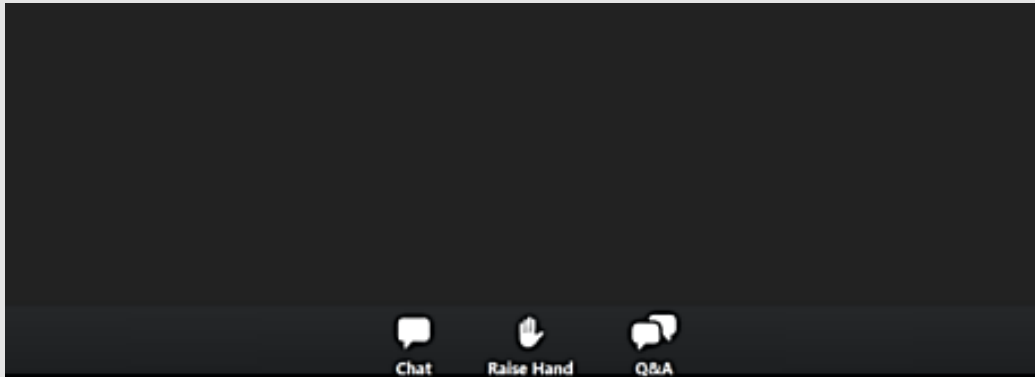




OLAW CONVERSATIONS
Humane Endpoints
Tuesday, July 26, 2022



Today's session



Troubleshooting tip when trying to speak after raising hand!
Zoom controls vary by device. If you are still muted after the host unmutes you, try looking for the speaker icon in the top right, turning off subtitles under the CC icon, or an audio settings menu on the bottom left of your screen.

- Session will not be recorded
- Slide PDFs and certificates will be sent to attendees
- 3 ways to interact:
 - Raise hand feature
 - Chat box
 - Q and A feature.
 - There is a checkbox in the Q and A feature that provides the option to submit questions anonymously



Leading the Discussion:

John Bradfield, DVM, PhD, DACLAM

Joseph D. Thulin, DVM, MS, DACLAM

Humane Endpoints in Animal Studies

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Overview

Responsibility

Requirements

Challenges

Development and Implementation

Euthanasia

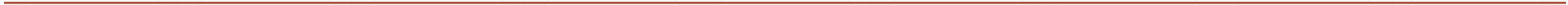
Two kinds of Endpoints.....

- The **experimental endpoint** of a study occurs when the scientific aims and objectives have been reached.
- The **humane endpoint** is the point at which pain or distress in an experimental animal is prevented, terminated, or relieved.



.....even if the experimental endpoints have not yet been achieved.....

Guide, p. 27



An example....



Pritchett-Corning, K., Girod, A., Avellaneda, G., Fritz, P. E., Chou, S., & Brown, M. (2011). Handbook of clinical signs in rodents and rabbits, 2nd Ed. Charles River, Wilmington. (Photo courtesy of LAVA, UK; www.digires.co.uk)

Ethical spectrum of animal use



Nothing is
acceptable

Everything is
acceptable

Most people fall somewhere in the middle....

“Some research with animals is ok, as long as there are limits....”

Societal expectation that there are limits.....

- Who is responsible for setting the limits?
 - Regulatory authorities?
 - PI?
 - Vet?
 - IACUC?
-

Requirements

- US Gov't Principle VI
- PHS Policy
- Animal Welfare Regulations (2.31,(d), V)

“Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure”.

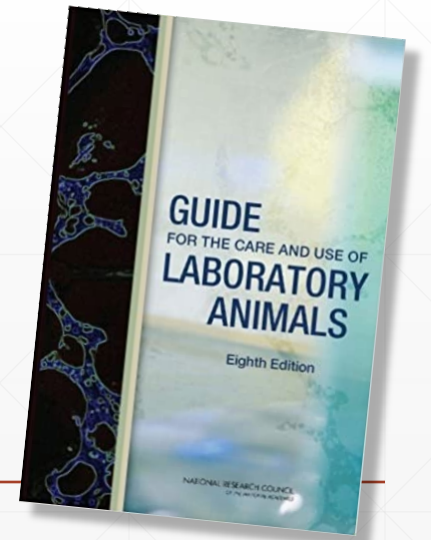


Requirements

Guide (p. 26)

