CCAC National Workshop 2015

Endpoints

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May 30, 2015 | Montréal, QC

CCAC Guidelines

The CCAC policy statement Ethics of Animal Investigation states:

"Animals must not be subjected to unnecessary pain or distress. The experimental design must offer them every practicable safeguard, whether in research, in teaching, or in testing procedures; ..."

(Ethics of Animal Investigation, CCAC, 1989)

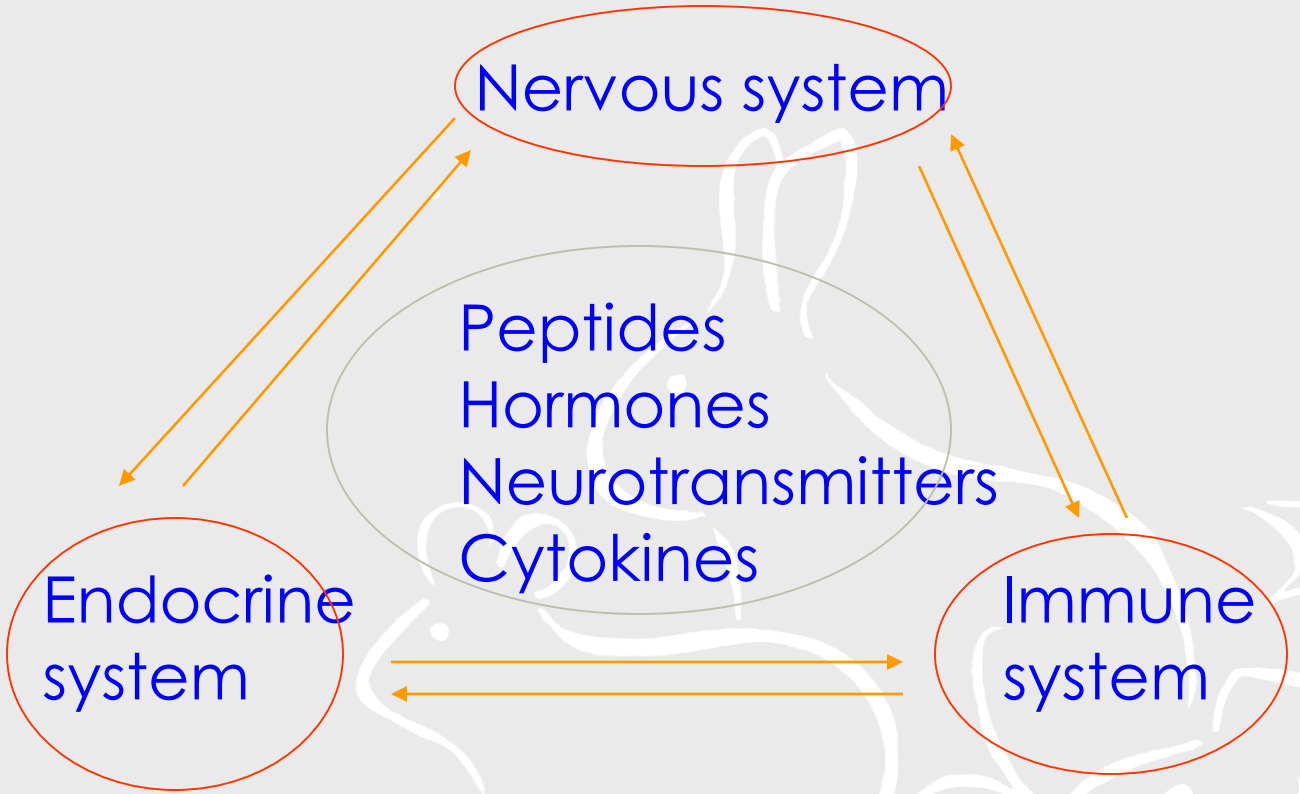
-requires consideration of 3Rs

http://www.ccac.ca/Documents/Standards/Guidelines/Appropriate_endpoint.pdf

Defining an Endpoint

- Humane endpoints used will be determined, in part, by the scientific goals of the study but should be set as the earliest possible time at which the scientific objectives can be reached
- Possible outcomes:
  - End the procedure and remove the animal from the study
  - Provide treatment or supportive care
  - Euthanize the animal to minimize pain and/or distress
- Moribund state or death must not be used as an endpoint whenever possible

