilt and rolling that prevents chinchilla from eating or drinking) or signs of distress (hunched posture, lethargy, squinting, poor hair coat or body weight loss >20% from baseline, will be euthanized.”

Assessment of Pain and Distress

- Know the baseline for your animals
- Components of evaluation:
  - View from a distance
  - Slowly approach and observe without handling
  - Hands-on examination
    - Physical examination
    - Veterinary consult and diagnostic workup
- Subjective vs. objective measures

Unexpected Outcomes

- Anticipate
  - Literature search – similar models in the same species, same model in a similar species
- Monitor
  - Lab members identify clinical concerns
- Consult
  - Investigators and veterinarians evaluate any unexpected outcomes
  - Consult as needed with veterinarian to manage individual cases
- Revise
  - If certain complications continue to occur, specific humane endpoints should be defined in the protocol.
  - Contact the ACUC if you are not sure whether something requires an amendment!

Signs of Pain in Rodents

- Lethargy, rapid or labored breathing, anorexia
- Lack of grooming pericollar and nasal papillae thinning
- Hunching, immobility, squinting, piloerection, weight loss
How can we be more objective?

- Vital parameters (TPR)
- Objective measurements – tumor size
- Bloodwork (CBC, chemistry)
- Body weight (% of baseline)
- Grimace scales and other pain scores
- Behavioral coding

How can we be more objective?

- Body condition score (BCS)

  **BC 1**
  - Mouse is emaciated.
  - Skull/parenychmal structures extremely prominent.
  - Tail or no tail present.
  - Vertebral column distinctly segmented.

  **BC 2**
  - Mouse is underconditioned.
  - Subcutaneous fat and more normal collateral muscle present.
  - Distal periosteal bone is easily palpable.

  **BC 3**
  - Mouse is well-conditioned.
  - Girth and muscle tone are appropriate.
  - Subcutaneous fat can be palpated with slight pressure.

  **BC 4**
  - Mouse is obese.
  - Girth and subcutaneous fat are prominent.
  - Visceral features/palpable organ masses.

  **BC 5**
  - Mouse is excessively obese.
  - Visceral features/palpable organ masses.

Tumors

- Allowable size varies by species
- ACUC tumor guidelines
- Tumor characteristics
  - Ulcerated, necrotic, infected
  - Tumor location
    - Impeding limb movement?
    - Impeding physiologic function?
  - Ocular tumors
  - Genital tumors
  - Visceral tumors
- Protocol-specific considerations – neoplasia models
  - Size, clinical complications, and criteria for intervention be specified in the protocol

Johns Hopkins University
Animal Care and Use Committee (ACUC)

**Purpose**

This document was developed to assist researchers in establishing criteria to ensure the welfare of animals involved in induced or spontaneous tumor studies.

**Guidelines**

- General: All studies in which mice or rats with tumors experimentally induced or hormonally induced tumors may be performed by approved staff. Clinical monitoring/study must cease once the tumor burden has reached the maximum size, according to the criteria below. Criteria for intervention and/or early termination must also be included. These details must also be included for tumors that are predicted to occur spontaneously in specific strains of rodents or genetically engineered rodents.

- Tumor size: The maximum allowable tumor size for a single spontaneous or implanted tumor that is visible without magnification is 3 cm in any dimension. The maximum size for a single tumor is determined by the maximum burden of a single tumor. Greater single- or combined tumor burdens may be approved by the ACUC with sufficient scientific justification. The maximum size that is a tumor can grow at location within the breast, thoracic cavity, or behind the eye that are monitored through imaging is more limited. Tumors at these locations may interfere with vital functions of the animal and result in morbidity or mortality even though the size may be much less than cited above.
Clinical Scoring Systems

