



ANIMAL TRACKS

A newsletter for the Duke research community



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<http://vetmed.duhs.duke.edu>

HUMANE ENDPOINTS FOR RESEARCH ANIMALS

Animal pain, distress, or suffering is generally not necessary for animal experiments. In fact, animals in pain, suffering or in distress can significantly complicate research data outcomes and may confuse the findings of an otherwise well designed study while wasting the time and resources of the researcher. Careful consideration of clear study endpoints, after which animals will be provided with analgesia, removed from the study, or euthanized will maximize the reliability of outcome data while assuring humane attention to creatures which can feel pain and can suffer. Employing humane endpoints in the design and performance of animal research activities is, for many reasons, the right thing to do!

According to federal regulations (USDA 9th CFR; PHS Policy; various federal laws), the Institutional Animal Care & Use Committee (IACUC) must consider whether the animals enrolled in a specific study are provided with appropriate analgesia, are assigned reasonable humane endpoints, and are protected from unnecessary pain or distress. In fulfilling its federal mandate, the IACUC will review all uses of animals which may involve procedures that cause clinical symptoms or morbidity in animals; more specifically, the IACUC must determine whether the researcher has sufficiently considered the impact of the study upon the animal being used in the study. For example, the IACUC will evaluate:

- The expected and possible adverse effects the research animals may experience in the study (e.g. pain, distress, illness, etc.);
- The most likely time course / progression of adverse effects (e.g. is tumor metastasis likely? Does infection often following this procedure?);
- The earliest or most predictive indicators of present or impending adverse effects (e.g. Is anemia likely 15 days after treatment? Does pneumonia generally occur after 20 days of this therapy?);
- The researcher proposed endpoints (e.g. what will occur of an animal reaches a specified painful condition); or
- Any argument that scientific requirements justify modification of the humane endpoints.

See Humane Endpoints Page 2

JUSTIFYING ANIMAL NUMBERS (Tips for Successful Protocol Approval)

The primary goal of estimating sample size in the planning of experiments is to estimate the minimum number of animals required to accomplish the research goal. A secondary goal is minimization of the numbers of animals used. Why is minimization of animal use not a primary goal? If we minimize the numbers of animals and do not accomplish the scientific objective, then all of the animals used have been wasted – the data is not usable or does not prove the thesis and more animal must be used. **TIP: When submitting an animal protocol or amendment, always assure you have requested sufficient animals to accomplish the scientific goal. Then try to reduce or consolidate groups of animals where possible (e.g. one control group serving two purposes).**

How do you know how many animals will be required? The basis for an appropriate justification of animal numbers depends upon the nature of the proposed activity. It is not possible to propose a 'one-size-fits-all' justification. There are at least five types of studies, and each has a special basis for justifying the animal numbers. For example:

- **Antibody or tissue production projects** use of animals to produce antibodies or specific tissue types. **TIP: A strong basis for justification is the amount of antibody/tissue required compared to ability of an individual animal to provide the needed amount, etc.** Clearly describing this relationship is critical justification for obtaining IACUC approval.

See Justifying Numbers Page 4

Upcoming Dates & Deadlines

October 2	SC meeting
October 6	New protocol deadline
October 6	SC amendment deadline
October 16	SC meeting
October 23	IACUC meeting
October 27	SC amendment deadline
November 3	New protocol deadline
November 6	SC amendment deadline
November 20	IACUC meeting

Deadlines are 5 PM on the date listed! No exceptions!

The effective use of study endpoints requires properly qualified individuals perform both general and study-specific observations of the research animals at appropriate time points. However, such performance requires a clear understanding of normal behavior in the animal and a reasonable expectation of the progression of disease or an increasingly infirmed condition of the animal.

Optimally, live animal studies are terminated when animals begin to exhibit clinical signs of disease; especially if this endpoint is compatible with meeting the research objectives. Termination of animal studies prior to causing the animal significant pain or distress is necessary both for minimizing unnecessary pain and distress in the research subject, and because such performance is the ethical and appropriate thing to do. Termination of the animal's participation in the study upon observation of animal pain or distress is preferable to death.

