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**OVERVIEW**

**Koch. Preventing Adverse Events at Research Facilities, pp. 660-669**

Domain: 1

SUMMARY: This article discusses the various types of adverse events that might occur, that actually have occurred, and that have been documented as noncompliances at research facilities regulated by APHIS, to assist institutions in creating proactive plans to prevent or mitigate such events in the future, as well as presenting some resources and ideas to help in making plans more effective in preventing or managing the wide variety of possible adverse events at research facilities. Under the Animal Welfare Act (AWA), APHIS regulates facilities that use animals in biomedical research, tests, experiments, or teaching. The implementing regulations of the AWA are established in the Code of Federal Regulations, Title 9, Chapter 1, Subchapter A, Parts 1 through 4. APHIS noncompliance data are documented by APHIS inspectors during periodic (at least annual) inspections. The identification, analysis, assessment, control, and avoidance, minimization, or elimination of unacceptable risks is known as risk management. Effective risk management typically requires an assessment of 2 factors: the likelihood that the risk will occur (probability) and the magnitude of the consequences when it does occur (effect).

APHIS defines serious adverse events as incidents that lead to significant injury or illness, unrelieved pain or distress, or the death of a regulated animal. When evaluating the magnitude of the effect of a possible adverse event, the effect on animal welfare is of primary concern, but additional potential factors might also be important to an institution, including loss of research data, negative media attention, Freedom of Information Act requests for details of the event by animal advocacy groups, and APHIS actions, such as citations or enforcement actions. An adverse event must result in noncompliance with an AWA regulation or standard to be cited on an inspection report. Noncompliances that currently (at the time of the inspection) have a serious or severe adverse effect on the health and wellbeing of the animal are categorized as direct noncompliances. However, not all direct noncompliances are associated with adverse events, and not all adverse events are documented as direct noncompliances (that is, when a past adverse event does not currently affect animal wellbeing, it is not a direct noncompliance). For that reason, in 2016, APHIS began documenting some citations as ‘critical noncompliant items.’ Noncompliant incidents noted to have had serious or severe animal welfare consequences previously but that pose no current risks to animals are ‘critical noncompliances.’ Critical noncompliances are even more serious when they are repeated noncompliances.

About 3/4 of the animals involved in noncompliances reported to OLAW are animals not regulated by APHIS. Most of the animals involved in direct noncompliances documented by APHIS were carnivores (33%), followed by ungulates (19%), rabbits (18%), and NHP and rodents (15% each).



Most noncompliances at research facilities are administrative or only slightly influence animal welfare. Of the research facility citations documented by APHIS from 2010 through 2014 (3587 total, averaging 717 per year), the greatest proportion of citations (39%) related to the administrative functions of IACUC. For APHIS, during 2010 through 2014, there were 149 direct noncompliances (about 4% of all citations) on 87 inspection reports involving 56 research facilities (about 5% of all registered research facilities). Of the direct noncompliances, veterinary care issues comprised about 44%, animal husbandry issues (monitoring, water availability, space issues, enrichment issues) were 34%, physical plant issues (ventilation, drainage, construction, maintenance) were 7%, and ‘other’ direct noncompliances (caused by human error, training failure, mishandling, and so forth) totaled 15%. As these data indicate, successful prevention and mitigation of adverse events require responsive monitoring of animals and enclosures. Many types of adverse events are preventable, but meticulous planning is required to establish a successful prevention and mitigation strategy.

QUESTIONS

1. What is the AWA definition of “animal”?

a. Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or such other warm blooded animal intended for use, for research, testing, experimentation or exhibition purposes, or as a pet, to exclude rats of the genus Rattus, and mice of the genus Mus bred for research purposes, and farm animals used for food/fiber/improving animal nutrition/husbandry

b. Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or such other warm blooded animal intended for use, for research, testing, experimentation or exhibition purposes, or as a pet, to exclude bird, rats of the genus Rattus, and mice of the genus Mus bred for research purposes, and farm animals used for food/fiber/improving animal nutrition/husbandry

c. Any live, vertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes

d. Any live, invertebrate animal used or intended for use in research, research training, experimentation, or biological testing or for related purposes

2. True/False: Per the AWA regulations, all noncompliances- including adverse events- are required to be reported to APHIS.

3. Which AWA amendment introduced environmental enrichment for the psychologic wellbeing of NHPs?

a. 1970

b. 1976

c. 1985

d. 1990

ANSWERS

1. b. Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or such other warm blooded animal intended for use, for research, testing, experimentation or exhibition purposes, or as a pet, to exclude bird, rats of the genus Rattus, and mice of the genus Mus bred for research purposes, and farm animals used for food/fiber/improving animal nutrition/husbandry.

