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**SPECIAL TOPIC SECTION**

***Experimental Use***

**Shomer et al. Review of Rodent Euthanasia Methods, pp. 242-253**

Domain 2

SUMMARY: The goal of this review is to provide guidance in rodent euthanasia based on a recent literature review. The overview will discuss evaluation of the effectiveness of euthanasia techniques, including a scoring rubric to assess euthanasia techniques.

Selecting the best method of euthanasia for a given study can be complicated and investigators must consider the effects that a given method may have on their data and also the impact of the method on the animal and on the ability of the other laboratories to reproduce the results of their studies. Published guidelines on euthanasia may help investigators choose euthanasia methods. The use of professional judgment and technical competence to make an assessment based on both the scientific requirements of the study and the welfare of the animals is primordial.

Euthanasia of large groups of rodents  when depopulation is required is done typically by inhalant CO2 or cervical dislocation, ideally in the animals’ home cages. Consider the CO2 gas exposure time for higher density of rats and mice: higher timeframe is required than for a single animal. If mixing animals of different ages, the CO2 exposure time must be sufficient for the least-susceptible animals.

Euthanasia of pregnant females is sufficient for euthanasia of the fetuses if they remain in utero. If the fetuses are removed from the amniotic sac after euthanasia of the dam and are able to breathe, they should be euthanized by an AVMA-approved method (cervical dislocation, decapitation, hypothermia, rapid freezing in liquid nitrogen or chemical anesthetic overdose). Age has the greatest effect on time to death after CO2 exposure (youngest animals require the longest exposure time); genetic background also influences the susceptibility of neonatal mice to CO2.

Euthanasia of rodents is frequently performed in an area separate from housing and breeding. Based on current literature, visual separation is not required for rats and mice. Professional judgment should be used to determine whether facilities offer sufficient separation to allow euthanasia to be performed in the same room with conspecifics.

CO2 narcosis and asphyxiation is recommended as a sole method of euthanasia if it can be delivered at a rate that rapidly induces loss of consciousness before inducing distress from the nociceptive and dyspneic effect of the gas. For small rodents (mice), AVMA recommends 10 to 30% fill rate but for some rodent models or strains, pre-anesthesia or faster CO2 delivery rate may be required to achieve human euthanasia. Recent studies support the humane use of fill rates of 30 to 70% to achieve faster loss of consciousness.

The CO2 gas displacement rate is critical to the human application of CO2 and an appropriate pressure-reducing regulator, flow meter, or restriction valve must be used. After the animal is unconscious (lost righting reflex or achievement of lateral recumbency), the flow rate can be increased to minimize the time to death.

The literature is not complete regarding euthanasia methods for all species, strains, and all ages of development. Until more conclusive research is available, due consideration should be given to minimizing pain or distress for the animal in question. Animals should be euthanized during research, teaching, testing or production in a way that ethically ensures the death is as painless and free of distress as possible.

QUESTIONS (True or False)

1. Mammalian embryos and fetuses are in a state of unconsciousness throughout pregnancy and birth; thus hypoxia does not evoke a response

2. Rats and mice have poor distance vision and have limited ability to clearly discern euthanasia or other procedures being conducted several feet away

3. CO2 prefilled chambers are acceptable method of rodent euthanasia

ANSWERS

1. True: even though fetal heartbeats may continue for an average of 30 to 60 minutes after euthanasia of the dam, the fetuses remain unconscious and are unable to experience pain or distress.

2. True

3. False

**Laferriere and Pang. Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents, pp. 254-263**

Domain 2: Management of Pain and Distress

Primary Species:Rat (*Rattus norvegicus*), Mouse (*Mus musculus*)

SUMMARY**:** Overdose with barbiturate is the only method of euthanasia in rodents approved by both the AVMA and CCAC and animal care guidelines in Europe, India and Australia. While intravenous injection is preferred, it is impractical for euthanizing rats and mice and is often administered by intraperitoneal (IP) injection. This article reviews literature about using IP pentobarbital for euthanasia in rats and mice and suggests potential alternatives.

IP Injection Technique: 2-person technique with one holder and one injector is considered more efficient with mice; reducing the misinjection rate. While in a horizontal position, the holder tilts the animal slightly downward, so the head is lower that its abdomen; creating more space in the caudal quadrants of the abdominal cavity.

Holding the right hindlimb with one hand, the injector injects into the caudal left quadrant at an injection angle approximately 10-20 degrees relative to the body wall in mice and 20-45 degrees in rats to a depth 4-5 mm.

Maximal injectable volume is 0.5-1.0 ml in mice and 5-10 ml in rats.

