Guidelines for Assessing the Health and Condition of Mice

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With the development of transgenic and targeted mutant (knockout) technologies over the past 10 years, there has been an increase in the number of investigators using mice; the number of mice used in biomedical research; the number of mice bred and maintained to their natural life spans; and the number of mice with potentially adverse or debilitating phenotypes. The authors present practical guidelines for assessing and monitoring the health of these mice.

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Transgenic and knockout technologies have caused a shift in research focus at many institutions. Increased numbers of mice—some with potentially debilitating phenotypes—are being maintained to their natural life spans, and this has challenged the laboratory animal community to devise effective strategies for monitoring and managing these colonies.

Aside from meeting essential obligations for ensuring the well-being (freedom from discomfort, distress, and pain) of the research mouse, effective management and supervision of mouse colonies is useful in fulfilling the "3 Rs" (replacement, reduction, refinement) put forth by Russell and Burch[1]. We also suggest a fourth essential "R," investigator responsibility, since investigators are increasingly monitoring and managing their own transgenic colonies. A well-monitored and -managed rodent colony will provide: more precise information on the progression of disease or phenotype; clear criteria for data collection; recovery of data potentially lost when unmonitored mice die; improved quality, and increased uniformity, of data due to standardized techniques for monitoring health; additional observations and characterization of phenotypes; possible decrease in number of mice needed to characterize a phenotype; and decreased costs of characterizing a phenotype.

The Importance of Endpoints

When there is potential for animal pain, distress, or suffering, investigators need to outline clearly the research objectives and procedures for assessing animal health. It is necessary to define appropriate experimental endpoints that allow for early intervention (*e.g.*, euthanasia), while attaining experimental objectives, minimizing data loss, potentially reducing animal suffering, and improving the quality of data collected.

In establishing criteria for assessing health and endpoint determination, we recommend that investigators avoid the terms "sick" or "moribund," which may have diverse meanings to different individuals. Rather, for effective communication, investigators and veterinary staff must find a common descriptive ground for monitoring criteria. Further, they must agree on when animals should be euthanized.

Specific criteria for determining suffering and predicting death are poorly described in both human and veterinary literature, and are commonly subjective and overly optimistic[2-4]. A number of publications have defined techniques for refined determination of experimental endpoints for specific types of studies, and have proposed criteria for monitoring animal health. Examples include: weight loss in a central nervous system tumor model[5]; hypothermia in septicemia models[6,7]; pain on abdominal palpation in a gallstone model[8]; and freezing and scratching behavior in arthritis models[9,10]. These criteria are helpful, and some may be applicable in other experimental scenarios.

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General monitoring criteria, which are both comprehensive and useful, have been described in the literature[11-29]. We have incorporated these and other empirically derived criteria into guidelines for investi-

gators, providing interpretations of their importance and recommendations for courses of action. Investigators receive these guidelines when they submit experimental protocols to the IACUC as an aid for formulating strategies for monitoring health.

The guidelines help to open lines of communication between investigative and veterinary staff, enabling development of specific criteria for monitoring and endpoint determination. Once staff members have better characterized a specific phenotype or experimental paradigm, they can institute specific scoring techniques, preventative or supportive strategies, and experimental endpoints.

The authors have found that investigators, as well as animal care and veterinary staff, have readily accepted the guidelines, using them as a training tool and for protocol development. Although mice are not covered under the Animal Welfare Act (AWA), and some institutions may not be subject to the principles in the *Guide*, most facilities adhere to standards described in these regulatory documents[30,31]. We have found that the guidelines in this article help both investigators and animal care staff meet the requirements espoused in those documents. We emphasize that investigators should contact the veterinary staff for further advice in applying these guidelines to their research needs; developing preventative and supportive strategies; determining appropriate endpoints; and for information on infectious causes and treatment of disease.

Our goal has been to establish techniques for monitoring that are not laborious or time-consuming, and to find practical methods that the investigator or veterinary staff can perform rapidly and effectively in various model applications. The following guidelines, including a technique for evaluating body condition (musculature and fat deposits over the sacroiliac bones), are useful and easy to implement in a wide variety of study paradigms. Experimental validation of this technique is described in the literature[32].