2. False: Noncompliances- including adverse events- are required to be reported to APHIS when the event results in the suspension of an animal activity by IACUC.

3. c. 1985

**ORIGINAL RESEARCH**

***Biology***

**Eshar et al. Reference Intervals for Plasma Biochemical Variables by Point-of-Care Testing in Captive Black-tailed Prairie Dogs (*Cynomys Ludovicianus*), pp. 670-678**

Domain 1, K1 - Diagnostic procedures

Tertiary Species: Other Rodents

SUMMARY: Clinical chemistry reference intervals for prairie dogs in the past have been derived from studies with small sample size of fewer than 20 animals. This study seeks to determine reference intervals for clinical chemistries in prairie dogs performed with a veterinary point-of-care (POC) analyzer (VS2, Abaxis, Union City, CA) which only requires 0.1 ml of whole blood, plasma, or serum. Heparinized plasma was collected from the cranial vena cava or jugular vein of 50 captive-raised, sexually intact prairie dogs (34 males, 16 females) under isoflurane anesthesia and tested with the POC analyzer. Samples from a subset of 17 prairie dogs (6 females, 11 males) were concurrently analyzed with an automated wet-biochemistry analyzer at the Kansas State Veterinary Diagnostic Laboratory. Reference intervals were determined according to ASVCP guidelines with modifications according to recommendations for small sample size (40-60 samples) by using a standard approach (mean +/- 2 SD) when normally distributed and a nonparametric approach when not normally distributed. Values were compared according to animals’ sex and age. Sex and age had significant effects on several plasma analytes. Female prairie dogs had significantly higher albumin than males and was significantly lower in older animals. Total protein and globulin levels were significantly higher in older animals. Juveniles had significantly higher ALP and ALT concentrations than adults. Older animals had significantly lower BUN and higher creatinine concentrations than juveniles. Younger animals had significantly higher glucose concentrations and significantly lower sodium concentrations compared with older animals. Comparisons between the POC analyzer and the laboratory reference biochemical analyzer only showed acceptable clinical agreement for calcium and BUN only. This study confirmed the need for method-specific reference intervals,

QUESTIONS

1. Prairie dogs are susceptible to developing which type of tumor that is associated with marked increases in ALP concentration when compared with healthy prairie dogs?
	1. Osteosarcoma
	2. Hepatocellular carcinoma
	3. Pheochromocytoma
	4. Hemangiosarcoma
2. In the group of healthy prairie dogs in this study, BUN concentrations \_\_\_\_\_\_\_ with age, and creatinine concentrations \_\_\_\_\_\_ with age.
	1. Increased, increased
	2. Decreased, decreased
	3. Increased, decreased
	4. Decreased, increased
3. According to the ASVCP reference interval guidelines, what is the optimal minimum number of animals in order to determine de novo reference intervals using nonparametric methods in veterinary species?
	1. 60
	2. 75
	3. 100
	4. 120
	5. 150

ANSWERS

1. b
2. d
3. d

***Reproduction***

**Majewski et al. Sterilization of Silastic Capsules Containing 17β-Estradiol for Effective Hormone Delivery in *Mus musculus*, pp. 679-685**

Domain 3: Research

Primary Species:Mouse (*Mus musculus*)

SUMMARY: Methods for estrogen delivery in animals include routine injections, oral gavage, commercially prepared pellets, and implantation of silastic capsules (which offer flexibility in dosage and convenience for sustained delivery of estrogen). Commercially prepared hormone pellets offer several advantages over other methods, including: 1) pellets are provided sterile and 2) they delivery consistent uniform subcutaneous release of hormone for specified length of time. However, several research groups have found that commercially prepared pellets elevate serum hormone levels to greater extent than projected and hormone delivery is not consistent.

When looking at the other methods for hormone delivery, preparing hormone compounds in reagent grade oils for subcutaneous injections offer flexibility for testing doses and sterility, but the daily injections can lead to skin lesions. Oral treatment does not require sterilization, but repeated handling of mice for gavage comes with risk for esophageal damage or accidental injection in lungs. Oral administration of estrogens may also have low bioactivity and systemic activity or elevated hepatic estrogenic toxicity can occur as well. Hormones packed in silastic capsules are convenient for extended delivery in mice since insertion and removal under isoflurane is easy and quick. Since these capsules are made in labs, sterilization is necessary prior to implantation, which may degrade the hormones. This study looked to show that silastic estrogen capsules can be sterilized by using ionizing radiation (IR) or ethylene oxide (EO) without diminishing efficacy.

