HORNOCKER WILDLIFE INSTITUTE, INC.
UNIVERSITY OF IDAHO

HORNOCKER WILDLIFE INSTITUTE
IMMOBILIZATION AND BIOLOGICAL SAMPLING PROTOCOLS

This information was compiled by Kathy Quigley D.V.M., Institute Veterinary Coordinator, using reference materials, and materials from the National Institute of Health. The purpose of this information is to aid U.S. and foreign field scientists in immobilization and biological sampling techniques. We are continually gathering and changing the information in this manuscript.

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ANESTHESIA INFORMATION

TIGERS/COUGARS

A. PHYSIOLOGICAL NORMALS

Heart Rate ..... 70-90 beats/minute
Respiration ..... 8-15 breaths/minute
Temperature .....100-104 degrees F, (38-40° C).

B. IMMOBILIZATIONS

1. Initial

The first drugs to be given to the cat, are a combination of Ketaset (Ketamine reconstituted at 100 or 200 mg/ml) and Rompun (Xylazine 100 mg/ml). Mix these drugs together just prior to darting the cat. Do not save drug that has been mixed. The dosages are as follows:

Rompun - 0.2-0.4 mg/pound I.M. (0.4-0.9 mg/kg)

Weight - pounds (kg)	Dosage Rompun
< 50 pounds (23 kg)	10-20 mg
50-100 pounds (23-45kg)	10-40 mg
100-200 pounds (45-91 kg)	20-80 mg
200-400 pounds (91-182 kg)	40-160 mg

Ketamine - 2.0-4.0 mg/pound I.M. (4.0-9.0 mg/kg)

Weight - pounds (kg)	Dosage Ketamine
< 50 pounds (23 kg)	100-200 mg
50-100 pounds (23-45kg)	100-400 mg
100-200 pounds (45-91 kg)	200-800 mg
200-400 pounds (91-182 kg)	400-1600 mg

*****NEVER GIVE MULTIPLE DOSAGES OF ROMPUN. THE FIRST DART SHOULD HAVE THE ENTIRE ROMPUN DOSE IN IT, WITH THE REST OF THE SYRINGE FILLED WITH KETAMINE. IF YOU THINK THE ANIMAL GOT ANY OF THE ROMPUN DOSE, YOU MUST NOT GIVE ANY MORE. AT THAT POINT YOU MUST GIVE ONLY KETAMINE. USING ONLY KETAMINE WILL GIVE YOU A VERY PROLONGED, "ACTIVE", ANESTHESIA, BUT IT IS MUCH SAFER THAN GIVING TOO MUCH ROMPUN.

2. Hands on the cat

Once the cat is anesthetized, and you can approach it safely, you want to assess the situation. Immediately take heart and respiration rates, and a temperature. They should all fall within the normals listed above. Then put ophthalmic ointment in both of the cat's eyes. The eyes will stay open with Ketamine anesthesia, so they must be lubricated to avoid damage.

Ketamine is a "dissociative" drug, so you can expect to see some or all of these other characteristics:

1. Increase in salivation
2. Muscle tremors
3. Cat will maintain swallowing and coughing reflexes
4. Most other reflexes i.e. corneal, pedal, etc.

**Note package inserts

a. Seizures

Many times wild cats will seizure under anesthesia. If this occurs, the drug to give is Valium (diazepam), at the dosage below:

Valium 5mg/ml (diazapam) 1.0 cc/100 pounds (45 kg). Given I.V.

*****You must remember however, that if the respirations are below 10/minute, give only half the dose. An I.M. dose will only prolong anesthesia, depress heart rate and respiration, and will do nothing for the seizures.

You may also see another seizure about 30-50 minutes into the anesthesia. Valium can be repeated at that time, again, following the same guidelines.

b. Maintaining anesthesia

You can supplement the anesthesia with hand held injections. The best option is to use Ketamine I.V (note: If using Ketamine at 200 mg/ml, switch to the 100 mg/ml at this point). Using the drug in this manner will yield a smooth anesthesia, and the cat will metabolize the drug more quickly than with the I.M. route.

