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***Contemporary Safety Topics in Animal Research***

**Bradfield et al.** [**Foreword and Introduction to This Issue on Contemporary Safety Topics in Animal Research**](https://academic.oup.com/ilarjournal/article/59/2/125/5490286)**, pp. 125-126**

Domain 5; T3

SUMMARY: Institutions must provide a safe work environment and mitigate the risks of hazards to acceptable levels.  Occupational health and safety programs include hazard identification and risk assessment, personnel training and protection, written procedures and policies regarding hazard use and monitoring, and medical evaluation and preventative medicine.  A successful program has coordination among medical and safety personnel, researchers, veterinarians, and the IACUC.  Guidance can be found in the *Occupational Health and Safety in the Care and Use of Research Animals*.

QUESTIONS

1. What are the 4 elements that occupational health and safety programs should include?
2. What is the guidance document that provides structuring an Occupational Health and Safety program?
3. Who must be involved to make a successful occupational health and safety program?

ANSWERS

1. Occupational health and safety programs include hazard identification and risk assessment, personnel training and protection, written procedures and policies regarding hazard use and monitoring, and medical evaluation and preventative medicine.
2. *Occupational Health and Safety in the Care and Use of Research Animals*
3. A successful program has coordination among medical and safety personnel, researchers, veterinarians, and the IACUC.

**Swearengen.** [**Common Challenges in Safety: A Review and Analysis of AAALAC Findings**](https://academic.oup.com/ilarjournal/article/59/2/127/5281127)**, pp. 127-133**

Domain 5: Regulatory Responsibilities; T3 - Provide advice to occupational health and safety programs; T4 - Provide advice on biological, chemical, and radiation hazards in an animal research program; K1 - Occupational Health and Safety in the care and Use of Research Animals (ILAR/NRC); K5 - occupational health and safety (including OSHA and allergens)

**SUMMARY**: AAALAC International (AAALACi) uses the 8th edition of the Guide for the Care and Use of Laboratory Animals (Guide) as a basis for its assessment of animal care and use programs.  Both Chapters 1 (Institutional Policies) and 2 (Animal Care and Use Program) include topics such as special qualifications for personnel using hazardous agents; personal hygiene; occupational health and safety of personnel; animal experimental involving hazardous agents; control and prevention strategies; hazard identification and risk assessment; facilities, equipment and monitoring; personnel training; personal protection; and medical evaluation and preventative medicine for personnel.  Therefore, evaluation of occupational health, the use of hazards, and personnel safety have become important in AALACi assessments.  The Guide requires that institutions that use animals for research and/or teaching must establish and maintain and OHS program as an essential part of the overall program of animal care and use and that the program is consistent with federal, state, and local regulations.  Components of such a program typically include administrative support, adequate resources, an implementation strategy, and appropriate risk assessment. Employees must be made aware of the risks involved in animal research via training. The institution is responsible for developing and maintaining the program and the IACUC is responsible for indirectly coordinating with the research program along with the attending veterinarian, the institutional official and administration, safety programs, and occupational health services. The elements of an occupational health program as described in the Guide are discussed and emphasis is placed on risk assessment, which may involve walk-throughs of laboratories and discussions with investigators, personnel training, and personal protective equipment. When institutions apply for AAALACi accreditation, they should include information about their occupational health program in the Program Description and this is described in great detail in this report.

Data from 1656 findings associated with OHS were identified during calendar years 2014 through 2016 and an analysis was performed to provide an overview of the most frequently observed OHS findings that occurred during this time span. Specific examples of mandatory findings and suggestions for improvement were provided. The 5 categories representing key OHS areas and the combined percentage of both mandatory findings and SFIs in each category included: workplace risk/safety assessment (37.3%; including animal allergen exposure training and scavenging of waste anesthetic gases); personnel protection (36.3%); personnel risk assessment (14.4%); hazard containment (9.4%); and medical services (2.6%). Occupational health programs continue to be an area of a program that can be improved and information on the most commonly observed OHS findings and associated trends may be helpful to animal care and use programs when conducting internal reviews of OHS programs and in preparation for AAALACi site visits when seeking accreditation.

**QUESTIONS**

1.   Look at the image below.  These individuals are preparing to enter a holding room containing non-human primates.  List 2 forms of PPE that they still need to don to comply with general recommendations for PPE requirements for Rhesus and cynomolgus macaques.





