

## Appendix H

### Production and Collection of Fluids

See RAR Guidelines for Immunization of Laboratory Animals

### 1. Immunization and Antibody Production

#### 1.1 List species:

Rhesus monkeys

#### 1.2 List antigen(s):

NA

#### 1.3 List or describe adjuvant(s):

Initial immunization:	NA
Subsequent immunizations:	NA

#### 1.4 List or describe injection:

List anatomic site, volume administered per injection site (maximum 0.05 ml CFA per site for mice and 0.1 ml per site for rabbits), total volume administered at one time, injection frequency and total number of administrations.

NA

#### 1.5 List or describe collecting blood or other body fluids:

List site, volume, frequency and method used. Indicate anesthetics, analgesics or tranquilizers to be used, if any, and duration of use.

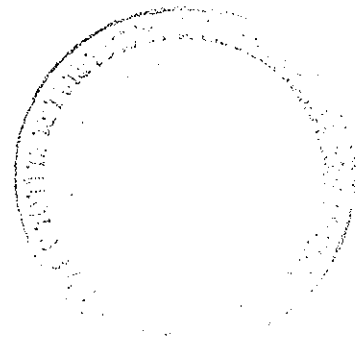
Females will be vaginally swabbed in some experiments to verify hormonal conditions and phase of the menstrual cycle. Samples will be obtained with a Q-tip dipped in saline and spread on glass slides with cover slips for future microscopic analysis. Slides will be stored in covered containers in a cabinet.

In both males and females blood samples will occasionally be drawn and analyzed at different time periods after the behavioral session, to measure estrogen levels (in females) and drug levels and compare plasma levels of the abused drug in males and females. The time periods for pharmacokinetic analysis will be 0, 30 and 60 min after the drug session. Animals will be anesthetized with ketamine (10 mg/kg), placed face down on a table in the anteroom, and 3 ml of blood will be drawn from the saphenous vein and immediately placed in a vacutainer. The blood will be centrifuged and stored in a secure container in a freezer in a locked refrigerator until it is sent off for analysis.

**2. Collection of Blood or Other Body Fluids (Other than ascites)**

2.1 Complete the table below.

Fluid Collection Table								
Fluid Collected	Frequency	Volume Collected	Method of Collection	Site of Collection	Anesthetization, Restraint, Sedation			List/Describe
					Anesthetic	Restraint	Sedative	
Vaginal smears	daily	0.5 ml	Q-tip swab	home cage	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Monkeys are trained to present
Blood	monthly	3 ml	Venopuncture	Anteroom	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	Ketamine (10 mg/kg)
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	



## Appendix I

### Pharmacologic/Toxicologic Studies

**1. Describe materials to be evaluated:** If hazardous materials are used, complete Appendix G.

Ethanol 8% wt/vol (p.o.); baclofen 0.5, 1.25, 2.5 mg/kg (i.p.); bremazocine 0.32, 1, 2.5 mg/kg (i.p.); cocaine 10 mg/kg (inhalation); heroin 0.06, 0.12, 0.25, 0.5, 1.0 mg/kg (inhalation); phencyclidine 0.06, 0.12, 0.25, 0.5, 1.0 mg/kg (p.o.); methadone 0.8 mg/ml (p.o.). Pentobarbital sodium 100 mg/kg will be kept on hand in the safe in case a vet needs to euthanize a monkey if they are very ill or injured.

**2. Describe the route and duration of administration:**

Route of administration is indicated above for each drug in item 1 and administration by oral or parenteral routes takes about 2-4 seconds. The self-administered drugs (ethanol, cocaine, methamphetamine, heroin, and phencyclidine) are available daily for 3 hr.; however, the monkeys typically self-administer the drug for only the first 1-2 hr. The monkeys have water continuously available, and self-administration of the drugs is voluntary. Thus, the monkeys control their own dose. The treatment drugs (baclofen and bremazocine) are administered i.m. before each daily session for 5 consecutive days, followed by a 5-10 day period of no treatment until behavior stabilizes. Each monkey will receive 4-5 series of injections over the 3-year period of this protocol.

**3. Describe the testing methods employed (e.g., tail flick, withdrawal, abdominal stretching assay, LD<sub>50</sub>, etc):** If blood or other body fluids are collected, also complete Appendix H.

For the treatment drugs, baclofen, bremazocine, brief intramuscular injections will be given once a day for 5 days followed by 5 or more days when no injections are given. The self-administered drugs will be taken orally by the monkey under a behavioral schedule that limits intake over the 3 hr session. Thus, intoxication is minimized and we have not seen signs of physical dependence.