Ketamine: .25 mg/pound (.55 mg/kg) I.V.

Ketamine: .5 mg/pound (1.0 mg/kg) I.M.

***Recovery is dramatically prolonged when Ketamine is given I.M. Also, it takes longer to see any effects from the drug, so if the cat is waking up. The I.V. route is better for achieving anesthesia quickly. An option you have as well is to give a small amount I.V., and the rest I.M.

c. Terminating anesthesia

There is no reversal agent for Ketamine, but there is one for Rompun. Ideally, you would have given Rompun at the first dose, combined with just enough Ketamine to get your hands on the cat. Then maintained the anesthesia with I.V. Ketamine. Finally, when the reversal agent is given, the cat has very little of either drug left in it's system. The drug and dosage is as follows:

Yohimbine (2mg/ml): 0.02 - 0.04 mg/pound I.V.
(0.04-0.09 mg/kg)

**This is usually given as 1/10 (0.10) the Rompun dose. It can be increased to 0.05 mg/pound, but it usually is not necessary. Expect to see effects of this drug within minutes of administering it.

C. EMERGENCY SITUATIONS

Ketamine/Rompun anesthesia is very safe, but still emergencies can arise, and you must be prepared to handle them.

a. Respiratory Arrest

Respiratory rate should be monitored every 5 minutes during anesthetic procedure. If the respiration rate drops to 4-5 breaths/minute, or if the cat stops breathing completely, the drug to give is Dopram.

Dopram (20 mg/ml) Dose: 0.25 mg/pound I.V. (.55 mg/kg)

**Give the dose I.V., then check for breathing. Push on the chest to stimulate. If no effect, repeat with a second dose. Give Yohimbine as well at the time of the first dose. If both of those procedures fail, begin artificial respiration. Place the endotracheal tube down the cat's throat, and blow air into it. Repeat Dopram dose again.

b. Cardiac Arrest

Heart rate should be monitored every 5 minutes during anesthetic procedure. If your find no heart beat, you must initiate emergency care. Although it is highly unlikely that you will ever need to use this drug, it is always a possibility. You may be anesthetizing a very old or sick animal, with metabolic problems that you cannot assess from just looking at it. This, of course, is a very serious situation, and if the anesthesia ever gets to this point, and the cat is not responding, the chances that it will respond with this drug are pretty slim. However, the drug you need is:

Epinephrine 1 - 5 ml given I.M. or subcutaneously

**The I.M. route is preferred. This can be repeated as well.

c. Shock/Dehydration

Sometimes, despite your best efforts, animals will have adverse reactions to anesthetic agents. If you have a cat that doesn't seem to be handling the anesthesia very well, and is not waking up from a normal or lower than normal drug dosage, you may want to give some fluids.

Or, if you have a cat that is injured, exhibiting signs of shock (i.e. pale or "bluish" gums, weak pulse, shallow sporadic respiration, cold extremities), or a cat that is dehydrated (sunken eyes, dry mucous membranes, skin lacking elasticity-does not return to normal in 2-3- seconds, when you pull up a patch of loose skin), you should administer fluids.

The best route for fluids is I.V., but that is also very difficult. I would recommend trying to give the fluids I.V., but if you are unsuccessful, it is still advantageous to give them subcutaneously.

Fluids: 40 ml/pound (88 ml/kg) if the cat is in shock. If the cat is just waking up slowly cut that back to 20 ml/pound (44 ml/kg).

If the cat is in shock, the fluids can be followed by the administration of dexamethasone, at the following dosage:

Dexamethasone (4.0 mg/ml): .25 - 1.0 mg/pound (.55 - 2.2 mg/kg) I.V. only

d. Body Temperature

Body temperature should be monitored every 5 minutes during the anesthetic procedure. If the body temperature falls below 99 degrees Fahrenheit (38 degrees C), the animal should be warmed up. Insulate it from the ground with a space blanket (if you haven't already done so), move animal into the sun, rub the animals body to stimulate circulation, and put a heat pack wrapped in cloth between its back legs. Check temperature every 10 minutes, and don't give any additional drugs when the temp is too low.