Interaction between stress, pain, and immunity



"Supersystem" model – Chapman, et al, J Pain, 2008

Principles guiding endpoint selection

- Endpoints are developed with knowledge of the scientific goals of the study
- They can be developed initially from a generic list but should be tailored to the specific protocol
- Endpoints should take into account the cumulative experience of the animals on that study, e.g., tumour induction, metastatic disease, repeated in vivo anesthesia and imaging, pain and sickness occurring with disease progression, wasting, biopsy, etc.

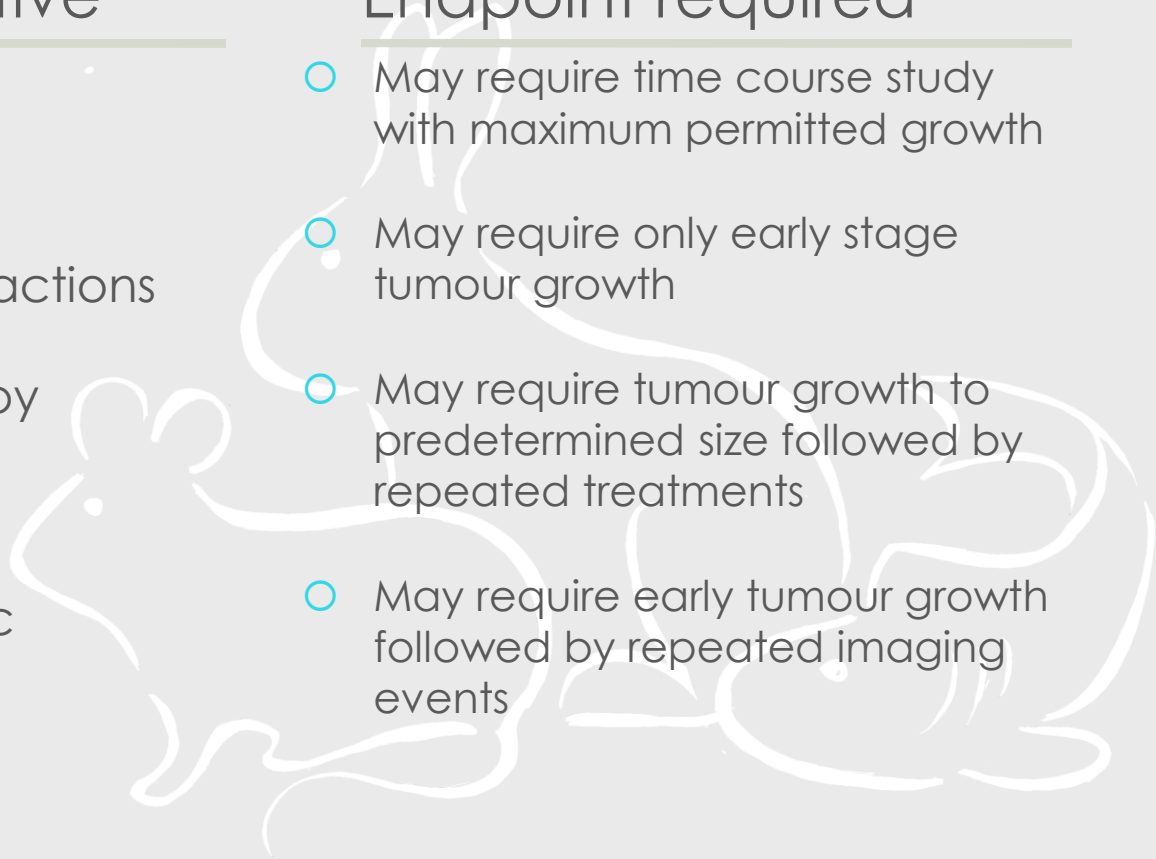
Example – Cancer studies

Scientific objective

- Tumour model characterization
- Host/tumour interactions
- Antitumour therapy
- Tumour diagnostic techniques

