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**OVERVIEW**

**Newsome et al. Compassion Fatigue, Euthanasia Stress, and Their Management in Laboratory Animal Research, pp. 289-292**

Domain 6

SUMMARY:People working with research animals are at an increased risk for compassion fatigue and euthanasia stress. Compassion fatigue is the reduced capacity in being empathetic and the consequent behaviors and emotions resulting from knowing about a traumatizing event experienced or suffered by a person, and is characterized by deep physical and emotional exhaustion. Euthanasia stress is a type of moral stress and is a significant contributor towards compassion fatigue.

Factors that contribute to the development of compassion fatigue include absence of personal community and societal support, the social stigma associated with animal research, desensitization to certain procedures such as euthanasia, and the complexity of the human-animal relationship in the research setting. Additionally, people engaged in laboratory animal care are often self-selected to be caring, compassionate, and empathetic, which increases their risk for compassion fatigue.

Symptoms of compassion fatigue occur on an individual and institutional level. An individual may suffer from bottled-up emotions, sadness/apathy, isolation, difficulty concentrating, and may feel chronically mentally and physically tired. Organizational symptoms include high employee turnover, lack of flexibility, inability to complete assignments/tasks, increased mistakes, and an effect on the quality of animal or medical care.

Strategies to address these risks are dependent on institutions recognizing them incorporating preventive and response measures. Such strategies may include: providing training in stress management and how to recognize signs of compassion fatigue, encourage open dialogue about euthanasia, identify and create social support systems, and providing access to counseling.

QUESTIONS

1. Which of the following does not contribute to compassion fatigue?
   1. Self-selection of animal care workers as empathetic and compassionate
   2. The social stigma associated with animal research
   3. Understanding the value of laboratory animal research
   4. Desensitization to certain procedures such as euthanasia
2. Select the definition of compassion fatigue
   1. The concept of being aware and psychologically challenged when faced with the task of euthanizing animals
   2. A state of exhaustion and biologic, physiologic, and emotional dysfunction resulting from prolonged exposure to compassion stress
   3. The unavoidable stress experienced when helping others in distress
   4. Being aware of the ethical principles at stake, but external factors prevent action
3. Match the contributing factor to compassion fatigue with an appropriate mitigation strategy

|  |  |
| --- | --- |
| a. Absence of community support | 1. Provision of access to knowledgeable counselors and compassion fatigue awareness training |
| b. Complexity of the human-animal relationship | 2. Round-table discussions involving all employees to support each other and exchange ideas regarding compassion fatigue |
| c. Inherent increased risk in empathetic individuals | 3. Offering to have another trusted colleague perform euthanasia aside from the primary caregiver |

ANSWERS

1. c

2. b

3. a-2, b-3, c-1

**ORIGINAL RESEARCH**

***Biology***

**Genzer et al. Hematology and Clinical Chemistry Reference Intervals for Inbred Strain 13/N Guinea Pigs (*Cavia porcellus*), pp. 293-303**

Domain 1

Secondary Species: Guinea Pig (*Cavia porcellus*)

SUMMARY: This publication establishes reference ranges for hematology and clinical chemistry parameters in 13/N inbred guinea pigs. Ranges were evaluated by sex and age. CBCs and chemistry panels were run on an automated analyzer. Blood smears were stained and a 7-way WBC differential count was performed. Overall, this strain follows similar age-related trends similar to those seen in other guinea pig strains (RBC, Hgb, and Hct). Males had higher RBC, Hct, Hgb, and percentages of Foa-Kurloff cells and monocytes than females. Glucose, albumin, and ALP decreased with age whereas BUN, creatinine, calcium, and amylase increased with age. This publication suggests age cutoffs of 0-150d for juveniles, 150-900d for adults, and >900d for seniors based on the trends in parameters.

QUESTIONS

1. Why should a manual WBC count be performed in guinea pigs?
2. What are 13/N strain guinea pigs particularly useful to study?