Photo from Colorado State EHS

Image link:  <http://ehs.colorado.edu/wp-content/uploads/2014/11/SodaCher2.jpg>

2.   Look at the image blow.  For which piece of equipment are these signs/stickers/placards most relevant for animal care personnel safety?



Sample decal image link   <https://s1.manualzz.com/store/data/047196242_1-7deada85f88526f34c4f8caabd87a38f.png>

a.   3 x 3 sized autoclave

b.  8 x 12 sized cage/rack washer

c.  Sonicator

d.   Bottle washer

e.  Food extruder

**ANSWERS**

1.   Eye protection and gloves

2.   b, 8 x 12 sized cage/rack washer

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**Colby and Zitzow.** [**Applied Institutional Approaches for the Evaluation and Management of Zoonoses in Contemporary Laboratory Animal Research Facilities**](https://academic.oup.com/ilarjournal/article/59/2/134/5239652)**, pp. 134-143**

Domain 4: Animal Care; T3 - Manage or provide indirect management/oversight of laboratory animal facilities

Domain 5: Regulatory responsibilities; T3 - Provide advice to occupational health and safety programs; K1l - Occupational Health and Safety in the Care and Use of Research Animals (ILAR/NRC)

SUMMARY:  The term zoonosis was coined by Rudolf Virchow when he was studying the life cycle of *Trichinella spiralis* in the 19th century and zoonotic organisms are responsible for up to 60% of known infections and 75% of emerging infectious diseases.   Common means of transmission of zoonotic diseases include fomites and vectors. As diseases can be transmitted between laboratory animals and investigators, and some infections are experimental in nature, prevention and control of zoonoses should be a primary focus of occupational health programs. Although, historically, animals with unknown health status were used and health monitoring was not performed, contemporary research programs purchase animals of known health status, have rigorous health monitoring programs, and are handled using microisolation technique. In order to provide adequate occupational health and safety to staff, a risk assessment should be performed including the type of infectious organisms and their transmission routes, training of personnel, animals used, and the physical structure and potential of the animal facility.  The usage of biological materials is also a risk for some agents.  All animals have the capability to transmit zoonoses (a partial list of agents is included in the document), including macacine herpesvirus-1, tuberculosis, and rabies.  The source of animals and type of research facility are important risk factors.  For example, international sources of non-human primates, and their transportation to the U.S., may pose additional risks compared to domestic sources and, in rodent facilities, the type of facility (conventional, biohazard, chemical hazard, or quarantine) may pose additional risks compare to barrier-housed animals. Experimental paradigms, such as the use of immunocompromised mice and humanized mice pose unique risks.   Employees can be both a source and a recipient of zoonotic infections.  If employees visit both a human hospital and a research vivarium, they may unwittingly carry in agents such as MRSA and *C. difficile*. Design of and equipment in facilities can minimize spread of infectious organisms with the HVAC design and air pressure differentials.  It is imperative that standard operating procedures be prepared and used to allow for correct use of equipment and PPE, as failure to properly operate and decontaminate may result in exposure to zoonotic pathogens. Pest control programs and occupational safety and health programs serve vial roles in protecting employees from injury and illness. Orientation to infectious models and animal use as well as safety training are invaluable for employees to help reduce risks of zoonotic transmission.

QUESTIONS

1.  The transmission and host of the zoonotic agent *Capnocytophaga canimorsus* includes

a.  Fecal-oral: cat

b.  Fecal-oral: mouse

c.  Bite wound: dog

d.  Bite wound: rat

e.  Needlestick: rabbit

2.   *Trichinella spiralis* is in which taxonomic phyla?

a.  Trichinellidae

b.   Nematoda

c.    Trichuridae

d.   Oxyurida

e.  Rhabditida

3.   Fill-in \_\_\_\_\_\_ can significantly impact building-wide airflow as movement can simulate a piston, drawing and pushing air between building floors.

a.   Air showers

b.   Loading dock doors

c.   Floor drains

d.  Cubicles for containment

e.  Elevators

ANSWERS

1.   c. Bite wound: dog (also cat); it is an oral bacterium

2.   b.  Hints to other answers below

  Nematoda is phylum of pinworms, hookworms, whipworms, and other roundworms like trichinella.  Trichinellidae is the family for trichinella.  Rhabditida is the order for hookworms. Oxyurida is the order for pinworms. Trichuridae is the family for whipworms.

