



The ACC Connection

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WELCOME to the 13th edition of the **ACC Connection**, a bi-monthly newsletter designed to help researchers with questions regarding animal research at the University of Connecticut Health Center. Our 13th issue is designed to help investigators establish humane endpoints for animal care and use protocols.

The establishment of humane endpoints is an important consideration when writing animal care and use protocol applications. Appropriate endpoints must be chosen based on consideration of the scientific requirements of the study, the expected and possible adverse effects the animals may experience, the expected time course and progression of adverse effects, and the earliest predictive indicators of adverse effects. It is **strongly encouraged** that a Principal Investigator consult with the attending veterinarian when determining appropriate endpoints for a scientific study. If a PI is using a USDA-regulated species (simply put, all warm-blooded vertebrate animals except mice, rats, and birds), this consultation is **required** by the Animal Welfare Act.

Regulations

The Animal Welfare Act, the Animal Welfare Regulations, Animal Care Policies (USDA), the U.S. Government Principles for the Care and Utilization of Animals Used in Research, Testing, and Training, and PHS Policy require that investigators establish appropriate and humane endpoints for their studies; in other words, establishing the criteria and the process for timely intervention and removal of animals from study. In addition, the methods used to eliminate or ameliorate pain and distress in animals on study must be described in the protocol(s) that are reviewed and approved by the ACC.

Animal Welfare Act 7 U.S.C. chapter 54, section 2143 regarding standards and certification process for humane handling, care, treatment, and transportation of animals states that (a)(3)(A) "the standards with respect to animals in research facilities, include requirements for animal care, treatment, and practices in experimental procedures to ensure that animal pain and distress are minimized, including adequate veterinary care with the appropriate use of anesthetic, analgesic, tranquilizing drugs, or euthanasia".

Animal Welfare Regulations expand this with 9 CFR 2.31 (e)(4) "A description of procedures designed to assure that discomfort and pain to animals will be limited to that which is unavoidable for the conduct of scientifically valuable research, including provision for the use of analgesic, anesthetic, and tranquilizing drugs where indicated and appropriate to minimize discomfort and pain to the animals" and (5) "A description of any euthanasia method to be used."

PHS Policy (IV.C.1.a-c) and US Government Principles (V, VI) state much of the same thing. In addition, the *Guide for the Care and Use of Laboratory Animals* states that a protocol must contain "criteria and process for timely intervention, removal of animals from a study, or euthanasia if painful or stressful outcomes are anticipated".

Establishing Humane Endpoints

Federal laws and institutional policies require that the criteria and process for timely intervention and removal of animals from study be specified in animal care and use protocols. In addition, the methods used to eliminate or ameliorate pain or distress in animals on study must be described in the protocol(s) that are reviewed and approved by the ACC. It is essential that properly qualified personnel monitor the animals at appropriate intervals to ensure adequate observation and care of the animals. It is preferable to use the earliest endpoints compatible with the scientific requirements of the study. It is also preferable to use quantitative endpoints rather than qualitative endpoints. Endpoints presented in this document are general guidelines; should you wish information specific to your experiment, please contact the attending veterinarian.

Assessment of Pain in Animals

Anything that would be considered painful or distressful to humans must be considered, by law, to be painful or distressful in animals unless the contrary has been established. Acute pain/distress in animals is generally rapid in onset and more intense than chronic pain. Chronic pain/distress may be more intermittent or less intense than acute pain/distress and is often more difficult to assess.

The following are signs that may be exhibited by an animal in acute pain/distress:

Guarding- animal protects painful area by moving away or biting the handler

Vocalization- animal may vocalize on movement or on palpation of the painful area

Self-mutilation- animal may repeatedly lick, bite, scratch, or shake the painful area

Restlessness- pacing, constant shifting or weight, repeated standing up or lying down

Abnormal ambulation- reluctance or difficulty in moving or risking from a lying to a standing position

Abnormal postures- hunched posture, head hanging down, and stiff legged, tucked abdomen

The following are signs that may be exhibited by an animal in chronic pain/distress:

General appearance- clinically evident body weight loss and/or dehydration

General behavior- Change in temperament or behavior towards handler

Physical appearance- Lack of self-grooming, as evidenced by ruffled or soiled hair coat

Abnormal ambulation- Reluctance to move or ambulate

Humane Endpoints in Non-Tumor Bearing Animal Models

There are some typical parameters that should be considered when establishing humane endpoints. These include the body weight of the animals as compared to age-matched, untreated cohorts or to a defined baseline; the general appearance of the animal; the behavior of the animal; the response of the animal to external stimuli; measurable clinical signs (e.g., body temperature, blood cell counts); the ability of an animal to gain access to food and water and normal eating/drinking habits; and the ability of an animal to ambulate.

