

Workshop in Laboratory Animal Medicine

Charles Louis Davis, D.V.M. Foundation

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Literature Review: 2009 JAALAS

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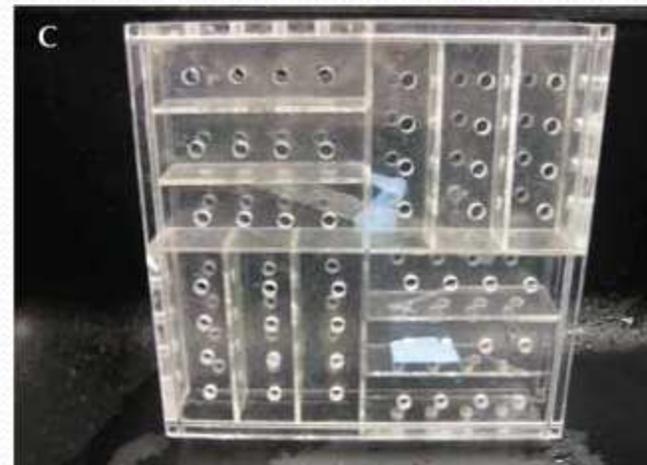
January 2009, vol. 48, No.1



Name the equipment and type of procedure that is taking place:

**Holding container
placed inside a drum of
an cesium irradiator**

Principles of Bone Marrow Transplantation (BMT): Providing Optimal Veterinary and Husbandry Care to Irradiated Mice in BMT Studies; JAALAS, vol. 48, No.1



Name the device:

Acrylic irradiation holding containers

January 2009, vol. 48, No.1

(AVMA) Panel on Euthanasia defines which of the following as “not acceptable” methods of euthanasia for rodents and other small mammals?

- a) barbiturates,
- b) carbon monoxide
- c) nitrogen
- d) microwave irradiation
- e) CO₂ generated by dry ice

Euthanasia of Neonatal Rats with Carbon Dioxide; JAALAS, vol. 48, No.1

This study evaluated the length of CO₂ exposure to euthanize 0 to 10 day old neonatal rats.

Did they find a difference between inbred and outbred stocks?

No.

Euthanasia of Neonatal Rats with Carbon Dioxide; JAALAS, vol. 48, No.1

Which of the following inversely varied with time of death by CO₂?

- a) Stock (F₃₄₄ vs. CD rats)
- b) number of animals in container to be euthanized
- c) Age
- d) litter size
- e) Activity during CO₂ exposure

Euthanasia of Neonatal Rats with Carbon Dioxide; JAALAS, vol. 48, No.1

Which of the following inversely varied with time of death by CO₂?

- a) Stock (F₃₄₄ vs. CD rats)
- b) number of animals in container to be euthanized
- c) **Age**
- d) litter size
- e) Activity during CO₂ exposure

Summary: The time of death increased steadily with increasing age. Euthanasia of day 0 animals required at least 35 min exposure to CO₂, a 5-min CO₂ exposure was sufficient for euthanasia at 10 d of age.

Suggested CO₂ exposure time for euthanasia of rats of various

<u>Appearance of animal time(min)</u>	<u>CO₂ exposure</u>
Nonhaired pups (0 to 6 d of age)	40
Haired pups, eyes closed (7 to 13 d of age)	20
Haired pups, eyes open, preweaning (14 to 20 d of age)	10
Weanlings and adults (21 d of age and older)	5

Note : The sample exposure chart presented here applies to the exposure chamber and filling parameters used in this study. The use of other chamber types and fill rates will require separate calibration. All euthanasia times recommended in this table should be validated at each facility, using current equipment and animals: table from paper K. Pritchett-Corning; **JAALAS; vol 48, No.1, pages 23-27**

JAALAS, vol. 48, No.1

Which of the following is listed by AVMA Guidelines as not “**conditionally acceptable**” for euthanasia of reptiles?

- a) penetrating captive bolt
- b) gunshot
- c) decapitation and pithing
- d) stunning and decapitation
- e) barbiturates

Use of Tricaine Methanesulfonate (MS222) for Euthanasia of Reptiles; JAALAS, vol. 48, No.1

The overall objective of the study was to evaluate the efficacy and practicality of intracoelomic injection of MS222 for euthanasia in reptiles.