3.  e

**Asfaw et al.** [**Managing Research Animal Specimens and Laboratory Safety**](https://academic.oup.com/ilarjournal/article/59/2/144/5257453)**, pp. 144-149**

Domain 5: Regulatory Responsibilities

SUMMARY: Testing procedures performed in veterinary diagnostic laboratories are associated with inherent risks due to the potential of personnel exposure to infectious disease (experimentally and naturally occurring), chemical, and radiologic hazards.   It is imperative that researchers, veterinary diagnostic laboratory personnel, and health and safety professionals work together to ensure adequate engineering controls, personal protective equipment (PPE), and laboratory procedures and training for personnel to properly and safely work with a wide range of potential hazards when handling research animals/specimens.  In order to address diverse hazards; laboratory design, safety equipment, PPE, laboratory procedures, personnel training, and occupational health programs should be based on risk assessments.  This article provides an overview of safety considerations in the operation of diagnostic laboratories handling research animal specimens.

QUESTIONS

1. Which of the following agents is known to be transmitted by the aerosol route?
2. Tuberculosis
3. Vaccinia virus
4. Adenoviruses
5. All of the above
6. BSL \_\_\_\_\_ is adequate for agents not known to cause diseases in immunocompetent adult humans.
7. 1
8. 2
9. 3
10. 4
11. BSL\_\_\_\_\_\_ is for agents known to cause disease in humans, and potential exposure can occur due to percutaneous injury, mucous membrane exposure, and ingestion.
12. 1
13. 2
14. 3
15. 4
16. Which of the following is NOT a BSL-2 agent?
17. *Staphylococcus aureus*
18. *Vibrio cholera*
19. *Clostridium botulinum*
20. *Francisella tularensis*
21. All of the above are BSL-2 agents
22. BSL\_\_\_\_\_\_\_ agents can be indigenous our exotic with potential aerosol transmission and the disease may have a serious or lethal consequence.
23. 1
24. 2
25. 3
26. 4
27. Which of the following IS a BSL-3 agent?
28. *Mycobacterium tuberculosis*
29. West Nile Virus
30. *Coccidiodes immitis*
31. All of the above are BSL-3 agents
32. BSL\_\_\_\_\_\_ agents are considered dangerous and exotic and pose high risk of serious or life-threatening diseases with risk of aerosol transmission or an unknown route of transmission.
33. 1
34. 2
35. 3
36. 4
37. Which of the following IS NOT a BSL-4 agent?
38. Marburg Virus
39. Rift Valley Fever
40. Ebola Virus
41. Lassa Virus
42. All of the above are BSL-4 agents
43. Which of the following developed a Globally Harmonized System of Classification and Labeling of Chemicals?
44. The Environmental Protection Agency
45. The United Nations
46. The Centers for Disease Control and Prevention
47. The National Institutes of Health
48. Which of the following IS considered a teratogen?
49. Formaldehyde
50. Xylene
51. Ethidium bromide
52. Toluene
53. All of the above
54. b and d
55. Which of the following IS a chemical category (according to the nature of the hazard they present) used by the United Nation’s Globally Harmonized System of Classification and Labeling of Chemicals?
56. Physical hazard
57. Health hazard
58. Environmental hazard
59. All of the above
60. \_\_\_\_\_\_\_\_\_\_\_\_ hazards include chemicals such as flammables, explosives, and oxidizers.
61. Physical
62. Health
63. Environmental
64. None of the above
65. Which of the following IS considered a mutagen?
66. Formaldehyde
67. Xylene
68. Ethidium bromide
69. Toluene
70. All of the above
71. b and d
72. Which of the following IS true regarding BSL-4?
73. All materials must be decontaminated and/or autoclaved before leaving the facility
74. Full body air-supplied positive pressure suits are required if Class I and II cabinets are used
75. A separate isolated zone or building with dedicated supply and exhaust system, vacuum, and decontamination system is required
76. All of the above are true
77. Laboratories handling specimens or using hazardous chemicals are required to comply with which of the following?
78. 19 CFR
79. 29 CFR
80. 39 CFR
81. 49 CFR

ANSWERS

1. d
2. a
3. b
4. d
5. c
6. d
7. d
8. b
9. b
10. f
11. d
12. a
13. c
14. d
15. b (29 CFR 1910.1450)

