POLICY FOR DETERMINATION OF HUMANE ENDPOINTS
FOR ANIMALS USED IN RESEARCH

Adopted April 17, 2007
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PURPOSE:

The obligation to minimize or eliminate unnecessary pain and distress that animals may experience when used in biomedical research is clearly stated in the USDA Animal Welfare Act and the PHS Policy on Humane Care and Use of Laboratory Animals. To that end, the LSU Health Sciences Center Animal Care and Use Committee (ACUC) reviews all proposed and current animal use to ensure that we, as an institution, meet both our legal and ethical obligations with regard to animal research.

Animal models used for the study of infectious diseases pose particular challenges as these animals may experience significant pain and/or distress as a consequence of the disease process being studied. However, the IACUC recognizes the importance of these types of studies to the improvement of human health care. Furthermore, the IACUC recognizes the difficulty in choosing appropriate humane endpoints for animals in these types of studies. Seeking earlier, more humane endpoints, prior to impending death, can result in significant reductions in the potential pain and distress experienced by these animals. However, if these endpoints do not accurately predict the outcome of the experiment, they may vastly alter the research results obtained. For example, animals becoming sick in an infection challenge/vaccine efficacy trial might be euthanized for humane reasons when in fact they could have survived the challenge, proving that the new vaccine was an effective treatment.

The following policy has been generated to provide guidelines to investigators performing these types of experiments. The specific intent is to minimize animal pain and distress while allowing the researcher to collect data that is meaningful and reliable. The decision to euthanize an animal must be made with appropriate clinical judgment, taking into account the severity of the condition, the amount of pain or distress, and the potential for alteration of or loss of valuable data. To accomplish this, we have developed suggestions for clinically scoring these animals and using humane endpoints to reliably predict morbidity. Due to the specific characteristics of disease caused by a particular infectious agent, it is not possible to have a list of all clinical signs that will occur in all experiments. Moreover, clinical signs can vary with the species, strain or age of animal and the method of recording. Therefore, use of pilot studies for individual cases is, highly recommended and may be required for endpoint determination. Prior experience by the investigator may also be utilized as justification for the chosen endpoints, but all endpoints must be approved by the IACUC.
GENERAL CONSIDERATIONS FOR ALL ANIMAL EXPERIMENTS:

- The earliest possible endpoints that are indicators of severe pain or impending death and do not compromise the value of experimental data should be used as indications for euthanizing an animal. These endpoints should be determined during a pilot study and should serve two purposes: 1) to determine humane endpoints and 2) to provide reasonable assurance to the investigator that early euthanasia or any supportive/therapeutic treatment is not altering final outcome (i.e. are animals being euthanized that would have ultimately survived without intervention).
- Studies should be terminated prior to their planned termination time if the objectives of the study have been satisfied, or it is obvious that they will not be achieved.
- Studies should build on existing knowledge about the agent or substance to be tested. This enables better prediction of the likely signs and timing of adverse effects, and allows those conducting the study to incorporate these endpoints into the experimental protocol.
- The successful application of humane endpoints is dependent on the involvement of all members of the study team. Personnel must be adequately trained and aware of their individual roles and responsibilities.

TYPES OF RESEARCH ENDPOINTS:

There are a number of studies in the literature that have evaluated various endpoints for their ability to reliably predict morbidity. These should serve as useful guidelines, but each combination of infectious agent and animal will require development of a specific set of clinical parameters to be followed and scored to determine which physiologic or behavioral changes best predict morbidity for that particular study. The decision to euthanize an animal must be made with appropriate clinical judgment, taking into account the severity of the condition, the amount of pain or distress, the prognosis, and the potential loss of valuable data. Specific endpoint criteria will be determined for each experimental model based upon the rationale provided by the investigator and approved by the IACUC Committee. The following are examples of criteria that could be used, either singly or in combination, to define endpoints:

1. **Body Temperature Change:**
   Hypothermia has been shown to be an important indicator of a deteriorating condition in several infectious disease and toxic states. Septic animals, especially rodents, lose their ability to maintain body temperature. A decrease of 4-6 °C has correlated with death as an outcome in several infectious disease models. Therefore, monitoring of body temperature should be an important part of clinical monitoring for any infectious disease or toxic shock model. This can be accomplished using infrared scanners or implanted thermometer microchips.

2. **Weight Loss:**
   One effect of illness on animals is the loss of appetite. In some models, weight loss may be a cardinal indicator of the severity of infection. The total amount of weight lost, as well as its duration and consistency, should be used to determine the endpoint.
for infectious disease animal models. Animals that are recumbent and have lost the
ability to reach food and water will lose weight rapidly. Depending upon the
particular experimental protocol, placement of moistened food in the cage bottom
may be required. A loss of more than 20% body weight from baseline is a commonly
used criterion for euthanasia.

