Title: Animal Nursing Care

Purpose:
Provide a framework and guidance for research personnel and ARC staff to carry out basic nursing care procedures for sick laboratory animals (laboratory mice and rats, Nile rats, and zebrafish). This document identifies some common clinical problems in our laboratory animal program and provides recommended nursing care procedures that trained personnel can initiate to resolve these problems. In all cases, the Campus Veterinarian or designee must be notified in a timely manner. In the case of an emergency or for specific conditions listed below (e.g., flooded cages, fight wounds, dystocia, and eye flushes), treatment should be initiated as soon as possible. Promptly notify the Campus Veterinarian if the problem is not improving or worsens during the treatment period.

1. Flooded rodent cage

Rodent cages infrequently flood due to a leaking water bottle or faulty Hydropac valve. Providing enough bedding in the cage to absorb the water will minimize the detrimental effects of flooding on the animals. Mice are especially vulnerable to hypothermia and dehydration from a flooded cage. If the animals are weak/inactive, dehydrated, and hypothermic, then they should be euthanized. Otherwise, the animals should be treated as follows and as soon as possible. You don't need to wait to notify the Campus Veterinarian to initiate this treatment.

a. Place the animals in a clean and dry cage with plenty of bedding and nesting material, and provide DietGel in a small paper portion cup on the bottom of the cage.
b. Place half the cage on a heating pad.
c. Monitor the animals closely for the next few hours. Check for signs of weakness or inactivity and dehydration.
d. Check the animals the next morning and return to regular housing if there are no abnormalities.

2. Fight wounds (mice)

Fighting between male mice is not uncommon, and seasonal variations have been reported/observed (peak in Summer). Fighting can result in wounds (usually on the base of the tail or dorsal pelvic region), and when this happens, the mice need to be separated as soon as possible. Check the prepuce carefully, as wounds in this area can lead to urethral obstruction and anuria, which can be fatal. Initiate treatment immediately; you don't need to wait to notify the Campus Veterinarian.

a. You may need to anesthetize the rodent to cleanse the wound thoroughly.
b. If there is swelling or ulceration of the prepuce, or substantial tissue necrosis (e.g., large turtle shell scab over the dorsal pelvis), then the animal should be euthanized for humane reasons.
c. Remove (using clippers) the hair from the affected area if the wound can't be easily and thoroughly cleansed.

d. Rinse the wound with sterile saline to remove any debris/contaminant (e.g., bedding chips).
e. Cleanse the wound(s) with a topical antiseptic wound care solution, Vetericyn Plus Antimicrobial Wound Care (Vetericyn solution)
f. Apply, at least once daily, Vetericyn Plus Antimicrobial Hydrogel (Vetericyn Hydrogel) or topical antibiotic ointment (Neosporin, or triple antibiotic: Bacitracin, Neomycin, and Polymyxin B) sparingly after cleaning the wound.

3. Fight wounds or self-trauma (Nile Rats)

Nile Rats are very aggressive; even the group-housed females may need to be separated after a certain age. Also, focus on group-housed animals after disrupting their environment (cage changes, removal, or separation for experimental procedures), as this may trigger aggression between cage mates. Separate the aggressor. Control any bleeding by covering the wound with gauze and applying gentle pressure. Wounds are generally minor, but if the wound is substantial or has bleeding that can't be controlled, the animal should be euthanized. Tail wounds are more commonly seen in Nile Rats than in other anatomical areas. Initiate treatment immediately; you don't need to wait to notify the Campus Veterinarian.

a. You will need to anesthetize the Nile Rat with Isoflurane.
b. Apply the wound care procedure described above (2).

4. Ocular foreign body in Nile Rats

Nile rats frequently have foreign material (dust, debris, nesting fibers) caught on their upper eyelashes and eyelids. As a non-domesticated animal, Nile rats are not well-adapted to captivity, and there may be extrinsic factors in the animal's environment that are not replicated in captivity resulting in this problem. When this condition is observed, the foreign bodies should be removed (flushed out with sterile saline) to minimize any potential discomfort they may cause the animal and because without treatment it may lead to more severe sequelae (i.e., blepharoconjunctivitis). Initiate treatment immediately; you don't need to wait to notify the Campus Veterinarian.

a. Anesthetize the animal.
b. Flush the affected eye and eyelashes with sterile saline until the foreign body is removed.
c. Recover the animal from anesthesia.
d. Replace the Nestlets in the cage with Enviro Dry paper nesting material, and the nesting should be permanently switched to Enviro Dry.

