

## Field Immobilization and Euthanasia of American Opossum

**Michael K. Stoskopf**<sup>1,2,5</sup> **Robert E. Meyer**<sup>1,3</sup> **Mark Jones**<sup>1,4</sup> and **Denton O. Baumbarger**<sup>1,4</sup> <sup>1</sup> Environmental Medicine Consortium, North Carolina State University, Raleigh, North Carolina 27606, USA; <sup>2</sup> Companion Animal and Special Species Medicine, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606, USA; <sup>3</sup> Anatomy, Physiology and Radiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606, USA; <sup>4</sup> North Carolina Wildlife Resources Commission, Raleigh, North Carolina 27604-1188, USA; <sup>5</sup> Corresponding Author (email:michaelstoskopf@ncsu.edu).

**ABSTRACT:** Seventeen recently trapped opossum, *Didelphis virginiana*, (median weight 2.45 kg; range = 1.6–5.0 kg; quartiles = 1.8–3.3 kg) were immobilized with either telazol (15 or 30 mg/kg) or a mixture of medetomidine (100 µg/kg), butorphanol (0.2 mg/kg), and ketamine HCl (10 mg/kg) based on estimated weights. Anesthetized animals were subjected to cardiac puncture for blood withdrawal and toe pinch. Euthanasia was accomplished by intracardiac administration of 1 ml of concentrated pentobarbital sodium/phenytoin solution. Weights were underestimated for 14 of 17 animals, but were within 0.5 kg of the actual weight. Both drug combinations provided rapid and calm immobilization. Median time to recumbency for the medetomidine–butorphanol–ketamine group ( $n = 5$ ) was 6 min (range = 4–10 min; quartiles = 6 and 8 min). The median time to recumbency was not statistically different for the low ( $n = 6$ ) and high dose ( $n = 6$ ) telazol groups, 3 and 3.5 min respectively (quartiles 3; 3.5 and 4; 5.5 min). The stronger heart beat with telazol immobilization facilitated cardiac puncture. All five animals administered the medetomidine–butorphanol–ketamine mixture and three of six animals given the low telazol dose reacted to cardiac puncture. Only one of six animals given the estimated 30 mg/kg dose of telazol reacted slightly to cardiac puncture. We conclude that 30 mg/kg telazol provides sufficient immobilization and analgesia to allow accurate cardiac puncture of the opossum if the procedure is performed within 5 to 10 min of recumbency. Intracardiac administration of concentrated pentobarbital sodium/phenytoin solution followed by bilateral thoracotomy provides appropriate euthanasia suitable for field situations.

**Key words:** American opossum, *Didelphis virginiana*, euthanasia, medetomidine, pentobarbital, phenytoin, telazol, tiletamine, zolazepam.

Humane euthanasia of animals killed in population manipulations is a major concern of wildlife biologists and veterinarians. The effectiveness, efficiency, and esthetics of the procedure are important to

the success of a professionally conducted study. To support a study on the effects of nest predation on quail and ground nesting songbirds, a literature search was conducted to determine appropriate immobilization and euthanasia protocols for American opossum (*Didelphis virginiana*) being trapped and removed from study sites compatible with the guidelines established by the American Veterinary Medical Association (Schaumburg, Illinois, USA). The American opossum is regarded as a significant nest predator, making their removal essential to the study. Relatively little has been published on anesthesia of the opossum, and we found no studies examining modern drug combinations. The most recent full publication reported success immobilizing opossum with an i.m. dose of 20 to 25 mg/kg ketamine HCl used alone and less satisfactory results using 5 to 6 mg/kg phencyclidine HCl i.m. (Feldman and Self, 1971). A later abstract suggested use of ketamine (10 mg/kg) combined with xylazine (2.0 mg/kg) delivered i.m. after atropine sulfate (0.4 mg/kg) worked well as an induction to allow intubation prior to maintenance on halothane and oxygen (Scott and Kolata, 1982).

