IACUC Guideline

FOOD & FLUID REGULATION IN MICE AND RATS

The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) has adopted the following policy to clarify the scenarios and procedures of regulating food and/or fluid in rodents. Food and/or fluid regulation is typically used in the research setting in (1) studies that use food/fluid consumption to motivate animals to perform novel or learned tasks, (2) studies of the motivated behaviors and physiologic mediators of hunger and thirst, and (3) homeostatic regulation of energy metabolism or food balance. This guideline will outline important issues to be considered and addressed in protocols that require food and/or fluid regulation/scheduling.

The objective when these studies are being planned and executed should be to use the least regulation necessary to achieve the scientific objectives while maintaining animal well-being. In order to accomplish this objective the following must always be considered and adequately described in the IACUC protocol:

- The necessary level of regulation
- Potential adverse consequences of regulation
- Methods to assess the health and well-being of the animals under regulation

Contact the Office of Animal Welfare (OAW) with any questions regarding this guideline or any general rodent use practices. This guideline will cover the following topics:

- Definitions
- Scientific justification
- Determining ad libitum values
- Determining the level of regulation
- Monitoring and documentation
- Endpoints
- Exemptions

DEFINITIONS

- Ad libitum: Rodents are offered access to a continuous supply of food and water and can eat and drink as much and as often as they want. This is the current husbandry practice at the University of Pennsylvania.
- Deprivation: Complete withholding of food or water.
- Regulation/Scheduling: The act of controlling the amount of water and food an animal receives and when the animal receives it. The amount is less than the average daily ad libitum water and food consumption.

SCIENTIFIC JUSTIFICATION

Scientific justifications should be provided for using regulation/scheduling of food or fluid in rodents. This justification should include an explanation as to why alternatives cannot be used and also provide rationale for the level and length of time the regulation/scheduling is proposed. In accordance with the Guide for the Care and Use of Laboratory Animals (2011), in the case of conditioned response research protocols, use of a highly preferred food or fluid as a positive reinforcement is recommended. The justification should also include reasons why positive reinforcement cannot be considered. A literature search must also be conducted for alternatives to food and or fluid regulation.
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DETERMINING AD LIBITUM VALUES
For any study requiring chronic (over 24 hour) regulation, the investigator should provide the normal range of ad libitum normals for the same background strain, sex and age group used in the study. Published values for the same age, sex, background strain, and weight may be used in lieu of in-house determination. See Appendix A for a sample of published data regarding common strains of mice used in research for water and food values.

DETERMINING LEVEL OF REGULATION
The goal of fluid regulation is to get required performance with the least restriction possible. In the procedure description, the investigator should provide the formula used for the proposed regulation/scheduling, e.g., “Food regulation equal to 70% of ad libitum intake until the rodent is at 80% body weight of peer controls.” Acute water deprivation up to 24 hours will result in clinical dehydration, but deprivation in excess of 24 hours is not permitted. Rodents may be placed under chronic water regulation of as much as 50% of the ad libitum daily ration, if it is imposed over 7 days.

Rodents should be acclimated for 7 days to new regulation/scheduling paradigms unless specifically approved by the IACUC. Consideration should be made to allow food and water to be available concurrently, as rodents typically do not eat without available water. For animals undergoing regulation/scheduling, access to food and/or water must be for 15 minutes at a minimum. Regulated levels of food should not be lower than 30% of ad libitum values. Animals undergoing surgery should not have either food or water restricted for at least 24 hours prior to surgery, and restriction cannot resume until the animal has fully recovered from the procedure.

Regulation is not recommended in rodents under 4 weeks of age.

MONITORING AND DOCUMENTATION
Animals on regulation/scheduling must be monitored daily and weighed at least twice weekly, but it is encouraged to do so as often as the critical aspects of the study permit. Please see Appendix B for an example form to use as a monitoring record sheet. Weight should be compared to either age and strain-matched controls or the baseline body weight (if an adult). Additionally, a body condition score (BCS) system should be used to evaluate the animals. Please see Appendix C for a diagram explaining the BCS system and how it differs between mice and rats.

