

# Field Use of Isoflurane for Safe Squirrel and Woodrat Anesthesia

WILLIAM T. PARKER, *Department of Forestry, Wildlife, and Fisheries, University of Tennessee, 274 Ellington Plant Science, Knoxville, TN 37996, USA*

LISA I. MULLER,<sup>1</sup> *Department of Forestry, Wildlife, and Fisheries, University of Tennessee, 274 Ellington Plant Science, Knoxville, TN 37996, USA*

REID R. GERHARDT, *Department of Entomology, University of Tennessee, 230 Ellington Plant Science, Knoxville, TN 37996, USA*

DORCAS P. O'ROURKE,<sup>2</sup> *Office of Laboratory Animal Care, University of Tennessee, 336 Ellington Plant Science, Knoxville, TN 37996, USA*

EDWARD C. RAMSAY, *Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996-4544, USA*

**ABSTRACT** We evaluated a chamber and nose cone method of isoflurane delivery for anesthetizing eastern gray squirrels (*Sciurus carolinensis*; summer  $n = 43$ , winter  $n = 48$ ) and Allegheny woodrats (*Neotoma magister*; summer  $n = 24$ , winter  $n = 13$ ) for use when pain or stress was possible from sampling procedures. Mean induction time for squirrels (from beginning of isoflurane administration to safe removal from trap), was  $4.63 \pm 0.58$  minutes. Squirrels awoke more quickly in summer ( $1.40 \pm 0.15$  min) than in winter ( $3.62 \pm 0.24$  min) after removal of the nose cone. We manually restrained woodrats and administered the nose cone for 0.5 minutes to each animal. Woodrats awoke after  $4.76 \pm 0.58$  minutes following the final dose of isoflurane for both seasons. These methods are useful for working with small mammals in the field and provide an appropriate anesthetic when there may be more than slight pain or distress. (JOURNAL OF WILDLIFE MANAGEMENT 72(5):1262–1266; 2008)

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Anesthesia reduces discomfort from punctures or cuts and allows blood and tissue samples to be collected more easily when handling wild animals (McCull and Boonstra 1999, Douglass et al. 2000, Hahn et al. 2005). Frase and Van Vuren (1989) noted that when using manual restraint, animals could harm themselves or the handler while trying to escape when samples were collected. There have been creative solutions for safe handling of small mammals without anesthesia such as a cloth cone bag to facilitate handling of tree squirrels (Koprowski 2002). However, anesthetics are necessary when a sampling procedure may cause more than slight or short-term pain even if the animal is suitably restrained (Gannon et al. 2007). What constitutes more than slight pain is often a judgment call and must be made by an Institutional Animal Care and Use Committee (IACUC; Dein et al. 2005) as outlined in the Animal Welfare Act (7 United States Code 2121, et seq.; amended 2002). Anesthetizing rodents also may lower the risk of disease transmission to researchers while minimizing animal pain (Parmenter et al. 1998). The University of Tennessee IACUC (UT-IACUC) would not allow tissue (ear notches) or blood sample collection on restrained wild rodents without anesthetics.

Many injectable anesthetic drugs have short induction times; however, recovery times can be from 2 hours to 6 hours (Arenz 1997, Belant 2004). Inhalation drugs such as methoxyflurane and halothane allow quick induction and

recovery times (Swann et al. 1997, Parmenter et al. 1998, and McCull and Boonstra 1999) but are rarely used now due to potential nephrotoxic and teratogenic effects (Plumb 2002). Another inhalation drug, isoflurane, is commonly used in veterinary practices. Isoflurane has not been extensively evaluated for use on wild mammals in the field.

Portable anesthesia machines with isoflurane-specific vaporizers have been used in beavers (*Castor canadensis*; Breck and Gaynor 2003) and big brown bats (*Eptesicus fuscus*; Wimsatt et al. 2005). However, these animals were easily and quickly transported to central processing facilities which facilitated the use of vaporizers. Isoflurane alone has been used in the field with a modified face mask and air (Belant 1995, Anstee and Needham 1996, and Lewis 2004). Although precision vaporizers and heavy equipment may be needed for more invasive procedures, most wildlife biologists need a simple, efficient, short-term anesthetic for sampling when pain or distress to animals is likely.

We evaluated isoflurane administered via nose cone for Allegheny woodrats (*Neotoma magister*) and simple chamber for gray squirrels (*Sciurus carolinensis*) to determine effectiveness and safety in a field setting. Our objective was to supply information on effects, induction times, physiological parameters, and potential adverse reactions of isoflurane when used to minimize pain from tissue collection in selected rodent species. We also wanted to evaluate anesthesia administration in summer versus winter because ambient temperature may affect the anesthetic response. This information is needed for researchers that must evaluate advantages and disadvantages of anesthetic options for field sampling (Dein et al. 2005).

