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# Evaluation of Zoletil and other injectable anaesthetics for field sedation of brushtail possums (Trichosurus vulpecula)

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## Abstract

*Ketamine has been used for many years for sedating possums captured in the wild in New Zealand, but its recent reclassification as a Class III drug under the Misuse of Drugs Act (1975) has made its continued use impractical. Consequently, four other injectable anaesthetic compounds (Zoletil, xylazine-butorphanol, medetomidine-butorphanol and Fentazin) were evaluated as replacements for ketamine. Zoletil (a combination of zolazepam and tiletamine) was the only effective alternative. Brushtail possums (*Trichosurus vulpecula*) were sedated adequately for general procedures, such as fitting radio-collars, at an intramuscular dose of 5 mg kg$^{-1}$. At this dose, possums were sedated on average in 3.6 min, and recovery took 65 min on average. Zoletil did not cause sudden arousal as seen with some other anaesthetics, but most possums showed involuntary chewing during recovery, and in some cases excessive salivation. In contrast, Fentazin and combinations of xylazine-butorphanol and medetomidine-butorphanol failed to produce sedation at doses known to be effective in other mammalian species. Zoletil proved similar to ketamine in both performance and cost, and is therefore recommended as a cost-effective anaesthetic and humane method for sedating possums captured in the wild.*

**Keywords**: *anaesthetics, animal welfare, brushtail possum, sedation, wildlife capture, Zoletil*

## Introduction

The brushtail possum (*Trichosurus vulpecula*), introduced from Australia to New Zealand in 1837 for the establishment of a fur trade, has become a serious pest over large areas of New Zealand due to its impact on indigenous fauna and flora, and its role as a vector of bovine tuberculosis (Montague 2000). Possum populations are researched extensively in the wild to underpin improved management through better understanding of impacts, movements, population dynamics, behaviour, disease transmission, and responses to control. Such work often requires that possums are restrained for purposes such as the attachment of radio-collars, collection of tissue samples, vaccination, or measurement of body parameters. Like most wild animals, possums are not innately docile, and vigorously resist prolonged physical restraint which is considered, therefore, both practically and ethically unacceptable. Rather, anaesthetics are used to sedate the animal so that movement and responsiveness are greatly reduced. Occasionally, researchers have needed to conduct more invasive procedures in the field, such as ovariectomy or castration, where the aims are to simulate potential effects of fertility control methods. Such procedures require induction of surgical anaesthesia where the animal becomes completely unconscious and loses all motor control.

The drug most commonly used to sedate possums was ketamine, but in December 2010 this compound was reclassified as a Class C substance under New Zealand's Misuse of Drugs Act (1975). The increased stringency of licensing, storage, and auditing requirements posed considerable additional cost and logistical constraints on using ketamine for field research. We therefore evaluated four other drugs used for sedation of mammals (ie single substances or combinations of substances) with the aim of identifying at least one that, at an appropriate dose, would provide sedation that was both rapid, to facilitate fieldwork, and 'smooth', to avoid injuries to possums that could also compromise scientific aims.

We selected four compounds for evaluation from those that were available for use by field biologists in New Zealand under veterinary prescription and regulatory oversight.

### Zoletil

Zoletil is an equal-parts combination of tiletamine, a dissociative agent that produces analgesia and anaesthesia, and zolazepam, a muscle-relaxing barbiturate that counters the convulsive seizures typically associated with tiletamine. Doses of Zoletil are generally given in the literature as the combined dose of tiletamine and zolazepam (eg a dose of 10 mg kg$^{-1}$ would contain 5 mg kg$^{-1}$ of each active). Researchers have used Zoletil routinely to anaesthetise

possums at intramuscular (IM) doses of 10–30 mg kg$^{-1}$ (McDowell *et al* 2009; Hufschmid *et al* 2010), but no dose-response data were located. Veterinary literature reported considerable variation in the responses of other marsupial species to Zoletil. For example, eleven marsupial species that received IM doses of 3–20 mg kg$^{-1}$ became sedated in 5 min or less, and while recovery generally took from 10 min to 3 h in most species, several took more than 12 h to recover, and two of three squirrel gliders (*Petaurus norfolcensis*) died after doses of 10 mg kg$^{-1}$ (Holz 1992). IM dose was varied in a study on grey kangaroos (*Macropus giganteus*) (King *et al* 2011) between 0.8 and 10 mg kg$^{-1}$: induction of sedation (1–12 min) was not related to dose, but time to recovery was not recorded.