If the cats body temperature is about 104 degrees Fahrenheit (40 degrees C), it needs to be cooled down. Move animal into the shade if possible. Cool animals abdominal area with snow (winter), or water (summer). If these procedures do not bring animals temperature down, give a cold water enema. Put a quart of water in a water bottle, lubricate tip with K-Y Jelly, and insert 3-4 inches into rectum. Hold water bottle above the animal, allowing gravity to feed water into animals rectum. Leave water in rectum for 5 minutes, then remove nozzle, and drain. Check temperature every 3-5-minutes. Repeat enema if temp does not go back down to the normal range.

d. Injury

If the animal has a laceration from the capture, squirt a small amount of Durapenn into the wound, and wrap lightly with gauze. If it requires sutures, clean area aseptically with an iodine solution, and suture wound. If the animal is injured seriously, contact the nearest Veterinarian, and the appropriate Project/Park officials.

If animals injuries are serious enough to warrant euthanasia, than Euthanol, at a dosage of 1.0 ml/10 pounds body weight should be given.
***Euthanol should only be given if animal is to be removed from the field.

SUMMARY

Remember that Rompun should be given only one time if any part of the initial dose is successfully administered to the cat.

Ketamine alone will take longer and not look as smooth, but it will be safer. Recovery will be really prolonged as well. Ketamine is pretty safe - the lethal dose is 5X the anesthetic dose, so when in doubt, give just a little more.

If you get to a point where the emergency drugs are necessary, be sure to look for clues as to why the cat may be having difficulty - i.e. thin, broken teeth, nasal discharge, diarrhea, etc. If the cat dies it may be just from the drugs, but that's not very common, especially with the safety margin of Ketamine. It does happen, but is just not common. Usually there is an underlying cause. You can always do a necropsy if it is allowed, and there are formalin jars for tissue samples. They should be collected before freezing.

ANESTHESIA INFORMATION
AMUR LEOPARD

LEOPARDS

A. PHYSIOLOGICAL NORMALS

Heart Rate ..... 70-90 beats/minute
Respiration ..... 8-20 breaths/minute
Temperature .....100-104 degrees F (38-40° C).

B. IMMOBILIZATION DRUGS

1. Initial

The drugs and protocol are the same for tigers and leopards, except for the fact that either Telazol or Ketamine/Rompun can be used for leopards. Telazol CANNOT be used in Siberian tigers. The dosages for leopards are as follows:

Ketamine/Rompun: same as listed for tigers.
Telazol: 2.0 - 3.0 mg/pound (4.4 -6.6 mg/kg) I.M.

2. Hands On

Again, the protocol is the same for tigers. Drug dosages are as follows:

a. Seizures

Valium: 0.5 - 1.0 ml (5 mg/ml) given I.V.

b. Maintaining Anesthesia

Ketamine: .25 mg/pound (.55 mg/kg) I.V.

Ketamine: .5 mg/pound (1.0 mg/kg) I.M.

*****OR, IF USING TELAZOL.....

Telazol: 1.0 mg/pound (2.2 mg/kg) I.M.

c. Terminating Anesthesia

Same as protocol for tigers when using Ketamine/Rompun (i.e. 1/10 Rompun dose). If using Telazol, there is no reversal agent.

C. EMERGENCY DRUGS

The protocol is the same as for tigers with all the emergency drugs. The only thing to remember is when a drug range is given, give the leopard the lower dose.

ANESTHESIA INFORMATION
BEARS

BEARS

A. PHYSIOLOGICAL NORMALS

Heart Rate ..... 60-90 beats/minute
Respiration ..... 15-30 breaths/minute
Temperature ..... 99.5-101 degrees F (37.5-38° C).

