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**POSITION STATEMENT**

**Association of Primate Veterinarians’ Position Statement: Cerebrospinal Fluid Aspiration for Nonhuman Primates in Biomedical Research, pp. 428-429**

Task 2: Management of Pain and Distress

SUMMARY: Cerebrospinal fluid analysis is used in a variety of ways including diagnostic [evaluate for neoplasia, hemorrhage, other] and research purposes [drug metabolization, biomarker identification, other]. Due to the inherent risk and need for this procedure the Association of Primate Veterinarians state that the use of this technique should be justified by either the veterinarian (diagnostic purposes) or approved by the IACUC (experimental purposes) in which case the benefits should always out-way the risks.

In order to perform a CSF tap, the animal should be properly anesthetized, placed in lateral recumbency, and with a sterile technique (including shaved hair, sterile preparation, and appropriately sterilized materials) the cisterna magna or lumbar vertebral space should be punctured. The needle size and length should be appropriate for the species. In addition, analgesia should be provided prior to the procedure and continued as needed post operatively. The APV members determined from the literature that this technique is being performed in many different ways and in some cases multiple frequencies per animal.

There have not been many complications reported in the literature. The authors mention that several complications can occur, including hemorrhage, herniation, infection, spinal/nerve damage, and discomfort.  Animals that undergo this procedure should be monitored post operatively for any changes including lumbar/spinal pain or head pressing. Animals should be monitored until they are able to stand on their own appropriately without support. Should any pain or discomfort be noted within the first 24 hours or more administration of analgesics is recommended. The article notes that this procedure in humans often results in spinal headaches which may be similar to behavioral, postural, or physiological changes in our NHP’s. Animals that undergo this procedure should have all relevant information noted in their medical record (including drug dose, preparation, CSF volume collected and recovery).

QUESTIONS

1. Which anatomic locations can cerebrospinal fluid be collected from?
2. What signs of discomfort might be noted from an NHP post CSF collection?
3. T/F: Spinal headache is commonly noted in humans after CSF collection?

ANSWERS

1. At the cisterna magna or between lumbar vertebral spaces.
2. Neurologic impairment including: lethargy, depression, obtundation, and head pressing.
3. True

**ORIGINAL RESEARCH**

***Husbandry***

**Moffitt et al. The Role of Emotional Contagion in the Distress Exhibited by Grouped Mice Exposed to CO2, pp. 430-437**

Primary Species: Mouse (*Mus Musculus)*

Domain 2

SUMMARY:Providing humane euthanasia to laboratory animals is federally mandated by the Animal Welfare Act. For Public Health Service (PHS) Assured Institutions, this requirement is also mandated by the Public Health Service*Policy on Humane Care and Use of Laboratory Animals.*These documents cite the AVMA Guidelines for the Euthanasia of Animals. The AVMA guidelines define euthanasia as “ending life of an individual in a way that minimizes or eliminates pain and distress.” The 2013 AVMA Guidelines consider CO2 inhalation to be acceptable with conditions for the euthanasia of small lab rodents. CO2 is known to have the potential to cause pain (due to formation of carbonic acid on mucous membranes) and distress (dyspnea) in rodents and these effects are mediated by concentration and flow rate (displacement of chamber volume per minute v/min). CO2 is also thought to control fear behavior because the amygdala is sensitive to hypercarbia and acidosis. Exposure to CO2 has been used and validated as a model of panic disorder in mice. Currently, the 2013 AVMA guidelines recommend a 10-30% v/min so that mice become unconscious before feeling pain. Most of the studies done to determine this flow rate was done in rats. More recent studies have shown that higher (30% v/min) have shown less distressful behaviors such as jumping or pawing at the face. Emotional contagion allows animals to perceive and share another’s affective state, even without the ability to recognize or understand the cause of emotion in the other. The current student explored behavioral and biochemical markers of pain and distress in stranger and cage mate mice exposed to 10%, 30%, and 50% v/min CO2. Conscious dyspnea and ataxia were used as markers of distress, pawing at the face was used as marker of pain, jumping interpreted as escape attempts and used as a marker of aversion, and plasma ACTH was used as a marker of stress.

