




THE RODENT SEMINAR SERIES

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

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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

Applications & Take Home Messages for investigators and lab members


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Humane endpoints

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4/27/22




Definitions

- Experimental endpoint: a point at which the scientific aims and objectives of the study have been reached¹
 - 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

1. Guide for the Care and Use of Laboratory Animals. 2011.

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Definitions

- **Humane endpoint:** a point at which an experimental animal's pain and/or distress is prevented, terminated, or relieved¹ 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

1. Guide for the Care and Use of Laboratory Animals. 2011.

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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-being²
- **Distress:** an aversive state where the animal has failed to adjust to stressors, and coping mechanisms have failed to re-establish homeostasis^{2,3}

1. International Association for the Study of Pain. 1979.
2. National Research Council Committee of Recognition and Alleviation of Distress in Laboratory Animals. 2008.
3. Guide for the Care and Use of Laboratory Animals. 2011.

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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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*“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)

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Importance & Benefits of Humane Endpoints



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



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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



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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)

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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

1. Guide for the Care and Use of Laboratory Animals, 2011.

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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

1. Guide for the Care and Use of Laboratory Animals, 2011.

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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

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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!

1. Guide for the Care and Use of Laboratory Animals, 2011.

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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

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JOHNS HOPKINS MEDICINE

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?*

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JOHNS HOPKINS MEDICINE

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

A Venn diagram with three overlapping circles. The top-left circle is labeled 'ACUC', the top-right circle is labeled 'PI & lab members', and the bottom circle is labeled 'Vets, vet techs, & care staff'. The central area where all three circles overlap is shaded in light green.

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JOHNS HOPKINS MEDICINE

Developing Humane Endpoints

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

A cartoon illustration showing an ostrich, a snake, and an elephant standing in front of a store sign that reads 'ONE SIZE FITS ALL STORE'. A yellow sign with the word 'SALE' is hanging from the ostrich. The cartoon is signed 'CARL STET'.

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JOHNS HOPKINS MEDICINE

Examples of Humane Endpoints

Head tilt


Tumor exceeding 2 cm and impairing mobility

SQ abscess refractory to treatment

Fight wounds

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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

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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.”

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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)

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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.

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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.”

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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



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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!

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Signs of Pain in Rodents



Lethargy, rapid or labored breathing, anorexia



Lack of grooming; periorbital and nasal porphyrin staining

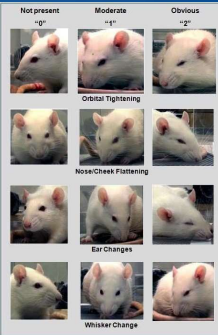


Hunching, immobility, squinting, piloerection, weight loss

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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



Rat Grimace Scale; Sotocinal et al., 2011. 29

Tumors

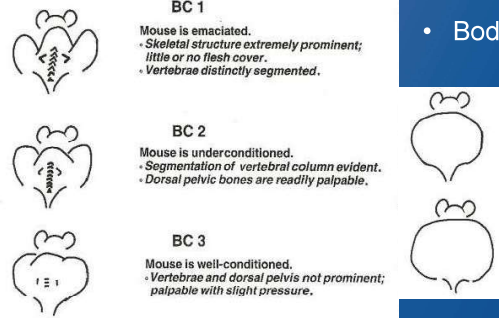
- Allowable size varies by species
 - ACUC tumor guidelines
- Tumor characteristics
 - Ulcerated, necrotic, infected
- Tumor location
 - Impeding limb movement?
 - Impeding physiologic function?
 - Ocular tumors
 - Genital tumors
 - Visceral tumors
- Protocol-specific considerations – neoplasia models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



Tumor impeding limb movement
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How can we be more objective?

- Body condition score (BCS)



BC 1
Mouse is emaciated.
• Skeletal structure extremely prominent; little or no flesh cover.
• Vertebrae distinctly segmented.

BC 2
Mouse is underconditioned.
• Segmentation of vertebral column evident.
• Dorsal pelvic bones are readily palpable.

BC 3
Mouse is well-conditioned.
• Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.

BC 4
Mouse is overconditioned.
• Spine is a continuous column.
• Vertebrae palpable only with firm pressure.

BC 5
Mouse is obese.
• Mouse is smooth and bulky.
• Bone structure disappears under flesh and subcutaneous fat.

Ullman-Culleré & Foltz, 1999. 30

Johns Hopkins University Animal Care and Use Committee (ACUC)

Tumor Study Guidelines: Mice and Rats¹

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 will have experimentally induced or implanted tumors must specify in the protocol the expected size, clinical complications (e.g., ulceration or necrosis), experimental endpoints, and potential metastatic spread or development of ascites. 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 imaging is ~2 cm in any dimension in adult mice and ~4 cm in any dimension in adult rats.² In cases where there are multiple tumors, they should not exceed 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 a tumor can grow at locations within the cranium, 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 animals and result in morbidity or mortality even though the size may be much less than cited above.