**Villano et al.** [**Safety Considerations When Working with Humanized Animals**](https://academic.oup.com/ilarjournal/article/59/2/150/5239650)**. ILAR J 59(2):150-160**

Domain 1

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Humanized mice are pivotal models in oncology and infectious disease research. They are characterized by being immunodeficient and accepting human engraftments in order to recreate a human immune system. Human peripheral blood, human CD34+ hematopoietic stem cells, fetal liver, fetal bone marrow or fetal thymus can be injected or implanted in immunodeficient mice in order to create different components of the human immune system. The highlight of these models is the study of the interaction between human immunocytes and human pathogens or human tumor cells. Examples of applications of humanized models include transplantation research, the study of growth of in vitro tumor xenografts or patient derived xenografts in order to personalized cancer therapies, and the study of Epstein Barr virus, HIV, dengue, mycobacterium tuberculosis and other infectious agents. Over the last years different immunodeficient models have been used in biomedical research. Not all of them are equally capable of accepting human engraftment. NOD-scid/gamma mice (NSG), and similar models (NOG, NRG, BRG), overcomes the limitations of other immunodeficient models and possesses a better engraftment rate. NSG mice are, therefore, one of the best choices for humanized models. Although mice are the most popular models, other animal species including rabbits and pigs are subject of study for the development of large and non-mouse humanized alternatives.

The creation of humanized models has a major impact on the health status and animal welfare of the animal. Immunodeficiency predisposes to opportunistic infection and residual mouse innate immunity and MHC activity can develop wasting disease or xenogenic-graft-versus-host-disease. That is why these models require special attention and care. The maintenance of a strict SPF barrier is mandatory while a proper supervision protocol with optimal oncology end-points criteria must be followed. Working with human derived substances is a critical point when using humanized models as it implies a risk for transmission of humans pathogens through blood, tissues and cell lines. Therefore, the implementation of a well-designed occupational health and safety program (OHSP) is required. In addition, the OHSP program must be under the umbrella of the standards guidelines when working with laboratory animals, regulatory documents and federal laws. All personnel in contact with human derived substances must follow proper standards in safe handling of sharps and wastes, hepatitis B vaccination, medical evaluation, safe animal manipulation and personal protective equipment management. Another important component is the risk assessment that determines the level of bio-containment and other measures depending on the type of activity to be conducted or the origin of cells and tissues. For example, when using cells from healthy patients or commercial cell lines (documented to be pathogen free) Biosafety level 1 can be used, while activities involving primary tissues or cells containing viruses (also vectors) must be conducted under a BSL-2. Despite of that, some institutions and guidelines recommends a BSL-2 for any sample coming from human subjects. Special considerations must be taken into consideration when working with animals. It is important to avoid actions that lead to aerosolization or spills and take caution with sharps material (needles for administration and surgical instruments). Safety implementation of activities with humanized mice requires the collaboration between users, vet staff, IACUC and occupational work experts in order to avoid unnecessary exposure of personnel to human derived substances.

QUESTIONS

1. Which is the cause why NOD/SCID have a short lifespan and therefore are not useful for long-term studies?

a. Development of thymic lymphoma

b. High incidence of infectious disease

c. Development of neurodegenerative disease

d. Early aging

2. Which of the following models do not have impairment of both B and T lymphocytes?

a. NOD/SCID

b. NSG

c. Athymic mice

d. NRG

3. BLT models are induced by implanting which of the following xenografts?

a. Human peripheral blood

b. Human CD34+ hematopoietic stem cells

c. Human fetal liver

d. Human spleen

4. Which of the following documents can be used as a standard for the establishment of an occupational work and safety program?

a. NIH guidelines

b. Bloodborne pathogen standard

c. Biosafety in microbiological and biomedical laboratories

d. All of them

5. Which of the following statements is false?

a. Personal protective equipment is the first line of defense in an Occupational health and safety program (OHSP)

b. Whenever possible the IACUC should incorporate an expert in environmental health and safety

c. The responsibility in an OHSP is shared among researches, veterinarians, IACUC, husbandry staff and experts in OH and safety

d. The risk associated to the manipulation of human derived substances may vary depending on the type of activity.