<sup>1</sup> E-mail: lmuller@utk.edu

<sup>2</sup> Present address: Department of Comparative Medicine, East Carolina University, Brody School of Medicine, 208 Ed Warren Life Science Building, Greenville, NC 27834, USA

## STUDY AREA

We trapped gray squirrels in Karns, Knox County, Tennessee (35°59'N, -84°08'W), and Maryville, Blount County, Tennessee (35°44'N, -83°57'W; Parker 2006), USA. Trapping sites were located in mixed hardwood stands. We trapped Allegheny woodrats at Royal Blue Wildlife Management Area (RBWMA) and Big South Fork National River and Recreation Area (BSFNRRRA) in Tennessee, as part of a larger ecological study (Parker 2006, Scheffel 2006). The RBWMA was located in the Cumberland Mountain region (36°20'N, -84°17'W) comprising 20,235 ha in Scott and Campbell counties, Tennessee. The BSFNRRRA was located on the Cumberland Plateau (36°29'N, -84°41'W) along the Tennessee and Kentucky state border and included McCreary County, Kentucky, and Pickett, Fentress, Scott, and Morgan counties, Tennessee. Both areas were comprised of mixed hardwood forests and temperate climates (Smalley 1984, National Park Service 2005).

## METHODS

We trapped eastern gray squirrels with Tomahawk live traps (TL201, 40.6 × 12.7 × 12.7 cm; and TL202, 48.3 × 15.2 × 15.2 cm; Tomahawk Live Trap, Tomahawk, WI) using animal procedures approved by the UT-IACUC (no. 1255). We trapped 3 days/season in summer (May–Jun) and winter (Nov–Jan) and checked each trap every 2–4 hours during the day. After retrieving captures, we kept squirrels in traps ≤1 hour (with cloth sheets over traps) before immobilizing them.

We used an anesthesia chamber composed of a clear plastic box (57.9 × 42.4 × 34.5 cm) with a snap-on lid and a petri dish containing 5 cotton balls. We added 15 ml of isoflurane (Abbott Laboratories, North Chicago, IL) to the petri dish approximately 10 minutes before we placed animals in the box. Once we captured gray squirrels, we placed the entire live trap inside the anesthesia chamber and placed the lid on the box. We observed squirrels for breathing, blinking, and loss of righting reflex. Once squirrels were unable to right themselves, we removed them from the trap using a leather glove.

We took blood samples from the ventral tail vein at 1 minute postimmobilization. We collected approximately 30–50 µl of blood from squirrels to determine La Crosse antibody prevalence in a companion study (Scheffel 2006). We administered additional doses of isoflurane prior to and during bleeding if the animal began to move. We gave added doses of isoflurane using a nose cone consisting of a 50-ml centrifuge tube (30 × 115 mm polypropylene; Becton Dickinson, Franklin Lakes, NJ) with 3 cotton balls containing 5 ml of isoflurane. Between 5 and 10 minutes after immobilization, we took rectal temperature digitally, measured pulse with auscultation, and measured respiration by observation of the diaphragm and body movements (15 sec). We marked animals with 2 uniquely numbered aluminum ear tags (1005-1; National Band and Tag Co.,

Newport, KY) and released animals after they recovered from the anesthesia.

We defined induction as the time when we could safely remove the squirrel from the trap. We measured induction time from when we placed the cage into the chamber until induction. We measured time until first administration of nose cone as the time from induction until the first additional dose of isoflurane was required. We measured time for additional dose as the time between additional isoflurane doses using the nose cone (occurred more than once for some squirrels). We measured recovery as the time from the end of the last anesthesia dose until the animal was alert and standing. We measured total down time as the time from induction until the animal was alert and standing.

We conducted Allegheny woodrat trapping on 19 sites located in RBWMA and BSFNRRRA between November 2003 and August 2005 using animal procedures approved by the UT-IACUC (no. 1200). We used Tomahawk live traps (TL201) to capture Allegheny woodrats. We trapped each site once during winter (Nov–Mar) and once in summer (Apr–Aug). We set approximately 10–20 Tomahawk live traps at each site depending on length of the rock outcrop or number of boulders or human structures. We baited and wired open each trap ≥2 days prior to trapping. We set traps and checked them in the morning for 2–3 consecutive days.

We placed captured Allegheny woodrats in cloth pillow cases and weighed them to the nearest 5 g. We manually restrained woodrats while in the cloth bag by scruffing the fur along the neck with a leather glove. We uncovered the head of the woodrat and placed a nose cone consisting of a 50-ml centrifuge tube with 3 cotton balls containing 5 ml of isoflurane over the nose. We administered the nose cone for 0.5 minutes for all woodrats, because it was difficult to determine the exact point of induction because the animals were manually restrained. We gave additional doses of isoflurane using the nose cone as needed. We measured time for additional dose, recovery, and down times as for the squirrels.