### Xylazine combined with butorphanol

Xylazine is an α-2 adrenoreceptor agonist, suppressing neurotransmitters throughout the central nervous system, sympathetic nervous system, and other body tissues, particularly the vascular and gastrointestinal systems. Butorphanol is a synthetic opioid analgesic derived from morphine and is used to enhance the sedation given by xylazine and reduce the excitation that occurs during sedation and recovery from xylazine (Greene & Thurmon 1988). This combination has been used in varying proportions in domestic (eg Geiser & Henton 1988; Ko *et al* 1998; Jacobson 2001) and wild animals (Kreeger *et al* 1989), although no previous reports of its use in marsupials were located.

### Medetomidine combined with butorphanol

Medetomidine is also an α-2 adrenoreceptor agonist, and the combination has the same mode of action as xylazine-butorphanol. While medetomidine is considerably more potent than xylazine and other α-2 adrenoreceptor agonists (Virtanen 1989), it is one of the safest sedatives available, with a range of animals known to tolerate 5–10 times IM overdosing (Fowler 1995). Dogs were sedated with IM doses of 10 µg kg$^{-1}$ medetomidine combined with 0.1 mg kg$^{-1}$ butorphanol (Bartram *et al* 1994). No examples of use in marsupials were found.

### Fentazin 5®

Fentazin 5® contains 0.4 mg ml$^{-1}$ fentanyl citrate (an opioid analgesic), 58.3 mg ml$^{-1}$ xylazine, and 3.2 mg ml$^{-1}$ azaperone (an antipsychotic that reduces or eliminates consciousness). This compound is used principally in deer species at IM doses of the combined solution of 0.01–0.02 ml kg$^{-1}$ (IVS 2011). No examples of use in marsupials were found.

## Materials and methods

Seventy-two possums, in an approximately equal sex ratio, were caught in the wild in apple-baited cage traps and transferred, in sacks, to a purpose-designed indoor facility. Following the recommended practice for acclimatising possums to captivity (Duckworth & Meikle 1995), they were housed for a minimum period of four weeks in individual cages (1,000 × 400 × 600 mm; length × width × height) that contained fibre glass nest boxes (350 × 200 × 230 mm), and were maintained on a diet of fruit, vegetables and supplementary feed pellets comprised of wheat, barley, soya meal, lucerne meal, molasses and minerals (Combined Rural Traders, Rolleston, New Zealand). Reticulated drinking water was available *ad libitum*. Ambient temperature was maintained within the range 18–22°C, and ventilation provided for complete refreshment of air every 4 min. Bodyweights of possums were 1.66–4.33 kg. Variable numbers of possums were used to evaluate the different anaesthetic compounds depending on the responses observed. For each anaesthetic tested however, doses were randomly assigned to possums, and possums were dosed only once.

In preparation for administering anaesthetics, possums were transferred from nest boxes to hessian sacks. While possums were calm, the thigh region of a hind leg was revealed and anaesthetic compounds were administered by injection into the thigh muscle using a 25-gauge needle on a 5-ml syringe. Most doses (64 of 72 given) were delivered at single sites in dose volumes of 0.01–0.25 ml kg$^{-1}$. Where larger dose volumes of 0.35 and 0.7 ml kg$^{-1}$ were required, these were divided between two separate thigh-muscle sites so as not to exceed the recommended maximal dose volume of 0.5 ml kg$^{-1}$ per site (Diehl *et al* 2001).

Initial doses described below were based on available data for other species. Depending on the observed response to the initial dose, further doses were either increased or decreased. After being injected, possums were placed in individual sacks on the floor of the animal facility and checked at intervals of *circa* 1 min by gently opening the sacks. Once sedated they were removed from sacks to facilitate physical testing of the depth of anaesthesia. Sedation was defined as a light plane of anaesthesia suitable for non-invasive animal manipulations, and was characterised by loss of consciousness and muscular control (ie no righting response, and no resistance to jaws being opened), and a lack of reflex responses to pinching between the toes and blowing gently on the face. Surgical anaesthesia, was characterised by a complete cessation of motor activity in addition to these signs of sedation. Recovery was defined as the stage at which all these functions were restored. As a general husbandry procedure, ten possums (all in the Zoletil-treated group) were ear-tagged for identification purposes (others being already ear-tagged) once they appeared to be sedated. Stainless steel 'Jiffy' 893 chick tags (National Band & Tag Co, Newport, Kentucky, USA) were fitted using specialised pliers, and possums' reactions to ear-tagging were noted.