As mandated by the *Guide* and the US Government *Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training*, investigators have an obligation to minimize pain, discomfort, and distress in the animals used in their research. Part of fulfilling that obligation is recognizing when an animal's well-being is compromised.

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We recommend evaluating individual mice at least once a week (in addition to the daily observation mandated by regulatory agencies) to identify mice that may need to be monitored more closely, or perhaps euthanized.

Body Condition Scoring

Body condition scoring (BCS) is a useful tool for evaluating overall condition of the mouse. Techniques such as obtaining body weights or temperatures regularly may be impractical, and investigators may be reluctant to perform them. When one is studying genetically altered animals, or any research mouse in danger of deteriorating over a long period, and monitoring large colonies with a spectrum of ages, BCS, as described below, offers a useful, rapid, practical, and objective alternative for health assessment. BCS is particularly helpful in cases where pregnancy, organomegaly, or tumor growth may interfere with body weight assessment.

BCS is simple to perform: when picking a mouse up at the base of the tail, note its body condition by passing your finger over the sacroiliac bones (back and pubic bones). The body condition can be scored on a scale of 1-5 as indicated:

5: The mouse is obese, and bones cannot be felt at all;
4: The mouse is well-fleshed, and bones are barely felt;
3: The mouse is in optimal condition. Bones are palpable but not prominent;
2: The mouse is becoming thin and bones are prominent. This category may be further divided subjectively as +2, 2, and -2. Euthanasia is recommended for BCS of -2.
1: Muscle wasting is advanced, fat deposits are gone, and bones are very prominent. Euthanasia is mandatory.

A body condition score of 2 or 1 suggests a decline in overall condition, and euthanasia is recommended. Considering BCS score and weight-loss may be useful. A weight-loss of 10-15% within a few days is a criterion for euthanasia. An overall weight-loss of 20% is also an indication for euthanasia.

Health Guidelines

Some investigators may have a background in biological systems, whereas others with backgrounds in molecular biology may have minimal exposure or experience in evaluating animal health. The guidelines provided below are simple interpretations of clinical signs for use by individuals with diverse backgrounds (*e.g.*, physicians, postdoctoral fellows, graduate students, and technicians). Additionally, these guidelines can be useful for aiding animal care staff observation of animals during the cage-changing process.

Obvious Health Problems

In addition to introducing investigators to BCS, we provide them with the following outline of obvious health problems:

Barbering: Mice often chew one another's hair in the grooming process. In some cases, this can become excessive, but is not a problem unless skin lesions result (*e.g.*, ulcerative dermatitis or bite wounds). Cage enrichment (*e.g.*, empty feed jars, PVC, nesting material) may discourage this behavior.

Fighting: Wounds from fighting are typically found at the tail base; along the back; on the prepuce or anus; or on the tail, feet,

or shoulders. Fight wounds will have scabs and may bleed. Fighting can be severe, and immediate separation is indicated. Wounded mice should be separated from the aggressor (the animal without wounds). Place mice in a clean cage so odors from previous battles do not provide continued stimulation for further fighting. It may be helpful to add a nestlet, nesting material, PVC tubing, or an empty feeding jar to the cage to distract the mice from fighting (if the fighting is not severe) or to discourage fighting among remaining cage mates when an aggressor is removed. Animals with severe wounds should be isolated and treated with an antibiotic as prescribed by the veterinarian. Certain backgrounds, such as FVB, BALB/c, and SJL, are noted for the aggressive tendencies of males. In these cases, preventative measures should be instituted (*e.g.*, only co-house litter mates, separate males >6-10 weeks of age, cull breeding males at 6-8 months old).