B. IMMOBILIZATION DRUGS

1. Initial

There are two drugging protocols to choose from. Telazol is the preferred drug.

a. Telazol: 2 - 3 mg/pound I.M. (4.4 – 6.6 mg/kg)

b. Ketamine/Rompun (2:1 mixture)

Black Bears
2.0 mg/pound Ketamine I.M. (4.4 mg/kg)
1.0 mg/pound Rompun I.M. (2.2 mg/kg)

Brown Bears
3.6 mg/pound Ketamine I.M. (7.2 mg/kg)
1.8 mg/pound Rompun I.M. (4.0 mg/kg)

*******Again NEVER give multiple doses of Rompun. See protocol for tiger anesthesia.**

2. Hands On

If you have used Telazol, you may supplement anesthesia with 1/2 the original dosage if bear is not completely anesthetized. However, if you have used Ketamine/Rompun you must supplement with only Ketamine. In addition, you should give the bear at least 10-15 minutes before deciding whether more drug is necessary.

a. Maintaining Anesthesia

1. Telazol
1 – 1.5 mg/pound I.M. (2.2 – 3.3 mg/kg)

2. Ketamine/Rompun

Black Bears
1.0 mg/pound Ketamine I.M. (2.2 mg/kg)

Brown Bears
1.8 mg/pound Ketamine I.M. (4.0 mg/kg)

b. Terminating Anesthesia

There is no reversal agent for Telazol. However, Dopram, a respiratory stimulant, is often given at the end of the anesthetic procedure. This drug will increase respiration rate and may help the bear clear the Telazol. The Dopram, however, must be given I.V. for it to have any effect.

Dopram (20 mg/ml) Dose: .25 mg/pound I.V. (.5 mg/kg)

If using Ketamine/Rompun, the reversal agent is Yohimbine for Rompun, at the following dosage. Again, this drug must be given I.V. for it to have effect.

Yohimbine (5 mg/ml)
Black Bears .05 mg/pound (.1 mg/kg) I.V.
Brown Bears .09 mg/pound (.2 mg/kg) I.V.

C. EMERGENCY DRUGS

The protocol for an emergency is the same as for tigers, cougars, or leopards.

KETAMINE/ROMPUN DOSAGES FOR BLACK BEARS AND BROWN BEARS
(2:1 Ratio)

Black bears - 300 mg/100 pounds (330 mg/50 kg)
Brown bears - 360 mg/100 pounds (396 mg/50 kg)

Ketamine/Rompun* Volume (cc's) I.M.

	Black Bears	Brown Bears
Weight (kg)	Ket./Rom. (100 mg/ml)	Ket./Rom. (100 mg/ml)
50 (23 kg)	1.0/0.50	1.8/0.9
100 (46 kg)	2.0/1.0	3.6/1.8
150 (68 kg)	3.0/1.5	5.4/2.7
200 (91 kg)	4.0/2.0	7.2/3.6
250 (114 kg)	5.0/2.5	9.0/4.5
300 (136 kg)	6.0/3.0	10.8/5.4
350 (159 kg)	7.0/3.5	12.6/6.3
400 (182 kg)	8.0/4.0	14.4/7.2
450 (205 kg)	9.0/4.5	16.2/8.1
500 (227 kg)	10.0/5.0	18.0/9.0
550 (250 kg)	11.0/5.5	19.8/9.9
600 (273 kg)	12.0/6.0	21.6/10.8

* Based on Ketamine and Rompun 100mg/ml.

RECOMMENDED YOHIMBINE DOSAGE FOR BEARS
DRUGGED WITH KETAMINE/ROMPUN
(.05 mg Yohimbine/1 mg Rompun)

Black bears - 1 cc/100 pounds (.1 mg/kg)
Brown bears - 1.8 cc/100 pounds (.2 mg/kg)

