University of Notre Dame
Institutional Animal Care and Use Committee
Humane Endpoints in Animal Experimentation

Background

A variety of studies involving animals have the potential for poor clinical outcomes for the animal which might result in substantial pain and/or distress. Examples include studies involving infectious agents, tumor growth, and studies specifying death as an endpoint. While many circumstances are appropriately addressed with judicious use of analgesics to relieve pain and discomfort, use of such measures is impractical in some cases and may skew experimental data in others. For this reason, investigators should consider, and describe in detail, specific endpoints requiring euthanasia when there is a reasonable chance for animals to experience pain and/or distress that is more than momentary (e.g., injection via needle and syringe) or transient (postoperative pain) that is relieved with appropriate use of analgesics. Such a prospective approach can optimize the humane treatment of animals used in these studies. Further, consideration of such approaches fulfills regulatory and ethical mandates (1-4).

Guidelines

The following guidelines are adapted from published recommendations (5). Specific criteria relating to the use of animals in experiments which may produce substantial pain and/or distress include:

1. Frequent Monitoring: Animals must be observed and checked at least daily by personnel trained and experienced in recognizing signs of pain and illness. Ideally experiments should be terminated prior to the animal becoming debilitated. Animals on studies with death as an endpoint must be observed and checked twice daily. Frequency should be increased when animals begin to experience pain and/or distress. Monitoring includes weekends and holidays.

2. Clinical Signs of Pain/Distress: A number of clinical signs can be used as benchmarks for establishing when animals are in substantial pain/distress. The criteria for establishing endpoints vary with the type of study. In general, a combination of signs is used to determine the endpoint. These include:

Body Posture	Abnormal, hunched, tucked-in posture, guarding of limb
Hair Coat	Rough, lack of grooming
Respirations	Labored, abnormal respiratory sounds
Body Temperature	Persistent hyperthermia (fever), hypothermia (low body temp.)
Appetite	Persistent anorexia and/or adipsia (not eating and/or drinking)
Behavior	Non-responsive to stimuli, lethargy, overly sensitive to touch/sound, self mutilation
Discharges	From orifices, surgical wounds, persistent diarrhea

3. Tumor-Bearing Animals: Tumor studies vary with the type of tumor as well as the focus of the investigation (tumor formation vs. treatment procedure). For this reason it is important that the investigator clearly define the purpose of the study and the endpoints. The clinical signs of pain and/or distress are included as criteria. In addition, experiments should be terminated before a tumor reaches a predetermined size, ulceration occurs or tumor invasion into surrounding tissues interfere with normal bodily functions. The table below contains typical criteria for euthanasia in tumor studies:

Tumor size	>20 mm in diameter and/or exceeds 10% of normal body weight
Locomotion	Interferes with ability to access food and water

Body weight	More than 20% weight loss or rapid weight loss (15% in a few days)
Body condition	Decrease in body condition - animal appears thin or emaciated, skeletal structures are prominent, little or no flesh cover.
Tumor condition	Ulceration or abrasion of tumor surface
Tumor location	Interferes with locomotion, with bodily functions or provokes self-trauma

Records

Written records will allow the investigator to demonstrate that humane practices are being followed. For this reason, it is advisable to keep written records of all monitoring sessions, indicating animal identity, protocol number, date/time of observations, clinical signs and condition of the animals, and any palliative treatments provided. Written records are required for any surgical procedures and protocols that require observation or treatment logs. These records must be available to the IACUC during the semi-annual inspections.

IACUC Animal Protocols

To properly evaluate an animal use proposal that involves the possibility of substantial pain and/or distress, the following information is needed:

1. A written justification which includes what alternative endpoints and models are available and why they cannot be used.

2. Justification should detail the sources (databases) consulted, keywords used (one of which must be "alternatives"), the date of the search, and the years of the database searched.

3. A written justification which demonstrates that the minimal number of animals is being used. Statistical calculations are particularly useful in establishing this justification.

4. Any palliative measures that will be taken, including specifying endpoints using the criteria described earlier in this document.

5. If analgesics cannot be used, a written justification, including supporting documentation, for why they cannot be used is required.

References

1. Flecknell P and Silverman J (2000). Pain and distress. In *The IACUC Handbook*. Silverman J, Suckow MA, and MurthyS (Eds.). CRC Press, Boca Raton, FL.
2. National Institutes of Health (1986). Public Health Service policy on humane care and use of laboratory animals. Office of Protection from Research Risks, NIH, Bethesda, MD.
3. Office of the Federal Register, Code of Federal Regulations, Title 7; Part 371.4(b)(2), Animal and Plant Health Inspection Service – Veterinary Services, Washington, D.C., 1989 (revised 1998).
4. National Research Council, *Guide for the Care and Use of Laboratory Animals* (1996). National Academy Press, Washington, D.C.
5. Hamm TE (1995). Proposed institutional animal care and use committee guidelines for death as an endpoint in rodent studies. *Contemporary Topics in Laboratory Animal Science* 34(3):69-71.