

Association of Primate Veterinarians Guidelines for Use of Fluid Regulation for Nonhuman Primates in Biomedical Research

PURPOSE

The Association of Primate Veterinarians (APV) recognizes fluid regulation may serve as a powerful behavioral modulator. The APV does not condone the use of fluid regulation in nonhuman primates (NHPs) as a routine and unjustified practice, and it supports alternatives where appropriate. The following guidelines have been developed to provide information to researchers, veterinarians and institutional animal care and use committees (IACUCs) on how to approach fluid regulation in a manner consistent with animal health and welfare while not compromising data collection.

BACKGROUND

Fluid regulation can be a powerful behavioral motivator, but it can be distressing to animals if not conducted appropriately. Its use in NHPs must be scientifically justified and approved by the IACUC and the veterinarian should closely monitor the animal well-being. The IACUC should carefully evaluate each proposal involving fluid regulation and consider the following issues:

1. Is fluid regulation essential to address the scientific objectives stated in the protocol? If so, how can fluid regulation be limited to the minimum required to meet the scientific objective?
2. How will the amount of fluid provided to each animal be determined? What are the limits of the regulation (i.e. daily volume, time frames, duration of regulation)?
3. Will there be opportunities to earn additional fluids once the animal has learned the task?
4. What is the plan to ensure the animal's physiologic and psychological well-being? What parameters will be used to monitor the health and well-being of the animal (e.g., body weight shifts and body condition scoring, changes in serum/plasma or urine osmolality, among others)?
5. What is the humane endpoint for fluid regulated animals?
6. What is the intervention plan for animals failing to meet the established health parameters (e.g., supplemental fluid or restoration of ad lib fluid consumption)?
7. How will "normal" fluid balance be re-established at the end of study or during prolonged periods of inactivity on the protocol?
8. Are references available that illustrate how fluid regulation has been used as a behavioral motivator for similar studies? Is this proposal consistent with those references?
9. Have alternatives been explored to determine if their use can further minimize or eliminate the need for fluid regulation (i.e. food treats, juice, etc.)?

When fluid regulation is determined to be scientifically justified, the transition from unrestricted to regulated fluid access is best accomplished through a gradual and systematic limitation of intake over a several day period (Toth 2000, NIH guidelines). Short periods with markedly reduced fluid intake may be required during the initial phases and the acclimation period will vary by species and hydration status. Larger NHP species may tolerate markedly reduced fluid intake but smaller species (e.g. squirrel monkeys), may be especially susceptible to

dehydration. It is preferable to first evaluate an individual animal's unrestricted fluid consumption and serum chemistry parameters, including serum & urine osmolality, *prior to* initiating a period of fluid regulation. Individual baseline fluid requirements under similar conditions (e.g., clinical health, environmental factors, level of physical exercise, etc.) vary depending on the species, gender, growth and development phase, body weight, social ranking and individual preferences (experience has shown that some animals may drink more juice under restricted conditions than water under *ad libitum* conditions).

GUIDELINES

There may be considerable individual performance differences with fluid regulation. The ability to learn a task assumes the task is not too complex for the age or ability of the animal. Additionally, animals may fail to learn a task for reasons unrelated to motivation (e.g., an ocular deficiency may impact a task that requires visual acuity or impaired cognition may occur secondary to dehydration) (Montain & Ely, Bar-David, Urkin & Kozminsky). The inclusion of an experienced animal behaviorist is encouraged to optimize animal welfare.

