

UBC ACC Guideline on Rodents with Ulcerated Subcutaneous Tumours: Protocol Requirements, Monitoring, Managing and Humane Endpoints (2018)

Background and Purpose

CCAC currently recommends that ulceration of subcutaneous tumours should be considered a humane endpoint (CCAC 1998). Traditional concerns for keeping research animals with ulcers are related to the impacts on animal welfare and data validity (e.g. changes to tumour growth patterns once ulcers form). The University of British Columbia previously followed this guideline but recognizes that scientific understanding of tumour models has progressed in recent years and there are exceptional circumstances where ulcers are scientifically justified. So in consultation with UBC researchers, the ACC developed this guideline. Note this guideline will likely evolve as our understanding increases.

These guidelines are intended to provide guidance to ACC reviewers, veterinarians, researchers and animal care staff on: 1) how to plan and evaluate ACC protocols requesting permission to keep animals with ulcerated tumours, 2) pathology of ulcers, 3) appropriate monitoring and care of animals with ulcerated tumours and 4) appropriate humane endpoints. They are intended to provide general guidance.

Evaluation and Planning of Protocols

Justification for keeping animals with ulcerated tumours must be evaluated on a model-dependent basis. Reviewers should look for evidence that every effort has been made to choose models that are not prone to ulceration. Where possible, when ulceration is characteristic of the tumour line, the aim of the study should be to complete the experiment in the latent period before ulceration.

If ulcers are justified then, researchers must make every effort to analyze data in a timely manner to ensure that experimental endpoints for animals with ulcerated tumours are reached as early as possible. See “Monitoring”, “Management” and “Humane Endpoints” (below) for specific criteria that must be included in the protocol.

In order to continue progress with respect to Refinement, it is imperative that the ACC collects data from researchers on how ulcers impact the welfare of animals, and the progression and severity of ulcers.

Potential Scientific Rationale and Justification for Keeping Animals with Ulcerated Tumours:

1. In order to better model human cancer, allowing ulcerated tumours may be important.
2. Some research areas e.g. immunotherapy, target cancer in its later stages which often present with ulcerated tumours.
3. Ulcerated tumours may heal with drug therapy and therefore if animals are pre-maturely euthanized one cannot know these results.
4. Some models are prone to ulcerate prior to completion of data collection.
5. Some patterns of ulceration cannot be predicted due to variations in the model and/or treatments.

Progress Reports

When protocols are renewed, information should be provided regarding the welfare of animals with ulcerated tumours, as well as whether keeping animals with ulcerated tumours turned out to be scientifically warranted. For example, looking back was the success of the study dependent on keeping animals with ulcerated tumours, what welfare issues arose, what percentages of animals had ulcers etc.?

See “Monitoring” for more details on information that should be gathered, summarized and reported to the ACC.

Planning your Study

- Where possible, each model should have historical data to guide monitoring; initial pilot studies with histopathology are informative.
- For each model, data should be collected to help develop a better understanding of the impacts on the welfare of the animal, the progression and the severity of ulcers (see “Monitoring” below).
- When submitting an application to the ACC, details on monitoring and record sheets are required. Justification for keeping animals with ulcers must be included.

Anatomy and Pathology

Potential Causes of Ulceration of Tumours

1. Certain types of tumours (e.g. papillomas) or cell lines.
2. Alterations in blood supply in regions of tumour (below to the epidermis). This results in a reduced (hypoxia) or lack of (ischemia) blood supply to the skin and subsequent cell death and sloughing of the epidermis or deeper layers.
3. Rapid tumour growth where it exceeds skin’s ability to stretch, causing rupture.
4. Tumours with haemorrhagic areas that are prone to ulceration.
5. Mechanical trauma – location of tumour results in constant friction with bedding or caging. For example tumours on the ventral surface of an animal’s body.
6. Self-induced trauma – rodent over-grooms or scratches the tumour due to irritation or discomfort.
7. Technique: Tracking of tumour cells into dermal layer.
8. Experimental therapy (phototherapy, irradiation, intra-tumoural injection etc.)