Malocclusion: Poor weight gain after weaning is often the first indication of a malocclusion. Note differences in size among litter-mates in the same cage. If you suspect a mouse is not gaining weight during a time that it should be growing, restrain it and look at its incisor teeth. Note if the top and bottom teeth are not hitting against one another, if the bottom teeth grow long and are easily seen, and if the top teeth tend to grow back into the roof of the mouth. You can trim both upper and lower incisor teeth with scissors provided by the veterinary staff. Identify the cage so the animal can be weighed and monitored. Because this is a hereditary trait, and these animals require life-long monitoring and treatment, we strongly urge euthanasia of these animals.

Rectal Prolapse: Rectal prolapse, or the protrusion of the rectum below the tail, may be common in some lines of mice, often with no recognizable cause. The prolapsed rectum is easily noticed when mice are picked up by the tail. Euthanasia may not be indicated unless the prolapse is large and necrotic. However, assessment of body condition and weight on a regular (bimonthly) basis is indicated because body condition tends to decline as more extensive bowel lesions develop.

Tumors and Masses: Tumors or masses can develop anywhere on the body. Observe mice for symmetry. If the mouse is not sleek and symmetrical, look more closely to see if there is a mass distorting its shape. Many times investigators have implanted tumors in mice by injecting cells under the skin of the neck, back, or flank area. Tumors that are >10% of the mouse's body size (~1 cm in diameter); ulcerated; that interfere with eating, drinking, urinating, defecating, or walking; or that result in loss of body condition are indications for euthanasia. Assessment of body weight may not be useful with a large tumor mass, and BCS is recommended in this case.

Ulcerative Dermatitis: Ulcerative dermatitis develops when inflammation causes erosions through all layers of skin, resulting in an open, oozing sore. These can develop anywhere on the body, and may be secondary to over-grooming, scratching at ear tags, infestations with parasites, or may have an unknown cause. Non-healing, spreading lesions with debilitating scars or contracture, or lesions covering 10-20% of the skin, are indications for euthanasia.

Vaginal or Uterine Prolapse: With hormonal stimulation, the vagina can become thickened and protrude from the vulva. Although vaginal hypertrophy is not a cause for euthanasia, it may limit usefulness of breeding females, and the condition of affected animals should be monitored for evidence of deterioration. The uterus may prolapse through the vagina and vulva if the mouse is straining (*e.g.*, after delivery of pups). A rectal prolapse and a uterine prolapse are not always easy to distinguish; check closely to determine through which opening the organ is protruding. Unlike vaginal and rectal prolapse, uterine prolapse generally has a poor prognosis, and euthanasia is recommended.

Subtle Health Problems

Activity/Behavior: When opening a cage, observe the behavior of the mice. Are they active and curious (*i.e.*, demonstrating normal open-field activity)? Do any seem thin, runted, lethargic, or in pain (especially in comparison to cage-mates)? Are any of the mice hunched up, or having difficulty breathing? Are some not grooming, over-grooming, scratching, licking, or mutilating themselves? All of these are indications for closer examination and evaluation.

Anemia: Anemia can be difficult to assess in pigmented mice, but observing the color of the foot pads is a quick, easy way to estimate anemia. You will be able to detect an anemic mouse because of the pallor or paleness of its footpads.

Dehydration: Dehydrated mice will have eyes that appear recessed in their heads, and the facial fur will appear fuzzier (due to piloerection). If you pick up the skin over their shoulder blades, it will not return quickly to its original shape, but instead will remain bunched. If fluids are administered under the skin, the mouse may recover; immediate euthanasia is generally recommended, because these mice will usually die quickly. Mice that are cool to the touch (hypothermic) are especially vulnerable and should be euthanized.

Diarrhea: Mice with diarrhea often don't have fluid feces; frequently, feces are just moist. The easiest way to detect this is to look at the contents of the cage. Is the bedding sticking to the feces or to the side of the cage? Notice if there is staining of the hair around, or feces sticking to, the rectum. In breeding cages, is the hair of the pups stained? Is there feces or blood around the rectum? Diarrhea may have an infectious cause and should be brought to the attention of the veterinary staff. Diligent assessment of hydration, body weight, and BCS is indicated.