- I. Whenever possible, fluid regulation should be introduced gradually through a gradual and systematic limitation of intake over a several day period (Toth 2000, NIH guidelines).
- II. Each animal should be provided with the opportunity to earn fluids to satiety during each work period. Animals failing to consume their calculated daily minimum fluid intake should be provided with supplemental fluids after the training session to ensure the minimal daily fluid intake level and hydration needs have been met.
- III. Ideally, fluids should be accessible at least twice a day. In the U.S., the USDA AWA Regulations (Section 3.83) states: "*Potable water must be provided in sufficient quantity to every NHP housed in the facility. If it is not continually available it must be offered as often as necessary to ensure health and well-being but no less than twice a day for at least 1 hour at a time. The attending veterinarian (AV) or an IACUC approved proposal may allow otherwise*". During experimental use of regulated fluid, access should be provided at least once daily at approximately the same time each day and given over an extended interval (e.g. several hours during training or data acquisition).
- IV. Once an animal has learned the required task, it should be given opportunities to complete the task with less fluid regulation. This is commonly done by increasing the fluid reward provided for each successful task.
- V. Caution must be used when returning some animals to *ad libitum* water as individual animals may gorge themselves to the point of serum electrolyte disturbances. Acute over hydration causes *hyponatremia* (serum Na<125 meq/uL) and may lead to mental confusion, disorientation, malaise, weakness, nausea, transient neurological deficits, seizures and coma (Montain & Ely). In addition, some animals may eat increased quantities of food resulting in bloat if "normal" fluid balance is restored too quickly. Gradual increase of fluid consumption to *ad libitum* access over several days during which the animal is closely monitored for food and fluid consumption is recommended when the animal is first returned to *ad libitum* water. A more abrupt transition from regulated to *ad libitum* water intake may be acceptable once an individual animal's response to these transitions has been evaluated.

VI. Fluid regulation may result in a decreased appetite for dry diets; therefore, to the extent possible, fluids should be given during meal times to encourage consumption of more food and reduce body weight loss (Prescott 2010). A “vacation” from regulated fluid intake should be considered and it is a period of time, ranging from a day to a few weeks in duration, when the animal is provided with unrestricted or a markedly increased fluids (commonly >1.5 to 3 times the regulated daily fluid intake). In addition, supplemental access to fluid for some period on days when research procedures are not scheduled should be considered unless scientifically justifiable reasons preclude such fluid (NIH Guidelines).

VII. Considerations for using fluid regulation as a motivator (weight and growth curves)

Between the ages of 4-6 years, rhesus macaques experience a growth spurt, during which time their body weight may normally increase by 2 kg in a 12 month period (Bourne 1975). Animals in this age range may be at increased risk of not maintaining body condition if they are concurrently on a fluid regulation regime. This period should be approached with increased vigilance. Species-specific growth and development patterns should be considered prior to beginning a fluid regulation regime. It should be noted the growth curve of an animal on a fluid regulated paradigm may not mirror animals provided with *ad libitum* access to water and food. Depending on the time of day and whether a sub-adult rhesus macaque has just been fed or watered, their weight can vary widely. Body weights should be obtained early in the day, ideally at the same time, and prior to receiving food or water. NHPs on *ad libitum* fluid intake may be fed twice daily to encourage them to eat all food provided and decrease wastage. Animals on fluid regulation will typically consume the majority of their dry food ration after they have consumed their fluid intake for the day.

VIII. Animals on fluid regulation may have to be exempted from some aspects of the institutional psychological well-being program (e.g., high fluid content fruits or vegetables puzzle boxes employing the use of water, etc.). Communication between the researchers, IACUC and animal care/veterinary staff to optimize participation of animals in all aspects of such program is essential. Food items with high water content may be given to contribute to the daily fluid intake by calculating fluid equivalents for produce (Bowes and Church’s 2005). This allows for use of highly desired produce to be used as positive rewards. The amount of produce used to meet the minimum daily water intake should not exceed 20% of the total daily fluid allotment (Food and Nutrition Board 2004). Provision of high water content foodstuffs (e.g. oranges, apples, grapes, etc.) must be tracked and controlled because they can impact the animal’s total fluid balance. It is recommended that clinically healthy animals on fluid regulation undergoing prolonged surgical procedures be returned to higher fluid balances (e.g. an increase from 20 ml/kg to 80 mL/kg or *ad libitum*) at least 48 hours prior to the surgery. To ensure appropriate drug metabolism and/or elimination, increased fluids should also be provided post-operatively depending on the analgesics, antibiotics (Prescott 2010) or anti-inflammatory agents used to treat the animal.