Endpoint required

- May require time course study with maximum permitted growth
- May require only early stage tumour growth
- May require tumour growth to predetermined size followed by repeated treatments
- May require early tumour growth followed by repeated imaging events

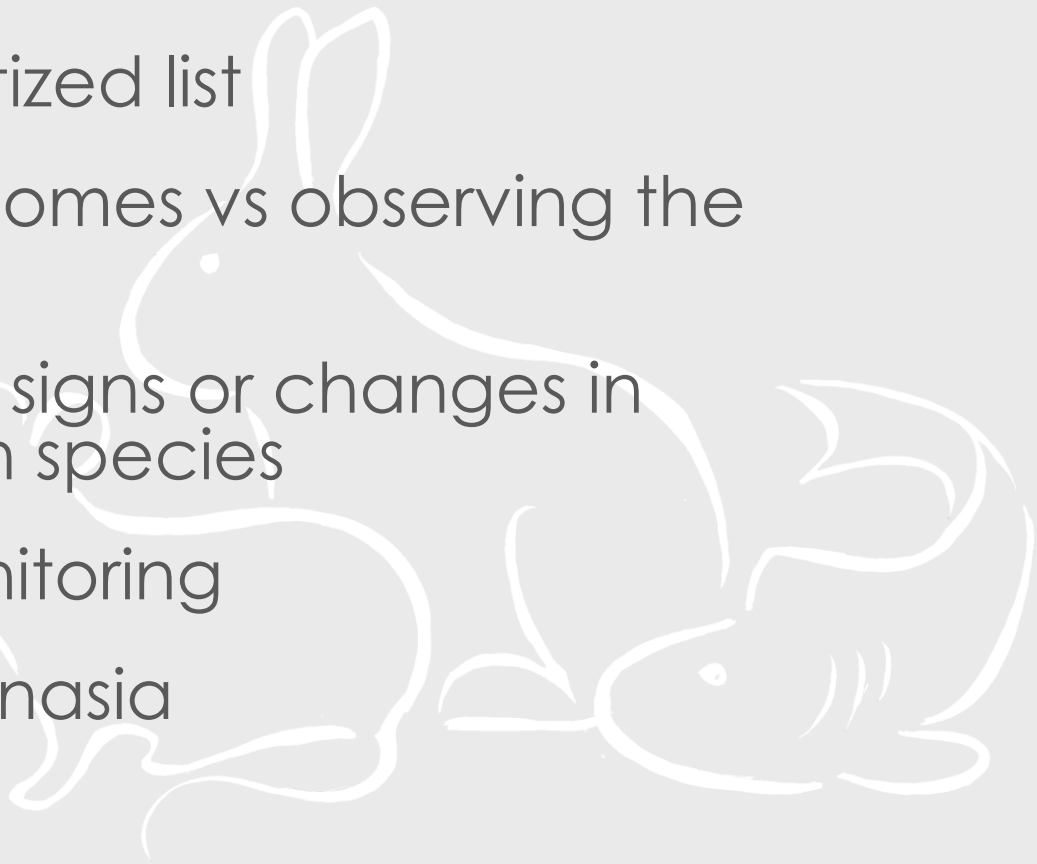


Category of Invasiveness and Study Endpoints

- Principles remain the same, regardless of the study type; however, the risk of potential harms experienced by the animal increases as the study invasiveness increases
- Emphasizes need for:
  - Increased frequency of monitoring
  - Well-defined endpoint criteria
  - Training of personnel to recognize species-specific signs of pain, distress, sickness, etc
- Where significant unknowns are present, pilot studies should be encouraged, e.g., dose-range finding studies, technique development studies, etc