Protocol Review

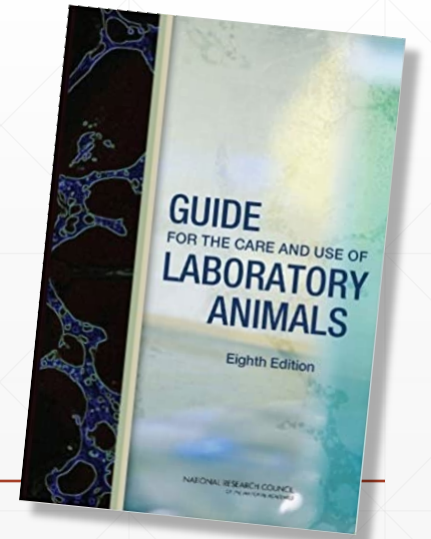
- *“impact of the proposed procedures on the animals’ well-being”*
- *“criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated”*



Requirements

Guide p. 27 (*humane endpoints*)

- *“...the point at which pain or distress is prevented, terminated, or relieved”.*
- *“...use of humane endpoints contributes to refinement....”*
- *“should be relevant and reliable”*



Requirements

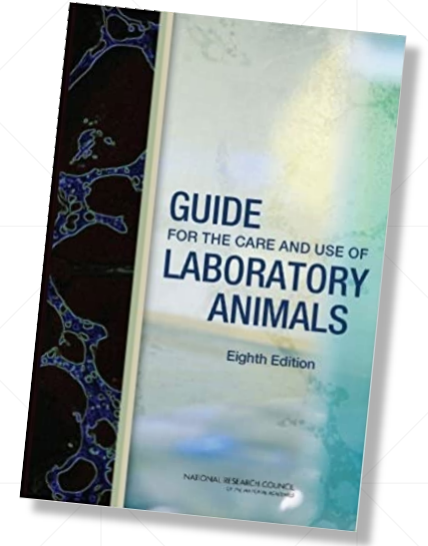
Guide (pp. 27, 28)

Protocol Review

- 1) Precise definition (assessment criteria)
- 2) Schedule of monitoring
- 3) Training of personnel (to recognize)
- 4) Required action

Determined by
PI, Vet, IACUC

Before the start
of the study.....



Role of the PI

- Ideally, have experience and familiarity with the model
 - Identify, specific, relevant, humane and experimental endpoints – and list those in the protocol
 - Ensure that laboratory staff are properly trained to monitor animals and identify humane endpoints
 - Set the culture/expectation in the lab that humane endpoints are important
-

Role of the Veterinarian

- Provide veterinary expertise on understanding and identifying animal pain/distress – *during protocol development and review*
 - Ensure that humane endpoints are accurate and relevant for the specific protocol/study (*e.g., consideration of “ramp-up” monitoring as the potential for pain and distress increases with progression of the model*)
 - Ensure that routine, cage-side monitoring is performed by animal care staff
-

Role of the IACUC

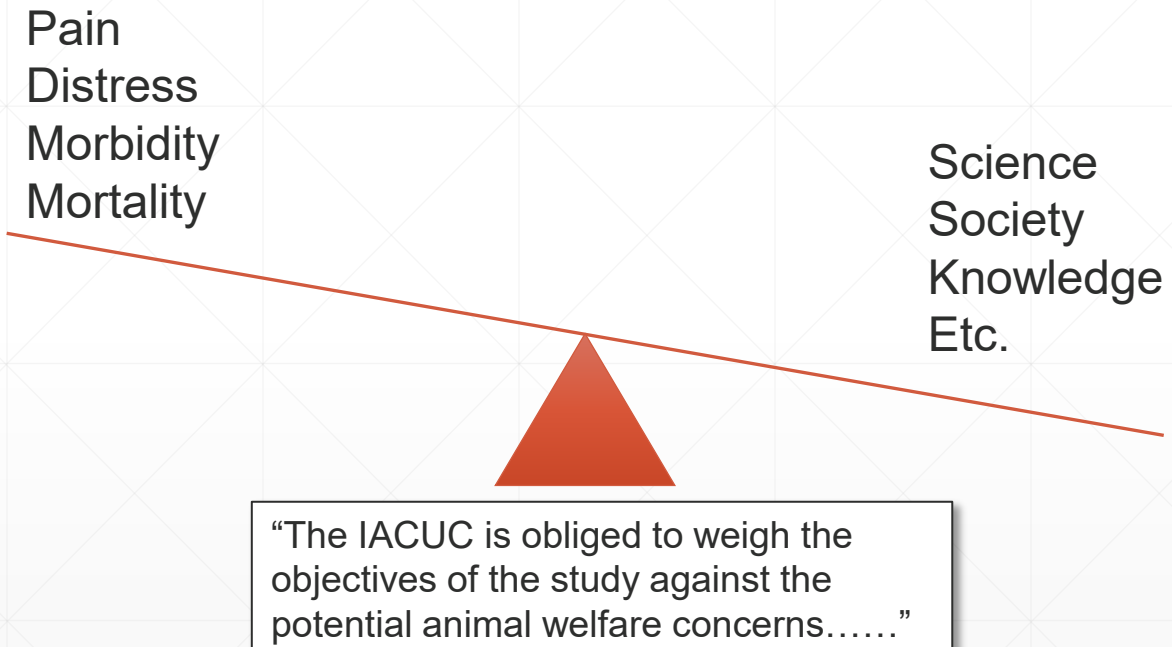
- Ensure protocols contain the 4 key elements of humane endpoints (*precise definition, schedule of monitoring, staff training, action required*)
 - If/when studies involve the potential for pain/distress - ensure that the humane and experimental endpoints are rational, logical, appropriately justified, and founded in proper scientific methodology
 - Conduct an ethical cost (harm): benefit analysis:
 - *Proper consideration of humane limits requires careful, thoughtful consideration of a rather nuanced issue – but when it's done well, the result is a significant improvement in animal welfare/ well-being.*
-

