Center for Biomedical Research / IACUC
Standard Operating Procedure – Guidelines

Humane Endpoints

Procedure:

Endpoints are a part of every Animal Care and Use Protocol, and the IACUC must review the endpoint for every animal on the study. It is ideal when the scientific aims and objectives of the study can be accomplished without adverse effects, pain, or distress to the animal. However, this is not always possible and careful consideration must be given to the scientific requirements of the study; the expected and potential adverse effects the research animals may experience; the most likely time course and progression of those adverse effects; and the earliest most predictive indicators of present or impending adverse effects. Where pain or distress is a necessary part of the study, a humane endpoint must always be used and is:

The IACUC-approved, earliest scientifically-justified point at which pain or distress in an experimental animal can be prevented, terminated, or relieved, while meeting the scientific aims and objectives of the study.

The effective use of endpoints requires that properly trained and qualified individuals perform both general and study-specific observations of the research animals at appropriate time points. The assessment criteria and required response must be clearly defined, and the use of study-specific animal assessment records should be considered. Studies must be designed to minimize pain and/or distress. If pain or distress is unavoidable, then a scientific justification must be reviewed and approved by the IACUC, and the earliest possible endpoint compatible with answering the scientific question must be employed. Such endpoints are preferable to death or moribundity since they minimize pain and distress.

When initiating a new set of experiments, the potential for pain and/or distress may be unknown and/or the nature and extent of resulting morbidity, moribundity, and/or mortality cannot be anticipated. Therefore, smaller pilot studies may be useful as they can be instrumental to the development of an appropriate endpoint.

Finally, investigators performing studies that include pain or distress should, during the conduct of the studies, try to refine the endpoint and eliminate the necessity for any morbidity and/or mortality.

Morbidity

Animal protocols that include morbidity as an endpoint or animal procedures that have the potential to cause adverse effects should include criteria that establish when the endpoint has been reached and a plan for monitoring the animals, providing care if appropriate, and increasing the level of monitoring as necessary.

Death or Moribundity

While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there may be studies that require moribundity or mortality as an endpoint. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. Commonly used signs of moribundity include, but are not limited to, lack of responsiveness to manual stimulation, immobility, and/or an inability to eat or drink. Animal protocols utilizing death or moribundity as an endpoint should contain the following information:

1. The scientific rationale for death or moribundity as an endpoint, including what alternatives were reconsidered; why morbidity as an endpoint cannot be used; the estimated number of animals that will be allowed to reach morbundity/death; and whether animals will be euthanized when moribund and if not, what information is to be gained in the interval between moribundity and death.

2. Assurances that the frequency of observation will be increased when animals exhibit signs of morbidity and that written records will be kept of the monitoring and observations.

Guidelines for Death as an Endpoint in Rodent Studies

Death as an endpoint in animal experiments must be appropriately justified by the investigator and approved by the IACUC. The earliest endpoint of an experiment must be chosen and the animal should be euthanized prior to death if possible. The following guidelines have been developed in the context of the current United States Regulations governing the humane care and use of animals in research:

To properly evaluate a proposal that involves death as an endpoint, the following information is needed:

1. A written justification, which includes the alternative endpoints that were considered and why death as an endpoint was selected.
2. A written justification for the numbers of animals proposed, which demonstrates that a minimum number is being used and that alternative endpoints other than death were considered and used whenever possible.
3. If analgesics cannot be used, a written justification for why they cannot be used whenever possible.

Investigators who seek approval from the IACUC to conduct an experiment that may lead to death must also monitor animals at least twice daily (in the early morning and the late afternoon including weekends and holidays) by personnel trained and experienced in recognizing signs of illness, injury, or abnormal behavior for at least the following:

- Abnormal appearance: abnormal posture, rough coat, head tucked into abdomen, exudate around eyes and/or nose, skin lesions, abnormal breathing.
- Abnormal activity: abnormal movement, decreased food or water intake, self-mutilation.

The frequency of observation should be increased once animals are found to be experiencing pain, distress, or death.

Any animal evidencing abnormalities must be removed from group housing situations and housed individually with easy access to food and water. Personnel with training to determine what should be done with these animals must be notified and make an assessment as soon as possible (within a few hours). If personnel directly responsible for the project cannot be contacted for some reason, the Attending Veterinarian should be contacted to provide care for the animals. It is good practice to determine from the investigator, in advance of an emergency, what procedures should be used. In all instances, the Attending Veterinarian should be notified that animals are showing clinical signs of disease. Animals showing any of the following signs must be euthanized by use of approved methods:

a. Prolonged inability to ambulate or maintain an upright position that prevents the animal's easy access to food and/or water.
b. Agonal breathing and cyanosis.
c. Severe muscular atrophy or other signs of emaciation.

Written records of all monitoring sessions must include:

1. Identity of the animals
2. IACUC protocol numbers
3. Date and time of the observations
4. Name of the person observing the animals
5. The number of animals evidence clinically abnormal behavior or death
6. Methods used to alleviate all problems observed

These records must be maintained for at least three years after the completion of the animal protocol and made available to the Attending Veterinarian and/or the IACUC on request.

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Behavior Score Sheet

Date / AM or PM	Body Posture	Activity Level	Eye Appearance	Score
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	
	0 1 2 3	0 1 2 3	0 1 2 3	

Body Posture:
0: normal posture
1: mildly hunched
2: moderately hunched
3: very hunched

Activity Level:
0: normal activity
1: slightly reduced activity
2: moving slowly
3: moving reluctantly/not at all

Eye Appearance:
0: eyes open, <25% closed
1: eyes 26-50% closed
2: eyes 51-75% closed
3: eyes 76-100% closed

References:

Adapted from NIH intramural guidelines:
http://oacu.od.nih.gov/ARAC/documents/ASP_Endpoints.pdf

Alternatives to Animal Testing on the Web (2004), Humane Endpoints Database.http://ocw.jhsph.edu/courses/HumaneScience/PDFs/CAATLecture7.pdf

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Canadian Council on Animal Care (1998), Guidelines on: Choosing an appropriate endpoint in experiments using animals for research, teaching and testing. Ottawa, Canada.Hendriksen CFM and Morton DB, ed. (1998), Humane Endpoints in Animal Experiments for Biomedical Research. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands. Laboratory Animals 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: No. 2

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Morton DB and Griffiths PHM (1985), Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Veterinary Record 116:431-43.

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Ray MA, et al (2010) Identification of Markers for Imminent Death in Mice Used in Longevity and Aging Research. JAALAS 48(3):282-288.Recognition and Alleviation of Pain In Laboratory Animals; NRC 2009.

Stokes WS (1999), Humane Endpoints in Animal Experiments for Laboratory Animals Used in Toxicity Testing Proceedings of the 3rd World Congress on Alternatives and Animal use in the Life Sciences, 31 August - 2 September 1999, Bologna, Italy.

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.

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