At the time of the initiation of the nest predation study, neither ketamine nor xylazine were considered controlled substances by the U.S. Federal Drug Administration (FDA, Washington, D.C., USA) or the North Carolina Drug Enforcement Agency (Raleigh, North Carolina, USA), reducing the paperwork and drug storage requirements for the field biologists. A combination of two parts ketamine to one part xylazine has been used successfully by the authors in a wide range of mammalian

species and seemed an appropriate choice for the opossum. Considering the ultimate outcome of euthanasia while under anesthesia in the study to be conducted, a dose double what is routinely used for field immobilization of free-ranging carnivores was selected. Field biologists working on the predatory study were instructed to deliver 9 mg/kg ketamine and 4.5 mg/kg xylazine intramuscularly by pole syringe to trapped opossum before handling them. Large amounts of blood were to be drawn from immobilized opossum via intracardiac puncture to obtain serum for disease prevalence studies and to assist in the euthanasia of the animals. Euthanasia was to be completed by bilateral thoracotomies creating a double pneumothorax.

Shortly it became apparent that this immobilization and euthanasia protocol was inadequate. Animals frequently failed to be completely immobilized by the first injection and induction times with repeated injections were prolonged. Immobilized animals frequently reacted to cardiac puncture and blood withdrawal. Time to death after bilateral thoracotomy was unacceptably long to the field biologists. The ketamine and xylazine dose was immediately increased to 18 mg/kg and 9 mg/kg respectively. A solution of 3% potassium chloride was dispensed to each biologist for delivery at a rate of 1 ml/kg intracardiac after drawing the required blood volume but prior to bilateral thoracotomy. This reduced the time required for immobilization and the number of animals requiring second injections, but reaction to cardiac puncture and speed of final euthanasia as judged by stoppage of the heart remained unsatisfactory to field biologists administering the protocol. This remained the situation even after the ketamine and xylazine doses were raised to 36 and 18 mg/kg respectively, and the potassium chloride dose increased to 2 ml/kg through the end of the first trapping period.

After the failure of the first dose combination, the authors interviewed colleagues with opportunities for experience

in the anesthesia of the American opossum, to identify unpublished protocols to improve the immobilizations and euthanasias in the predator study. Few veterinarians or zoologists had actual experience in anesthesia of opossum. Those with experience invariably preferred inhalation agents delivered in various ways. Numerous anecdotal reports about the difficulty of adequately euthanizing opossum characterized the species as remarkably resistant to anesthetic drugs and tenacious to life. Although field use of inhalation agents can be accomplished, the dispersed nature of the study sites in the nest predation study would have made implementation unwieldy and increased the pack weight for the field biologists. The close of the first trapping year of the predator study coincided with activity on the part of the FDA to define ketamine HCl as a controlled substance in category III. The potential for this change in regulatory category combined with the unsatisfactory results of using non-controlled substances to euthanize the opossum directed our efforts toward identifying injectable agents suitable for field immobilization and euthanasia of opossum regardless of control status. Toward that end, all opossum presented to the Veterinary Teaching Hospital (VTH, Raleigh, North Carolina, USA) for euthanasia were included in a study to evaluate the efficacy of either tiletamine HCl and zolazepam (Telazol, Ft. Dodge Laboratories Inc., Ft. Dodge, Iowa, USA; 50 mg/ml tiletamine plus 50 mg/ml zolazepam) or medetomidine (Domitor, Pfizer, Lee's Summit, Missouri, USA; 100 µg/ml) and ketamine HCl (Ketaset, Ft. Dodge Laboratories Inc.; 100 mg/ml) with butorphanol tartrate (Torbugesic-SA; Ft. Dodge Laboratories Inc.; 2 mg/ml). A concentrated solution of pentobarbital sodium and phenytoin sodium (Beuthanasia-D, Schering-Plough, Kenilworth, New Jersey, USA; 390 mg/ml pentobarbital plus 50 mg/ml phenytoin) was evaluated for use as a final euthanasia agent.