Results:CO2 flow rate of 10% v/min resulted in longest time to unconsciousness in both cage mate and stranger mice and resulted in significantly longer mean duration of conscious dyspnea compared with 30% v/min and 50% v/min (no significant difference between 30 and 50%). In casemates, 10% v/min CO2 resulted in significantly longer mean duration of ataxia compared with 30% v/min and 50% v/min and in strangers 10% v/min resulted in significantly longer mean duration of ataxia compared to 50% v/min. neither plasma ACTH levels nor face pawing differed significantly between any of the study groups.

Conclusion:More potential for distress exists at 10% v/min CO2 than at higher flow rats (30% or 50%), which agrees with previous studies that found that duration of dyspnea in mice undergoing CO2 euthanasia was inversely related to CO2 flow rate. Emotional contagion in context of group CO2 euthanasia may be mediated by CO2 flow rate.

QUESTIONS

1.  Contact of CO2 with the nasal mucosa leads to the production of intracellular \_\_\_\_\_\_\_\_\_\_\_\_\_\_, which decreases pH and thus is thought to cause pain.

a.  Hydrocarbon

b.  Acetic acid

c.  Carbonic acid

d.   Benzoic acid

2.  The 2013 AVMA Guidelines for the Euthanasia of animals defines euthanasia as\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_.

a.  “Good death”

b.  “Humane destruction of an animal accomplished by a method that produces rapid unconsciousness and subsequent death without evidence of pain or distress, or a method that utilizes anesthesia produced by an agent that causes painless loss of consciousness and subsequent death”

c.  “Ending life of an individual in a way that minimizes or eliminates pain and distress”

d.   “The act of inducing humane death in an animal by methods that induce rapid, unconsciousness and death with a minimum of pain or distress”

ANSWERS

1. c

2. c (FYI, choice b is the AWA definition of euthanasia).

**Taitt and Kendall. Physiologic Stress of Ear Punch Identification Compared with Restraint Only in Mice, pp. 438-442**

Domain 2: Management of Pain and Distress

Primary Species – Mouse (*Mus musculus*)

SUMMARY

Background: In the USA and Europe ear punching is the most common way to identify rodents. Other species (calves) have shown that ear notching can induce stress behaviors and increase cortisol which indicate acute pain as a result of the procedure. Previous studies have found increased grooming at the expense of locomotion and marked decrease in burrowing as behavioral indicators of pain for rodents. Behavioral assays are subjective and thus it is helpful to measure relevant physiological parameters simultaneously. This study examined physiologic and behavioral responses of mice to ear punching vs restraint only and routine handling.

Materials/Methods: 6 female 9 wk old Swiss-Webster mice were implanted with radiotelemetry devices. Telemetry collection began during acclimation and continued throughout the study and included heart rate, temperature, and activity levels. Baseline levels were collected the day prior to handling manipulation.

Day 1 Manipulation – Removal from home cage and placement on top of cage for restraint for 3 sec.

Day 2 Manipulation – Restrained by scruffing for 3 sec during which time either the right or left ear was punched once using standard ear punch.

Day 10 Manipulation – handled for routine husbandry

Behavior analysis from the telemetry device had a 3-axis accelerometer that could calculate the total activity in counts per minute and was measured at 30min, 1 hr, and 24 hr intervals. Focal grooming assays (5 min each) were performed immediately after ear punch and 1 hr later.

2-way repeated measures ANOVA was used to analyze heart rate, body temp, and activity level. One-way repeated measures ANOVA was used to analyze the focal grooming data.

Results: Ear punching caused transient changes in heart rate and body temp. HR up 26.9% relative to baseline for 30 min and returned to baseline at 1 hr. Body temp elevated over 30min in ear punching. Restraint only and routine handling heart rates and body temps were not significant. Activity measurements over 1 hr did not follow a trend. Restraint only had decreased activity at 30 min and became significant at 1 hr. Time spent grooming after ear punch did not significantly differ between immediately after to 1 hr.