Role of the IACUC

Ethical Cost (harm): Benefit analysis

Pain
Distress
Morbidity
Mortality

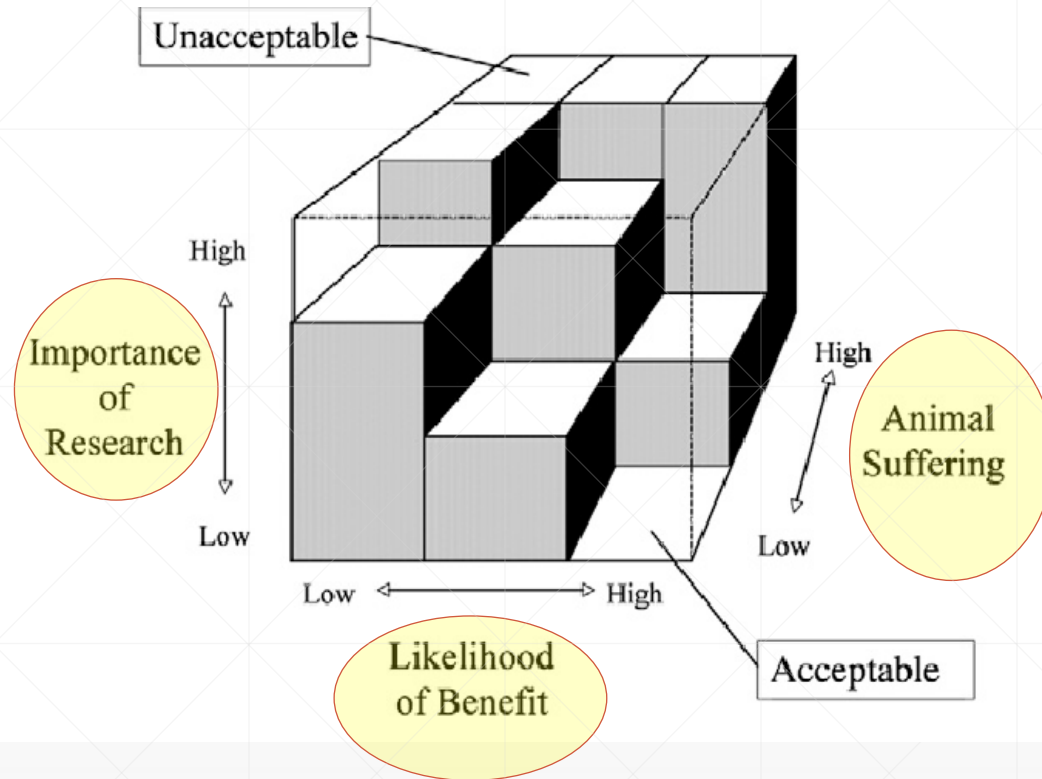
Science
Society
Knowledge
Etc.



“The IACUC is obliged to weigh the objectives of the study against the potential animal welfare concerns.....”