Terminology

It is useful to present terminology used to describe pathology of tumours so that everyone is accurate in their descriptions of clinical signs.

Dermatitis: Inflammation of skin. Inflammation can be characterized according to the type of pathology and includes the following lesions.

Ulcer: Loss of epidermis and at least the superficial portion of the dermis (McGavin & Zachary 2011). In tumours, ulcers typically form from cell death (necrosis). Common terms used to describe ulcer appearance when they are sunken are “pitted” or “cratered”. Both imply an ulcer that is deep, invaginated (sunken in the middle) with sharply defined walls much like a volcanic “crater”. These can be called “cratered” ulcers.

Scab: Material formed by drying of exudate or secretion (fluid, cells, debris from blood vessels) on the skin surface. For scab to be present, there has to be an ulcer. The scab formation is part of the skin’s attempt to heal. The correct term for scab is “fibrin crust”.

Necrosis: Death of cells in a living animal. The mechanism leading to cell death varies. In tumours cell death would typically be caused by “oncotic necrosis” which is death following irreversible cell injury by hypoxia, ischemia, and membrane injury. This may or may not be visible grossly.

Typically when an ulcer and/or scab is visible this would be called “ulcerative dermatitis”. However, sometimes the skin appears black without an ulcer and this would be called “necrotizing dermatitis”. The black colour indicates necrotic cells are present. Note that necrotizing dermatitis can progress to ulcerative dermatitis so one may see a combination. If there is bleeding from the ulcer, this would be called “haemorrhagic ulcerative dermatitis”. Sometimes tumours have shiny thin skin. This likely indicates that the epidermis is separated from the dermis and in these cases there is an increased risk of ulceration.

Epithelialization: Epithelialization is an essential component of wound healing. The various cells in the skin form a lattice (granulation tissue) where the epidermal cells can migrate over top to create the normal epidermal barrier. In the absence of re-epithelialization, a wound cannot be considered healed.

Histology of Normal and Ulcerated Skin

Figures 1 and 2 (Conti et al. 2004) illustrate the normal anatomy of mouse skin. Compared to humans the rodents have a very thin epidermis. Thus, the epidermis can easily be damaged when tumours are prone to ulceration.

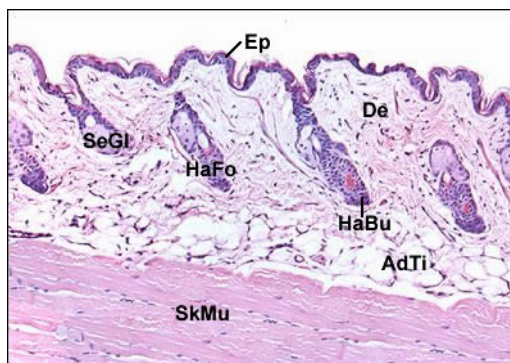


Figure 1 Skin of mouse 10x magnification

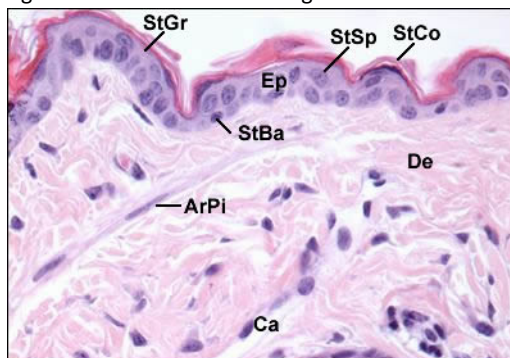


Figure 2 Skin of mouse 40x magnification (Conti et al. 2004)

Ca	Capillary
De	Dermis
Ep	Epidermis
AdTi	Adipose Tissue (subcutaneous space)
ArPi	Arrector pili muscle
HaBu	Hair Bulb
HaFo	Hair follicle
SeGl	Sebaceous Gland
SkMu	Skeletal Muscle
StBa	Stratum basale
StCo	Stratum corneum
StGr	Stratum granulosum
StSp	Stratum spinosum

Figures 3 to 4 illustrate mouse skin with ulcers that developed on the surface of subcutaneous tumours that were implanted with Shionogi tumour cells. Note in Figures 3b-4c there is no evidence of epithelialization, indicating that the skin is currently not healed. As the skin responds to the ulcer by trying to fill the gap where epidermis is lost, certain cells are deposited and the tissue is called granulation tissue. Granulation tissue is highly vascular, so there is increased risk of bleeding and blood loss when granulation tissue is present.