6. Which biosafety level of containment is mostly recommended when working with humanized models?

a. BSL-1

b. BSL-2

c. BSL 3 and  BSL-4

d. BSL-1 and BSL-2

7. Which of the following measures is not considered to protect personnel when administering animals with human cells?

a. The use of anesthesia

b. The use of a restraining device

c. Proper staff training in animal manipulation

d. The use of the recommended maximum volumes for substance administration

ANSWERS

1. a

2. c

3. c

4. d

5. a

6. d

7. d

**Edwards et al.** [**Agricultural Animals as Biomedical Models: Occupational Health and Safety Considerations**](https://academic.oup.com/ilarjournal/article/59/2/161/5196514)**, pp. 161-167**

Domain 3: Research; K3 - Animal models

Domain 5:Regulatory Responsibilities; K5 - Occupational health and safety

SUMMARY: With the use of agricultural animals in biomedical research is increasing, there are some special considerations with regards to occupational health and safety including personnel training, handling equipment, physical plant infrastructure, and potential zoonoses. This increase is due in part to some limitations of rodent animal models, such as their dissimilarities to human cardiovascular, gastrointestinal, respiratory, reproductive, and immune systems. Agricultural animals such as swine and cattle have longer gestation periods similar to humans and may produce research results that more readily translate to human medicine. While rodents offer advantages with genome manipulation, this technology is more recently being used to create transgenic pigs for larger animal models of human disease. There are disadvantages, however, to agricultural animals used in biomedical research due to their large size, such as increased cost of husbandry, veterinary care, and carcass disposal, personnel safety concerns, and the requirement of special handling techniques.

Husbandry issues for agricultural animals include physical space limitations of biomedical research facilities. Normal sized adult swine and sheep require at least 1.5 square meters per animal, which is difficult to achieve in facilities designed to house rodents. Other housing considerations include room drains that may not be large enough to accommodate cleaning of large animal waste and flooring that may be a slipping hazard to large animals. Walls and panels designed for rodent or companion animal rooms may not be strong enough to withstand the physical stress placed on them by large animals. Another husbandry consideration for agricultural animals is environmental enrichment. Most agricultural animals are social species, so the allowance for social interaction amongst conspecifics and with humans is needed. It may be worthwhile and necessary to consider higher housing densities that are more in line with agricultural settings, as long as this is approved by the IACUC. These species also need to become acclimatized to handling by husbandry personnel and research staff in order to produce reliable data, and this can be achieved through positive reinforcement training with nutritional enrichment.

There are several occupational health and safety concerns to consider when working with agricultural animals in the biomedical research setting due to their large size. The greatest hazard to humans is being kicked or physically run over by an agricultural animal. Operating equipment used to handle farm animals also poses a safety risk to the human and the animal if not handled properly. These safety risks can be mitigated through thorough training of personnel and choosing breeds with less excitable temperaments. Because agricultural animals can carry several zoonotic diseases, there are husbandry controls such as personal protective equipment to mitigate these health risks. For instance, research and husbandry personnel should be aware of the risk of Q fever and brucellosis from exposure to placental tissue or amniotic fluid and wear appropriate PPE to mitigate this risk when assisting with delivery from ruminants. Proper vetting of disease status and animal handling training of personnel can also mitigate many of the risks of zoonotic diseases.

There are many examples of agricultural animals that can be used for animal models of human disease. Female reproduction in cattle has been used as an animal model with nuclear transfer and in vitro fertilization, and the longer gestation period of cattle is a critical factor in these studies. Goats have joint anatomy that closely resembles human joints, and thus are an important orthopedic model. Sheep are an excellent model for respiratory diseases such as cystic fibrosis, respiratory distress syndrome in infants, and respiratory syncytial virus infection. Swine have proven to be invaluable for cardiovascular research in diseases such as atherosclerosis, aortic aneurysm, and heart failure. It is clear that agricultural animals will gain a more significant role in animals studies of disease that affect humans, due to the fact that they may more closely resemble human diseases than rodent models. As their use increases, training and infrastructure must be in place to ensure proper care of these animals.

QUESTIONS

1. Which breed of swine has naturally occurring type 2 diabetes?
	1. Yucatan
	2. Sinclair
	3. Hampshire
	4. Gottingen
	5. Ossabaw
2. True or False: *Coxiella burnetti*is a select agent that can be spread through direct contact with reproductive tissues such as placenta and amniotic fluid or airborne transmission.
3. What is the recommended minimum space in square feet for a singly housed pig weighing 25-50 kg according to the *Guide for the Care and Use of Laboratory Animals*?
	1. 10
	2. 12
	3. 15
	4. 18
	5. 20
4. True or False: The use of electric prods in cattle does not greatly elevate the levels of stress hormones in these animals.