Discussion: On the severity index, ear punching was found to be around 10 to 15 compared to 4,7,5, and 9 for IP injection, Intradermal injection, tail venous puncture, and tail tipping respectively. Ear punching caused a transient but significant physiologic response in mice that was greater than restraint only and routine handling. The physiologic findings were more informative than the behavioral data. The total activity data was odd in that the restraint only group showed a trend but the ear punch group was restrained for the same amount of time and did not show a similar trend. This inconsistency shows that activity in itself is not the most informative metric of postprocedural distress. There was a decreasing trend in focal grooming at 1 hr compared to immediately after. This suggests discomfort or pain associated with the procedure which is consistent with other studies. Overall, ear punching caused slight to momentary pain (similar to human children) based on the data and thus does not support the use of analgesics in the procedure.

QUESTIONS

1. What is the most common way to identify rodents?

a. Toe clipping

b. Ear punching

c. Tattoos

2. True/False: Pain experienced from ear punching is similar between rodents and larger species.

3. What type of analgesic is recommended for mice ear punching?

a. Topical lidocaine

b. Meloxicam

c. Buprenorphine

d. None

ANSWERS

1. b. Ear punching

2. False – more painful in larger species due to the thickness of the ear cartilage and the amount of pressure required to achieve a full thick-ness cut. Calves are given a topical analgesic for pain (vapocoolant spray)

3. d. None – the procedure only cause momentary to slight pain and thus does not indicate the use of analgesics

**Taylor et al. Evaluation of a 16-week Change Cycle for Ventilated Mouse Cages, pp. 443-449**

Domain 4

SUMMARY: For the overall wellbeing of the animals and to reduce possible negative effects on research a study was conducted comparing the standard cage change schedule (once every 2 weeks) to changing cages once every 16 weeks. The control group had the cage bottoms replaced with new cages and bedding every 2 weeks. In the test group, the cage bottoms were not changed for 16 weeks. Instead an innovative method was used that allowed 75% of the bedding to be scooped from each cage every 2 weeks and replaced with fresh bedding. For both groups, the wire-bar lids and filter tops were changed after 16 weeks. All bedding and supplies were autoclaved to maintain sterility. Half of each group contained corncob and the other contained paper bedding. Intracage ammonia, temperature, humidity, CO2, and ATP were assessed to compare the intracage environments of each group. Animal behavior, animal appearance, bedding condition, cage condition, cage accessories condition, and mouse cage location were also assessed. Data demonstrated that scoop cages, regardless of the type of bedding, had slightly higher levels of ammonia than the cages that were replaced every 2 weeks. The average levels of ammonia never reached the arbitrary limit of 25 ppm, except at the 2 week time point in cages containing corncob bedding (although this was thought to be an equipment error). No other parameters differed significantly. The authors state that these results successfully prove that cage conditions in which the bedding is partially removed every 2 weeks over a 16 week cycle are at least comparable to those of a standard cage change cycle of every 2 weeks.

QUESTIONS

1. According to the Guide, how often should enclosures and accessories (i.e. tops) be sanitized?

2. According to the Guide, how often do solid bottom caging, bottles, and sippers require sanitization?

ANSWERS

1.  Once every 2 weeks

2. Once every week

**Esvelt et al. Variation in Bacterial Contamination of Microisolation Cage Tops According to Rodent Species and Housing System, pp. 450-455**

SUMMARY: Changing cage tops more frequently than may be necessary might affect management practices, including increasing labor costs associated with housing research rodents and decreasing equipment lifespan.  Bacterial loads on microisolation cage tops were evaluated at serial time points to 90 days from static cages housing mice or rats and from rat and mouse cages on several different ventilation systems.  Across all ventilation systems, bacterial counts at 14 days were significantly higher on rat microisolation cage tops compared with mouse microisolation cage tops.  Most organisms identified were gram positive and were commensals that would be unlikely to negatively affect the animals.  Mice and rats were also found to have very different patterns of bacterial contamination of microisolator cage tops, with mice having 20 unique species of bacteria and rats having 11 species.  They concluded that 14 days is an appropriate sanitization time point for rat microisolation cage tops, but the interval at which mouse microisolation cage tops are cleaned can be extended past the 14 days recommended in the Guide.

