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**ORIGINAL RESEARCH**

***Biology***

**Sciurba et al. Reference Intervals for Total T4 and Free T4 in Cynomolgus Macaques (*Macaca fascicularis*) and Rhesus Macaques (*Macaca mulatta*), pp. 380-387**

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

Primary Species: Macaques (*Macaca spp.*)

**SUMMARY:** Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), are crucial for several physiologic processes. Typically, these hormones are bound to plasma proteins in circulation, but unbound thyroid hormones are the active forms (free T3 & free T4). Hypothyroidism can manifest clinically as several non-specific clinical signs including alopecia, a common problem in captive nonhuman primates. T3 levels in circulation are less consistent at any given time; therefore, total T4 and free T4 are considered more reliable indicators of thyroid hormone status. The aim of this study was to establish reference intervals for total T4 and free T4 for 2 species of NHP commonly utilized in biomedical research. Immunoassays were utilized to measure the quantity within serum. Samples from 172 rhesus and 133 cynos were collected and used to establish RI. Total T4 levels were not different between rhesus and cynos; however, cynos demonstrated higher free T4 concentrations than rhesus. Significant differences were seen between rhesus macaques of Chinese vs. Indian origin with Chinese having higher TT4 and FT4. Cynos of Mauritian origin have lower TT4 than cynos from other Asian backgrounds. Additionally, young rhesus exhibited significantly higher FT4 and TT4 concentrations than older rhesus.





**QUESTIONS**

1. Thyroid releasing hormone is produced by which organ?

a.  Hypothalamus

b. Neurohypophysis

c.  Adenohypophysis

d.   Thyroid

2. In cases of primary hypothyroidism, which of the following hormone profiles would be expected?

a.  Low TT4, FT4, TRH, TSH

b. Low TT4, high FT4, TRH, TSH

c.  Low TT4, FT4, high TRH, TSH

d.  Low FT4, high TT4, TRH, TSH

**ANSWERS**

1. a

2. c

***Reproduction***

**Shirzeyli et al. Assessment of Mitochondrial Function and Developmental Potential of Mouse Oocytes after Mitoquinone Supplementation during Vitrification, pp. 388-395**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Vitrification affects the mitochondrial homeostasis and causes oxidative stress-related changes which causes low fertilization rates and poor development of oocytes.  Mitoquinone is an antioxidant that has been shown to protect mitochondrial membranes from oxidative stress and reactive oxidative species.  This twofold study was conducted to determine the appropriate concentration of mitoquinone during vitrification to protect mouse embryos and to determine the effect of mitoquinone on oocyte survival rate and embryo development.

To determine the most optimal concentration of mitoquinone, 120 mouse embryos were divided into 4 groups.  Each group received one of four concentrations: 0, 0.01, 0.02, and 0.04 uM of mitoquinone supplemented into basal medium.  The survival rate was evaluated in vitrified oocytes.  To determine the effect of mitoquinone on embryonic development, 20 oocytes from each group (control and treated) were in-vitro fertilized.  The cleavage rate was evaluated at 24 hours post fertilization and the blastocyst formation rate was determined at the end of the fifth day.

The optimal dose of mitoquinone was determined to be 0.02 uM based on cleavage rates for that group were 87% compared to 72% for the control group, 70% for the 0.01uM group and 73% for the 0.04uM group.  Fertilization and cleavage rates in the mitoquinone-treated oocytes were significantly higher than in the untreated controls.

QUESTIONS

1. What is vitrification?

2. How does mitoquinone protect the mitochondria?

3. What is the optimal dose of mitoquinone, according to the article, which produced the highest survival rate and highest developmental competency in the fertilized oocytes?

ANSWERS

1. An ultrarapid cooling method that is recommended for oocyte and embryo cryopreservation.

2. Mitoquinone resided in the mitochondrial inner membrane and reduces lipid peroxidation which decreases cellular oxidative stress.  It also helps to increase glutathione levels which also helps protect the cells from the nuclear and cytoplasmic damage that occurs during vitrification.