Figure 3a - Photo of mouse with tumour depicted in Figures 3b-3c

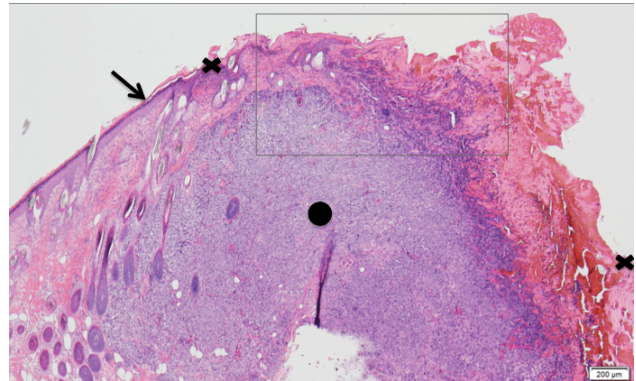


Figure 3b: Black arrow shows normal epidermis. Cross shows start of ulcer where normal epidermis is lost. Area between crosses represents ulcer surface. Pink and red stained cells represent fluid, inflammatory and necrotic cells, and debris from blood vessels. Circle (with purple staining) represents area of tumour cells. 200 microns

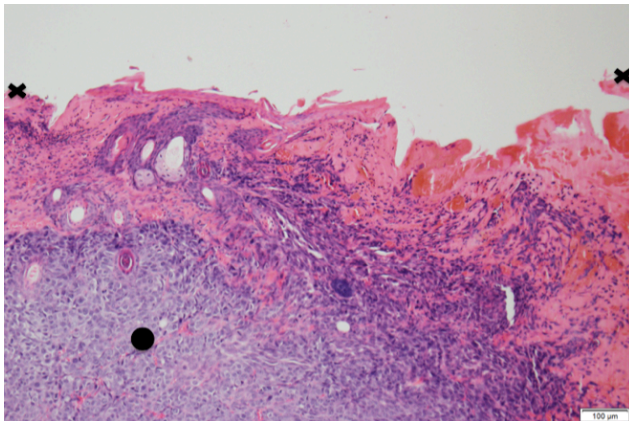


Figure 3c: Close up of insert from Figure 3a to illustrate the nature of cells present in the ulcer and the depth of ulcer. 100 microns

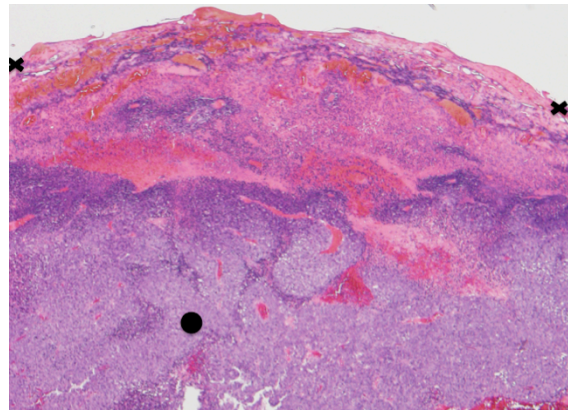


Figure 4a: An example of a tumour with an extensive and deep ulcer (full thickness from subcutaneous layer up to epidermis). The area between the crosses is the crust with no epidermis but with large numbers of necrotic and inflammatory cells and large amounts of fibrin and haemorrhage (blood cells, red staining) are present. 200 microns