	Yohimbine* Volume (cc) I.V.	
weight-pound (kg)	Black Bears	Brown Bears
50 (23 kg)	0.5	0.9
100 (46 kg)	1.0	1.8
150 (68 kg)	1.5	2.7
200 (91 kg)	2.0	3.6
250 (114 kg)	2.5	4.5
300 (136 kg)	3.0	5.4
350 (159 kg)	3.5	6.3
400 (182 kg)	4.0	7.2
450 (205 kg)	4.5	8.1
500 (227 kg)	5.0	9.0
550 (250 kg)	5.5	9.9
600(273 kg)	6.0	10.8

*Based on Yohimbine 5mg/ml

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ANESTHESIA & HANDLING
BEAR SPECIES

Avoid handling bears when the temperature is above 90 degrees Fahrenheit (32 degrees Centigrade). With anesthesia of any wild animal, the number of people involved and noise levels (talking, vehicle engines, etc.) should be kept to a minimum. It is important to try and alleviate stress and fear in an animal prior to anesthesia, thus reducing the need for multiple drug doses.

The drug Telazol, and the drug mixture Ketamine/Rompun are recommended for use on bears. Telazol and Ketamine are dissociative anesthetics. They are absorbed into the bloodstream from the injection site, and reach the brain where, based on their molecular size, pH, and electrical charge, act at various sites to depress nervous tissue activity, thus producing anesthesia. The tranquilizers Zolazapam HCL (found in Telazol) and Xylazine HCL (found in Rompun) help relax the animal.

I. Telazol

In the past fifteen years, thousands of bears have been immobilized with Telazol. Bears tolerate it well, it has a wide margin of safety, and a small amount of drug is necessary for anesthesia (as compared to Ketamine/Rompun). It is felt that Telazol is safer than Ketamine/Rompun because the bear awakens from anesthesia more slowly than with K/R. Also, there have been no documented incidents of explosive arousal with this drug. There is no reversal agent for Telazol. However, although it is of questionable value, Dopram, a respiratory stimulant, is often given at the end of a procedure. Dopram will increase respiration rate, and may, therefore, help the bear clear the Telazol more quickly. However, this drug must be given intravenously for it to have any effect.

With Telazol, induction time is generally 3 to 8 minutes. Brown bears remain anesthetized for 45 to 211 minutes (dose dependent), and recover from the drug fairly slowly. Bears drugged with Telazol should be tested every 10 minutes for signs of arousal. Signs of recovery include head and paw movement, lip and nose twitching, and reaction to sound. Recovery usually occurs approximately 20 to 30 minutes after the bear begins to raise its head.

II. Ketamine/Rompun

The combination Ketamine/Rompun (K/R) can be used for most trapping and handling situations. The induction period for brown bears is roughly 8-10 minutes, depending on the individual bear. The major limitation to this mixture is the prolonged depressant effects of Rompun upon the temperature regulatory system. However, Yohimbine (Antaganol) can be used as an antagonist to Rompun, and thus speed recovery. The mixture of R/K is inexpensive, has a wide margin of safety, and is a rapid immobilizing drug. With K/R, the depth of anesthesia must be monitored constantly. Recovery from this drug mixture can be immediate. When a bear shows the first signs of arousal, the area should be evacuated promptly or an additional half dose of Ketamine HCL should be given. Signs of recovery are the same as described for Telazol recovery. Occasionally, bears will "sleep off" this anesthesia without exhibiting these early signs of recovery.

SAMPLE COLLECTION

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FIELD COLLECTION OF BLOOD SAMPLES

It is always important to try and collect blood samples from each captured animal. The procedure for collection and storage is as follows.

Procedure:

1. Using a new sterile syringe and needle, or a vacutainer system, collect at least 15ml of blood from the anesthetized animal.
2. Have ready four sterile storage tubes, each containing 13.5 ml lysis buffer. Remove caps.
3. Carefully, in a sterile manner, add 1.5ml whole blood to each of the four (4) tubes.
4. Replace caps, and invert each tube a number of times to thoroughly mix the blood and buffer.
5. Check that the tubes are closed tightly, and are not leaking. If any are leaking, wrap the outside of the cap with parafilm.
6. Label, and store at room temperature.
7. Follow the procedure for *serum samples* with the remaining blood.