3. 0.02uM

**de Carvalho et al. Cryopreservation and Preparation of Thawed Spermatozoa from Rhesus Macaques (*Macaca mulatta*) for In Vitro Fertilization, pp. 396-406**

Domain 3: Research

Primary Species - Macaques*(Macaca spp.)*

SUMMARY: Spermatozoa cryopreservation and recovery of viable sperm after thawing is a major concern in NHP assisted reproductive technologies. The objectives of the study were to:

1) Compare 2 methods for cryopreservation of sperm from rhesus macaques with regard to sperm viability characteristics after thawing;

2) Compare 4 different methods of removal of cryopreservation reagents and sperm isolation after thawing

3) Evaluate the overall efficacy of the 2 best sperm isolation methods for use in IVF.

Comparison 2 Methods For Cryopreservation Of Sperm From Rhesus Macaques With Regard To Sperm Viability Characteristics After Thawing

* Vitrification (process of obtaining a glass-like solid to avoid ice crystal formation)
	+ Straw packaging system was submerged directly in liquid nitrogen for 5 s and stored until analysis after thawing
* Slow-freezing
	+ Diluted semen was loaded into 0.25 mL French straws an inserted into cool water bath - cooling rate of -0.5 °C/min from 22 °C to 4 °C.
	+ After equilibration, samples were submitted to nitrogen vapor for 10 min, with a cooling rate of approximately -220 °C/min from -10 °C to -70 °C, by placing the straws on a 1-cm thick Styrofoam “boat”
	+ The straws were then plunged into liquid nitrogen and stored until analysis after thawing
* A total of 16 semen samples were used for this experiment. Frozen-thawed sperm had significantly lower quality (P< 0.0001) in all parameters evaluated, including percent total motility, progressive motility, intact plasma membrane, and intact acrosome, as compared with fresh sperm, regardless of the freezing method.
* However, the slow-freezing method yielded significantly better sperm quality (P < 0.0001) for all parameters evaluated as compared with the vitrification method.

Comparison 4 Different Methods Of Removal Of Cryopreservation Reagents And Sperm Isolation After Thawing (Performed With Slow-Frozen Sperm)

* Simple washing was significantly better than glass wool filtration and did not significantly differ from direct swim up or density gradient centrifugation for the total number of sperm and the number of sperm with an intact plasma membrane.
* The preparation methods did not differ in terms of the number of motile sperm, percentage of sperm with progressive motility, or number of sperm with an intact acrosome
* Simple washing
	+ Two cycles of dilution (with medium - (modified Tyrode solution with albumin (3 mg/mL), lactate, and pyruvate; TH3) and centrifuging
* Direct swim-up
	+ Incubation followed by one cycle dilution and centrifuging
* Density gradient centrifugation
	+ 1 mL of ISolate Stock Solution (90% density gradient medium, added to a 15-mL conical tube and layered with 1 mL of 45% ISolate (Isolate Stock Solution diluted 1:1 with TH3) on top. Diluted semen (1 mL) layered on top of the second layer, before centrifuging
	+ Followed by cycles of dilution and centrifuging with TH3
* Glass wool filtration
	+ Incubation with glass wool followed by one cycle dilution and centrifuging

Evaluation The Overall Efficacy Of The 2 Best Sperm Isolation Methods For Use In IVF (Performed With Sperm Recovered By Simple Washing Or Density Gradient Centrifugation

* Oocytes were inseminated with a pool of frozen-thawed sperm prepared using the 2 different methods.
* A fresh sperm sample was used as control for standard IVF
* The fertilization rates were 44 ± 16% (MI) and 41 ± 4% (MII
* The blastocyst rates were 11 ± 7% (MI) and 65 ± 14% (MII)
* For MI stage oocytes at the time of insemination that had become MIIs by the time of the first assessment after insemination, fresh sperm had a significantly higher fertilization rate than SW, but did not significantly differ from DGC (P <0.05).
* The blastocyst rate did not differ significantly between treatments. For the oocytes that were at the MII stage at the time of insemination,
* The fertilization rate for fresh sperm was significantly higher (P < 0.05) than that of frozen-thawed sperm regardless of the treatment used (MII stage)

QUESTIONS

1. Why was the sperm for 4 all the male animals pooled when comparing methods of removal of cryopreservation reagents and sperm isolation?

2. When is sexual maturity reached in male rhesus macaques?

3. What are typical success rates in rhesus IVF?

4. What do MI and MII represent?

ANSWERS

1. Semen from all 4 males was pooled to reduce biologic variation due to possible differences in the sperm tolerance to freezing among the males, a phenomenon that has been previously described in both rhesus macaques and other species.