### Zoletil

Forty possums were injected with Zoletil 100® (Virbac New Zealand Ltd, Auckland, New Zealand) in groups of four per dose. The initial dose of Zoletil, 0.1 ml kg$^{-1}$, delivered 10 mg kg$^{-1}$ of the combined actives per kilogramme bodyweight, and was the lowest dose used in reported studies for routine sedation of possums (Hufschmid *et al* 2010). Doses were then progressively reduced in further groups to 7.5, 5, 4, 3, 2.5, 1.5 and 1.25 mg kg$^{-1}$ in anticipation of identifying a 'failure point', and one group was dosed at 20 mg kg$^{-1}$ to examine safety



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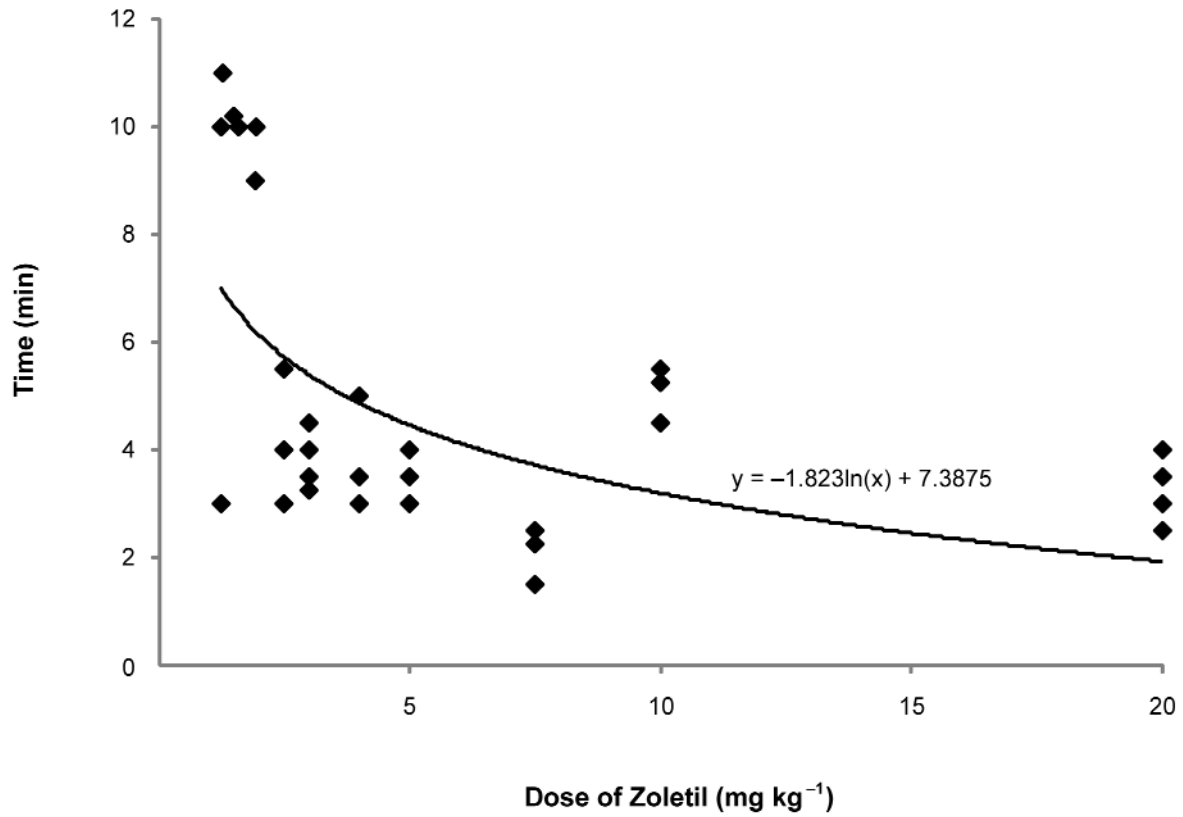
**Figure 1**




Relationship between Zoletil dose and time to sedation. A logarithmic regression model gave the best fit to the data, as described by the regression equation.

margins. An additional group of four possums was given a standard dose of 5 mg, which equated to weight-adjusted doses of 1.3–2 mg kg$^{-1}$. Bodyweight-adjusted dose volumes were 0.08–1.44 ml. Dose data were log-transformed and analysed using the linear models procedure in the R statistical computing environment (version 2.11.1) (R Development Core Team 2010).