QUESTIONS

1. According to the Guide, how often should rodent enclosures and accessories, such as tops, be sanitized?
	1. At least once every week
	2. At least once every 10 days
	3. At least once every 2 weeks
	4. At least once every 30 days
2. According to the Guide, how often should solid-bottom caging, bottles, and sipper tubes be sanitized?
	1. At least once every week
	2. At least once every 10 days
	3. At least once every 2 weeks
	4. At least once every 30 days



1. What does the instrument in the picture monitor?
	1. Colony forming units
	2. ATP activity
	3. Light absorption
	4. Microbiological counts

ANSWERS

1. c
2. a
3. b

**Tasaki et al. Creating a Stable Short-term Housing Environment for Rabbits in a Cargo Van, pp. 456-461**

Domain 3: Research; K3 – Animal models

**Primary Species:**Rabbit (*Oryctolagus cuniculus*)

SUMMARY:This study describes the creation of a stable and adequate environment for laboratory rabbits at the gyrotron facility that allowed the performance of reliable and reproducible animal tests evaluating ocular damage due to Millimeter waves (MMW). Because the gyrotron system is composed of large and immobile equipment, the rabbits had to be brought to the gyrotron facility to perform experiments. No laboratory animal facility for rabbits was available at that site. Because rabbits are prone to transportation stress, it was important to keep them on site. A suitable environment was created in a cargo van without changing the original configuration of the vehicle. Environmental factors were monitored to ensure that they met the criteria for conventional animal facilities. The adaption period required by the laboratory rabbits were also assessed. 6 Dutch rabbits were singly housed (to prevent aggravating any eye injuries that may result after MMW exposure) for a maximum of 6 days. To control the interior environment, a window air conditioner, humidifiers, dehumidifier, photocatalyst deodorizer and LED lamp were placed in the cargo area. Urine and feces were collected by using pet pads in catch pans cleaned daily. All environmental conditions including microbial air monitoring were within appropriate levels. The animals were found to be fully recovered from the initial transportation after 2 days, allowing ocular studies to start after this adaptation timeframe (the rabbits lost 6.4% of their initial body weight during transportation).

QUESTIONS (True or False)

1. Rabbits are prone to transportation stress, rarely eating or drinking during transportation.
2. New Zealand white rabbits are known to be more aggressive than Dutch rabbits

ANSWERS

1. True
2. False

**McIntosh et al. Refinements of Equipment and Methodology to Reduce Risk during Pole-guided Chair Transfer of Nonhuman Primates, pp. 462- 468**

Domain 3

Primary Species: Macaques (Macaca spp)

SUMMARY: This article look at methods of transferring Nonhuman Primates for chair transfer.  Pole-collar method transfer has been used in the past with history of trauma or some type of injury (bites, scratches, equipment related incidents), and even escape of the primate.   For this study chair training was required to get 8 primate to transfer from their home cage to the charge via pole and collar and once in the chair they had to be trained to lift their head so a neck plate could be placed around their neck.  These primate was trained using the docking chair transfer method.

In this study the researchers refine the transfer of the NHP into a chair design to reduce the risk by maintaining a constant barrier between NHP and handler while providing control to the handler to facilitate chairing. These modifications was accomplished using a commercial, manual, hydraulic lift table as a transfer device to bring the chair into the NHP facility and raise it to the height of the

cage door.

These modifications did not compromise existing features of the chair, they did not require training time in addition to that for the standard chairing method in our facility, and they improved safety.

QUESTIONS

1.   It this study the improvement of the transfer on NHPs into a chair design to reduce the risk by maintaining a constant barrier between NHP and Handler? This is an example of

a.  Refinement

b.  Reduction

c.   Replacement

d.   All the above

2.   These refinements to a commonly used chair and transfer methodology support

a.  Rapid habituation

b.  Safe transfer

c.  Reduced stress for both animal and handler.

d.  a, b, c

3.  True or False: The refinements described using a hydraulic lift table as a transfer device to bring the chair into the NHP facility and raise it to the height of the cage door decreasing possible injury mitigate the potential risk of harm during NHP transfers and thus advance animal welfare.