Food and water availability should be recorded daily (and weights at least twice weekly) during any time period that regulation/scheduling is being conducted. If animals are not undergoing regulation then documentation is not necessary at that time. The records must be available for the ULAR staff, IACUC members or outside inspectors at all times. The records should contain the following information:

- General information (i.e. protocol number, animal identification number, Principal investigator, contact person, contact phone number)
- Baseline weight (before restriction period began)
- Date (daily documentation is necessary)
- Indication that water was given (daily)
- Indication that food was given (daily)
- Weight and BCS (twice weekly)
- Health (including hydration status)
- Initials of observer/recorder
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ENDPOINTS
Specific humane endpoints that would require intervention must be clearly stated in the protocol.

For food regulation, a rodent may not lose more than 20% of age-strain-sex matched controls or baseline body weight (if adult). If greater than 20% weight loss has occurred (the animal weight is below 80% of the baseline weight or that of matched controls), the daily food allowance should be adjusted to maintain the animal’s weight at or above the 20% cut off. Regulation cannot be attempted again until the animal weighs at least 80% of its original weight.

Rodents on fluid regulation/scheduling with a weight loss of 10% of baseline weight should be considered clinically dehydrated and should be treated as outlined below.

1. Any rodent appearing dehydrated (e.g. displaying listlessness/inactivity, increased “skin tent”, and/or sunken eyes) must have drinkable fluid support provided immediately by supplying a measured volume of fluid. Enough fluid should be provided to allow the animal to drink freely without interruption.
2. In addition, alternative fluid sources (e.g. hydrogel, moistened food, 0.5-2 mL subcutaneous sterile lactated ringers or saline (0.9% NaCl)) must be administered.
3. Lab members involved in fluid regulation must be trained to be able to identify dehydration and be comfortable giving subcutaneous fluids.
4. If a rodent appears dehydrated, listless, hunched, or showing signs of pain/distress, contact the ULAR clinical care staff in addition to providing supplemental food/water. An assessment table with signs for pain and distress is in Appendix D.
5. If an animal’s weight loss progresses from 10% to 20%, despite treatment, it should be humanely euthanized.

EXEMPTIONS
If there is a scientific necessity to manage food and fluid regulation in your model in such a way that is different from the parameters described above, please submit an Exemption providing justification as to why this guideline cannot be followed. All Exemptions must be submitted in ARIES by navigating to “My submissions > Create > Exemption request”. Exemptions must be reviewed and approved by the IACUC prior to implementation.

REFERENCES


Appendix A

Published Graphs of Water and Food Intake of 28 Common Mouse Strains


- Add ranges from above reference paper: 3.1 +/- 0.1 g/mouse (SPRET/Ei) to 6.3 +/- 0.3 g/mouse (RBF/DnJ)
- Overall strain mean: 4.4 +/- 0.1 g/mouse, n=28
- Add additional charts from reference to clarify: include mL/mouse and g/mouse charts
Fluid and Food Restriction Record Sheet

Currently Being Restricted: Water  Food  Both

Protocol #: ___________________   PI: ___________________

Total Length of Regulation Period (as approved in protocol): __________ days/weeks/months

Regulation Start Date: __________    Regulation End Date: __________

Contact individual: ___________________
Contact phone #: ___________________
Contact email:   ___________________

Animal ID: ___________                Baseline Weight: ________ g

<table>
<thead>
<tr>
<th>Date</th>
<th>Water Given (Y/N)</th>
<th>Food Given (Y/N or grams)</th>
<th>Body Weight (g)</th>
<th>Currently Regulated (Y/N)</th>
<th>Health Observations (normal/dehydrated, body condition score, other observations)</th>
<th>Initial</th>
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*“Ad lib” is acceptable to place in “Water Given” and/or “Food Given” columns*
Appendix C
Body Condition Scoring (BCS) Charts for Mice and Rats

BC 1
Mouse is emaciated.
- Skeletal structure extremely prominent; little or no flesh cover.
- Vertebrae distinctly segmented.