Serum E2 levels in normally cycling mice range between 5 and 60 pg/ml and do not differ between age‑matched virgin and parous mice. They saw that the commercial implants had considerable increases in serum E2 and that while they thought the 14d commercial pellet would lead to normal levels of E2 by day 28, the pellets continued to deliver high concentrations longer than 14d. Removing the pellet at day 14 was required to allow normal estrous cycling. When looking to make sure that sterilization did not affect hormone effects, ELISA kits were used. They found that neither sterilization method affected E2 serum levels by day 14. They also saw that the sterilized capsules produced mammary glands with alveolar development similar to that of pregnant mice. Levels of Progesterone receptor gene (Pgr) expression did not differ between sterilization methods.

One thing to note, in some mice treated with 0.05mg E2 capsules lead to serum E2 levels to fall below the established threshold on day 14. After more testing, they saw that by day 9, serum E2 was decreased and below what would be seen during pregnancy and were more consistent with normal estrous cycling. Lastly, E2 capsules resulted in increased ductal clusters and alveogenesis in the mammary glands of treated mice.

Commercially available pellets work well in elevating serum E2 levels and removing the pellets on day 14 will resolve the issue of extended hormone delivery, but these pellets are chalky, difficult to remove, and carry the risk of retained hormone secreting pellet fragments. The silastic capsules result in a supraphysiologic serum E2 spike at day 3, but the interanimal variation with these in house made capsules was more modest than the commercial pellets. The authors also found that E2 is reasonably stable and resistant to radiolysis by γ irradiation at doses between 5 to 26.6 kGy. Not all 0.05mg silastic E2 capsules secrete enough hormone to maintain serum E2 for treatment period, which is in contrast to commercially pellets that still secrete significantly increased amounts of estrogen after 65d. Even though 0.05mg E2 capsules did not sustain serum E2 levels above those necessary to maintain pregnancy, they were sufficient to induce pregnancy like changes in mammary glands of treated mice. Finally, they stated that either of these terminal sterilization methods (IR or EO) can be used to ensure both sterility and efficacy of silastic capsules.

QUESTIONS

1.   What is one disadvantage of commercially prepared pellets for hormone secretion?

2.   How long is the estrus cycle in mice?

3. According to the Office of Laboratory Animal Welfare (OLAW), what is the requirement when using pharmaceuticals in animals?

4.  When is it okay to use nonpharmaceutical grade substances in animals?

ANSWERS

1.   Hormone release is not consistent among different animals

2.   4-5 days

3.  “Pharmaceutical-grade chemical compounds should be used when available, for all animal-related procedures”

4.  With IACUC approval and when demonstrated that the compounds are reasonably safe in terms of grade, purity, pH, and sterility.

***Husbandry***

**Levy et al. Effects of Extruded Compared with Pelleted Diets on Laboratory Mice Housed in Individually Ventilated Cages and the Cage Environment, pp. 686-694**

Domain 4: Animal Care

Primary Species: Mouse (*Mus Musculus*)

SUMMARY

Introduction: Diets for laboratory animals are available in different types and physical forms with pelleted being the most common. The pelleted diet is manufactured by injecting steam into a mixture of ground ingredients, which are then forced through a die. Advantages to a pelleted diet include their ease of handling, storage, and usage; reduction of dust in animal facilities, and reduced wastage compared with meal or ground or meal from diets. One disadvantage is that they are difficult to alter or add test compounds to once they are manufactured. The extruded diet form is similar to the pelleted diet. Both are produced by injecting steam into ground meal, however with the extruded diet, pressure and higher temperature are used to force the meal through a die. Extruded diets are not as dense as pellet diets and preferred by some larger animal species. Due to formulation and decreased density, extruded diets break into chunks instead of powdered fines observed with pelleted diets. Powdered fines observed in pelleted diets accumulate at the bottom of cage and lead to increased soilage, and consequently may increase cage changes and labor costs. Disadvantages of extruded diets are that they have a higher price point per unit compare to pelleted diets. This study hypothesized that compared with mice that consumed a pelleted diet, those fed an extruded diet would maintain cages with less soilage, ultimately resulting in lower intracage ammonia concentrations. In addition, researchers wanted to evaluate the effects of extruded and pelleted diets on feed disappearance, cage weight, and body weight. Lastly, they wanted to evaluate whether any effects of diet form were influenced by stock or strain or sex.