### Xylazine and butorphanol

Twenty possums were injected, in groups of four per dose, with a combination of solutions of 2% xylazine (Phoenix Pharm Distributors, Auckland, New Zealand) and 1% butorphanol (Lloyd Laboratories, Iowa, USA). The initial dose of xylazine, 1.5 mg kg$^{-1}$, was the mean dose of the range recommended for dogs (1–2 mg kg$^{-1}$) (IVS 2011), and this was combined with 0.3 mg kg$^{-1}$ butorphanol. A similar mixture (2 mg kg$^{-1}$ xylazine and 0.4 mg kg$^{-1}$ butorphanol) was found effective in wolves (*Canis lupus*) (Kreeger *et al* 1989). Four further groups received doses of xylazine at 2.25, 3.0, 5.0, and 10.0 mg kg$^{-1}$, combined, respectively, with butorphanol at 0.45, 0.6, 1.0, and 2.0 mg kg$^{-1}$. Bodyweight-adjusted dose volumes of the combination were 0.28–2.7 ml.

### Medetomidine and butorphanol

Eight possums were injected with solutions of 0.1% medetomidine (Vetpharm, Auckland, New Zealand) and 1% butorphanol (Troy Laboratories, Auckland, New Zealand). One group of four possums received 0.1 mg kg$^{-1}$ of medetomidine and 1 mg kg$^{-1}$ of butorphanol, and the second group received 0.2 mg kg$^{-1}$ and 2 mg kg$^{-1}$, respectively. The manufacturer's recommended dose of medetomidine for moderate sedation of cats is 0.05–0.1 mg kg$^{-1}$, while butorphanol is recommended at 0.4–0.8 mg kg$^{-1}$ as an analgesic. Bodyweight-adjusted dose volumes of the combination were 0.4–1.5 ml.

### Fentazin 5®

In a pilot trial, individual possums were injected with Fentazin 5® at doses of 0.02, 0.04, 0.08 or 0.16 ml kg$^{-1}$.

### Ethical note

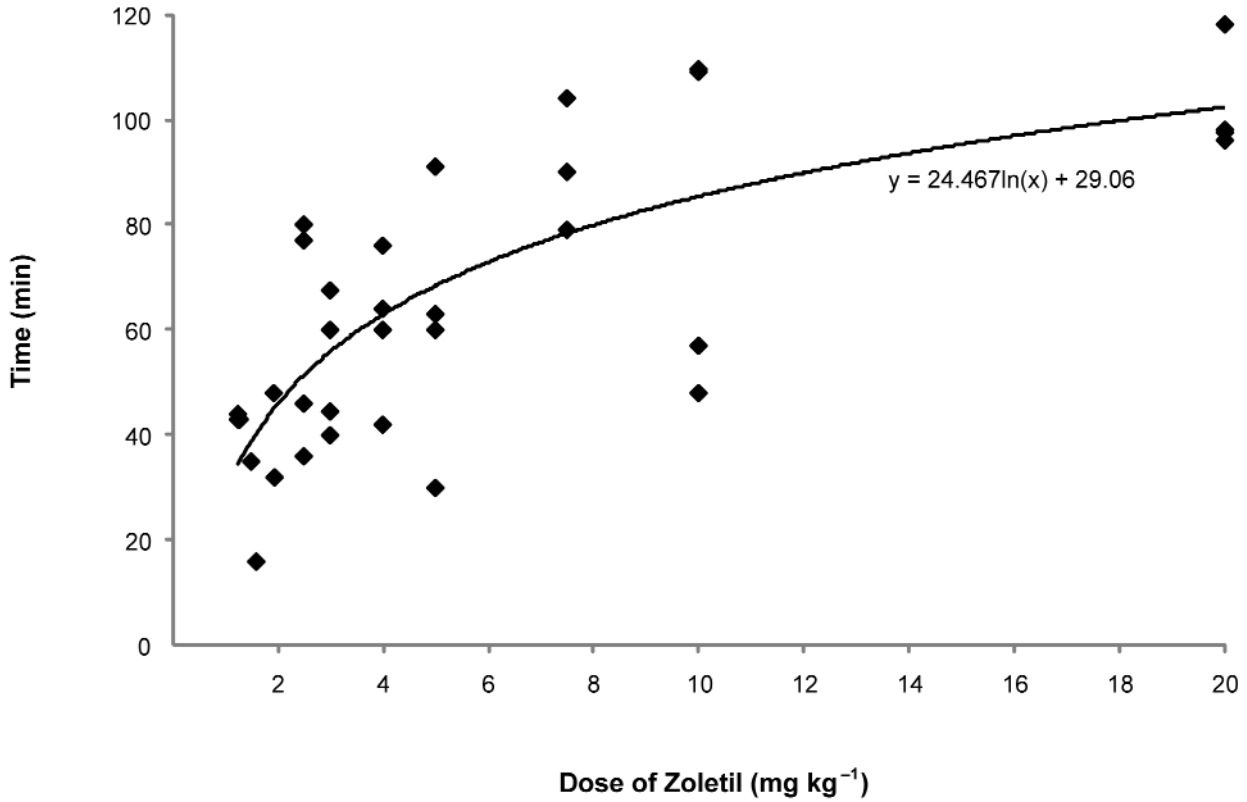
The study was conducted with the approval (No 11/04/03) of the Landcare Research Animal Ethics Committee and the hands-on involvement of a practicing veterinarian (DA).

