

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

## Purpose

The Association of Primate Veterinarians recognizes that fluid regulation may serve as a powerful behavioral modulator. APV does not condone the use of fluid regulation in nonhuman primates (NHPs) as a routine and unjustified practice, and it supports and encourages alternatives where appropriate. The following guideline has 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, approved by the IACUC, and the veterinarian should closely monitor the animal's 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) Have alternatives to fluid regulation been considered, such as the use of preferred fluid reward, novel food reward, or different reward interval schedule (that is, 'fixed' versus 'variable,' in terms of timing and size of the reward)?
- 3) How is the amount of fluid provided to each animal determined? What are the limits of the regulation (that is, daily volume, time frames, duration of regulation)?
- 4) Are there opportunities to earn additional fluids once the animal has learned the task?
- 5) What is the plan to ensure the animal's physiologic and psychological well-being? What parameters are used to monitor the health and well-being of the animal (for example, changes in body weight and body condition score, changes in serum/plasma or urine osmolality)?
- 6) What is the humane endpoint for fluid regulated animals?
- 7) What is the intervention plan for animals failing to meet the established health parameters (for example, supplemental fluid or restoration of ad lib fluid consumption)?
- 8) How is "normal" fluid balance re-established at the end of study or during prolonged periods of inactivity on the protocol?
- 9) 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?

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 period of several days.<sup>29,48</sup> Short periods with markedly reduced fluid intake may be required during the

initial phases, and the acclimation period varies by species and hydration status. Larger NHP species may tolerate markedly reduced fluid intake, but smaller species (for example, 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 and urine osmolality, *prior to* initiating a period of fluid regulation. Individual baseline fluid requirements under similar conditions (for example, clinical health, environmental factors, level of physical exercise, etc.) vary depending on the species, sex, growth and development phase, body weight, social ranking, and individual preferences (for example, 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 (for example, an ocular deficiency may impact a task that requires visual acuity or impaired cognition may occur secondary to dehydration).<sup>7,26</sup> The inclusion of an experienced animal behaviorist is encouraged to optimize animal welfare.

- 1) It is preferable to begin fluid regulation by gradually reducing the amount of water available to the animal each day over several days to allow the physiologic changes that conserve water to take place and the animal to adapt.
- 2) 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.
- 3) Ideally, fluids should be accessible at least twice a day. In the United States, the USDA Animal Welfare Act Regulations (AWAR), Section 3.83 state: "*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.*"<sup>6</sup> If fluid is provided only once per day this practice should be scientifically justified and approved by the IACUC as a deviation from the USDA AWAR. Under these experimental conditions it is recommended that animals be provided with the opportunity to earn water at approximately the same time each day and ideally allowed to work to satiety over an extended interval (for example, several hours during training or data acquisition).
- 4) 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 successfully performed task.

5) 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 overhydration causes hyponatremia (serum Na < 125 mEq/ $\mu$ L) and may lead to mental confusion, disorientation, malaise, weakness, nausea, transient neurological deficits, seizures, and coma.<sup>26</sup> 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 availability to ad libitum access over several days during which the animal is closely monitored initially for food and fluid consumption is recommended. A more abrupt transition from regulated to ad libitum water intake may be acceptable once an individual animal’s response to this transition has been evaluated.

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

Between the ages of 4 and 6 y, rhesus macaques experience a growth spurt, during which time their body weight may normally increase by 2 kg in a 12-mo period.<sup>9</sup> Animals in this age range may be at increased risk of poor body condition if they are concurrently on a fluid regulation regimen. This age period should be approached with increased vigilance. Species-specific growth and development patterns should be considered prior to beginning a fluid regulation regimen. It should be noted the growth curve of an animal on fluid regulation may not mirror animals provided with ad libitum access to water and food. Additionally, intake of dry biscuits is related to the volume of fluid given. Depending on the time of day that feed and water are offered, body weight can vary widely in subadult rhesus. 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 typically consume the majority of their dry food ration after they have consumed their fluid intake for the day. Fluid regulation may also result in a decreased appetite for dry diets; therefore, to the extent possible, fluids should be given during mealtimes to encourage consumption of more food and reduce body weight loss.<sup>35</sup> In addition, supplemental access to fluid for some period of days when research procedures are not scheduled should be considered unless scientifically justifiable reasons preclude such fluid.<sup>29</sup>

*Considerations for use of fluid regulation and NHP psychological well-being.*

Animals on fluid regulation may have to be exempted from some aspects of the institutional psychological well-being program (for example, 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 a 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.<sup>45</sup> 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.<sup>13</sup> Provision of high-water content foodstuffs (for example, 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 (for example, an increase from 20 ml/kg to 80 mL/kg or ad libitum) at least 48 h prior to the surgery. To ensure

appropriate drug metabolism and/or elimination, increased fluids should also be provided postoperatively depending on the analgesics, antibiotics,<sup>35</sup> or anti-inflammatory agents used to treat the animal.

