



## STANDARD OPERATING PROCEDURE 306 FOOD AND WATER REGULATION IN RODENTS

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### 1. PURPOSE

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This Standard Operating Procedure (SOP) describes food and water regulation guidelines in rodent experiments.

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### 2. RESPONSIBILITY

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Principal investigator (PI) and their research staff.

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### 3. INTRODUCTION

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- 3.1. Food and water regulation includes any deviation from usual rodent husbandry procedures (*ad lib* food and water). The regulation process may consist of strict food and water monitoring and control and scheduled access to food or fluid sources performed by the research staff.
- 3.2. Practical reasons for introducing food and water regulation include but are not limited to, studies that use food or water as a motivational tool in training rodents to perform tasks required for a research protocol.
- 3.3. Rodents have a very high metabolism and fast gastrointestinal transit time. Therefore, **food or water regulation affects them quickly and significantly**.
- 3.4. Particular attention should be given to ensure that animals consume a balanced diet. Food consumption decreases with water regulation.
- 3.5. In conditioned-response experiments, a palatable food or fluid reward is recommended as a positive reinforcement instead of regulation.
- 3.6. Before proceeding with food and water regulation, consider the following idea:
  - 3.6.1. Finding alternatives to food regulation (ex., food reward, acetic acid water) whenever possible.
  - 3.6.2. If the PI can demonstrate that there are no suitable alternatives to food regulation, **minimal** regulation to achieve experimental results needs to be used.
  - 3.6.3. Food regulation needs to be selected over water regulation whenever possible.
  - 3.6.4. Potentially adverse outcomes and **criteria for removing the animal from the experiment** must be addressed.
  - 3.6.5. **Methods used to assess animal health** and well-being must be determined, especially, body weight, body condition score, and hydration status.

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## 4. PROCEDURES

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- 4.1. **Scientific justification for food or water regulation must be provided** in the Animal Protocol and approved by BGU Animal Care and use Committee.
- 4.2. Purpose and planned food or water regulation duration **must be specified**. The justification must address the principles listed in section 3.6.
- 4.3. **Research staff must be trained and competent to evaluate the animal's condition**.
- 4.4. The goal is to establish the minimal food and water regulation necessary to produce the required behavioral performance or experimental results for the study while maintaining animal well-being.
- 4.5. Establishment of food and water consumption baseline:
  - 4.5.1. The animal's baseline body weight must be recorded before food or water regulation **is commenced**. All other weights will be compared to this baseline body weight.
  - 4.5.2. The estimated daily fluid maintenance requirement in rodents (mouse and rat) is 60 ml/kg/day. Each animal's hydration status must be evaluated daily on water regulation.
  - 4.5.3. **No rodent can be deprived entirely of water/fluids for more than 24 hours**.
  - 4.5.4. For scheduled access to water, the animals are given *ad libitum* access to water for a minimum of 1 hour, at least once every 24 hours.
  - 4.5.5. The estimated food consumption for a **mouse** is 180-200g/kg/day of a nutritionally balanced diet containing 16-20% protein and 5-25% fat, which can be calculated as 4.5-5g of food for a 25g mouse. The estimated food consumption for a **rat** is 80-100/kg/day of a nutritionally balanced diet containing 12-27% protein and 5-25% fat, which can be calculable to 25-30g of food for a 300g rat. However, species and strain variation are common, and the body condition score must be evaluated frequently for each animal on food regulation.
  - 4.5.6. **Food should be provided concurrently with water** as the animal benefits from the simultaneous availability of food and water.
- 4.6. Food/Water regulation in young or growing animals:
  - 4.6.1. **Young or growing animals** (<14 weeks) are **susceptible** to fluid regulation and malnutrition. A particular concern for their health and minimum growth requirements must be met.
  - 4.6.2. **Body weight should not be compared with the animal's baseline body weight**. Instead, animals should be **maintained within a specific percentage** (i.e., 85%) of an age/sex/strain-matched control littermate with *ad libitum* food and water. Vendor growth charts can also be compared if no control littermates are present.
- 4.7. Implementation of food or water regulation:
  - 4.7.1. Animals undergoing surgeries must be fed *ad libitum* **for one week before** the food or water regulation starts, i.e., from one week after the procedure.
  - 4.7.2. Animals must gradually acclimate to the new regulation paradigm for 3 to 7 days.
  - 4.7.3. The research staff must frequently assess body condition and hydration status. **Animals must be weighed at least twice a week**.
  - 4.7.4. **Animals may be separated during feeding** to avoid competitive behavior in socially housed conditions. If an additional cage is needed for separation, the new cage must be labeled with an appropriate cage card.
  - 4.7.5. Investigators are encouraged to offer alternative rewards (e.g., fruit juices, raisins, peanuts, etc.), allowing the animal to increase the regulation baseline while maintaining task performance.
- 4.8. Withdrawal of food or water control:

- 4.8.1. Once the regulation protocol is no longer required, animals must gradually be brought back to ad libitum food and water **for three days**. During this time, the animals should be monitored closely (daily) for deleterious effects of fluid overload and gastrointestinal problems.
- 4.9. Ongoing health monitoring:
  - 4.9.1. **Chronic** food or water regulation results in the establishment of new physiological set points.
  - 4.9.2. The following parameters must be monitored as specified below:
    - 4.9.2.1. Body weight: Animals should be weighed at least twice weekly, in no less than 48-hour intervals. The body weights must be obtained as consistently as possible (e.g., at the same time each day).
    - 4.9.2.2. Body condition and hydration status: research staff should assess through qualitative observation of body condition score, presence of fresh feces, and general mental status. (See Appendix 2 and 3: Body Condition Score Outline for rats and mice).
    - 4.9.2.3. Animals exhibiting clinical signs of ill health must be reported to veterinary care staff.
- 4.10. Record keeping:
  - 4.10.1. Each cage must be identified with a detailed specific card with a "Food or Water Regulation" label (i.e., fed by investigator – water given by investigator).
  - 4.10.2. Food or water regulation Rodents must **be monitored daily**, and food or water intake must be **recorded daily in the Food or Water Regulation Log** (see Appendix 1) **and kept in the animal holding room**. A log sheet is required for all regulation lasting more than 12 hours.
  - 4.10.3. If the log sheet is not signed for the previous day food pellets or water are given to the animal on regulation,
  - 4.10.4. Individual records must be maintained and include the following:
    - 4.10.4.1. Body Weights
    - 4.10.4.2. Food or water consumption data: Number of pellets/grams of food or ml of water consumed daily or amount of time permitted with free access to food or water.
- 4.11. Termination of food or water regulation:
  - 4.11.1. The following criteria will necessitate evaluation by the veterinarian:
    - 4.11.1.1 Significant weight loss (e.g., exceeding 20% of baseline body weight) at any time throughout the study.
    - 4.11.1.2 Body condition score <2.
    - 4.11.1.3 Abnormal behavior (increased aggression, ruffled fur, recurrent episodes of self-harming, depression) that has not improved despite veterinary intervention.
  - 4.11.2. Food or water regulation may be reduced or stopped upon consultation with the veterinarian.
  - 4.11.3. Food or water regulation can only resume with approval from the veterinarian.

**Appendix 1 (SOP306b)**

**LOG FOR ANIMALS ON RESTRICTED FOOD/WATER**











INVESTIGATOR:	PROTOCOL:
PRIMARY CONTACT:	START DATE/TIME:
TELEPHONE:	END DATE/TIME:
CELL:	EMERGENCY PELLETS:
EMAIL:	EMERGENCY WATER:
CAGE LOCATION:	

DATE	FOOD/WATER PROVIDED BY	AC CHECK	EMERGENCY FOOD/WATER PROVIDED

DATE	FOOD/WATER PROVIDED BY	AC CHECK	EMERGENCY FOOD/WATER PROVIDED

**Animal Care staff, will check every morning that the animals were fed on the previous day and sign the log.**  
 Please immediately alert the Veterinary Care staff if the log was not signed for the previous day.  
 If the contact person cannot be reached by the end of the day, the emergency number of pellets or volume of water will be given.