Materials and Methods: 4 wk inbred mice (athymic nude (n=60 male, n = 60 female), (C57BL/6NHsd n=60 male, n=60 female), and outbred ICR (n= 60 male, n= 60 females) were randomly assigned to be fed a diet of no. 5053 Picolab Rodent Diet (P1), no. 5R53 Picolab Rodent Diet 20 Extruded (E1), no.2918X, Teklad Irradiated Global  18% protein rodent diet (P2), and no.2918X, Teklad Irradiated Global 18% protein extruded diet (E2) for 14 days. On days 0, 7, and 13 intracage ammonia levels were monitored for every cage. On days 0 and 14 each mouse and cage were weighed individually. Feed disappearance was calculated as the difference between the weight of the feed added and the feed remaining, divided by the number of animals per cage. A single observer assessed the housing environment and scored all cages using a Likert scoring scale. All cages were assigned a score of 0-4 (0= Fresh cage; no urine or feces, 1 = Clean; little to no urine or feces; no saturation; cage change not needed, condition: Great, 2 = slightly soiled; little urine or feces, 25% saturation; cage change not needed. Condition: good, 3 = Soiled moderate urine and feces; 50% saturation; cage change needed. Condition poor, 4 = extremely soiled; copious urine and feces; 75% saturation; cage change needed. Condition: very poor. Cage soilage reflected amount of gross fecal and urine soiling inside the cage as well as any feed that fell into the cage bottom.

Results

Body Weight:Type of diet was not found to have a significant effect on body weight.

Feed Disappearance:Feed disappearance was dependent on diet type and mouse strain or stock, and was the greatest in cages of mice that consumed the P1 diet. However, accumulation of powdered fines were noted at the bottom of the cages of mice fed a P1 diet which could account for the greater feed disappearance. The greatest feed disappearance was observed in the ICR strain which can be explained by their larger size and need to consume more feed.

Cage Weight:Cages containing C57BL/6 female mice that consumed the E1 diet were heavier than those housing C57BL/6 male mice on the same diet. When compared with other C57BL/6 mice, those that consumed the P1 diet had the heaviest cages. Similarly, cages containing C57BL/6 females were heavier than those that consumed E2 or P2. But no differences were seen in female C57BL/6 mice that consumed E1 or P1. Female ICR mice on the P1 diet had heavier cages than male ICR mice on the same diet. Overall, both male and female ICR mice that consumed the P1 diet generated greater cage weights compared to male and female ICR mice on any of the other diets. Nude mice cage weights were greater for both male and female mice that consumed the P1 diet than any of the other three diets. Increased cage weight seen with the P1 diet were also consistent with the presence of powdered fines

Intracage Ammonia Concentration:In cages that house ICR mice, ammonia concentrations were higher in cages where mice consumed P2 than E1. On day 13 ICR mice that consumed P2 had lower ammonia levels compared to mice that consumed E2. The greatest ammonia concentration occurred in the cages of nude mice given P1 compared with nude mice on any of the other 3 diets. Interestingly, cages housing C57BL/6 mice did not show any increases in ammonia levels after day 7 on any of the diets.

Cage Score:C57BL/6 mice and ICR mice had higher cage scores than did nude mice, with nude male mice having a higher score compared to nude females. Male mice that consumed E2 diets had higher cage scores compared to females.

Discussion:Although not always statistically significant, mice that consumed P2 diets maintained that lowest level of cage soilage throughout the study. Findings were consistent with the overall intracage ammonia concentration seen with both nude and ICR mice that consumed P2 diets. P2 diets also did not have to be topped off during the study indicating decreased wastage. Cage score and ammonia concentration data further support the idea that P2 diets provide greater absorption than other diets. The conclusion disproves hypothesis that extruded diets would maintain cages with less soilage and lower ammonia concentrations.  Study did reveal that the physical form of the diet does affect cage weight, feed disappearance, cage soilage, and intracage ammonia levels. This study demonstrated the importance of considering physical form of diet when considering experimental design.

QUESTIONS

1. Advantages of Pelleted diets include all except:
	1. Ease of handling and storage
	2. Reduction of dust in facilities
	3. Ease of adding test compounds
	4. Reduced wastage compared to meal and ground diets
2. Powdered fines observed in extruded diets can accumulate at the bottom of the cage and lead to increased soilage. True or False?
3. The Pelleted diet form is similar to the extruded diet. Both are produced by injecting steam into ground meal, however with the pelleted diet, pressure and higher temperature are used to force the meal through a die. True or false?