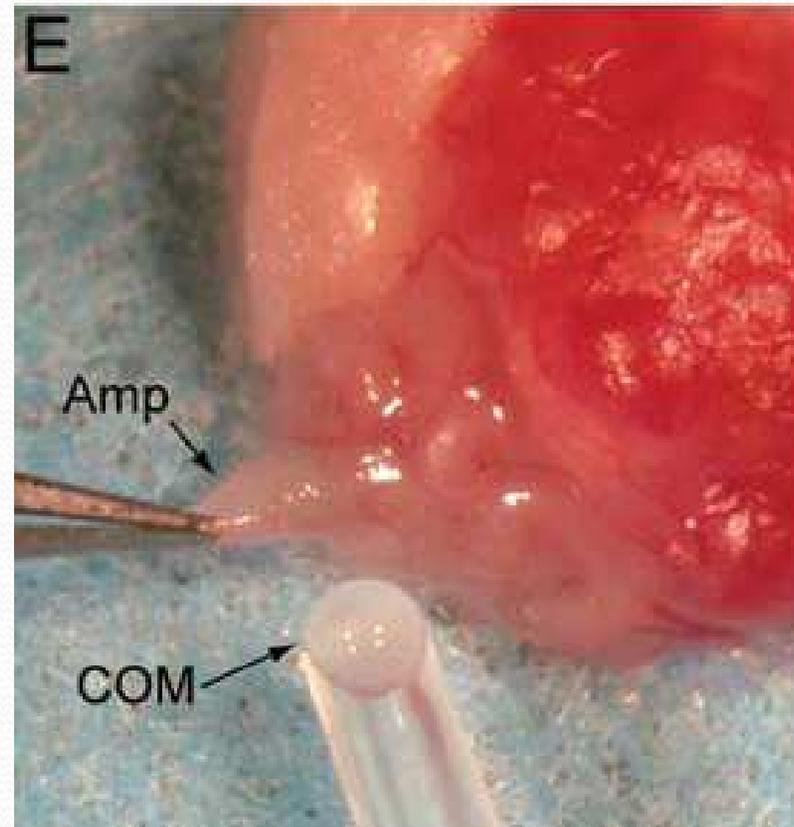
The study concluded that intracoelomic injection of 250 to 500 mg/kg of 0.7% to 1% sodium bicarbonate-buffered MS222 solution resulted in rapid loss of consciousness in all reptiles tested. Respiratory and cardiac functions remained intact until intracoelomic injection of 0.1 to 1.0 ml *unbuffered* 50% (v/v) MS222 solution

Sperm Freezing and In Vitro Fertilization in Three Substrains of C57BL/6 Mice; JAALAS, vol. 48, No.1

- Which of the following is not true regarding raffinose?
 - a) acts as a cryoprotectant in freezing media
 - b) Incapable of crossing cell membranes
 - c) Raffinose-skim milk method predominates in labs for cryopreserving and storing mouse sperm.
 - d) is a macromolecule that acts as a buffer and diluent to stabilize or assist in stabilizing the cell membrane

What procedure is occurring in the image?

- **Surgical oocyte retrieval:**
Cumulus oocyte mass is removed for mature oocyte retrieval



Surgical Oocyte Retrieval (SOR): a Method for Collecting Mature Mouse Oocytes Without Euthanasia; JAALAS, vol. 48, No1.

- Surgical oocyte retrieval (SOR), facilitates the collection of ovulated oocytes, does not require euthanasia and removing the oviduct , and preserves reproductive potential.
- The surgery involves a small incision in the ampulla region of the oviduct, through which the cumulus oocyte mass is removed with a gel-loading pipette.
- More dead oocytes were recovered when tribromoethanol was used than when isoflurane was used.

An Effective Venipuncture Technique and Normal Serum Biochemistry Parameters of the Captive Fat-Tailed Jird (*Pachyuromys duprasi*); JAALAS; Vol. 48, No.1



- Common Name?
 - Fat-tailed jird or fat tailed duprasi
- *Genus species?*
 - *Pachyuromys duprasi*
- *Models:*
 - thermoregulation, audiology, reproductive physiology, and infectious diseases.