There are some endpoints that would require notification of the attending veterinarian and initiation of treatment, removal or animals from study, and/or euthanasia of the animals. These include, but are not limited to, the following: rapid body weight loss (>10% in 48 hours or >20% over the course of the experiment); debilitating diarrhea (>48 hours in duration); self-induced trauma, bleeding from any orifice, neurological signs incompatible with maintenance of normal life functions (e.g., inability to eat, drink, or ambulate); excessive or prolonged (>24 hours) hyper- or hypothermia; respiratory difficulties (e.g., labored breathing, nasal discharge, coughing); ambulatory difficulties, jaundice or anemia; change in behavior (e.g., lethargy, hunched posture); inability to gain access to food or water and eat/drink normally; non-responsive to external stimuli; and moribund condition.

Any animal found moribund should be euthanized immediately or the attending veterinarian should be notified for consultation on immediate medical intervention plans. For group-housed animals, consideration should be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely.

Humane Endpoints in Tumor-Bearing Animal Models

Special consideration should be given to tumor-bearing animal models when establishing humane endpoints.

Subcutaneous Tumors- The following are endpoints typical of procedures involving subcutaneous tumors: tumor volume in excess of 2000mm³; tumor necrosis (purulent discharge, necrotic area, ulceration with bleeding); self-mutilation of tumor implant sites; and the tumor inhibiting normal movement or normal physiologic function.

Intraperitoneal Tumors- The following are endpoints typical of procedures involving intra-peritoneal tumors: body weight gain or loss of >20% as compared to age-matched, non-treated cohorts; formation of excessive fluid in the abdomen characterized by gross abdominal distension, respiratory difficulty, or inability to ambulate; and the tumor inhibiting normal movement or normal physiologic function.

Metastatic or intravenous Tumors- The following are endpoints typical of procedures involving metastatic tumors or tumors resulting from IV administration: body weight loss of >20% as compared to age-matched, non-treated cohorts; respiratory difficulty as evidenced by cyanosis, open-mouthed breathing, or other evidence of labored respiration; and the tumor inhibiting normal movement or normal physiologic function.

Monitoring of Animals

Any animal experiencing adverse clinical signs should be monitored a minimum of twice each day. Monitoring and clinical care should be provided and documented similarly on weekends and holidays.

Written records of all monitoring and treatments must be maintained in order to assure that adequate care is being provided to the animals. Checklists/score sheets may be helpful in ensuring appropriate observations are being made, consistently interpreted, and properly documented. Personnel should be identified who are responsible for the evaluation, record keeping, and notification of the Principal Investigator and/or attending veterinarian.

The plan for the parameters to be monitored, the frequency of monitoring, the names of the qualified personnel who will perform the evaluations, and the criteria for euthanasia must be described in the animal care and use protocol submitted to the ACC.

Death as an Endpoint

Studies should be terminated when animals begin to exhibit adverse clinical signs, if this endpoint is compatible with meeting research objectives, since such endpoints minimize pain and distress. If a study requires moribundity or mortality as an endpoint (this is **NOT** euthanasia, but the natural death of the animal as a direct result of the experimental procedures being performed), this must be specifically described and approved in the animal care and use protocol submitted to the ACC. Animal care and use protocols that propose the use of death as an endpoint must include the following information: the scientific rationale for death as an endpoint; considerations of alternative endpoints; why pain/distress relieving medications and/or treatments cannot be utilized; number of animals to be used and why this is the minimum number required; plan that details the parameters to be monitored, the timetable and frequency of monitoring, the personnel responsible for making recorded observations; and what information is to be gained in the interval between moribundity and death.

Under no circumstances will death as an endpoint will be allowed simply because it is an easy and unambiguous criterion to apply.

Frequently Asked Questions

How should I develop appropriate endpoints for my study?

This can be difficult- endpoints need to be in line with the scientific requirements of the study and also be humane for the animals being used. Consultation with the attending veterinarian can often help to identify appropriate and humane endpoints for an animal study. It is necessary to evaluate the procedures being done to the animals so that expected outcomes can be addressed.

What should I do if I come across some adverse effects that I didn't anticipate?

All unanticipated adverse effects involving the use of laboratory animals must be reported to the Animal Care Committee. Please refer to the ACC policy on reporting these events on the ACC website: <http://clacc.uchc.edu/ACC/Policies/MorbidityMortality.htm>.

Upcoming Training, March 2008 – April 2008

The following is the list of classes currently scheduled by the ACC office; please contact the website for any unanticipated changes.

New Animal Users Initial Basic Core Training

Monday, March 17	9:00 am – 12:00 pm	Building 20 conference room
Monday, April 21	9:00 am – 12:00 pm	Building 20 conference room

New Institutional, State, or Federal Regulations

Institutional

ACC Policy	2/28/08	Assigning Reviewers to New protocols. To view policy, go to: http://clacc.uchc.edu/ACC/Policies/AssignReviewers.htm .
ACC Policy	2/28/08	Animal Transfers to Approved Protocols- USDA Species. To view policy, go to: http://clacc.uchc.edu/ACC/Policies/AnimalTransfers.htm .

State

None

Federal

Chimp Haven Home Act	Signed into law by President Bush on December 26, 2007. This law eliminates the possibility that chimpanzees retired into the National Chimpanzee Sanctuary System could be returned to research.
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Next Issue: Reporting Animal Welfare Concerns

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