

General Animal Use Training (2021)

Laboratory Animal facility, HKUST (CWB)

Contents:

Part 1 Animal Ethics

Part 2 Occupational Safety when Working with Animals

Part 3 Animal Works in Your Research Laboratory

Note: This material serves as part of the training program for animal users of Laboratory Animal Facility, HKUST (CWB). The rest of the training program includes

- Animal Handling (on the species required)
- LAF User Manual
- Hands-on training and orientation in the facility, and
- Biosafety course (provided by HSEO)

Part 1

Animal Ethics, Pain and Distress, and the 3Rs

Keystones in Animal Ethics

Aristotle (384 - 322 BC):

- Animals lack reason, humans are at the top of the natural world

Rene Descartes (1596 - 1650)

- Only humans are conscious
- Animals are living organic creatures, but they are automata, like mechanical robots

Jeremy Bentham (1748 - 1832)

- The ability to suffer that should be the benchmark of how we treat other beings
- If rationality were the criterion, he argued, many humans, including infants and the disabled, would also have to be treated as though they were things
- The question is not *Can they reason? nor, Can they talk?* but, *Can they suffer?*

Richard Martin (1754 - 1834)

- In 1822, Richard Martin pushed for the first anti-cruelty bill in the UK parliament

Peter Singer (1946 -)

- In his book *Animal Liberation* (1975), he criticizes testing on animals but is willing to accept such testing when there is a clear benefit for medicine

Keystones in Animal Ethics

Consensus of animal experiment

- It is the ethical obligation of all personnel involved in the usage of animals in research to **reduce or eliminate pain and distress in research animals** whenever such actions do not interfere with the research objectives

Pain and Distress

Discomfort:

- Discomfort is viewed as a mild form of distress

Stress:

- Biological responses that an animal exhibits in an attempt to cope with a threat to its homeostasis (Carstens and Moberg 2000)

Pain and Distress

Pain:

- is an unpleasant sensory and emotional experience
- is associated with actual or potential tissue damage
- elicits protective motor and vegetative reactions
- results in learned avoidance behavior
- may modify species specific behavior (Zimmerman, 1986)

Pain and Distress

Distress:

- Distress is a state at which normal biological responses are no longer sufficient to achieve return to homeostasis
- Normal biological functions may be disrupted as the animal must devote substantial effort or resources to challenges emanating from the environmental situation
- Distress may result in disease or pathological changes

The 3 Rs of Humane Animal Experimentation

The 3 Rs:

- The 3 Rs stand for ***Reduction, Replacement*** and ***Refinement***
- In the book *The Principles of Humane Experimental Technique*, published in 1959
- The authors Russell and Burch proposed that all research using animals should be evaluated to see if the 3 Rs could be applied

The 3 Rs of Humane Animal Experimentation

Replacement

- Methods which avoid or replace the use of animals

I. Absolute replacements

- The use of an inanimate system as an alternative (mathematical and computer models)

II. Relative replacements

- The use of “less sentient” vertebrates or invertebrates, such as zebrafish, fruitfly and nematode

The 3 Rs of Humane Animal Experimentation

Reduction

- Methods which minimize the number of animals used per experiment with no loss of useful information
- Examples
 - reducing the number of variables through good experimental design
 - improving statistical analysis
 - sharing data and resources
 - using genetically homogeneous animals (preferably using a number of distant inbred strains over an outbred strain)
 - the use of technology, e.g. imaging to enable longitudinal studies in the same animals

The 3 Rs of Humane Animal Experimentation

Refinement

- Methods which minimize suffering and improve animal welfare
- A change in some aspects of the experiment resulting in
 - a reduction of pain, stress or distress that animals may experience
- Examples:
 - using appropriate anesthetics and analgesics
 - avoiding stress by training animals to cooperate with procedures
 - use of non-invasive techniques
 - use of enrichments that improve living conditions
 - establish a scientifically sound earliest endpoints for a study that has the potential to cause pain or distress

Ref:

Training module of Canadian Council on Animal Care
National Centre for the Replacement, Refinement & Reduction of Animals in Research

Local Legislation & Guideline

- [*The Hong Kong Code of Practice for Care and Use of Animals for Experimental Purposes*](#)
- Animals (Control of Experiments) Ordinance Cap. 340
- Prevention Of Cruelty to Animals Ordinance, Cap. 169
- Genetically Modified Organisms (Control Of Release) Ordinance, Cap. 607

