

STANDARD OPERATING PROCEDURE (SOP) FOR ASSESSING TUMOR BURDEN AND APPROPRIATE TIME FOR EUTHANASIA IN ANIMALS:

PURPOSE: To establish a North Carolina State University (NCSU) Institutional Animal Care and Use Committee (IACUC) SOP for assessing tumor burden in animals and the appropriate time for euthanasia of these animals. This SOP should be used by investigators at NCSU for naturally-occurring and experimentally-induced tumors in animals. These guidelines may be used as written when preparing an Application for Vertebrate Animal Use (AVAU), as well as for an amendment to an approved protocol. Exceptions to these guidelines may be requested. However, the scientific rationale for an exception must be clearly stated and approved by a convened quorum of the IACUC.

PROCEDURES: Optimally, studies are terminated when animals begin to exhibit clinical signs of disease, i.e., when they become morbid (criteria for morbidity are defined in the next section). However, this endpoint may not be compatible with the research objectives. All efforts should be made to ensure that death or moribundity is not an endpoint. It is incumbent upon the Principal Investigator to clearly explain and justify scientifically why animals should be allowed to progress past the point where clinical signs of disease are manifest. Animals must be monitored according to the plan described in the approved protocol and all monitoring must be documented. Checklists and/or scoring sheets help to ensure that appropriate observations are made, consistently interpreted, and properly documented. A sample scoring sheet is described in this SOP and you are encouraged to use it. You may use an alternate scoring system, but **some scoring system must be used**. An alternate scoring should be described in your protocol and must be approved by the IACUC. Animals that are approved to progress past the point where clinical signs of disease are manifest must be monitored at least once daily, including weekends and holidays.

PROTOCOLS IN WHICH MORBIDITY IS THE ENDPOINT FOR EUTHANASIA: For protocols that include morbidity, the following signs should be observed to determine that the endpoint for euthanasia (morbidity) has been met:

1. Body weight: Animals should be weighed at least once a week.
2. Clinical observations including changes in general appearance, skin and hair, eyes, nose, mouth and head, respiration, urine, feces and locomotion.
3. Clinical signs, depending on severity and duration, that may constitute an endpoint include:
 - a. Rapid weight loss
 - b. Diarrhea (if debilitating)
 - c. Progressive dermatitis or rough coat
 - d. Hunched posture, lethargy or persistent recumbency
 - e. Coughing, labored breathing, nasal discharge
 - f. Jaundice and/or anemia

- g. Neurological signs which may be subtle (tics, twitches, changes in gait, repetitive behavior, etc.)
- h. Bleeding from an orifice or the presence of blood on the animal
- i. Self-induced trauma
- j. Any condition interfering with drinking or eating, including impaired ambulation, paresis (weakness) or paralysis (inability to move).
- k. Excessive or prolonged hypothermia or hyperthermia
- l. Presence of observable tumors.
- m. Tumors that ulcerate and become necrotic or infected.

PROTOCOLS IN WHICH MORIBUNDITY IS THE ENDPOINT FOR EUTHANASIA:

For those protocols in which **moribundity** has been approved as the endpoint for euthanasia, the following signs indicate that an animal is very ill or dying:

- 1. Comatose, hypothermic, and/or unable to remain upright
- 2. Severely impaired ambulation (unable to reach food or water)
- 3. Breathing rate very slow, shallow and labored
- 4. Any obvious prolonged illness including such signs as difficult to arouse, reluctant to move, inactive, lack of mental alertness, inappetence (no appetite)/anorexia (not eating) for more than a 24 hour period, pronounced signs of central nervous system disturbance (e.g., tremors, convulsions, repetitive movements, etc.), chronic diarrhea or constipation and muscle atrophy, or emaciation.

SAMPLE CHECKLIST AND IMPACT NUMBER SCORING SYSTEM TO DETERMINE TUMOR BURDEN AND ITS EFFECTS ON THE ANIMAL. Note that the higher the impact number, the greater its effect will be on the disposition of the animal. Also note that any animal found to be moribund or severely cachectic must be immediately euthanized.

- **General appearance (includes skin, orifices; the impact number is in parenthesis):**
 - (0) Normal
 - (2) Not well groomed and less active, but otherwise normal posture and gait
 - (3) Skin or hair coat in poor condition (scruffy) and less active and more poorly groomed than in previous bullet
 - (6) Skin or coat in very poor condition, dehydrated (skin remains “tented” when gently pulled up over dorsum), emaciated, inactive
 - (18) Moribund or severely cachectic (**immediate euthanasia)
- **Tumor size and degree of necrosis:**
 - (0) Tumor very small or nonexistent
 - (1) Tumors present
 - (4) Tumors present and some or all are necrotic

- (12) Tumor large (≥ 18 mm in any two dimensions) and interfere with locomotion, eating, drinking and other activities
- **Weight loss:**
 - (0) No weight loss
 - (2) 5-10% loss in body weight compared to pre-inoculation weight of the same animal
 - (4) ≥ 10 -15% loss in body weight compared to pre-inoculation weight of the same animal
 - (8) $> 15\%$ loss in body weight compared to pre-inoculation weight of the same animal

<u>Total Impact Number Score</u>	<u>Action to be taken</u>
0-7	Routine daily monitoring.
8-13	Daily monitoring. May put food on cage bottom and/or provide supportive therapy if permitted by the study objectives.
14-17	Consult veterinarian. Consider euthanasia.
≥ 18	Euthanize immediately.

The impact scoring system is quite subjective. Therefore, investigators should strive to have the same individual make and score the observations.