ANSWERS

1. Guinea pigs have Foa Kurloff cells containing large intracytoplasmic vacuoles that can exceed the size of the nucleus. This may interfere with obtaining accurate automated WBC counts.
2. 13/N inbred guinea pigs are used to study highly pathogenic viruses that cause hemorrhagic fevers in humans. They are more susceptible to Lassa and Lujo viruses than mice or outbred guinea pigs. 13/N guinea pigs uniformly succumb to infection and mirror human disease without the need for rodent-adapted variants

***Reproduction***

**Zhang et al. Effect of Predator Stress on the Reproductive Performance of Female Mice after Nonsurgical Embryo Transfer, pp. 304-310**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Mice exposed to a variety of stressors show incidences of failure to maintain pseudopregnancy, degenerated embryos in oviducts, implantation failure, and post implantation losses. Plus, pregnant mice rodents exposed to predator odors during the peri-implantation period may give birth to small litters. However, few studies have demonstrated the effect of predator stress through the entire length of gestation on reproduction performance. The aim of the study was to identify the effect of maternal stress on embryo development in vivo by examining levels of pregnancy-related hormones such as progesterone and cortisol, and outcomes from embryo transfer.

Mice were assigned randomly to three different groups, auditory, visual + auditory, and control. For each group, 8 mice were euthanized at 6.5 days postcoitum to evaluate implantation sites, by visualizing the morphologic features of the uterus, and serum cortisol and progesterone levels; the remaining mice were used to assess reproductive parameters. After nonsurgical embryo transfer (NSET), mice were placed in a cage and transferred to the cat rearing room. For auditory stress group, mice cages were placed beside the cat cage after the side was covered with an opaque fabric squares to block visual stimuli. All cages from visual + auditory group were placed inside the cat cage throughout the gestation. Cat noise was measured every 0.5 hours by using a sound-level meter; the meter was placed inside the mice cage to help to gauge sound heard by the stressed mice. Depending on the group, mice were exposed to cat noise daily after NSET throughout the gestation length. Cardiocentesis was performed after euthanasia by CO2 inhalation for blood collection; serum cortisol and progesterone were measured.

There were no significant change in the pregnancy rates of mice in the auditory and visual + auditory groups in comparison with the controls; however, there was a decrease in implantation rate in both the auditory and visual + auditory groups (41.7% and 22.9 % respectively) compared with the control group (87.5 %). Reduction in the live pup rate, measured by embryo death, was observed in the auditory group (25.5%) and visual + auditory group (22.5%) compared with the control group (50.1%). Moreover, gestation period was significantly extended in mice exposed to visual + auditory stimuli compared with the control group, which was not different from the auditory stress group. Significant decrease in neonatal birth weight was observed in both groups, auditory and visual + auditory compared with the control group. The concentration of serum cortisol was significantly higher in the auditory and visual + auditory group compared with the control group. No significant stress-related changes were noted among the different groups.

QUESTIONS

1.   (T/F) Data showed the exposure of pregnant mice to predator stress significantly decreased the implantation and live pup rates, suggesting that embryo losses occurred during implantation or early post-implantation stages.

2.  What NSET stands for? And how this procedure is performed in mice?

ANSWERS

1.  True

2. NSET stands for nonsurgical embryo transfer. After mice are anesthetized, the cervical opening is visualized by inserting a speculum into the vagina, and then blastocysts are loaded on the NSET device and deposited in one uterine horn.

***Husbandry***

**Chen et al. Effects of Sodium Lighting on Circadian Rhythms in Rats, pp. 311-320**

NOTE: This article is being retracted with the support of all 3 coauthors due to methodological and authorship issues. (see JAALAS 58(5):606).

***Management***

**Kelly et al. Teaching Surgical Model Development in Research by Using Situated Learning and Instructional Scaffolding, pp. 321-328**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Domain 3: Facilitate and Provide Research Support

SUMMARY: Situated learning and instructional scaffolding are useful skill-building tools. The stages of learning include cognitive, associative, and autonomous stages. The goal of this overview was to detail a schema for training research surgeons to skillfully develop, master, and execute surgical models. They describe a method of adapting standardized teaching approaches to surgical training. Once trainees have progressed through these learning stages, they should be both competent in the process of model development and proficient in surgical methods specific to their studied model.

