A. Definition: For the purpose of these guidelines, food or fluid regulation/restriction includes any deviation from normal husbandry procedures for the species.

Food or fluid regulation/restriction may be required in order to achieve a variety of research objectives. It has been shown that some methods of food and/or fluid restriction may be physiologically and/or psychologically stressful. In addition, if restriction is allowed to exceed acceptable levels, it can be physically harmful to an animal. The goal of these guidelines is to ensure that dietary restrictions are scientifically justified and necessary for study objectives, and that animals subjected to dietary restriction are appropriately monitored.

Food or water restrictions must be conducted with care and be tailored to the feeding patterns and nutritional requirements of the strain and species as well as to the requirements of the study. Specialized diets should be assessed to assure that they are palatable enough that the animal will eat sufficient quantities to maintain body weight and condition.

Specific Guidelines:

1. The degree of food or water restriction used must be the minimum level that will achieve the scientific objective. Restriction must be based on a measurable parameter such as percentage of ad libitum intake, percentage of body weight compared to an unrestricted control animal, percentage of body weight compared to an established growth curve, or the amount of time the animal is allowed access to food/fluid per 24 hours. Complete food deprivation should be avoided for mice and may not exceed 48 hours for rats. Complete water deprivation for more than 24 hours should be avoided. Consideration must be given to alternative methods and/or modifications to food and/or fluid restriction. Life stage (growth, pregnancy, lactation, and senescence), demands of the task, and state of health must also be taken into consideration in determining requirements.

2. The investigator must indicate the percentage changes that define the target weight as well as the floor weight in the protocol. The floor weight is defined as the weight below which the investigator must immediately notify the BRF veterinary staff and provide supplementary feeding of the animals. Weight loss beyond the floor weight should be one of the defined humane endpoints (see below) that will trigger early removal of the animal from the study.

3. Unless scientifically justified, food restriction leading to a body weight loss of greater than 10% should be introduced incrementally to allow for physiological and psychological adaptation.

4. Water restriction should be avoided unless scientifically justified. Water restricted animals should be allowed sufficient time each day to consume food while water is available (an acceptable time is 20-60 minutes) since water-deprived animals generally do not consume food.

5. For all dietary restriction studies, humane endpoints (triggering a decision for early
removal of the animal for humane reasons before the study is concluded) must be specified in the protocol. Examples of appropriate humane endpoints include:

- For adults: Loss of 20% of the body weight of age-matched unrestricted control animals; for developing animals: failure to gain weight within 80% of normal controls.
- For any animal, a poor body condition score including signs of emaciation (evident segmentation of vertebral column or readily palpable dorsal pelvic bones or signs of significant dehydration).

6. Monitoring and documentation must be done. The investigator must monitor parameters such as body weight, hydration status, body condition, and food consumption with suitable frequency, as approved by the IACUC.

- Animals should be singly housed to eliminate competition for limited food/water resources.
- Weight records must be regularly recorded and should be available in the animal housing room for monitoring by the animal care staff as well as the PI’s laboratory staff.
- Animals on food/fluid restriction protocol must be clearly identified by a dedicated label on the cage. The duration (experiment start date and time, termination date and time) must be stated on the cage label as well as any other relevant information.
- The investigator must specify that calibrated scales will be used to weigh animals on a regular basis, the scale is validated with a known weight to ensure accuracy at a monthly minimum, and calibration records are maintained for inspection purposes.

7. In behavioral studies (e.g. conditioned response research), the use of highly preferred food as positive reinforcement can often be used instead of food restriction. This should be considered when possible. However, dietary restriction may be justified in some cases, depending on the species, the behavioral task, and the requirements of the research protocol.

Whenever an animal obtains any portion of its diet through positive reinforcement (food reward), the investigator must ensure that the sum of the nutritional value of the food earned through reward and of the food provided “free” (without necessity of earning it) is sufficient to maintain the animal in a healthy state.

8. Food and/or water restriction studies conducted in healthy rodents will be considered pain/distress “Category C” if:

- the floor weight is not less than 85% of the body weight from established growth curves of unrestricted control animals;
- the target weight is not less than 85% of this established body weight; and
- the experimental treatment is not expected to produce further weight loss than the intake restriction alone.

Studies deviating from these recommendations will automatically be assigned to pain/distress “Category E” (unalleviated pain or distress) and require more extensive scientific justification and consideration of alternatives.
B. Special Considerations For Obesity Research: The experimental induction of obesity through diet, drugs or genetic models has special animal welfare issues. The PI should be cognizant of these and discuss these in the protocol.

1. One of the considerations is that of potential cage overcrowding. Because obese animals weigh significantly more than the average rodent (e.g. twice as much), frequent monitoring is needed to assure that the minimum space/caging requirements are met. Placing fewer animals per cage is often necessary.

2. Because diabetes can accompany the obese phenotype, bedding and cage type should be a consideration. Increased urine production with diabetes will necessitate more frequent bedding changes. Alternatively, wire-grid hanging cages (with platforms or plastic pipes included to provide a few areas of solid flooring) may be considered for diabetic animals.

3. For an animal with diabetes, end-organ effects/failure can occur over time (retinopathy, nephropathy, uncontrollable hyperglycemia, etc). Obese animals, even without diabetes, can develop pododermatitis (paw lesions), hypertension, renal problems and respiratory depression. Thus, careful monitoring to assure that animals are humanely euthanized before developing morbidities associated with diabetes or extreme obesity is essential. Both the monitoring protocol and the humane endpoints must be clearly delineated in the protocol.