2. 3 to 4 years

3. Fertilization rates from 50% to 89% and blastocyst rates from 48% to 61%

4. Oocyte maturity. M= metaphase

***Husbandry***

**Islam et al. Weight Gain, Glucose Tolerance, and the Gut Microbiome of Male C57BL/6J Mice Housed on Corncob or Paper Bedding and Fed Normal or High-Fat Diet, pp. 407-421**

Domain 3: Research

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Husbandry practices can influence the reproducibility of research results. This study was conducted to understand the possible influence of bedding on dietary obesity studies. The effects of paper and corncob bedding on weight gain, metabolism and gut microbiota (GM) of mice (C57BL/6J) fed a high fat or normal diet was studied. Fecal and cecal samples were collected after euthanasia to study the impact of bedding and diet on the GM.

The gut microbiota has been shown to influence human health and disease conditions. Organisms in the GM provide metabolic benefits, protect against foreign bacteria, and promote homeostasis by providing immune responses. This makes the state of the GM important in mice models for human disease and disease states affecting metabolism.  The GM of mice can vary between where studies are located, and leads to lack of reproducibility between facilities. Since housing changes can affect the GM, this study endeavored to understand the influence of some housing changes on metabolism, weight gain and the GM.

Diet has a major impact on changing GM, and a diet that promotes obesity is related to dysfunctional microbiota and dysbiosis. Most animal GM are composed of the same 5 phyla (Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrocomicrobia). The most prominent bacteria (approximately 90% of the total) are Bacteroidetes, and Firmicutes. An increase in the Firmicutes to Bacteroidetes ratio is associated with obesity and increased food intake.

Results showed that bedding type did not have a significant effect on weight gain and metabolism. The results showed that bedding did not affect the species richness of the GM but did influence the composition of the GM. Bedding type may need to be taken into account in metabolic studies, where the composition of the GM could influence the animals’ metabolism.

QUESTIONS (True or False)

1.  The most prominent bacteria in the normal mouse microbiome are Bacteroidetes, and Firmicutes.

2.   Bedding type was found to cause changes in metabolism such as weight gain.

ANSWERS

1. True

2. False

***Management***

**Schlanser et al. Compassion Fatigue and Satisfaction in US Army Laboratory Animal Medicine Personnel, pp. 422-430**

**Domain** 3: Research

SUMMARY:

Definitions

* Compassion Fatigue (CF) - mental weariness resulting from exertion that is associated with attending to the emotional and physical pain of others.
* Burnout (BO)– feeling of hopelessness at work and difficulty in carrying out one’s job effectively.
* Secondary Traumatic stress (STS) – work-related secondary exposure to extremely stressful or traumatic events that cause the worker to experience an extreme state of tension and preoccupation with suffering in the medical setting.
* Compassion Satisfaction (CS) – feelings of pleasure that result from contributing positively to one’s work or the greater good of society.
* Euthanasia Distress – occupational stressor in vet med and varies with different species and the levels of attachment the caregiver has to an animal.

CF consists of 2 components: BO + STS. Lab animal vets and techs are at risk of CF due to the human animal bond developed while caring for animals while at the same time having to perform duties that may require the death of animals or cause pain to the animals. Army lab animal vets and techs have the added stressors including geographical separation, fluctuation of personnel, and delayed team cohesion due to new military assignments. CF causes a decrease in work satisfaction, mental health, and overall performance. In animal care work environments specifically it can lead to high rate of absenteeism, turnover, lack of team cohesion, aggressive behavior, unwillingness of staff to respect deadlines/rules, increased negativity, increased mistakes, safety violations, excessive work comp claims, increased stress by research animals, and decreased quality of animal or medical care.

It was hypothesized that euthanasia distress, self-reported difficulty in working with primary investigators, high levels loneliness, working with NHPs, and supporting category E studies would create low levels of CS and high levels of CF. The study explored a cross-sectional survey of experiences and perceptions among personnel serving at the DOD animal research environments.

Out of 150 possible respondents to the survey 65 responded with 37 veterinarians and 28 animal care specialist. 52% reported high CS and 48% low to moderate CS. 37% reported moderate BO and 63% were low BO. 15% reported moderate STS and 85% reported low STS. Overall the survey found job-related stressors contributed to decreased CS and increased BO and STS. The high CS reported may counterbalance the BO and STS. The high CS may be unique to the military due to programs and resources designed to support occupational and behavioral health. Additionally the government provides other benefit to increase job satisfaction including little to no student debt, free comprehensive healthcare, and opportunity for career advancement.