IX. Social housing of animals on fluid regulation is recommended based on availability of suitable social partners. Procedures to ensure that each individual animal in the social group receives its daily fluid intake allocation must be implemented. If a single animal in the group is on fluid regulation, access to fluids may be withheld from the entire social group until the time that the fluid regulated animal has been separated from the group, after which the non-fluid regulated animals may be provided with *ad libitum* access to

water until the fluid regulated animal is returned to the group. Alternatively, some IACUCs have approved the implementation of fluid regulation for all cohorts in a social group, even animals not currently under study, to maintain social housing.

X. Health monitoring during fluid regulation studies:

1. Prior to placing an animal on fluid regulation, the animal should be given a complete physical examination by a veterinarian. Attention should be given to the animal's body condition and amount of body fat. Baseline clinical chemistry panels, serum/plasma *osmolality*, urinalysis, urine osmolality and other tests should be conducted as needed to establish baseline data and determine the animal's renal function and readiness for study.
2. When on fluid regulation, regardless of whether actively working or not, the body weight of each animal should be recorded prior to fluid consumption at least once weekly for animals >5 kg, and ideally, 2 to 3 times per week for animals <5 kg.
3. Intake of dry biscuits will be related to the volume of fluid given. Animals with normal body fat often do not lose more than 10% of their starting weight during fluid regulation. The weight loss humane endpoint for obese animals may exceed 10% while still maintaining appropriate muscle mass and adequate body condition scores. The veterinarian should carefully note an animal's body condition at the beginning of any fluid regulation paradigm (Clingerman & Summers 2005).
4. Different approaches in regards to the minimum daily fluid volume have been used at various institutions. Some institutions take an empirical approach and specify the minimum daily fluid requirement. The methodology chosen often depends on the degree of fluid regulation required to meet the research objectives and the animal itself. **To date, no one metric or minimum daily fluid requirement has proven ideal for all animals in all research situations.** Examples of various metrics that have been used include the following:
 - a. Assessment of the minimum fluid volume that maintains individual normal values of plasma *osmolality* from increasing 5.8% +/- 0.4% and plasma *sodium concentration* from increasing 5.6% +/-1.4%. These parameters correlate with significant cellular dehydration seen in rhesus monkeys following 24 hours of water deprivation. Normal serum *osmolality* in rhesus and cynomolgus macaques offered *ad libitum* water average 296 mosm/kg H₂O (+/- one standard deviation of 9 mosm/kg H₂O) and 304 mosm/kg H₂O respectively (+/- one standard deviation of 5 mosm/kg H₂O) (International Species Information System. *ISIS physiological reference values for Macaca mulatta and Macaca fascicularis*. Apple Valley, Minn: International Species Information System, 2002). This increase in *osmolality* is far above the 2.3% +/- 0.2% *osmolality* and 2.9% +/- 0.7% sodium increase corresponding with the drinking/thirst threshold (Wood et al 1982).
 - b. Assessment of *ketonuria* (> 2 mg/day) which is above the maximum normal value, and elevated *urine specific gravity* of 1.038 which is above the maximum normal value and two standard deviations above the published mean value (Bennet *et al* 1998) and elevated total *plasma protein* of 9.4 mg/dl which is two standard deviations above the published mean value (Fox & Loew 1984).

- c. Setting a daily minimum fluid intake of 20 ml/kg/day. A minimum daily water allocation of roughly 20 ml/kg/day has been shown to maintain osmotic state (a 2.5% increase in serum *osmolality* when on *ad libitum* water) by measuring serum *osmolality* in rhesus macaques (Yamada et al 2010).
 - d. Monitoring for clinical signs of dehydration, such as drinking urine, anorexia, scant or no urine output (i.e. oliguria), scant hard feces, lethargy, incoordination, dry mucous membranes and corneas, reduced skin turgor or other changes in behavior (poor study performance) should be in place for animals with follow up veterinary assessment for hematological parameters (e.g. increased serum osmolality, serum protein and hematocrit) if dehydration is suspected.
5. A physical examination by a clinical veterinarian with attention to the animal's body condition and assessment of clinical chemistry profiles (serum chemistry, osmolality, and complete blood count and differential) should be performed at least every 6 to 12 months with urine concentration, ketones and osmolality monitored more often as clinically possible or as needed.
 6. Each animal should be observed daily during periods of fluid regulation. Special emphasis should be placed on food intake, consistency of stool, amount of urine and behavior. Animals manifesting signs indicating dehydration, such as drinking urine, anorexia, scant or no urine output, scant hard feces, lethargy, incoordination, dry mucous membranes and corneas, reduced skin turgor or other changes in behavior (poor study performance) should be reported immediately to the clinical veterinarian.
 7. In situations where it is believed that an animal lacks sufficient motivation to accomplish a more difficult task, investigators are encouraged to first test the animal using a less difficult known task. Unmotivated, stubborn animals will often perform the known, easier task, whereas acutely dehydrated animals will commonly fail at both tasks. In cases of acute dehydration, further fluid restriction is unproductive and potentially distressful to the animal. So as not to confuse poor performance or lack of motivation with loss of cognition and mental acuity secondary to acute dehydration (Mountain & Ely, 2010, Bar-David, Urkin, Kozminski 2009), investigators and veterinary staff must be observant for signs of acute dehydration.