ANSWERS:

1. e
2. True
3. c
4. False

**O’Rourke et al. 2018.** [**Nontraditional Laboratory Animal Species (Cephalopods, Fish, Amphibians, Reptiles, and Birds)**](https://academic.oup.com/ilarjournal/article/59/2/168/5194097)**, pp. 168-176**

Domain 3: Research

Domain 4: Animal Care

Domain 5: Regulatory Responsibilities

SUMMARY: This review article focuses on the challenges associated to the housing and caring of nontraditional laboratory animal species (birds, reptiles, amphibians, fish and cephalopods) in contemporary research facilities. It gives an extensive overview of the factors to consider when developing occupational health and safety programs, mainly: environmental hazards, hazards associated with handling and zoonosis.

Many of these species are housed in environments that are high in moisture and that can be therefore associated with major risks such as slips, development of dermatitis, electric shock and exposure to sharp surfaces (glass enclosures and wet surfaces).

In zebrafish facilities, ultraviolet sterilizers are frequently used to disinfect water and generate ultraviolet light: if the light is suspended (unshielded) it may lead to development of acute erythema and be a long term potential for photo carcinogenesis.

Chemicals commonly used in aquatic facilities, as for example disinfectants for the preparation of food baths as well as the anesthetic tricaine methane sulfonate (MS-222), should be considered as hazards if the personnel handle them without the use of proper PPE. The first one may cause acute inhalation toxicity while MS-222 has been reported to be retinotoxic and mucous membrane irritator.

Allergy to laboratory animals is a well-documented occupational hazard and precautions should be taken when working with nontraditional species; as a general principle, the exposure should be minimized with the proper use of PPE. Allergy has been documented in individuals exposed to birds: hypersensitivity pneumonitis (Bird’s Fancier Lungs) mimics pneumonia and may occur several hours after exposure to bird allergens.

Allergic reactions to reptiles have also been documented and mostly due to venoms from *Hemachatus hemachatus* (rinkhals), Crotalus, Ophiophagus Hannah (king cobra) and iguanas. A few reports have documented reactions to bites as well as dermal hypersensitivity (there is a similar pattern seen in arthropod-bite reactions).

Both for amphibians and fish, there are only limited reports of allergic reactions and the majority involves food allergy. Crickets (*Grullidae*) and mealworm beetle (*Tenebrio molitor*) colonies are maintained in animal facilities to produce feed for frogs, reptiles and birds and have been associated to allergy symptoms in a few animal care personnel.

With regards to the hazards associated with handling of nontraditional species, trauma (bites, electric shock, toxins and venoms) is a well-known problem and it appears of primary importance that personnel working with such species is well trained on restraint and immobilization approaches/techniques. The head of the animals and also other appendages, either arms with claws, talons, venomous spines (or just the fact of being massive and powerful) may represent a hazard. It may be interesting to mention for example that among the amphibians, members of the genus *Ceratophrys* show a short and ossified jaw, with ossified mandibular symphysis that together with a recurving tooth structure may inflict serious bites to handlers.

Another interesting example of hazards associated to bites is the beak of many birds (many parrots) that can inflict severe wounds with secondary infection caused by the introduction of rickettsial and mycobacterial microorganisms.

Electric shock is a hazard peculiar to aquatic facilities since some of the fish that can generate dangerous electric fields are also used in research settings:  a few examples are *Electriphorus electricus*, electric catfishes and the marine electric rays. Even though strong electrical discharges from these fish are unlikely to kill a healthy person, they could incapacitate a person, sufficiently to cause them to fall/to drown.

Venomous snakes can of course be source of intoxication and was not part of this review to give information on the safety procedure to manage and handling them.

Among species used in research settings, It’s interesting to mention that frogs of the genus *Dendrobates*produce neuromuscular blocking compounds (curare-like substances) that are generated through metabolizing precursors ingested in the native diet (ants).

Toads such the cane toad (*Rhinella marina*) synthesize cardioactive steroids (bufdienolides) that are derived from cholesterol and have similar activity to plant cardenolides  (inhibitors of membrane bound Na+/K+ ATPase).