During the cognitive stage of learning, surgical trainees must learn basic surgical theory, vocabulary, and concepts. Textbooks, lectures, and electronic (e-) learning are useful as tools to develop procedural knowledge and learn relevant background information such as vocabulary, concepts, and anatomy. First, each trainee should identify a surgical mentor. The consultation should include a discussion on the primary desired outcomes of the model, the animal species used, any previous relevant experience of the novice surgeon, and the generation of a timeline to estimate the duration of training. Discussions also focus on anesthetic and analgesic plans, pre and post-operative care, location for surgery, recordkeeping, and humane endpoints. The model is then brought before the IACUC for approval and supplies, such as suture material, and surgical equipment, are obtained in consultation with the surgical mentor.

During the associative stage of learning, the novice surgeon practices their surgical skills. Having a mentor present is critical for this stage as they can specifically address any gaps in learning and motor skills unique to individual trainees. Training can be facilitated using inanimate models such as skeletons and/or cadavers, non-survival surgery, and performing survival surgery with aseptic technique under direct supervision of a mentor to give real-time constructive feedback. Mentors gradually decrease personal involvement and verbal instruction leading to trainee autonomy. Mentors assess competency by observing trainees perform a given task within the surgical environment and determining whether the task was performed correctly.  Proficiency is commonly assessed by someone other than the original mentor to mitigate bias and ensure objectivity. Surgical trainees also learn appropriate post-surgical care at this time.

During the autonomous stage, the trainees should be competent in the procedure and the mentor´s presence should no longer be required. A marker of proficiency is the demonstrated ability of now-autonomous surgeons becoming mentors themselves and training new surgeons in the model. A surgical assistant is recommended for ensuring that aseptic technique is maintained and for monitoring anesthesia, administering medications, monitoring recovery and maintaining records.

In summary, the combination of situated learning and instructional scaffolding is an ideal strategy for educational training of surgeons in model development and greatly improving the chance of achieving the desired surgical outcome and optimizing animal welfare.

QUESTIONS

1. The stages of learning include:

a.   Textbook, E-learning, and Hands on training

b. See one, Do one

c.   Cognitive, Associative and  Autonomous stages

2. Dermestid beetles are useful for:

a. Determining anesthetic depth

b.   Eating dead flesh off cadavers

c.  Facilitating post-surgical recovery

3. Practicing surgical technique on cadavers can be done prior to IACUC approval:

a.  True

b.  False

c.  Depends on the animal model

ANSWERS

1. c

2. b

3. b

***Animal Health Surveillance***

**Estes et al. Effectiveness of Various Floor Contamination Control Methods in Reducing Environmental Organic Load and Maintaining Colony Health in Rodent Facilities, pp. 329-338**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Mouse (*Mus musculus*)

SUMMARY: This paper compares four floor contamination control strategies. The strategies employed were shoe covers (ShCv), contamination control flooring (CCF), a combination of the two (ShCv + CCF), and neither strategy. Each strategy was evaluated as a means of preventing bacterial transfer, reducing organic load on floors, and maintaining murine colony health status.

Shoe covers are expensive, detrimental to the environment, and present a hazard from an ergonomic perspective (i.e. bending and twisting to apply). Additionally, shoe covers have been shown to be a possible source of contamination of gloves and in a clinical setting have shown to be not helpful in decreasing the length of stay or have any significant effect on mortality on human ICU patients. CCF is a popular alternative due to its proposed longevity, its ability to be recycled after the effective life is reached thereby reducing waste, cost effectiveness, and increased energy efficiency.

In the study the group hypothesized that ShCv and CCF would prevent the transfer of bacteria most effectively and reduce the organic load on the floors of the facility. The group used a combination of bacterial transfer using *S. xylosus*, organic load quantification using ATP testing, PCR analysis of the floors for murine pathogens, and exhaust air duct sampling to look for the effect on mouse colony health status.

The group found that CCF was less effective than donning new ShCv for preventing bacterial transfer and reducing organic load on the floors of animal facilities. Neither method resulted in any changes to the animal health status. They reported a 50% decrease in ATP levels from a facility where neither strategy was employed. In this area, they suggested that other factors affected the amount of organic material on the floors including a different pattern of foot traffic. Their findings were suggestive that floor contamination control methods did not markedly affect the health status of mice housed in IVC facilities. If additional controls are needed for biocontainment, ShCv in conjunction with CCF presented a better level of contamination control.

QUESTION

1. What is the most common site for *S. xylosus*?

ANSWER

1. *Staphylococcus xylosus* is an environmentally persistent commensal bacterial strain found on the skin of humans and animals.

