

Comparison of Intrarenal and Intravenous Injections of Sodium Pentobarbital for Euthanasia in New Zealand White Rabbits (*Oryctolagus cuniculus*)

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Peripheral venous access in rabbits can be difficult to obtain. When failure occurs, there is a dire need for alternative vascular access routes to be available. The AVMA categorizes intrarenal injection of pentobarbital as acceptable with conditions for euthanasia. Animals must be in an unconscious state, and only minimal studies using intrarenal administration have been reported. A total of 53 rabbits were used to conduct 3 separate analyses to assess and measure the efficacy, efficiency, and validity of the intrarenal route for euthanasia in New Zealand White rabbits by assessing the time to cardiopulmonary arrest (TCPA). Animals were sedated with 40 mg/kg ketamine and 50 µg/kg dexmedetomidine intramuscularly into the lumbar muscles, and timing started at the beginning of the injection and ended when cardiac and respiratory arrest were observed. Cardiac and respiratory arrest following intravenous injection of pentobarbital was significantly quicker (cardiac, 6 to 24 s, median 9 s; respiratory, 6 to 19 s, median 9 s; $P < 0.001$) than for the intrarenal route (cardiac, 40 to 900 s, median 411 s; respiratory, 23 to 900 s, median 120 s; $P < 0.001$), with no negative animal reactions observed during euthanasia injection performance. Four animals did not achieve TCPA within 15 min after administration. Although TCPA was longer with intrarenal compared with intravenous euthanasia ($P < 0.001$), this study demonstrates that the intrarenal approach under anesthesia is a feasible alternative to the intravenous approach, as it can be reliably performed without observed animal distress or alterations in organ pathology. The overall information from this study can help guide both laboratory and practicing clinicians considering this technique. Still, factors such as variable times to cardiopulmonary arrest and technical skill should be considered.

Abbreviations and Acronyms: EBD, Evans blue dye; IC-EBD, intracardiac with 2% EBD; IR-EBD, intrarenal with 2% EBD; IV-EBD, intravenous with 2% EBD; TCPA, time to cardiopulmonary arrest

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Introduction

Euthanasia is a routine procedure performed by clinical and laboratory animal veterinarians. The most common and preferred method for small mammals is through an intravenous injection of a barbiturate overdose of sodium pentobarbital.¹ Sodium pentobarbital, a barbituric acid derivative, has a high affinity for serum proteins, allowing it to stay within the vasculature, resulting in rapid distribution to the brain and neural centers with its primary mechanism of action causing accelerated depression of the CNS, loss of consciousness, and apnea followed by cardiac arrest.¹ In general, barbiturates are the drug of choice due to being inexpensive, providing rapid and smooth

delivery, being nonirritating, and having reduced or minimal discomfort to the animal.¹ The intravenous administration route is considered one of the most rapid and reliable methods and is commonly the most desirable route among clinicians.^{1,2} In laboratory rabbits, peripheral venous access is most commonly obtained using the marginal ear vein largely due to prominent visualization for ease of access.^{3–5} Although other peripheral venous sites are available, obtaining access could require sedation and can be challenging due to their small size, shallow nature, and vasoconstriction mechanisms. Difficulty accessing intravenous sites of common laboratory animal species could be due to inexperience with the species, as well as smaller than normal peripheral vasculature. The intracardiac route is routinely used with the animal under heavy sedation or anesthesia for terminal procedures. The benefits of the intracardiac route include that it is quick, direct, and allows for large terminal blood volume collections, as well as providing a direct vascular access route for euthanasia. Still, the intracardiac method is not as routinely performed in the private clinical setting.^{2,6–8} Intracardiac injection presents some technical difficulties, and this route may not be preferred due to the potential for iatrogenic accumulation of artifactual changes observed during histopathology, which has been observed in other species.⁹ In addition, this route of

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administration may be considered least aesthetically appealing in a clinical scenario when owners are present. Thus, when traditional routes, such as intravenous or intracardiac, cannot be used, other methods of drug delivery need to be considered.

According to the 2020 AVMA *Guidelines for the Euthanasia of Animals*, intrarenal injection is an acceptable-with-conditions method of euthanasia for mammals, with the prerequisite for this technique being that the animal is anesthetized or in an unconscious state. The kidney is a unique organ because of the large circulating blood flow delivered from the aorta, and blood vessels are well integrated throughout with arcuate arteries. It is estimated that 20% to 25% of the total circulating blood flow is through the kidneys at any given time.³ The rationale for use of intrarenal injection is based on this large blood supply as a way to deliver drugs systemically. Currently, the only published studies exploring the efficacy of intrarenal euthanasia injection have been conducted in domestic cats, and those authors concluded that the technique and method are appropriate by measurement of the time of injection to time to cardiopulmonary arrest (TCPA).⁴ For intrarenal administration to be successful, there needs to be correct injection placement within the renal cortex or medulla, as it is these sections that have the adjacent blood supply through the parenchyma capillaries and arcuate arteries. In contrast, injection into the renal pelvis would deliver drugs into the bladder, negate vascular delivery, and successful euthanasia would not be achieved. There are currently no published studies in any species regarding the use of advanced imaging for successful confirmation of correct intrarenal injection into cortex or medulla. However, most practitioners reported a palpable increase in organ size, also referred to as 'kidney swelling,' as a confirmation of successful placement, and published feline literature supports that a palpable increase in organ size also correlates with a faster time to death following intrarenal administration of sodium pentobarbital.⁴

Thus, the intrarenal injection route has potential to provide an alternative method for administration of euthanasia drugs when traditional routes of administration are not feasible. The objective of this study was to determine the efficacy and practicality for intrarenal administration of sodium pentobarbital for euthanasia in the rabbit when compared with administration by a traditional intravenous route. We hypothesized that the intrarenal route would be a viable alternative to the intravenous route in rabbits, and that this route would not have any adverse effects when evaluating gross or histologic findings.