Pharmacokinetics: Sodium pentobarbital, an oxybarbiturate ligand of the gamma-aminobutyric acid (GABA) A receptors, increases chloride conductance through receptor channels causing neural hyperpolarization and CNS depression.

Absorption across the visceral peritoneum and omentum is the predominant route of absorption after IP injection. Other routes: absorption via the parietal peritoneum and lymphatic drainage. Existence of varied routes of absorption affects the consistency in timing of physiologic responses after injection.

Euthanasia in rats, a dose of 800 mg/kg of sodium pentobarbital IP is associated with greater consistency and speed of effect than 200 mg/kg; the commonly used dose. At 800 mg/kg, the time taken to achieve loss of righting reflex and apnea should not exceed 2.5-4.5 minutes unless misinjected.

Euthanasia in Mice: 150 mg/kg IP of sodium pentobarbital at a concentration of 50 mg/ml has been the suggested dose. Studies suggest a higher concentration of 390 mg/ml at a dose greater than 1300 mg/kg for males and 1680 mg/kg for females, greatly shortened time to unconsciousness and death.

Disadvantages of IP Injections of Sodium Pentobarbital: Variability (2 categories): inherent variability and misinjection.

Inherent Variability: Existence of different pathways of absorption and distribution.

Misinjection: Incorrect placement of administered substances which can lead to a marked delay in drug onset of action, irritation, inflammation, perforation of organs and respiratory distress.

In rats, common sites of IP misinjection: cecum, small intestine, subcutaneously, retroperitoneally and into the urinary bladder.

In mice, frequent sites for misinjection: stomach, small intestine, uterine horn, and subcutaneous.

About 20% of rats and mice have been identified as having misinjections on necropsy. 2-person technique reduces this number.

Histopathologic and Physiologic Changes: Pentobarbital can damage local tissue and superficial cells of organs near the injection site and splenomegaly from smooth muscle relaxation.

Pain Associated with IP Pentobarbital: Writhing, an abnormal posture in both rats and mice after IP injection recognized as a behavioral response to abdominal pain. Rats contract their abdomen and extend their hindlegs backward. Incidence of writhing varies due to difficulty assessing motor behavior while using an anesthetic.

Using c-fos and Fos as neuronal markers for measuring pain via immunohistochemical staining led to use of FLI (fos-like immunoreactive) neurons in the spinal cord.

c-fos: an early response protooncogene, rapidly expressed in specific pain receptive neurons of the dorsal horn after noxious stimulation.

c-fos gene encodes for protein Fos: an intermediary between extracellular events and long-term intracellular changes. Quantification of FLI neurons has been used to infer pain. FLI neurons increased after IP pentobarbital.

Adding lidocaine to IP pentobarbital lowered the number of FLI neurons in the spinal cord. Using analgesics reduced occurrence of writhing behavior and concurrently reduced the number of FLI neurons.

Relationship between FLI neuron activity and cortical perception of pain is still unclear and is only used to draw inferences about presence of pain.

Stress: Has been inferred using ACTH levels and plasma corticosterone but results varied.

IP injection has been associated with hyperthermia and tachycardia in rats and mice.

Stress from handling can be reduced by habituation.

Alternatives to Intraperitoneal Pentobarbital

*Ethanol*: Currently described in AVMA guidelines as “acceptable with conditions”

100% ethanol, at 15.3-15.8 g/kg (0.5 ml) IP in mice causes similar onset of respiratory and cardiac arrest as similar volume of pentobarbital; while not a controlled substance.

Not in rats as the volume was too large and time from injection to respiratory arrest slow.

Retroorbital injection of overdose of ketamine and xylazine in mice has resulted in rapid death, but not evaluated in rats.

Intrahepatic injection technique has been used in cats but not yet in rodents.

Best-Practice Recommendation:

1. Within an institution establish a standard dose, volume, and formulation of sodium pentobarbital.

2. Ensure that users are proficient in steps required to perform IP injection (drug calculation, restraint, injection, signs of death)

3. Consider monitoring key outcomes as a means of tracking consistent practice.

QUESTIONS

1. The preferred technique for administering sodium pentobarbital IP in rodents is the:

1. One-person technique
2. 2-person technique
3. 3-person technique

2.  When administering an IP injection, the rodent is maintained in a vertical position (T/F)

3.  Sodium pentobarbital causes death by:

1. Causing an influx of potassium depolarizing the myocardial membrane, resulting in a diastolic arrest
2. Causing hypernatremia, resulting in cerebral edema and subsequent death
3. Increasing chloride conductance through receptor channels. causing neural hyperpolarization and CNS depression