ANSWERS

1.c.  Ease of adding compounds

2. False: They are observed in pelleted diets

3. False: Extruded diets are produced this way

**Peveler and Hickman. Effects of Music Enrichment on Individually Housed Male New Zealand White Rabbits, pp. 695-697**

Domain 1: Management of Experimentally Induced Diseases and Conditions

Domain 3: Animal Care

Secondary Species: Zebrafish (*Danio rerio)*

SUMMARY: Body condition scoring (BCS) is a simple, rapid, noninvasive way to assess body condition in animals; requiring minimal training. In aquaculture and fish industries, body condition is often calculated according to physical measurements of weight and standard length (snout to caudal peduncle fin base) which is impractical when working with large populations of small fish such as zebrafish. This BCS system was developed using 2 criteria: the ability to perform visual health assessments by tank side exam and common anatomic sites for white adipose tissue depots in adult fish. The visual body condition scoring system (score, 1 through 5) assessed fish as thin to obese. The BCS system focuses on the size of the head and shape of the abdomen to classify animals within the 5 categories. The 2 anatomic sites assessed are the area of the head between the eye and operculum and the abdominal width. Laterally, the abdominal area of interest is halfway between the pectoral and anal fin; dorsally, halfway between pectoral fin to the dorsal fin. The cranial attachments of each fin are used as start and end points.

For BCS 1: The head is larger than the body (big head); laterally, concave ventrally; dorsally, the body is narrower than head and linear. The fish is considered thin (emaciated).

For BCS2: The head and body are of equal size; laterally, there is a flat ventral surface; dorsally, the head and width of abdomen are equal. This fish is considered under-conditioned.

For BCS 3: The body is larger than the head; laterally, there is a slightly convex ventral surface; dorsally, the head is slightly smaller to a fusiform body. The fish is considered well-conditioned.

For BCS 4: The body is significantly larger than the head; laterally, the body is moderately convex on the ventral surface and symmetrical; dorsally, the head is visually smaller to a moderately distended abdomen. This fish is considered over-conditioned.

For BCS 5: The body is significantly larger than the head; laterally, the body has a significantly convex ventral surface; dorsally, the head is visually smaller to a significantly distended abdomen. The fish is considered obese (large).

After a 20 minute hands-on training session and receiving a visual BCS chart showing lateral and dorsal views of adult zebrafish, 5 people rated 95 adult zebrafish.  Their results were combined. A Pearson correlation coefficient between the average BCS and expected BCS (according to their defined categories), the calculated predictive power of BCS, and intraclass coefficient confirmed that expected BCS definitions were appropriate and that there was excellent agreement between the raters.

QUESTIONS

1. According to the described BCS system, a well-conditioned zebrafish has a BCS of:

a.  BCS 2

b.  BCS 3

c.  BCS 4

2. The abdominal area of interest when assessing BCS is:

a.   Halfway between the pectoral and anal fin

b.   Near the pectoral fin

c.   Near the anal fin

3. The area of the head evaluated when determining BCS is:

a.   By the mouth

b.   Behind the operculum

c.   Between the eye and operculum

ANSWERS

1. b.  BCS 3

2. a.   halfway between the pectoral and anal fin

3. c.   between the eye and operculum

***Management***

**Kurtz et al. Acrylamide Production in Autoclaved Rodent Feed, pp. 703-711**

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Rodent feed is sterilized to eliminate the introduction of potentially pathogenic microorganisms.  Current feed sterilization methods are steam autoclaving and irradiation.  Neither method will sterilize all loads 100% of the time because sterilization depends on the size of the load and the ability for adequate quality steam or radiation penetration.

Both methods can affect the quality of feed. Autoclaving feed can increase pellet hardness; decrease vitamins A, B1, and D; affect protein quality and availability; and alter isoflavone content.  Irradiation has little effect on the physical quality but may change the chemical composition.

Recent studies have shown that heating foods with high levels of starches caused a chemical reaction which results in increased levels of acrylamide.  Acrylamide is widely used in the chemical industry for various purposes such as paper production, grouting agents, water treatment, and cosmetics.  It also occurs in cigarette smoke and is considered a neurotoxin, genotoxin, and carcinogen.   Glycidamide is a metabolite of acrylamide that reacts in vivo with glutathione, hemoglobin, and DNA.  This study tested the effect of autoclaving at various temperatures on the physical and chemical quality of NIH31 rodent diet, and the biological sequelae to the mice to which it was fed.