Materials and Methods

Animals. A total of 53 New Zealand White rabbits (26 males and 27 females, weight range 2.49 to 4.4 kg) were used. In support of the 3Rs (replacement, reduction, and refinement), all animals were opportunistically acquired from either training protocols, which involved minimal handling and restraint, or from another study that had used the animals as a surgical model. These animals were used for a series of 3 analyses, with the data from some animals being used for multiple experiments as detailed in the experimental design.

All animals originated from Charles River Laboratories (Saint-Constant, QC, Canada) and maintained in a climate-controlled facility. According to the vendor's health monitoring report, all animals were determined to be free of reovirus, *Bordetella bronchiseptica*, *Helicobacter* spp., *Lawsonia* spp., *Pasteurella* spp., *Salmonella* spp., *Treponema* spp., Tyzzer disease pathogens, CAR bacillus, *Cheyletiella parasitovorax*, *Leporacarus gibbus*, *Psoroptes cuniculi*, *Eimeria* spp., *Eimeria stiedae*, and other intestinal protozoa, as well as *Encephalitozoon cuniculi*. Rabbits

were housed in accordance with the *Guide for Care and Use of Laboratory Animals* with adherence to the Animal Welfare Act at North Carolina State University's College of Veterinary Medicine Laboratory Animal Resources Facility (Raleigh, NC). This facility is accredited by AAALAC International, and all work was approved by the Institutional Animal Care and Use Committee.

The first analysis consisted of 12 rabbits (6 males and 6 females). Forty-nine rabbits (25 males and 24 females) were used for the second analysis, and the third analysis consisted of 27 rabbits (13 males and 14 females).

Rabbits were housed individually in semisolid cage banks (29 × 28 × 19 in., Tecniplast, West Chester, PA). As previously stated, all animals were obtained from use under other IACUC-approved protocols and were exempt from social housing for individual medical or research monitoring. The animals were then immediately transferred for euthanasia to the study described here. Environmental conditions were maintained at temperature of 61 to 72 °F (16 to 22 °C) and 30% to 70% humidity with a 12-h light/12-h dark light cycle (lights on at 0600 and lights off at 1800). Water was provided ad libitum by the city of Raleigh, NC municipality via sanitized water bottles, and rabbits were fed a pelleted rabbit diet (5L3M PicoLab certified high-fiber rabbit diet; Lab Diet, Richmond, IN) once a day with adequate autoclaved Timothy hay (Twins Oak Feed and Supply, Willow Spring, NC) replenished daily. Environmental enrichment was provided in the form of sanitized toys, food treats, social interaction with humans, and sanitized huts. Prior to studies, all rabbits were determined to be healthy by means of physical examination by a veterinarian and serum biochemical panels prior to inclusion as described below.

Experimental design. This study consisted of 3 analyses. The first analysis compared the routes of intravenous, intracardiac, and intrarenal for euthanasia and used a 2% (0.06 g) Evans blue dye (EBD) powder dissolved into 3 mL of euthanasia solution (EBD powder, Thermo Fisher Scientific, Waltham, MA) to verify kidney parenchymal placement (intravenous with 2% EBD [IV-EBD; $n = 4$], intracardiac with 2% EBD [IC-EBD; $n = 4$], and intrarenal with 2% EBD [IR-EBD; $n = 4$]). The aim of the second analysis was to determine whether the dye influenced the TCPA when comparing routes of intravenous to intrarenal administration. The second analysis also included animals that were administered euthanasia solution with dye and without dye: intravenous ($n = 14$), IV-EBD ($n = 10$), intrarenal ($n = 13$), and IR-EBD ($n = 12$). The third analysis analyzed intravenous ($n = 14$) and intrarenal ($n = 13$) administration of pentobarbital alone in the absence of the dye mixture. All animals were randomly assigned to groups by using an Excel (Microsoft, Redmond, WA) randomization tool. All techniques were performed by the same personnel as described below. A summary of experimental design is provided in Figure 1.

Screening. Prior to study inclusion, all rabbits had a biochemical serum analysis for routine screening of potentially complicating factors, such as kidney disease. All samples were evaluated using a chemistry analyzer (VetScan VS2; Abaxis, Union City, CA). Prior to administration of the euthanasia solution, an ultrasound (IBEX EVO III; E.I. Medical Imaging, Loveland, CO) evaluation was performed on the right and left kidneys of all rabbits to assess for adequate kidney parenchyma of the renal cortex and medulla and to rule out any ultrasonographic renal conditions that could result in an inappropriate parenchymal placement.