4.   True or False: The picture above demonstrates a commercial, manual, hydraulic lift table as a transfer device to bring the chair into the NHP facility and raise it to the height of the cage door decreasing possible injury to the primate and handler.

ANSWERS

1. a

2. d

3. True

4. True

***Management***

**Davis et al. Postapproval Monitoring Practices at Biomedical Research Facilities, pp. 469-474**

Domain 5: Regulatory Responsibilities

SUMMARY: The USDA and OLAW require ongoing oversight of IACUC-approved animal activities. The first mention of postapproval monitoring (PAM) is in the Guide. There are no defined specific processes for conducting PAM and therefore each institution can design and implement its own. There is therefore a diversity of methods used across institutions but the PAM programs are all compliance-focused risk management. Most researchers collaborate if they feel it ensure the success of their research programs and institutional reputation. Appropriate PAM programs balance regulatory burden with compliance. This publication reports similarities in PAM programs and how institutions are achieving set goals. Of the top 100 funded research institutions invited to participate, 55% completed the survey. All institutions were AAALAC accredited; 74% managed over 300 IACUC protocols, 91% had more than 10 IACUC members and 76% exceeded 10,000 cages of rodents.

Several components of the PAM program were consistent among most institutions but were variable in the observation of technical activities. Routine PAM activities included reviews of: protocols; medical, procedure and training records and observation of procedures; animal condition; husbandry procedures; drug storage and records; animal transportation; laboratory safety and occupational health records.

Findings included:

* + 42% of the institutions had formalized PAM programs into IACUC-approved written policies.
	+ 59% had dedicated staff to perform PAM reviews.
	+ 62% felt researchers valued the PAM process.
	+ 57% allowed corrective measures for minor items which were not formally reported to the IACUC.
	+ PAM findings were reported directly to the full IACUC (57%), Director of IACUC office (26%), designated IACUC subcommittee (10%) or attending veterinarian (4%).
	+ The frequency with which protocols underwent PAM was based on risk to the institution or PAM was performed at a set frequency e.g. annually. Risks included previous history of non-compliance, procedures with potential for pain or distress, protocols requiring satellite housing locations, or protocols approved for multiple survival surgeries.
	+ Most institutions achieved their PAM goals (over 80% for all species) with similar mechanisms and programs being applied across species.
	+ Although institutions considered observation of procedures as critical to assessing a laboratory’s compliance with proper techniques, only 1/3 of institutions reported observing a surgical procedure for all protocols that included surgery.
	+ Few institutions performed PAM of veterinary (10%) or husbandry (16%) programs beyond semiannual inspections and program reviews.

Recommendations included:

* + A need for senior institutional leadership to support IACUC review and approval of a formal PAM program.
	+ Use dedicated staff that are not part of IACUC or the veterinary team for PAM to encourage a favorable culture of compliance without the perception of being ‘policed’ or undermining trust between researchers and veterinary staff.
	+ Seek IACUC input to clarify what issues would be considered inconsequential and those that would need formal reporting.
	+ Clarify various categories of risks to ensure effective oversight at a locally defined frequency of PAM sessions.
	+ Perform a PAM on the veterinary and husbandry programs to avoid overlooking critical issues.
	+ Use PAM to communicate institutions IACUC policies and program expectations with researchers.
	+ Share institutions’ shortcomings as well as successes.

QUESTION

1. According to Davis et al’s 2019 article on postapproval monitoring (PAM), which 2 methods were reported as most commonly used by institutions for addressing concerns or non—compliances:

a.   Follow-up observational visit

b. Stopping problematic procedures

c.   Retraining

d.   Contacting regulatory authorities

e.   Protocol amendment

ANSWER

* 1. c and e. Concerns or non-compliances noted during a PAM were addressed by retraining (90%), protocol amendment (95%), follow-up observational visit (80%), stopping problematic procedures (73%), modifying SOPs (55%); other measures included suspending a protocol, contacting regulatory authorities and initiating compliance investigations when findings were serious enough to warrant more aggressive action.