Establishing endpoints

- Important for ACC and research team to be clear about research goals and to understand if achievable – should be a conversation
- Generic list vs prioritized list
- Mathematical outcomes vs observing the animal
- Ability to recognize signs or changes in condition in a given species
- Invasiveness of monitoring
- Treatment vs euthanasia



Which signs should be observed?

- Changes in body weight (and related changes in food and water intake);
- External physical appearance;
- Measurable clinical signs (e.g., changes in heart rate, in respiratory rate, and in their nature);
- Changes in unprovoked behavior; and,
- Behavioural responses to external stimuli (Morton & Griffiths, 1985)
- Plus specific conditions related to model or manipulations
- Etc.... (should be based on model knowledge and literature search)

Developing endpoints for 'difficult to monitor' species

- Examples of endpoints for fish
  - Slower than normal rate of gain (weight loss is extremely slow for fish in suboptimal environment or health)
  - Change in feeding activity or consumption
  - Physical appearance
  - Fin and skin condition
  - Mucus production
  - Color change
  - Increased or decreased respiratory rate
  - Posture in the tank
  - Social interaction
  - Hyper or hypoactivity
  - Avoidance to mechanical prod or light beam

Avoiding the Moribund state

- The moribund condition is a clinically irreversible condition leading inevitably to death
- Moribund animal may actually be past suffering - it is important for intervention to occur as early as possible
- Commonly used signs of moribundity: severely impaired mobility, laboured breathing and cyanosis (blue color to skin or mucous membranes), clinical dehydration and/or prolonged decrease food intake (more than 72 hours), severe, rapid weight loss (15% or more), muscle atrophy, lethargy, low body condition score , lack of responsiveness to manual stimulation, prolonged abnormal posture, serum chemistry values indicating organ failure, self-mutilation, unconsciousness, prolonged inability to urinate or defecate

Managing endpoints

- Documenting endpoints and access to records
- Who conducts monitoring?
- How long are endpoints monitored in the life of a protocol?
- What happens when the scientific endpoint is not reached at predetermined endpoint?
- What happens when unexpected outcomes occur on a study and is the ACC notified of these events?
- PAM discussions – opportunities for endpoint refinement, use of surrogate endpoints, numbers of animals moribund/found dead on protocol, have the endpoints made a difference to animal welfare?

Example endpoints – Infectious disease study

- Hypothermia of 34-36° C (predictive of mortality from bacterial pathogens)
- Ruffled coat
- Weight loss
- Ocular discharge
- Lethargy
- Hunched posture
- Ataxia
- Tremour
- Cyanosis

Clinical Assessment Score Sheet

For AUP# _____

Preamble (taken from AUP):

The primary viremia and associated clinical signs are expected to be most intense within the first 2-3 days of the study. For the dose range finding study, animals dosed with live virus will be at monitored least 4 times daily for the first 4 days post-infection, and more often as necessary. From Day 5 to 14, animals will be observed at least twice daily or more often, as necessary. An assessment sheet will be completed for each virus-treated rabbit. Control (vehicle only) rabbits in the definitive study will be observed once daily and a monitoring log form will only be completed if animals experience unexpected clinical problems.