Sedation, euthanasia, and death confirmation. All injections were given at a rate of ~1 mL/s for consistency. All rabbits were

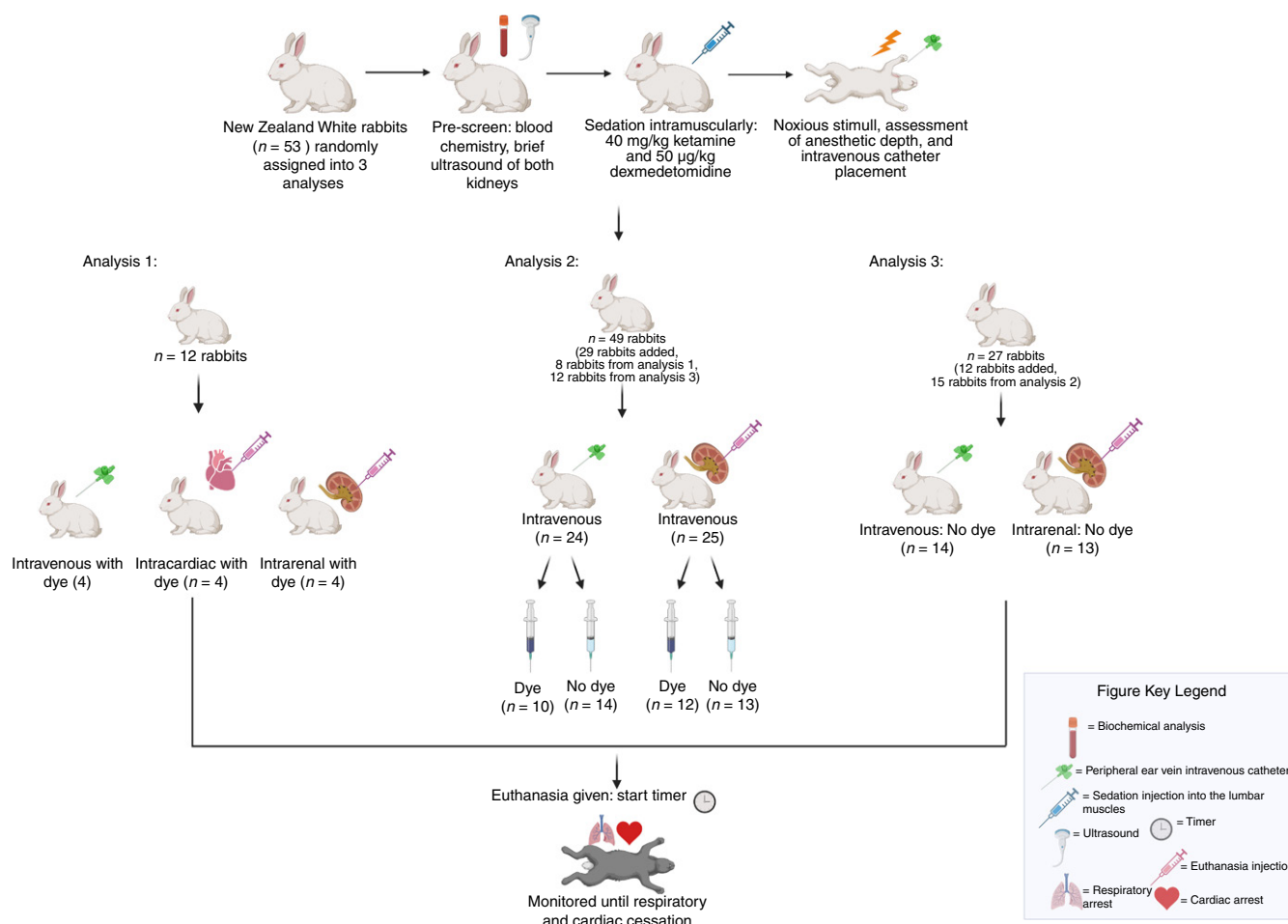


Figure 1. Experimental design summary. All animals were randomly assigned to intravenous or intrarenal administration groups and prescreened for study enrollment with biochemical analysis and parenchyma ultrasound of both kidneys. Animals were then sedated intramuscularly with 40 mg/kg ketamine and 50 µg/kg dexmedetomidine. All animals were confirmed to be in the correct anesthetic plane with lack of response to noxious stimuli, and a peripheral ear vein catheter was placed. Animals were then euthanized according to their assigned group and timed from the moment of injection and ending at cardiac and respiratory arrest with each value recorded separately.