4. The predominant route of absorption after IP injection is:

1. Via the parietal peritoneum and lymphatic drainage.
2. Via the visceral peritoneum and omentum
3. Via the gastric mucosa

5. Writhing is:

1. Reduced ambulation in rats and mice; recognized as a behavioral response to abdominal pain
2. Abnormal posture in rats and mice; recognized as a behavioral response to abdominal pain
3. Eye squinting and changes in ear and whisker position in mice and rats; recognized as a behavioral response to abdominal pain

6. Quantification of FLI neurons in the dorsal horn of the spinal cord has been used to infer pain (T/F)

7. Misinjections have been identified in approximately \_\_\_\_\_ % of rats and mice at necropsy:

1. 40-50 %
2. 30-40 %
3. 20-30 %
4. 10-20 %

8. 100% Ethanol has potential as an acceptable alternative for euthanasia in:

1. Rats
2. Mice
3. Both
4. Neither

9. Retroorbital injection of overdose of ketamine and xylazine has resulted in rapid death in:

1. Rats
2. Mice
3. Both
4. Neither

10. Stress is mice and rats have been associated with:

1. Trembling
2. Alopecia
3. Hyperthermia and tachycardia
4. Tachypnea

ANSWERS

1. b

2. False

3. c

4. b

5. b

6. True

7. d

8. b

9. b

10. c

**Laferriere et al. Evaluating Intrahepatic and Intraperitoneal Sodium Pentobarbital or Ethanol for Mouse Euthanasia, pp. 264-268**

Domain 2: Management of pain and distress; T4. Euthanatize (Euthanize) and K7. euthanasia

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The goal of this study was to determine if intrahepatic (IH) injection of pentobarbital (PB) led to a more humane euthanasia than intraperitoneal (IP) injection.  Other factors considered in the study were the use of isoflurane prior to injection and the use of ethanol as opposed to pentobarbital.  Comparisons were made for accuracy (number of missed injections as determined by necropsy), time from injection to apnea, and time to cessation of heartbeat (CHB).

The IP misinjection rate varies from 10-20%, with the most common sites of misinjection being the GI (stomach or intestine), the uterine horn, and the skin (accidental SQ).  Barbiturates can be challenging to acquire (DEA controlled, limited production), while ethanol (ET) is more readily available.  Ethanol is mentioned in the AVMA guidelines on euthanasia, but little research has been conducted using it as an alternative to pentobarbital, and current published times indicate a prolonged period to death.  Intrahepatic injection of barbiturates is approved in unconscious dogs and cats.

Study design:  prospective, randomized, blinded study.  80 adult SPF, CD1 male and female mice which required euthanasia for another study.  PB dose: 5.4 g/kg.  ET dose:  96%.  Approximate volume of each injection: 0.55mL.

Treatment Groups (Phase 1, 15 Per Group, Anesthetized With Iso Prior To Dosing)

* IH injection of PB (IH PB)
* IH injection of ET (IH ET)
* IP injection of PB (IP PB)
* IP injection of ET (IP ET)

Treatment Groups (Phase 2, 10 Per Group, No Anesthesia)

* IP injection of ET (IP ET awake)
* IP injection of PB (IP PB awake)

Results

* Misinjections with anesthetized IP:  3/40
* Misinjections with anesthetized IH:  28 of 40
* Misinjections awake IP:  1/20
* 1 IP misinjection failed to result in death within 5 minutes.  Those that did die were after 146, 154 and 265s
* IP ET was superior to IP PB in quickly achieving apnea and CHB regardless of the presence of anesthesia
* Time to apnea was achieved more quickly in anesthetized animals in comparison to awake animals

Conclusions

* IH injection not recommended
* IP ET can be used as an alternate to PB.  Death resulted faster than previously published.
* Isoflurane anesthesia decreased the time to death for both IP PB and ET.

QUESTIONS

1. Per the 2020 AVMA guidelines on Humane Euthanasia, IP injection of Sodium Pentobarbital in rodents is considered to be:

* 1. Acceptable
	2. Acceptable with conditions
	3. Unacceptable
	4. The guidelines are silent on this issue

2. Per the 2020 AVMA guidelines on Humane Euthanasia, IP injection of 70% ethanol in rodents is considered to be:

1. Acceptable
2. Acceptable with conditions
3. Unacceptable
4. The guidelines are silent on this issue

3. True or False:  Barbiturate combinations (such as Euthasol) can be administered IP in rodents since the combination decreases pain associated with the injection.