Isoflurane Waste Anesthetic Gas Concentrations Associated with the Open-Drop Method; JAALAS 2009 Jan;48(1):61-4

- Which group has established the permissible exposure limit for anesthetic gases?
 - a) Occupational Safety and Health Administration (OSHA)
 - b) National Institute for Occupational Safety and Health (NIOSH)
 - c) American Conference of Governmental Industrial Hygienists (ACGIH)
 - d) Environmental Protection Agency (EPA)
 - e) Both B & C

Waste Anesthetic Gas Concentration Guidelines

- NIOSH has a recommended exposure limit (REL) that the average concentration of halogenated anesthetic agents should not exceed 2 ppm during any one hour period
- American Conference of Governmental Industrial Hygienists (ACGIH) has recommended the threshold limit value (TLV)-time weighted average (TWA) of 75 ppm over an eight hours/day and 40 hours/week

Isoflurane Waste Anesthetic Gas (WAG) Concentrations Associated with the Open-Drop Method

- **Summary:**
 - The open-drop technique is used frequently for anesthetic delivery to small rodents. This study was conducted to determine whether administration of isoflurane by the open-drop technique generates significant WAG concentrations.
 - At operator levels, WAG concentrations were always at or near 0 ppm. The WAG concentration associated with using the 500 ml container was marginally to significantly greater than that for the 1000 ml jar.

Comparison of Buprenorphine and Butorphanol Analgesia in the Eastern Red-Spotted Newt (*Notophthalmus viridescens*); JAALAS, Vol. 48, No.2



Courtesy of Connecticut Department of Environmental Protection Agency

- Common Name?
 - *Eastern Red-spotted Newt*
- Genus Species?
 - *Notophthalmus viridescens*
- Summary:
 - Buprenorphine by intracoelemic injection and butorphanol in tank water allowed resumption of normal behavior following limb amputation

Positive Reinforcement Training to Enhance the Voluntary Movement of Group-Housed Sooty Mangabeys (*Cercocebus atys atys*); JAALAS, Vol. 48, No.2



Photo credit: Yerkes NPRC

- Common Name?
 - *Sooty Mangabey*
- Genus Species?
 - *Cercocebus atys*
- Models: SIV, leprosy
- Summary:
 - In this study, PRT was used as the sole training method to teach sooty mangabeys to move throughout their enclosure on cue (group 90% compliance rate).

Apparatus for Collection of Fecal Samples from Undisturbed Spiny Mice (*Acomys cahirinus*) Living in a Complex Social Group; JAALAS, Vol. 48, No.2



Photo credit: Oregon Zoo

- Common Name?
 - *Egyptian Spiny mice*
- *Genus Species?*
 - *Acromys cahirinus*
- Models: congenital erythrocytic porphyria; obesity & diabetes due to overeating; Leishmaniasis.

Effects of Fenbendazole on the Murine Humoral Immune System: JAALAS, Vol. 48, No. 3

- FBZ-medicated feed may alter B cell function in rodents, results from this study indicated which of the following?
 - a) Effects were more pronounced in young mice and mice should not be used in experiments until 6 weeks after the end of treatment.
 - b) Effects were more pronounced in old mice and mice should not be used in experiments until 6 weeks after the end of treatment.
 - c) Effects were more pronounced in young mice and mice should not be used in experiments until 8 weeks after the end of treatment.
 - d) Effects were more pronounced in old mice and mice should not be used in experiments until 8 weeks after the end of treatment.

A PCR-Based Strategy for Detection of Mouse Parvovirus: JAALAS, Vol. 48, No. 3

Which of the following is true?

- a) Swabbing of multiple cages in rows in which only 1 cage contained MPV-infected mice was effective.
- b) Comparison of cage-bottom swab PCR, fecal PCR, and soiled bedding sentinels revealed that all 3 methods were 100% sensitive through PID 14.
- c) C57BL/6 mice are more susceptible to and sustain a more robust MPV infection than Balb/c mice.
- d) Fecal PCR was 100% sensitive through PID 33 and detected infection through PID 56, confirming that this method was the most sensitive for detecting infection.
- e) Both b & d are true.

Previous studies have suggested that images of conspecifics are useful for environmental enrichment for nonhuman primates and may act as a positive sensory reinforcement .