Most of salamanders secrete toxins from skin glands and some being very potent toxins including tetrodotoxin.

Regarding zoonoses, bacterial pathogens are the most commonly described zoonotic agents associated with nontraditional research species.

Chlamydia *psittaci*, most commonly found in birds (parrots, cockatiels, budgerigars, other psittacines but also documented in turkeys, ducks and chickens) can be transmitted to humans by inhalations of infected nasal discharge or aerosolized dried feces and symptoms include fever, headache, pneumonia.

Mycobacterium *marinum* (and related species: M. *fortuitum*, M. *ulcerans*, M. *chelonae*) is a zoonotic bacteria associated with aquatic species; humans may contract the disease via a preexisting wounds, typically when handling fish or cleaning tanks without PPE.

Salmonella is a gram negative bacterium that can be present as part of the normal gut flora in some species and that can cause outbreaks in presence of crowding, stress, and in general with poor husbandry conditions.

Reptiles (particularly turtles), iguanas, frogs, salamanders and birds have been implicated in the transmission of Salmonella to humans and this occurs through contact with feces or contaminated surfaces.

Vibrio*vulnificus*is found in aquatic environments (warm temperature and low salinity) and can cause hemorrhagic and ulcerative disease in fish, while in humans it is associated to necrotizing infections of preexisting skin wounds and even septicemia.

Streptococcus*inane*infects fish with clinical signs associated to lesions of the central nervous system and in humans can cause cellulitis if the organism infect wounds. In certain cases it can also cause systemic infections (endocarditis and meningitis).

*Erysipelothrix* *rhusiopathie*is found in birds, reptiles, fish and cephalopods (hemorrhagic septicemia) while in humans the disease may manifest as a. localized and painful cutaneous infections, b. cutaneous cellulitis and c. septicemia.

*Dermatophilus* *congolensis*causes exudative skin lesions in crocodilians while in humans the disease manifests as a self-limiting pustules or eczematous lesions.

As for the viral zoonoses, it’s important to mention the following disease that interest birds: New Castle Disease (paramyxovirus), avian influenza (orthomyxovirus) and West Nile Virus.

Fungal zoonoses can also occur: Histoplasma *capsulatum* and *Microsporum* *gallinae*andare both associated with birds.

QUESTIONS

1.   Describe the mechanism of action of the bufdienolides produced by toads

2.  What are the main considerations associated to Mycobacterium infections humans working in a zebrafish facility?

3.  What are the main clinical signs in birds affected by West Nile Virus?

ANSWERS

1.  Bufdienolides are cardioactive steroids similar in structure to digoxin; they are derived by cholesterol and are inhibitors of the membrane bound Na+/K+ATPase. They are secreted by skin glands, especially parotid glands. Like digoxin, they are known to increase vasoconstriction, vascular resistance and blood pressure.

 Interestingly, they have been shown to be a potential source of new cytotoxic and antitumor molecules, showing biological action on hematological, solid, sensitive and/or resistant human tumor cell lines.

2. Mycobacterium can be found both in fish and environment (aquaria, biofilm). Fish infected with M. *marinum* can develop visceral granulomas and skin ulcerations. Humans contract the disease thorough contamination of preexisting wounds; in a healthy human the disease usually manifests as a self-limiting localized granuloma, while in immunocompromised individuals it can become more invasive and treatment will be necessary (antibiotic therapy and surgical excision of the granuloma). The risk of transmission of mycobacteria can be reduced by an effective fish colony management and use of PPE.

3.  Various neurological signs (ataxia, paresis, seizures)

**Kendall et al. 2018.** [**Replacement, Refinement, and Reduction in Animal Studies With Biohazardous Agents**](https://academic.oup.com/ilarjournal/article/59/2/177/5298775)**, pp. 177-194**