***Animal Health Surveillance***

**Benga et al. Current Distribution of Rodentibacter Species Among the Mice and Rats of an Experimental Facility, pp. 475-478**

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

SUMMARY: Pasteurella pneumotropica, among the most prevalent infections in lab animals, and other rodent Pasteurellaceae have been reclassified under the new genus Rodentibacter. The uncertain taxonomy has made it difficult to understand the pathogenesis, epidemiology, diagnostics, and control of infections. In mice and rats these organisms are considered host specific and typically do not result in clinical disease and are considered opportunistic. This study documented which of the newly described Rodentibacter spp. are present within their mouse and rat colonies. The mouse and rat colonies are housed in either open cages or IVC in microbiologic units with varying microbiologic status. Swabs of the nasal cavities, oropharynx, and genital mucosa were obtained during routine microbiologic monitoring and were cultured on Columbia blood agar and MacConkey agar plates for 48 hours at 37C under aerobic and anaerobic conditions. Molecular identification was done by PCR analysis and 16S rDNA sequencing. Further phenotypic identification of rat isolates were done with the API 20E kit. This screening of 50 microbiologic units of mice and rats yielded 51 Rodentibacter isolates. Among 16 of 31 mouse isolates were R. heylii and the remaining 15 mouse isolates were R. pneumotropicus. Among the 20 rat isolates, 16 were classified as R. ratti and 4 were R. heylii. The presence of R. heylii in both mice and rats suggest that some strains can be found in closely related species while most appear to be host specific. In conclusion, 3 of the newly described Rodentibacter were documented in laboratory rodents and their diagnosis contributes to the understanding of this group of bacteria.

QUESTIONS

1. Which of the following genus is the former rodent Pasteurellaceae classified in*?*

* 1. Mycobacterium
	2. Rodentibacter
	3. Salmonella
	4. Echinococcosis

2. T/F: Infection of mice and rats with Rodentibacter spp results in clinical disease?

ANSWERS

1. b

2. False

**Baker et al. Management of Multiple Protozoan Ectoparasites in a Research Colony of Axolotls (*Ambystoma mexicanum*), pp. 479-484**

Domain 1

Tertiary Species: Amphibians

SUMMARY: Axolotls are an endangered species of neotenic salamander with their use in research commonly relating to studies of regeneration and stem cells. There is limited information available concerning the treatment of ectoparasites in axolotls. This clinical case study concerned a colony of axolotls which presented with multifocal, white chalky to gray skin lesions with a diffuse whitish to blue color to the skin and friable gill filaments. Skin scrapings were performed and demonstrated protozoan ectoparasites (Chilodonella, Ichthyobodo, and trichodinid species). Please note, of the 25 axolotls examined prior to any treatment being instituted, 19 (76%) had at least one of the three protozoal organisms. Only 1/19 had all three organisms. Treatment consisted of changes in the husbandry regimen (elimination of bloodworms from the diet, implementation of a glove change between each cage, implementation of a net disinfection protocol between cages, and the water in which the axolotls resided was changed from treated tap water to RO water which also underwent particulate filtration and UV irradiation prior to adding the salts) as well as an 8-hour static immersion bath containing 0.025 mL/L of 37% formaldehyde. After one such immersion bath, the Chilodonella was eliminated, and the Ichthyobodo burden decreased but not to a significant level. However, the trichodinid burden substantially increased and the number of axolotls containing at least one of the protozoal organisms increased from 19/25 to 24/25. Two additional 8-hour immersion baths (on consecutive days) ensued this time containing 0.05 mL/L of 37% formaldehyde. This produced no additional reduction in the Ichthyobodo burden, but it did eliminate the trichodinid burden in all but one of the axolotls under treatment with the one axolotl remaining positive for trichodinid species but having a much-reduced burden. The source of the protozoal organisms was not determined although the live bloodworms were suspected.

