I. PURPOSE

1. This SOP outlines the proper procedures to be followed for food and fluid regulation in mice and rats.

2. Food and/or fluid regulation may be used in studies (a) to motivate animals to perform novel or learned tasks, (b) of the behaviors and mediators of hunger and thirst, and (c) of the homeostatic regulation of energy metabolism and its influence on disease pathogenesis.

3. This SOP will outline important issues to be considered and addressed in IACUC protocols that require food or fluid regulation.

II. RESPONSIBILITY

1. It is the responsibility of the Principal Investigator to ensure the research staff are properly trained, and follow both the IACUC protocol and this SOP.

III. BACKGROUND & SCOPE

1. The objective when food and fluid regulation studies are 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 be considered and described in the IACUC protocol:
   a. The level of food and/or fluid regulation
   b. Potential adverse consequences of the regulation
   c. Methods to assess the health and well-being of animals under regulation

2. Definitions
   a. Ad libitum: Rodents are offered access to a continuous supply of food and water.
   b. Deprivation: Complete withholding of food or water.
   c. Restriction: Acute (<12hr) withholding of food or water prior to imaging, behavior testing, etc.
   d. Regulation/Scheduling: Controlling the amount of, and when water or food is offered to an animal; an amount less than the average daily ad libitum consumption.

3. A scientific justification must be provided in the IACUC protocol when proposing food and/or fluid regulation. This justification should provide a rationale for the level and length of time the regulation is proposed.
IV. PROCEDURES

1. Protocols requiring chronic (i.e., >24 hrs) regulation should indicate that either a strain-specific-published or in-house-determined *ad libitum* intake normal range will be used.

2. Protocols should provide the formula used for the proposed regulation (e.g. 70% of *ad libitum* intake food regulation to achieve 80% body weight of controls). Typically, food regulation should not be lower than 30% of *ad libitum* intake.

3. Note that acute water deprivation of up to 24 hours will result in clinical dehydration (e.g. listless, inactive, “skin tenting”, sunken eyes). Chronic water regulation of up to 50% of *ad libitum* offering may be imposed gradually over 7 days.

4. Consideration should be made to allow food and water to be available concurrently, as rodents typically do not eat without available water. During regulation, access to food and/or water must be for 15 minutes at a minimum.

5. Records must be kept of rodents on regulation, including the baseline body weight, records of daily monitoring ensuring the availability of food and water, at least twice weekly body weights, a weekly body condition score (BCS, see Appendix A), and other observations (e.g., hydration status).

6. Individual animal assessments must be made on CMDC #245 entitled *Food and Fluid Regulation - Individual Record*. Assessments for rodents may also be kept as batch records using CMDC #270 entitled *Food and Fluid Regulation - Cohort Records*. Records must be kept in the housing room book while animals are present, and be made available for review.

7. If animals are undergoing Restriction, then documentation (CMDC #245 or 269) is not necessary at that time.

8. Specific clinical endpoints that require intervention must be clearly stated in the protocol (e.g., food regulated rodent may not lose >20% of baseline body weight without adjusting the daily food allowance) (e.g., fluid regulated rodent may not lose >10% of baseline body weight without adjusting the availability of fluid).

9. Rodents that appear dehydrated must immediately be provided with a measured volume of drinkable fluid *ad libitum*, and alternative fluid sources (e.g. hydrogel, sterile subcutaneous fluids).

Approved: Date:
Appendix A

Body Condition Scoring (BCS) Charts for Mice and Rats

<table>
<thead>
<tr>
<th>BCS</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BC 1</strong></td>
<td>Rat is emaciated.</td>
<td>- Skeletal structure extremely prominent; little or no flesh cover. - Vertebrae distinctly segmented.</td>
</tr>
<tr>
<td><strong>BC 2</strong></td>
<td>Rat is underconditioned.</td>
<td>- Segmentation of vertebral column evident. - Dorsal pelvic bones are readily palpable.</td>
</tr>
<tr>
<td><strong>BC 3</strong></td>
<td>Rat is well-conditioned.</td>
<td>- Vertebrae and dorsal pelvis not prominent; palpable with slight pressure.</td>
</tr>
<tr>
<td><strong>BC 4</strong></td>
<td>Rat is overconditioned.</td>
<td>- Spine is a continuous column. - Vertebrae palpable only with firm pressure.</td>
</tr>
<tr>
<td><strong>BC 5</strong></td>
<td>Rat is obese.</td>
<td>- Mouse is smooth and bulky. - Bone structure disappears under flesh and subcutaneous fat.</td>
</tr>
</tbody>
</table>

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