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Paralysis grading system and disposition of animals			
	Clinical signs	Monitor freq.	Intervention
Grade 0	Normal, no signs of neurological disease	1x/day	- No intervention
Grade 1	Flaccid paralysis of the tail; partial or no tail muscle tone. Mouse is unable to curl tail around finger or pencil. No significant gait abnormalities	1x/day	- Remove mouse house.
Grade 2	Incomplete hind limb paralysis: Hind limb paresis, weak or wobbling gait, impaired righting reflex.	1x/day	- 4-8 food pellets in bedding - Slice of Napa nectar on cage floor in Petri dish lid - Monitor for progression - Remove mouse house
Grade 3	Bilateral hind limb paresis. Mouse drags its hind limbs over flat surface. Mouse exhibits incontinence.	1x/day	- Same as for grade 2 - Monitor for progression to grade 4 or 5
Grade 4	Quadriparesis/quadriparalysis: Hind and forelimb paralysis. Mouse barely moves around.	2x/day	- Same as grade 2 and - Subcutaneous 5% dextrose saline solution administration (40 ml/kg/24 hours). - Animal to be monitored for four days, to confirm the grade reliably for two consecutive days plus two additional days for monitoring potential amelioration of the disease. - Euthanized by the end of day 4 if no disease regression.
Grade 5	Moribund (paralysis in all 4 limbs and trunk with labored or reduced breathing).		- Immediate euthanasia

* Supportive care should be provided once every 24 hours at approximately the same time each day.
- Napa nectar and moistened food should be removed daily and replaced with fresh.




Clinical Scoring Systems

Experimental Allergic Encephalomyelitis (EAE)

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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.

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Category E Protocols



- Unalleviated pain or distress
 - Pain without administration of analgesia
 - Moribundity: 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

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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.


Expected Experimental Outcomes, Phenotypes, and Endpoints						
Animal Information		Expected Experimental Outcomes and Phenotypes per Approved ACUC Protocol			Severe Endpoints (ACUC-approved Cat. E)	
PI	Protocol	Racks	Description	Management	Moribund=Endpoint?	Death=Endpoint?
PI	RA22MXX	2,3	Model of XYZ Syndrome; expect mice to develop neurologic signs (seizures, ataxia)	Lab to provide hydrogel, feed on cage floor	No	✓

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Non-Research Related Endpoints




Penile prolapse




Ocular mass




Spontaneous tumors



Ulcerative dermatitis refractory to treatment



Severe fight wounds with bone exposure



Necrotic/hemorrhagic rectal prolapse

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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 tx log!
- Keep cage cards updated with **emergency phone number!**
 - Responsible person must be reachable at all times – after hours, holidays, weekends – and available to come to facility to assess, treat, or euthanize animals

Prompt clinical calls improve clinical outcomes, enhancing animal welfare and supporting research

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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



Investigator: [redacted] State: 4/15/22

Contact Person: [redacted] Telephone: [redacted]

Species: Mice Protocol #: [redacted]

Building: [redacted] Room#: [redacted] Rack / Cage / Animal #: [redacted]

Clinical Problem: 2/5 Dermatitis

Notes: 2/15 read up in [redacted] + @
2/15 [redacted] [redacted]
2/15 [redacted] [redacted]

Veterinarian: 4/17 Telephone: [redacted]

Animal Health Assistance


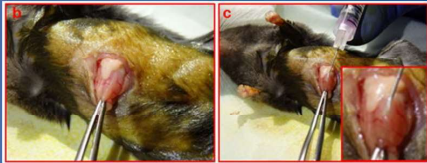
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SToP Form

- 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!

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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

Busuttill RA et al. 2018

What is the next step?

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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?




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Case Study 1



What topics would you discuss with the vet?



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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?

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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


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 2



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


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



Older animals have additional considerations for age-related humane endpoints.

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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 euthanasia 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 RAR 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.



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MEDICINE**

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?



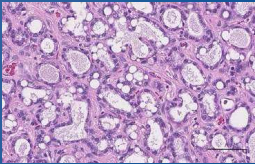
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 **JOHNS HOPKINS
MEDICINE**

Case Study 2


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

Early recognition, reporting, and intervention improves clinical outcomes




Veterinarians can facilitate performing diagnostics to inform decision-making about an animal.

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 **JOHNS HOPKINS
MEDICINE**

What topics would you discuss with the vet?

- Differential diagnoses:
 - Abscess
 - Benign tumor
 - Malignant tumor
 - Cyst
- How long do you need to keep this animal?
 - Endpoint is in 3 months
- Treatment options
 - Surgery?
 - Monitoring?



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 **JOHNS HOPKINS
MEDICINE**

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!

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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 Endpoints: ACUC Office Perspective

Kinta Diven **Jon Harrold**
ACUC Sr. Training & Compliance Specialists

4/27/22

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.