sedated with 40 mg/kg ketamine (ketamine hydrochloride injection, 100 mg/mL; Dechra, Overland Park, KS) and 50 g/kg dexmedetomidine (Dexased, 0.5 mg/mL; Pivotal Veterinary Supplies, Loveland, CO) intramuscularly in the lumbar muscles prior to euthanasia. Once sedated, all rabbits had a 24-gauge catheter placed into the marginal ear vein. This catheter was used for intravenous administration in the intravenous pentobarbital control group. This catheter was also to be used if a second dose of euthanasia solution was needed for those animals within the intrarenal or intracardiac groups who failed to die within the allotted timeframe. All animals were determined to be at a surgical plane of anesthesia prior to euthanasia, confirmed by a lack of a withdrawal reflex from toe pinch and lack of palpebral reflex. Sodium pentobarbital (Fatal-Plus, 390 mg/mL; Covetrus, Columbus, OH) was given at a dosage of 3 mL/rabbit (~88.6 to 156.6 mg/kg). Only the left kidney was used for intrarenal administration and the right kidney was used for a postmortem control comparison. An elapsed time of 15 min as described was considered the maximum time point. For those animals that reached the maximum allotted time, but did not meet the definitions of death, those deaths were recorded at 15 min, and a second dose of 3 mL of sodium pentobarbital was given through the intravenous ear catheter. Confirmation of death was defined as a lack of spontaneous respiration, heart beat as determined by auscultation, and lack of corneal reflex.

Isolation of kidneys and intrarenal injection placement. Kidneys were isolated by manual traction with the animal positioned in sternal recumbency. This was done by starting at the xiphoid process of the sternum and firmly pressing inward toward the middle of the animal's abdomen, then pressing in an upward and outward motion, walking along the caudal rib cage, moving in a caudolateral direction. This walking technique isolated the kidney in a dorsocaudal position located caudal to the ribcage (Figure 2). Intrarenal injection into the renal cortex/medulla was confirmed visually at the time of necropsy.

Personnel roles and responsibilities. All analyses used 3 primary personnel: a timer/recorder, an observer, and an injector. The injector, a veterinarian trained in intrarenal injections but who had not regularly performed them in daily practice, conducted all of the injections for sedation and intrarenal administrations. The timer/recorder recorded all pertinent observations communicated by the observer as well as timing the TCPA for each animal. Timing began at the start of euthanasia injection and ended when the observer noted cardiac and respiratory arrest. Cardiac and respiratory arrest were noted as 2 separate data observations. The observer was responsible for animal observations and continuously monitored or auscultated the animal starting at the time of euthanasia injection and ending at the cessation of monitored heart/respiratory activity. The observer noted any apparent changes in respiratory or cardiac

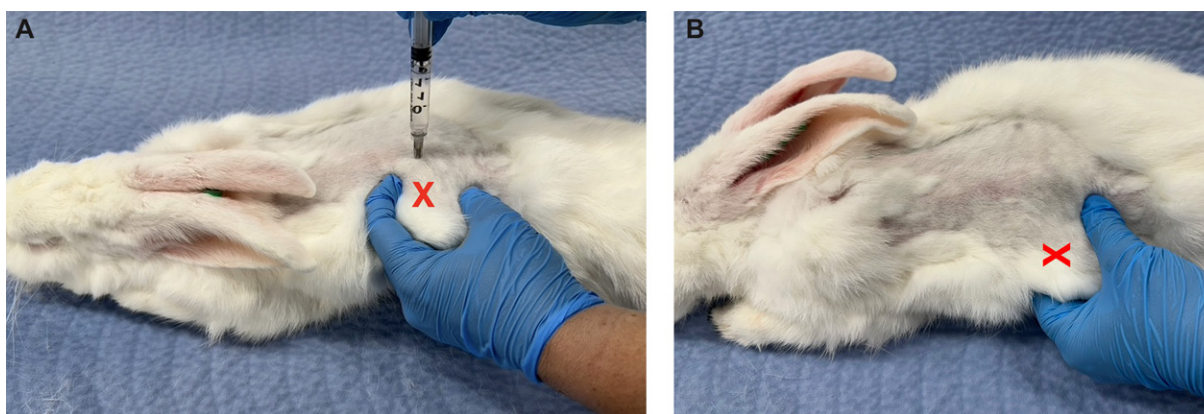


Figure 2. Intrarenal injection procedure in an anesthetized rabbit. Starting at the xyphoid process of the sternum and using a walking technique, the kidney can be isolated by manual traction in a dorsocaudal position. (A) Isolation of the left kidney (red X) in dorsocaudal position (between thumb and forefinger) caudal to the rib cage with injection demonstration. (B) Dorsal view of left kidney isolation (red X) located between thumb (dorsal) and forefinger (ventral).

rates, depths of anesthesia, or adverse animal reactions. The observer stated aloud any observations to be recorded.

Necropsy, histology, and sample analysis. A veterinarian performed a gross necropsy on all animals after confirmation of death. Kidneys for a subset of the intrarenal group animals ($n = 24$) were evaluated grossly as well as histologically on cut surfaces for misalignment of injection and any other visual or observable abnormalities. Samples from the left (injected) kidney were taken at the point of insertion, and included ~4 to 5 mm of surrounding tissue for evaluation. A sample of the same approximate size and location was also taken from the right kidney for contralateral comparison and used as the control sample. All samples were placed in 10% neutral buffered formalin for fixation for 1 to 2 wk before being routinely processed and embedded with paraffin wax using an automated tissue processor (Tissue-Tek VIP; Sakura Finetek USA, Torrance, CA). The paraffin-embedded tissue blocks were then sectioned into 5- μ m slices and stained with routine hematoxylin and eosin and coverslipped. A veterinary pathologist blinded to treatment group used a subjective grading scale to assign scores ranging 0 to 4 (0, no abnormalities observed; 1, minimal; 2, mild; 3, moderate; 4, marked) reflecting the presence of lymphoplasmacytic inflammation, interstitial mineralization, and hemorrhage. The results were peer reviewed by a second blinded veterinary pathologist.