Materials and Methods:Ninety male C57Bl/6NCrl mice were part of this study. The NIH31 diet was autoclaved for 20 minutes sterilization exposure time, a drying time of 5 minutes, and a purge time of 1 minute at either 230, 250, 260, or 270 degrees Fahrenheit.  The irradiated diet received an exposure of 20 kGy and was obtained from Envigo.  The irradiated diet was the control.

Another group of mice was fed NIH31 pelleted diet that was autoclaved at 270 degrees Fahrenheit; NIH31 pelleted diet that was autoclaved but ground; NIH31 ground plus 200 ppb acrylamide (AA-low); and NIH31 ground plus 1000 ppb (AA-high) to test for feed intake and body weight gain.  Parameters tested were acrylamide concentrations in the feed; acrylamide and glycidamide concentrations in the plasma and urine; and hepatic glycidamide-DNA adducts that were quantified from the liver.

Results

Feed Intake and Body Weight:  Study diets were confirmed to be free of bacteria and fungi.  Feed intake did not differ among groups except that the AA-high group had a higher feed intake than the irradiated group.  All groups gained weight during the study without any differences in body weight gain noted.

Plasma Acrylamide and Glycidamide Analysis: No differences in the plasma acrylamide levels between the irradiated control and the diets autoclaved at 230, 250, or 260 degrees Fahrenheit.  However, the acrylamide levels were elevated when the control was compared to the diets autoclaved at 270 degrees Fahrenheit (both pelleted and ground) and both of the ground AA supplemented diets.  Measurable glycidamide levels were detected in all study groups, but the groups fed the irradiated diet or any autoclaved diet all had mice with undetectable levels.

Urine Acrylamide and Glycidamide Metabolite Analysis:  Metabolites of both agents were increased in all groups as compared to the control group.

Liver DNA Adducts:  All groups that received the autoclaved diets and AA spiked diets showed a significant increase in the number of glycidamide-DNA adducts as compared with the control.

Discussion: This study shows that acrylamide levels in these diets increases after autoclaving, and the levels produced are positively correlated with the temperature.  It is also important that institutions that cannot convert to an all irradiated diet to consider using sterilization temperatures at or below 250 degrees Fahrenheit to minimize the potential effects of acrylamide on autoclaved rodent feed.

QUESTION (True or False)

1.  Autoclaving has no effect on the levels of acrylamide in rodent feed

2.   Usually autoclaving has no effect on the hardness of pelleted feed

3.   Usually irradiation has no effect on the hardness of pelleted feed.

4.   Sterilization rarely affects the chemical composition of feed.

ANSWERS

1.  F

2.  F

3.   T

4.   F

**Gaafar and Fahmy. Effects of Laboratory Animal Science Training on Scientists’ Attitudes and Practice in Egypt, pp. 712-714**

Domain 6: Education

SUMMARY: This study was conducted to determine the effect of a training course offered to researchers by the IACUC at Cairo University in Egypt. The training course focused on laboratory animal science and ethical issues involved in animal use. The course was intended for a wide range of personnel involved with animal research, including PIs, research staff, student volunteers, graduate students, postdoctoral fellows, etc. Participants of the training course were surveyed using a self-administered questionnaire before and after they completed the training course. The survey results revealed that the scientists’ knowledge of animal welfare issues increased effectively after the completion of the training course. For instance, before the course, only about 10% of course attendees were able to correctly identify the 3Rs principles. After attending the course, almost all participants were able to correctly identify the 3Rs. The study reveals the importance of IACUC training for researchers working with laboratory animals at Cairo University. The authors state that these training courses must be mandatory for all animal users and other Egyptian universities and research centers because it is a valuable means of educating and raising awareness regarding animal welfare.

