

Guidelines for Fluid Restriction in Conjunction with Behavioral Studies of Nonhuman Primates

Background:

Behavioral research often requires that a nonhuman primate (animal) perform a task whereby it receives food or fluid rewards for correct behavior. This situation is not unlike conditions in the wild, in which animals must forage, travel, solve problems, or otherwise work to obtain their food and water. In the judgment of many investigators, performing a task for such rewards is behaviorally enriching for laboratory animals, especially nonhuman primates. This document provides both policy and guidelines for fluid restriction in neuroscience and behavioral studies, and can be referenced by IACUC protocols that entail fluid restriction of nonhuman primates. As stated in the *Guide*, “the least restriction that will achieve the scientific objective should be used”.

Policy:

Whenever an animal obtains any portion of its fluid requirements through fluid rewards in neuroscience and behavioral testing, the investigator must ensure that the daily total fluid intake is sufficient to maintain the animals' health and well-being.

The development of animal protocols that involve the use of food or fluid regulation requires the determination of three fundamental details:

- 1) the necessary level of regulation,
- 2) the potentially adverse consequences of regulation,
- 3) methods for assessing the health and well-being of the animals. Consideration of each of those details facilitates the establishment of interventional endpoints to maintain the animals' health and well-being.

Combined food/fluid restriction for the purpose of neuroscience and behavioral studies may not occur without scientific justification and IACUC approval.

Guidelines:

To accomplish this objective, the animal must:

- 1) be permitted to earn fluids to satiety each day during behavioral training and testing,
- 2) be given *ad libitum* chow, and treats (e.g., pieces of individual's favorite fruit) following each daily behavioral training session.
- 3) be returned to *ad libitum* fluids when not being tested
- 4) be given a water bottle (containing the metabolic requirements) for interruptions in testing (<4 days)
- 5) be closely monitored, with written records, by both the investigator(s) and the Veterinary Clinical Services (VCS) staff.

A nonhuman primate is to be considered on a fluid restriction regimen and thus subject to the policy outlined here whenever potable water is not being offered *ad libitum* at least twice daily for at least 1 hour each time (see Animal Welfare Act 3.83) and is not being consistently offered at normal maintenance levels of daily fluids (approximately 65 ml/kg per day for adult macaques).

These guidelines were developed for old world monkeys of the genus *Macaca*, especially *Macaca mulatta*. These four principles are elaborated below. Although they apply to all animals, there can be variation in their application to individual animals, as well as variation due to the specifics of a particular experiment.

- 1) During the preliminary behavioral training and subsequent experimentation, the animal's access to fluid can be restricted in that they will not have *ad libitum* access to water in their home cage. Instead, they will obtain fluids to satiety each day by performing tasks while restrained in a chair. Typically, each correct trial is rewarded by 0.10-0.25 ml drops of the animals preferred liquid, and animals typically perform their tasks daily to earn 100-500 ml. Under normal conditions (non-testing), these animals consume approximately 65 ml/kg.
- 2) When an animal ceases to initiate trials, the experiments should be terminated, and the animal should be returned to its cage, where it will be given *ad libitum* chow and treats (fruits, vegetables, peanuts, etc.). Whenever an

animal cannot be allowed to work to complete satiety because the experiment must end for other reasons (e.g., the laboratory is scheduled for another experiment) the amount of free fluids provided should be equal to the amount typically consumed by the animal when it is permitted to eat fluids to satiety. Supplemental fluids must be given afterwards if indicated by Veterinary Clinical Services (VCS) judgements discussed in 4B) and 4C) below. In addition, animals on water restriction may be fed moistened chow if needed to help maintain normal levels of chow intake and body weight during the course of the fluid restriction.

- 3) Cessation and resumption of liquid restriction. Testing schedules vary (i.e. daily, every other day, Monday through Friday, etc.) and are reviewed on a case-by-case basis. Before brief periods (<4 days) during which they will not be tested (e.g., weekends, vacations) they must be given *ad libitum* water or given a water bottle(s) with sufficient volume to meet their metabolic needs. The use of water bottles during brief interruption in testing provides continuity in the water source. NHPs accustomed to water bottles may have an initial adjustment period to the automatic watering valve, which could impact their behavior. In cases when the water bottle(s) are used, they will be filled at least twice daily offered for one hour each time to replenish consumed water or water lost by disruption of bottle. Thus, it is feasible that the bottle will be emptied between the twice daily refill interval for reasons other than thirst.
- 4) If they will not be tested for five consecutive days they should be returned to *ad libitum* water, preferably by an automatic watering system, until testing resumes. Thus, whenever behavioral testing is suspended for more than four day the animal should be immediately returned to a period of *ad libitum* water. The animal's daily fluid intake record must reflect when the animal is not undergoing testing. On the day before such testing resumes (e.g., Sunday), a reduced fluid allotment is permitted, if necessary, to re-establish adequate motivation for testing (e.g., Monday).
- 5) Assessment of adequacy of fluid intake-monitoring and record keeping. Even though animals should meet their daily fluid requirement by earning fluids to satiety during the daily testing sessions, additional precautions must be taken to avoid the possibility of acute dehydration or chronic fluid deficiency:
 - A. Daily records of fluid intake, both earned and free, must be maintained by the investigators and be available for review by the veterinary staff and by regulatory agencies and bodies. These records should also document daily food intake (number of biscuits and treats (e.g., fruits) given).
 - B. Each animal under fluid restrictions must be observed daily for health status and adequate hydration by VCS as well as by the investigators. If VCS determines that an animal is dehydrated on the basis of either physical examination (e.g., general appearance, skin turgor, fecal output/consistency) or clinical measurements (e.g., specific gravity of urine), the animal's fluid intake must be immediately increased by an appropriate change in regiment, ranging from:
 - i) giving additional supplemental fluids immediately following each daily testing,
 - ii) having *ad libitum* water available in the home cage for an hour each day following testing, or
 - iii) returning to completely *ad libitum* water in the home cage.
 - iv) Increasing fruit offering
 - C. A small but chronic fluid deficiency could escape detection by physical observation, but such chronic deficiencies result in a loss of body weight due to reduced food consumption. Therefore, as a precaution against chronic deficiency, the animal's weight should be recorded weekly (and documented on the daily fluid intake records). If an animal's body weight drops more than 15%, fluids should be increased (see item #2 above). Exceptions to this policy will be allowed only if the VCS determines that an animal is adequately hydrated and that the weight loss will not endanger its health. This information along with the animal's fluid intake, food intake and other clinical signs will be used to judge adequacy of hydration.
- 6) Nonhuman primates with evidence of clinical disease, infection, or injury or sub-clinical conditions as determined by VCS will be returned to *ad libitum* water until resolution of the condition. Additionally, nonhuman primates will receive *ad libitum* water during administration of chemotherapeutic agents or supplements incompatible with water restriction.

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