FIELD COLLECTION OF SKIN BIOPSIES

Procedure:

1. Shave a 2" square area on inside of thigh.
2. Cleanse area three times each with Betadyne and alcohol. (Alternate between Betadyne and alcohol).
3. Obtain a 1.0cm2 piece of clean, shaved, skin using either a punch biopsy, scalpel, or scissors. (Only the skin is needed, so don't make the cuts too deep. The sample should not be larger than 0.5 to 1.3 grams).
4. Have ready a 15ml sterile tube filled with 13.5ml lysing buffer.
5. Remove top from sterile tube, and drop tissue sample into buffer solution. Replace cap.
6. Once you return to the field station, the following steps should be taken. Using a flame, sterile a forceps.
7. Carefully remove cap from tissue sample tube. Remove tissue sample with sterile forceps.
8. Remove lid from a sterile petri dish, and place tissue sample into the dish.
9. Use sterilized scissors and forceps, or a sterile scalpel blade, to macerate the tissue sample. Cut sample into numerous small pieces.
10. Return the macerated tissue to the tube containing the buffer. Invert tube many times to thoroughly mix the tissue and buffer.
11. Make sure all tissue is submerged in buffer.
12. Label and store at room temperature.

****The skin must be kept as sterile as possible before freezing, so please keep your hands clean. Wash with 70% alcohol, or wear gloves and wash them with alcohol. Prepare a clean surface to work on – keep air movement to a minimum – do not work near open windows or doors. Keep the petri dish lid, scissors, blades, and forceps sterile – do not set them down on a son-sterile surface and then use them again. Keep forceps and scissors in a sterile solution if possible. Flame instruments before use if you are not sure they are sterile.

FECAL SAMPLE
COLLECTION PROCEDURE

Fecal samples for parasite analysis must be collected "fresh" (less than 24 hours old) for this assay. Although fresh samples are difficult to obtain, often they may be successfully collected from a captured animal. Trapped animals usually defecate when approached by a human. If no sample is found at the trapping site, you may retrieve one rectally using a gloved finger.

1. Use the collection spoon in the fecal collection kit to scoop a small amount of feces.

2. Place it in one container.

3. Repeat process, and place same amount of feces in second container.

4. Invert to mix feces with media.

5. Label both containers with animal identification number.

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SERUM SAMPLE
COLLECTION PROCEDURE

You should fill at least one redtop collection tube with blood from each animal captured. Red-topped tubes are sterile, but have no anticoagulant in them, so the blood will clot normally.

1. Let sample sit at room temperature for 15 - 20 minutes.

2. Centrifuge at 2500 rpm for 10 minutes, until the sample is separated into two distinct fractions. A clotted red cell mass will be at the bottom of the tube, and the clear straw colored serum will be in the top half of the tube.

3. Carefully draw the serum into a sterile syringe, taking care not to touch the red cell mass.

4. Fill as many cryo-tubes as possible with 2 cc's of serum each.

5. Label each cryotube with the appropriate animal identification numbers.

6. Carefully place cryotubes into the freezer in an upright position.

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HEMATOCRIT (PCV)
COLLECTION PROCEDURE

You should fill one small lavender topped collection tube with blood for each animal captured.

1. Carefully mix blood by slowly inverting tube 10 times. Also make sure that there are no clots present. (If the sample is clotted, throw it away).

2. Remove lavender stopper.

3. Hold sample tube at a 35-degree angle, and place the tip of one microhematocrit tube in the blood.

4. Capillary action will fill the microhematocrit tube.

5. Holding the microhematocrit tube horizontally, carefully clean any blood off the outside with gauze. Then seal the "bottom" end with critoseal clay.

6. Repeat process.

7. Place each sealed microhematocrit tube into a clean centrifuge tube that has been prepared with a cotton ball in the bottom of it.

8. Centrifuge at the highest speed for 5 minutes.

9. Using a hematrocrit chart (or a ruler if necessary), determine percentage of red cell mass in total volume of blood in the tube (Red cells and plasma).