Statistical analysis. Data from all experiments for animals who received the same treatment were also included in all analyses. All statistical methods were performed using IBM SPSS Statistics for Windows version 29.0.2.0 (IBM, Armonk, NY) and GraphPad Prism version 10.2.3 (403) for Windows (GraphPad Software, Boston, MA). Data for all experiments were not normally

distributed; therefore, Mann–Whitney U tests were used to compare the groups in all analyses. A 2-sided P value ≤ 0.05 was considered statistically significant for all statistical analyses.

Results

Screening. All biochemical analyses fell within normal reference ranges; thus, no animals were excluded from the study.

First analysis: comparison of IV-EBD, IC-EBD, and IR-EBD. All animals were successfully euthanized by all 3 routes of pentobarbital administration, thereby demonstrating feasibility of intrarenal administration. In contrast, correct delivery of material using the intracardiac route was found to be difficult to confirm using the dye mixture, as it produced a deep, navy blue to black coloring, resulting in inability to confirm, by means of a flash of blood in the syringe, successful intracardiac placement.

Second analysis: comparison of intravenous, intrarenal, IV-EBD, and IR-EBD. When dye was used, a trend for delayed time to TCPA was observed; this was statistically significant for the intravenous administration group but not for the intrarenal group (Table 1). One of 25 animals (4%) within the intrarenal group needed a second dose of pentobarbital, as death was not achieved within the 15-min timeframe and their arrest time was recorded at 15 min (900 s). There were no major gross abnormalities on initial necropsy, and successful injection placement was confirmed with and without the use of EBD (Figure 3).

Third analysis: comparison of intravenous with intrarenal administration. Animal reactions and visual observations. The most common observed reaction followed the sedation injection, with 19 of 27 animals (70%) demonstrating tensing of the dorsal muscles. There was no observable reaction during the intravenous

Table 1. Summary of time to cardiac arrest for New Zealand White rabbits euthanized using the intrarenal and intravenous routes and with or without the use of 2% Evans blue dye

Outcome	Group	EBD status	n	Median (s)	Minimum (s)	Maximum (s)	z	P
Cardiac arrest	Intrarenal	Dye	12	325	48	1200	1.09	0.295
		No dye	13	411	40	900		
	Intravenous	Dye	10	12.5	10	27	2.8	0.004
		No dye	14	9	6	24		
Respiratory arrest	Intrarenal	Dye	12	325.5	22	1200	0.71	0.503
		No dye	13	120	23	900		
	Intravenous	Dye	10	11	8	27	2.24	0.026
		No dye	14	9	6	19		

The use of Evans blue dye (EBD) shows a general trend toward elongated time to death, and the intrarenal group demonstrates greater variability in time to death than for the intravenous route when no EBD was used.

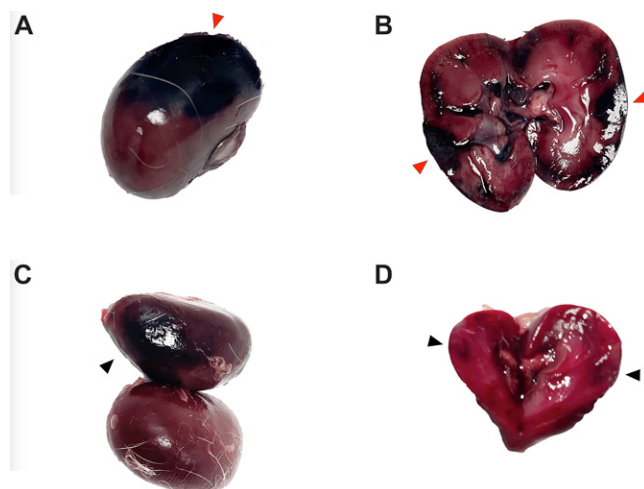


Figure 3. Injection confirmation into the renal cortex/medulla on gross and cut surface during necropsy of New Zealand White rabbits following intrarenal injections of pentobarbital. First and second analyses confirmed correct intrarenal placement into the renal cortex/medulla. Correct placement (C and D) confirmed that use of 2% EBD resulted in dark blue pyramidal shapes within the renal cortex/medulla (black arrows). Those without the use of EBD (A and B) were confirmed successful placement with areas of hemorrhage (red arrows).

or intrarenal euthanasia solution injections. Four of 13 animals (30%) experienced an initial increase in heart rate noted by auscultation at 16, 18, 28, and 120 s after intrarenal injection.

Isolation of kidneys and injection confirmation. Both kidneys were successfully palpated in all animals. For animals that were over-conditioned, the physical isolation of the kidney was noted to be more difficult but still obtainable. There were no observed complications associated with any of the intravenous or intrarenal injections of euthanasia solution. All animals in the intrarenal administration group were confirmed to have had correct intrarenal placement as evidenced by varying degrees of hemorrhage within these areas and that did not extend into the renal pelvis (Figure 3). Four of 13 animals administered solution without dye (30%) were noted to have palpable organ enlargement during treatment administration.