Seventeen opossum (9 males and 8 fe-

males), recently trapped and transported to the VTH were immobilized and euthanized in the study. All animals were alert and active upon arrival. Their median weight was 2.45 kg (range = 1.6 – 5.0 kg; quartiles = 1.8–3.3 kg). Initial weights of the animals were estimated, as would be the case in the field. Actual weights to the nearest 0.1 kg were measured after euthanasia. Immobilization doses based on estimated weights to the nearest kg were delivered by pole syringe to simulate field delivery conditions. Time to recumbency was recorded in minutes when the recumbent injected animal failed to respond to gentle prodding with a pole. Withdrawal reflex was judged by pinching a toe firmly with a mosquito forceps. Blink and aural twitch reflexes were judged by gentle placement of the tip of the forceps near the medial canthus of the eye or into the aural canal respectively.

After an animal was judged recumbent, cardiac puncture was accomplished by feeling the beating heart through the thoracic wall with index finger and thumb placed on opposite sides the chest and directing an 18 gauge 3.8 cm needle attached to a 35 ml syringe into the heart. Twenty to 35 ml of blood was withdrawn except in the first animal receiving a given dosage regimen. For that animal, blood was withdrawn until the mucous membranes became pale and blood could no longer be withdrawn, or until the animal began to open and close its jaws, move its legs, or adjust position, whichever occurred first. When blood drawing was completed, the needle was left in place and one ml of concentrated pentobarbital sodium/phenytoin solution administered by a separate syringe through the same needle. Movement of the disconnected needle was then monitored to determine the time required for the heart to stop beating. If the heart continued to beat for 5 min, an additional 1 ml of pentobarbital sodium/phenytoin was administered intracardiac. Cessation of heart beat was confirmed by bilateral thoracotomy using a

disposable scalpel blade. This procedure resulted in suitable, confirmed euthanasia within 1 to 4 min.

Three animals that were very reactive to cardiac puncture or blood withdrawal were given 2 ml concentrated pentobarbital sodium/phenytoin intraperitoneally to evaluate this as an alternative approach in field situations where initial immobilization with the first intramuscular injection failed. Reactivity was judged over time in these animals by evaluating withdrawal reflexes and subjective assessment of the palpability of the heart's contractions through the chest wall. Two of the three animals which received this treatment became quiet within 5 min, and tolerated intracardiac puncture before succumbing to administration of 1 ml of concentrated pentobarbital sodium/phenytoin intracardiac. The third animal remained reactive to toe pinch at 5 min and was given a second intraperitoneal dose of 2 ml which provided good analgesia for euthanasia by intracardiac injection.

A total of 12 animals received telazol. Six opossum (three males and three females) received an estimated dose of 15 mg/kg telazol. The actual median dose delivered based on actual body weights was 16.2 mg/kg (range = 6.0–18.75 mg/kg). Six opossum (two males and four females) received an estimated dose of 30 mg/kg telazol. The actual median dose delivered in those animals was 28.6 mg/kg (range = 22.0–37.5 mg/kg). Five (four males and one female) animals received a mixture of medetomidine, butorphanol and ketamine HCl at an estimated rate of 100 µg/kg medetomidine, 0.2 mg/kg butorphanol and 10 mg/kg ketamine HCl. The actual median doses delivered were 91 µg/kg medetomidine (range = 94–80 µg/kg); 0.18 mg/kg butorphanol (range = 0.16–0.19 mg/kg); and 9.1 mg/kg ketamine HCl (range = 8.0–9.4 mg/kg).

Statistical comparisons between groups used the Wilcoxon Rank Sum Test for nonparametric data and evaluation for correlations employed the Olmstead and Tu-

key's Corner Test for Association (Sokal and Rohlf, 1981).

