

**University of Notre Dame Institutional Animal Care and Use Committee  
Policy for the Use of Animals for Blood Feeding Mosquitoes**

**Purpose**

Mice, rats, gerbils, hamsters, and rabbits are all used for the purpose of blood feeding mosquitoes. The welfare of the animal must be assured during the process through the use of anesthesia, careful monitoring of the animals' health and adherence to the guidelines set forth in this policy regarding the amount of blood that can be taken during each feeding and the frequency of feedings. Records must be kept to document the use of the animals, including the use of anesthetics, verification of recovery from anesthesia, the date of use, and the initials of the technician. The formula used to determine the number of mosquitoes allowed to feed on an animal at a time is:

**BW (g) x .06 (per cent of BW that is blood) x .20 (20% blood can be lost without death) = maximum amount in ml that can be taken at one time.**

This is a maximum monthly figure so for weekly feedings, this figure is divided by 4 so that animals can recover from the weekly blood loss without becoming anemic. The final figure is divided by 0.003 ml, the average volume of blood taken by a mosquito, which provides the maximum number of mosquitoes to be used per animal. Based on this formula, the following table lists the recommended number of mosquitoes to be fed on each species. If an accurate weight, in grams, is available, the number of mosquitoes allowed to feed equals the weight in grams.

Species	Max. Numbers of Mosquitoes Fed	Frequency
Hamster	120	Weekly
Hamster	50	3 consecutive days
Gerbil	80	Weekly
Guinea Pig	1000	Weekly
Rat - adult	200	Weekly
Rat - weanling	unlimited	Once non-survival
Mouse	25	Weekly

**Procedures**

Once the animal reaches the desired infection level, as determined by blood smears, it is anesthetized. The number of mosquitoes is calculated and the animal is introduced to the mosquitoes to take a blood meal. All animals are transferred to and from the facility according to the IACUC Animal Transportation Policy. The freight elevator is recommended for moving animals between floors.

**Hamsters:**

1. Hamsters that are infected with various malarial parasites are used to blood feed uninfected mosquitoes to perpetuate the infection in the mosquitoes.
2. Anesthesia Used: Ketamine 100-200 mg/kg Xylazine 5-10 mg/kg given IP or Ketamine 150mg/kg Acepromazine 5mg/kg given The anesthesia is based on the weight of the animal and on the tolerance that the animal has developed.
3. Frequency: See the above chart. The dates of use and anesthesia dosage are recorded on the back of the cage cards.
4. Maximum number of mosquitoes: See the above chart

**Gerbils:**

1. Gerbils that are infected with various blood parasites are used to blood feed uninfected mosquitoes to perpetuate the infection in the mosquitoes.
2. Anesthesia Used: Once the gerbil reaches the desired infection level, as determined by blood smears, it is anesthetized with a combination of Ketamine (100 mg/ml) and Xylazine (20 mg/ml), prepared by mixing 0.35 ml Ketamine with 0.15 ml of Xylazine and dosing this combination at 0.08 - 0.1 ml per animal and administered subcutaneously.
3. Frequency: The gerbils are used no more than once every seven days. Dates of use and anesthesia dosage are recorded on the back of the cage cards.

4. Maximum number of mosquitoes: See the above chart.

**Guinea pigs:**

1. Identification: all guinea pigs are micro-chipped prior to being used for blood feeding.
2. Anesthesia used: The guinea pigs are given 0.3-0.6 cc SQ of Guinea Pig Cocktail. Guinea Pig Cocktail is made of 2 parts Ketamine (100 mg/ml), 1 parts Xylazine (100 mg/ml), and 3 parts Acepromazine (10 mg/ml). The lowest dose of Guinea Pig Cocktail is used and increased in 0.05 increments as anesthetic tolerance develops to provide the depth of anesthesia required for bloodfeeding.
3. Frequency: Guinea pigs are used no more than once every seven days. Individual records are maintained for each guinea pig. Date of use and anesthetic dosage are recorded.
4. Maximum number of mosquitoes: See the above chart.
5. When all guinea pigs are fully awake, they are returned to their primary enclosure.

**Rabbits:**

Rabbit blood is used to blood feed mosquitoes. Only trained FLSC animal technicians withdraw blood utilizing vessels in the rabbit's ear, as per the FLSC guidelines. This blood is then used in artificial feeding devices. No mosquitoes are fed directly on rabbits.

**Rats:**

1. Anesthesia used: Ketamine(100mg/ml)/Xylazine (20mg/ml) at a 2:1 mix. Dosage is weight dependent, with a typical dosage being 0.012 - 0.03cc/10 gm BW given IM. Anesthetics will be administered by trained FLSC animal technicians.
2. Frequency: Rats are used no more than once per week.
3. Maximum number of mosquitoes: See the above chart.
4. Rats are housed singly until fully recovered from anesthesia.
5. Weanling rats that are culled from the LOBUND-Wistar colony can be used once prior to euthanasia. Dosage is weight/age dependent, with a typical dosage of Rodent Cocktail being 0.3 to 0.4 cc IP. This dosage approximates 90 mg/kg Ketamine + 18 mg/kg Xylazine + 3 mg/kg Acepromazine. Since this is non-recovery procedure, there is no limit on the number of mosquitoes that can be fed on these animals.

**Mice:**

1. Anesthetic Used: Mice are anesthetized with Rodent Cocktail given IP, containing 9 parts Ketamine (100 mg/ml) + 9 parts Xylazine (100 mg/ml) + 3 parts Acepromazine (10 mg/ml) + 79 parts saline. The dosage is calculated as  $BW \times 100 \pm 50$  to give the amount of anesthetic in a microliter volume. As a general rule 0.3cc (300 microliters) is sufficient for 30 minutes or longer immobilization. This dosage approximates 90 mg/kg Ketamine + 18 mg/kg Xylazine + 3 mg/kg Acepromazine. The anesthetized mice are taken to the insectories and placed on a mosquito cage for 20-30 minutes. Anesthetized mice should never be left unattended.
2. Frequency: To avoid anemia of the mice, the mice are used no more than once every 7 days. The date of usage must be recorded on the cage card.
3. Maximum number of mosquitoes: See the above chart.
4. Returning mice to their room in FLSC
  - a. The number of mice in the cage must be the same as indicated on the cage card.
  - b. Never place anesthetized mice in with mice that are awake. It may lead to cannibalism.
  - c. The cages must be properly docked on ventilated racks or the water bottle replaced correctly to allow mice access to water.
  - d. Do not replace enrichment devices in the cages until all animals have completely recovered from anesthesia.