## Results

### Zoletil

Zoletil was the only one of the four compounds tested that gave effective sedation in possums. Onset of sedation occurred smoothly with no involuntary excitation during this phase although a few possums defaecated and/or urinated as normal responses to being handled. Once possums were sedated they showed no unusual symptoms.

**Figure 2**




Relationship between Zoletil dose and time to recovery. A logarithmic regression model gave the best fit to the data as described by the regression equation.

The mean time to sedation across all doses was 4.7 min (range: 1.5–11 min) and time to sedation was inversely related to log-dose (Figure 1). However, as indicated by the low correlation coefficient ($r^2 = 0.32$, $P < 0.001$), variability in the data was poorly explained by the curvilinear relationship which nevertheless suggests that little further shortening of induction time occurs above a dose of 4 mg kg$^{-1}$. Two of the four possums dosed at 1.25 mg kg$^{-1}$ and three of the four dosed at 1.5 mg kg$^{-1}$ became unco-ordinated but not unconscious. All other doses (2.5–20 mg kg$^{-1}$) were effective in sedating all possums. Surgical anaesthesia was achieved in only two of four possums dosed both at 20 and 10 mg kg$^{-1}$, and in one of four at 2.5 mg kg$^{-1}$. Six possums ear-tagged after dosing with 2.5–4 mg kg$^{-1}$ showed a strong reaction (ie vocalised and pulled away), while four possums ear-tagged at higher doses showed only a mild reaction (ie brief tensing of the body).

Possums recovered fully in an average time of 65.1 min (range: 16–118 min). Time to recovery was positively correlated with dose ($r^2 = 0.60$, $P < 0.001$) (Figure 2). The four possums that received the highest dose (20 mg kg$^{-1}$) showed the longest mean recovery time (102.4 min). During recovery, all possums chewed their sacks. This mild excitation behaviour was an autonomic response that started when animals were clearly still unresponsive to stimulation, and continued until consciousness was regained. No other excitation or other unusual behaviours, such as shivering, were noticed during recovery, although excessive salivation was seen in a few possums.

### Xylazine and butorphanol

No possums became sedated. All possums became excited within a few minutes of dosing at all doses, particularly if stimulated by touch or noise, but also without stimulation. Excitation manifested as a series of jerky or jumping movements while possums were in sacks. Of the 20 possums dosed, only one progressed beyond excitation, becoming subdued yet still responsive.

### Medetomidine and butorphanol

No possums were sedated at either dose tested. All possums appeared subdued but were easily aroused and responded with violent movement. One possum in the higher dose group became excited, without stimulation, 6 min after dosing.

### Fentazin 5®

All four dosed possums remained fully alert following injection of Fentazin 5®.



