

## WVU IACUC Policy: Pain and Distress Recognition – Humane Endpoints

### Background

The purpose of this document is to provide guidance for the establishment of humane endpoints in research, teaching and testing. Humane endpoints are essential to minimize pain and distress that may be encountered during experimental manipulations. Investigators should consult veterinary staff for guidance on appropriate humane endpoints for their specific study. Study endpoints requiring animals to reach a moribund condition or death are strongly discouraged and require scientific justification and IACUC approval. When possible, the use of clinical, behavioral, and physiological signs which may predict death, or a moribund state, should be used. Humane endpoints *must* be included for all studies.

### Policy

Compliance with all governing regulations involving humane care and use of animals is mandatory at WVU. The 8<sup>th</sup> edition of *The Guide for the Care and Use of Laboratory Animals* (page 27) indicates that “while all studies should employ endpoints that are humane, studies that commonly require special consideration include those that involve tumor models, infectious diseases, vaccine challenge, pain modeling, trauma, production of monoclonal antibodies, assessment of toxicologic effects, organ or system failure, and models of cardiovascular shock.” WVU is committed to ensuring the welfare of all animals used on campus. In line with the 3R’s (replacement, refinement, reduction) it is our policy to consider procedural changes that can improve animal well-being and minimize pain and/or distress when possible. All personnel involved in using animals on campus *must* consider the pain and distress involved in procedures performed and take steps to address these concerns. Any animal that has reached a humane endpoint, as outlined in an approved IACUC protocol, *must* be euthanized, or treated/managed as deemed appropriate by the veterinary staff. Adherence to this policy is essential to ensuring appropriate animal welfare. All exceptions to this policy require scientific justification, review and approval by the IACUC.

### Definitions

- **Experimental Endpoints:** Endpoint of a study which occurs when the scientific aims and objectives have been reached (*Guide* p.27).
- **Humane Endpoints:** The point at which pain and distress in an experimental animal is prevented, terminated or relieved.
- **Morbidity:** A condition of being unhealthy or diseased.
- **Moribund:** A severely debilitated clinical state that precedes imminent death.
- **End-stage illness:** Signs of a debilitated state where death is imminent and treatment ineffective.
- **Death as an endpoint:** Studies in which animals are allowed to die without intervention.

### **Establishing Humane Endpoints**

1. Humane endpoints are often model and study specific and should be established in consultation with the veterinarian.
2. Current literature and potential refinements/replacements that could reduce pain and distress should be considered when planning a study.
3. Scoring systems are a useful tool to quantify clinical parameters and provide a consistent mechanism to determine if an endpoint has been reached. Scoring systems should be created based on the models and study needs. (*see Appendix E for examples*)
4. Pilot studies may be requested by the IACUC if there are concerns regarding pain and distress in a model in where endpoints do not already exist. Results of the pilot study should be reported back to the IACUC, with the proposal for a full study.
5. When working with animal models where endpoints or scoring criteria are not well defined, personnel should monitor animals with sufficient frequency to avoid unrelieved pain or distress. A complete description of monitoring frequency and supportive care should be outlined in the IACUC protocol.
6. Criteria commonly used to evaluate animal health and develop humane endpoints include:
  - a) Body weight and/or body condition score (*see Appendix C*)
  - b) Decreased food/water consumption or inability to reach food/water
  - c) Behavioral changes
  - d) Activity changes
  - e) Tumor size
  - f) Imaging findings
  - g) Blood test results
  - h) Changes in body temperature
  - i) Respiratory distress
  - j) Neurological changes (e.g. paralysis, paresis, head tilt, seizures, etc.)
  - k) Signs of unresolved pain
  - l) Skin lesions or self-trauma
7. **The Attending Veterinarian (AV) is responsible for the health and welfare of all animals on campus. The AV and WVU designated veterinary staff can intervene when unexpected signs of pain or distress are observed. In such cases, medical care or euthanasia will be provided on an emergency basis under the direct recommendation of an AV/clinical veterinarian. When these situations arise, veterinary staff will be communicating with the IACUC.**
8. Pain evaluation: A combination of several tools can be used to evaluate pain.
  - a) Pain specific changes in behavior, e.g. ethogram assessment for species/model (*see Appendix B*).
  - b) Grimace Scale (*see Appendix D for examples*).
  - c) Nest building behavior or time to integrate material in nest (mice).

