

## *Endpoint Guidelines for Animal Use Protocols*

Animal use protocols (AUP) are reviewed by the IACUC in accordance with the Animals Welfare Act, *The Guide for the Care and Use of Laboratory Animals*, and other federal regulations. To be in accordance with these regulations, all research, testing, and teaching that utilize animals must be performed to minimize discomfort, distress, and pain in relation to scientific aims. Federal Guidelines require that endpoints be determined for animal use and require the consideration of (4) areas:

1. Anticipated adverse effects the research animals may experience (e.g. pain, distress, illness).
2. Most likely time course and progression of these adverse effects.
3. Earliest most predictive indicators of present or impending adverse effects.
4. Justification of the humane endpoint to meet the scientific requirements of the study.

Conditions or procedures that cause more than momentary or slight pain or distress should be performed with species appropriate sedation, analgesia, or anesthesia. Discomfort to animals must be limited to that which is unavoidable for the conduct of scientifically valuable research, and unrelieved pain or distress can continue only for the duration necessary to accomplish the scientific objectives.

Research procedures involving unrelieved pain or distress may be approved by providing appropriate scientific justification on the Animal Use Protocol (AUP) form. The justification must describe how interventions to relieve pain or distress would interfere with the scientific objectives of the study.

While each AUP is unique, the IACUC has established the following set of guidelines to assist principal investigators in looking for clinical signs in rodents indicative of a humane experimental endpoint. Given that these guidelines cannot be articulated satisfactorily to cover every research proposal, the IACUC evaluates AUPs on an individual basis.

### **HUMANE EXPERIMENTAL ENDPOINTS**

Humane experimental endpoints are criteria used to end experiments on individual animals in order to avoid or terminate unrelieved pain and/or distress. Once a humane endpoint is reached, the animal should be immediately euthanized or treated as described in the approved protocol.

The presence of one or more of the following criteria below may be indications for euthanasia. The clinical signs, depending on severity and duration, that may constitute an endpoint include, but are not limited to:

- ***Hunched posture, lethargy, persistent decumbency, or inability to rise or ambulate*** – This condition would indicate that an animal would not be able to reach for food / water. Animals should be euthanized within 4 hours of not being able to rise.
- ***Rough, unthrifty or stained hair coat*** – Healthy rodents fastidiously groom their hair coats and coat deterioration is a sign of distress. Rats secrete porphyrin that stains around eyes and nostrils when stressed.
- ***Dyspnea*** – Labored breathing. A humane endpoint may be reached when animals show an altered respiratory rate and/or effort. Labored breathing is often accompanied by a strong abdominal component to breathing.

- **Dehydration** – As evaluated by skin turgor. Severe dehydration is manifested when an animal’s skin loses elasticity. Skin pinched over the back that does not return to normal is called “tenting”. The presence of significant dehydration is considered a humane endpoint.
- **Anorexia/Weight loss** – A 20% weight loss over a few days would be considered rapid. This requires frequent monitoring. A gradual weight loss over an extended period of time (weeks to months), which leads to emaciation, would also be grounds for euthanasia. Weight loss should be evaluated based on pre-experimental weight, based on an untreated control experimental cohort, or published weights for species/strain. If weight loss is expected, the degree of weight loss and monitoring frequency should be defined and described in the AUP.
- **Tumor size** – In rodents the occurrence of tumors can be either an unintended consequence or an experimental goal. In either case, tumor burden should not exceed 2cm diameter in any direction in adult mouse or 4cm in an adult rat. Any tumor growth that interferes with normal ambulation, breathing, or sleeping should be taken as an experimental endpoint. Tumor endpoints should also take into account the location of the tumor and the ability of the animal to ambulate. In addition, an endpoint is reached if the tumor ulcerates or becomes necrotic, or infected, or produces signs of discomfort such as increased scratching or rubbing. For tumor studies, please refer to the UMSOM [Rodent Tumor Production and Monitoring Guidelines](#) for additional guidance.
- **Abdominal Distension** – Certain conditions will lead to enlarged abdomens (i.e., hydro-abdomen) and may lead to a difficulty in breathing and/or ambulating.
- **Other clinical signs that may lead to an humane endpoint, depending on severity and duration, include:**
  - Diarrhea (2-3 days of duration, can lead to dehydration)
  - Progressive alopecia, caused by disease, that covers >25% of body surface area.
  - Jaundice and/or uncorrected anemia.
  - Neurologic signs (e.g., ataxia, circling or head tilt, limb paralysis)
  - Bleeding from any orifice
  - Self-trauma
  - Any condition interfering with eating or drinking (e.g., dental overgrowth that can not be resolved)
  - Excessive or prolonged hyperthermia or hypothermia
  - Prolapse of genitals or rectum
  - Lack of response to external stimuli
  - Unrelieved pruritus

**Monitoring Plan** – The AUP should contain a plan for monitoring animals on experimental study. At a minimum, animals should be monitored 3 times a week.

