University at Buffalo Institutional Animal Care and Use Program
Policy on Humane Interventions and Endpoints

Purpose

This policy describes the expectation for the application of humane interventions to minimize pain and distress, sets criteria (humane endpoints) that should be used to prompt early euthanasia and describes the expectations for approval of toxicity experiments.

Definitions

- **Humane Interventions:** Actions designed to prevent or relieve unnecessary pain or distress.
- **Humane Endpoint:** Criteria that, once reached, animals must be euthanized unless otherwise described in an approved protocol.
- **Moribund:** The condition of an animal that is near death or in the process of dying.
- **LD50:** The dose of a toxin or infectious agent required to kill 50% of the animals.

Policy

1. **Humane Interventions**:
    a. The following actions are expected whenever **unmitigated pain or distress** are observed in research animals participating in IACUC-approved experiments:
        i. Provide any protocol-required analgesia or supportive therapy (ex. subcutaneous fluids, wet food).
        ii. Contact LAF Veterinary Staff if unexpected pain or distress is observed. A veterinarian is always on-call. Follow LAF SOPs to report concerns.
        iii. Increase the frequency of animal observations.
        iv. Consider modifications to housing and husbandry that may improve animal comfort. Coordinate with LAF Staff to make any modifications (ex. supplemental heating, separation of cage mates).
        v. Stop any painful or stressful procedures that are causing pain or distress above what is expected and described in the IACUC protocol.
        vi. Euthanasia may be necessary.

2. **Humane Endpoints**:
    a. **Unless otherwise described in an approved protocol**, IACUC expects that animals will be promptly euthanized when one or more of the following general conditions are observed:
        i. Chronic weight loss exceeding 20% of baseline bodyweight. Baseline should be measured prior to applying interventions that cause weight loss.

Effective Date: 2/1/2025
Next Review Date: 2/2028
First Approved: 1/21/2025
Revised: --

ii. For growing animals, failure to maintain normal weight gain within 15% of age-matched control animals.
iii. Body Condition Score (BCS) of less than 2 (out of 5 point scale).
iv. Facial Grimace Scale greater than 2. (NC3Rs: Grimace Scales)
v. Uncontrollable seizures, incoordination or paralysis.
vi. Hunched posture that is combined with lethargy and lack of grooming.
vii. Decreased mobility that interferes with normal eating, drinking or grooming.
viii. Weak response to external stimuli.
ix. Respiratory distress, as indicated by increased or decreased breathing rate, labored breathing or cyanosis.
x. Pale eyes, ears or extremities.
xi. Uncontrolled bleeding.
xii. Self-mutilation.
xiii. Mass that is ulcerated, necrotic and/or impairing normal function.
xiv. Tumors greater than 4 cm (widest diameter) in rats and tumors greater than 2 cm (widest diameter) in mice.

3. Monitoring Animals

a. Normal, healthy experimental animals must be observed at least once a day by qualified personnel. LAF Staff provide this service per LAF SOPs.
b. Animals in studies involving expected pain and/or distress will often require more frequent observations by Research Staff to effectively determine the time at which a specific, humane endpoint has been reached.
c. An appropriate monitoring schedule must be specified in the IACUC protocol for each study. This includes frequency, duration, parameters and expected actions.
d. Dependent on the study, monitoring charts may also be required.
    i. When required, specific monitoring charts are approved with the IACUC protocol and should be used as described.
    ii. At the conclusion of each experimental cohort, copies of monitoring charts should be sent to the IACUC.
    iii. Any changes to monitoring requirements and charts should be made via amendment to the IACUC protocol.

4. Moribund Animals

a. Animals found in this condition are near death of dying and should be euthanized.
b. Moribund animals may be comatose (unresponsive to stimuli) and past the point of having awareness of suffering.
c. Animals may experience pain and/or distress prior to reaching a moribund state. Therefore, stating that an animal will be euthanized when they become moribund is not appropriate, as euthanasia at this late stage does not reduce or alleviate suffering

that is likely experienced prior to reaching this state. Earlier humane endpoints should be used.

d. Commonly used signs of moribundity:
    i. Lack of responsiveness to manual stimulation (no righting reflex when placed on its side).
    ii. Complete immobility (does not move when touched).
    iii. Inability to eat or drink even if food/water are placed in close proximity.

5. Death as an Endpoint

a. With the goal of minimizing animal pain and distress, investigators are required to administer euthanasia in death endpoint experiments, rather than allowing the animal to die on its own. Surrogate criteria must be used that predict death (decreased surface body temperature, for example).
b. If euthanasia itself will compromise experimental validity, this must be scientifically justified in an approved protocol. Moreover, animals in these experiments must be monitored more frequently throughout the entire study (including holidays and weekends).
c. For death to be used as an endpoint, **4x per day monitoring (~every 6 hours including overnight)** is required. **This is non-negotiable.**
d. If a prolonged period of time (multiple days) is expected before the development of any abnormal clinical signs (i.e. animals are completely normal), this may be explained (and references must be given to substantiate this expectation). In such cases, a minimum of 1x per day monitoring is expected until animals are expected to develop abnormal clinical signs (when 4x per day monitoring MUST begin).
e. For experiments where scientific validity requires allowing an animal to die as an endpoint, the experimental design must reflect a rigorous effort to minimize the number of animals used.