BC 2
Mouse is under-conditioned.
- Segmentation of vertebral column evident.
- Dorsal pelvic bones are readily palpable.

BC 3
Mouse is well-conditioned.
- Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.

BC 4
Mouse is over-conditioned.
- Spine is a continuous column.
- Vertebrae palpable only with firm pressure.

BC 5
Mouse is obese.
- Mouse is smooth and bulky.
- Bone structure disappears under flesh and subcutaneous fat.

A "+" or a "-" can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2, 1-).

BC 1
Rat is emaciated
- Segmentation of vertebral column prominent if not visible.
- Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
- Segmentation of caudal vertebrae prominent.

BC 2
Rat is under-conditioned
- Segmentation of vertebral column prominent.
- Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
- Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.

BC 3
Rat is well-conditioned
- Segmentation of vertebral column easily palpable.
- Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
- Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.

BC 4
Rat is over-conditioned
- Segmentation of vertebral column palpable with slight pressure.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae palpable.

BC 5
Rat is obese
- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
- Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
- Thick fat store over tail base, caudal vertebrae not palpable.
Appendix D
Assessment of Pain/Distress in Rodents

### VISUAL ACTIVITY

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<tbody>
<tr>
<td>1</td>
<td>Inactive</td>
<td>a</td>
<td>b</td>
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<tr>
<td>2</td>
<td>Isolated</td>
<td>Y</td>
<td>N</td>
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<td>3</td>
<td>Walking on tiptoe</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>Hunched posture</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>5</td>
<td>Pinched face</td>
<td>a</td>
<td>b</td>
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<td>6</td>
<td>Unkempt coat</td>
<td>a</td>
<td>b</td>
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<tr>
<td>7</td>
<td>*Type of breathing</td>
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<tr>
<td>8</td>
<td>Twitching</td>
<td>a</td>
<td>b</td>
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<td>9</td>
<td>Licking at Incision (if present)</td>
<td>a</td>
<td>b</td>
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<tr>
<td>10</td>
<td>Stretching</td>
<td>a</td>
<td>b</td>
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### ON HANDLING

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<tr>
<td>11</td>
<td>Not inquisitive and alert</td>
<td>a</td>
<td>b</td>
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<tr>
<td>12</td>
<td>Not eating</td>
<td>a</td>
<td>b</td>
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<tr>
<td>13</td>
<td>Not drinking</td>
<td>a</td>
<td>b</td>
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<td>14</td>
<td>Vocalization on gentle palpation</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>15</td>
<td>Pale or sunken eyes</td>
<td>a</td>
<td>b</td>
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<tr>
<td>16</td>
<td>Dehydration</td>
<td>a</td>
<td>b</td>
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<tr>
<td>17</td>
<td>Distended abdomen/swollen</td>
<td>a</td>
<td>b</td>
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<tr>
<td>18</td>
<td>** Diarrhea 0 to 3 (mucus/blood)</td>
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</table>

| 19 | Nothing abnormal detected |   |   |

**Diarrhea Scoring:**

0 = normal; 1 = loose feces on floor; 2 = pools of feces on floor; 3 = running out on handling
+m = mucus; b = blood

Y = Yes; N = No

### Scoring Details

*Breathing: R = rapid; S = shallow; L = labored; n = normal; include normal mouse respiration rate = 80-230bpm

**0 = normal; 1 = loose feces on floor; 2 = pools of feces on floor; 3 = running out on handling

+m = mucus; b = blood

### Scoring Symbols

a. Mild
b. Moderate
c. Serious

### Humane Endpoints and Actions

1. Any animal showing signs of coma within the first 24-48 hr will be euthanized.
2. Any animals losing more than 20% of its start weight at any time will be euthanized.
3. Any animal showing tiptoe or slow ponderous gait will be euthanized or staff will confer with the veterinarian staff.
4. Inform veterinarian and principal investigator if more than one clinical sign occurs.

ILAR Journal V41(2) 2000 Humane Endpoints for Animals Used in Biomedical Research and Testing