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.

*Health monitoring during fluid regulation studies.*

Prior to placing an animal on fluid regulation, the animal should be given a complete physical examination by a veterinarian. The veterinarian should carefully note an animal’s body condition at the beginning of any fluid regulation paradigm.<sup>11</sup> 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.

When on fluid regulation, regardless of whether actively working or not, the body weight of each animal should be routinely recorded prior to fluid consumption and at least once weekly for animals > 5 kg. For animals < 5 kg, a weight monitoring plan should be addressed with the institution’s veterinarian. Additional factors should be considered as below in the New World primate section. In addition to body condition scoring, attention should be paid to the amount of body fat, in that animals with normal body fat often do not lose more than 10% of their starting weight during fluid regulation, but overly conditioned (for example, obese) animals may lose more than this cutoff and be physiologically normal.

Different approaches regarding 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:

1) Assessment of the minimum fluid volume that maintains an individual animal’s serum osmolality and serum sodium concentration (Rhesus:  $148 \pm 3$  mmol/L and Cynomolgus:  $151 \pm 3$  mmol/L)<sup>14</sup> within the normal range for that animal. Normal serum osmolality in rhesus and cynomolgus macaques offered ad libitum water average  $296 \pm 9$  mOsm/kg H<sub>2</sub>O and  $304 \pm 5$  mOsm/kg H<sub>2</sub>O, respectively.<sup>19</sup> An increase in serum osmolality of  $2.3\% \pm 0.2\%$  or serum sodium of  $2.9\% \pm 0.7\%$  above baseline corresponds to the threshold for drinking/thirst, consistent with adequate motivation for seeking fluid rewards. Increases in excess of 5.8% above baseline serum osmolality or 5.6% above baseline serum sodium concentration correlate with significant cellular dehydration in rhesus monkeys following 24 hours of water deprivation.<sup>53</sup>

2) Assessment of the minimum fluid volume that maintains an individual animal's ketonuria below 2 mg/d, urine specific gravity below 1.038,<sup>8</sup> and total plasma protein below 9.4 mg/dL,<sup>14</sup> which are two standard deviations above the published mean values.

3) Setting a daily minimum fluid intake of 20 mL/kg/d. A minimum daily water allocation of roughly 20 mL/kg/d has been shown to maintain an osmotic state with a 2.5% increase in serum osmolality compared to ad libitum water.<sup>56</sup>

A physical examination by a clinical veterinarian with attention to the animal's body condition and assessment of clinical chemistry profiles (serum chemistry and osmolality, and complete blood count and differential) should be performed at least every 6 to 12 mo with urine concentration, ketones, and osmolality monitored more often as needed.

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, behavior, and clinical signs of dehydration. 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 with follow up veterinary assessment of hematological parameters (for example, increased serum osmolality, serum protein, and hematocrit) if dehydration is suspected.

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 often perform the known, easier task, whereas acutely dehydrated animals 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,<sup>7,26</sup> investigators and veterinary staff must be observant for signs of acute dehydration.

#### *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. The weight loss humane endpoint for obese animals may exceed 10% while still maintaining appropriate muscle mass and adequate body condition scores. 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. Body condition scoring (BCS) is a valuable tool to monitor animals and aid in decision-making.<sup>11</sup> 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% body weight 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 subadult macaques that are undergoing growth spurts as they may present with low BCS and still be normal.<sup>11</sup> 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.

#### *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 (for example, oranges, apples, grapes, etc.) fed to the animal should also be recorded in fluid equivalents if used to meet minimum daily fluid requirement.<sup>45</sup> 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 postregulation 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 (for example, water or juice).

5) The results of behavioral training and testing (for example, poor, satisfactory, good), including the length of time required to acquire specific skills.

#### *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.

#### *Special considerations for New World primates and other small species.*

Most of the above recommendations can be similarly applied to New World primates and smaller species; however, there are several important considerations for these species, especially when investigators are adopting a new model or transitioning from work with macaques, mice, or rats to these smaller primate species.