**Experimental Allergic Encephalomyelitis (EAE)**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Intervention</th>
<th>Age of onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal, no signs of neurological deficits</td>
<td>No intervention</td>
<td>1-2 days</td>
</tr>
<tr>
<td>2</td>
<td>Flaccid paralysis of the tail; partial or no tail movement; no signs of neurological deficits</td>
<td>Remove mouse house</td>
<td>1-2 days</td>
</tr>
<tr>
<td>3</td>
<td>Severe hind limb paralysis; hind limb pals, weak or weakly walking, inability to bear weight</td>
<td>Remove mouse house</td>
<td>1-2 days</td>
</tr>
<tr>
<td>4</td>
<td>Severe hind limb paralysis; hind limb pals, weak or weakly walking, inability to bear weight</td>
<td>Remove mouse house</td>
<td>1-2 days</td>
</tr>
</tbody>
</table>

*Updated: Model should be terminated once any 34 hours or approximately the same time over 24 hours.*

*Pups vector and maintained should be removed daily and replaced with fresh food.

**Mark your Category E Cages!**

- Some protocols have animals in many different categories.
- Veterinary staff need to be able to identify the approved endpoint for a given animal.
  - Avoid premature euthanasia of animals and loss of data.
- We will soon have stickers available in animal rooms to allow easy marking of Cat. E cages. In the meantime, please write on the cage cards (e.g., "endpoint = death"). Although this takes additional time, it is very beneficial to research and clinical evaluation.

**Category E Protocols**

- Unalleviated pain or distress
  - Pain without administration of analgesia
  - Moribund: severely debilitated and preceding imminent death
  - Death
- Justification:
  - Scientific rationale and justified animal numbers
  - What alternatives have been considered and why not suitable?
  - Literature search demonstrating no alternatives, or written description of experience in the field
- Strict monitoring plan
- Veterinary pre-approval review for Cat. D and Cat. E

**Coming Soon:**

**Expected Experimental Outcomes Log**

- We will be implementing this log in animal rooms to more easily disseminate information about the expected experimental outcomes for different models, how those cases are to be managed by the lab per the protocol, and to identify category E protocols.

<table>
<thead>
<tr>
<th>Expected Experimental Outcomes, Phenotypes, and Endpoints</th>
<th>Severe Endpoints (should occur in Cat. E)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Information</td>
<td>EAE</td>
</tr>
<tr>
<td>Exploitable Outcome &amp; Phenotypes</td>
<td>EAE</td>
</tr>
<tr>
<td>Protocol</td>
<td>EAE Protocol</td>
</tr>
<tr>
<td>Description</td>
<td>Clinical Score</td>
</tr>
<tr>
<td>Measurement</td>
<td>EAE</td>
</tr>
</tbody>
</table>

| R | RA238692 | 1.3 | Model of EAE syndrome; expect mice to develop neurological signs (stupor, ataxia) | Lab to provide hydrogel, feed on cage floor | Yes |
Non-Research Related Endpoints

- Perineal prolapse
- Ocular mass
- Necrotic/malignant rectal prolapse
- Spontaneous tumors
- Ulcerative dermatitis refractory to treatment

How you can facilitate an effective clinical call process!

- Know what is “normal” for your animals
  - Baseline
  - Expected experimental outcomes
- Assess your animals frequently to detect clinical calls
- Communicate with vet staff about clinical concerns
- Monitor and treat animals
- Keep good records – including orange clinical call card & log!
- Keep cage cards updated with emergency phone numbers!
- Responsible person must be reachable at all times – after hours, holidays, weekends – and available to come to facility to assess, treat, or euthanize animals

Non-Research Related Endpoints

- Clinical cases - will vary based on age, strain, genotype, etc.
- Management and endpoints determined in consultation with veterinarian
  - Endpoints do not need to be specifically described in the protocol

SToP Form

- Special Treatments or Procedures (SToP) for rodents
- Utilized to implement conditions that differ from standard husbandry
- Form submitted to supervisors
- Monthly monitoring sheet used by lab when necessary
- Examples of procedures for which SToP Form can be utilized:
  - 1) Procedures that require ACUC approval
    - Delayed weaning
    - Food/water restriction
    - Single-housing
  - 2) Procedures that require RAR approval
    - Feeding on cage floor
    - Lab performing husbandry
- See RAR website for more information!
Case Study 1

Researchers contacted veterinarians when they noted unanticipated complications with the animal model.