5. Dystocia (mice)

Determine the due date, if possible, and if the animal is >2 days past the due date, then the prognosis is very poor, and it should be euthanized. If the mouse is inactive, debilitated (weak), dehydrated, or if there is any obstruction or prolapse of the uterus, then immediately euthanize the animal and save the carcass for a necropsy by the AV. If the mouse is alert and active, not dehydrated, and in good body condition, provide

the following supportive care for the next 24 hrs. Initiate treatment immediately; you don't need to wait to notify the Campus Veterinarian.

a. Place the cage on a warm heating pad. Place the cage half-on and half-off the heating pad so the animal can behaviorally regulate its temperature (i.e., choose a spot in the cage).
b. Offer DietGel, NutriGel (preferred), or moistened rodent chow on the bottom of the cage.
c. Carefully monitor the animal over the next 24 hrs; euthanize if pups are not delivered or the dam's condition has worsened.

6. Growth retardation (aka small pups or runts)

Reproductive performance data from JAX indicates that their C57BL/6J strain (B6/J) has an average litter size of 4.9 pups born, a wean:born ratio of 0.8, or 3.9 pups weaned per litter, and an average body weight at 3 weeks of age of 10.1 ± 1.7 g (females; average ± SD) or 10.7 ± 1.9 g (males; average ± SD). In our rodent breeding program, we tend to see larger litter sizes (≥6) and smaller pups (~7 g) for this strain. There are many potential causes for small pups in a litter, and in general larger litters will tend to have smaller pups.

Inbreeding is well-known to concentrate harmful mutations. Additionally, animals nearing the end of their productive breeding period have a higher incidence of dystocia and producing litters with developmental abnormalities. Growth retardation can also be an expected phenotype in some mutant lines, which should be described in the approved protocol along with a detailed humane endpoint monitoring plan. Exposure to toxicants (teratogens) or infectious agents during the embryonic or postnatal period can also lead to malformations or even death. Nutritional deficiencies or malnutrition are another potential cause, although they are less of a concern if the mice are fed a balanced diet (rodent chow) and they can access food and water (i.e., juvenile mice and the dam need to be able to reach the feeder and water lix-it valve) and nurse (i.e., the pup is able to nurse).

When a small/runt pup is discovered, the following supportive care should be provided and if it's more than a one-off occurrence, consider weaning later (at 28 days of age). Ensure that food and water are available and accessible in the cage to all the animals. Assess the general condition of the pups, looking for common congenital malformations (malocclusion, hydrocephalus, etc.), an ability to suckle (do they have a milk spot in their abdomen), and any persistent inactivity or dehydration in the pups. Euthanize any pups with these conditions for humane reasons. Review the breeding record and determine the dam's age, body condition, and general health. If the dam is healthy and in good body condition, and the pups are nursing or eating, otherwise active with no other observable abnormalities (other than their size), then place the cage on a heating pad, and offer the pups and dam DietGel/NutriGel or LoveMash on the bottom of the cage. If the dam is not healthy, or if multiple pups in the cage are affected, notify the Campus Veterinarian. Monitor the small pup and euthanize it if it's not gaining weight, or worse if it's losing weight after receiving additional nutritional

support. The carcass of any animal that is euthanized should be saved in the necropsy refrigerator for examination by the Campus Veterinarian.

7. Ear tag wounds (mice)

Occasionally, mice develop ulcerative wounds around their ear tags. These often occur when the ear tag is placed incorrectly. You may need to anesthetize the mouse to remove the ear tag with hemostats or needle-nose pliers. You may need to cut the ear tag first with wire cutters. After removing the ear tag, trim the nails on the hind paws. Apply Vetericyn hydrogel (HG) once daily to the wound.

8. Ulcerative wound (Nile rats)

Ulcerative skin wounds are commonly observed in diabetic Nile rats, especially on the ears. Ulcerative skin wounds on the ear(s) of Nile rats can be treated but the prognosis is guarded because of the underlying problem (poor circulation due to uncontrolled diabetes). The animal should be euthanized for humane reasons if the wounds are extensive or severe or the animal is very pruritic (constantly scratching at the wound). Plan: Start treatment pending decision on humane endpoint. Apply Vetericyn HG once daily to the wound.