***Anesthesia***

**Dunbar et al. Preliminary Evaluation of Sustained-release Compared with Conventional Formulations of Meloxicam in Sheep (*Ovis aries*), pp. 339-345**

Domain 2: Management of Pain and Distress

Secondary Species: Sheep (*Ovis aries*)

SUMMARY: In this study using sheep two different drug delivery was studied. One was Sustained-release (SR) meloxicam and the other convention multiple injection of meloxicam. Using SR meloxicam refined current analgesic regimens by alleviating the need for multiple sessions of handling and restraint and by reducing the local tissue irritation that can occur due to repeated injections. This study used HPLC–MS to measure the plasma concentrations of 2 formulations of meloxicam—conventional and SRM.

Plasma levels of SRM were higher than conventional meloxicam (CM) throughout the initial 24 h, remained variably elevated until 60 h after injection, but failed to sustain presumed therapeutic levels of 400 ng/mL for the full 72 h across all animals in this study. The characteristics of SRM dosage were less consistent that CM over all sheep throughout the 72 hour period.  Further investigation is warranted to determine the safety and clinical efficacy of SRM in sheep.

This study concluded when SRM is used in sheep, it is recommended the combination of a preemptive and multimodal analgesia regimen with clinical assessments throughout the postoperative period.

QUESTIONS

1. SR drug meloxicam compare with that of conventional multiple injection of meloxicam for adequate analgesia is an example of \_\_\_\_\_\_*?*

a.   Reduction

b.  Replacement

c.  Refinement

d.  a, b, c

2. T or F: Sustained-Release meloxicam can be administered as a one-time dose that would reach and maintain therapeutic plasma concentrations at least 400 ng/ml over 72 h, thus avoiding the time dependent fluctuations in drug concentration that occur with once daily dosing of conventional meloxicam for 3 days

3. Sheep are commonly used for \_\_\_\_\_\_\_ and \_\_\_\_\_\_\_ studies, because their anatomy and physiology render them suitable as models of human disease.

4. T or F: The characteristics of SRM dosage were less consistent that CM over all sheep throughout the 72 hour period.

5. T or F: When SRM is used in sheep, it is recommended to use a combination of a preemptive and multimodal analgesia regimen with clinical assessments throughout the postoperative period.

ANSWERS

1. c

2. False

3. Soft tissue, orthopedic

4. True

5. True

**Malinowski et al. Butorphanol-Azaperone-Medetomidine for the Immobilization of Rhesus Macaques (*Macaca mulatta*), pp. 346-355**

Domain 3 – Research

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: BAM injectable (IM) combination for chemical restraint in common use for wildlife, group assessed for use in Rhesus macaques.

* Butorphanol tartate (27.3 mg/mL) – opioid agonist-antagonist with analgesic (variable), antitussive and antiemetic properties
* Azaperone tartate (9.1 mg/mL) – butyrophenone tranquilizer with antipsychotic, sedative and antiemetic properties
* Medetomidine hydrochloride (10.9mg/mL) – potent alpha-2 adrenergic agonist sedative with analgesic, anxiolytic and muscle-relaxant properties; only one of three that solely produces recumbency and is driver for immobilization

Following pilot studies, pursued two groups, concentrations (16 and 24 uL/kg) in rhesus macaques (n=12 per group - 6 male/6 female, age-matched 3 yrs. of age).  Actual injection volume is quite small overall. Assessed physiological parameters (mucus membrane color, CRT, pulse ox, heart rate, indirect MAP, temp, resp rate), induction quality, anesthetic depth (e.g. pedal reflex) and recovery quality scored separately. The group established study endpoints for all parameters that would result in intervention prior to serious physiological compromise as a result of BAM administration.

Induction, quality of sedation (i.e. spontaneous movements) and recovery were generally very smooth for both treatment concentrations and resulted in immobilization for 26 +/- 15 minutes with an induction time of 4 mins (+/- 1 min) and recovery time of 12 mins (+/- 9 mins). Cardiovascular parameters were initially concerning with the majority of animals, 17 of 24 reached experimental end points for bradycardia or hypotension or both, with neither dose increasing the risk of reaching the experimental end points. All but one animal that reached experimental endpoints for bradycardia were normotensive (MAP > 50 mmHg). All animals were initially hypoxemic (SPO2 <89%) at initial reading but all recovered to normal oxygenation with supplementation rapidly. No differences found between genders overall.