Which of the following was true about displaying movies to Japanese macaques?

- a) Response duration was longer when monkeys were included in the movies.
- b) Response duration was longer for males than females.
- c) Response duration was negatively correlated with decreasing age in males
- d) All are true

- 
- What rodent environmental enrichment device was found to activate the aryl hydrocarbon receptor ?
 - a) Cotton balls
 - b) Corn cob bedding
 - c) Nestlets
 - d) Sunflower seeds

Activation of Aryl Hydrocarbon Receptor Signaling by Cotton Balls Used for Environmental Enrichment; JAALAS, vol. 48, No. 1

Activation of Aryl Hydrocarbon Receptor Signaling by Cotton Balls Used for Environmental Enrichment

Summary:

- The term 'dioxins' refers to halogenated aromatic hydrocarbons that are structurally similar and share a common mechanism of toxicity.
- Cotton balls and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) **increased** Cytochrome P₄₅₀ 1A₁ (Cyp1A₁) in the livers of animals.
- Induction of Cytochrome P₄₅₀ (Cyp1A_{1/2}) occurs in response to increased activity of the aryl hydrocarbon receptor (AhR).
- Contamination of animal bedding material with very small doses of dioxins can cause wasting syndrome, tumor development, and reproductive and immune system function, and ultimately death.

Which of the following has been associated with murine urologic syndrome in mice?

- a) B6C₃F₁ mice
- b) Housing on wire bottoms
- c) Euthanasia by ether overdose
- d) Ketamine and medetomidine anesthesia
- e) All the above

Urethral Obstruction by Seminal Coagulum is Associated with Medetomidine–Ketamine Anesthesia in Male Mice on C57BL/6J and Mixed Genetic Backgrounds; JAALAS, Vol. 48, No. 3

Construction Noise Decreases Reproductive Efficiency in Mice: JAALAS, Vol. 48, No.4

- Study analyzed the effects of construction noise on mouse gestation and neonatal growth. Which of the following was influenced by construction noise?
 - a) incidence of cannibalism
 - b) incidence of barbering
 - c) incidence of stillborn pups
 - d) rate of neonatal weight gain
 - e) All of the above

Summary:

Noise decreased reproductive efficiency by decreasing live birth rates and increasing the number of stillborn pups.

Assessment of Immune Activation in Mice before and after Eradication of Mite Infestation: JAALAS , Vol. 48, No.4

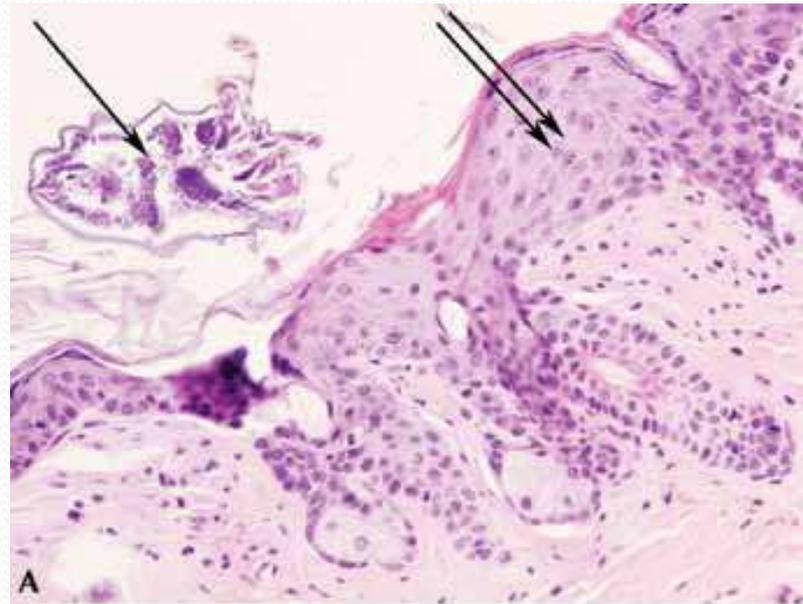
This study compared cytokine levels and histologic findings in mite-infested, treated, and mite-negative mice. What was discovered in mite-infested mice?