In answering the questions below, please use terminology that will be understood by a non-specialist. Spell out abbreviations on first use.

9. OBJECTIVE(S): Briefly explain the overall purpose of the project.


10. IMPORTANCE OF RESEARCH: What is the relevance of this work for human or animal health, the advancement of knowledge, or the good of society?

11. RATIONALE FOR ANIMAL USE: Why are live animals necessary for this study? Include the reason a non-animal approach such as mathematical models or computer simulation cannot be used.

12. SPECIES SELECTION: Explain why you selected this species as opposed to another.

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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.

13. NUMBERS OF ANIMALS AND RATIONALE: Explain how many animals (or range of animals) are needed for each experimental condition (e.g., sexes, sites) and estimate the total number of animals for the 3 years covered by this protocol (e.g., numbers of groups or experiments). State how you determined that this number per experimental condition is appropriate (e.g., power analysis, previous studies, FDA request, etc.) Use a table showing experimental and control groups if it aids communication. If the total number of animals will not match up with the total for question 17a, please explain.

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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 incision dehiscence, need to be anticipated here. In addition other indicators of general poor health need to be listed.
 - Defining what 'withdrawal from the study' means should be included.
 - Consideration for consultation with a veterinarian should be included.

16c. Give the health conditions and/or criteria under which early euthanasia or withdrawal of an animal from the study will be considered. These include, but are not limited to, general signs of distress such as hunched posture, lethargy, anorexia, dehydration, rough hair coat etc. and/or those that are directly related to the experimental procedures (e.g. tumor ulceration, dislodged/unrepairable headcap, etc.)

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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
- 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.

16a. PLANNED ENDPOINT/EUTHANASIA: 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. Save method of euthanasia for 16d.

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Protocol Form: Question 16d




- Content:
 - The method(s) of euthanasia
- Purpose:
 - To 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

16d. Which method(s) of euthanasia will be used by laboratory personnel? State how death will be verified before disposal (give two methods if euthanasia is other than decapitation). See JHU's Euthanasia Guideline at web.jhu.edu/animalcare for suggested methods.

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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.

17a. PAIN/DISTRESS: Indicate in the table below the number of animals that will fall in each Pain/Distress category. In addressing pain and distress, please consider all aspects of the study (e.g., surgery, phenotype of the animal, induced disease, tumor burden, behavioral procedures). For protocols that involve more than one procedure, place animals in the category that pertains to the greatest degree of pain, distress and/or discomfort to which the animal will be exposed. Do not count an animal more than once. The total number of animals entered must agree with the total animals in question 13 unless the discrepancy is explained there. The total should not include the number in answer to question 4b (euthanasia rack). Use of anesthesia for rodent tail snips or purely for restraint (e.g., for imaging) does not require placement in Category D.

Categories	Examples	Total Number of Animals for 3 Years (Entered into ACUC database at time of protocol approval)
B—Breeding - number of males and females to be used for breeding	e.g., breeding only, no other procedures	
C—Procedures cause momentary, slight, or no pain/distress in absence of analgesia or anesthesia	e.g., injections, euthanasia, blood collection, brief restraint, imaging	
D—Procedures potentially are painful but anesthetics and/or analgesics are given	e.g., surgery, blood collection by invasive routes	
E—Procedures involve pain/distress that will not be alleviated by drugs	e.g., toxicity studies, pain or stress studies, some disease models	

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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.

17c. Are there alternative methods to those named in 17b that could be used that would produce less pain and distress and achieve the same experimental purpose? EITHER state approaches that might seem to be reasonable alternatives to the ones in 17b and explain why they will not accomplish the experimental objective with less pain/distress OR carry out a Keyword/Literature Search. DO NOT DO BOTH

If you choose the first approach, state the reasons you can be confident that you have relevant and up-to-date information on the topic. Reasons could include: 1) consultation with an expert in the research area (give name/qualifications), 2) regular attendance at scientific meetings (names of organizations), 3) regular attention to the scientific literature on the topic (cite sample journal names), and/or 4) personal experience with the alternative method.

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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.

17b. Which procedure(s) or other elements in this protocol fit the definition of Category D and/or E as given in the chart above?

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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?

If you do a Keyword/Literature search provide the following information:

Date (day, month, year) search was performed: _____

Years covered by search: _____

Keywords used in search: These must be in relation to alternatives to the procedure(s) named in 17b, not in relation to use of animals.


Number of hits: _____

Databases searched (check all that apply):
 MEDLINE /PUBMED _____ AWIC _____ TOXLINE _____ AGRICOLA _____ Other (describe) _____

Did the literature search reveal one or more alternatives that cause less pain/distress than the methods proposed but accomplish the same scientific purpose? Yes No If "Yes", explain why the alternative is not being used.

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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](#)

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Next seminar:

Rodent analgesia, anesthesia, and euthanasia

May 25th, Wed, 3-4 PM



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References






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Thanks for your attention!

Questions?

Raffle & Prizes

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