### **Documentation**

Studies with monitoring parameters outlined in the IACUC protocol are expected to maintain appropriate records. The records may be reviewed by the IACUC and AV upon request.

**Death as an Endpoint**

1. Studies requiring death as an endpoint are discouraged and require robust scientific justification.
2. Refinements *must* be considered including alternative endpoints, analgesics, sedatives or anesthetics.
3. Details including monitoring and supportive care *must* be provided.

**Appendix A**

The following criteria can be used to help differentiate between a morbid state and a moribund state in rodents.

RODENTS	
Morbid (ill)	Moribund (near death)
Hunched posture/sunken eyes	Impaired mobility
Ruffled hair coat and lack of grooming behavior	Lethargy/lack of activity
Sunken eyes, with or without discharge	Inability to remain upright
Acute diarrhea or constipation (<48 hr)	Severe self-mutilation or injury
Respiration rate increased or decreased	Labored breathing/cyanosis
Lameness or ataxia	Lack of response to external stimulus
Weight loss (<20 %)	Weight loss ≥20%; BCS <2/5
Decreased food/water intake	Pain which cannot be managed with analgesics
Mild dehydration	Inability to reach food/water/ambulate normally
Urinary output concerns	
Ulcerative dermatitis/skin lesions	
Ulcerated tumors	
Moderate to severe fight wounds	

**Morbid animals should be reported to the veterinary staff for examination and treatment recommendations. Conditions constituting a moribund state necessitate euthanasia unless there is sufficient scientific and ethical justification, and IACUC approval. If research personnel cannot be reached, Office of Laboratory Animal Resources (OLAR) staff will humanely euthanize moribund animals.**

**Appendix B**

Below are some behavioral signs associated with pain/morbidity in the species indicated.

RODENTS	
Decreased activity	Porphyrin staining (“red tears”) – esp. albino rats
Piloerection	Reduced or absent food and/or water intake
Rapid, shallow respiration	Weight loss / loss of overall condition
Vocalization (pitch may be above human range)	Unkept, “scruffy” fur / lack of grooming
Abnormal aggression	Social isolation
Abnormal posturing, including hunched posture	Self-mutilation

<b>RABBITS</b>	
Vocalization – loud /squealing, crying	Tonic immobility (playing dead)
Unusually anxious or apprehensive	Reduced or absent food and/or water intake
Unusually quiet, dull or inactive	Weight loss / loss of overall body condition
Bruxism (teeth grinding) and/or salivation	Increased respiratory rate
Restless	Licking or chewing of a body area (localized pain)
Hunched posture	Perineal soiling (hind end pain/arthritis)
Hypersensitivity	
<b>BIRDS (NOTE: THESE SIGNS IN BIRDS INDICATE A MORE ADVANCED STATE THAN WOULD BE THE CASE IN LABORATORY RODENTS)</b>	
Vocalization	Eyes partially or entirely closed
Restlessness /excessive movements	Decreased interest in surroundings
Weight loss/loss of overall body condition	Inactivity
Reduced or absent food and/or water intake	Wings flat /drooped
“Fluffed appearance”	Neck retracted
Laying down more than normal	
<b>FISH</b>	
Lethargy or listlessness	Agitated swimming
Decreased appetite	Rapid gill or mouth movements
Loss of body condition	Rapid operculum movements
Dull or darker coloring	Abnormal orientation
Circling or drifting	Social isolation
<b>SWINE</b>	
Dull or depressed mentation	Reluctance to stand and/or move
Abnormal posture (e.g. head drooped)	Abnormal gait
Rapid, shallow respiration	Abnormal or persistent vocalization
Grunting	Decreased appetite or anorexia
Bruxism (teeth grinding)	Tucked abdomen
Abnormal aggression	Social isolation
<b>RUMINANTS</b>	
Dull or depressed mentation	Decreased food and/or water intake
Lethargy	Weight loss / loss of overall body condition
Abnormal postures (e.g. rigid, neck extended)	Persistent vocalization
Rapid, shallow respiration	Social isolation
Grunting	Decreased milk yield
Bruxism (teeth grinding)	Increased body temperature
Persistent licking or kicking at a body area	Abnormal gait and/or weight-shifting
Restless (e.g. repeatedly standing then lying down)	Reluctance to stand or move

## Appendix C

Mouse Body Condition Score (BCS for other species available by request)



### **BC 1**

Mouse is emaciated.