Time to arrests. When comparing animals without dye, statistical differences were seen for the cardiac and respiratory arrests between intravenous and intrarenal groups (Mann-Whitney test, $z = 4.43$, $P \leq 0.001$; Table 2, Figure 4). For cardiac arrest, the intravenous group ranged from 6 to 24 s, with a median of 9 s (0.15 min). The intrarenal group had greater variability in the time to cessation, ranging from 40 to 900 s, with a median of 411 s (6.85 min). For respiratory arrest, the arrest median following intravenous administration was 6 s (0.1 min), ranging from 6 to 19 s, and the median was 120 s (2 min), ranging from 23 to 900 s following intrarenal administration. Four of 13 intrarenal administration group animals (30%) needed a second dose of pentobarbital, as death was not achieved within the 15-min timeframe.

Gross necropsy and histology. Varying degrees of hemorrhage were seen along the kidney capsule and retroperitoneal space with no compromise to anatomic visualization (Figure 5). Intrarenally injected kidneys had a more gelatinous consistency on gross examination. On histology, most animals (18 of 24; 75%) scored a 0 in all categories. Two of 24 animals (8%) scored a hemorrhage severity of grade 1, 1 of 24 animals (4%) scored a grade 2 for hemorrhage, 1 of 24 animals (4%) scored a grade 2 for interstitial mineralization, 1 of 24 animals (4%) scored a grade 1 for interstitial mineralization, and 1 of 24 animals (4%) was noted to have a grade 1 for lymphoplasmacytic inflammation.

Discussion

The results of this study demonstrate that intrarenal injection of sodium pentobarbital offers an alternative route of administration in the rabbit. According to the 2020 *AVMA Guidelines for the Euthanasia of Animals*, intrarenal injection is an acceptable-with-conditions method of euthanasia for mammals with a dose recommendation of 3 mL/4.5 kg (10 lb). To simplify this study, we chose to give 3 mL/rabbit, as none of the animals weighed >4.5 kg (10 lb). Because death was not achieved for 30% of animals, it is possible that 3 mL/4.5 kg is not an appropriate dosage for intrarenal administration in this species and that a dosage increase may be needed to achieve adequate death, though further research is needed to support this hypothesis. In an anesthetized New Zealand White rabbit, the kidney is easy to isolate and intrarenal injection provides a straightforward and consistent approach as an alternative when intravenous or intracardiac techniques cannot be performed.

The first 2 analyses addressed potential adverse effects of the intrarenal route and led to modification of the third analysis. First, the administration of 2% EBD + sodium pentobarbital mixture proved that intrarenal administration was a feasible and successful route of euthanasia. Next, we compared the use of pentobarbital alone to pentobarbital mixed with 2% EBD. Interestingly, we found that inclusion of EBD was associated with a trend toward prolonged time to death. Because intrarenal delivery relies on parenchymal absorption, a thicker viscosity of the pentobarbital mixture with dye could delay absorption uptake.¹⁰ We also speculated that other pharmacokinetic properties could have had drug uptake interference; however, this was not explored in the present study. For example, both EBD and pentobarbital are highly serum protein bound, which could alter drug delivery to target tissues.^{5,11} Due to the findings of delayed euthanasia in these studies, EBD would not be recommended for use in future euthanasia studies, as newer technologies are available.^{5,11} Refinements from the first 2 studies resulted in the third analysis, which compared intravenous and intrarenal injections without the use of EBD. Postmortem evaluation confirmed the successful intrarenal injection placement on the cut surface of the organ at the time of necropsy for all animals, which suggests that the use of advanced imaging may not be necessary for future successful injection placements.

This study demonstrates that the intrarenal route is an acceptable euthanasia alternative to the intravenous route in

Table 2. Influence of administration route on time to cardiac and respiratory arrest

Outcome	Group	n	Median (s)	Minimum (s)	Maximum (s)	z	P
Cardiac arrest	Intrarenal	13	411	40	900	4.43	<0.001
	Intravenous	14	9	6	24		
Respiratory arrest	Intrarenal	13	120	23	900	4.43	<0.001
	Intravenous	14	9	6	19		

This table shows a significant difference between intravenous and intrarenal routes ($P < 0.001$).

