

ENDPOINTS IN ANIMAL STUDY PROPOSALS

Clinical Signs or Stages of Morbidity as Endpoints

Experimental studies often involve procedures that cause clinical symptoms or morbidity in animals. Optimally, studies should be terminated when animals begin to exhibit morbid signs or clinical signs of disease if these endpoints are compatible with meeting the research objectives. Such endpoints minimize pain and distress and are preferable to death or moribundity as endpoints which involve considerably more pain and suffering.

Selection of earlier endpoints should address the following:

1. A method or set of criteria establishing an endpoint for euthanasia. There are several examples in the literature that might be considered including:
 - a. Evaluation of five aspects of an animal's condition as described by Morton and Griffiths. These are body weight, physical appearance, measurable clinical signs, unprovoked behavior and response to external stimuli.
 - b. Clinical observations used in cancer research and toxicological studies as described by Montgomery. Parameters include changes in general appearance, skin and fur, eyes, nose, mouth and head, respiration, urine, feces and locomotion.
 - c. General clinical signs including, but not limited to:
 - i. Rapid weight loss or total weight loss exceeding 20% of body weight (bwt) as compared to controls or starting weight.
 - ii. Diarrhea, if debilitating or prolonged (2-3 days), usually accompanied by dehydration.
 - iii. Spreading alopecia caused by disease that covers 25% or more body surface area.
 - iv. Excessive scratching or inability to rest due to dermal changes.
 - v. Rough hair coat, hunched posture, head tucked into abdomen, lethargy or persistent recumbancy.
 - vi. Persistent coughing, labored breathing, or nasal discharge.
 - vii. Jaundice and/or uncorrected anemia.
 - viii. Neurological signs.
 - ix. Bleeding from any orifice.
 - x. Self-induced trauma.
 - xi. Excessive or prolonged hypothermia or hyperthermia.
 - xii. Any condition that interferes with normal water and food intake or elimination.
 - d. Additional signs in neoplasia studies including, but not limited to:
 - i. A tumor burden greater than 10% bwt in rodents, or in the adult mouse, a size greater than 2 cm diameter .
 - ii. Any tumor growth that interferes with normal ambulation, breathing, or sleeping.
 - iii. Tumors that ulcerate, become necrotic or infected, or produce signs of discomfort such as increased scratching or rubbing.
 - iv. Hydrothorax or hydro-abdomen development to the point of respiratory compromise.
 - e. Any animal found unexpectedly to be moribund or to be cachectic.
2. A plan for monitoring the animals both before and after a change in any of the above aspects. This would generally increase from once a day to twice or more a day until endpoint(s) is/are reached.
3. Designation, 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.

ENDPOINTS IN ANIMAL STUDY PROPOSALS

Death or Moribundity as an Endpoint

Although infrequent, death or moribundity as endpoints may be necessary for some research projects and therefore included on an animal study proposal. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. In these studies, animals are permitted to die or become moribund, as a result of experimental procedures, without use of pain relieving measures, because such measures may compromise the experimental integrity of the study. 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 the individual Animal Care and Use Committees at NIH in reviewing proposal with death or moribundity as an endpoint.

Animal Study Proposals utilizing 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 utilized.
 - 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. Designated personnel, including the attending veterinarian, should be notified as soon as animals show signs of disease and an assessment made as soon as possible of the animals' condition and what actions are to be taken.
 - c. Consideration should be given to removing animals to individual cages when their condition deteriorates to a point where cannibalism is likely. Dead animals must be promptly removed.
 - d. Written records should be kept of all monitoring sessions.

Guidelines for Experimental Neoplasia Studies

To limit the discomfort, pain or distress which animals may experience in studies involving induction or treatment of neoplasia, the following guidelines should be considered by the Principal Investigator and addressed in the experimental design of the animal study proposal:

1. Frequency of Observation and Reporting: Animals should be observed daily after observable tumor growth has begun. Observations may be performed by either the investigative staff or animal care staff. When clinical deterioration is likely to be rapid, the investigative staff should be notified promptly and the investigative staff, along with the veterinarian(s) and care taking staff should agree on a responsible, monitoring and reporting regime. Animal exhibiting clinical symptoms should be promptly reported to the principal investigator.

ENDPOINTS IN ANIMAL STUDY PROPOSALS

2. Endpoints: Alternative endpoints to death or moribundity are encouraged. The following conditions generally fall into Category C:
- Subcutaneous Tumors: A tumor burden in rodents not exceeding 10% of body weight and in an adult mouse, a tumor diameter not exceeding 2 cm.
 - Multiple Site Tumor Inoculation or Metastasis: Endpoints in rodents that do not result in the development of significant clinical symptomatology as described in the Endpoint Guidelines under Morbidity as an Endpoint.
 - Anti-neoplastic Treatment: Endpoints in rodents that do not result in signs of toxicity due to the treatment modality. Toxic signs include those described in the Endpoint Guidelines under Morbidity as an Endpoint.

These guidelines may not be applicable to all neoplasia types or treatment modalities, but it is incumbent upon the Principal Investigator to define alternative endpoints, which will balance the requirements of the study with the well-being of the animals.

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, urine stain, 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, rales 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
- 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
- Institute for Laboratory Animal Research Journal. (2000). Humane Endpoints for Animals Used in Biomedical Research and Testing, 41(2).
- Morton and Griffith (1985), *Veterinary Record* 116:431-436.
- Montgomery (1990), *Cancer Bulletin* 42:230-237.
- Stokes. (1999). Humane Endpoints in Animal Experiments for Laboratory Animals Used in Toxicity Testing Proceeding of the 3rd World Congress on Alternatives and Anima Use in the Life Sciences, 31 August – 2 September 1999, Bologana, Italy
- Toth (1997). *Contemporary Topics*, 36:44-48.

ENDPOINTS IN ANIMAL STUDY PROPOSALS

General Endpoint References (cont'd) :

United Kingdom Co-ordinating Committee on Cancer Research. (1997). UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia, 2nd ed. London, England.

Tumor Size References:

Bullard et al. (1981), *J. Neuropath. Exp. Neurol.* 40:410-427.

Hamm (1995), *Contemporary Topics* 34:69-71

Sung et al. (1993), *Cancer Research*, 53:2092-2099.

Tomayko and Reynolds. (1998), *Cancer Chemother. Pharmacol.* 24:148-154

Welch et al. (1994), *Oncogene*, 9:255-262.

Modified from the ARAC Guidelines, National Institutes of Health, Office of Animal Care and Use