- Scenario: lab received ACUC approval for an amendment to perform gut injections in mice, but noted premature and unanticipated mortality after the initial procedures
- Lab members did not have much experience with this technique
- Lab contacted veterinary team for assistance

What is the next step?

Busuttil RA et al. 2018

Case Study 1

What topics would you discuss with the vet?

- Why are the mice dying?
  - Gut perforation?
  - Anesthetic complication?
  - Complication with study drug?
- How can we improve survival?
  - Can I give any treatments to prevent death?
  - Should I modify the surgical procedure?
- What new endpoints should we implement?
  - Do I need an amendment?

Case Study 1

What topics would you discuss with the vet?

- What signs are you observing in the mice prior to death?
  - How soon after surgery are you observing these signs?
- What is the experimental drug?
- What is your anesthesia protocol?
- Do the animals receive any pain medication?
- How is the surgical procedure being performed?
  - Can I observe the procedure?
**Case Study 1**

- Using the clinical signs that mice were exhibiting prior to death, veterinarian and lab members determined humane endpoints for these mice
  - Hunched posture, lethargy, abdominal distension
  - Animals were euthanized prior to death and submitted for necropsy
  - Necropsy identified gut perforation

**Case Study 2**

- Scenario: caretaker notes a large mass on the neck of a mouse and places a clinical call

**Consultation between vet and lab member resulted in identification of humane endpoints.**

**Implementation of humane endpoints prevented animals from being found dead.**

**Necropsy on euthanized animals, rather than animals that are found dead, is more diagnostic.**

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**Case Study 1**

- Lab members consulted with another investigator for suggestions on performing the procedure based on their experience
- Veterinarian observed lab performing anesthesia and surgery
- Veterinarian instituted further surgical training using animals from euthanase racks
- Lab contacted ACUC to amend the existing protocol to reflect changes in surgical technique
  - ACUC provided expedited review
- Improved survival for study animals
  - Benefits animal welfare and scientific results

**Consulting with experienced labs benefits animal welfare and reduces animal use.**

**Training provided by RAB resulted in improvement of surgical technique and better outcome for animals.**

**Timely amendment of ACUC protocols is necessary.**

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**Case Study 2**

- Vet assesses mouse. Mass is firm and approximately 1.3 cm in diameter
- Vet checks the protocol; tumors are not an expected part of the model
- Vet contacts lab to inform them of clinical concern.

Care staff will place clinical calls if they have concerns about an animal.

Older animals have additional considerations for age-related humane endpoints.
What topics would you discuss with the vet?

- What is this mass?
- Will this mass interfere with my study endpoint?
- What should we do to manage this animal?
- Is this a humane endpoint?
- Do I need an amendment?

Case Study 2

- Vet recommends surgical resection of the mass before it gets larger and becomes more difficult to remove.
- Lab performs post-operative monitoring of mouse and vet performs weekly rechecks until incision is healed.
- Mouse survives to desired endpoint.

Summary

1. Scientific, regulatory, and ethical imperatives to develop appropriate humane endpoints
2. Endpoints need to be well-defined and study-specific
3. Implementation of endpoints needs to be timely
4. Benefits scientific research and animal welfare
5. Modified approaches to special cases
6. Team effort!
**Acknowledgements**

- Dr. Jason Villano
- ACUC members – Jonathan Harrold and Kinta Diven
- Dr. Jessica Plunkard, Dr. Amanda Maxwell, Dr. Caroline Krall, Tina McKim, Alicia Bukowski – photographs and resources

**Thank you!**

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**The ACUC Role**

The Guide mentions “endpoint” 59 times.

- 2nd Paragraph of the overview:
  - “Discussions of the latter include institutional animal care and use committee (IACUC) functions, protocol and Program review, postapproval monitoring (a new section), and considerations such as humane endpoints.”