9. Excoriative or ulcerative dermatitis (mice)

Spontaneous skin disease (dermatopathy) is highly prevalent in laboratory mice. The typical skin lesions include alopecia, pruritus, erythema, crusting or scaling, excoriations or ulcerations, and necrosis. Excoriative dermatitis is the most common and is typically seen around the base of the skull or the nape of the neck. Ulcerative dermatitis can occur anywhere on the mouse's body. Both conditions are common, multifactorial skin diseases and highly prevalent in C57BL/6 and C57BL/6-background strains. Ulcerative dermatitis in mice has a very poor prognosis, and the animal with this problem should be euthanized. Excoriative dermatitis on the nape of the neck or base of the skull of mice can be treated as follows unless the lesions are extensive or severe or the animal is very pruritic (constantly scratching). Plan: Separate the sick mouse from any cage mates and trim the nails on its hind feet. Clip and remove the hair from around the skin lesion, cleanse the wound with Vetericyn Wound Care solution and apply antimicrobial ointment (SilverHoney) after cleaning. Repeat this treatment at least once daily and until the problem has resolved. If there is any evidence that the problem is worsening or not improving with treatment, notify the Campus Veterinarian immediately.

10. Hyperkeratotic dermatitis

Hyperkeratotic dermatitis is a common opportunistic infection of hairless immunodeficient mice and rats (i.e., athymic nude mice and rats). The infection is caused by the bacterium Corynebacterium bovis (Cb), which infects rodents, cows, and humans. However, only isolates from rats and mice are believed to be associated with disease outbreaks in contemporary laboratory animal colonies. The typical clinical signs are numerous white or tan scales or flakes adherent to the skin of the mouse (dorsum mainly). Severe erythema of the affected areas is also observed. In severe

cases, the mice will show non-specific symptoms of distress such as hunched posture, inactivity, weight loss, and poor body condition. Haired mice and rats are typically asymptomatic carriers, but they can demonstrate clinical signs, especially if the animal is immunodeficient. The typical signs in these mice are limited to the eyes (periocular alopecia, erythema, and thickening of the skin) and ears (white or tan scales or flakes and skin thickening). Mice or rats exhibiting these symptoms should be screened (pelt/skin swab and PCR testing; IDEXX "Skin PCR" profile) for Cb. Affected immunodeficient mice should be euthanized, and the colony/source should be quarantined.

11. Barbering

Barbering (plucking or trimming the hair or whiskers of cage mates) is a common and complex neurobehavioral problem in mice, especially female B6 mice. Barbering most often occurs in the head/face/neck areas and can be attributed to dominance behavior, sexual over-grooming, maternal behavior in lactating mice, or stress-evoked behavior. If the exposed skin of the barbered animal(s) is intact and normal in appearance, then separate the dominant or barber animal if it can be identified (i.e., the mouse with all its hair) and it is not nursing a litter. If there are any skin wounds or lesions, apply Vetericyn HG once daily until the wound has healed (approximately seven days).

12. Wound dehiscence (opening of a surgical skin incision)

Dehiscence of a surgical wound (incision) is a preventable problem. The surgeon must understand and perform appropriate incision and wound closure technique. If the problem is occurring with an unacceptable frequency, retraining is required. If the dehiscence is fresh (<6 hrs) and there is no evidence of gross contamination or extrusion of underlying tissues, then closure of the wound by a trained and qualified individual can be pursued and the wound should then heal by primary intention. If the dehiscence is not fresh, or if the wound is contaminated, then do not close the wound. Instead, carefully rinse the wound with copious amounts of sterile fluid (sterile saline for irrigation), clean skin around the wound margins with Vetericyn skin and wound care solution, and apply topical antimicrobial ointment (e.g., SilverHoney) to the wound. The wound should then heal by secondary intention. If there is any evidence that the wound is not healing normally (swelling, redness, pus), notify the Campus Veterinarian immediately.

13. Skin lesions around surgical implants (rats)

SC implants are generally well-tolerated in rats. However, in some cases, skin lesions develop along the margins of chronic implants include alopecia, pruritus, erythema, crusting or scaling, and excoriations or ulcerations. Treating these lesions can be difficult, and complete resolution may not be possible without removing the implant. Before initiating treatment, it's important to ensure the implant is intact (undamaged) and not leaking (if it's a catheter port). Removing hiding devices (tubes) or overhanging feeders, individual housing, and nail trimming are supportive treatments that may aid in the resolution of the problem. Plan: For mild cases, clip/trim the hair in the area, and cleanse the skin with a topical antiseptic wound care solution, Vetericyn Plus