4. Per the 2020 AVMA guidelines on Humane Euthanasia, proper positioning for intrahepatic injection in cats is:

* 1. Dorsal recumbency
	2. Lateral recumbency
	3. Upright position with the forequarters elevated
	4. Upright position with the forequarters lowered

5. Per the 2020 AVMA guidelines on Humane Euthanasia, ethanol injected IP in rodents should be greater than:

* 1. 60%
	2. 70%
	3. 80%
	4. Ethanol is not an approved method of euthanasia.  Only Tribromoethanol (Avertin) is.

ANSWERS

1. a

2. b

3. False

4. c

5. b

**Galex et al. Evaluation of Effective and Practical Euthanasia Methods for Larval African Clawed Frogs (*Xenopus laevis*), pp. 269-274**

Domain 2: Management of Pain and Distress; Task 3: Euthanize

Secondary Species: African clawed frogs (*Xenopus spp.)*

SUMMARY: The current AVMA Guidelines for the Euthanasia of Animals does not provide specific guidance for the euthanasia of amphibian larvae. This group investigated the efficacy of three methods of euthanasia on larval Xenopus at various developmental stages (NF stages 46, 47, and 49). The methods investigated were immersion in 6 g/L solution of MS222, immersion in 800 uL/L eugenol, and rapid chilling via immersion in an ice bath at 2 to 4°C.

Developmental stages 46, 47, and 49 represent NF stages in which external gills were present, but the lungs are still developing, and their functionality is likely variable.

Immersion in MS222 at the described concentration for a period of 15 minutes consistently resulted in heartbeat cessation. The use of Eugenol had varying success based on the age of the tadpoles. Eugenol was also found to have varying effectiveness based on the dose and exposure time. Rapid chilling was not found to be effective. This group found that not only did the animals exposed to rapid chilling not have cessation of heartbeats, but they all regained movement over time once placed in the recovery tank.

This study shows that immersion of groups of tadpoles in MS222 for 15 minutes at a concentration of 6 g/L is effective as a euthanasia method.

QUESTIONS

1.   T or F: The developmental stages of xenopus are called Nieuwkoop and Faber stages.

2.   What is the mechanism of action of MS222?

3.   What is the mechanism of action of eugenol?

4.  T or F: Eugenol, isoeugenol, and clove oil are acceptable euthanasia methods for frogs.

ANSWERS

# 1.  True: Illustrated in the text Normal table of Xenopus laevis (Daudin) : a systematical and chronological survey of the development from the fertilized egg till the end of metamorphosis

2. MS222 is a local anesthetic that works through stabilization of cellular membranes, by inhibiting transient increases in sodium ion permeability thereby decreasing excitability and blocking impulse conduction.

3.   The exact mechanism of action is unknown. It has been shown to interrupt action potentials by inhibition of voltage-gated sodium channels.

4.   False: Although they have all be used successfully as anesthetics in frogs the AVMA has only established eugenol as an acceptable euthanasia method in finfish.

**ORIGINAL RESEARCH**

***Biology***

**Strumpf et al. Hematologic Values of Jamaican Fruit Bats (*Artibeus jamaicensis*) and the Effects of Isoflurane Anesthesia, pp. 275-281**

Domain 3: Research

Tertiary Species: Other Mammals

One-Line Summary:This paper provides information on the baseline hematological parameters in a captive population of Jamaican fruit bats (JFB) (*Artibeus jamaicensis*).

SUMMARY: The objectives of the paper were two-fold, 1. to establish baseline parameters for a captive population of JFB and 2. to compare the hematologic values of physically restrained (unsedated) and anesthetized bats.  The animals were captured, restrained or anesthetized and their wings held close to an infrared heat lamp.  The heat from the lamp induced vasodilation of the propetigial (cephalic) vein. Then using a 26-gauge intradermal needle, a small laceration was made in the vein, which allowed the free flow of blood.  The blood was collected using heparinized microhematocrit capillary tubes.

The results showed significant differences in hematologic parameters between physically restrained (unsedated) and anesthetized bats, particularly with the WBC parameters, showing lower total WBC, lymphocyte and monocyte counts in physically restrained bats.  Additionally, a higher lymphocyte-to-granulocyte ratio was observed.

