

## Policy for the Use of Animals for Blood Feeding Sand Flies

### Purpose

Mice, rats, hamsters and birds are all used for the purpose of blood feeding sand flies. The welfare of the animal must be assured during the process through the use of anesthesia, careful monitoring of the animal's 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.

### Background

Because sand flies have a shorter proboscis than mosquitoes, they are able to access only the most peripheral capillaries in the skin. This may require shaving areas of the animal to allow easy access to the skin or the use of the ears and/or the tail. There is also the possibility of localized irritation of the skin surrounding a sand fly bite. All irritation, swelling or erythema is noted on the back of the cage card along with the date. If signs persist beyond 2 days, the attending veterinarian must be notified and a Report of Animal Illness/Injury filled out.

### Calculations

The formula used to determine the number of sand flies 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 the animal can recover from the weekly blood loss without becoming anemic. The final figure is divided by 0.00025 ml, the average volume of blood taken by a sand fly, which provides the maximum number of sand flies to be used per animal. Based on this formula, the following table lists the recommended number of sand flies to be fed on each species.

Species	Max. Numbers of Sand Flies Fed	Minimum Frequency Interval
Hamster	1400	Weekly
Hamster	460	3 consecutive days
Rat - adult	3000	Weekly
Rat - weanling	unlimited	Once non-survival
Mouse	300	Weekly
Mouse	150	every 3 days for 2 weeks
Birds	100 sand flies per gram body weight	Weekly

### Procedure by Species

#### Hamsters

1. Hamsters that are infected with various blood parasites are used to blood feed uninfected sand flies to perpetuate the infection in the sand flies.
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 blood fed 3 consecutive days. The dates of blood feeding and anesthesia dosage are recorded on the back of the cage cards.
4. Maximum number of sand flies: Based on the weight of 120 grams, the maximum number of sand flies allowed to feed on one hamster is 1400, if bled once weekly. If blood fed for 3 consecutive days, the maximum number of sand flies allowed per animal is 460 per day.

## **Rats**

1. Rats that are uninfected are used to blood feed sand flies infected with various blood parasites to transfer the infection to the animals.
2. 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.
3. Frequency: The rats are used no more than once a week.
4. Maximum number of sand flies: The maximum number of sand flies allowed to feed on each rat is 2400. This is based on an average weight of 200 grams.
5. Rats are housed singly until fully recovered from the anesthesia.
6. Weanling rats that are culled from the LOBUND-Wistar colony can be used once prior to euthanasia. Since this is non-recovery procedure, there is no limit as to the number of sand flies fed on these rats.

## **Mice**

1. Mice that are uninfected are used to blood feed sand flies infected with various blood parasites to transfer the infection to the animals.
2. Anesthetic Used: The mice are anesthetized with Ketamine (100mg/ml)/Xylazine (20mg/ml) prepared as follows: 10 ml Ketamine + 1 ml Xylazine to produce the stock solution. The stock solution is diluted 1:4 with sterile normal saline. The dosage is 0.15 – 0.20 ml diluted Ketamine/Xylazine given IP. This anesthetic should give up to 45 minutes of anesthesia. The anesthetized mice are taken to the insectory and placed on a sand fly cage for 20-30 minutes. Anesthetized mice should never be left unattended.
3. Frequency: To avoid anemia of the mice, the mice are used no more than once every 7 days. For some experiments, the mice must be every 3 days. After two weeks of blood feedings (~6 times) the mice are not used again due to the production of antibodies to sand fly saliva. The date of usage must be recorded on the cage card.
4. Maximum number of sand flies: The maximum number of sand flies allowed to feed per mouse is 300, based on an average weight of 25 grams. Again, this is to avoid anemia of the animal.

## **Birds**

1. Birds are used for blood feeding when working with species specific blood parasites, viruses or when working with a species of mosquitoes that feeds exclusively on avian blood. Birds used for blood feeding include wild caught species and domestic chickens (chicks and adults).
2. Birds are not anesthetized for blood feeding. For this reason, anyone using birds must remain with the animal during the blood feeding. Birds are manually restrained, placed on a restraint board, or placed in netting tubes.
3. Birds manually restrained are positioned so that areas with relatively few feathers are exposed or the feathers are parted. The sand fly cages are then placed directly against the animal's skin.
4. Birds placed on a restraint board are positioned so that areas with relatively few feathers are exposed or the feathers are parted. The bird is secured with rubber bands or tubing. The sand fly cages are then placed directly against the animal's skin.
5. Birds placed in netting tubes can be introduced into the sand fly cage to allow access to the animal for blood feeding. The netting tube must be small enough to prevent excess movement and large enough to allow for expansion of the abdomen. Constriction of the abdomen of birds can result in suffocation. The netting is closed and secured using a clip, twist tie or rubber band.
6. The number of sand flies fed on the bird will be determined by the weight of the animal; 10 sand flies per gram of body weight.