QUESTIONS

1.  What are the 3Rs?

a.  Reduce, reuse, recycle

b.  Reduction, refinement, repurpose

c.  Reduction, repetition, refinement

d.  Replacement, reduction, refinement

e.   Replacement, reduction, repetition

2.  According to The Guide for the Care and Use of Laboratory Animals, who is responsible for providing oversight and evaluating the effectiveness of the training program at an institution?

a.  The Institutional Official (IO)

b.   The Institutional Animal Care and Use Committee (IACUC)

c.   The Attending Veterinarian (AV)

d.   The Chief Executive Officer (CEO)

e.   The Principal Investigator (PI)

ANSWERS

1. d. Replacement, reduction, refinement

2. b. The IACUC

***Experimental Use***

**Fiebig et al. Evaluation of Infrared Thermography for Temperature Measurement in Adult Male NMRI Nude Mice, pp. 715-724**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (Mus musculus)

SUMMARY: Ten male retired breeder Rj:NMRI nude mice were used to investigate the accuracy of an infrared camera as an alternative method of body temperature measurement and compare its accuracy with established methods of temperature measurement (rectal, subcutaneous, and intraperitoneal).

Methods of temperature measurement were obtained via the following methods:

* Subcutaneous transponders (ST) were placed between the shoulder blades using a needle applicator.
* A freely moving sterilized data logger (DL) was surgically placed within the peritoneal cavity.
* For subcutaneous measurement, a noncontact infrared camera (IRC) was mounted on tripod and positioned 50cm over a clean dry cage.
* Rectal temperatures were measured using the stand technique.

In addition to body temperature measurement, body weight was recorded 3 times a day for 14 days.

Rectal temperature measurements generated data which were unevenly distributed among mice and covered a wide temperature range (34.01 to 38.87℃).  Overall, rectal measurements produced the highest number of outliers of any technique.  When compared to IRC, a significant correlation was not established - rectal and IRC temperatures increase at differing rates. IRC measurements within each mouse were similar and had a small standard deviation with the average temperature measurement ranging from 36.57 to 38.05℃. ST measurements had the largest range from 35 to 40℃.  DL measurements showed lowest median values of all the methods used, with measurements ranging from 33.21 to 37.76℃.

DL are not recommended for body temperature measurement due to several factors: surgical requirement, largest number of outliers, inability to be fixed within the peritoneal space, removal required for data acquisition, and its inability to show a stress-related influence on body temperature.

ICR method proved to be a reliable method of body temperature measurement due to its ability to accurately reproduce the published physiologic body temperature of mice (37.0 to 37.2℃) at the level of the doral skin.  Measurements were comparable to those obtained with ST and rectal methods.  ST and IRC were the only methods to demonstrate temperature fluctuations within the physiologic range; rectal and intraperitoneal methods were less suitable for showing these fluctuations.  In addition, the IRC method does not require survival surgery and measurements can be obtained without contacting the mice and within standard housing conditions, thus minimizing the need for potentially stressful handling.  A significant relation was seen between body weight and IRC however this was not seen in the other methods.  The authors recommend further refinement of the set-up when using IRC, as sufficient resolution is required to account for the small size and swift movement of mice - a minimum resolution of 640 x 480 is required for this system.

QUESTIONS

1. The NMRI mouse is traditionally used in what fields of research?
2. Increased body weight is most likely to have an effect on which method of body temperature measurement?
	1. Rectal
	2. Subcutaneous
	3. Intraperitoneal
	4. Cutaneous

ANSWERS

1. Toxicology, pharmacology, general biology/physiology, oncology, and teratology
2. d

**Hickman. Interpreting Neuroendocrine Hormones, Corticosterone, and Blood Glucose to Assess the Wellbeing of Anesthetized Rats during Euthanasia, pp. 725-728**

Doman 1

Primary Species: Mouse (Mus musculus)

SUMMARY:In this article authors discuss the current preferable recommendations for assessing animal wellbeing during euthanasia: measuring neuroendocrine hormones (i.e., ACTH, noradrenaline, and adrenaline). This is mainly because of the sensitivity of neuroendocrine hormones to the acute stress associated with rapid methods of euthanasia (when compared to corticosterone and blood glucose.) However, authors note how neuroendocrine hormones can be stimulated in ways that confound interpretation of welfare assessment in euthanasia studies (specifically the route/agent used).

Experimental Design: 24 subjects (n=12 male, 12 female rats) were anesthetized with a combination of ketamine–xylazine. Once animals reached a surgical plane of anesthesia, they were exposed to O2, CO2, or isoflurane, followed by terminal blood collection to assess concentrations of ACTH, noradrenaline, corticosterone, and blood glucose.

Results/Findings: Compared with animals exposed to O2 or isoflurane, rats exposed to CO2 had significant increases in their serum concentrations of ACTH and noradrenaline. Additionally, blood glucose and corticosterone levels did not differ between groups.

Take Home Message: authors recommend that noradrenaline and ACTH should be used with caution to assess overall animal wellbeing when the method of euthanasia might confound that assessment.