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## Discussion

Zoletil was the only compound of the four tested that proved suitable for sedating possums at the doses tested. We found that a minimum dose of 2.5 mg $kg^{-1}$ ensured all possums were sedated, but since some possums clearly showed a pain-response to ear-tagging at doses up to 4 mg $kg^{-1}$, we suggest a dose of 5 mg $kg^{-1}$ (deliverable by Zoletil 100® at 0.1 ml $kg^{-1}$) as being optimal in minimising induction and recovery times while maximising cost-effectiveness. Induction of sedation at this dose (mean = 3.6 min) was similar to that produced by ketamine at a dose of 100 mg $kg^{-1}$. The cost of these doses was less for Zoletil (approximately NZ$1.50 per 3-kg possum) than ketamine (NZ$1.90). Recovery following dosing with 5 mg $kg^{-1}$ generally took 65 min on average. Routine field procedures requiring sedation, such as fitting radio-transmitters, can usually be completed well within this time.

Our recommended dose of 5 mg $kg^{-1}$ Zoletil for field sedation of possums will be appropriate when procedures necessitating sedation are no more painful than ear-tagging. Zoletil may not, however, be suitable for more invasive field procedures that may require surgical anaesthesia, as this was not consistently produced at the highest dose tested, 20 mg $kg^{-1}$. This dose exceeds that recommended in the manufacturer's product leaflet for surgical anaesthesia in several eutherian species (eg dogs, leopards, deer) and also the dose of 13 mg $kg^{-1}$ (Degerfeld 2005) that was found suitable for surgery on another marsupial species, the red-necked wallaby (*Macropus rufogriseus*). Possums, therefore, appear relatively insensitive to the drug, and would need higher doses for consistent attainment of surgical anaesthesia. However, since no significant adverse signs were observed following the highest dose, and all animals resumed normal food intake within 24 h, we conclude that Zoletil used at 5 mg $kg^{-1}$ in possums has a wide safety margin. Excessive salivation, which was seen in a few possums, may cause respiratory difficulty and can be treated, if required, with an intramuscular injection of 0.5 mg atropine (Creed & McDonald 1970).

The three other compounds tested were all ineffective in inducing sedation at the doses tested. Higher doses of xylazine and medetomidine, both in combination with butorphanol, may prove effective but would be disadvantaged by the likely significant excitation during recovery that could endanger possums, as well as high cost. Fentazin, which was used at up to eight times the recommended dose for sedating deer, with no effect, is clearly unsuitable for sedating possums.

### Animal welfare implications

In seeking a suitable method for chemically restraining possums, we have shown that injection of Zoletil is a rapidly acting, humane, safe and cost-effective method that is suitable for use in field research. Notably, it lacks the significant excitation phase and hypothermia-inducing characteristics of some other anaesthetics which could endanger possums recovering in the wild. Nevertheless, as there is no reversal drug for Zoletil, it would be advisable to ensure that possums left unattended during recovery in the field are always placed at a site where they would not stumble into danger (eg cliffs, streams, pools) if they were to move while still partially sedated. Sites should also be shaded because possums' eyes remain open during Zoletil anaesthesia. There is little risk of possums being disturbed or predated by other animals during recovery as there are no naturally occurring predators of possums in New Zealand.

Hypothermia may complicate recovery of humans and animals following general anaesthesia and surgery (particularly if the body cavity is opened) (English *et al* 1991), and adrenoceptor agonists such as medetomidine and xylazine used in wildlife research are known to induce dose-dependent hypothermia which can be managed with a reversal agent (Arnemo & Søli 1992). However, hypothermia is an unlikely consequence for possums in the wild during light anaesthesia with Zoletil for non-invasive manipulations. We observed no shivering in possums during recovery in the present study, and while body temperature was not monitored, other medium-sized mammals, including red foxes (*Vulpes vulpes*) (Travaini & Delibes 1994), raccoons (*Procyon lotor*) (Pitt *et al* 2006), wolves (Kreeger *et al* 1990) and crested porcupines (*Hystrix cristata*) (Massolo *et al* 2003), showed little deviation from normal body temperatures during Zoletil anaesthesia over a wide range of ambient temperatures in the field.

Although negative results were obtained with the three other compounds tested, we believe it is important to report these findings to help avoid wasteful use of animals and duplication of effort by other researchers.

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