Use of NHPs was reported to contribute to strong moral struggles. This study found that those who have worked with NHP vs those that did not were associated with higher BO and STS. This study found that different species of animals may invoke different levels of attachment from those who work with them depending on how similar to humans the animal is perceived to be or the experiences they have had with specific animals in the past. This finding recommends assessing workplace risks with CF in NHP settings. Category E studies may be associated with higher levels of BO and STS but this finding was not statistically significant (likely due to the small sample size). Difficulty working with primary investigators found statistically significant associations with increased BO/STS and decreased CS. This could be due to the fact that investigators are motivated to generate quality data while animal personnel are focused on animal wellbeing, thus better understanding and communication between PI and animal personnel should be encouraged.

Higher loneliness scores were significantly associated with higher BO and STS and lower CS. This finding recommends that military leadership should increase opportunities for camaraderie and team building to combat loneliness. Euthanasia distress were significantly associated with higher BO/STS and lower CS. Veterinary personnel considered euthanasia to be a main cause of occupational stress. Little research has gone into the emotional distress of those who also provide care to the animals they are euthanizing as well as the frequency of euthanasia. This is an area that leadership should focus and provide adequate behavioral support and counseling to help with this stressor.

Study limitations included the small number of survey responses (45% response rate) and there could have been nonresponse bias. Those that did respond may be more or less unhappy working in research environments than those who did not respond which could cause the data to be skewed. However, even with the small sample size there were still indication of moderate BO and STS. This is an indication that intervention programs could be useful in this research environment.

QUESTIONS

1. What is the definition of Compassion Satisfaction?

a.  Mental weariness resulting from exertion that is associated with attending to the emotional and physical pain of others.

b.   Feeling of hopelessness at work and difficulty in carrying out one’s job effectively.

c.    Work-related secondary exposure to extremely stressful or traumatic events that cause the worker to experience an extreme state of tension and preoccupation with suffering in the medical setting.

d.   Feelings of pleasure that result from contributing positively to one’s work or the greater good of society.

1. T/F: This study found that there were moderate levels of both BO and STS with low CS reported amongst military animal personnel?
2. T/F: The Frequency of euthanasia also has a statistically significant effect on the level of CF.

ANSWERS

1. d
2. False – high levels of CS
3. False – this study did not evaluate the frequency of euthanasia as if related to CF

**Collins et al. Reuse of Disposable Isolation Gowns in Rodent Facilities during a Pandemic, pp. le Isolation Gowns in Rodent Facilities during a Pandemic, pp. 431-441**

Domain 3: Research

SUMMARY: The recent SARS CoV-2 (COVID) pandemic has significantly impacted the supply chain resulting in a shortage and price increases for much needed personal protective equipment. In this study, the authors examined the reuse of disposable gowns after being worn while changing rodent cages or performing research tasks, with the goal of establishing the gown's ability to continue to provide protection to personnel from exposure to contaminants such as laboratory animal allergens.

Fluorescent powder (XR7) was used as an identifier of contamination in the study. Mouse cages were treated with XR7 prior to manipulation for husbandry or research tasks. Disposable gowns were labeled and placed in a designated area within rodent housing areas with minimal drafts and traffic. Gowns used for husbandry tasks, were reused by a single individual at 3 different time points to change a total of approximately 340 cages. To simulate research tasks, a single gown was worn and reused by the same individual to access 3 cages daily for 5 days.

The authors found that disposable gowns can be reused a maximum of 5 times for research tasks and a maximum of 1 day for facility staff, without contamination of underlying clothing or arms and hands.

Additionally, the authors investigated the use of 5 vaporized hydrogen peroxide to decontaminate the disposable gowns and extend their use; they found that the gowns were adequately decontaminated, and that this could provide a safe reuse option for their staff.

QUESTIONS​​​​

1. According to NIOSH what is the estimated number of lab animal workers that will develop asthma?
	1. 1%
	2. 5%
	3. 10%
	4. 20%
2. Which of the following is not considered an engineering control?
	1. Biosafety cabinets
	2. Individually ventilated caging
	3. HEPA filtered cage dump station
	4. Personal protective equipment
3. T/F: Fel d 1 allergen is found in the urine.
4. T/F: Mus M1 is 100x more concentrated in the urine compared to the serum.
5. T/F: Male mice secrete 4x as much Mus m 1 in the urine than females.