Antimicrobial Wound Care (Vetericyn solution). Apply topical antibiotic/analgesic (Neo-Predef) or antimicrobial ointment (SilverHoney) to the lesion at least once daily. Moderate cases may require parenteral antibiotics. A bacterial culture swab should be collected in those cases for Aerobic Culture and MIC sensitivity testing. Until those results are received, and based on previous experience, initiate treatment with ampicillin 50 mg/animal SC once daily (rat) or 100 mg/kg SC bid (mice) for 10 days. If the problem is not improving, or if it's worsening, notify the AV. Careful attention to ensuring the sterility of the implant, aseptic technique during surgery, and routine care of the implant-skin margins after surgery are required to prevent or at least minimize the incidence of this iatrogenic problem.

14. Skin lesions around cranial implants (mice and rats)

The typical skin lesions that develop along the margins of chronic cranial implants include alopecia, pruritus, erythema, crusting or scaling, and excoriations or ulcerations. A recently published survey of researchers performing this surgical procedure in rats and mice found the most commonly reported adverse effect at any point post-surgery was scabbing (crust/scale buildup) around the head cap (Barkus C. et al., 2022). Treating these lesions can be difficult, and complete resolution may not be possible without removing the implant. Careful attention to ensuring the sterility of the implant and aseptic technique during surgery are required to prevent or at least minimize the incidence of this post-surgical problem. Plan: For mild cases, clean the lesioned skin daily with a topical antiseptic (5% povidone-iodine ophthalmic solution). Apply topical antimicrobial (SilverHoney) or ophthalmic antibiotic ointment to the wound after cleaning. If the wound is not improving with the topical treatment or worsening, notify the Campus Veterinarian.

15. Swelling underneath VAP implants (rats)

SC swellings may develop underneath or around interscapular vascular access ports (VAP) surgically implanted in rats. These may be a soft fluid-filled cyst (i.e., seroma) or a firm coalescing abscess. Serosanguineous transudate (seroma) or suppurative exudate (abscess) may leak from the VAP margins. Treating an infected cyst or abscess is very difficult without removing the implant. Parenteral antibiotic therapy targeted to the infectious agent (based on bacterial C/S results) should be initiated.
Coagulase-negative Staphylococcus and Enterococcus species bacteria are most commonly isolated in our colonies. Enterococcus species have been resistant to enrofloxacin. Baytril 10-20 mg/kg SC daily for 14 days or ampicillin 50 mg/animal SC once daily (rats) or 100 mg/kg SC bid (mice) for 10 days has generally been effective in preventing systemic complications and reducing the size of the swelling and exudative discharge. If the symptoms do not improve following treatment, or if there is any pain on palpation of the swelling, then the animal should be euthanized for humane reasons. Careful attention to gentle tissue handling and minimal blunt dissection, ensuring the sterility of the implant and all catheter flush solutions, aseptic technique during surgery and catheter flush/maintenance, and routine care of the implant-skin margins after surgery are required to prevent or at least minimize the incidence of this iatrogenic problem.

16. Swollen forelimb and axillary area (rats)
Edema of the forelimb, chest, and axillary region is occasionally observed in rats following jugular vein catheterization, especially if the catheter placement is occluding the venous drainage from the cephalic or facial vein. In some cases, facial swelling may also be observed, depending on what vein(s) (i.e., jugular, cranial vena cava) has been compromised/occluded by the catheterization surgery. This swelling is diffuse and soft, and there should be no signs of pain on palpation. The swelling/edema should gradually reside over the next 7-14 days. Plan: Check to make sure that the catheter is patent (i.e., not leaking into the perivascular space). Re-training is recommended for the surgeon to correct catheter placement and reduce the incidence of this complication. Monitor the animal for any distressful complications: pain on palpation, firmness to the swelling, and weakness or paralysis of the affected limb.

17. Missing cranial implant (mice or rats)
Occasionally, the animal will dislodge or remove their cranial implant. When this happens, the resulting wound may bleed and is usually contaminated with bedding/debris, which could lead to a serious infection. In nearly all cases, implant loss means the animal is no longer suitable for the experimental aim. Any infection or distress caused by the loss of the implant is also a serious animal distress or health concern. Control any bleeding by applying gentle pressure with sterile gauze. Notify the lab as soon as possible. The lab should euthanize the animal unless there is a specific scientific reason for maintaining the animal and the treatment plan is described in the approved protocol.

