

**Mouse and Rat Fluid  
Regulation  
IACUC Standard Procedure  
Effective Date: June 2023**

**General:** This standard procedure is for investigators who use fluid restriction and/or fluid deprivation in healthy rodents for experimental reasons such as behavioral, physiological and neuroscience studies. Due to the variability in experimental design and due to the compensatory action known as “dehydration anorexia”, close monitoring and provision of supportive care are necessary for fluid-regulated animals.

**Description of procedure:** At UCSF, fluid regulation is implemented through two main distinct regimens that may sometimes be used in conjunction:

- **Fluid deprivation:** refers to the withholding of water for a definite period of time. At the end of the designated timeline, animals are tested and then either euthanized or most commonly returned to *ad libitum* water consumption. Scientific justification for a particular duration must be provided within the context of potential discomfort and physiological harm.
- **Fluid restriction:** refers to a prolonged water restriction (e.g.: 1ml/per day). The chronic nature of this regimen can lead to adverse effects on animals’ health and well-being.

**Classification**

List as Category E:

- Animals that will be deprived of water for approximately 24 hours. The total duration can occasionally increase to 26 hours but not more than once weekly.
- Animals that will undergo chronic fluid regulation with anticipated weight loss of > 20% of *ad libitum* weight.

**Description of procedure**

- Preferably, animals should be allowed to gradually acclimate to fluid restriction (e.g.: 3-5 days transition period).
- Animals must not be water regulated for the first three days following surgery unless the protocol states otherwise
- Fluids offered as rewards should be very palatable (e.g.: sucrose solution, evaporated milk, soy milk); alternatives to plain water as a reward must be considered and justification must be provided if only plain water is offered.
- Prior to initiating fluid deprivation/restriction, a baseline weight must be recorded.
- Animals on fluid regulation should be weighed 3 times weekly (and weights must be recorded) until a drop of **10% *ad libitum* weight** is observed.
- *Ad libitum* weight is defined as:
  - > 8 weeks old, the animal’s pre-restriction weight
  - < 8 weeks old, the *ad libitum* weight of age-matched controls or of strain-specific growth curves.
- Once the animal exhibits 10% weight loss, more frequent weighing is required and the increased frequency must be described in Section J. of the approved IACUC protocol.
- The next critical threshold requiring intervention is any of the following (if an animal

reaches a critical threshold, LARC veterinary staff must be contacted immediately):

- **20%** weight loss relative to ad lib weight as defined above
- The animal has a ruffled hair coat and hunched posture or any other signs of distress (i.e. decreased activity).

### **Training and Recordkeeping**

1. The assessment, care and documentation for fluid-regulated animals are the responsibility of the Principal Investigator. These responsibilities are required 7 days a week, including weekends and holidays.
2. Training in health assessment, monitoring and supportive care is available through LARC veterinary staff upon request.
3. All weights and volumes administered must be recorded and maintained by the laboratory. Supportive care provided to water regulated animals should be documented. Records must be presented to LARC or IACUC upon request.

### **Scoring systems**

1. Scoring systems are helpful in assessing health and supportive care requirements for animals under water regulation. If scoring systems are used, they must be included in the approved protocol.
2. See [scoring system](#) example.

### **Literature search**

Literature searches were performed for development of this Standard Procedure in December 2022 and May 2023

<b><u>Key Words</u></b>	<b><u>Search Sites</u></b>	<b><u>Years Covered</u></b>
Mouse, Fluids, Deprivation, Regulation, Restriction, Guidelines, Welfare	PubMed	1970-2022
Rat, Fluid, Water, Restriction, Regulation, Deprivation, Behavior, Motivation, Alternative, Refinement	PubMed	1969-2023

### **References**

Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG. Food intake, water intake, and drinking spout side preference of 28 mouse strains. *Behav Genet.* 2002 Nov;32(6):435-43. doi: 10.1023/a:1020884312053. PMID: 12467341; PMCID: PMC1397713.

Drucker, Ackroff, K., & Sclafani, A. (1994). Nutrient-conditioned flavor preference and acceptance in rats: effects of deprivation state and nonreinforcement. *Physiol Behav*, 56(4), 701–707. [https://doi.org/10.1016/0031-9384\(94\)90230-5](https://doi.org/10.1016/0031-9384(94)90230-5)

Nigel Foreman, Frederick Toates & Tom Donohoe (1990) Spontaneous and learned turning behaviour in food- or water-restricted hooded rats, *The Quarterly Journal of Experimental Psychology Section B*, 42:2, 153-173, DOI: 10.1080/14640749008401878

Guo ZV, Hires SA, Li N, O'Connor DH, Komiyama T, Ophir E, et al. (2014) Procedures for Behavioral Experiments in Head-Fixed Mice. *PLoS ONE* 9(2): e88678. <https://doi.org/10.1371/journal.pone.0088678>

National Research Council. *Guide for the Care and Use of Laboratory Animals: Eighth Edition.*

Washington, DC: The National Academies Press, 2011. Pp 30-31.

Rowland NE. Food or fluid restriction in common laboratory animals: balancing welfare considerations with scientific inquiry. *Comp Med*. 2007 Apr;57(2):149-60. PMID: 17536615.

Thunhorst, & Johnson, A. K. (2003). Thirst and salt appetite responses in young and old Brown Norway rats. *American Journal of Physiology.*, 284(2), R317–R327.  
<https://doi.org/10.1152/ajpregu.00368.2002>