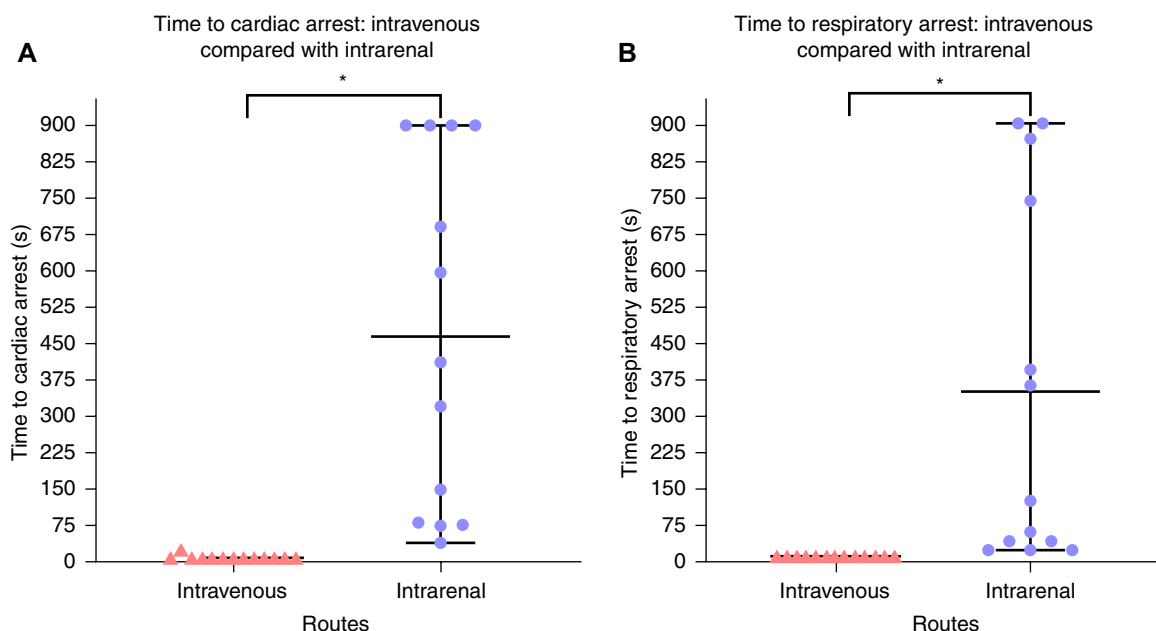


Figure 4. Clinical assessment for timepoints for cardiac and respiratory arrest comparing the intravenous and intrarenal routes of pentobarbital in rabbits. Animals that were noted to exceed the 15-min allotted timeframe were recorded at 15 min (900 s) and a second dose of euthanasia solution was administered. The black lines indicate the median with range. The intrarenal route demonstrates statistically significant differences, elevated times, and variability to arrest. *, $P \leq 0.001$.

the anesthetized rabbit, as most animals within the intrarenal administration group experienced a TCPA within a 1- to 10-min timeframe. This aligns with findings in cats in which 21% of cats died within 1.5 to 8 min following intrarenal injection.⁴ The most common reaction we observed in rabbits was tensing of the dorsal muscles in 19 of 27 animals (70%), noted at the time of sedation. We attributed these reactions to ketamine's acidic pharmaceutical composition, designed for increased shelf-life storage, and previous publications have described the occurrence of pain upon intramuscular injection of ketamine.¹² Although all animals were confirmed to be in a deep anesthetic plane prior to the pentobarbital injection, 4 of 13 animals (30%) demonstrated an initial increase in heart rate immediately after intrarenal injection. We believe that this could have been due to a physiologic or compensatory response. This effect has also been noted in an equine euthanasia study.¹³

We found that the intrarenal method of pentobarbital administration is associated with longer times to TCPA than the intravenous method, and it has greater variability. These disparities could be explained by the need for parenchymal absorption into the bloodstream and individual animal variations; and it can also be speculated that some degree of euthanasia solution may have leaked into the retroperitoneal space in some animals, resulting in a reduced dose entering the kidney. Although we were able to confirm correct parenchymal placement at necropsy, for those animals that needed a second dose, it could also be possible that a decrease in drug uptake from the intrarenal route was due to other factors such as pharmacologic property differences that could have interfered with pharmacodynamics; however, we did not explore the individual pharmacologic composition, interactions, and their relationship with the intrarenal route. It is also possible that the recommended 3 mL/4.5 kg dosage of

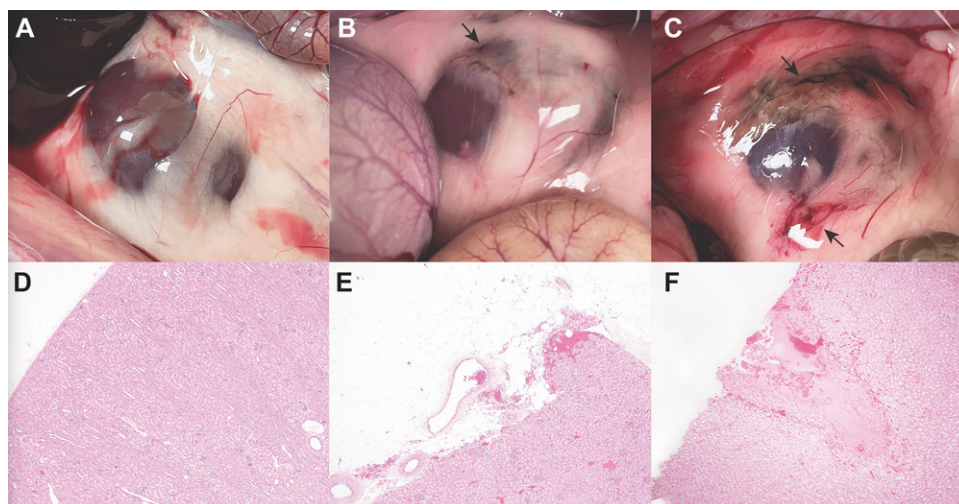


Figure 5. Gross and histologic necropsy findings of the left kidney after intrarenal injection with pentobarbital in rabbits. Varying amounts of hemorrhage within the retroperitoneal space and fat pads (black arrows) are present (A, B, C). Histology represents the image of a grade 0 (D), 1 (E), and 2 (F) hemorrhages, all taken at 40× original magnification.