18. Abdominal swelling/distension (mice or rats, but more common in mice)
Marked abdominal distension may be observed in non-pregnant mice with hydro/mucometrium. Neoplasia (e.g., lymphoma and enlarged spleen), peritonitis, polycystic kidney disease (PKD), or hydronephrosis can also produce an abdominal distension in either sex. In the case of hydro/mucometrium, the vagina will be closed and covered with an imperforate vaginal membrane, but the animal may appear and behave normally. Hydronephrosis is relatively common according to the literature, and regarded as an incidental congenital malformation. If only one kidney is involved, and it has not ruptured, the animal may not show other abnormal clinical signs and will behave normally. The other conditions, neoplasia, peritonitis and PKD, typically result in severe and debilitating clinical signs such as, inactivity/lethargy, piloerection, hunched posture, dehydration, very thin or emaciated body condition. The prognosis is very poor in all of these cases (mucometrium, neoplasia, peritonitis, PKD, or hydronephrosis), and the animal should be euthanized for humane and diagnostic reasons.

19. Perineal swelling
Young or juvenile female mice, usually on a B6 strain/background, occasionally present with a soft but prominent swelling of the perineal area. It may appear that the female mice have a scrotum. The mice are otherwise normal, and the swelling is typically soft

and non-painful upon palpation. The mice will have a closed vaginal opening, and the swelling is due to a retention of fluid/mucus from the uterus resulting in the visible swelling in the perineal region. This is an inherited abnormality, and the parents of the mice with this condition should be identified, culled, and replaced (with unrelated mice). Any mouse with this condition should similarly be euthanized for humane reasons.

20. Ulcerative lesion on prepuce (mice)
Genitourinary obstruction syndrome (aka mouse urologic syndrome and obstructive uropathy) has been reported in B6 mice, and while the etiological diagnosis has not been determined, the chronic form of this syndrome/condition develops in breeding males with and without a history of fighting, and S. aureus is commonly isolated bacterial agent in the resulting ascending urinary infection. There is no treatment. Plan: Euthanasia for humane reasons.

21. Scrotal swelling
Scrotal swelling has been observed in young/juvenile male mice treated with tamoxifen. The scrotal swelling is soft and easily reducible on palpation, and there is no evidence of pain on palpation. Necropsy and histopathological results of previous cases indicated that the swellings arose from either a thinning of the inguinal body wall (omphalocele) or ascites and fluid accumulation in the scrotal sac. Inguinal or abdominal hernias in male mice are reported in the literature for mice treated with tamoxifen, especially when treated as pre-weanling mice. Plan: Monitor the mice for any change in the firmness of the scrotal swelling, behavioral signs of pain, abdominal distention, and any evidence of urethral obstruction (decreased or no urine production).

22. Prolapsed rectum
Rectal prolapse is not uncommon in mice because of the unique anatomy of their rectum and because it's a sequela in rodent colonies endemically infected with Helicobacter sp. bacterium. When identified, unless it is a very mild case of rectal prolapse (≤1 mm of prolapsed tissue visible), the affected animal should be promptly euthanized for humane reasons.

23. Prolapsed penis (paraphimosis)
The penis is normally not visible, but occasionally, in mice, especially breeding males and group-housed males, the penis will be visible, but this is not a clinical problem. If the penis is visible for an extended period (hours), the likelihood that it will spontaneously retract is very low. This condition is potentially very painful, and challenging to treat, therefore, the animal with this condition should be euthanized for humane reasons.

24. Hydrocephalus (mice)
Hydrocephalus is an untreatable congenital/developmental abnormality frequently seen in B6 mice. The prototypical appearance is a pre-weanling or juvenile mouse with

a dome-shaped skull. Depending on the severity, the animal may exhibit distressful symptoms such as persistent inactivity, dehydration, thin body condition, and occasionally ataxia is also observed. There is no treatment, and the mouse should be euthanized for humane reasons before (or at least by the time) the distressful symptoms are identified.

25. Incisor malocclusion and overgrowth (mice)

Incisor teeth continuously grow in mice, and the teeth will overgrow if malocclusion occurs (i.e., the upper and lower incisors are not aligned). Mice with overgrown teeth will not be able to eat, and overgrown incisors can also cause a painful condition because the teeth will grow into and damage the hard palate. These mice will be malnourished and have a very thin or emaciated body condition. There is no humane treatment for this condition. Continuously trimming the teeth is a painful, life-long treatment and should not be performed. Mice with overgrown incisors should be euthanized for humane reasons. This problem has a hereditary component and is relatively common in B6 mice.