ANSWERS

1. c
2. d
3. False - this feline allergen coats the hair and is produced in saliva.
4. True
5. True

**Walker et al. Evaluation of Rodent Cage Processing Using Reduced Water Temperatures, pp. 442-450**

Domain 4:Animal Care

SUMMARY:The purpose of this study was to compare the sanitization performance of different wash and rinse temperatures and then compare this to the more traditional wash cycle temperature of 180oF. Experimental temperatures used were 125oF, 140oF, and 180oF. Highly soiled cages, half of which were autoclaved were used to mimic some of the most difficult cages to clean. The outcome to establish sanitization standards was a visual inspection, ATP monitoring, and replicate organism detection and counting (RODAC). A study aim in addition to verifying sanitization standards was to estimate time savings and quantify cost savings in both electrical and steam-boosted heating. After being used under normal conditions cages were selected for the study based on visually looking heavily soiled. Half the cages were autoclaved at 250oF for 30 minutes. Conditions for cleaning ranged from a low of wash at 125oF and rinse at 125oF to as high as wash at 180oF and rinse at 180oF. Visual assessment of the cages for cleanliness did not predict ATP or RODAC and thus did not predict overall passing results. For a cage condition to be considered clean it first had to be visibly clean with no debris. In addition for ATP and RODAC to pass respectively there had to be less than 30 relative light units (RLU) and less than 15 cfu. Of the 9 conditions that were tested 2 passed. One was the institutional standard (condition 6) of wash/rinse at 180oF/180oF with wash/rinse times of 120/25 seconds and condition 9 with a wash/rinse at 140oF/140oF and a wash/rinse time of 450/50 seconds. Condition 6 took 27 minutes and condition 9 took 12 minutes. With this information condition 6 can run 29 cycles per day and condition 9 can run 13 cycles per day. Based on the current cost of power condition 6 was estimated to cost $38,549.24/year and condition 9 was estimated at $17,971.22/year.

QUESTIONS

1. What does RODAC stand for?
2. What three conditions would establish a cage as being sanitized
3. What cycle other than the traditional 180oF/180oF passed as being sanitized and provided a significant cost savings?

ANSWERS

1. Replicate organism detection and counting
2. The outcome to establish sanitization standards was a visual inspection, ATP monitoring, and replicate organism detection and counting (RODAC).
3. 140oF/140oF and a wash/rinse time of 450/50 seconds.

**Baldauf et al. Thermal Inactivation of Carcasses of Mice and Rabbits Infected with Pathogens of Risk Groups Two to Four, pp. 451-461**

Domain 5: Regulatory Responsibilities

Primary Species: Mouse (*Mus musculus*)and Rabbit (*Oryctolagus cuniculus*)

SUMMARY: Because BSL2 containment level or higher is required for most human pathogens and many animal pathogens, biosafety considerations are important during all steps of research.  Thermal inactivation of the majority of infectious agents is achieved using moist heat sterilization at 121oC for 20 minutes, but standard cycle times may not be sufficient to reach the required sterilization temperature throughout an infected animal carcass.  Depending on the facility, incineration may be the method of choice to treat large volumes of infectious waste or animal carcasses.  Alkaline hydrolysis and alkaline hydrolysis-based tissue dissolvers may not be suitable for every facility due to safety issues or insufficient space.  As an autoclave is a prerequisite for a containment facility, this study served to provide a template to evaluate autoclave procedures to safely and effectively inactivate mice and rabbits infected with risk group 2 to 4 pathogens.  Biologic indicators or temperature sensors were used to monitor the autoclaving process.  Temperature sensors were placed in the brain and abdomen of fresh rabbit carcasses weighing 3.23-3.77 kg, while biologic indicators were placed inside the carcass and between the carcass and bedding material underneath the carcass.  Temperature sensors were also placed in various locations in the bodies of frozen mice, as well as 2-3 biologic indicators placed below the lowest mice in the pile.  Fractionated prevacuum stage pulses alternatively caused injection of steam and removal of exhaust air, leading to effective and even distribution of heat within the load.  The authors were able to reach 121oC within 180 minutes for rabbits and 51 minutes for mice through use of a porous goods program instead of a liquid waste program.  This gave them the advantage of including a fractionated prevacuum to allow steam to penetrate into porous and hollow goods.  The authors’ protocol was deemed suitable only for separately placed thawed or frozen mice with additional safety margins recommended when piling up thawed or frozen mice.  While larger rabbit carcasses would need further validation, the number of rabbits autoclaved at the same time did not appear to affect the efficiency of the sterilization process.