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



### **BC 2**

Mouse is underconditioned.

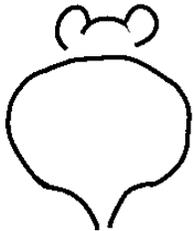
- *Segmentation of vertebral column evident.*
- *Dorsal pelvic bones are readily palpable.*



### **BC 3**

Mouse is well-conditioned.

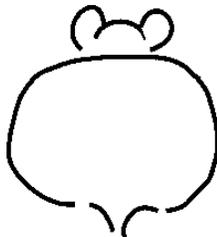
- *Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.*



### **BC 4**

Mouse is overconditioned.

- *Spine is a continuous column.*
- *Vertebrae palpable only with firm pressure.*



### **BC 5**

Mouse is obese.

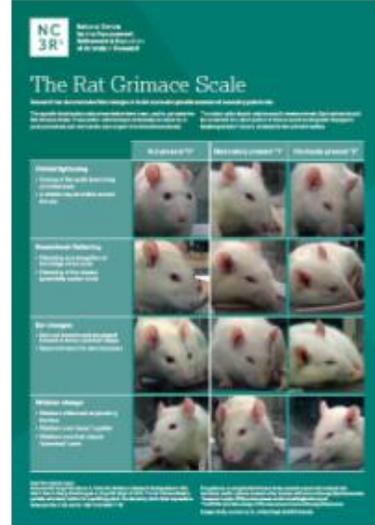
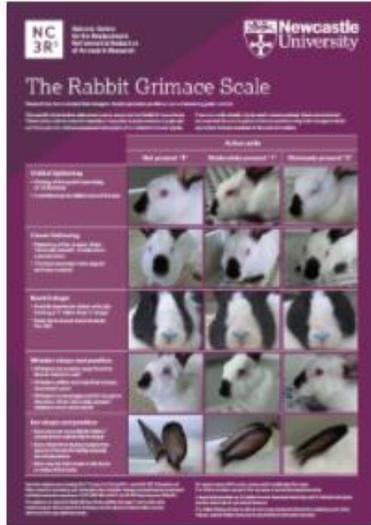
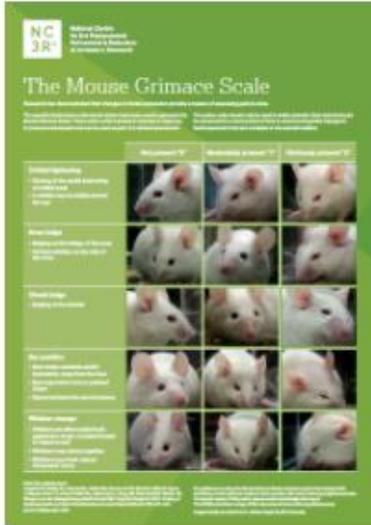
- *Mouse is smooth and bulky.*
- *Bone structure disappears under flesh and subcutaneous fat.*

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

## Appendix D

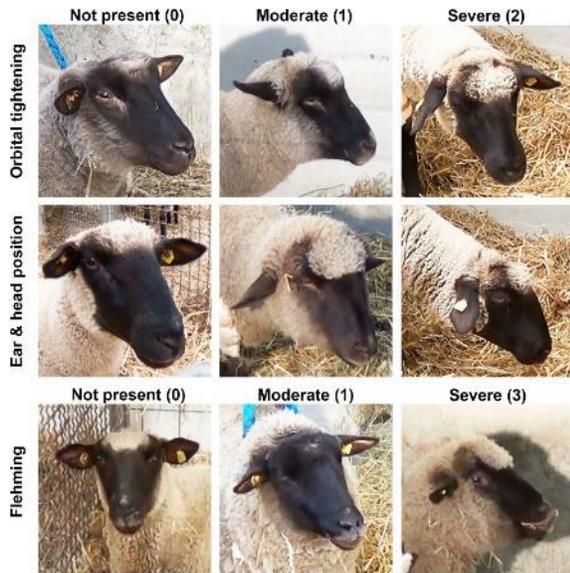
Grimace scales for mouse, rabbit and rat can be found on the National Centre for the Replacement Refinement & Reduction of Animals in Research (NC3Rs) website:

<https://www.nc3rs.org.uk/grimacescales>.