QUESTIONS

1.   Why are axolotls given to be neotenic salamanders?

2.   T/F: The treatment with the formalin as well as the husbandry improvements proved to significantly reduce but not completely eliminate the protozoa burden while also resolving the clinical signs.

3.   T/F: The formalin (37% formaldehyde) preparation used for treatment is an FDA-approved product for finfish.

ANSWERS

1.   Neoteny is a condition in which an organism reaches maturity while still maintaining some of its juvenile characteristics. As it relates to axolotls, it refers to their retention of gills as an adult.

2.   True

3.   True

***Anesthesia***

**Hutson et al. Analgesia during Monkeypox Virus Experimental Challenge Studies in Prairie Dogs, pp. 485-500**

Domain 2: Management of Pain and Distress

Task 2: Minimize or eliminate pain and/or distress

Tertiary Species: Other Rodents

ONE-LINE SUMMARY:Buprenorphine proved to be a more effective analgesic for pain associated with Monkeypox Virus (MPXV) challenge than meloxicam in Prairie Dogs.

SUMMARY: The authors of this paper sought to determine which, of buprenorphine or meloxicam, was the more effective analgesic agent to be used for 8 month old Prairie dogs challenged with Monkeypox virus.  Both drugs were given via subcutaneous injections daily after inoculation.  There were 5 experimental groups and 2 control groups.  The experimental groups included Meloxicam only, Buprenorphine only, MPXV only, MXPV and meloxicam and MPXV and Buprenorphine. The authors collected data on morbidity, mortality, clinical lesions, weights, blood chemistry values, virus-tissue infectivity, serological response, gross and histopathological changes.

From all observations and tests done they concluded that Meloxicam-treated animals showed increasing trends of morbidity and mortality compared with the other groups.  While the use of buprenorphine for short-term pain relief would likely result in few or no differences in pathogenicity.  In response to their findings they modified their pain scale for such studies to include the use of buprenorphine in hopes of improving overall animal welfare.

QUESTIONS

1. What viral genus does Smallpox and Monkeypox viruses belong to?

2.   What is NFKB?

3.  TRUE/ FALSE  Orthopoxviruses have been shown to encode multiple proteins that act in various ways to inhibit NFKB activity.

4.   Name two viral clades of MPXV.

5.  TRUE/ FALSE   Meloxicam analgesia have been shown to cause temperature deregulation in rats.

ANSWERS

1.   Orthopoxvirus

2.  NFKB (nuclear factor kappa-light-chain-enhancer of activated B cells) - is a protein complex that controls transcription of DNA, cytokine production and cell survival.  It is a mediator of pro-**inflammatory** gene induction and plays a key role in regulating the immune response, including to viral infection.

3.   TRUE

4.   Two viral clades are West African and Congo Basin.

5.  FALSE. Opioid analgesics cause the temperature deregulation and not meloxicam.

**Mackiewicz et al. Pharmacokinetics of a Long-lasting, Highly Concentrated Buprenorphine Solution after Subcutaneous Administration in Rhesus Macaques (*Macaca mulatta*), pp. 501-509**

Domain 2: Management of Pain and Distress

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Buprenorphine is a commonly used analgesic in non-human primates due to its high potency, long duration, and wide safety margin. Previous studies have published pharmacokinetic data on the use of 2 commercially available buprenorphine formulations in rhesus macaques; this study focuses on a 3rd formulation- highly concentrated buprenorphine solution (HCBS). HCBS (1.8 mg/ml) is administered subcutaneously and has been FDA-approved for use in cats (SimbadolTM), where it may last 24 hours when dosed at 0.24 mg/kg SC and 0.72 mg/kg SC. HCBS, unlike other commercially available buprenorphine solutions, is not compounded and therefore not subject to state laws that may limit or prohibit the purchase of compounded controlled substances.