Overall, it is the opinion of the authors that BAM at these doses is a potentially useful tool for immobilization of Rhesus macaques, but that cardiovascular parameters should be monitored carefully and supplemental oxygen be available. Other age groups and health status could be evaluated in future. All of the drugs in the BAM cocktail are lipophilic and metabolized by the liver so body fat composition and liver function may influence drug effects.

QUESTIONS

1. (T/F) Azapirone, a component of the BAM cocktail, is a tranquilizer

2. Which of the following would be an appropriate reversal for the immobilization effects of BAM?

a. Suggamadex

b. Atipamezole

c. Flumazenil

d. All of the above

3. An investigator wishes to sedate a mouse for a brief scan in an MRI/PET unit and wishes for there to be minimal respiratory side effects. What anesthetic combination would be the best choice for this purpose?

a. Ketamine/Xylazine

b. Ketamine

c. Ketamine/Dexmedetomidine

d. Any of the above would be equally successful

ANSWERS

1. False -- azapirone (though it close in name to Azaperone - the actual drug in BAM) is in fact an anxiolytic/antidepressant. Azaperone is a tranquilizer

2. b. Atipamezole; a = reversal for neuromuscular blockers (e.g. rocuronium) c = reversal agent for benzodiazepines,

3. c. Ketamine alone

**Smith et al. Evaluation of Analgesic Patches in Cynomolgus Macaques (*Macaca fascicularis*), pp. 356-361**

Domain 2: Management of Pain and Distress

Primary Species: Macaques (*Macaca spp.*)

SUMMARY

Goal:Determine whether opioid transdermal patches (TDP) attain therapeutic concentrations in NHP, by assessing the pharmacokinetics of fentanyl (25 µg/h) and buprenorphine (10 and 20 µg/h) TDP in naïve, adult, male cynomolgus macaques

Background: Cynomolgus macaques are valuable animal models for pharmaceutical development, organ transplant medicine, and neuroscience. Fentanyl is a pure µ-opioid receptor agonist indicated for the control of moderate to severe pain, with a rapid onset of action (less than 5 min) and a duration of action of 15-30 min when administered IV. Fentanyl is formulated as an injectable solution, transdermal solution, transmucosal lozenge, and transdermal patch. TDP is described to deliver fentanyl to cats and dogs at a controlled rate over 72 h. Buprenorphine is a semisynthetic, partial µ-opioid receptor agonist indicated for treatment of mild to moderate pain, with an onset of action at 30-60 min and duration of 8-12 h. Buprenorphine is formulated as an injectable, sustained release injectable, transmucosal solution, and a TDP. Buprenorphine TDP are primarily used in human medicine for the treatment of osteoarthritis and cancer pain and have controlled drug delivery for 7 d. Both opioids and ketamine are metabolized through CYP450 enzyme pathways in the liver. Although metabolized primarily by CYP3A4, opioids can also be degraded by alternative CYP enzymes and by glucuronidation when the primary CYP pathway is saturated.

Methods:Fentanyl and buprenorphine (low and high dose) TDP were placed individually in 3 locations on the ventral thorax of the macaques. Macaques wore jackets throughout the study to protect the patches from removal. Endpoints included drug levels, weight, food consumption, fecal and urinary outputs, activity level, respiratory depression, presence of vomit, general attitude, rectal temperature, heart rate, and respiratory rate. Fentanyl plasma sample analysis was conducted by using UPLC with single-quadrupole MS detection. Plasma sample analysis for buprenorphine concentration was determined by using an automated liquid handling workstation and analyzed by LC–tandem MS.

Results:

* No adverse effects noted throughout the study.
* Minimal therapeutic concentrations for fentanyl and buprenorphine were achieved in all macaques within 8 h of fentanyl and 24 h of buprenorphine TDP application.
* Therapeutic levels for the fentanyl and low- and high-dose buprenorphine patches were maintained for 96, 120, and 144 h, respectively.
* 25-µg/h fentanyl patches should be replaced every 4 d, and the low and high-dose buprenorphine patches should be replaced every 5 and 6 d, respectively.