26. Aged breeders

Mice that are beyond their productive breeding period should be culled and replaced. As a general rule, younger (6-8 weeks of age) female mice breed better than older ones, and they should be replaced by 30 - 34 weeks of age (i.e., B6/J = 30 weeks). The breeding pair should also be replaced if they fail to produce a litter after 60 days, or if they fail to wean any pups for two successive litters.

27. Anophthalmia or microphthalmia

Anophthalmia and microphthalmia are usually unilateral (only one eye is affected) and are common heritable abnormalities of B6 mice. If blepharospasm (constant blinking or squinting) is also observed, then this would suggest that there may be discomfort or pain associated with the problem. Blepharitis (redness and hair loss around the eye), conjunctivitis (redness of the conjunctiva and whitish discharge), and corneal opacity (gray/white spot on the cornea) are often sequelae of a smaller globe. If blepharospasm, blepharitis, or conjunctivitis are observed, then the animal should be euthanized. If no signs of discomfort or infection are observed, then the lab should determine if the animal with this condition (anophthalmia or microphthalmia) is a valid experimental model and if not, it should be culled.

28. Blepharitis or conjunctivitis

Ulcerative blepharitis and suppurative conjunctivitis are occasionally observed in some inbred strains, including 129 and BALB/c, and may be associated with Corynebacterium infection. In other strains, opportunistic infection with Rodentibacter bacterium has been known to cause conjunctivitis. If there is no blepharospasm (constant blinking or squinting) to suggest that there may be discomfort or pain associated with the problem, then the following treatment may be attempted for valuable experimental animals: ophthalmic antibiotic ointment, at least twice daily for at least one week, and Baytril, 10 mg/kg, PO or SC daily for at least one week. Breeder

animals with this condition should be culled to reduce the prevalence and spread of opportunistic bacterial agents in the rodent colony.

29. Spontaneous ocular opacity (mice)

Ocular abnormalities are common in the B6 mouse, and GEM (genetically-engineered mice) on a B6 background. Lenticular opacities (i.e., cataracts) are an especially common age-related problem in this strain. Spontaneous corneal opacities are also especially common in B6 and other strains (e.g., 10% of BALB/c can develop keratopathy). Iatrogenic causes can also result in ocular opacity. Corneal opacities are frequently observed in anesthetized mice that have damaged their delicate corneas (i.e., no ophthalmic lubricant was placed on the animal's corneas). Due to the small size of their eyes, treatment (e.g., corneal transplant) is not an option for mice. Extra-label treatment with topical antibiotics or steroids is similarly ineffective and can result in adverse effects because of the high rate of systemic absorption. Plan: Keep the animal in the colony if there are no clinical signs of discomfort/pain (i.e., blepharospasm), and the lab determines that an animal with this condition (i.e., essentially blind in the affected eye) is still a valid experimental model. However, if the animal is going to be used as a breeder, or if it manifests or later develops any signs of pain/distress or infection (i.e., ocular discharge, alopecia around the eyes, or redness swelling of the eyelids or conjunctiva), then that animal should be euthanized for humane reasons.

30. Ocular porphyrin discharge/staining (rats)

Unilateral or bilateral ocular discharge and staining of the skin and hair with red porphyrin pigment may indicate ocular irritation or inflammation caused by environmental agents, such as dust or debris from bedding. Other potential causes include experimental stressors and respiratory infections. In the case of respiratory infections, the discharge is usually bilateral and typically occurs in younger animals. Animals with porphyrin discharge/staining should be carefully examined for signs of upper respiratory infection, including sneezing, nasal discharge, facial swelling, weight loss and inactivity. If any of these signs are present, the animal should be euthanized for humane and diagnostic reasons. If environmental factors are suspected, consider decreasing stocking density (e.g., placing the animals in a larger cage), replacing the bedding, and monitoring the animal for any changes in clinical symptoms. It is important to rule out experimental factors (e.g., recent blood collection) when possible.

31. Hunched, unkempt, weak, inactive, and dehydrated

There are many potential causes of this classic "sick" rodent appearance. Assess the cage condition to ensure that food and water are available to the animal. Check the water valve on the Hydropac to ensure it's working (toggle it, and a drop of water should come out). Assess the animal's body temperature. Does it feel cool to the touch? Assess hydration status by gently pinching the skin around the scruff of the neck. If the animal is dehydrated, the skin will tent and not immediately return to normal shape. Another sign the animal is dehydrated is if its eyes appear sunken and dry. Plan: Separate the affected animal(s) from unaffected cage mates. If its body temperature is low (hypothermia), place its cage on a warm heating pad.