pentobarbital may not be adequate enough to produce death in this species and that an increase in dosage may be needed when choosing this route, though further research is needed to support this. The intrarenal administration technique was easy to perform; however, in animals that were over-conditioned, more manual effort was needed to physically isolate the kidney, as the surrounding perirenal and intraabdominal fat deposits were increased. In most cases, performing the intrarenal injection in anesthetized rabbits was not technically challenging, but it can be difficult to confirm 100% injection accuracy without visual confirmation. Because intrarenal administration utilizes the kidney's blood supply for systemic delivery, a prolonged TCPA could be expected if there was not correct parenchyma placement.

Death was not achieved in 15 min for 4 of 13 animals (30%) in the intrarenal administration group. Mechanistically, barbiturates cause rapid CNS depression, followed by apnea, and then cardiac arrest.³ For the animals that did not meet the definition of death within 15 min, a second dose of pentobarbital was given intravenously. The time of death of these animals then resulted in values consistent with those of the intravenous administration group. It is possible that these animals would have had cessation in all parameters given a longer timeframe. We recommend considering the variability in the time lapse when performing the intrarenal technique, as it may not be appropriate in all situations. Future studies are needed to evaluate the effect of higher dosages of intrarenal pentobarbital administration on the TCPA in rabbits.

Another observation made using the intrarenal technique was a palpable organ enlargement upon injection. This enlargement, or more commonly known as 'kidney swelling,' has been anecdotally used as confirmation of successful injection placement but is not predictive of timely death.⁴ Only 4 of 13 animals were noted to have palpable kidney swelling at the time of injection. The TCPA values for these 4 rabbits varied greatly, suggesting that palpable kidney swelling does not correlate with shorter TCPA times. This is in contrast to a feline study that confirmed that a palpable organ enlargement was correlated with a quicker time to death.⁴

Gross necropsy and histology were conducted to assess whether intrarenal delivery produced any lesions that would impact research results. On gross examination, intrarenally injected kidneys took on a more gelatinous consistency but continued to retain normal histologic architecture. The most common observation was varying degrees of hemorrhage; however, the hemorrhage observed was not significant enough to obscure research results. This is an important correlation, as this route can be used even if the kidney is the target organ of research interest. The interstitial mineralization and lymphoplasmacytic inflammation were considered age-related and incidental in this study, as inflammation resulting from the intrarenal injection would have resulted in a dense heterophil population. The sample size was too small to make any correlations and determine whether these findings directly impacted intrarenal absorption, correlating with the varying times to death.

Some strengths of this study included the fact that, in the anesthetized rabbit, the kidney is successfully and easily palpated and isolated, injection can be done with relative ease, and there are relatively few gross or histologic distortions. It is our opinion that the success of intrarenal injection highly depends on whether the kidney can be isolated, and we recommend using the intrarenal technique in those species where the kidney can be readily and adequately palpated.

This study had several limitations. First, one individual performed all injection techniques to reduce variation and provide

a consistent comparison between the study groups. However, this individual could present a bias for technique due to preference or skill level. This individual was also not blinded to the administration groups, as it would have been difficult to have a blinded observer for data collection. Second, all animals were obtained from prior studies in support of the laboratory animal 3Rs (replacement, reduction, and refinement). Consequently, we did not control for factors such as age or variable body condition scores with respect to impact on TCPA. Finally, while comorbidities, such as renal disease, did not appear to interfere with time to death in cats, the efficacy of intrarenal administration of pentobarbital for euthanasia of rabbits with renal disease is unknown and requires further research.⁴ Challenges with this procedure can include kidney isolation difficulty on over-conditioned animals, difficulty in holding the kidney during the entire duration of the injection, or accidental injection into another abdominal organ or peritoneal space. As this is a technical skill, performance requires practice so that those conducting this procedure have both confidence and competence. Future directions include evaluating differences in sex, age, and body condition score effects on the pharmacokinetics of intrarenal pentobarbital, evaluating whether 3 mL/rabbit is an optimal intrarenal dosage to achieve death, and adding imaging guidance to its relationship to efficacy, practicality, and TCPA.

In conclusion, we determined that the intrarenal route is an effective alternative method for euthanasia of anesthetized rabbits with pentobarbital when intravenous or intracardiac access cannot be obtained. The intrarenal technique resulted in minimal histopathologic or gross renal changes. These experiments evaluated the ease and efficacy of the technique and the overall TCPA when performing this technique in the rabbit compared with the traditional intravenous route. Although intrarenal euthanasia has been previously performed on cats, it is apparent that the technique is not performed widely by practitioners, and consideration should be given to the possibility for species differences.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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