- The monitoring frequency should be increased in protocols with a higher probability of the development of a condition that leads to pain, distress, morbidity or mortality.
- The PI should identify, in the AUP, endpoints that will be consistent with the study goals and appropriate monitoring frequency to assure these endpoints can be identified. *If the AUP is written to allow the study to continue after certain endpoints are reached, the monitoring frequency is expected to increase (i.e., daily, twice-daily) until final endpoint(s) is/are reached.*
- The AUP should contain the designation of responsibility for this monitoring, training and coordination of the personnel responsible for: evaluations, written reports, notification of the investigator or their staff, and/or veterinarian(s) and persons responsible for euthanasia.

**NOTE:** Daily monitoring = monitoring animals 7 days a week, including weekends and holidays.

## DEATH OR MORIBUNDITY AS AN ENDPOINT

Although infrequent, death or moribundity as endpoints may be necessary for some research projects. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. In studies requiring such endpoints, animals are permitted to die or become moribund as a result of experimental procedures to reach the scientific aims. Examples of some research proposals that may have death or moribundity as an endpoint include: infectious disease studies, drug and toxicity studies, and cancer research. The following guidelines are suggested as a starting point to assist investigators with justifying death or moribundity as an endpoint.

### AUP's in which death or moribundity as an endpoint should contain the following information:

1. The reason why death or moribundity was selected as an endpoint including:
  - a. What alternatives were considered and how alternatives will be used whenever possible.
  - b. Number of animals to be used and why this is the minimal number of animals required.
  - c. Why pain relieving measures cannot be used.
  - d. Whether animals will be euthanized when moribund, and if not, what information is to be gained in the interval between early moribundity and mortality.
2. A statement of acceptance of the following animal care and monitoring procedures:
  - a. Animals involved in experiments that may lead to a moribund condition or death *will be monitored daily* by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior) for *at least* the following:
    - i. Abnormal appearance: abnormal posture, rough coat, head tucked into abdomen, exudates occurring around eyes, nose, and/or urogenital regions, or skin lesions.
    - ii. Abnormal activity: difficulty with ambulation, decreased food or water intake, abnormal breathing, difficulty/inability to eliminate, or self-mutilation.
  - b. The frequency of observation should be increased to *twice daily* when animals are found to be experiencing pain, distress or death.
    - i. Designated protocol personnel are to be notified to assure increased monitoring occurs as soon as animals show signs of disease.
      1. An assessment should be made as soon as possible of the animals' condition and what actions are to be taken.
      2. A consult with a Vet Resources (VR) veterinarian should be considered if personnel are unsure of moribundity status. The professional judgment and decision of the VR Veterinarian will be final.
  - c. Consideration should be given to removing animals to individual cages when their condition deteriorates to a point where harm from cage mates is likely. Dead animals must be promptly removed.
  - d. Written records should be kept of all monitoring sessions.

## UNEXPECTED EXPERIMENTAL ENDPOINT

When conducting a study and an unanticipated adverse affect occurs due to the experimental manipulation (*morbidity, moribundity, mortality, pain or more than momentary distress not approved to occur in the AUP*), the animals must be immediately euthanized or evaluated by a Vet Resources (VR) Clinical Veterinarian to avoid noncompliance as to IACUC unapproved pain and distress. If recommended veterinary clinical treatment would affect the experimental results, the animals must be euthanized. The clinical veterinarian has the final decision as to continuing treatment or requiring euthanasia at all times.

A protocol amendment must be submitted for IACUC review and approval to continue any experiment with the unanticipated adverse outcome which results in pain or distress.

## UNEXPECTED NON-EXPERIMENTAL RELATED ENDPOINT

Conditions may arise in “normal” animals, breeders or others during the conduct of research that are unexpected and unrelated to the research being conducted. Conditions may be specific, such as a spontaneous tumor, or may be more of a general deterioration of health/quality of life. These conditions can still have a significant impact on animal welfare and experimental results and must be addressed appropriately. Any animal found unexpectedly to be moribund, cachectic, or unable to obtain food or water must be euthanized in a timely manner. In less severe cases that may include pain or distress, the unexpected/unrelated condition should be assessed for the impact on animal welfare and experimental results. If it impacts experimental results, the animal should be euthanized. If it does not affect the experimental results (*this would also be the case with a breeder or a normal untreated animal*), a request for a VR Clinical Veterinarian consult must be submitted to determine appropriate treatment (medical or euthanasia). If recommended veterinary clinical treatment would affect experimental results, the animals are to be euthanized. If the condition does not respond or worsens while under veterinary treatment, the animal should be euthanized. In some animal models, such as specific phenotypes in Genetically Modified Animals (GMAs), conditions that impact animal welfare can be expected to continue or reoccur. If not already present these should be included in an amendment to the AUP as “expected resultant affects” to alert animal care staff and specify appropriate additional animal care and/or euthanasia.