- a) higher levels of inflammatory cytokines at draining lymph nodes (axillary) as compared with mice without mites
- b) higher levels of inflammatory cytokines systemically as compared with mice without mites
- c) histologic evaluation of skin revealed significant inflammation in mite-infested mice still present in the skin of mice at 6 to 8 wk after treatment of mites
- d) mite infestation caused marked and possibly permanent damage to the skin in these mice
- e) All the above.

Assessment of Immune Activation in Mice before and after Eradication of Mite Infestation: JAALAS, Vol. 48, No. 4

Summary:

- In this study, mice with mite infestation had higher levels of inflammatory cytokines (e.g. $\text{TNF}\alpha$, $\text{MIP}\alpha$, and β) both at draining lymph nodes (axillary) and systemically (spleen), as compared with mice without mites.
- Inflammatory changes were still present in the skin of mice at 6 to 8 wk after treatment, despite absence of detectable infestation at that time



Name the rat parasite

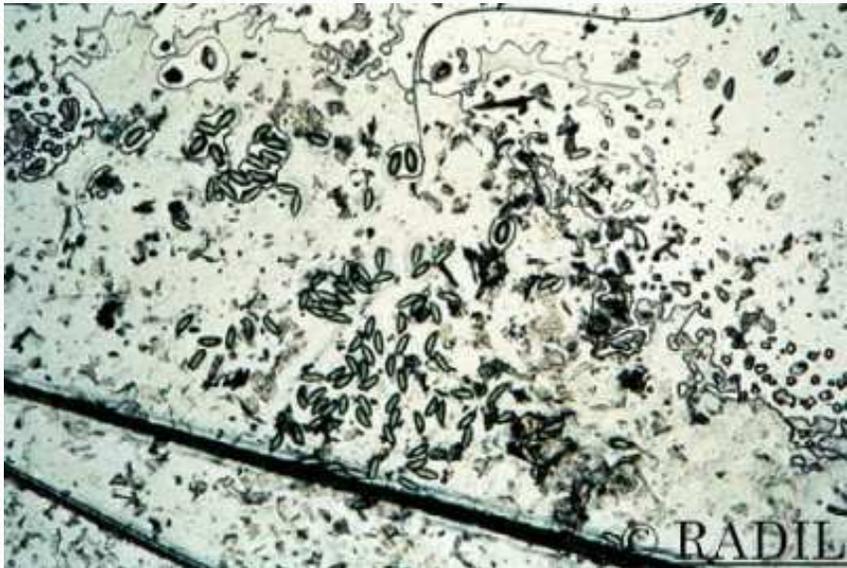


Photo of a tape test of a rat infected

Syphacia muris

Name the mouse parasite



Syphacia obvelata, *Aspicularis tetraptera* are expected pinworm species in mice

- Adults / larvae in cecum - colon
- *Syphacia* → asymmetric eggs on perineum
→ tape test detection
- *Aspicularis* → symmetric eggs in/on feces
→ fecal float detection

Summary: Sensitivity of Perianal Tape Impressions to Diagnose Pinworm (*Syphacia* spp.) Infections in Rats (*Rattus norvegicus*) and Mice (*Mus musculus*); *JAALAS* vol.48, no.4; The sensitivity of perianal tape impressions for detecting *Syphacia muris* infections in rats was 100%, and for detecting *Syphacia obvelata* in mice was 85.5%. Caution the use of perianal tape impressions alone for *Syphacia* spp. screening in sentinel mice and rats because intermittent shedding of pinworms. **Photo courtesy of RADIL .**



Nursery rearing is the single most important risk factor in the development of severe forms of abnormal behavior, such as self-biting, in rhesus macaques.

Which variation in peer socialization most exhibited 'floating limb' and self-biting behavior at significantly higher frequencies than any other strategy?

- a) continuously paired
- b) intermittently paired
- c) continuously paired rotationally (partners rotated within the group once a week)
- d) intermittently paired rotationally

Note: Continuously pairing was most effective in reducing abnormal behaviors.



Which variation in peer socialization significantly reduced partner clinging and geckering than any other strategy?

- a) continuously paired
- b) intermittently paired
- c) continuously paired rotationally (partners rotated within the group once a week)
- d) intermittently paired rotationally.

