Preclinical Research Center
Ben-Gurion University of the Negev

STANDARD OPERATING PROCEDURE 306

FOOD AND WATER REGULATION IN RODENTS

1. PURPOSE

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

2. RESPONSIBILITY

Principal investigator (PI) and their research staff.

3. INTRODUCTION

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 ensuring 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.

4. PROCEDURES

4.1. The Animal Protocol must provide scientific justification for food or water regulation and be 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 60ml/kg/day. Each animal's hydration status must be evaluated daily on water regulation.

4.5.3. Acetic acid 2% in water can replace water regulation.

4.5.4. No rodent can be deprived entirely of water/fluids for more than 24 hours.

4.5.5. 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.

4.5.6. The estimated food consumption for a mouse is 200g/kg/day of a nutritionally balanced diet containing 16-20% protein and 5-25% fat, which can be calculated as 5g of food for a 25g mouse. The estimated food consumption for a rat is 100g/kg/day of a nutritionally balanced diet containing 12-27% protein and 5-25% fat, which can be calculable to 30g of food for a 300g rat. However, species and strain variation are common, and each animal's body condition score must be evaluated frequently on food regulation.

4.5.7. 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 return 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 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 consistently (e.g., simultaneously daily).

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 the investigator – water given by the 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 regulations 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

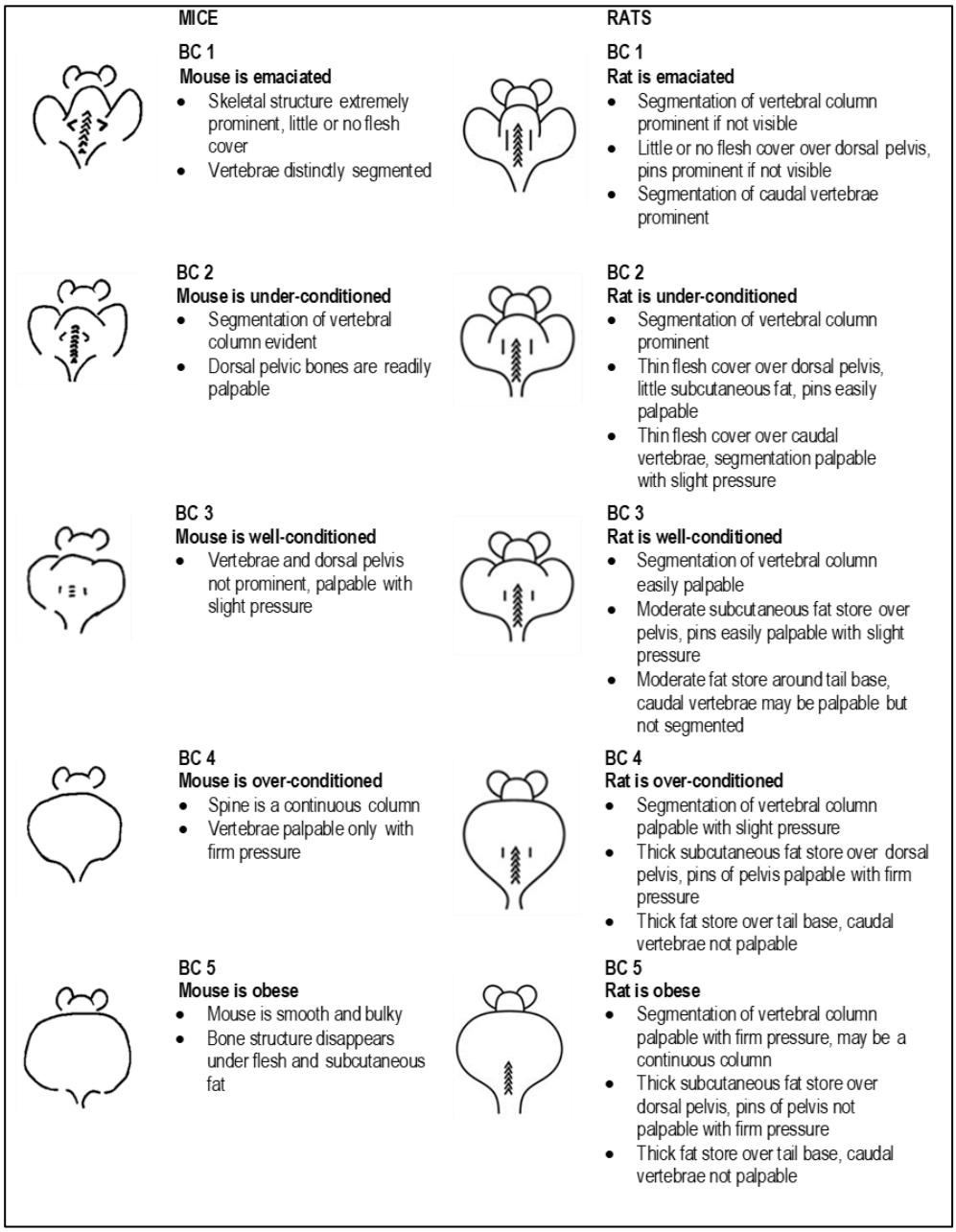
DATE	FOOD/WATER PROVIDED BY	AC CHECK	EMERGENCY FOOD/WATER PROVIDED

Animal Care staff will check every morning that the animals were fed 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



Appendix 3

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

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

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