Hypothermia: Mice that are cool to the touch or have a body temperature of less than 98°F (36.5°C) require immediate attention. If the hypothermia is severe, the animal will be sluggish or possibly non-responsive. One may attempt to warm the animal (*i.e.*, provide supplemental heat). If no supportive care (warming) is provided, the mouse should be euthanized.

Icterus: Icterus, or yellowing of the skin, develops when the liver or bile system is

not working properly, or when there is destruction of red blood cells. The ears are probably the easiest site for detecting icterus. When comparing and observing mice, note mice that have a yellow tinge to their ears.

Preputial or Vaginal Discharge: These may be found under a variety of circumstances (*e.g.*, infections or tumors of the urogenital tract). Is there white material or bloody urine oozing from the prepuce or vagina? The veterinary staff should examine these animals.

Additional Health Observations

Abnormal Breathing: Dyspnea (difficulty breathing), rales (noisy breathing from congestion in the lungs), and tachypnea (rapid breathing) are nearly always a serious sign and euthanasia is recommended.

Abnormal Locomotion: Ataxia (lack of coordination), circling, and weakness are common to conditions that are slow or rapidly progressive. Close evaluation of the mouse's ability to eat and drink, and of weight-loss or body condition, is indicated.

Eye Abnormality: Opacity, dilated pupils, constricted pupils, exophthalmia (bulging eye), and enophthalmia (sunken eye) are important if noticed along with other signs (*e.g.*, loss of condition). Conjunctivitis (inflammation of the ocular mucous membranes), abnormal secretions or crusting, or corneal ulcers may result in some cases; if these do not respond to treatment, euthanasia may be indicated.

Head Tilt: Head tilt is simply when a mouse consistently holds its heads to one side. This clinical sign can have multiple causes, and close evaluation and monitoring are indicated. In severe cases, animals may be unable to stand, eat, or drink, in which case euthanasia is indicated.

Hyperactivity: Hyperactivity is characterized by abnormal, excessive activity or frantic behavior. It is a nonspecific clinical sign, and should be monitored closely (daily). Hyperactivity may be related to phenotype, reflecting a nervous system abnormality. Some strains of mice are normally hyperactive, and behavior may not be significant.

Lethargy: A lethargic mouse may exhibit sluggish behavior, stupor, coma, hypoactivity, prostration, or a hunched posture. These are important clinical indicators of a serious illness. Supportive care or euthanasia is indicated.

Paresis: Paresis is indicated when the mouse is weak, unable to support its body weight, but can move its legs. This condition is clinically important because it may progress to paralysis, and should be monitored closely.

Paralysis: Paralysis is the loss of voluntary muscle movement in the legs or tail. Depending on underlying cause (*e.g.*, tumor infiltration of spinal cord), this is an indication for euthanasia. In some cases (*e.g.*, experimental allergic encephalomyelitis models), mice may recover from paralysis. In such cases, supportive care (*e.g.*, nesting and food provided on floor, easy access to water, bladder expression) is indicated. Excessive weight loss (>25%), decubital ulcers (bedsores), and self-mutilation are indications for euthanasia.

Ruffled Fur: An unkempt and ungroomed appearance indicates a mouse is not feeling well. If noticed along with other signs, such as dehydration or loss of body condition, this may be an indication for euthanasia.

Tremors: Frequent and severe tremors, involuntary shaking, convulsions, or seizures should be monitored closely, and may be indications for euthanasia.

Conclusion

We have found the general criteria presented above to be useful for evaluating animal health, and for determining appropriate endpoints in a variety of model systems: *e.g.*, use of foot-pad pallor and loss of body condition in inflammatory bowel models; open-field activity, weight loss, and loss of body condition for bone marrow transplant models; ulcerative dermatitis and loss of body condition in selectin-deficient mice.

The veterinary and animal care staff can play an important role in assisting the investigative staff take responsibility for its role in ensuring the well-being of research mice. For this reason, it is critical to establish effective communication and cooperation with investigators, and to ensure that the laboratory animal staff is seen as a useful resource. Providing guidelines such as the ones presented above is the first step in meeting this objective.

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