Discussion:The results support the use of fentanyl and buprenorphine TDP as feasible and practical analgesic options in NHP pain management protocols. Fentanyl and buprenorphine patches maintain adequate and consistent therapeutic plasma levels in cynomolgus macaques for a clinically significant duration. Transdermal drugs are absorbed along a concentration gradient through the epidermis and into the dermal layer, where large capillary beds take up the drug and distribute it to the systemic circulation. Several factors contribute to absorption variations between subjects and between drugs. In humans, stratum corneum thickness, skin hydration, underlying skin disease or injuries, body temperature, and genetics all could cause variable drug absorption rates. Opioid TDP have the advantage of decreasing animal stress, because they require only a single application rather than repeated injections or continuous infusion. Opioid TDP avoid peaks and troughs caused by injectable formulations, which, in humans, are associated with increased incidence of side effects including nausea and vomiting, respiratory depression, and sedation. Continuous drug delivery of the TDP decreases the likelihood of intermittent lapses in analgesic coverage, and TDP— unlike topical solutions or sustained-release injectable formulations—can be removed quickly if adverse effects do occur.

QUESTIONS

1. What is the mechanism of action (receptor) for fentanyl as an analgesic agent?
2. What is the mechanism of action (receptor) for buprenorphine as an analgesic agent?

ANSWERS

1. Pure µ-opioid receptor agonist
2. Partial µ-opioid receptor agonist

***Experimental Use***

**Serensen et al. Time-dependent Pathologic and Inflammatory Consequences of Various Blood Sampling Techniques in Mice, pp. 362-372**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY:This paper compares histopathologic, local and systemic inflammatory and welfare effects of 6 commonly used blood collection methods: lateral tail incision, tail-tip amputation, sublingual puncture, submandibular puncture, retro-orbital (RO) puncture and saphenous vein puncture.

4 female C57BL/6NTac mice were sacrificed for each blood collection method and at 9 time points (starting at 6h, up to 12 days post-collection). Control mice were anesthetized with isoflurane and sacrificed at 6h or 1 day post-anesthetic event. All mice were unanesthetized except for RO bleeding performed under isoflurane anesthesia. Mice were only sampled once with 50-100uL collected at time of sampling – no analysis was done on this blood. At each time point, mice were anesthetized with an unreported dose of Hypnorm (fentanyl and fluanisone) and midazolam prior to euthanasia with pentobarbital and terminal sampling: blood collection via RO bleed, stomach contests weighed and dissection of local sampling site for either histopathology or PCR for expression of inflammatory genes. Mice were weighed prior to initial sampling and at euthanasia.

All mice lost weight 6 and 10h post-sampling with mice undergoing RO puncture losing the most weight, associated with lower weight of stomach contents. All mice had elevated cortisol levels that may have been attributed to Hypnorm/midazolam anesthesia. Significantly higher plasma corticosterone [ ] were found in mice sampled by RO or sublingual puncture, but were comparable to mice that only underwent isoflurane anesthesia. Systemic inflammation as measured by serum haptoglobin, IL6 and IL1b was not evident in any of the groups.  Mice that underwent lateral tail incision or tail-tip amputation took longer to heal locally and, along with saphenous vein puncture, had higher local gene expression of *S1008/9A*, *Cxcl2*, *Il1b* and *Nlrp3*. However, microabscesses, trichogranuloma were occasionally found in mice undergoing RO or sublingual puncture.

In light of these results, the authors concluded that RO and sublingual puncture had the greatest effect on animal welfare and recommended anesthesia for sublingual bleeding. They suggest researchers to choose their blood sampling method carefully while considering the parameters they want to measure.

QUESTIONS

1. What is the typical blood volume of a mouse?

a.  10 ml/kg

b.   30 ml/kg

c.  60 ml/kg

d.   100 ml/kg

2.  What is the generally accepted, maximum blood volume allowed to be collected from mouse in a 2-week period?

a. 1% body weight

b.   3% body weight

c.  1% blood volume

d.  3% blood volume

ANSWERS

1.       c

2.       a

**Dutton III et al. Assessment of Pain Associated with the Injection of Sodium Pentobarbital in Laboratory Mice (*Mus musculus*), pp. 373-379**

Domain 2: Management of Pain and Distress; K1 - assessment of pain and distress (e.g., behavior which is a sign of pain and/or distress; physiologic changes; pain and distress scoring systems)

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The primary advantages of using sodium pentobarbital as a euthanasia agent are its reliability to induce rapid unconsciousness followed by medullary depression, cardiac and respiratory arrest, and subsequent death. Administration of sodium pentobarbital is one method of euthanasia that is listed as acceptable in laboratory rodents per AVMA Guidelines on Euthanasia.