3. Other Behavioral and Physiological Changes:
Decreased activity (lethargy) and alertness, a rough hair coat, and hunched posture
are other direct signs of illness, pain, or distress. Detailed observational checklists
(see Appendices 1 and 2 for examples) should be devised for each individual study in
consultation with the veterinarian. Clinical scoring sheets are recommended to help
assess the animal’s condition and determine appropriate and humane endpoints.

RESEARCH ENDPOINT POLICY:

Any investigator proposing a study involving the induction of a diseased state where pain
and/or distress is anticipated must prepare a statement on research endpoints that is
included in the Animal Protocol and incorporates the following elements:

1. The investigator should be familiar with the clinical signs described in Appendix 1
   that may result from performance of the proposed experiments. Furthermore, the
   investigator must ensure that research staff are familiar with the endpoint statement
   and conduct their research adhering to the criteria.
2. One of the laboratory animal veterinarians must be consulted in the design of the
   endpoint statement.
3. Animals should be monitored at least 2 times daily during the window of time in
   which illness and mortality are anticipated. This window should be predetermined
   based on investigator experience or a pilot study and discussed with the veterinarian.
4. Humane endpoints must be established and clearly defined in the animal use protocol.
   This may require the completion of a pilot study or provision of previously
   collected/published data by the investigator. For example, justification of greater
   than 20% weight loss may require documentation that conclusions derived from
   particular experiments will be substantially altered if this endpoint is used. In any
   event, deviance from the guidelines set forth in this document will require IACUC
   approval and the guidance of the LSUHSC-S Veterinary Services.
5. Any significant deviation from the policies outlined here must be specifically justified
   by the investigator, preferably using pilot study data.
APPENDIX 1

List of clinical signs and conditions where euthanasia may be appropriate:

1. **Any condition resulting in a prolonged or irreversible inability to eat or drink**, e.g., prolonged immobility, obstruction of the oral cavity, missing or abnormal teeth. Rodents should not go more than 24 hours without access to food or water, unless scientifically justified.

2. **Diseases or conditions indicating severe pain, distress, or suffering**, e.g., fractures, self-induced trauma, abnormal posture or movements, open wounds or ulcers, or abnormal vocalization that is not relieved by anti-anxiety or analgesic agents.

3. **Rapid or continuous weight loss**, e.g., 20% or greater body weight over a few days, or gradual but continued weight loss.

4. **Generalized decrease in grooming and abnormal appearance over an extended time period**, e.g., rough hair coat, extensive alopecia, prolonged diarrhea, urine-stained hair coat, swollen limbs, paralysis, or other neurological disturbances (convulsions, abnormal head carriage, circling behavior, prostration).

5. **Severe or continuing respiratory distress**, e.g., coughing, sneezing, bloody nasal discharge, increased respiratory rate, labored breathing.

6. **Frank bleeding** that is uncontrollable.

7. **Evidence of microbial infections or other diseases** that interfere with the experimental protocol or cause any of the above and based on veterinary judgment are not treatable.
APPENDIX 2

Example of response variables and scoring system in rodents:

The total score should be tallied with every observation time point and a score equal to or above a certain predetermined amount would warrant euthanasia or evaluation by a laboratory animal veterinarian. If all of the criteria listed below were included in a clinical scoring sheet, a total combined score above 8 would warrant euthanasia. Any mice with 2 or more individual maximum scores (e.g., a 3 for activity and a 3 for weight) should also be euthanized. Notes for technicians should be included at the bottom of all score sheets, providing guidance on how to record qualitative clinical signs, criteria for humane endpoints, and instructions for euthanasia and carcass disposal, as well as emergency phone numbers for laboratory personnel.

Body weight changes

0 - Normal
1 - <10% weight loss
2 - 10-15% weight loss
3 - >20% weight loss

Physical condition

<table>
<thead>
<tr>
<th>Haircoat</th>
<th>Eyes and Nose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, well-groomed</td>
<td>Normal</td>
</tr>
<tr>
<td>Rough haircoat,</td>
<td>Eyes closed or squinted – no discharge</td>
</tr>
<tr>
<td>Rough coat, hair loss,</td>
<td>Eyes closed or squinted – discharge or porphryin staining</td>
</tr>
<tr>
<td>ungroomed</td>
<td></td>
</tr>
</tbody>
</table>

Behavior

<table>
<thead>
<tr>
<th>Activity</th>
<th>Posture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Decreased activity, locomotion</td>
<td>Sitting in hunched up position</td>
</tr>
<tr>
<td>after slight stimulation</td>
<td></td>
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<tr>
<td>Inactive, less alert, locomotion</td>
<td>Hunched posture/ head on cage floor</td>
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<tr>
<td>after moderate stimulation</td>
<td></td>
</tr>
<tr>
<td>Self-mutilation, either very restless</td>
<td>Lying prone on cage floor</td>
</tr>
<tr>
<td>or immobile, or no locomotion after</td>
<td></td>
</tr>
<tr>
<td>moderate stimulation</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES:

3. CCAC [Canadian Council on Animal Care]. CCAC guidelines on choosing an appropriate endpoint in experiments using animals for research, testing, and teaching. Ottawa, Canada; 1998.