We took blood samples from the marginal ear vein, ventral tail vein, or saphenous vein at 1 minute postimmobilization for future hormone analysis (Parker 2006). We collected approximately 30 µl of blood using a 100-µl microcapillary tube. We also collected a 5 × 2-mm ear notch for potential population genetic determinations (Castleberry et al. 2002). We aged woodrats based on a combination of reproductive condition, weight (juv ≤175 g, subad 176–225 g, and ad >225g), and pelage color (Mengak 2002). Between 5 and 10 minutes after immobilization, we measured rectal temperature, pulse, and respiration as with the squirrels. We marked animals with a uniquely numbered aluminum ear tag and released them after recovery from anesthesia.

We performed data analysis for both delivery methods of isoflurane using SAS Version 9.1 (SAS Institute, Cary, NC). We calculated mean values by species. We used transformations (log and square root) for variables that were not normal. All transformations produced data with a normal distribution and equal variance. We used a *t*-test on

**Table 1.** Mean time to induction, recovery time (animal standing and alert after last dose of isoflurane), down time (time from induction until animal was standing and alert), and physiological parameters (measured 5–10 min following induction) by season for eastern gray squirrels anesthetized with isoflurane (chamber method) in eastern Tennessee, USA during December 2004 and May 2005.

Parameters	Winter ( <i>n</i> = 48)		Summer ( <i>n</i> = 43)		<i>P</i> -value <sup>a</sup>
	$\bar{x}$	SE	$\bar{x}$	SE	
Induction (min)	4.61	0.61	4.64	1.04	0.75
Recovery (min)	3.62	0.24	1.40	0.15	<0.001
Down time (min)	9.47	0.75	9.21	1.09	0.60
Temp (° C)	39.2	0.2	39.7	0.1	0.006
Pulse (beats/min)	373.2	18.5	324.6	7.7	0.009
Respiration (breaths/min)	116.3	6.6	144.5	5.7	0.002

<sup>a</sup> *P*-values for differences between seasons. Before analysis, we used natural log transformations on induction time and down time to ensure normality of data.

transformed values to determine differences by season for each species. When we did not detect differences by season, we calculated the total mean.

## RESULTS

We trapped 48 squirrels in winter (Dec 2004) and 43 in summer (May 2005). We had also captured 18 of the 43 summer-trapped squirrels the previous winter. Overall mean time to induction was  $4.63 \pm 0.58$  minutes. Out of the 48 winter-trapped squirrels, 30 needed 1 or 2 additional administrations of anesthesia ( $\bar{x} = 0.81$  doses/squirrel). All 43 squirrels trapped in the summer required 1–6 additional administrations of anesthesia ( $\bar{x} = 2.7$  doses/squirrel). Different people were responsible for monitoring squirrel anesthesia in summer and winter. Mean time for additional doses of isoflurane using the nosecone was  $1.33 \pm 0.09$  minute. Recovery time differed by season ( $P < 0.001$ ), with squirrels recovering more quickly in summer (1.40 min) than in winter (3.62 min) after removal of the nose cone (Table 1). Mean pulse rate was greater in winter than summer ( $P = 0.009$ ), whereas mean body temperature ( $P = 0.006$ ) and respiration rate ( $P = 0.002$ ) were less (Table 1).

Two squirrel fatalities occurred during the study. We were not able to determine cause of death for either animal during necropsy. In one case, during winter, the squirrel showed no problems until procedures were finished and we placed the animal back in the cage. The animal showed a slow respiration rate and died shortly afterwards. In the second fatality, during summer, the squirrel was very aggressive and injured itself trying to escape from the trap. The animal died during induction while still in the trap.

We captured 13 woodrats during 2 winter seasons and 24 during 2 summer seasons from November 2003 to August 2005. We recaptured 3 winter-trapped woodrats during the second winter season, 2 summer-trapped woodrats during the second summer season, and 3 previously captured woodrats in both summer and winter seasons. We considered woodrats trapped in different seasons as independent observations. We trapped 8 juvenile, 11

**Table 2.** Mean time to induction, time until first additional isoflurane dose, time until additional isoflurane doses (administered by nose cone method), recovery time (animal standing and alert after last dose of isoflurane), down time (time from induction until animal was standing and alert), and physiological parameters (measured 5–10 min following induction) by season for Allegheny woodrats anesthetized with isoflurane (nose cone method) in eastern Tennessee, USA between November 2003–August 2005.