QUESTIONS

1.  What diseases are the Jamaican Fruit Bats animal models for?

2.  What is the weight range of a Jamaican Fruit Bat?

3.  What are the typical characteristics of a stress leukogram?

4.   What are the effects of isoflurane on the cardiovascular system?

ANSWERS

1.  Middle East Respiratory Syndrome virus (MERS), Dengue virus, Zika virus, Tacaribe virus and Rabies virus.

2. Between 40 and 60g.

3.   A stress leukogram id characterized by mature neutrophilia and lymphopenia.

4.  Alteration of cardiac output, decrease in stroke volume and an increase in heart rate.

***Reproduction***

**Chesney et al. Using Vaginal Impedance Measurement to Identify Proestrus in Rats Given Luteinizing Hormone Releasing Hormone (LHRH) Agonist, pp. 282-287**

Domain 3 and 4: Research and Animal Care

SUMMARY: Vaginal cytology is considered the “gold standard” for staging the estrous cycle and is commonly used in rats to determine the best time for timed-mating.  Vaginal impedance measures naturally occurring changes in electrical resistance of the vaginal mucosa during different stages of the estrous cycle.  A value of >3 kiloohms (kΩ) is indicative of proestrus while the other 3 stages of estrus have a value lower that 3 kΩ.  The goal of the current study was to evaluate the accuracy of vaginal impedance measurements in determining when a female is in proestrus.  The study compared vaginal impedance measurements with vaginal cytology in female Sprague Dawley rats that were given 40 μg LHRH agonist 72 hours prior to sampling.

Two studies were conducted with 36 rats each.  The first study compared a single vaginal impedance measurement directly with vaginal cytology results.  Females were paired with a male on day 4 following the LHRH (the expected day of estrus).  The second study conducted sequential vaginal impedance measurements to determine when the 3.0 kΩ threshold was reached.  Females were paired with a male 24 hours after the reached the proestrus threshold reading.

In the first study, the vaginal impedance readings and vaginal cytology were in agreement of proestrus 78% of the time (14/18 rats); however, only 5 rats were confirmed pregnant at necropsy.  The vaginal impedance and vaginal cytology had the same false positive (28%) and false negative (31%) proportions.  In the second study, 29 had a vaginal impedance reading > 3kΩ on the expected day of proestrus and 24 were confirmed pregnant at necropsy.  Females that reached the threshold were more likely to be confirmed pregnant than those that did not. The study demonstrated that vaginal impedance had similar false positive and false negative rates to vaginal cytology and can potentially be used as an alternative method in monitoring proestrus.  Sequential impedance testing did result in higher numbers of pregnant animals.

QUESTIONS

#### 1. What stage of the estrous cycle is characterized by round, nucleated epithelial cells of uniform size?

* 1. Diestrus
	2. Proestrus
	3. Estrus
	4. Metestrus

2.  T/F: Vaginal impedance is limited in that it can only distinguish proestrus from the other 3 stages of estrus.

3.  T/F:  A single dose of LHRH (luteinizing hormone releasing hormone) was sufficient to induce estrous synchronization in this study.

ANSWERS

1.     b

2.     True

3.     False

***Husbandry***

**Carpenter et al. Effects of Trio and Pair Breeding of Mice on Environmental Parameters and Nasal Pathology and Their Implications for Cage Change Frequency, pp. 288-297**

Domain 4

Primary Species: Mouse (*Mus musculus*)

SUMMARY: There are concerns with air quality when mice are stocked at high housing densities. Although the Guide provides space recommendations for housing, cage change frequencies are left up to professional judgement. Here male and female CD1 mice were housed in pairs or trios. Cages were microisolator cages housed on individually ventilated racks or static-free standing wire shelving racks. The caging systems were alternated for a total of 3 breeding rounds. Ammonia and carbon dioxide were measured daily starting when pups were born until weaning. The amount of urine-soaked bedding was scored, and hair coat, eyes and posture were evaluated. Nest quality was scored. Ventilated cages were changed every 14 days and static every 7 days. Tissues were obtained from weanlings 21-23days of age for nasal pathology. Mean ammonia and carbon dioxide were non significantly higher for static cages. There were no significant differences between trios and pairs in either static or ventilated housing. Mean intracage ammonia levels increased over time after cage change for all breeding and housing scenarios whereas carbon dioxide did not increase. All groups had carbon dioxide levels greater than the 5,000 threshold as soon as 1 day after cage change. Static cages were significantly more humid and exceeded the 70% maximum and bedding took significantly less time to become wet. Pups born to paired breeders in static cages were smaller and pups born to trio breeders in ventilated cages were larger than pair breeders in ventilated cages. Nasal lesions in weanlings increased in severity and incidence with increasing ammonia and weanlings from static trio groups had the most severe nasal lesions overall.

