University of Louisville
Institutional Animal Care and Use Committee
Policies and Procedures

Humane Endpoints

Policy: Humane experimental endpoints that minimize pain, distress, or discomfort by choosing the earliest endpoint that is compatible with the scientific objectives of the research project must be identified within UofL Institutional Animal Care and Use Committee (IACUC) *Proposals*.

Rationale: The UofL IACUC is responsible for evaluating and ensuring that research projects are conducted in accordance with the Animal Welfare Act and Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals. These regulations and policies require that any research, testing and teaching that uses animals must be performed in such a way as to minimize discomfort, distress and pain consistent with sound research design. Some experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. Studies that cause pain and/or distress are categorized as Class D (Formerly Class II) or Class D (Formerly Class III) studies. Further, the investigator and the IACUC must consider the selection of the most appropriate endpoints. This requires careful consideration of the scientific requirements of the study, the expected possible adverse effects the research animals may experience (pain, distress, illness, *etc*.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. The effective use of endpoints requires that properly qualified individuals perform both general and study-specific observations of the research animals at appropriate time points. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity since they minimize pain and distress.

Procedures, Guidelines, and Exceptions:

1. **Standard removal criteria**
   The following criteria constitute standard humane endpoints for all research *Proposals* at UofL when applicable to the species used. If one or more criteria are met, the principal investigator is responsible for removing the animal, treating as approved in the *Proposal*, or consulting with a veterinarian to discuss other treatment options. Exceptions to these endpoints must be scientifically justified and require prior IACUC approval.
   a. **Weight loss**: Loss of 15% body weight compared to baseline or age-matched control
   b. **Poor body condition score (BCS):** BCS < 2/5 in rodents (Ullman-Culleré and Foltz, 1999; Hickman and Swan, 2010) or equivalent in non-rodents based on species-specific body scales
   c. **Inability to obtain food or water**: Inability to rise or comfortably ambulate to get to food and water; lesions that interfere with eating or drinking; consistent abnormal swimming or loss of equilibrium in aquatic species
   d. **Hypo/Unresponsive**: Severely diminished or lack of response to normal stimuli/physical manipulation

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e. **Dehydration**: Persistent clinical dehydration non-responsive to treatment, which may be observed as prolonged skin tenting, dry mucus membranes, increased capillary refill time, and sunken eyes
f. **Tumor burden (study-related)**: Cumulative tumor burden > 15 mm diameter in an adult mouse or > 40 mm in an adult rat; tumors impacting normal movement; or evidence of tumor ulceration, necrosis, or infection
g. **Surgical site complications**: Surgical site complications such as infection, dehiscence, or abnormal discharge
h. **Infection**: Clinical signs of infection that are not treated or unresponsive to medical management
i. **Unrelieved pain/distress**: Signs of significant pain and/or distress which are unresponsive to analgesics, or as determined by a veterinarian. Pain may be evaluated using species-specific changes in biochemical and behavioral parameters including grimace scales (Langford et al. 2010; Sotocinal et al. 2011; Keating et al. 2012).
j. **Organ dysfunction/failure**: Clinical signs of organ dysfunction with a poor prognosis or non-responsive to treatment. In addition to hematologic and biochemical values, this may include:
    i. **Respiratory**: Labored breathing; cyanosis; excessive opercular movements or gasping in aquatic species
    ii. **Cardiovascular**: Anemia/pallor, ascites, shock
    iii. **Gastrointestinal**: Chronic diarrhea, constipation, or vomiting unresponsive to treatment, rectal prolapse
    iv. **Urogenital**: Renal failure, urinary tract obstruction, bladder rupture, uterine or vaginal prolapse
    v. **Nervous**: Protracted seizures, ataxia, spontaneous paralysis, neurologic abnormalities, hydrocephalus, neurological swimming behaviors in aquatic species (e.g. vertical, spinning, tank diving)
    vi. **Musculoskeletal**: Severe lameness or mobility issues; scoliosis, extreme bloat, or lethargy/laying on the bottom of the tank in aquatic species
    vii. **Integument**: Non-healing or severe wounds, self-mutilation, or progressive dermatitis; cutaneous hyperemia and discoloration in reptiles and amphibians; pigment changes or gas bubble formation in aquatic species
k. **Moribund**: The term moribund describes a severely debilitated state that will ultimately lead to death. Moribund animals often present unresponsive to environmental/physical stimulation, unable to rise or ambulate, hypothermic, emaciated and/or agonal breathing/cyanotic. However, timely identification of animals for euthanasia prior to moribund condition improves animal welfare and allows for meaningful data collection. This can be done with validated markers of imminent death based on the model, such as low body temperature, weight loss, or inability to move, in order to minimize terminal distress.

2. Expectations for monitoring

Proposals with expected adverse effects should include a plan for appropriate animal monitoring and care by investigative staff. This monitoring is in addition to the daily checks performed by animal care or veterinary staff unless otherwise agreed upon. Certain procedures/experiments may require monitoring from weekly up to several times per day. Personnel responsible for

animal evaluation, record keeping, notification of the investigator and/or veterinarian, and euthanasia should be identified prior to study initiation.

3. **Scoring Systems**
   a. Scoring systems may be useful to provide an objective format for deciding when an animal's condition meets humane endpoints.
   b. If used, a checklist or score sheet system should be prepared specifically for each scientific procedure since the expected sequelae of experiments can vary greatly. For example, the effects of an abdominal surgical procedure will differ significantly from a cranial implant. Qualitative signs such as limping may be assigned scores according to severity. Score sheets provide a method to obtain an overall impression of well-being and should be considered as a part of the IACUC *Proposal* review process when available.
   b. The Comparative Medicine Research Unit veterinarians are available for individual consultation and development of score sheet systems; an example may be found in the IACUC Information Sheet "Pain Scoring using Response Variables."

References:

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5. Institute of Laboratory Animal Resources, National Research Council (2011). *The Guide for the Care and Use of Laboratory Animals*. National Academies Press, Washington, DC.
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8. Montgomery CA (1990), Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. Cancer Bulletin 42:230-237.
9. National Research Council Committee on Recognition and Alleviation of Pain in Laboratory Animals (2009). *Recognition and Alleviation of Pain in Laboratory Animals*. National Academies Press, Washington, DC.
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12. Ullman-Culleré 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.
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