Table 1. Selected Clinical Observations Used in Cancer Research and Toxicology Studies

Parameter	What to Look for
General Appearance	Dehydration, decreased body weight, missing anatomy or fractured appendages. Abnormal posture, swelling of tissues or masses. Prolapses or paraphimosis. Hypothermia
Skin and Fur	Discoloration of fur/ hair, urine stain, porphyrin staining (rats) pallor, redness, cyanosis, icterus, wound(s), sore, abscess, or alopecia or ruffled fur
Eyes	Exophthalmos, microphthalmia, ptosis, reddened eye, increased lacrimation or colored discharge. Opacity to the eye or cellular or blood accumulation in the eye.
Nose, Mouth and Head	Head tilted, nasal discharge, malocclusion of teeth or jaw. Salivation or malodor associated with any orifice.
Respiration	Sneezing, dyspnea, tachypnea, harsh audible respiratory sounds or abdominal distension interfering with respiration.
Urine	Discoloration or blood in urine. Polyuria or anuria
Feces	Discoloration or blood in feces, softness / diarrhea or mucoid stool
Locomotor	Hyperactivity, hypoactivity, coma, ataxia, circling, tremors, convulsions, paralysis, or prostration

Montgomery, C.A. Jr. (1990). *Cancer Bulletin* 42(4): 230-237

### General Endpoint References:

Canadian Council on Animal Care. (1998). Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching or testing. Ottawa, Canada

Everitt, J. I. (2014). The Future of Preclinical Animal Models in Pharmaceutical Discovery and Development: A Need to Bring In Cerebro to the In Vivo Discussions. *Toxicologic Pathology*, 43, 70–77.

Franco, N. H., Correia-Neves, M., & Olsson, I. A. S. (2012). How “humane” is your endpoint?-refining the science-driven approach for termination of animal studies of chronic infection. *PLoS Pathogens*, 8(1).

- Hendriksen & Morton, ed. (1998). Humane endpoints in animal experiments for biomedical research. Proceeding of the International Conference, 22-25 November 1998, Zeist, The Netherlands. Laboratory Animal Ltd, by Royal Society of Medicine Press Limited, London, England
- Hooijmans, C. R., Leenaars, M., & Ritskes-Hoitinga, M. (2010). A gold standard publication checklist to improve the quality of animal studies, to fully integrate the Three Rs, and to make systematic reviews more feasible. *Alternatives to Laboratory Animals : ATLA*, 38(2), 167–82.
- Institute for Laboratory Animal Research Journal. (2000). Humane Endpoints for Animals Used in Biomedical Research and Testing, 41(2).
- Madden, J. C., Hewitt, M., Przybylak, K., Vandebriel, R. J., Piersma, A. H., & Cronin, M. T. D. (2012). Strategies for the optimisation of in vivo experiments in accordance with the 3Rs philosophy. *Regulatory Toxicology and Pharmacology*, 63(1), 140–154.
- Morton and Griffith (1985), *Veterinary Record* 116:431-436.
- Montgomery (1990), *Cancer Bulletin* 42:230-237.
- Schuppli, C. A., Fraser, D., & McDonald, M. (2004). Expanding the three Rs to meet new challenges in humane animal experimentation. *Alternatives to Laboratory Animals : ATLA*, 32(5), 525–32.
- Stokes. (1999). Humane Endpoints in Animal Experiments for Laboratory Animals Used in Toxicity Testing Proceeding of the 3<sup>rd</sup> World Congress on Alternatives and Anima Use in the Life Sciences, 31 August – 2 September 1999, Bologana, Italy
- United Kingdom Co-ordinating Committee on Cancer Research. (1997). UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia, 2<sup>nd</sup> ed. London, England.
- Veterinary & Animal Ethics: Proceedings of the First International Conference on Veterinary and Animal Ethics, September 2011 - Wiley Online Library. (n.d.).
- Wathes, C. M., Corr, S. A., May, S. A., McCulloch, S. P., & Whiting, M. C. (Eds.). (2012). *Veterinary & Animal Ethics*. Oxford, UK: Blackwell Publishing Ltd. doi:10.1002/9781118384282

### **Tumor Size References:**

- Bullard DE, Schold SC Jr, Bigner SH, Bigner DD (1981), Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell lines. *J Neuropath Exp Neurol* 40:410-427.
- Hamm (1995), Proposed institutional animal care and use committee guidelines for death as an endpoint in rodent studies. *Contemp Top Lab Anim Sc* 34:69-71.
- Sung C, Dedrick RL, Hall WA, Johnson PA, Youle RJ (1993), The spatial distribution of immunotoxins in solid tumors: assessment by quantitative autoradiography. *Cancer Research* 53: 2092-2099.
- Tomayko MM and Reynolds CP (1989), Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol* 24:148-154.
- Welch DR, Chen P, Miele ME, McGary CT, Bower JM, Stanbridge EJ, Weissman BE (1994), Microcell-mediated transfer of chromosome 6 into metastatic human C8161 melanoma cells suppresses metastasis but does not inhibit tumorigenicity. *Oncogene* 9: 255-262.
- Workman P, et al; Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer* (2010) 102, 1555-1577