Ethical Cost (harm): Benefit Analysis

Sir (Paul) Patrick Bateson - ethologist



Bateson P. When to experiment on animals. *New Sci.* 1986 Feb 20;109(1496):30-2. PMID: 11655736
Pound, Pandora; J. Nicol, Christine (2018): Bateson's cube. *PLOS ONE*. Figure. <https://doi.org/10.1371/journal.pone.0193758.g001>

Bateson Cube

Bateson Method.....

1. “Importance of research” = **scientific merit**
2. “Likelihood of benefit” = **proper experimental design and conduct**
3. “Animal suffering” = **consideration of the endpoints**

Proper consideration and implementation of humane endpoints reduces animal pain/distress and thereby improves the ethical cost benefit analysis.....

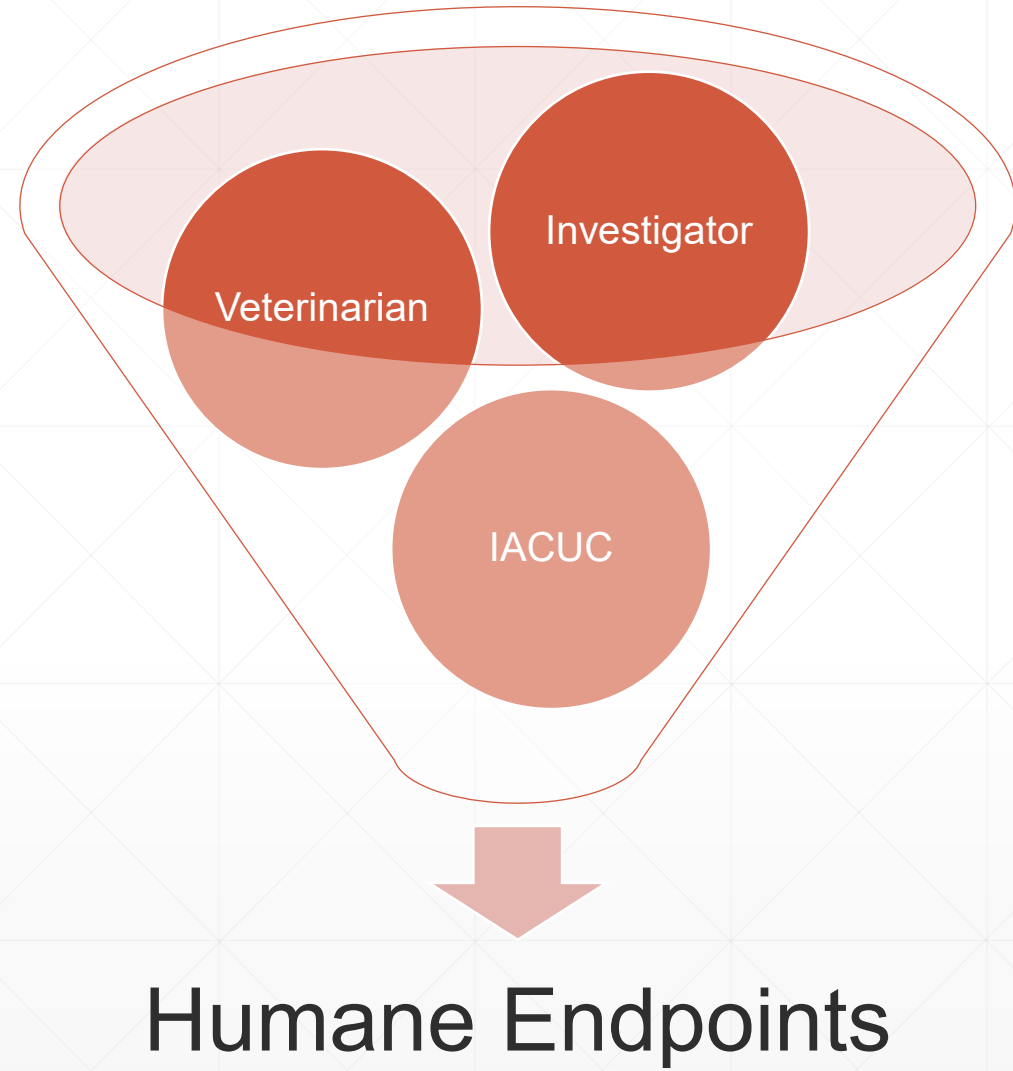
Challenges

When experimental and humane endpoints are closely linked.....