QUESTIONS

1. What is generally used as a guideline for the maximum ammonia threshold in rodent cages?

a. 25ppm

b. 60ppm

c. 80ppm

d. 100

2. T/F: Mean intracage ammonia levels increased after cage change over time for all scenarios whereas carbon dioxide did not increase over time.

3. Which of the following weanlings had the most severe nasal lesions?

a. Static pairs

b. Static trios

c. Ventilated pairs

d. Ventilated trios

ANSWERS

1. a

2. True

3. b

***Anesthesia***

**LaTourette et al. Effects of Standard and Sustained-release Buprenorphine on the Minimum Alveolar Concentration of Isoflurane in C57VL/6 Mice, pp. 298-304**

Domain 2: Management of Pain and Distress

T1. Recognize pain and/or distress

T2. Minimize or eliminate pain and/or distress

T3. Administration of anesthesia

SUMMARY

**Introduction:** Many opioids reduce the minimum alveolar concentration (MAC) of isoflurane required to maintain mice at a surgical plane of anesthesia. Multimodal anesthetic protocols minimize isoflurane side effects of hypotension, hypothermia, and respiratory suppression. Isoflurane MAC is the concentration of isoflurane needed for 50% of mice to reach a surgical plane of anesthesia. This study compares the isoflurane MAC sparing effects of subcutaneously injected 1) standard form buprenorphine 0.1mg/kg (Bup), 2) sustained-release buprenorphine 1.2mg/kg (Bup-SR), and 3) crystalloid fluid (control). Terminal laparotomies were performed on a subset of each treatment group at the minimum 100% effective for surgical plane isoflurane concentration for each treatment group.

**Materials and Methods:** 51 C57BL/6J (36 male, 27 female ) 8-16wk

Exp 1: MAC was determined for each drug by 61 anesthetic events (36 male, 27 female) which included 2 randomized isoflurane doses. Each trial included a 20min acclimation period at the lower isoflurane concentration before the first 300-g noxious stimulus test followed by 10 minutes to acclimate at the second isoflurane concentration and the last 300-g noxious stimulus test.  MAC was determined using both bracketing analysis and quantal analysis. Bracketing analysis estimated the isoflurane concentration each mouse transitioned from a positive response to noxious stimuli to negative. Quantal analysis graphed each isoflurane concentration used versus the percentage of mice at a surgical plane at that concentration. HR, RR, and genders also compared.

Exp 2: Mice were randomly assigned terminal laparotomy or sham laparotomy and procedure performed under 2.0% isoflurane for the control group and 1.7% isoflurane for both buprenorphine groups due to the 15% decrease in MAC values found in Exp 1 through bracketing analysis.

**Results**

Exp 1:

* Bup significantly decreased isoflurane requirements 25.5% and decreased MAC to 1.34% ± 0.08%.
* BupSR significantly decreased isoflurane requirements 14.4% and decreased MAC to 1.54% ± 0.09%.
* Gender had no significant effect on HR or RR during MAC determination in Exp 1.

Exp 2:

* All laparoscopic procedure mice successfully reached surgical plane at 1.7% isoflurane.
* Time to surgical plane more variable with buprenorphine: control 9.8 ± 0.9min, Bup 14.6 ± 9.0min, BupSR 16.9 ± 11.0min.
* Gender had no significant effect on HR or RR;  significant effect due to time.
* Greenhouse-Geisser analysis significant differences in RR versus drug, procedure type, and time.

**Discussion:**

* Bup and BupSR exhibit MAC sparing effects in mice undergoing isoflurane and successfully be maintained at a surgical plane.
* Factors that could affect time to effect include individual response to drugs and potential microbiome differences.
* MAC values may be affected by age, strain, health status, nature of noxious stimulus, and site of noxious stimulus (tail less sensitive than hindlimb requires less anesthetic).
* Individual mouse response to isoflurane is affected by consciousness state when administered due to neuronal inertia.

QUESTIONS

1. What is the duration of analgesia for standard form buprenorphine and for suspended release buprenorphine?
2. Describe the bracketing analysis method used in this paper.
3. What is generated by the quantal analysis and used to determine MAC?
4. What are some factors that can affect MAC values?

