I. **General:**

This Standard Procedure is for investigators who use food regulation in non-USDA mice and rats for experimental reasons such as studies of homeostatic regulation of energy metabolism or fluid balance, studies of the motivated behaviors and physiologic mediators of hunger or thirst, and studies that regulate food consumption to motivate animals to perform novel or learned tasks. It does not apply to pre surgical fasting prior to anesthesia and surgery or food restriction to animals to maintain optimal, healthy body weight. or fasting periods less than 24 hours prior to assays. As stated in the *Guide*, “the least restriction that will achieve the scientific objective should be used.” The animals need to be closely monitored to assure their nutritional needs.

II. **Definitions:**

- **Ad libitum:** Animals are offered access to a continuous supply of food and can eat as much and as often as they want.

- **Baseline Body Weight:** The average weight prior to beginning the restriction period.

- **Removal of food:** Food is removed for < 24 hours for reasons scientifically justified in the protocol (e.g., prior to sample collection, etc.), after which animals are returned to ad lib food.

- **Regulation:** A deviation from the standard husbandry practices in the amount or availability of food. It can include Scheduling and Restriction as defined below. Special diets are not inherently considered Regulation.

  - **Restriction:** The provision of rations such that the total volume of food is strictly monitored and controlled. Restricted feeding typically limits the total volume of food consumed for the purpose of reducing the animal’s weight to a level lower than that expected for an ad libitum fed animal.

  - **Scheduling:** Limiting of the number of times or length of periods during which the animal has access to food so that the animal consumes a normal portion (as much as desired), but at intervals or durations that differ from standard husbandry practices. This definition only applies if food is removed for a period of greater than 24 hours. Scheduled feeding should not result in a subnormal body weight. For example, rodents are given ad lib access to food at least once every 24 hours for at least one hour. After the study session is completed, the rodent is returned to the home cage with ad lib food.

III. **Considerations:**
1. The goal for body weight should be no greater than 15% weight loss when compared to an age- and sex- matched ad lib fed control unless scientifically justified in the IACUC protocol.

2. In the case of conditioned-response research protocols, use of highly preferred food as positive reinforcement, instead of restriction, is recommended. However, exceptions to this recommendation or alternatives may be allowable if scientifically justified.

3. Efforts should be made to match an animal’s typical eating schedule with circadian variables. Rodents are nocturnal and eat and drink primarily at night, therefore, removing food in the evening for overnight restriction usually results in restriction lasting 24 hours.

4. Research staff responsible for monitoring animals on food regulation studies should be trained and competent to evaluate the animal’s condition.

5. Food regulation is not recommended in rodents less than 8 weeks of age. Severe food restriction studies in rodents younger than 14 weeks of age is detrimental to their health. Young or growing animals are especially sensitive to food restriction and should be evaluated with a concern for their health and minimum growth requirements.

IV. **Pain Category Classification:**

Any animal without access to food for approximately more than 24 hours must be listed in Category E. Food deprivation must not exceed 48 hours. Additionally, if scientific needs require a weight loss of > 20%, list animals in Category E.

V. **Description of procedure:**

1. Any sort of food regulation must be included in the IACUC protocol, scientifically justified and approved before being implemented.

2. Cages containing rodents which are on a food regulation study must be labeled in accordance with LARC guidelines.

3. Animals should be acclimated to a regulated feeding schedule over a period of time.

4. Animals are likely to have the most significant weight fluctuations during acclimation to the food regulation. To ensure that animals are adjusting appropriately during this period, they should be weighed 3x/week.

5. Any animal that exceeds the % allowable weight loss or displays any clinical signs of distress such lethargy/ruffled hair coat/hunched posture must be temporarily or permanently removed from the study and fed ad libitum.

6. Assessment and documentation* are required for:
   - Feeding restrictions of any kind; and,
   - Scheduled feeding where animals are without food > 24 hours:
     a. Record daily food consumption for each animal. This can be derived by recording:
        i. Animal’s baseline weight prior to food regulation
        ii. Daily amount food provided and food consumed
     b. Record body weights at least once per week. More frequent body weight recording will be required for greater restrictions. The body weight monitoring plan must be described and approved in the IACUC protocol.
     c. Clinical parameters must be monitored and recorded as described and approved in the IACUC protocol. Examples include,
        i. Activity level (Active versus quiet, lethargic)
        ii. Hair coat (Groomed versus ruffled)
        iii. Posture (normal versus hunched)
*7. Recordkeeping:
   a. The Principal Investigator is responsible for the assessment, care and
documentation for food-regulated animals.
   b. The lab must record all body weight measurements, quantitative health
assessments, scoring, and supportive care. Records must be presented to LARC or
IACUC upon request.
   c. Records must include protocol number, principal investigator’s name, date of entry,
and pertinent information.

VI. Literature search words required:

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<tr>
<th>Key Words</th>
<th>Search Sites</th>
<th>Years Covered</th>
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Literature search was performed for development of this Standard Procedure in June 2020.

VII. References:

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   KCl, CaCl2 and NH4Cl Solutions by 28 Mouse Strains." Behav Genet 32.6 (2002): 445-
   57.
   "Dehydration Parameters and Standards for Laboratory Mice." JAALAS 52.3 (2013):
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4. Boston University Food Regulation and Restriction in Rodents Policy, Review dated:
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7. Goodrick, C. L. et al. (1983). Effects of intermittent feeding upon growth, activity, and
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