Except in the very few cases where the IACUC has approved a 'death as an endpoint' study, no animal should be allowed to 'die naturally.' In research where there is a high likelihood of animals dying if the disease is allowed to proceed unmitigated, there will routinely be a point where the data obtained from the animal begins to decline in quality – a point research data is no longer useful. The researcher should determine what level of animal condition is likely to cause less reliable inferences, and at that point, euthanasia must be considered.

Moribund: Of all terms describing animal condition, moribundancy is one of the most difficult to accurately define. The moribund condition is defined as a clinically irreversible condition leading inevitably to death. As a general rule, proposals where moribundancy is a potential outcome for the animal subject should consider the following:

- **Criteria that establish when the endpoint has been reached:** There are several examples that might be considered as an outline for selection of clear end point criteria:

- **Body Condition (BC) Scoring for determining moribundancy:**

- **BC1: Emaciated**

- Skeletal structure prominent
- Ribs prominent
- Little to no fat covering
- Vertebrae distinctly segments



NOTE: BC 1 would be classified as moribund and would require euthanasia!

BC2: Under conditioned:

- Skeletal structure observable
- Ribs observed but soft appearing
- Minimal fat
- Vertebrae evident; pelvis palpable



BC 3: Well conditioned:

- Skeletal structure not seen
- Ribs not seen
- Smooth rounded appearance
- Vertebrae / pelvis palpable with slight pressure



BC 4: Over conditioned

- Rounded body shape
- No skeletal structure observed
- Vertebrae / pelvis palpable with firm pressure



BC 5: Obese

- Bulky body shape



- **General Appearance Assessment for determining moribundancy:** Moribund (requiring euthanasia) could be classified as have 5 or more of the following signs:

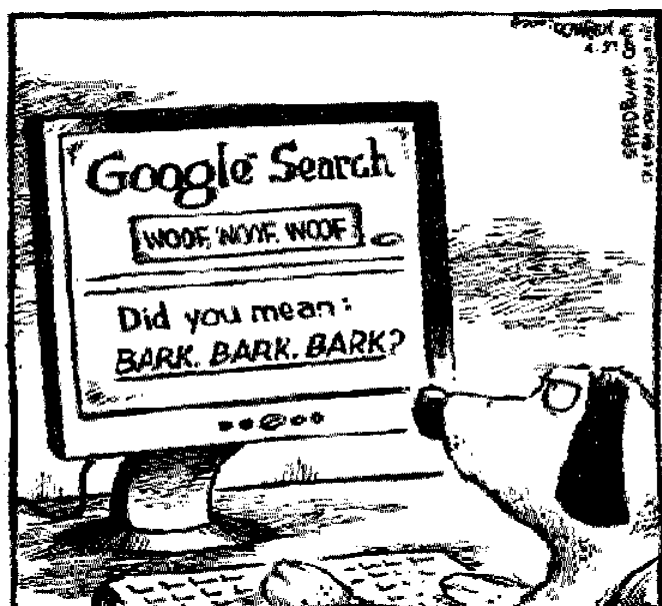
- Loss of skin turgor (dehydrated)
- Ruffled, unkempt fur
- Dull eyes,
- Dry cracked nose or mouth
- Rapid abdominal respiration
- Zero to minimal urine over a 24 hour cycle
- No feces within 24 hours
- No locomotion or painful locomotion
- Body weight less than 60% of normal
- Measurable clinical signs, depending on severity and duration, that may independently constitute an endpoint, or might be included in the assessment criteria. These include, but are not limited to:
 - Sudden unexpected weight loss (tumor studies may see weight gain)
 - Diarrhea, especially if debilitating
 - Progressive dermatitis, especially if pruritic
 - Rough hair coat, hunched posture, lethargy or persistent recumbency.
 - Coughing, labored breathing, nasal discharge.
 - Jaundice and/or anemia
 - Neurological signs
 - Bleeding from any orifice
 - Self-induced trauma (often an indicator of pain)
- Unprovoked behavior of biting or vocalizations
- Strong adverse response to external stimuli
- Any condition interfering with eating or drinking (e.g. difficulty with ambulation)
- Excessive or prolonged hyperthermia or hypothermia.