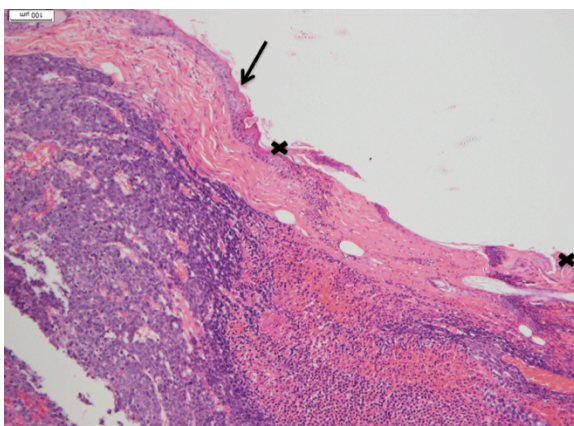


Figure 4b: Ulcer from Figure 4a showing edge where epidermis is lost (area between crosses). The area between the crosses shows an area of crust with no evidence of epithelialization. This could be in the process of healing but it is not healed. 100 microns

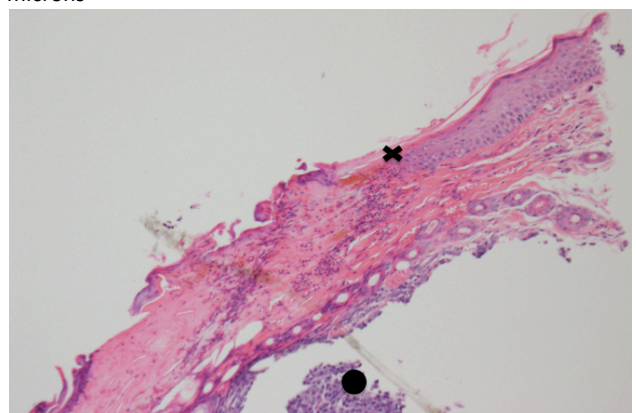


Figure 4c: Ulcer from Figure 4a showing edge where epidermis is lost (area at cross) and no evidence of epithelialization. 100 microns

Figures 5a-5c illustrate visible signs of healing in an ulcer on a Nude mouse. Signs include evidence of granulation tissue (Figure 5a), contraction of the skin around the ulcer (Figure 5b) and ultimately covering of the ulcer with epithelium (Figure 5c). The time it took to heal was 4 weeks.



Figure 5a



Figure 5b



Figure 5c

Monitoring and Welfare

Major animal welfare concerns related to keeping research animals with ulcers include concerns for:

1. Loss of body fluid
2. Infection
3. Pain and discomfort experienced by the animal

Criteria for Monitoring:

Monitoring ulcers will depend on: a) what is already known about the tumour model and ulcer development in the species under study and b) the type of experimental treatment. These criteria must be defined and approved in the protocol.

See example of monitoring sheet (Table 1) for information that should be collected and Grading Sheet (Table 2) for examples of how to grade animal with ulcers.

Features Important to Monitoring:

- **Size of ulcer** – Changes in ulcer size (area and depth) should be monitored. Ulcers that are enlarging fast (e.g. daily) should be monitored more frequently. Frequency will depend on progression and current monitoring frequency. Larger ulcers (e.g. 20% surface area) will increase the risk for fluid loss, infection and pain. So increased monitoring is critical.
- **Evidence of healing** – Extensive ulcers that persist without evidence of healing (see Figures 5 a-c for examples of healing ulcers) should be considered grounds for humane endpoint. Healing may be a goal for some studies so researchers need to work with their clinical veterinarian to develop criteria for healing and assessment of pain or discomfort.
- **Discharge (amount, type)** – Open ulcers with discharge are at risk of infection and animals are at risk of dehydration. Typically wet discharge indicates that damage to skin is acute and active with minimal or no healing. The nature of the discharge can be assessed visually, however this can be challenging because rodents groom away discharge, fur may cover the area, and wet discharge will dry. Evidence of wet discharge includes: wet appearance and surrounding fur that is discoloured and clumped. Assessments of discharge along with signs of general health of the animal are critical.
 - Type of discharge: blood, purulent (pus), clear (serum). Blood loss may result in anemia so animals may show pale extremities and may display signs consistent with systemic illness (e.g. lethargy and poor grooming). Note that in some cancer models anemia of cancer is an expected outcome. Pus would indicate local infection with risk of systemic infection.
 - Fluid loss from discharge may result in dehydration (skin tent > 2 seconds and sunken and dry eyes). The degree of dehydration should be monitored.
 - Larger volumes of discharge (e.g. from cratered ulcers) with longer duration of active discharge put animals at risk of developing anemia or dehydration. These animals would be considered to be at a greater risk of declining health than animals with dry, shallower ulcers.
- **Signs of inflammation surrounding the ulcer** – Signs would include redness and swelling around the tumour and ulcer.
- **Evidence of self-mutilation** – This may appear as scratches or gouges into and around the tumour. In addition you may observe the animal licking or scratching but this typically requires a longer period of observation. These signs indicate that the animal is feeling some discomfort (pain or irritation). In these cases, animals must be monitored for signs of pain (e.g. grimace scale).
- **Signs of Pain** – There is some consensus that ulcerated tumours are painful in humans. Some tumours, such as those grown in sensitive sites or that develop extensive necrosis, may be painful. Historically we have not monitored for pain in animals with tumours nor tumours with ulcers. In some studies, the treatment of pain may not be permitted because of concerns for the effect of the analgesia on data. At this point it would be useful to monitor for pain to gain a better understanding and researchers may consider testing the effects of analgesia. Where permitted, analgesia should be administered when behavioural signs of pain are present.
- **Overall Health and Welfare** – It is essential to take into account the overall condition of the animal (e.g. clinical health score). Clinical health should include behaviour, appearance, dehydration, body weight, tumour burden and signs of pain. General health should be monitored throughout the study so that potential effect of ulceration can be tracked. If clinical health of animal is declining then the frequency of monitoring should increase or euthanasia should be considered.

Frequency of Monitoring:

Monitoring frequency will depend on the researcher's understanding of tumour model, characteristics and progression of the ulcer, the individual animal's general health clinical score and risk of deterioration of the animal's health and welfare (see UBC Policy 004). In new models, this information can be generated in the first group of animals. For general health, the frequency could follow UBC Facilities Grading System and Response Guide for Rodents. However, when an ulcer occurs or there is evidence that an ulcer is likely to occur, then specific ulcer monitoring should begin.

- For tumours where ulcers develop very rapidly (e.g. some melanoma models), animals should be monitored 2 times a day (facility monitoring is sufficient for one time point but animal technicians need to see the tumour and the ulcer). For increased visibility of ulcers on furred animals, shaving the tumour area can help.
- For tumours with small and dry ulcers that are known to remain stable, then monitoring every second to third day is likely sufficient. Researchers can justify a monitoring frequency based on their understanding and experience with their specific model.
- When models and treatments are unknown, then extra monitoring may be warranted until caregivers and researchers are confident the animals are not at risk of deterioration of their health and welfare.

Management

There are few known treatments to prevent the development of ulcers, however treatments and changes in husbandry can be implemented to reduce the potential negative impacts on animal welfare. These might include:

- To reduce potential for rubbing and causing further mechanical damage to the skin, remove huts and food hoppers (place food on floor). Ensure sufficient nesting material is present as a substitute for huts.
- To reduce damage caused from scratching, trim tips of toenails.
- If showing signs of declining health: e.g. decrease in body weight, body condition score, clinical signs of ill health (e.g. Grade 2-3 on UBC Facilities Grading System and Response Guide for Rodents) consider supplemental food (e.g. bacon softies, hydrogel, diet gel). For reducing variability across treatment groups, it may be useful to provide the same supplemental food to all animals in an experiment.
- Treatment with local antibiotic or anti-inflammatory to prevent infection and inflammation. E.g. Hibitane Cream, Polysporin (topical triple antibiotic; polymyxin B, bacitracin gramicidin), Flamazine (topical antibiotic cream; silver sulfadiazine cream), Calendula cream (plant extract for treatment of wounds), Duoxo Mousse (antibiotic and anti-inflammatory; Phytosphingosine).
- Shaving fur may keep area dry, which reduces risk of bacterial infection and enables better visualization.
- Pain: Currently Metacam (NSAID) is very effective in rodents and likely would be a good analgesic choice. This could be administered initially daily for 3-5 days and then the frequency of dosing could be reduced over time. Other analgesic drugs include Buprenorphine or tramadol suspension in the drinking water or flavored meloxicam-containing gel. Veterinarian consultation is recommended for finding a drug choice and dosing schedule that animals can tolerate over longer periods of time.


- Application of topical anesthetic (e.g. EMLA cream: 2.5% lignocaine and 2.5% prilocaine) might reduce potential discomfort and is recommended at some institutions. However, potential for oral ingestion, toxicity and effectiveness need to be considered. In a full thickness burn model in rats, topical application of EMLA has been shown to reduce mediators associated with pain and inflammation (Yregård et al. 2003). However in mice the application of EMLA prior tail vein injections did not reduce behavioural signs of pain (David et al. 2014). Note that bioavailability of lignocaine and prilocaine are increased when EMLA is applied to damaged skin (Al-Musawi 2012).

Humane Endpoints Related to Ulcerated Tumours

Humane endpoints must consider a combination of signs (general health of animal and ulceration characteristics). Humane endpoints related to tumour size and burden endpoints must be followed, independent of ulcer grading (see UBC ACC Monitoring Policy 017 Appendix IB). All exceptions must be justified in the protocol. A clinical veterinarian can approve temporary permission but the protocol must be amended as soon as possible.



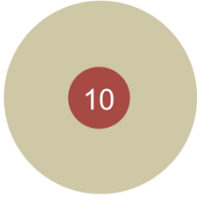
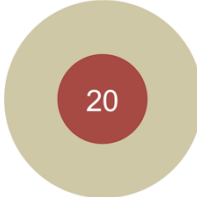
- Pus reflects infection and animals should be treated with antibiotic or be euthanized. If no response to an appropriate antibiotic treatment in 3 days, then animals must be euthanized.
- Frank blood (active bleeding) increases risk of infection, dehydration and anemia. If active bleeding is visible and the animal is showing signs of anemia (pale extremities such as footpads in non-pigmented mice) and dehydration (skin tent > 2 seconds, sunken and dry eyes), then the animal must be euthanized.
- If the tumour is known to be highly vascular and/or there is a known risk of excessive bleeding, then the animal should be euthanized as soon as visible active bleeding begins.
- If there is clear and wet discharge and the animal is dehydrated (skin tent > 2 seconds, sunken and dry eyes) with no resolution after 3 days of supportive care, then the animal must be euthanized.
- If there is evidence of self-mutilation such as gouges into the tumour tissue, bleeding, scratches or one can observe persistent scratching, then the animal must be euthanized or analgesia and other treatments must be implemented and monitored for success of treatment. If no improvement in 3 days, then the animal must be euthanized.
- If an animal displays signs of pain (facial grimace or other known signs) or interferes with normal functions with no resolution despite analgesia treatment, then the animal must be euthanized.
- An animal whose health and welfare (e.g. score 4) cannot be sustained beyond 3 days without extensive supportive care (frequent rehydration, special foods and heat) must be euthanized.
- If an animal has an ulcerated tumour in addition to a progressive increase in severity of clinical signs (e.g. score 3-4 on the UBC Facilities Grading System and Response Guide for Rodents), the animal must be euthanized unless supportive care improves their clinical signs.
 - Example 1: If a mouse with an ulcerated tumour is piloerect (grade 2) in the morning and then hunched and lethargic (grade 4) in the afternoon, then that mouse should be euthanized.
 - Example 2: If a mouse with an ulcerated tumour becomes lethargic (no longer Bright Alert and Responsive or scores grade 3-4 in appearance and behaviour) and develops pale toes, it should be euthanized. In contrast, if a mouse had pale toes consistently throughout the study, before tumour ulceration, then euthanasia may not be warranted.