Parameters	Winter ( <i>n</i> = 13)		Summer ( <i>n</i> = 24)		<i>P</i> -value <sup>a</sup>
	$\bar{x}$	SE	$\bar{x}$	SE	
Induction (min)	0.5 <sup>b</sup>	0	0.5 <sup>b</sup>	0	<sup>b</sup>
First additional dose (min)	1.13	0.22	2.16	0.20	0.001
Additional dose (min)	2.25	0.20	2.63	0.14	0.13
Recovery (min)	4.68	1.09	4.80	0.70	0.81
Down time (min)	17.81	3.30	14.40	1.42	0.34
Temp (° C)	35.7	0.3	36.6	0.3	0.09
Pulse (beats/min)	360.0	36.9	347.5	16.5	0.72
Respiration (breaths/min)	79.3	12.2	78.2	4.3	0.92

<sup>a</sup> *P*-values for differences between seasons. Before analysis, we used square-root transformations on first additional dose, recovery, and down time to ensure normality of data.

<sup>b</sup> We administered the nose cone for 0.5 min for all woodrats because it was difficult to determine exact timing of induction. Woodrats stopped moving and closed their eyes when the nose cone was placed over their mouth.

subadult, and 18 adult woodrats. One technician was responsible for anesthesia of all woodrats. Time until first administration of nose cone after induction was longer in summer (2.16 min) than in winter (1.13 min; Table 2). Recovery time ( $P = 0.81$ ), total down time ( $P = 0.34$ ), temperature ( $P = 0.09$ ), pulse rate ( $P = 0.72$ ), and respiration rate ( $P = 0.92$ ), did not differ by season in Allegheny woodrats immobilized with the isoflurane nose cone delivery method (Table 2).

## DISCUSSION

The chamber isoflurane delivery method was effective in immobilizing squirrels. The entire trap could be placed inside the chamber, which allowed for less handling before immobilization. Minimal handling was important because squirrels were aggressive and stressed in the traps before anesthesia. We were able to watch squirrels in the immobilization chamber and determine when loss of reflexes occurred. The nose cone worked well for the docile woodrats that were easily manipulated by manual restraint and showed minimal stress when captured.

Short induction times reduce risk of handling stress and chance of injury. Isoflurane provided quick induction times and allowed control over the depth of anesthesia in the animal. Even without a precision vaporizer, we were able to maintain adequate anesthesia. Additional doses of isoflurane were easily provided as needed to keep both squirrels and woodrats immobilized during sampling.

Squirrels showed a decrease in respiration rate and temperature and an increase in pulse rate during winter. Because isoflurane is primarily eliminated by the lungs, decreased respiration rates cause the isoflurane to be washed from the body more slowly and results in longer recovery

times (Muir et al. 2000). The decrease in body temperature may be a side effect of anesthesia. Although not statically significant ( $P = 0.09$ ), woodrats also exhibited lower body temperatures during winter. Small mammals can rapidly lose heat when anesthetized (Edling 2003), which can become important in winter. Animals may need to be wrapped in a towel or a heating pad during sampling procedures to reduce amount of heat loss. The increase in heart rate of squirrels during winter may be a response to heat loss, because heart rate increases to circulate blood to the extremities and to maintain core body temperature.

Immobilization cost using 20 ml of isoflurane in the chamber method was (United States currency) \$2.20/animal. The cost of isoflurane (5 ml) using the nose cone was \$0.55/woodrat. We used new cotton balls and new isoflurane for each animal for consistency. However, using the chamber and nose cone multiple times before adding additional isoflurane, keeping the chamber lid closed unless inserting or removing a trap, and capping the nose cone between animals could reduce costs.

The disadvantages of isoflurane use were the cumbersome container required for the chamber method and having to transport isoflurane to potentially remote areas. Without a precision vaporizer, isoflurane cannot be completely regulated. However, we did not feel this was a problem because we could gauge the level of anesthesia and adjust treatment as needed. Researchers may need to be prepared to provide heat for small mammals when using isoflurane during cold ambient temperatures. Additionally, personnel need to be trained on use of isoflurane. Technicians must understand effects of chemical immobilization on humans and animals and must be able to recognize potential complications (Roffe et al. 2005). Ideally, one person would be in charge of monitoring anesthesia while another person conducts sampling. Handling devices have been developed that only require one person to conduct small mammal sampling (Koprowski 2002). Isoflurane is considered a legend drug; however, it is not a scheduled control substance. Therefore, fewer regulations are involved in purchase, use, and record-keeping of isoflurane.

### Management Implications

Isoflurane caused rapid immobilization and animals recovered quickly after administration. Animals can safely be immobilized in summer or winter with acceptable physiological parameters to ensure health. Researchers required to use an anesthetic for sampling procedures such as tissue collection (ear notch) or blood sampling may effectively and safely use isoflurane in field settings.

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