Domain 3

SUMMARY: Animal use in biohazard studies requires careful consideration of animal welfare. PIs and IACUCs are ethically obligated to apply the 3Rs (replacement, reduction, and refinement) to minimize the impact of the infectious agent on animal well-being. Replacement is arguably the most challenging of the 3Rs to apply in infectious disease studies as many aspects of disease processes cannot be fully mimicked with a whole-body response; nevertheless, in vitro, mathematical, and computer models expand the ability to study biohazardous agents while minimizing the use of animals. Relative replacement have included the use of less sentient species, including amoeba, wax moths, and cockroaches. Refinement to experimental procedures should be used to improve the well-being of animals in infectious disease studies, such as assessments to help identify humane end points precluding prolonged distress. These assessments can include the use of biomarkers, temperatures  and thermography, clinical signs and score sheets, and ethograms. The ability to reduce the number of animals used in infectious disease models has been substantial given the significant technological advancements in animal research, namely telemetry and imaging modalities that allow for longitudinal evaluation of animals over time. The benefits of these modalities on research and animal use are apparent but the special circumstances surrounding working with biohazardous environments must be taken into consideration when deciding to implement their use in projects. Facility space, environmental containment, and the ability to sanitize or sterilize the equipment after use and between experiments must be carefully planned. While there is no single method available to universally apply to all infectious disease models, several techniques to replace, refine, and reduce the number of animals used in infectious disease research are available and should be considered when developing animal models.

QUESTIONS

1. Which organizations promote the use of alternative in animal research?



     a. 

     b. 

     c. 

     d. 

2. What is the difference between absolute replacement and relative replacement?

3. **True/False:** A decrease in body temperature of more than 4-6 degrees Celsius correlates to impending death after multiple types of immune system challenges in mice.

ANSWERS

1. d 

2. Absolute replacement replaces animals with inanimate systems; relative replacement replaces animals with an animal lower on the phylogenic scale

3. True

**Roble et al. 2018.** [**Disaster Planning for Animals in Hazardous Agent Containment Units**](https://academic.oup.com/ilarjournal/article/59/2/195/5481801)**, pp. 195-202**

Domains 4 and 5: Animal Care and Regulatory Responsibilities

SUMMARY: Disaster response planning for laboratory animal facilities is a time- and personnel-intensive process.  The size of the facility, number of facilities managed by the program, the type of species housed, and the most common types of disaster that might affect the facility must all be taken into account when developing an individualized, facility specific disaster plan.  Disaster response for the containment unit will often be a small component of the overall larger facility plan.  Planning teams for the disaster plan should include subject matter experts such as the biological safety officers, veterinarians, and principal investigators as well as first responders, government and institutional officials, IACUC representatives, and animal caretakers.

Situational understanding is a key component in disaster response.  Risk assessment is one of the first steps in situational understanding.  Two of the most common risks that must be considered is the human risk and species risk.  Human error is the most common risk that must be accounted for and considered.  The type of agents used in the facilities and the types of chemical hazards must also be considered in the disaster plan.  Appropriate situational awareness determines the goal and objectives needed to eliminate the risk.  One of the most common goals of a facility is to have appropriately trained personnel.  Plan development is broken down into 3 levels: strategic, operation, and tactical.  Once the plan is developed, it should be practiced to ensure it will work when needed.  There are two types of rehearsal exercises used: discussion and operation-based.  All personnel who work in biocontainment areas should have annual training.  Humane endpoints must be considered and included in the disaster plan.  Appropriate supplies for euthanizing all animals in a containment unit must be available in the event of a disaster and depopulation is the determined to be the required course of action.

Media responses should be prepared and practiced so the public remains appropriately informed during and after a disaster response.  Animal facility leadership should be capable of presenting a statement to the press.

QUESTIONS

#### 1.  What are the most difficult species to handle in containment during or following a disaster?

* 1. Genetically modified rodents
	2. Agricultural animals
	3. Nonhuman primates
	4. Dogs
	5. a & c
	6. b& c

2. Which definition appropriately describes the strategic level of planning

* 1. Aim to identify the roles and responsibilities of people identified in situational awareness and goal setting
	2. Aim to examine the larger picture in a response including allocation of resources
	3. Aim to focus on the personnel and auxiliary resources required in the actual response

3. Which planning level is the most critical for success in a disaster response?

* 1. Strategic
	2. Operation
	3. Tactical

4. (T or F) Shelter-in-place is most likely the only option for containment level animals since movement is often too risky.

5. (T or F) For both ethical and humane reasons, disaster depopulation planning should rest with the attending veterinarian.

6. (T or F) Risk assessment is an ongoing process

7. (T or F) Many decisions during or after a disaster will come from an Incident Command System (ICS).

ANSWERS

1.  f

2.  b

3.  c

4.  False

5.  False

6.  True

7.  True