ANSWERS

1. Standard form buprenorphine (Bup) 3-6 h in mice depending on dose

 Suspended release buprenorphine (Bup-SR) Greater variability, some studies say up to 12 h, 24 h, or up to 48 h

1. The isoflurane concentrations between positive and negative responses were averaged. This provided an estimate of isoflurane concentration at the transition.
2. Dose response curve



1. Age, strain, health status; nature of noxious stimulus (compression, surgical incision, intubation); and site of noxious stimulus (tail less sensitive than hindlimb requires less anesthetic to eliminate reflex)

**Mills et al. Pharmacokinetic Profiles of Gabapentin after Oral and Subcutaneous Administration in Black-tailed Prairie Dogs (*Cynomys ludovicianus*), pp. 305-309**

Domain 2: Management of Pain and Distress

Tertiary Species: Other Rodents

SUMMARY: Black-tailed Prairie Dogs (*Cynomys ludovicianus*) are used a biomedical model for infectious diseases, hepatobiliary disease, oxygen consumption and hibernation research.

Gabapentin is a commonly prescribed analgesic for neurological and chronic pain. However, little is known about gabapentin in black-tailed prairie dogs. Studies involving meloxicam and buprenorphine have shown that prairie dogs may have atypical pharmacokinetic responses to standard rodent doses. For example, standard doses (0.2-0.4mg/kg) of meloxicam result in sub therapeutic plasma concentrations. However, higher doses resulted in insufficient elimination.

This study investigated the pharmacokinetic profile of gabapentin. 24, male and female, two-year-old, wild caught prairie dogs where allocated to 4 treatment groups:

* High dose oral gabapentin
* Low dose oral gabapentin
* High dose subcutaneous gabapentin
* Low dose subcutaneous gabapentin

The half-life of gabapentin in other species is 2-7 hours. In prairie dogs, the half-life varied due to both initial dose and route of administration:

* High dose oral gabapentin – half-life = 4.98 hours
* Low dose oral gabapentin – half-life = 7.39 hours
* High dose subcutaneous gabapentin – half-life = 3.58 hours
* Low dose subcutaneous gabapentin  – half-life = 3.18 hours

Only the low dose oral gabapentin was effective in maintaining the concentration of gabapentin required to produce analgesia in other rodent species without exceeding it.

QUESTIONS

1. What viral infectious disease is studied with the black-tailed prairie dog model?
2. By what mechanism does gabapentin reduce pain?
3. Why is gabapentin used in nerve injury models?

ANSWERS

1. Monkey pox
2. Binds to α2δ ligands of calcium-voltage-gated subunit receptors, thereby reducing excitatory neurotransmitter secretions e.g. Substance P
3. Neuroprotective properties e.g. axonal regeneration and anti-inflammatory activity via release of antioxidants

***Experimental Use***

**Gehling et al. Investigation of Various Intramuscular Volumes Delivered to the Semimembranosus Muscle of *Cavia porcellus*, pp. 310-321**

Domains 2 and 3

Secondary Species: Guinea pig (*Cavia porcellus*)

SUMMARY: Guinea pigs are one of the most commonly used animal models in vaccine research against important viral diseases such as Lassa and Ebola viruses. Inaccuracies in administration of IM injections in these preclinical vaccine studies could lead to distorted findings and thereby, complicate translational application of these results in human patients. Previous studies lack accurate recommendations on intramuscular injection volumes in this species. Thus, authors of the current study designed a series of experiments to provide accurate quantitative data on ideal volume IM injection in Hartley guinea pigs that weigh between 320 to 410 grams. Through Computed Tomography (CT) imaging, dispersion leakage of various volumes of radiocontrast agent (iohexol) was investigated. The results of this experiment show that IM volumes of 150 and 300 μL remain within the target muscle without leakage. Due to the potential of Iohexol causing acute renal toxicity and local tissue necrosis, saline (0.9% NaCl) injections were used to assess pain and tissue pathology of various volumes of IM injections. There was no correlation between volume and pain, or activity levels assessed 72 hours post-injection. However, the potential pain induced by larger volumes at the time of injection could not be assessed because these injections were done in sedated animals. Injection site pathology at 72 hours post-injection was related to needle track than the volume of injectate. Although many other factors such as injectate pH, viscosity, temperature and osmolarity of the injectate and needle size (25g needles were used in the current study) could affect the tissue distribution, pain, distress and tissue pathology, which were not evaluated in this study but the current study concluded that the ideal volume of isotonic injectate for IM injection in the semimembranosus muscle of guinea pigs should be less than 500 μL.