The following slides highlight how ACUC committee members evaluate criteria relevant to endpoints while keeping in mind: the needs of a study, how results advance knowledge within a given field of research, and the welfare of the animals involved.

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**Protocol Form: Questions 9-12**

- Content:
  - Objective
  - Importance of research
  - Rationale
  - Species selection
  - Purpose:
    - Briefly explain the purpose of a project, its relevance to humans and/or animals, and why a particular species must be utilized.
  - Evaluation:
    - The answers to these questions are not to be cut and pasted from a grant but should be brief and can be understood by a non-specialist.
Protocol Form: Question 13

- Content:
  - Numbers of animals rationale

- Purpose:
  - Explain how many animals (or range of animals) are needed for each experimental condition (e.g., group size) and estimate the total number of animals for the 3 years covered by this protocol (e.g., numbers of groups or experiments).

- Evaluation:
  - We understand that in some cases they are estimates but the rationale should be clear.

Protocol Form: Question 16a

- Content:
  - Planned Endpoint / Euthanasia

- Purpose:
  - State the timepoint or other criterion in the experiment at which euthanasia will occur for each animal or experimental group if the study goes as planned.
  - If this has been provided in 14a, please refer reader back to that section. Here method of euthanasia for 16a

- Evaluation:
  - The information here should be consistent with question 14a. Only the planned endpoints should be stated.
  - An endpoint does not have to be euthanasia. Depending what the experiment involved, an animal can be transferred to and other protocol or adopted out.

Protocol Form: Question 16c

- Content:
  - Criteria for early euthanasia or withdrawal

- Purpose:
  - Give the health conditions and/or criteria under which early euthanasia or withdrawal of an animal from the study will be considered.

- Evaluation:
  - Unexpected conditions that could arise from the experiments, i.e., fast tumor growth or metabolic changes, need to be anticipated here. In addition other indicators of general poor health need to be linked.
  - Defining what 'withdrawal from the study' means should be included.
  - Consideration for consultation with a veterinarian should be included.

Protocol Form: Question 16d

- Content:
  - The method(s) of euthanasia

- Purpose:
  - List physical methods, drug routes and doses
  - Include multiple methods for flexibility when appropriate
  - Describe how death will be verified

- Evaluation:
  - The methods should be age specific.
  - Need to be consistent with the guidelines
Protocol Form: Question 17a

- Content:
  - Address the animals by pain category.

- Purpose:
  - List the number of animals by pain category.

- Evaluation:
  - The numbers in the pain categories are consistent with the use of anesthesia and analgesia in previous questions.

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Protocol Form: Question 17b

***Answer 17b and c below only if any animals fall into Category D or E.***

- Content:
  - List of protocol procedure(s) or other elements that fit the definition of Category D and/or E.

- Purpose:
  - That alternatives are being considered for all category D and E procedures.

- Evaluation:
  - All category D and E procedures described in the protocol are listed.

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Protocol Form: Question 17c

***Answer 17b and c below only if any animals fall into Category D or E.***

- Content:
  - To address alternatives.

- Purpose:
  - Ensure that the PI has properly considered alternatives.

- Evaluation:
  - The PI’s experience and supporting resources are relevant and complete.

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Protocol Form: Question 17c Continued

***Answer 17b and c below only if any animals fall into Category D or E.***

- Content:
  - Literature search.

- Purpose:
  - Another approach for alternatives.

- Evaluation:
  - Are the keywords appropriate?
  - Are the keywords inclusive enough?
Links to Resources

- JHU ACUC Website
  - New / 3rd year renewal protocol form
  - ACUC Guidelines (including tumor guidelines)
- RAR Website
  - SToP Form
- Guide for the Care and Use of Laboratory Animals (8th Ed.) (2011)
- University of Michigan End-Stage Illness Scoring System

References


Next seminar:
Rodent analgesia, anesthesia, and euthanasia
May 25th, Wed, 3-4 PM

Thanks for your attention!

Questions?
Raffle & Prizes