- Tumor models
 - Infectious diseases
 - Vaccine challenge
 - Pain modeling
 - Neurodegenerative disorders
 - Some behavior studies
 - Toxicology studies
 - Inflammatory conditions (arthritis)
 - Drug dependency
-

Development and Implementation of Humane Endpoints

A Collaboration



Humane Endpoints

The protocol form – A tool for collaboration

Should address:

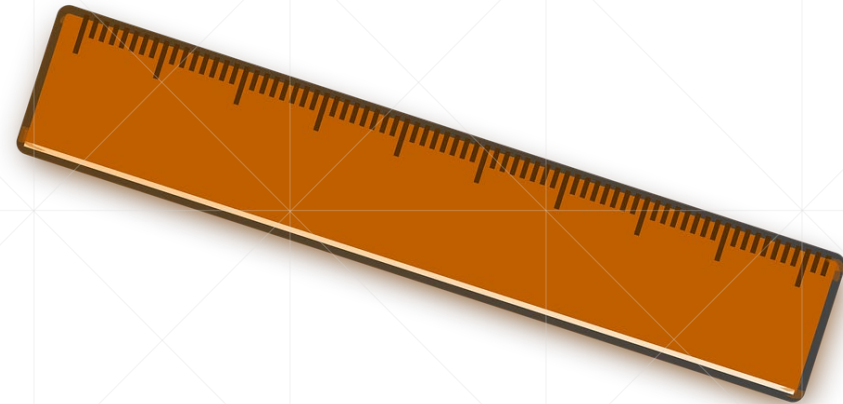
- Definition of the humane endpoint
- Animal observations
- Identification and training of personnel
- The response/action to be taken



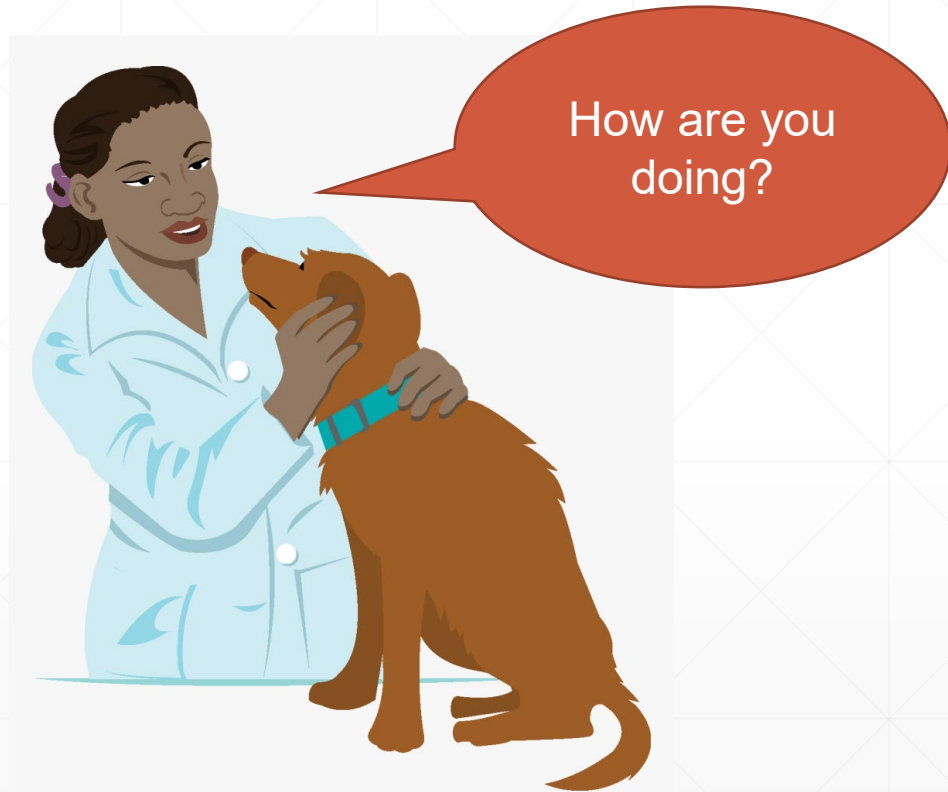
“Engineering” versus “Performance” Endpoints

Engineering endpoints

- Clear, objective, measurable criteria
- Little or no professional judgement involved
- Decreased chance of disagreement
- Not necessarily direct measure of animal state of health or wellbeing