QUESTIONS

1. What BSL containment level is SARS-CoV2?
	1. BSL1
	2. BSL2
	3. BSL3
	4. BSL4
2. Which of the following bacteria is commonly used as a biologic indicator for autoclaves?
	1. *Geobacillus stearothermophilus*
	2. *Geobacillus vulcani*
	3. *Pyrolobus fumari*
	4. *Thermus thermophilus*
3. Which of the following terms describes the time that is required to kill 90% of the microbial population?
	1. Reduction value
	2. D-value
	3. P-value
	4. Thermal death time
4. Which of the following is commonly used for sterilization of medical devices to effectively remove air from porous or hollow loads and replace it with steam to allow for effective steam penetration?
	1. Liquid waste process
	2. Pulsed vacuum process
	3. Gravity cycle conditioning
	4. Steam flush pressure pulse

ANSWERS

1. c
2. a
3. b
4. b

***Anesthesia***

**Levinson et al. Pharmacokinetic and Histopathologic Study of an Extended-Release, Injectable Formulation of Buprenorphine in Sprague-Dawley Rats, pp. 462-469**

Domain 3

Primary Species: Rat (*Rattus norvegicus)*

SUMMARY: This article examined a novel extended release buprenorphine (BUP-XC) which is made with a lipid-encapsulated, low viscosity BUP suspension indicated for SC injection to control pain. The pharmacokinetics were evaluated for two different dosages: 0.65mg/kg and 1.30mg/kg in both male and female Sprague-Dawley rats at specific time points: 6, 24, 48, 72, 96 and 168 hours.  Injection sites were also evaluated  via physical exams and with histopathology.  Animals were either euthanized on day 8 or day 15.

Prior to the study the animals were handled for health status evaluation and acclimation.  During the study, hands on evaluations took place twice daily, looking for adverse reactions which include: GI or urologic abnormalities, nausea, food intake aberrations, distended abdomen, excessive grooming or chewing on forelimbs and pica.

Results: All test rats survived until euthanasia. Mean body weights of each test group reflect normal weight gains for the period of study time. In the low-dose test group, none of the males developed clinical signs. In the remainder of test groups, the most prevalent clinical sign was urinary sedimentation (high dose male 5/6 rats, low dose female 3/6 rats and high dose female 4/6 rats.) Other clinical signs observed in these groups were: forelimb lameness, ear tag inflammation (females only), vaginal discharge (females only), abdominal mass and injection site dermatoses (males only) and paw dermatoses and forelimb scab or alopecia (females only).

Male and female rats in the high dose groups had a significantly greater C-max and Mean AUC values than did the low dose groups. The t1/2 of the high dose group was 53% longer than the low dose groups. The mean volume of distribution was 20-27% greater and the mean elimination rate constant was approximately 6-fold greater in the high dose groups.

Mean plasma BUP concentration peaked at 6-24 hours in all test groups. The low dose groups, whether male or female, had lower mean plasma BUP levels at all time points as compared with the high dose groups, and female rats had lower mean plasma BUP levels than male rats at all time points.

Within 6 hours after administration all groups had mean plasma concentrations >1ng/mL, which were maintained in the high dose male rats for at least 96h. Detectable levels of BUP were present in 11/12 male rats until the 168h time point and in 10/12 female rats at 96 h.

Mild to moderate SC granulomatous inflammation was macroscopically observed at the injection site for 6/12 low dose rats and 7/12 high dose rats. Microscopically, injection site changes for the low dose included minimal perivascular infiltration of lymphocytes and plasma cells. For the high dose group, injection site inflammation involved formation of granulomas associated with lipid material.

Results indicate that a single BUP-XR dose at either dose concentration can reliably provide plasma levels of BUP reported in the literature to e therapeutically relevant for up to 72 hours, with lower plasma BUP levels anticipated in female rats over male rats.