QUESTIONS

1.  The ideal volume of IM injection for precise and accurate delivery in semimembranosus muscle of Guinea Pig weighs between 320 to 410 grams.

a. 150 to 300uL

b. 300 to 500uL

c. 500 to 1000 uL

d. 1000 to 1500 uL

e. all the above

2. T/F: Guinea Pigs are one of the most commonly used laboratory animal species for vaccine research.

3. T/F: Iohexol can cause local tissue necrosis and acute renal toxicity

ANSWERS

1. a

2. True

3. True

**Hoareau et al. Reference Intervals for and the Effects of Sample Handling and Sex on Rotational Thromboelastometry in Healthy Adult Pigs, pp. 322-327**

Domain 3: Research

Primary Species: Pig (*Sus scrofa*)

SUMMARY:This paper sought to establish reference intervals for rotational thromboelastometry (ROTEM) values in Yorkshire cross pigs. In addition, the authors wanted to determine the effect of several different factors (e.g. sex, sampling order, preanalytical sample agitation, hematocrit, fibrinogen, platelet count) on ROTEM results.

The reference intervals for all parameters are provided in the publication. Sex, preanalytical sample agitation (using a rocker), hematocrit, and platelet count did not significantly affect ROTEM results. Sampling order affected clot formation time and α angle. Fibrinogen concentration influenced clot firmness after 10 minutes and 20 minutes, maximum clot firmness, α angle, and clot formation time.

QUESTIONS

1. True/False: Rotational thromboelastometry is considered a viscoelastometric method of assessing coagulation

2. Which of the following is the benefit of rotational thromboelastometry over traditional coagulation tests (e.g. PT, PTT)?

a. It only evaluates the plasma components of coagulation

b. It evaluates the entire coagulation cascade (including platelet function and fibrinolysis)

c. It only provides static information and not information on the functionality of coagulation components

d. It only evaluates the cellular components of coagulation

3. Which of the following is true when comparing coagulation in pigs vs. humans?

a. Pigs exhibit a more procoagulant profile

b. Pigs exhibit weaker clots with poor viscoelastic properties

c. No differences exist

d. Humans have higher serum fibrinogen concentrations

ANSWERS

1. True

2. b

3. a

***Animal Health Surveillance***

**Luchins et al. Detection of Lactate Dehydrogenase Elevating Virus in a Mouse Vivarium Using an Exhaust Air Dust Health Monitoring Program, pp. 328-333**

Domain 4

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Lactate dehydrogenase-elevating virus (LDV), an arterivirus and common cell culture contaminant, does not usually cause clinical signs in mice but can impact immunologic research conducted in infected mice. Live-animal sentinels are not a reliable means of detecting the presence of the virus in an animal facility. Exhaust air dust (EAD) health monitoring has been demonstrated to be effective for detecting various murine pathogens and has demonstrated increased sensitivity compared to soiled-bedding sentinels. This is the first report to demonstrate the use of environmental PCR analysis for detection of LDV infection in a mouse vivarium.

Initial EAD PCR was positive for LDV in 2 adjacent rooms, however, confirmatory testing was negative, and the initial nucleic acid copy number was low, therefore, it was assumed that the initial results were false positives. At the next quarterly test, one of the racks was again positive for LDV and again confirmatory PCR was negative. The diagnostic lab advised that a sample is considered to be LDV-positive for any copy number detected, given that the virus is uncommon, and the assay is very specific for it, and an outbreak of LDV on the rack was presumed. Mice housed on the rack had been inoculated with various PDX cell lines, all of which tested positive for LDV via PCR. Animals receiving contaminated tumors were culled. Breeding animals housed in the rack PCR performed on oral swabs and all animals tested negative. After all animals exposed to contaminated tumors were culled, the rack was sanitized, and new EAD collection media was placed. After sanitizing the rack, EAD PCR was again positive for LDV, despite no additional use of the contaminated cell lines. The rack was depopulated and blood samples from one mouse per cage were submitted for PCR. PCR indicated that 94% of the tested animals were positive for LDV. After sanitization of the rack and placement of new EAD collection media, quarterly PCR results were negative.

QUESTIONS

1.    How is LDV primarily transmitted in laboratory mice?

a.   Transplacentally

b.   Horizontally through direct contact and aerosols

c.   Through tumor stocks, cell lines, and mouse-derived biological materials

d.    Fecal-oral transmission

2.   What is the long-term outcome for animals infected with LDV?

a.   Animals are transiently viremic then clear the organism, after which they are resistant to re-infection

b.    Animals become persistently viremic

c.    Immunocompromised mice, such as NSG mice, quickly succumb to infection and die within 5-7 days after infection

d.  Animals persistently excrete virus in their saliva

3.   How is LDV best characterized?

a. RNA virus, enveloped, family *Arteriviridae*

b.   RNA virus, enveloped, family *Arenaviridae*

c.   RNA virus, unenveloped, family *Picornaviridae*

d.  RNA virus, unenveloped, family *Caliciviridae*

ANSWERS

1. c

2. b

3. a