QUESTIONS

1. True or False: Regarding the mechanism of action for CO2 overdose, as blood concentrations of CO2 increase (hypercapnia and hypercarbia), serum pH levels are driven down, initiating respiratory acidosis.

2. What is the drug classification for Ketamine?

3. What is the drug classification for Xylazine?

ANSWERS

1.  True

2. NMDA receptor antagonists

3.  Alpha2-agnosits

**Hickman. Home Cage Compared with Induction Chamber for Euthanasia of Laboratory Rats, pp. 729-733**

Domain 2

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: This study compared the behavioral and physiological changes in outbred Sprague Dawley rats and inbred Brown Norway rats that were euthanized using 30% volume displacement of CO2 in either their home cage or induction chamber. Both males and females were pair housed by sex and one rat from each cage was assigned to either the induction chamber or home-cage euthanasia. The two treatment groups for each sex was: (1) induction chamber rat euthanized first, home-cage euthanasia rat euthanized second and (2) home-cage rat euthanized first, induction chamber rat euthanized second. Fasting blood glucose and serum corticosterone and noradrenaline were analyzed using blood collected from cardiac puncture at euthanasia. The number of times a rat reared, jumped, or exhibited digging behavior was recorded. These behaviors were extrapolated from published rat ethograms as being reflective of anxiety, agitation, or escape behaviors suggesting distress.

Results:There was a significant difference in rearing frequency, showing significant increase in induction chamber and in Sprague Dawley rats. Comparison for the mean dig frequency showed a significant difference in the overall model with significant increases in home cage and in Brown Norway rats. There was no overall difference between groups in jumping behavior.  Sprague-Dawley and Brown Norway rats showed no significant differences in selected neurophysiologic measurements between induction chamber and home cage euthanasia. The absence of jumping and digging behavior in the induction chamber seems to support the conclusion that rats did not experience increased agitation associated with euthanasia in the chamber vs. the home cage.

Conclusion: Both home cage and induction chamber are acceptable for induction of inhalant anesthesia or euthanasia in rats in regard to animal well-being.

QUESTIONS

1.  A catalog or table of all different kinds of behavior or activity observed in an animal is referred to as :

a. Harmonogram

b.  Anagram

c.  Ethogram

d.  Monogram

e.  Stereogram

2.  What are some behaviors that are thought to be associated with anxiety and distress in rats, as noted in published rat ethogram studies?

ANSWERS

1.  c

2.   Rearing, jumping, digging

**Pritchett Corning et al. Using Hysterectomy Rederivation to Produce Guinea Pigs (*Cavia porcellus*) Free of Guinea Pig Cytomegalovirus, pp. 734-737**

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

Primary Species: Guinea Pig ( *Cavia porcellus*)

summary: Guinea pigs give birth to precocial young and have a discoidal, hemomonochorial placenta. Maternal antibodies readily transfer from dam to offspring and the newborn is not reliant on antibody transfer through milk. Guinea pig cytomegalovirus (GPCMV) is an enveloped, DNA beta herpesvirus that can be transmitted both vertically and horizontally. Embryo transfer is commonly used in mice and rats to eliminate vertically transmitted agents; however, this method is difficult in guinea pigs due to the long estrus cycle, few embryos produced per mating event, oviduct anatomy, and lack of hormonal priming protocols.

It was hypothesized that offspring of guinea pigs positive for GPCMV antibodies but negative by PCR during pregnancy would be protected from in utero infection. These pups would be removed from the uterus via terminal hysterectomy then tested by serology and PCR to confirm GPCMV status. Of the five dams used, pups survived from four. The survival rate for pups at the appropriate stage of gestation was 64%, three litters were antibody positive and none were PCR positive for GPCMV.

The best candidate dam to establish a GPCMV-free colony would be one that was both seronegative and PCR negative. Seronegative animals should be single housed and tested repeatedly to confirm status. Seropositive dams could serve as a source of GPCMV-negative offspring provided that the offspring are confirmed either to lack antibodies to the virus or are sampled to ensure waning of maternal antibody.

QUESTIONS

1. What is the placentation of guinea pigs?

a. Zonary epitheliochorial

b. Discoid hemomonochorial

c. Cotyledonary epitheliochorial

d. Diffuse endotheliochorial

2. What is the taxonomy of cytomegalovirus?

ANSWERS

1. b

2. Enveloped DNA virus in the family *Herpesviridae*, subfamily *Betaherpesvirinae*