Summary

- Nursery rearing is the single most important risk factor in the development of severe forms of abnormal behavior, such as self-biting, in rhesus macaques.
- Independent of peer-rearing condition, infants reared without maternal contact show an increased propensity to develop self-sucking, self-clinging, partner clinging, decreased exploration in novel environments, and insufficient social behaviors. **All 4 treatment groups developed abnormal behavior traits to some degree.**
- Solitary incubator environment may be a trigger for the development of abnormal behaviors.
- What is geckering?
Distress vocalizations of rhesus macaques in the wild

Molar Malocclusions in Pine Voles (*Microtus pinetorum*) JAALAS;
48(4): 412–415

Name the disease:
Molar Malocclusion



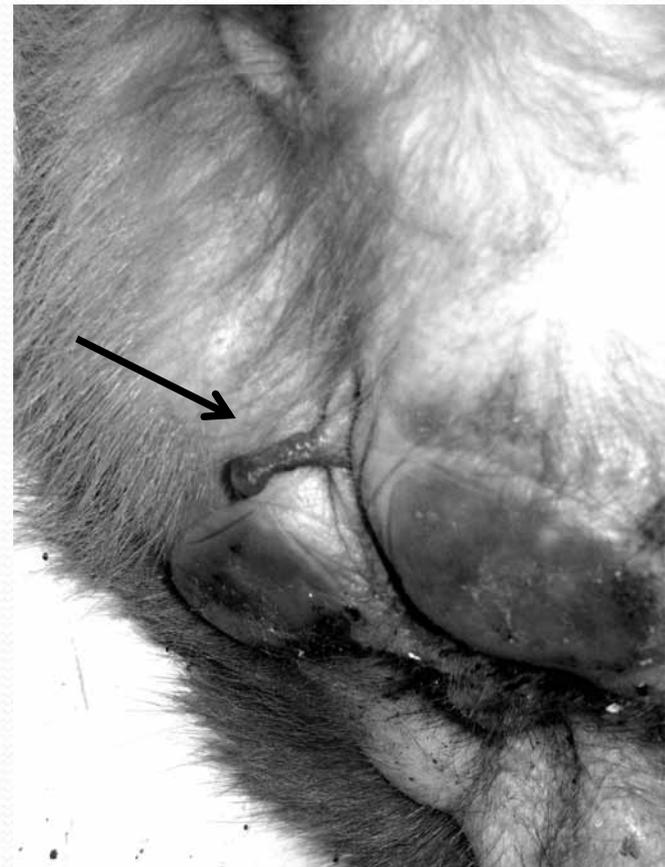
Molar Malocclusions in Pine Voles (*Microtus pinetorum*) JAALAS;
48(4): 412–415

- Difficulty in mastication resulted in poor body condition necessitating euthanasia of 5 Pine voles.
- Postmortem examination of oral cavity revealed grossly elongated mandibular and maxillary molars with abnormal wear at occlusal surfaces.
- How was the colony health problem addressed successfully?
 - by adding autoclaved hardwood sticks to each cage as an enrichment tool.

Name the structure

Long clitoris of rhesus macaque

- Does clitoral length affect female fecundity of macaques?
- No.



Variation in Clitoral Length in Rhesus Macaques (*Macaca mulatta*); JAALAS; Vol. 48, No.5

- Clitoral length was highly variable and had no relationship to fertility.
- Statistical evaluation revealed no association in the distribution of daughters with and without clitoris between mothers with and without clitoris.
- Even when mated with several female monkeys, some male macaques produced primarily daughters without clitoris.



Decreased Blastocyst Production in Mice Exposed to Increased Rack Noise; JAALAS; Vol.48, No.5

- Study was conducted to investigate the possible effect of rack type on the blastocyst yield of mouse embryo donors. Which of the following is true?
 - a) Donors housed on static racks produced more blastocysts compared to mice housed on ventilated racks
 - b) Serum corticosterone concentrations did not differ between static and ventilated racks
 - c) There was no difference between donors housed on static racks produced more blastocysts compared to mice housed on ventilated racks

Summary:

The number of blastocysts per donor was highest in mice housed on static racks in rooms without ventilated racks not in the same room. Number of blastocysts per donor was negatively correlated with increased mean rack noise levels.