## Appendix 2

MICE	RATS
 <p><b>BC 1</b> <b>Mouse is emaciated</b></p> <ul style="list-style-type: none"> <li>Skeletal structure extremely prominent, little or no flesh cover</li> <li>Vertebrae distinctly segmented</li> </ul>	 <p><b>BC 1</b> <b>Rat is emaciated</b></p> <ul style="list-style-type: none"> <li>Segmentation of vertebral column prominent if not visible</li> <li>Little or no flesh cover over dorsal pelvis, pins prominent if not visible</li> <li>Segmentation of caudal vertebrae prominent</li> </ul>
 <p><b>BC 2</b> <b>Mouse is under-conditioned</b></p> <ul style="list-style-type: none"> <li>Segmentation of vertebral column evident</li> <li>Dorsal pelvic bones are readily palpable</li> </ul>	 <p><b>BC 2</b> <b>Rat is under-conditioned</b></p> <ul style="list-style-type: none"> <li>Segmentation of vertebral column prominent</li> <li>Thin flesh cover over dorsal pelvis, little subcutaneous fat, pins easily palpable</li> <li>Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure</li> </ul>
 <p><b>BC 3</b> <b>Mouse is well-conditioned</b></p> <ul style="list-style-type: none"> <li>Vertebrae and dorsal pelvis not prominent, palpable with slight pressure</li> </ul>	 <p><b>BC 3</b> <b>Rat is well-conditioned</b></p> <ul style="list-style-type: none"> <li>Segmentation of vertebral column easily palpable</li> <li>Moderate subcutaneous fat store over pelvis, pins easily palpable with slight pressure</li> <li>Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented</li> </ul>
 <p><b>BC 4</b> <b>Mouse is over-conditioned</b></p> <ul style="list-style-type: none"> <li>Spine is a continuous column</li> <li>Vertebrae palpable only with firm pressure</li> </ul>	 <p><b>BC 4</b> <b>Rat is over-conditioned</b></p> <ul style="list-style-type: none"> <li>Segmentation of vertebral column palpable with slight pressure</li> <li>Thick subcutaneous fat store over dorsal pelvis, pins of pelvis palpable with firm pressure</li> <li>Thick fat store over tail base, caudal vertebrae not palpable</li> </ul>
 <p><b>BC 5</b> <b>Mouse is obese</b></p> <ul style="list-style-type: none"> <li>Mouse is smooth and bulky</li> <li>Bone structure disappears under flesh and subcutaneous fat</li> </ul>	 <p><b>BC 5</b> <b>Rat is obese</b></p> <ul style="list-style-type: none"> <li>Segmentation of vertebral column palpable with firm pressure, may be a continuous column</li> <li>Thick subcutaneous fat store over dorsal pelvis, pins of pelvis not palpable with firm pressure</li> <li>Thick fat store over tail base, caudal vertebrae not palpable</li> </ul>

## Appendix 3

Variable		Score
<b>Body Weight Changes</b>		
0	Normal	
1	< 10 percent weight loss	
2	10-15 percent weight loss	
3	> 20 percent weight loss	
<b>Body Condition Score (see diagram for details)</b>		

0	Body condition score >3	
1	BCS >2 and < 3	
2	BCS >1 and <2	
3	BCS of 1 or less	
<b>Physical Appearance</b>		
0	Normal	
1	Lack of grooming	
2	Rough coat, nasal/ocular discharge	
3	Very rough coat, abnormal posture, enlarged pupils	
<b>Measurable Clinical Signs</b>		
0	Normal	
1	Small changes of potential significance	
2	Body temp change of 1-2 ° C, cardiac and respiratory rates ↑ up to 30%	
3	Body temp change of 1-2 ° C, cardiac and respiratory rates ↑ up to 50%, or markedly reduced	
<b>Unprovoked Behavior</b>		
0	Normal	
1	Minor changes	
2	Abnormal, reduced mobility, decreased alertness, inactive	
3	Unsolicited vocalizations, self-mutilation, either very restless or immobile	
<b>Behavioral Responses to External Stimuli</b>		
0	Normal	
1	Minor depression/exaggeration of response	
2	Moderately abnormal responses	
3	Violent reactions, or comatose	
		<b>TOTAL:</b>

*Note: This scoring template should be modified for specific species and designed to fit each protocol and animal model. In this example, a score is assigned to each variable, from 0 (normal or mild) to 3 (severe). The cumulative score indicates the likelihood of the animal experiencing pain or distress. Humane endpoints can be established based on these criteria. **A total score of >5 or a score of 3 in any one variable, regardless of the total score, should warrant mandatory evaluation/decision by a veterinarian or humane euthanasia***

**SOP 306 FOOD AND WATER RESTRICTION IN RODENTS**

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Approved by the BGU Animal Policy and Welfare Oversight Committee