Additional signs for neoplasia studies: These signs may constitute an endpoint include, but are not limited to:

- Mice with tumors 2000 mm³ in size (which is roughly 10% baseline body weight) or greater, or rats with tumors 5000 mm³ in size or greater. NOTE: Tumors may be measured using the following formula: $TV = [(Width)^2 \times Length] / 2$.
- Tumors that are ulcerated. If an exemption is provided for this condition, then the affected animals are required to be single housed (may require protocol amendment and / or alternate environmental enrichment or medical treatment).
- Tumors where the animals chew on the lesion or pay undue attention to the ulcer.
- Tumors that interfere with 'normal' animal functions (e.g. eat, drink, or ambulate).

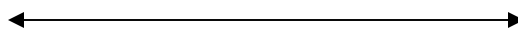
A plan for monitoring the animals both before and after the period where the above signs may be observed: Daily monitoring is the baseline for all animal care and use. Daily monitoring also includes assessment on weekends and holidays. During periods when signs of disease or progression of disease are likely, the frequency of assessment must be increased. The plan for monitoring must be included in the animal protocol proposal.

Identification of personnel responsible for evaluation of humane endpoints: The IACUC will want to know who will serve as the responsible individual to assure humane endpoints are maintained as approved by the IACUC. Checklists, spreadsheets, or score sheets may be helpful to ensuring the required observations are performed as approved; interpreted as required; and documented as necessary.



HUMANE ENDPOINT REFERENCES

- Alternatives to Animal Testing on the Web, Humane Endpoints Database. (<http://apps1.jhsph.edu/altweb/humane/>) John Hopkins Center for Alternatives to Animal Testing. Baltimore.
- Canadian Council on Animal Care, Guidelines on: Choosing an appropriate endpoint in experiments using animals for research, teaching and testing. Ottawa, Canada.
- Hendriksen CFM and Morton DB, ed, 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.
- Montgomery CA, Oncological and toxicological research: Alleviation and control of pain and distress in laboratory animals. Cancer Bulletin 42:230-237.
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- Stokes WS, 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.
- Toth L, The moribund state as an experimental endpoint. Contemp Top Lab Anim Sc 36:44-48.
- Ullman-Culleré MH and Foltz CJ, 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, UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia, 2nd ed. London, England.



TUMOR SIZE REFERENCES

- Bullard DE, Schold SC Jr, Bigner SH, Bigner DD, Growth and chemotherapeutic response in athymic mice of tumors arising from human glioma-derived cell lines. J Neuropath Exp Neurol 40:410-427.
- Hamm, 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, The spatial distribution of immunotoxins in solid tumors: assessment by quantitative autoradiography. Cancer Research 53: 2092-2099.
- Tomayko MM and Reynolds CP, Determination of subcutaneous tumor size in athymic (nude) mice. Cancer chemother Pharmacol 24:148-154.

- **Exploratory studies** may use of animals to demonstrate a simple up or down success (or failure) of a desired goal as one would have with a simple question of can we create offspring with our specific transgene, or not. **TIP: These studies may be justified by a simple probability of success of the experimental procedure.** For example, if male and female of a certain known genotype are bred, we would expect 'x' % of the offspring to demonstrate the desired genotype.
- **Pilot studies** generally involve use of small numbers of animals for a preliminary study which will help identify/clarify the proposed hypothesis, identify problem areas with the research plan, or develop specific procedures for a successful outcome. In pilot studies, the numbers are not usually sufficient to apply firm statistical tests, and not enough is known to allow an estimation of variances or probability. **TIP: In these cases, the animal numbers requested may be based upon prior literature or the investigators' professional judgment. It is always a wise step to propose capturing the pilot study numbers as part of the more extensive experimental protocol.**
- **Studies requiring statistical analysis** include those uses of animals where there exists a designed approach for answering a specific hypothesis, which will result in inferences from the data to a larger group. **TIP: Most often this is accomplished through inferential statistical procedures (e.g., analysis of variance, regression, categorical data analysis). Proposed numbers justifications should use a power analysis.** The IACUC expects to see values for α , β , s , and the effect size used in the power analysis.
- **Teaching projects** where animals are used for the purpose of teaching students. **TIP: Consider animal numbers based upon a specified student-to-animal ratio. The choice of the specified ratio must be explained in the justification statement, and must make sense. Generally, 3-4 students per animal is easier to justify than 1 animal per student.**