XI. Endpoints

If an animal loses 10% or more of optimal body weight while on fluid regulation, the clinical veterinarian should assess the animal's body condition and physical well-being and compare it to the records of the animal at the start of the regulation period. If the animal was obese at the beginning the veterinarian may determine the new body weight is "normal" and establish a new "starting/optimal" body weight. If the veterinarian determines the animal's condition warrants medical intervention, the investigator should be notified and the animal's diet adjusted or supplemented with high calorie treats and fluid soaked monkey biscuits. Optimal body weight can be determined on the basis of body condition scoring (BCS) (Weindruch 1996). If an animal's weight remains below 85% of optimal body weight for 24 h despite intervention, the animal should be given *ad libitum* access to water until its weight has increased to greater than 90% of starting or optimal body weight. Clinical diagnostics, such as serum *sodium concentration* and *osmolality* may also be used in determining animal well-being.

Animals should be given unrestricted access to fluid if there is >15% BW loss from baseline, the BCS is <2.5/5, significant abnormal behaviors have developed or clinical chemistry parameters are significantly out of normal range. Note that judgment must be used when evaluating juvenile macaques that are undergoing growth spurts as they may present with low BCS and still be normal (Clingerman & Summers 2005). The animal may be returned to study when improvements in body weight, BCS, and/or behavior have been made. Animals should be permanently removed from a fluid regulation study if they continue to have significant problems in any of the above identified areas after being returned to fluid regulation more than twice.

XII. Record keeping

Daily records of fluid intake should be kept for each animal and made available to the veterinarian or IACUC upon request. The veterinarian or their designee should review the records on a regular basis. The recorded fluid intake should be the sum of the earned fluid and any supplemental fluid provided. In addition, any high fluid foodstuffs (e.g. oranges, apples, grapes, etc.) fed to the animal should also be recorded in fluid equivalents if used to meet minimum daily fluid requirement (Bowes & Church 2005). Fluid regulation records should include:

1. Veterinary assessment of an animal's well-being prior to study, including a complete physical exam, body condition scoring, clinical chemistry, serum or plasma osmolality, urinalysis and osmolality, etc.
2. The pre-, during and post-regulation total daily consumption of fluid, inclusive of supplemental fluid sources and any high fluid food provided.
3. The duration of the regulation and results of routine daily monitoring parameters, such as body weight, BCS, behavioral assessments, quality/quantity of urine and fecal material, appearance of visible mucous membranes and corneas, skin turgor and laboratory data. Body weight should be logged a minimum of once each week when an animal is on fluid regulation.
4. The individual animal's preferred positive fluid type (e.g., water or juice).
5. The results of behavioral training and testing (e.g. poor, satisfactory, good), including the length of time required to acquire specific skills.

XIII. Reporting

The veterinarians should observe animals and the IACUC or post-approval monitoring program should review records regularly to ensure that animals are properly hydrated. The IACUC should regularly evaluate the animal records more often if there is evidence of fluid regulation related issues. Animals that become dehydrated or that physically or mentally deteriorate as a result of fluid regulation must be removed from study. This removal may be temporary if treatment is available or permanent if the condition is declared untreatable. Untreatable cases should be reported to the IACUC.

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