When historical reproductive and growth data were compared for colonies of mice and rats maintained in open-topped cages in either single-species or dual-species barrier rooms. Which of the following was affected?

- a) Litter size at birth for mice
- b) Missing pups at weaning for mice
- c) Growth rate for rat pups
- d) Body weights of mice growing in rooms with rats
- e) None of the above

Breeding and Housing Laboratory Rats and Mice in the Same Room Does Not Affect the Growth or Reproduction of Either Species; JAALAS; vol. 48, No.5

Euthanasia Method for Mice in Rapid Time-Course Pulmonary Pharmacokinetic Studies; JAALAS, Vol. 48, No.5

- When various methods of euthanasia were compared with regard to preparation time, utility, tissue distribution, and time to onset of euthanasia. Which of the following was the most rapid method of euthanasia?
 - a) Intravenous ketamine–xylazine
 - b) Retroorbital ketamine–xylazine
 - c) CO₂ gas
 - d) intraperitoneal pentobarbital
 - e) Retroorbital pentobarbital

Evaluation and Refinement of Euthanasia Methods for *Xenopus laevis*; JAALAS, Vol. 48, No.5

- The most common method of euthanasia for *Xenopus* species is by immersion in tricaine methane sulfonate solution (MS222). What dose of MS222 was recommended for adult *Xenopus*?
 - a) MS222 (0.5 g/L) for 1 hour
 - b) MS222 (1 g/L) for 1 hour
 - c) MS222 (5 g/L) for 1/2 hour
 - d) MS222 (5 g/L) for 1 hour
 - e) None of the above doses worked.

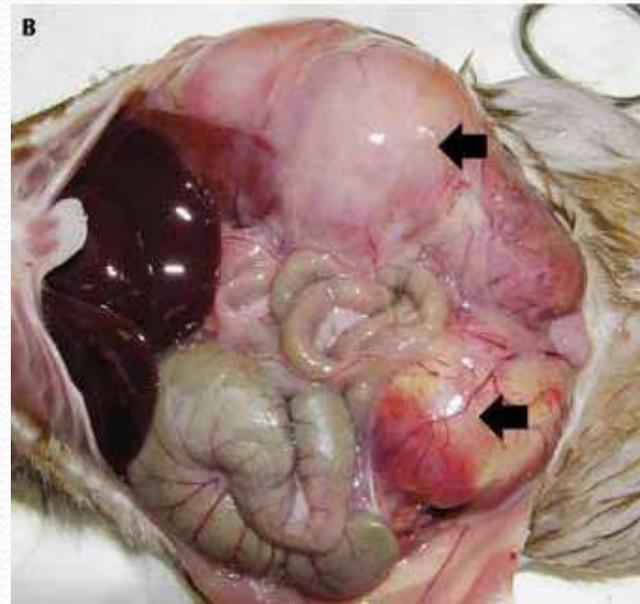
Evaluation and Refinement of Euthanasia Methods for *Xenopus laevis*; JAALAS, Vol. 48, No.5

- Summary,
 - Necessary to euthanize adult *X. laevis* and ensure complete cessation of the heartbeat without recovery:
 1. Immersion for at least 1 h in a 5-g/L buffered solution of MS222
 2. Intracoelomic injection of 1100 mg/kg sodium pentobarbital with sodium phenytoin (equivalent to 0.3 mL solution per frog) or ventral cutaneous application of 182 mg/kg benzocaine (equivalent to a 2 cm × 1 mm of 20% benzocaine gel)
 - These doses are considerably higher than those previously recommended for this species.