TIP: Visit the ILAR Journal's extensive review of this topic at URL: http://dels.nas.edu/ilar_n/ilarjournal/43_4/

TIP: Visit this interactive web tool which helps determine the correct kind of analysis for your data. The URL is: <http://www.microsirris.com/Statistical%20Decision%20Tree/>

Another site (from Bill Trochim @ Cornell) to help determine the proper statistical assessment tools can be found at URL: <http://www.socialresearchmethods.net/selstat/ssstart.htm>

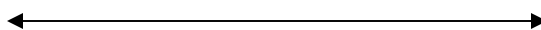
STANDARD OPERATING PROCEDURE REQUIREMENTS FOR ABSL2 CONTAINMENT AT DUKE

The Principal Investigator (PI) has the responsibility to inform the laboratory personnel of the appropriate research procedures. When using hazardous or regulated biological agents the PI must prepare a written Standard Operating Procedure (SOP) outlining the necessary precautions to safely conduct research. An SOP is a set of specific guidelines designed to address the methods that will be used and the safe handling of biological agents. The SOP must be available in the laboratory.

The SOP is a valuable tool and worth the preparation time. A well-written SOP can be used to satisfy several compliance requirements. SOP should be written for all procedures that pose an identified potential risk to the health and safety of the laboratory personnel, although a separate SOP does not need to be written for each individual experiment, procedures with the same hazards can be combined into one SOP.

The process of writing SOPs requires an individual to think through all steps of a procedure and perform a risk assessment before work has begun. The best approach to writing an SOP is to do it, write it and test it. Be brief and succinct; the shorter the better. A SOP template is available on OESO Biosafety Web Site:

<http://www.safety.duke.edu/BioSafety/Animals.htm>



OESO HAS SEVERAL GUIDELINES FOR SOP DEVELOPMENT OF HAZARDOUS AGENT USE IN ANIMAL PROTOCOLS

OESO Biosafety Division has a great web site which assists researchers with specific SOP development! For example:

- [Guide for Developing an SOP for the use of Bio-hazards in Animals](#)
- [Guide for Developing SOP for the use of Hazardous Drugs](#)
- [SOP for the use of Toxic Chemicals in Animals](#)
- [Guidelines for the Safe Handling of Animals Exposed to LPS in Research](#)
- [Radiation Safety Animal Care and Use Protocol Wizard](#)

You can reach this site and use these links by going to the OESO Biosafety site at:
<http://www.safety.duke.edu/BioSafety/Animals.htm>



OAWA's Brown Bag Seminar

Monday, October 6th, 2008

Noon – 1 p.m.

Bryan Research Building: Room 103

Ms. Tina Tyson, JD

Chief Compliance Officer of the Duke
School of Medicine will be presenting:

Hi! I'm From Compliance, and I'm Here to Help You Really!

Tina Tyson, J.D. and Chief Compliance Officer of the School of Medicine will provide a review of the SOM Compliance Office and the present compliance environment. During her presentation, Ms. Tyson will review some "mishaps" that have occurred on university campuses and how each of us can prevent such mishaps; or if they do occur, how we can effectively manage them to discourage significant negative fall-out. She will end her session with a period of Q&A, allowing the attendees to pose questions surrounding preferred compliance outcomes within their laboratory.

The presentation will be on **Monday, October 6th, 2008 from noon to 1 p.m.**

The session will be held in room **103 of the Bryan Research Building**, located at 421 Research Drive, on Duke University's West Campus.

Attendees are encouraged to bring a lunch. OAWA will provide drinks and desserts.

Please plan on arriving prior to noon in order to get refreshments, sign in, and be seated.

For those who will be coming from off campus, driving directions and parking information can be found at the following link: <http://neuro.duke.edu/Links/map.htm>

This session will count for 1 CEU of AALAS In-house Training Credit