For this study, 6 adult rhesus macaques (3 males and 3 females) were randomly assigned into 2 dosage groups- low (0.24 mg/kg SC) or high (0.72 mg/kg SC). Injections were given in the left shoulder after shaving. Animals were trained to present their forearms for cage side blood sampling from the cephalic vein prior to injection (time 0) and then 0.5, 1, 2, 6, 12, 24, 48, 72, and 120 hours after injection. After a 21 day wash-out period, animals received the other dose and repeated the study. Buprenorphine levels in plasma were determined via HPLC-tandem mass spectrometry. 0.1 ng/ml was used as the (estimated) minimal therapeutic threshold, based on extrapolation from previous studies.

Plasma buprenorphine levels were > 0.1 ng/ml for both doses within 0.5 hours of injection and remained significantly above this threshold for 48 hours. The high dose maintained levels > 0.1 ng/ml for 72 hours. Levels decreased to <0.1 ng/ml for both doses by 120 hours. Plasma levels were significantly higher in animals receiving the high dose for 24 hours after injection; levels were not significantly different for the high and low doses after this point. Female macaques had significantly lower levels of plasma buprenorphine than males at both the low and high doses 0.5, 1, and 2 hours after injection, although levels were still >0.1 ng/ml. Elimination half-life was 19.6 hours for the low dose and 20.6 hours for the high dose.  ⅙ macaques appeared lethargic after injection and ⅙  was pruritic at the site of injection for both doses; clinical signs resolved within 2 hours after injection, and no other adverse effects were noted in study animals. The authors concluded that HCBS is a safe and viable analgesic for use in rhesus macaques at 0.24 and 0.72 mg/kg SC, particularly in facilities where purchase of other buprenorphine solutions may be difficult.

QUESTIONS

1. Buprenorphine is a \_\_\_\_\_ opioid with \_\_\_\_\_\_ dissociation rate from the u receptor.
	1. Full u-agonist, slow
	2. Full u-agonist, fast
	3. Partial u-agonist + partial k-antagonist, fast
	4. Partial u-agonist + partial k-antagonist, slow
2. T/F: Naloxone only partially reverses the effects of buprenorphine.
3. T/F: HCBS is labeled as a veterinary drug, and therefore less likely to be affected by the current withdrawals of human opioids from the market.

ANSWERS

1. d

2. True

3. True

***Experimental Use***

**Bartley and Johnson. Human Infant Pants for Postoperative Protection during Social Housing of New Zealand White Rabbits (*Oryctolagus cuniculus*), pp. 510-516**

Domain 4: Animal Care

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY:The goal of this retrospective study was to compare methods of incision site protection (following 2 angioplasty procedures) in an effort to increase social housing success in 154 male intact New Zealand white rabbits. The authors were primarily interested in the utilization of commercial infant pants instead of traditional E-collars, since E-collars are known to restrict the expression of normal species-typical behaviors and inhibit coprophagy. The rabbits were divided into 2 groups: group 1 (n = 72), E-collars ; group 2 (n = 82), infant pants for postoperative incision protection. Group 1 animals were separated from cage mates postoperatively for 10 days, and re-housing with previous casemates was attempted on day 11 following E-collar removal. Group 2 animals were socially housed immediately after recovering from anesthesia, and infant pants were removed on post-op day 11. If pants were removed/destroyed in the postop period, rabbits were provided either a new pair of pants or an E-collar at the discretion of the facility veterinarian, but social housing was maintained. Social housing success was greater in group 2 for both surgical procedures, rate of failure of infant pants was significantly different between the first (62%) and second surgery (37%), self-mutilation was minimal for both groups, and wound healing did not differ between groups. The authors concluded that human infant pants are a feasible method to increase social housing success in adult NZW male rabbits with no detriment to wound healing, as compared to E-collars. Infant pants may also be more cost-effective than E-collars.

QUESTIONS

1. The male rabbits in this study weighed an average of 3 kg. What is the minimum floor space AND interior height that must be provided for a singly-housed rabbit from this study, according to the Animal Welfare Act?
2. What two specific vitamins are abundant in cecotrophs?

ANSWERS

1. Floor Space: 0.28 m2/3.0 ft2 ; Interior Height: 35.56 cm/14 in

2. Vitamins K and B