Mice were euthanized with three concentrations of IP sodium pentobarbital and observed for writing, peritoneum-directed behaviors (PDB), loss of righting reflex, and time to death. In a second set of experiments, the same solutions/concentrations were injected into the plantar hind paw and measured for latency until first lick and the number of licks to determine time until irritancy which was compared to time of unconsciousness when used for euthanasia.

Writhing at any dose for IP injections did not occur. There was no difference between saline and high dose groups for PDB and high dose groups had quicker onset of loss of righting reflex and death. Hind paw testing showed that animals lose conscious more rapidly than the onset of pain seen in the paw-lick test. Authors conclude that sodium pentobarbital is capable of causing inflammation, thought euthanasia via the IP route is rapid enough where it does not cause overt signs of pain.

QUESTIONS

1. Which of the following methods is an acceptable method of euthanasia per the AVMA Guidelines on Euthanasia of Animals?

a. Inhaled CO2

b. Ketamine with xylazine

c. Barbiturates

d. Potassium Chloride

2. Pentobarbital has a similar mechanism of action to all of the following agents except:

a. Propofol

b. Diazepam

c. Tolazoline

d. Barbituric acid

3. Sodium pentobarbital shares the same DEA Drug Schedule with which other agent?

a. Fentanyl

b. Ketamine

c. Diazepam

d. Buprenorphine

ANSWERS

1. b, c

2. d

3. a (pure sodium pentobarbital is a class II; Combination phenytoin and sodium pentobarbital mixtures are class III)

**Falkenberg et al. Clinical, Physiologic, and Behavioral Evaluation of Permanently Catheterized NMRI Mice, pp. 380-389**

Domain 3: Research

Primary Species: Mouse (Mus *musculus*)

SUMMARY: Blood sampling is commonly performed in many laboratory species but has been shown to show a stress response that can be confounding. The stress response is related to handling, restraint, vascular puncture, blood loss, +/- anesthesia. To overcome some of these issues, a permanent vascular catheter can be used, however, the catheter could cause stress from surgery and social isolation. This study investigated the clinical, physiologic and behavioral parameters impacted by long-term arterial catheterization in mice, and how they affect the animal’s overall welfare. Body weight and food and water intake were measured. Fecal corticosterone metabolites (FCM) were collected to measure stress. Oxidative damage (due to bacterial infection, trauma, tissue necrosis and presence of foreign body) was studied by assessing urinary levels of DNA and RNA fragments. Behavioral effects were studied by scoring overall welfare and nest building.

Mice with permanent catheters implanted in the right common carotid artery were compared to sham-operated mice (carotid ligated) and non-operated control mice. The study failed to identify a difference in the biomarkers of oxidative damage between the groups. There was no effect on FCM from surgery or catheterization compared to controls (this may have been insensitive to mild stressors).  As expected, the body weights of sham-operated and catheterized mice decreased (insignificantly) but by day 24 after surgery no longer differed from control mice. Impaired nest building is considered indicative of reduced animal welfare. There appeared to be some effects on where the mice built their nests immediately post-surgery, but overall nest quality did not appear to be significantly affected. The animal welfare assessment (AWA) did not detect any differences due to catheterization or sham surgery. Overall this study showed minimal effects of carotid catheterization on animal welfare of the mouse model used. This may differ for less robust mice strains.