6. LD50 Experiments and Alternative Approaches

a. The traditional LD50 (lethal dose 50) experiment to measure toxicity is widely criticized as inhumane and wasteful of animals. This procedure involves groups of animals that are exposed to increasing doses of a toxic substance or infectious agent to determine the dose required to kill 50% of the animals. UB's IACUC has established rigorous standards that must be met before an LD50 study can be approved. Both this policy and a corresponding **"LD50 Animal Study Proposal Checklist"** (focusing on where information should be included in Click) should be used.
b. Those investigators whose work requires death as an endpoint must review current statistical approaches for reducing animal numbers and apply these methods whenever possible.

c. As reviewed in the references below, alternative methods have been developed that reduce the number of vertebrate animals used in LD50 experiments without compromising experimental reliability. Investigators should plan to work with a research librarian to perform a comprehensive alternatives search. The bibliography of the search must be included in the IACUC protocol.
d. One approach that has been used to derive median values with relatively few samples is the up-and-down procedure. Whereas conventional methods using a dose-response experimental design generally employ 24 to 40 animals, LD50 values calculated through the up-and-down procedure can use as few as six animals. The up-and-down procedure yields essentially identical LD50 values as the conventional dose-response experiments. It must be considered, and if it cannot be used, this must be explained in the IACUC protocol.

7. IACUC Approval of Protocols Including Death as an Endpoint and/or LD50 Experiments
    a. Protocols must be submitted for Full Committee Review after all required information has been added. Refer to the "LD50 Animal Study Proposal Checklist".
    b. The IACUC must conduct and vote on a Harm-Benefit Analysis for such protocols at a fully convened IACUC meeting.
    c. Protocols will be reviewed annually, at which time the number of animals used in LD50 Experiments must be explicitly stated.
    d. Submission of monitoring charts is a firm expectation at the conclusion of each experimental cohort. Failure to properly complete and/or submit monitoring charts is considered noncompliance and could result in protocol suspension.

References:

1. Erhirhie E.O., Ihekwereme C.P., Ilodigwe E.E. 2018. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip. Toxicol. 11(1):5-12.
2. Saganuwan S.A., 2017. Toxicity studies of drugs and chemicals in animals: An overview. Bulg. J. Vet. Med. 20 (4): 291–318.
3. Parasuraman, S. 2011. Toxocological screening. J. Pharmacol. Pharmacother. 2(2):74-9.
4. Aldred A.J., Cha M.C., Meckling-Gill K.A. 2002. Determination of a humane endpoint in the L1210 model of murine leukemia. Contemporary Topics 41(2):24-27.
5. Rispin A., Farrar D., Margosches E., Gupta K., Stitzel K., Carr G., Greene M., Meyer W., McCall D. 2002. Alternative methods for the medial lethal dose (LD50) test: The up-and-down procedure for acute oral toxicity. ILAR J. 43(4):233-43.
6. Warn P.A., Brampton M.W., Sharp A., Morissey G., Steel N., Denning D.W., Priest T. 2002. Laboratory Animals 37:126-131.
7. Sass N. 2000. Humane endpoints and acute toxicity testing. ILAR J. 41(2):114-23.
8. Lichtman A.H. 1998. The up-and-down method substantially reduces the number of animals required to determine anti-nociceptive ED50 values. J. Pharmacol. Toxicol. Methods 40:81-5.

9. Hamm T.E. 1995. Proposed Institutional Animal Care and Use Committee guidelines for death as an endpoint in rodent studies. Contemporary Topics 34(3):69-71.
10. Laboratory Animal Welfare Bibliography, prepared by Scientists Center for Animal Welfare for the USDA National Agriculture Library, December 1989.
11. Tomasovic S.P., et. al. 1988. IACUC evaluation of experiments requiring death as an endpoint. Lab Animal 17:31-34.
12. UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia. 1988. Laboratory Animals 22:195-201. Reprinted in ILAR News 31:16-23 (1989) with Commentary.
13. Spinelli J.S., Morrish R.H. 1987. Pain and discomfort: How to recognize and manage pain associated with animal research. Invest. Radiol. 22:348-352.
14. Morton D.B., Griffiths P.H.M. 1985. Guidelines on the recognition of pain, distress, and discomfort in experimental animals and an hypothesis for assessment. The Veterinary Record 116:431-436.
15. Association of Primate Veterinarians' Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research. 2022. J Am Assoc Lab Anim Sci. 2020 Jan;59(1):6–8.
16. Humane endpoints in laboratory animal experimentation. 3Rs-Centre Utrecht Life Sciences. Accessed 10/2024.
17. Guidelines for Endpoints in Animal Study Proposals. Accessed 10/2024
18. Silverman J, Suckow MA and Murthy S. (2014) *The IACUC Handbook*. CRC Press. https://doi.org/10.1201/b17109