

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

I. Purpose:

Mice, rats, gerbils, hamsters, rabbits, chicken, and non-human primates 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 animal 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
Non-Human Primate	24,000 - 28,000	Once
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

II. Procedures:

A. Non-human Primates:

1. Non-human primates that have been infected with malarial parasites are used to blood feed uninfected mosquitoes in order to perpetuate the infection in the mosquitoes.
2. Once the animals reach the desired level of infection as determined by blood smears, the blood feeding procedure must be coordinated with the Freimann veterinary staff.
3. Anesthesia used: Non-human primates are immobilized with Ketamine (10mg/kg) given IM. The animal is then placed on inhalation Isoflurane anesthesia via face mask.
4. Frequency: The infected primates are usually blood fed one time only and then cured. Other needs must be discussed with the Attending Veterinarian.
5. Maximum number of mosquitoes: The average rhesus will weigh 12-15 pounds (6-7 kg). The maximum number of mosquitoes to be fed on one rhesus is 24,000, for a one time feeding.
6. All aspects of this procedure must be recorded in the individual's medical record.

B. 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: Once the hamster reaches the desired infection level, as determined by blood smears, it is anesthetized with a combination of Ketamine and Xylazine, as per the FLSC guidelines for anesthesia. The anesthesia is based on the weight of the animal and on the tolerance that the animal has developed. The anesthesia is given IP.
3. Frequency: The hamsters are used no more than once every seven days. For some experiments, the hamsters must be fed 3 consecutive days. The date of use and anesthesia dosage are recorded on the back of the cage cards.
4. Maximum number of mosquitoes: Based on the weight of 120 grams, the maximum number of mosquitoes allowed to feed on one hamster is 120, if bled once weekly. If bled for 3 consecutive days, the maximum number of mosquitoes allowed per animal is 50 per day.

C. 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 gerbil, given subcutaneously.
3. Frequency: The gerbils are used no more than once every seven days. Date of use and anesthesia dosage are recorded on the back of the cage cards.
4. Maximum number of mosquitoes: Based on the weight of 80 grams, the maximum number of mosquitoes allowed to feed on one gerbil is 80.

D. 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 interferes with the animal remaining anaesthetized for the desired period.
3. Frequency: The guinea pigs are used no more than once every seven days. Individual records are maintained on each guinea pigs. Date of use and anesthesia dosage are recorded.
4. Maximum number of mosquitoes: Based on the average weight of 1000 grams, the maximum number of mosquitoes fed per guinea pig is 1000.
5. The guinea pigs are transferred to and from the facility in a “Transport and Recovery Cage” using the freight elevator. When all guinea pigs are fully awake, they are returned to their proper enclosure.

E. Rabbits:

Rabbit blood is used to blood feed mosquitoes. The trained FLSC animal technicians withdraw the blood from the vessels in the rabbit's ear, as per the FLSC guidelines. This blood is then used in artificial feeding devices.

F. Chickens:

1. Chickens are used for blood feeding when working with avian malaria or when working with specific species of mosquitoes that only feed on avian blood.
2. Restraint methods
 - a. The young chickens are manually restrained or placed on a restraint board. Mosquito cages are then placed against the animal's body.
 - b. Large chickens are placed on a restraint board. Mosquito cages are then placed against the animal's body.
 - c. The number of mosquitoes fed on the chicken will be determined by the weight of the animal; one mosquito per gram of body weight.

G. Rats

1. Anesthesia used: Ketamine(100mg/ml)/Xylazine (20mg/ml)at a 2:1 mix. Dosage is weight/size dependent, with a typical dosage being 0.012 - 0.03 cc/ 10 gm BW given IM. It will be administered by trained FLSC animal technicians.
2. Frequency: The rats are used no more than once a week.
3. Maximum number of mosquitoes: The maximum number of mosquitoes allowed to feed on each rat is 200. This is based on an average weight of 200 grams.
4. Rats are housed singly until fully recovered from the anesthesia.
5. Weanling rats that are culled from the LOBUND-Wistar colony can be used once prior to euthanasia. The anesthesia used is the Rodent Cocktail. The dosage is 0.3 to 0.4 cc , given IP. This dosage approximates 90 mg/kg of ketamine + 18 mg/kg of xylazine + 3 mg/kg of acepromazine. Since this is non-recovery procedure, there is no limit as to the number of mosquitoes fed on these rats.

H. Mice:

1. Anesthetic Used: The mice are anesthetized with a Rodent Cocktail, which consists of 9 parts Ketamine (100 mg/ml), 9 parts Xylazine (100 mg/ml), and 3 parts Acepromazine (10 mg/ml) to 79 parts saline. The dose is 0.3cc I.P. This dosage approximates 90 mg/kg of ketamine + 18 mg/kg of xylazine + 3 mg/kg of 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: The maximum number of mosquitoes allowed to feed on one mouse is 25, based on an average weight of 25 grams. Again, this is to avoid anemia of the animal.
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 placed properly on the rack to allow the mice access to water.
 - d. Do not replace enrichment devices in the cages until all animals have completely recovered from anesthesia.

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