QUESTIONS

1. What is buprenorphine’s mechanism of action?
	1. Mu partial agonist
	2. Mu antagonist
	3. K antagonist
	4. a & c are correct
	5. b & c are correct
2. T or F, This study reported that male rats had lower plasma BUP levels than female rats

ANSWERS

1. d
2. False

***Experimental Use***

**Xu et al. Strength and Sterility of Stock and Diluted Carprofen Over Time, pp. 470-474**

Domain 3: Research

Domain 4: Animal Care

Domain 5: Regulatory Responsibilities

SUMMARY

* FDA approved drugs are not routinely labeled for small animals, rodents or exotic species. Dilution of manufactured drugs is often necessary to obtain the dose and appropriate volume for safe administration of a drug in these species. The United States Pharmacopeia (USP) publishes standards for Pharmaceutical compounding and Sterile Preparation (USP General Chapter 797), which is a legally enforceable standard. This standard states that a compounded sterile preparation must have a beyond use date (BUD) after which the drug must not be stored transported or administered and therefore must be discarded. There are several assays that can be used to determine the optimal storage or BUD dating for compounded preparations; those assays are as follows: product specific quantitative strength assays (i.e. HPLC), validated stability- indicating methods, sterility testing, and bacterial endotoxin testing.
* The objective of this study was to evaluate the BUD of carprofen 50mg/mL (Dechra Veterinary Products), which when diluted to 0.5-1mg/mL with 0.9% sterile saline (Hospira) is currently approved for storage under refrigeration for 28 days once the vial is punctured.

Categories Tested (4)

* Dilution (diluted carprofen 1mg/mL vs. stock 50mg/mL)
	+ All refrigerated samples fell within 90-110% of labeled claim for strength, freezer samples varied
* Storage container (sterile vial with rubber stopper vs. conical tube)
	+ Sterile vial with rubber stopper maintained strength longer than conical tube for room temperature samples
* Storage temperature (room temperature vs. refrigeration)
	+ All samples were below the <5 EU/mL for endotoxin growth
* Time point since dilution (0-180 days)
	+ Both the frozen and refrigerated samples showed a downward trend in strength overtime

Conclusion

* Sterility and strength of diluted / stock solutions of carprofen can be maintained longer than 28 days
* For long periods of time: stock or diluted carprofen can be kept at refrigerated conditions in sterile vials for up to 180 days.
* For short periods of time: diluted carprofen can be kept in room temperature conditions in conical tubes for up to 60 days.

QUESTIONS

1.   Which organization publishes the guidelines for tests, and reference standards for drugs, biologics and compounding preparations (sterile and non-sterile)?

a. The Guide  for the Care and Use of Laboratory Animals

b. The United States Pharmacopeia (USP)

c. Food and Drug Administration (FDA)

d. Environmental Protection Agency (EPA)

2.  T/F: According to a recent journal article, dilutions of carprofen should be stored in the freezer to maintain strength of the drug overtime.

ANSWERS

1. b
2. False, freezing creates freeze-thaw artifacts such as crystallization or container damage and this decreases the strength of the drug overtime

**Rhynd et al. Efficacy of Fenbendazole and Ivermectin against *Trichuris* spp. in African Green Monkeys (*Chlorocebus sabaeus*) in Barbados West Indies, pp. 475-483**

Domain 3: Research

Tertiary Species: Other Nonhuman Primates

ONE LINE SUMMARY:The authors’ demonstrated usage of Fenbendazole alone and a combination of Fenbendazole and Ivermectin resulted in a 100% fecal egg count (FEC) reduction of *Trichuris spp*. by day 60 post-treatment compared to 86% FEC reduction in African Green Monkeys (AGM) treated with Ivermectin alone.

SUMMARY: Trichuris spp. are a common gastrointestinal nematode, with a direct life cycle, in mammals. Upon ingestion of an embryonated egg, the released L1 larvae will travel to the cecum and colon, where it buries into the epithelia and develops into sexually mature adults. Trichuris spp. have a high prevalence in Old World non-human primate populations, and in efforts to promote parasite-free AGMs in research facility settings, different antihelminth treatment regimens have been explored regarding effectiveness as a treatment.