**4. Describe criteria that will be used to ensure that the animal does not experience pain or distress and methods to monitor the animals:**

Monkeys will be observed for signs of local irritation (scratching, licking) after injections. We have worked with these drugs before, and have observed no signs of pain or distress at the used.  
NA

**5. If pain or distress are anticipated, how will they be minimized? Describe methods, including dose and route of administration, if appropriate.**

NA

**6. What is the endpoint of these studies, e.g. time points:**

When the necessary behavioral data are collected, usually in 3-4 months.

**Appendix J****Dietary Manipulations or Fluid Restriction**

See RAR Food and Dietary Restrictions

**1. Describe any dietary manipulations or special feeding requirements:**

Monkeys will be fed standard high protein monkey chow with occasional access to preferred substances such as trail mix, fruit, vegetables, or other small nutritious treats to vary their diets.

**2. Describe caloric or nutrient restrictions or excesses:**

This is a summary of the guidelines that have been used over the last 17 years in our laboratory to determine the amount of food the monkeys receive depending on their age:

**Adult monkeys**

**Restricted feeding.** For most experiments, adult monkeyeys are kept at 85% of their free-feeding weight. The free-feeding weight is determined by letting them free feed (never run out of food) until there is no longer an increasing trend in body wieght, and then an 85% value is calculated. This is usually between 9 and 12 kg. We then feed the monkeys an amount each day that keeps them at their 85% weight. That amount is usually between 100 and 200 g depending upon thye monkey's age and activity level.

**Free feeding.** In some of our experiments the goal is to compare restricted feeding (85% of free feeding) with ad lib feeding with respect to how hard the animals will for drugs during behavioral and pharmacological treatments. Thus, occasionally, monkeys are maintained in the free-feeding condition. When changing from restricted feeding to free feeding we increase the amount of food by about 50 g per day until the onlkey is leaving food in the ccage between feedings. We then adjust the aouint so that there is minimal hoarding and wasting of food. They are fed after their daily drug session, so that the next morning before the drug session begins, the small amount of food remaininig can be removed so that they do not ingest it while intoxicated.

**Adolescent monkeys**

**Restricted feeding.** The procedure used with these monkeys is to feed them amoiunts that are similar to what the adult restricted feeding monkeys receive, 120-200 g per day, with the criterion that their weight should be steadily increasing. Since these monkeys are still growing, it is impossible to keep them at a fixed weight. The amount of food they receive is gradually increased until their weights increase, but they are generally not fed any more than 200 g per day. When they reach adulthood and are over 10 kg in body weight, the procedure described above for adult monkeys is used for restricted feeding.

**Free feeding.** The procedure for free feeding is identical to that described for adult monkeys.

**3. Describe length of time animals will be on experimental diet:**

NA

**4. Will neonates or rodents be fasted beyond 3 hours or other animals beyond 48 hours?**

Yes.  No.

If yes, provide a scientific justification:

[Empty rectangular box for scientific justification]

5. Will animals be provided less than ad lib fluids or drinking water for experimental reasons?

Yes.  No.

If yes, provide details including amount/day, monitoring of animals, criteria used to determine well-being of animals, and scientific justification:

[Empty rectangular box for details]

6. Will fluids be withheld for more than 24 hours or more than 5 hours for rodents?

Yes.  No.

If yes, describe this aspect of the study, including the rationale and monitoring methods:

[Empty rectangular box for description]

**Appendix O**  
Source of External Funding

*If this project will be funded with external funds, answer all of the following questions and provide original signature at the bottom of this appendix.*

*If this project is funded under multiple grants, complete an Appendix O for each source of funding.*

*In addition, include the 5-point vertebrate animal section of the grant application with this application.*

**1. Project:**  has been  will be submitted to the following funding agency:

Name of Sponsor:	National Institute on Drug Abuse
Address:	600 Executive Boulevard, Bethesda, MD
Contact Person:	Dr. Cora Lee Wetherington

**2. Funding decision:**  is pending.  has been awarded.

**3. Agency-assigned grant number(if available):**

RO1 DA02486-25

**4. Nature of funding source:**

**Federal grant.** Submit a copy of the grant with your IACUC application, please flag the page containing the five-point vertebrate animal description.

**Other grant or contract.** Specify:

*If you would like certification of IACUC approval sent to the funding agency, provide the name and address of the contact person at the agency and any identifying codes:*

Name:		Phone:	
Address:		Fax:	
		Codes:	

***This Section is required if this research is externally funded.***

Federal Regulations require that you submit to the IACUC a copy of any and all grants associated with this IACUC application. The application you submit to the IACUC must precisely match the animal work described in the corresponding grant or contract. Your signature below assures that this IACUC application and the corresponding grant application involve identical animal care and use plans. In addition, your signature assures your acknowledgement and understanding that you will notify your funding agency of animal care and use changes made to your protocol as a result of IACUC review and approval.

*Marilyn E. Carroll*  
  
 Signature

*10/20/04*  
 Date