Administer parenteral fluids to treat dehydration. Warm the fluids (LRS or saline) before administering them to the animal. Inject, SC, a volume of up to 5% of the animal's body weight (1 g = 1 ml). For example, a 300 g rat would receive 15 ml, and a 20 g mouse would receive 1 ml. Offer DietGel (preferred) or moistened rodent chow on the bottom of the cage. Antibiotic therapy can be attempted, but the prognosis is guarded to poor. The antibiotic commonly used to treat experimental CNS infections (e.g., meningitis) and sepsis in rodents is ceftriaxone. The dose for ceftriaxone is 100 mg/kg, IP, every 12 hours for five days. Reconstitute a 1g/vial ceftriaxone vial with 9.6 ml of sterile saline for injection. The concentration of ceftriaxone in the vial will equal 100 mg/ml. A 25g mouse should receive a 25µl injection of this solution every 12 hours for five days. A 250g rat should receive a 0.25 ml injection of this solution every 12 hours for five days. Monitor the animal closely over the next 24-hour period and euthanize the animal if any complications arise (i.e., rapid or labored breathing) or the animal is not showing improvement.

32. Seizures or epilepsy

Generalized uncontrolled motor and non-motor (absence) activity can be observed in a few common inbred mouse strains (e.g., audiogenic seizures in DBA/2J) but are more common in GEM with mutations (targeted and spontaneous) in genes important in epileptogenesis. The clinical symptoms are often triggered during the cage change procedure or experimental manipulations/handling, that is, periods of stress for laboratory animals. These mice will manifest the following behaviors: freezing, falling over uncontrolled motor activity, and hyper-salivation. The seizures are often brief; afterward, the animal appears and behaves normally. Unfortunately, there is no practical treatment for this problem, and the animal with this problem should be euthanized for humane reasons. Valuable and irreplaceable experimental mice with seizures should be cared for as follows:

a. Identify the seizure condition on the cage or sick card.
b. Identify the animal (e.g., tail mark with a Sharpie) and monitor the animal's general health during each cage change.
c. Handle the animal with care and as little as possible, and document any seizure activity that occurs when the animal is handled (during cage change or experimental manipulation) on the sick card.
d. If the seizure activity increases in severity, frequency, or duration, or if the animal’s general condition deteriorates, the animal should be euthanized.

33. Circling, spinning, head tilt, ataxia, paralysis or hyperactivity

Any of these neurological symptoms can occur at any time and are not typically triggered by a cage change, unlike seizures. These problems are often found in GEM models (e.g., AD models), which is why it’s crucial to always have accurate strain nomenclature on the cage card. Some of these problems can also be caused by an infectious disease (e.g., contamination of drinking water and infection with Burkholderia sp. can lead to a head tilt, spinning, and ataxia) or experimental treatment (EAE can cause ataxia and hindlimb paralysis). Unfortunately, there is no practical

treatment for any of these problems, and an animal with one or more of these problems should be euthanized for humane reasons.

34. Lameness
A rodent can become lame for many reasons, including traumatic injuries that damage soft tissues (muscles, tendons, or ligaments) or bones (fractures). Infection involving the joints can also cause the animal to become lame. The prognosis is usually very poor, and the condition is very distressful and painful (fractures). Therefore, the animal should be euthanized for humane reasons.

35. Amputation
Juvenile mice presenting with healed amputating lesions, missing phalanges or extremities. If the mice received tattoos as pups on the missing limbs, then review the procedure and ensure that it's being performed aseptically. If other external cutaneous lesions such as conjunctivitis or swollen extremities are present, or there is no history of tattooing, then euthanize the mouse, perform a necropsy including collecting blood from the mouse for comprehensive serology to rule out mouse pox virus infection.