Considerations for animal monitoring include:

- general attitude (bright & alert, depressed or withdrawn, reactive to environment or non-reactive to environment)
- haircoat (well groomed, unkempt) and skin (swelling, vesicles)
- respiration and mucous membrane colour (normal, increased and shallow, stridor, cyanosis)
- hydration status (skin fold – normal, mild or or moderate dehydration)
- pyrexia (daily rectal temperature from Days 1-4, weekly thereafter)
- body weight - done twice weekly on Tuesdays and Fridays unless animals are showing clinical signs, then it may be done more frequently

Any rabbit that shows any one (or more) of the following criteria will be humanely euthanized:

- ongoing daily weight loss greater than 10% per day over 3 days (i.e., >20% total weight loss);
- persistent respiratory distress (over 2 monitoring periods) as detailed below
- persistent lack of responsiveness to caregivers

Clarification of Assessment Scores in Table:

- **Breathing** is monitored as follows: **Normal** respiration means no visible signs of distress, and breathing is not obvious. **Rapid rate** means that breathing is obvious. **Distress** means that the rabbit is showing signs of dyspnea such as open-mouthed breathing, cyanosis (blue discolouration of ears and lips), or stridor (expiratory noise).

Personnel Responsible for Clinical Monitoring:

Action(s) to be Taken Based on Assessment Score Sheet Results:

Mild or moderately dehydrated animals may be supplemented with warmed LRS SC at up to 30 mL/injection site (up to 4 sites), using a 22g butterfly catheter. Animals with reduced feed intake or body weight loss may be supplemented with additional hay or alfalfa cubes, fresh vegetables or fruit.

If any endpoint is reached, contact xxxx. If they cannot be contacted, the rabbit is to be humanely euthanized by sedating with acepromazine, 1 mg/kg and then administering Euthansol by lateral ear or cephalic vein (dosage 1 ml/4.5 kg body weight). The carcass should be immediately refrigerated (not frozen).

PLEASE USE ONE ASSESSMENT SHEET PER RABBIT

Rabbit ID#:

Day of Study (Add Day of Week)	Date (mm/day/yr) Time (24-hr clock)	General Attitude 0 – bright & alert 1 – depressed 2 – unresponsive	Hydration Status Normal Mild* Moderate* Note: supplement with fluids	Hair and Skin Normal Unkempt Swelling (site) Vesicles (site and number)	Rectal Temperature	Body Weight Collected once weekly or more often, as necessary	Respiration 0 – normal 1 – rapid 2 – distress (see note)
-1							
1	Date: Pre-injection:				x	x	
	+4 h:				x		
	+8 h:						
	+12 h:				x		
	+16 h:				x		
2	+4 h:				x		
	+8 h:						
	+12 h:				x		
	+16 h:				x		
3	+4 h:				x		
	+8 h:						
	+12 h:				x		
	+16 h:				x		
4	+4 h:				x		
	+8 h:						
	+12 h:				x		
	+16 h:				x		
5							

Resources for endpoint development

- CCAC 3Rs microsite: http://3rs.ccac.ca/en/
- NC3Rs: https://www.nc3rs.org.uk/humane-endpoints
- Laboratory Animals (UK) training site: http://www.lal.org.uk/publications/this-is-a-test-subject/humane-endpoints/
- Assessing the health and welfare of laboratory animals (pictures, video): http://www.ahwla.org.uk/
- Humane endpoints (pictures, papers): http://www.humane-endpoints.info/eng/index.php?lang=en

Discussion and questions

1. At your institution, who establishes endpoints(PI) ?
2. Endpoints are selected from a pre-established list? ?
3. Endpoint monitoring is done by the research personnel, the animal facility personnel, other?
4. Who evaluates toxicity and makes endpoint decisions during toxicology protocols?
5. Are analgesia contraindications well-justified?
6. How are endpoint decisons made for aging models?
7. What is the ACC's role in endpoint development and approval?
8. Are face to face meetings encouraged between the research and animal care personnel for D and E protocols?
9. How are endpoints established for teaching protocols? Do you limit category of invasiveness for these types of protocols?
10. How does your ACC reviewanimal health status for noncommercial vendors (e.g., farms, local breeders, etc)?
11. Does your ACC review endpoints of protocols during annual renewals as part of PAM?

Thank-you!