Many New World primates and a few Old-World primate species under 5 kg adult body weight tend to have an increased body surface area to volume ratio, fewer body reserves, and a

faster metabolic rate. This means that these animals are more sensitive to environmental changes (for example, temperature, relative humidity), and do not tolerate prolonged periods of fasting as well as the larger primate species that are more commonly used in behavioral research. Changes happen quickly in such animals and the window to intervene can be quite narrow; therefore, enhanced monitoring is recommended along with a more proactive approach to intervention. Considerations should be given for more frequent weighing based on size of species. The weight of stomach contents is often significant when compared to the weight of the animal, which makes it crucial that animals be weighed at the same time each day, ideally prior to provision of food or water. If changes in body weight are noted, it is recommended that investigators or caretakers have a pre-approved intervention plan so that appropriate fluid or caloric supplementation can be implemented without delay. This strategy can help to stabilize the animal while it awaits veterinary assessment.

Visual system characteristics for each species should be considered when designing studies or tasks or when adapting tasks from macaques to other primate species. For example, most macaques have trichromatic or true color vision; however, a wide range of variation in color vision exists for many New World species. In particular, male marmosets have dichromatic vision while females may have either dichromatic or trichromatic vision, and the nocturnal owl monkeys may have only one type of cone and is thus a monochromat.<sup>20,21</sup>

General species attributes may require a revision of training approaches when compared to traditional macaque species. For example, tamarins have a relatively short attention span, marmosets slightly longer,<sup>41</sup> and owl and squirrel monkeys somewhere in between these 2 species.<sup>38</sup> In general, owl monkeys and marmosets are naturally more fearful and hesitant to engage with humans than the more outgoing squirrel monkey, tamarins, and cebus species. Investigators should anticipate this and plan for more desensitization or acclimation time up-front to train animals and adapt the length of initial training to match species attention span. Shorter, more frequent sessions likely will be more productive in species with a shorter attention span.<sup>41</sup>

Species-typical behaviors should also be considered in study design and implementation. Owl monkeys are nocturnal, thus, training and testing should ideally take place during the dark phase of the light cycle. Investigators should consider housing animals under a reverse light cycle to facilitate research work with this species. In contrast, marmosets are diurnal and generally consume minimal to no food or fluid after the onset of the dark cycle.<sup>50</sup> For marmosets, testing must take place during the light phase and if the animals are to receive all fluids during testing, they must be returned to their home cage at least one hour before the onset of the dark phase to allow them sufficient time to eat prior to entering the nest box for sleep. Marmosets generally do not eat well if all food is dry and hard,<sup>10</sup> some amount of moisture in the diet should be provided and considered even when on fluid regulation. Due to their pronounced diurnal cycle marmosets emerge from the nest box in the morning hungry and thirsty. Researchers should strive to take advantage of this and schedule testing to take place as early in the light phase as possible. Many animals under this paradigm do not require fluid restriction but will readily work for fluids early in the day and may then be given ad libitum access to a full bottle for several hours that day. If the water bottle is removed in the afternoon, animals will be thirsty again the next morning. Similarly, marmosets are highly motivated by sweet flavors<sup>17</sup> and may work

very well for dilute sweetened solutions even when plain water is available ad libitum in the home cage.<sup>12,34,43</sup>

The current lack of availability of marmosets makes it tempting to use limited animal resources in more than one way (for example, both as breeding and as experimental animals) and has inspired many facilities to begin their own breeding colonies.<sup>30</sup> Pregnancy, lactation, and growth place immense metabolic demands on smaller species especially callitrichids which routinely twin and for whom the combined weight of infants is often at or above 15% of maternal body weight. As such, any amount of fluid restriction for pregnant, lactating, or young, juvenile animals should only be undertaken with extreme caution and include astute monitoring. The need for such restriction during these phases of production should be carefully evaluated by the IACUC and accompanied by rigorous scientific justification. Example considerations to minimize the potential for adverse outcomes include: train the dam on performance of the behavioral task before she becomes pregnant such that all acclimation is complete and usual performance established before mating/pregnancy; reduce fluid control to the minimum necessary to achieve stable performance or consider manipulation only of the timing of fluid delivery without volume restriction during pregnancy and lactation; monitor animals for body condition routinely in addition to weight; and monitor feed consumption, and urine and fecal output and character. Failure to gain weight should be the humane endpoint and/or trigger veterinary evaluation especially for pregnant or juvenile animals. For lactating animals, infant weights should be monitored and failure of the nursing infant to gain weight should trigger not only fluid supplementation for the dam on study, but also supplemental milk replacer to be given to the infant(s).

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