Table 1. Example of monitoring record sheet specific to ulcers

Date	Area (mm)*	Depth (mm) or What is visible	% of Tumour Surface Area Ulcerated*	Discharge	Appearance	Self-Mutilation or Scratching	Overall Health (score)	Photo	Other
Dec 10, 2016	2 x 4	1	5	Dry	Scab	No	0		
Dec 15, 2016	3 x 7	Tumour tissue visible	10	Wet, red	Cratered	No	2		

* Note that ulcer area can be measured or estimated visually. Images are useful for estimating percent surface area (see example grading sheet below). Estimate of surface area is sufficient.

Table 2. Example of Grading Sheet (to harmonize with the UBC Facilities Grading System and Response Guide for Rodents)

Signs	Score			
	2	3	4	5 (Humane Endpoint) *
% Tumor surface area Red/dark area = ulcer on tumour Number = % area	< 5 	5 - 9 	10 - 15 	>20 
Discharge	None, but ulcer likely	Dry, scab	Wet: blood; signs of infection (controlled by treatment)	Wet: Pus (infection) that has not responded to treatment for 3 days. Fresh blood with signs of anemia and/or dehydration. Fresh blood in known vascular tumour. Clear discharge with signs of dehydration with no resolution in 3 days.
Depth	< 1 mm	1-2 mm	3-4 mm, cratered	> 4 mm, tumour tissue visible or loss of tumour tissue.
Signs of inflammation in surrounding area	Mild hair loss	Skin red, hair loss	Skin red and swollen	Skin severely damaged (infected, necrotic) or open to muscle or bone.
Scratching	Possible scratching but not observed	Occasional scratching	Frequent scratching	Evidence of severe self-mutilation and/or constant scratching (even when disturbed) with no resolution with treatment in 3 days.
Behaviour	Slightly slow moving; still interested in environment.	Less interested in the environment, interacts less with cage mates, disregards observer. Occasional abnormal gait e.g. limping or "tip-toed" gait.	Isolated from cage mates, minimally active; does not readily move when cage disturbed. When nudged, reluctantly moves. Frequent limping or "tip-toed" gait.	Immobile or hyperactive; not moving when nudged; animal cannot right itself.
Appearance	Piloerection/ruffled (< 10% of body).	Piloerection (25% of body e.g. base of neck) and dull fur (not shiny or smooth). Slight hunching in back.	Piloerection (50% of body), matted and un-groomed. Whiskers barbered. Severe hunching in back, even when walking.	Piloerection (>75% of body), matted and un-groomed with other severe signs of illness.
Dehydration	Mildly sunken eyes (appear >75% open).	Skin tent > 2 seconds (decrease in skin elasticity). Sunken eyes (appear half closed).	Skin tent > 5 seconds. Completely closed or severely sunken eyes; tail feels square. Cool to touch.	Animal unresponsive and cold to touch. Severe skin tent (> 10 seconds) or dehydration with no resolution after 3 days.
Pain	Facial Grimace: Narrow or closed eyes, bulge on top of nose (mice), flattening of bridge of nose (rat), cheek (between eye and whiskers) bulge (mice), cheek sunken (rat), ears back or flat, whiskers pointing back or "standing out on end". Other: Muscle twitching or flinching, staggering, back stretch (like cat), abdominal writhing or pressing.			Persistent signs of pain that interfere with normal functions or that cannot be alleviated with analgesia.

* If an animal has an ulcerated tumour in addition to a progressive increase in severity of clinical signs (e.g. score 3-4 on the UBC Facilities Grading System and Response Guide for Rodents), the animal must be euthanized unless supportive care improves their clinical signs.

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