QUESTIONS

1. T/F: Impaired nest building is considered indicative of reduced animal welfare in mice.

2. Causes of oxidative damage post-surgery include all of the following except:

a.  Bacterial infection

b.   Trauma

c.   Tissue necrosis

d.   Presence of foreign body

e.   Good aseptic technique

3. T/F: permanent catheterization in mice appears to have a negative impact on their overall welfare.

ANSWERS

1. True

2. E. good aseptic technique

3. False

**Deebani et al. Effect of MS222 on Hemostasis in Zebrafish, pp. 390-396**

Primary Species: Zebrafish (*Dario rerio*)

Domains 2 & 3

SUMMARY: Tricaine methane sulfonate (MS222), a routinely used general anesthetics for vertebrate animals such as fish, has many side effects. For example, cardiac depression and failure, hyperglycemia, hypertaxia and tachyventilation and gill bleeding in fish. MS222 acts mainly by blocking sodium channels which decreases nerve conduction, and causes muscle relaxation. Current study investigated the undesirable effects of MS222 on blood parameters. Using chemical and mechanical bleeding techniques, authors showed that non-anesthetized fish bled significantly more, and authors collected more blood compared to zebrafish anesthetized with MS222. In contrast, the blood collected from the anesthetized zebrafish group showed increased percentage of RBCs and hematocrit compared to non-anesthetized zebrafish. In addition, higher number of total blood cells without change in thrombocyte counts were seen in anesthetized zebrafish groups. However, thrombocyte aggregation using plate tilt assay was not affected among these two groups. Lastly, authors demonstrated increased activation of intrinsic pathway of clot formation (kPTT) in anesthetized group compared to non-anesthetized zebrafish group. Thus, authors concluded that investigators should be aware of the undesirable effects on blood parameters while working with MS222 anesthesia in fish.

QUESTIONS

1.  What concentrations of Tricaine methane sulfonate (MS222) used for zebra fish anesthesia?

a. 0.15 mg/mL

b. 0.3 mg/mL

c. 0.5 mg/mL

d. 0.015 mg/mL

2. What are the undesirable effects of MS222 anesthesia in fish?

a. Cardiac depression and arrest

b. Alteration blood parameters (e.g., decreased bleeding, activation of blood clotting)

c. Gill bleeding

d. Hyperglycemia

e. All the above

3. T/F: Combination of MS222 and mechanical euthanasia is recommended for euthanasia in reptiles and fish.

ANSWERS

1. a

2. e

3. True

**Christe et al. Modified Dose Efficacy Trial of a Canine Distemper-Measles Vaccine for Use in Rhesus Macaques (*Macaca mulatta*), pp. 397-405**

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Measles virus, a member of the genus Morbillivirus  and family Paramyxoviridae, is a highly contagious virus. Exposure to humans is the major risk factor NHP’s. Since the decline in human use of the measles vaccination has put the human population below the threshold for herd immunity, there is a concern for NHP’s used in research facilities to contract this disease from humans. Measles vaccination programs for NHP’s have been developed but are difficult to implement in large facilities due to financial concerns, feasibility concerns, and lack of availability for some vaccines. An attenuated modified live bivalent canine distemper-measles vaccine (Vanguard DM, Zoetis, Parsippany, NJ) has been proven to be safe and efficacious for measles vaccination in NHP’s. The recommended protocol is to receive 2 doses of 1.0 mL IM. This study compared the use of one full-dose of vaccine (1.0 mL IM) to one half-dose (0.5mL IM) and one quarter-dose (0.25mL IM) in previously unvaccinated male and female juvenile Rhesus macaques. Antibody levels for all groups were compared at 0, 6 and 12 months following vaccination. The binding antibody and neutralizing antibody levels declined with time for all groups. At 12 months, the quarter dose group had significantly lower (p=0.05) binding antibody levels and low, although not significant (p=0.06), neutralizing antibody levels, when compared to the full and half dose groups.

The authors of this paper conclude that the half-dose of vaccine can be used with a similar effect as the full-dose vaccine. The use of the half-dose vaccine would help reduce cost and make vaccination practices more practical to implement.

QUESTIONS

1. Which of the following diseases is NOT from the genus Morbillivirus?
   1. Rinderpest
   2. Distemper
   3. Blue tongue
   4. Peste des petits ruminants
2. Infection with measles virus (or vaccination with live measles virus) can lead to a false negative test result when performing a  \_\_\_\_\_\_\_\_\_\_\_\_\_\_ test because \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.
3. T/F: NHP’s have been shown to be susceptible to infection by canine distemper virus through both experimental vaccination and natural occurrence.

ANSWERS

1. c
2. Infection with measles virus (or vaccination with live measles virus) can lead to a false negative result when performing a Mycobacterium tuberculosis skin test because the measles virus causes immunosuppression that can interfere with delayed-type hypersensitivity reactions.
3. True