You can also find examples of a sheep grimace scale online

(<https://doi.org/10.1371/journal.pone.0175839>).



## **Appendix E**

### Example scoring systems

<b>Variable</b>	<b>Score and description</b>
<b>Appearance</b>	0- Coat is smooth
	1- Patches of hair piloerected
	2-Majority of back is piloerected
	3-Piloerection may or may not be present, mouse appears "puffy"
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<b>Level of consciousness</b>	0- Mouse is active
	1- Mouse is active but avoids standing upright
	2- Mouse activity is noticeably slowed. The mouse is still ambulant.
	3- Activity is impaired. Mouse only moves when provoked, movements have a tremor
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<b>Activity</b>	0- Normal amount of activity. Mouse is any of: eating, drinking, climbing, running, fighting
	1- Slightly suppressed activity. Mouse is moving around bottom of cage
	2- Suppressed activity. Mouse is stationary with occasional investigative movements
	3- No activity. Mouse is stationary
<hr/>	
<b>Response to stimulus</b>	0- Mouse responds immediately to auditory stimulus or touch
	1- Slow or no response to auditory stimulus; strong response to touch (moves to escape)
	2- No response to auditory stimulus; moderate response to touch (moves a few steps)
	3- No response to auditory stimulus; mild response to touch (no locomotion)
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<b>Eyes</b>	0- Open
	1- Eyes not fully open, possibly with secretions
	2- Eyes at least half closed, possibly with secretions
	3- Eyes half closed or more, possibly with secretions
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<b>Respiration rate</b>	0- Normal, rapid mouse respiration
	1- Slightly decreased respiration (rate not quantifiable by eye)
	2- Moderately reduced respiration (rate at the upper range of quantifying by eye)
	3- Severely reduced respiration (rate easily countable by eye, 0.5 s between breaths)
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<b>Respiration quality</b>	0- Normal
	1- Brief periods of labored breathing
	2- Labored, no gasping
	3- Labored with intermittent gasps
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	4- Gasping

Shrum et al. BMC Research Notes 2014, 7:233

Variable		Score
<b>Body Weight Changes</b>		
0	Normal	
1	< 10 percent weight loss	
2	10-15 percent weight loss	
3	> 20 percent weight loss	
<b>Body Condition Score (see diagram for details)</b>		
0	Body condition score >3	
1	BCS >2 and < 3	
2	BCS >1 and <2	
3	BCS of 1 or less	
<b>Physical Appearance</b>		
0	Normal	
1	Lack of grooming	
2	Rough coat, nasal/ocular discharge	
3	Very rough coat, abnormal posture, enlarged pupils	
<b>Measurable Clinical Signs</b>		
0	Normal	
1	Small changes of potential significance	
2	Body temp change of 1-2°C, cardiac and respiratory rates ↑ up to 30%	
3	Body temp change of > 2°C, cardiac and respiratory rates ↑ up to 50%, or markedly reduced	
<b>Unprovoked Behavior</b>		
0	Normal	
1	Minor changes	
2	Abnormal, reduced mobility, decreased alertness, inactive	
3	Unsolicited vocalizations, self mutilation, either very restless or immobile	
<b>Behavioral Responses to External Stimuli</b>		
0	Normal	
1	Minor depression/exaggeration of response	
2	Moderately abnormal responses	
3	Violent reactions, or comatose	
<b>TOTAL:</b>		

IACUC Guideline Humane Endpoints for Laboratory Animals, University of Pennsylvania

### Reference Documents

- A Review of Pain Assessment Methods in Laboratory Rodents. Comparative Medicine. 2019. December; 69(6): 451-467.
- Shrum et al. BMC Research Notes 2014, 7:233.
- IACUC Guideline Humane Endpoints for Laboratory Animals, University of Pennsylvania.
- Ullman-Cullere MH and Foltz CJ (1999) Body condition scoring: a rapid and accurate method for assessing health status of mice. Lab Anim SC 49:319-323.