Name the condition in rats

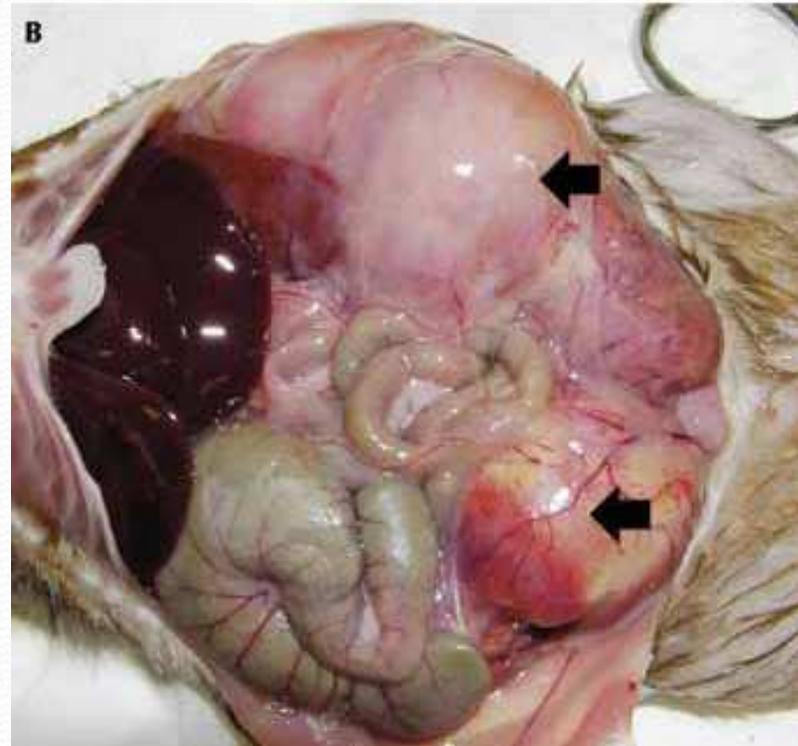


Pyometra



Interstrain Differences in the Development of Pyometra after Estrogen Treatment of Rats; JAALAS, Vol. 48, No.5

- Name the condition in rats
 - Pyometra
 - What infectious agent is well documented cause of the condition in rats?
 - Mycoplasma pulmonis
 - Estrogen supplementation predisposed what strain to pyometra?
 - Brown Norway



Ultrasonographic Diagnosis of Pregnancy in Rats: JAALAS, Vol.48, No.6

The study determined that ultrasonographic evidence of pregnancy was present in the rat by day_____ post coitum.

- a) 6
- b) 7
- c) 8
- d) 9
- e) 10

Summary: Diagnosis of pregnancy was confirmed by detection of the embryo heart beat by day 12. Embryo characteristics were ultrasonographically measurable between days 9 and 16.

Response of C57BL/6J and BALB/cJ Mice Increased Housing Density; JAALAS, Vol.48, No. 6

- Which male inbred strains are characterized as very aggressive.
 - a) C57Bl/6
 - b) FVB
 - c) Balb/c
 - d) SJL
 - e) SJL/J and BALB/cJ

Summary:

Findings indicate that mouse housing density can be increased 50% to 100% above GUIDE recommendations with few apparent affects. Decreased weight gain, increased fecal corticosterone levels, increased barbering behavior, and increased adrenal gland weight when housing density is increased (e.g. 8 per pen).

Comparison of Selamectin and Imidacloprid plus Permethrin in Eliminating *Leporacarus gibbus* Infestation in Laboratory Rabbits (*Oryctolagus cuniculus*): JAALAS vol. 48. No.6

Name the ectoparasite:

Leporacarus gibbus= rabbit fur mite
formerly *Listorphorus gibbus*

Summary:

Both Selamectin and Imidacloprid plus Permethrin were 100% effective by day 13 but Selamectin was effective by day 3.



**Photograph / Copyright: Dermod Malley
FRCVS**

Effects of Cage-Changing Frequency and Bedding Volume on Mice and Their Microenvironment: JAALAS vol. 48. No.6

- This study demonstrated that a 2-wk interval between cage changes for ICR female mice housed in IVC caging and aspen chip bedding did not affect measures of animal well-being. What was the most significant predictor of intracage ammonia concentration?
 - a) Temperature
 - b) Humidity
 - c) Bedding volume
 - d) use of nestlets
 - e) Fecal corticosterone levels

Effects of Cage-Changing Frequency and Bedding Volume on Mice and Their Microenvironment: JAALAS vol. 48. No.6

- **Summary:**

- Study showed that low volume of bedding within cages produced the highest levels of ammonia due to the rapid saturation of bedding by ammonia waste. This saturation aids bacterial proliferation, resulting in greater ammonia production. Temperature and humidity were not significant predictors of ammonia levels.
- Bedding volume and days after last cage change are most significant factors on production of ammonia levels.

Good Luck!