Body weight was underestimated in 14 of 17 animals, although weight estimates were generally within 0.5 kg of the actual weight. Both drug combinations provided rapid and calm immobilization with the longest time to recumbency being 10 min in one animal receiving the medetomidine–butorphanol–ketamine mixture. Median time to recumbency for the medetomidine–butorphanol–ketamine group was 6 min with a range of 4 to 10 min and quartiles of 6 and 8 min. The median time to recumbency was not statistically different for the low and high dose telazol groups, 3 and 3.5 min respectively (quartiles 3; 3.5 and 4; 5.5 min). Time to recumbency was not completely correlated with actual dose delivered in any treatment group. This could have been because of the use of the pole syringe, making precise delivery to the same muscle mass and injection depth unlikely.

A marked response to cardiac puncture was noted in all five animals administered the medetomidine–butorphanol–ketamine mixture. This was characterized by a sharp twitch of the body upon needle insertion, often followed by jaw flexing, muscle twitching and/or purposeful twisting of the body. The heart beat was quite weak in this group of animals, making it more difficult to accurately position the needle for cardiac puncture. By comparison, the strong jump at needle insertion was not present, nor were the purposeful muscle movements nearly so strong or extensive in the low dose telazol group, although three animals of six responded in some way during cardiac puncture and blood withdrawal. The heart beat was uniformly very strong and easily palpated. These advantages over the medetomidine–butorphanol–ketamine mixture, combined with the easier logistics associated with telazol's availability as a prepared commercial mixture led us to abandon the medetomidine–butorphanol–ketamine mixture and evaluate a higher dose of telazol.

The withdrawal of blood until reaction in one animal of each dose group was based on assessment of the reactions observed in the animals immobilized with ketamine and xylazine mixtures in the first year of the predator study. It appeared that these were likely undirected autonomic movements potentially mediated by high sympathetic tone, possibly in response to acute loss of blood volume. In these studies, withdrawal of 60 to 70 ml of blood (40 to 50% of circulating blood volume assuming a total blood volume of 57 ml/kg, range = 44.5–69.8 ml/kg) (Burke, 1954 cited in Altman and Dittmer, 1974), induced jaw opening and closing, muscle twitching and movement of the limbs in an undirected manner regardless of the drug mixture or dose administered. Similar movements were seen with removal of as little as 35 ml of blood in one other animal. Although further physiologic studies would be necessary to confirm it, our impression is that these movements are indeed unconscious autonomically mediated responses to rapid loss of blood volume. To avoid these responses, the field protocol has been modified to collect only 30 ml of blood from each individual.

One animal of six in the high dose telazol group reacted slightly during the cardiac blood withdrawal procedure. That animal received one of the lowest doses based on actual weight in the group (23.1 mg/kg). It also was handled approximately 10 min later after recumbency than other animals in the high dose telazol group. Subjective assessment of the results of the 17 immobilizations conducted in these trials suggests that a 30 mg/kg dose of telazol administered intramuscularly should provide sufficient immobilization and analgesia to allow accurate cardiac puncture of the opossum, particularly if the procedure is performed within 5 to 10 min of the time when the animal becomes completely recumbent. This recommendation is only for animals destined for euthanasia without recovery. It is likely that a lower dose would be more appropriate for animals be-

ing immobilized for recovery procedures. Additional studies with more complete physiologic monitoring and recovery assessment are needed to evaluate that situation.

#### LITERATURE CITED

- ALTMAN, P. L., AND D. S. DITTMER. 1974. Biology Data Book, 2nd Edition. Vol. III. Federation of American Society for Experimental Biology, Bethesda, Maryland, 1847 pp.
- FELDMAN, D. B., AND J. L. SELF. 1971. Sedation and anesthesia of the virginia opossum, *Didelphis virginiana*. *Laboratory Animal Science* 21: 717-720.
- SCOTT, H., AND R. J. KOLATA. 1982. Anesthesia for the North American opossum (*Didelphis virginiana*). *Laboratory Animal Science* 32: 433.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry, The Principles and Practice of Statistics in Biological Research*, 2nd Edition. W.H. Freeman and Company, New York, New York, 859 pp.

*Received for publication 31 May 1998.*