36. Injection Site Reaction
Injection site reactions (ISRs) commonly occur with parenteral (e.g., SC) administration of therapeutic or experimental drugs due to chemical irritation or toxicity and infrequently due to immune-mediated hypersensitivity. The abnormal clinical symptoms that develop can vary and include swelling, erythema, pruritus, and pain around the site of injection. In severe cases, ulceration and necrosis of the injection site are also observed. Almost all local ISRs to subcutaneously administered drugs can be prevented by changing the injection technique or drug formulation. Treat mild to moderate ISRs by following the wound care procedures described above (2). The animal should be euthanized for humane reasons in severe cases (extensive necrosis or ulceration) or if there is any systemic abnormality (inactivity, lameness, respiratory distress). Again, prevention is key, and all drug solutions administered parenterally must be sterile, pharmaceutical-grade, and biocompatible (pH between 4.5 and 8.0, and osmolality between 150 and 600 mOsm/kg). Additional information is available from this IACUC guideline.

37. Pendulous or distended abdomen (zebrafish)
A pendulous/distended abdomen with no other symptoms (e.g., swimming abnormality, ulceration of body wall, loss of neutral buoyancy) is not uncommon in a zebrafish breeding colony and is of no colony health significance. Most commonly, the abdominal distention is due to enlargement of the testis (seminoma) or non-specific, egg-associated inflammation. However, if additional symptoms, including loss of neutral buoyancy (unable to swim off the bottom of the tank), "dropsy" or "pinecone scales'' (i.e., generalized edema), or ulcerations of the body wall, are observed, then the animal should be promptly euthanized for humane and diagnostic reasons. Preserve the animal carcass in buffered formalin. These distressful conditions may be

caused by opportunistic bacterial infections (e.g., mycobacterium and bacterial aerocystitis).

38. Ulcerations (zebrafish)
Ulceration of the body wall, usually of the abdomen or around the cloaca, is a common manifestation of opportunistic and granulomatous inflammation/infection with Mycobacterium bacteria. When animals with these symptoms are identified, they should be euthanized for humane and diagnostic reasons.

39. Abnormal swimming behavior or loss of equilibrium (LOE)
Fish lying at the bottom of the tank or floating or persistently swimming at the tank's surface should be promptly euthanized for humane and diagnostic reasons. If the abnormal swimming pattern involves multiple fish in the tank, especially if their respiration rates are increased or their gills are very red, then this may be a symptom of poor water quality (low oxygen saturation). Immediately check the dissolved oxygen levels in the tank water and ensure that the water flow has not been interrupted in the tank or elsewhere on the rack (e.g., the sump tank).

40. Emaciation and musculoskeletal deformities (scoliosis or lordosis)
Zebrafish with an emaciated body and curved spines are observed sporadically in our zebrafish breeding colony. These clinical symptoms can be seen in fish with microsporidiosis (Pseudoloma neurophilia). When animals with these symptoms are identified, they should be euthanized for humane and diagnostic reasons.

41. Craniofacial malformation (small opercula, deformed jaws)
Congenital or phenotype-associated malformations have been observed infrequently in our transgenic zebrafish. This condition has no colony health significance. However, it's recommended that these animals be euthanized and culled from the colony to reduce the incidence of these heritable malformations.

42. Exophthalmia or buphthalmia (zebrafish)
A congenital malformation or trauma may cause unilateral enlargement or malformation of the eye. If it's due to trauma, then with time, the symptoms may spontaneously resolve. If it's a congenital or dietary problem, usually the eyes are malformed, not just enlarged or bulging, or other ocular structures (lense) will be affected. It's recommended that these animals be euthanized and culled from the colony to reduce the incidence of these heritable malformations. If both eyes are affected, or there is red/gray discoloration of the eye or surrounding tissues, it may indicate an opportunistic infection or neoplasia (cholangiocellular carcinoma; spindle cell tumor). When animals with these symptoms are identified, they should be euthanized for humane and diagnostic reasons. Preserve the animal carcass in buffered formalin for histopathology. If there is a visible gas bubble in/behind one or both globes, this may be caused by gas supersaturation in the water. Check the water pump to make sure that there are no gurgling noises or leakage. Check the oxygen saturation levels in the water to ensure they are not too high.

43. Age-associated diseases

Zebrafish mount a robust granulomatous response to infection (i.e., they wall them off), and many of their infections are subclinical (no clinical symptoms). However, over time, they will develop non-specific symptoms of these diseases; as such, aged zebrafish with any clinical symptoms, especially those with poor body condition (thin), make for good sentinels of the diseases in the colony. Zebrafish in the breeding colony should be culled and replaced before 1.5 years of age. Any aged zebrafish with abnormal clinical signs (ulcerations, abdominal distension, spinal deformities, loss of natural buoyancy or equilibrium) should be euthanized, especially those aged zebrafish in poor body condition.

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