“Engineering” versus “Performance” Endpoints



Performance endpoints

- Professional judgement & subjectivity
- Greater chance for disagreement
- Assess impact on overall animal health or wellbeing
- May be basis for subsequent engineering endpoints

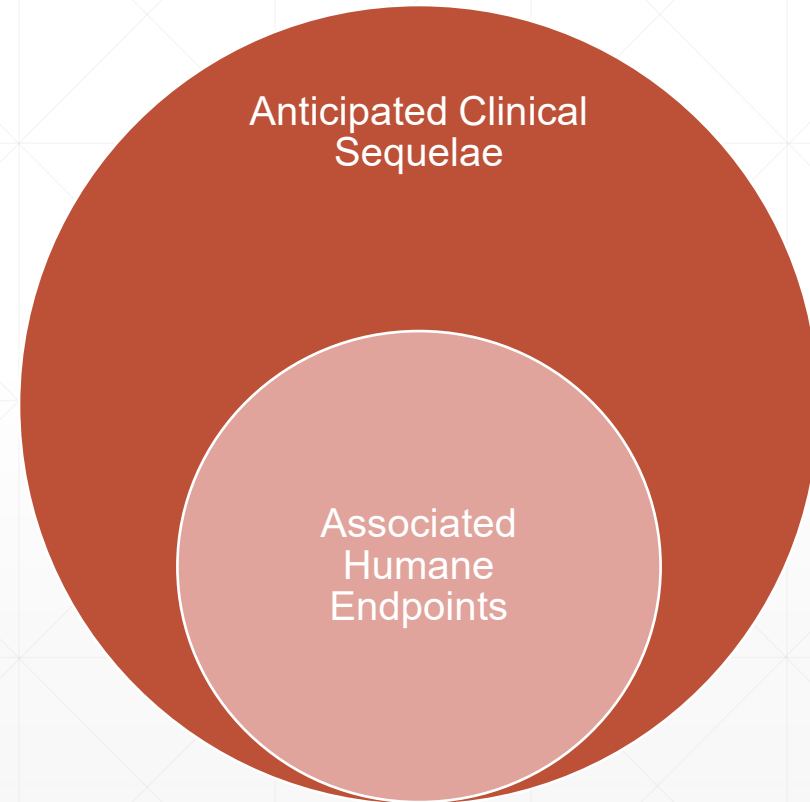


Poll Question

Does your IACUC require inclusion of humane endpoints for protocols in which no adverse outcomes are anticipated?

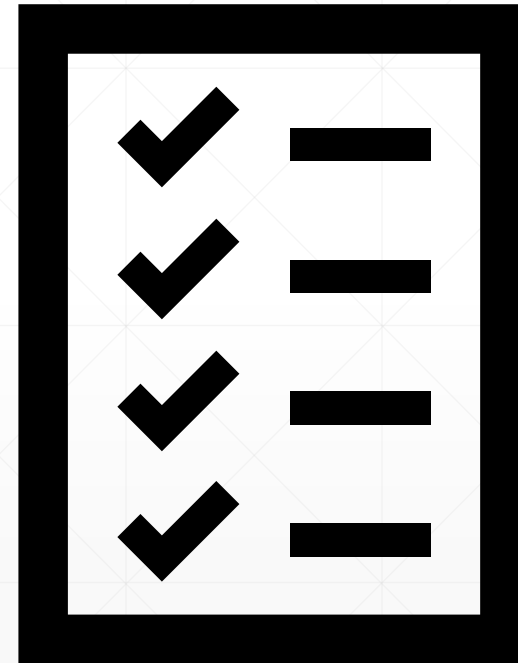
The Relationship between Outcomes and Endpoints

- “[C]riteria and process for timely intervention, removal of animals from a study, or euthanasia **if painful or stressful outcomes are anticipated**” (Guide p. 26)



Standard Humane Endpoints

- Standard text offered for use in animal protocols
- Communicate minimum expectations
- Help establish greater consistency
- Not study-specific, so may need additional or alternative criteria
- Useful for studies for which there are no painful or stressful outcomes anticipated



**For whom are the
endpoints described?**

The Animal Care/Veterinary Staff

- Frequently the only ones in the program specifically tasked with daily observations
- Frequently the ones to make first determination as to whether an animal has reached a humane endpoint
- Ready access to the approved protocol is essential