All fecal samples used to analyze Trichuris spp. involved usage of 3g (wet weight) of feces from naturally infected AGM. The feces were analyzed with Modified McMaster Flotation method using a saturated sugar solution containing a specific gravity of 1.27. AGM were maintained in elevated stainless-steel cages and cleaned daily to reduce any potential for auto-infection. Fecal samples were collected 7d, 4d and d0 prior to treatment start date, and the baseline FEC was established from the mean FEC of all three dates. A population of 65 AGM were used and broken into the following groups: 50mg/kg PO SID d0 – d3 Fenbendazole only (16 AGM); 200ug/kg SC d0 Ivermectin only (16 AGM);  Fenbendazole + Ivermectin (15 AGM) and untreated control (18 AGM). Fecal samples were collected and FEC measurements were performed on d7, d14, d28 and d60 post-treatment. AGM treated with Fenbendazole alone showed a rapid decline in shedding probability, with minimal to no shedding detected by d28 post-treatment. AGM treated with the combined therapy had low shedding probability across each sampling time points, with the greatest shedding probability occurring 14d post treatment. Lastly, AGM treated with Ivermectin alone were able to demonstrate a consistent decline in shedding but not to the extent seen with AGM treated with Fenbendazole or with the combined treatment regimen.

The authors concluded usage of Fenbendazole alone or a combined Fenbendazole and Ivermectin therapy proved to be more effective than Ivermectin alone, based on the rapid decrease in shedding rates and the FEC of Trichuris spp. Of the two, the combined treatment therapy appeared to be most effective treatment therapy.

QUESTIONS

1. Which of the following drug classes does Ivermectin belong to?
	1. Benzimidazoles
	2. Imidazothiazoles
	3. Macrocyclic Lactones
	4. Organophosphates
	5. Salicylanilides
	6. Tetrahydropyrimadines
2. Which of the following Trichuris species is commonly to infect both humans and NHPs?
	1. *Trichuris muris*
	2. *Trichuris serrata*
	3. *Trichuris suis*
	4. *Trichuris trichiura*
	5. *Trichuris vulpis*
3. Which of the following characteristics is true regarding Fenbendazole?
	1. Acts on Cys-loop family ligand-gated ion channels*.*
	2. Binds cytoskeletal protein tubulin
	3. Easily absorbed in the gastrointestinal tract
	4. Promotes influx of chloride ions resulting in long-lasting hyperpolarization, and decreases onset of action potentials

**Answers**:

1. c (Macrocyclic Lactones)
2. d (Trichuris Trichiura)
3. b (Binds cytoskeletal protein tubulin)

**Lutz. Effect of Pregnancy and Age on Alopecia in Adult Female Baboons (*Papio hamadryas* spp), pp. 484-488**

Domain 4: Animal Care

Secondary Species: Baboon (*Papio* *spp.*)

SUMMARY: Alopecia is a multifaceted condition that is affected by a variety of both intrinsic and environmental variables including species, sex, age, social rank, pregnancy, housing, season, behavior, stress, hormonal changes, nutritional deficits, infection, and parasites. Because coat quality and alopecia can be impacted by multiple factors, identification of potential causes can be challenging. In addition, its persistence may result in management or clinical responses that are not consistent with animal wellbeing. However, a better understanding of the etiology of alopecia in captive NHP will help to identify welfare concerns. Because alopecia commonly occurs in pregnant women and NHP, the purpose of this study was to further assess the incidence of alopecia in NHP during pregnancy. Due to its association with alopecia, age was also included as a variable.

Adult female olive baboons (*Papio hamadryas anubis*) and olive/yellow baboon (*P. h. cynocephalus*) crosses were evaluated for this study (note: gestation period lasts approximately 6 months). Assessments were conducted when the animals were sedated for routine physical examinations. Alopecia was scored on a scale of 0 to 5, with 0 being no alopecia and 5 being severe alopecia. Results showed pregnant females had significantly more moderate alopecia than did control females, there was no effect of age on alopecia, and un unexpected outcome was that among nursing females, more of those with female infants had moderate alopecia than did those with male infants.

In summary, pregnancy was found to be a risk factor for alopecia in captive baboons. However, in the current population, age did not contribute to alopecia, which may be unique to baboons, as compared to other NHP species like the macaque. Moderate alopecia was shown to be more common in mothers with female infants than in those with male infants. This difference could be due, in part, to greater contact with female infants or to increased maternal investment directed toward daughters.

QUESTIONS

1.   True/False: Telogen effluvium is a disorder characterized by significant hair shedding resulting from a disruption of the hair cycle and excessive loss of telogen hairs.

2.   Name the phases of cyclic hair regrowth.

ANSWERS

1. True

2.   Anagen, catagen, and telogen phases, followed by hair shedding