Response required upon reaching the humane endpoint

- Commonly think of euthanasia as the intervention when the endpoint is reached
- But other interventions may be appropriate and preferred
 - Analgesia
 - Nursing intervention
 - Removal of the physical or behavioral stressor



Some Practical Considerations

Case 1: Generic study in rats



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<https://www.pngall.com/mouse-animal-png/download/17698>

- Humane endpoint:
 - 20% loss of body weight from weight at study start.
- Observations:
 - Weekly body weights
- Response:
 - Euthanasia

Weight Loss as a Humane Endpoint

- Probably the most commonly listed endpoint
 - 15% - 20% most frequent
- Complicating factors
 - Continuous growth of rodents
 - Loss relative to starting weight or average weight of controls?
 - Some study outcomes contribute to body weight
 - For example, tumors and ascites
 - How often are the animals going to be weighed?



Body Condition Score Chart



BC 1

Mouse is emaciated.

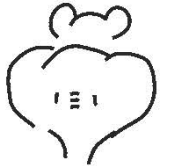
- *Skeletal structure extremely prominent; little or no flesh cover.*
- *Vertebrae distinctly segmented.*



BC 2

Mouse is underconditioned.

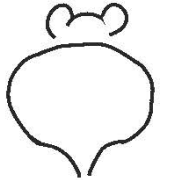
- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*



BC 3

Mouse is well-conditioned.

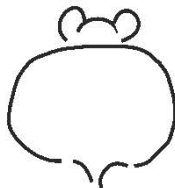
- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*



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.*

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 2-...)

Case 2: Graft-Versus-Host Disease Model in Mice

Anticipated outcomes:

- “Progressively severe clinical disease and potentially death in some animals.”

Humane Endpoints:

- “When they are observed to be moribund.”

Observations:

- “Animals will be assessed once daily.”

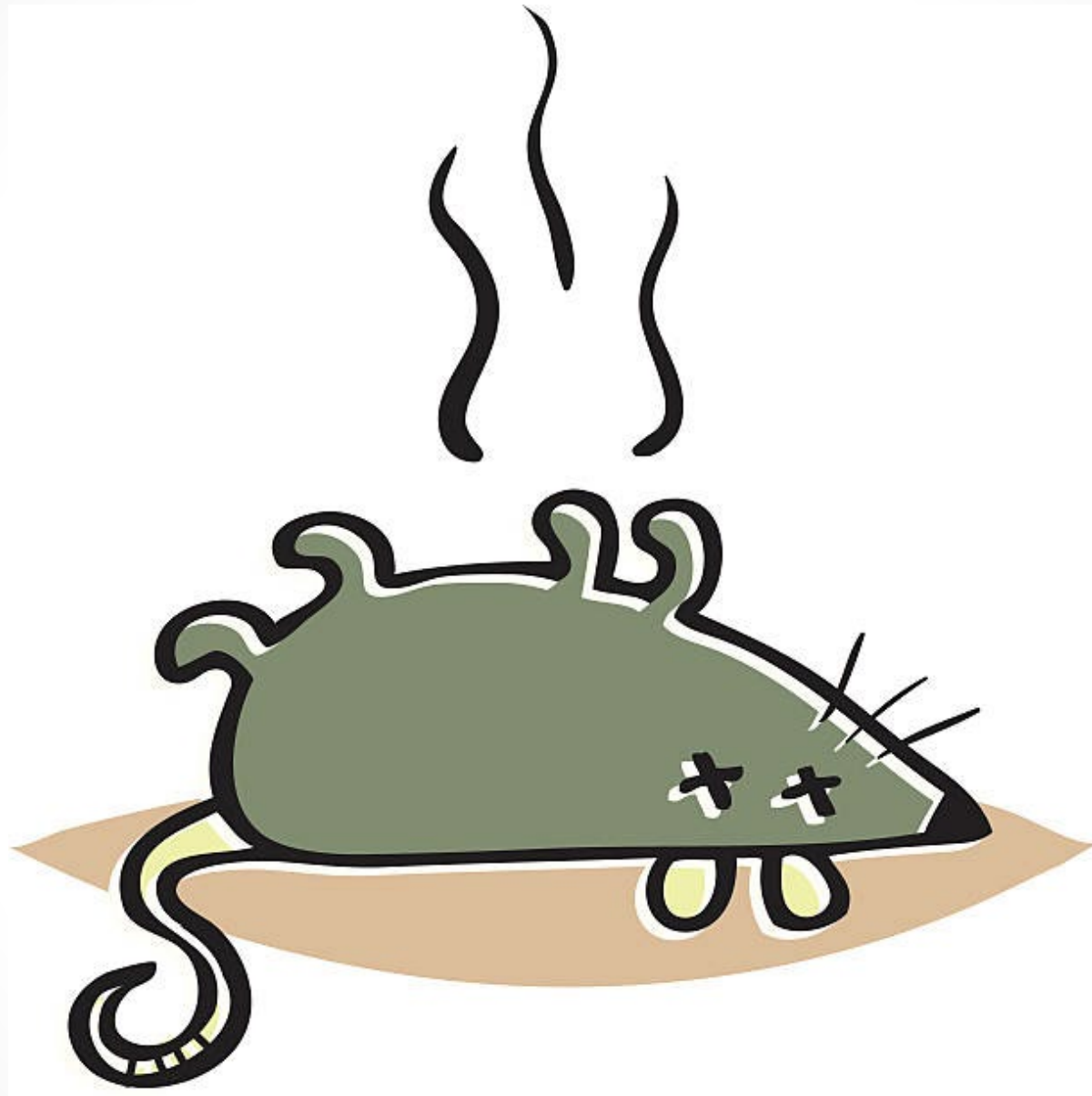
Response:

- Euthanasia
-



Poll Question

Is "moribund" or "moribundity" an adequate definition of a humane endpoint?



Poll Question

Is death ever an acceptable endpoint?

Clinical Scoring Example

GvHD Clinical Scoring System

Grade	Weight Loss	Diarrhea	Posture	Activity	Fur Texture	Skin Integrity
0	<10%	No	Normal	Normal	Normal	Normal
1	>10% to <25%	Yes	Hunching at rest	Mild to moderate decrease	Mild to moderate rufflings	Scaling of paw and tails
2	>25%		Severely hunching	Severe decrease	Severe rufflings	Obviously denuded

Case 3: Tumorigenesis in Mouse Model

Anticipated Outcome:

- Development of subcutaneous tumors.

Humane Endpoints:

- Tumors reaching or exceeding 2 cm diameter

Observations:

- Tumors measured 3 times per week

Response:

- Euthanasia



Public domain
<https://visualsonline.cancer.gov/details.cfm?imageid=1967>

Case 3: Tumorigenesis in Mouse Model

- The investigator contacts the AV to request she be able to keep the animals longer because the animals look fine.
- The AV examines the animals, and agrees the animals appear perfectly healthy other than the presence of bilateral subcutaneous tumors.



Public domain
<https://visualsonline.cancer.gov/details.cfm?imageid=1967>



Poll Question

Does the investigator have to euthanize the animals despite the absence of evidence of negative impact of the tumors on the animals?

Euthanasia

The Decision to Euthanize

- *“Criteria for euthanasia include protocol-specific endpoints (such as degree of a physical or behavioral deficit or tumor size) that will enable a prompt decision by the veterinarian and the investigator to ensure that the endpoint is humane and, whenever possible, the scientific objective of the protocol is achieved.”*
- *“In the case of a pressing health problem, if the responsible person (e.g., investigator) is not available or if the investigator and veterinary staff cannot reach consensus on treatment, the veterinarian must have the authority, delegated by senior administration...and the IACUC, to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary.”*

AVMA Guidelines for the Euthanasia of Animals: 2020 Edition*

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*The AVMA Panel on Euthanasia develops the content of the guidelines, with support from its working groups. The panel is required to do a comprehensive review and update of the report at least every 10 years, although more frequent major revisions are possible based on substantive information gleaned from new research and experience with practical implementation. To ensure the guidelines remain as up-to-date as possible, interim revisions (reflecting substantive updates, but of a less extensive nature than a major revision) are also accommodated.

Euthanasia

Ending the life of an animal in a way that minimizes or eliminates pain and distress

Humane technique minimizes pain, distress, and negative effect to the animal

A good death is tantamount to the humane termination of an animal's life.



Consider impact on personnel

Do you have a plan for that?



Questions/Discussion

References

- U.S. Government Principles. OER Home Page – Grants Web, Office of Laboratory animal Welfare: PHS Policy on Humane Care and Use of Laboratory animals Available from <https://olaw.nih.gov/policies-laws/gov-principles.htm>
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Thank you!
Next Session: TBD