Part 2

Occupational Safety when Working with Animals

Major hazards in working with animals

- Allergens
- Zoonotic disease
- Protocol related hazards

Allergy Risk

Facts

- Up to 44% of the individuals working with laboratory animals report work-related allergic symptoms
- Of those who become symptomatic, 4 to 22% may eventually develop occupational asthma that can persist even after exposure ceases

Low risk:

- Procedures on unconscious animals

High risk:

- Handling live moving animals

Ref:

Bush and Stave (2003) Laboratory animal allergy: an update. ILAR J;44:28–51

Major Source of Allergens

- Hair, dander, urine and saliva
- By contact or respiratory (on <5micron particles)

Table 2 Laboratory animal allergens^a

Animal	Allergen	MW ^b (kD)	Source	Biological function
Mouse (<i>Mus musculus</i>)	Mus m 1 (prealbumin)	19	Hair, dander, urine	Lipocalin-odorant binding protein
	Mus m 2	16	Hair, dander	Unknown
	Albumin		Serum	Serum protein
Rat (<i>Rattus norvegicus</i>)	Rat n 1A/Rat n 1B	18.7	Hair, dander	Lipocalin-pheromone binding protein
	(α_{2u} -globulin)		Urine, saliva	
	Albumin		Serum	
Guinea pig (<i>Cavia porcellus</i>)	Cav p 1		Hair, dander, urine	Unknown
	Cav p 2		Hair, dander, urine	
Rabbit (<i>Oryctolagus cuniculus</i>)	Ag 1 (Price and Longbottom 1990 ^c)	17	Hair, dander, saliva	Possible lipocalin
	Ag 2 (Warner and Longbottom 1991)		Hair, dander, urine	
Cat (<i>Felis domesticus</i>)	Fel d 1	38	Hair, dander, saliva	Unknown
	Albumin		Serum	Serum protein
Dog (<i>Canis familiaris</i>)	Can f 1	25	Hair, dander, saliva	Lipocalin cysteine protease inhibitor
	Can f 2	19	Hair, dander, saliva	Lipocalin
	Albumin		Serum	Serum protein

^aAdapted from Wood RA. 2001. Laboratory animal allergens. ILAR J 42:12-16.

^bMW, molecular weight.

^cSee text for complete references.

Ref:

Bush and Stave (2003) Laboratory animal allergy: an update. ILAR J;44:28–51

Symptoms of allergy

Contact urticaria

- redness, itchiness of skin, welts and hives

Allergic conjunctivitis

- sneezing, itchiness, clear nasal drainage, nasal congestion

Allergic rhinitis

- sneezing, itchiness, clear nasal drainage, nasal congestion

Asthma

- cough, wheezing, chest tightness, shortness of breath

Anaphylaxis

- generalized itching, hives, throat tightness, eye or lip swelling, difficulty in swallowing, hoarseness, shortness of breath, dizziness, fainting, nausea, vomiting, abdominal cramps, diarrhea

Minimizing Allergen Exposure

Procedural Control

- Replace open-top cages with individually ventilated cages or static cages
- Handle animals in biological safety cabinet or animal transfer station
- Segregate animal works from other works
- Minimizes aeroallergen levels and prevents spread of allergens into the environment (e.g. wherever appropriate, use female or juvenile animals instead of adult males, as male animals secrete more allergens in their urine)

Personal Control

- Correct use of personal protective equipment
- General hygiene

Ref:

Gordon and Preece (2003) Occupational Medicine 2003;53:371–377

Response to allergy

- Stop animal works
- Report to your supervisor and seek clinical assistance
- Ask for clinical consultation on your fitness to continue animal work

Zoonotic Disease

Zoonotic disease

- Any disease that may be transmitted from an animal to a human under natural conditions
- Animals used for laboratory research or teaching purposes in LAF include mice, rats and rabbits

Transmission of zoonotic disease is primarily by

- fecal-oral route
- inhalation of aerosolized materials
- indirect contact with invertebrate vectors
- contaminated inanimate objects

Risk of Zoonotic Disease

Imported animals

- Laboratory rodents are generally maintained free from specific pathogens. Risk of zoonotic disease is low
- Agriculture, Fisheries and Conservation Department request imported animals were maintained in colonies tested free from specified zoonotic pathogens, i.e. hantavirus, lymphocytic choriomeningitis virus, rabies and monkeypox

Wide rodents

- The presence of zoonotic agents are more likely

Pet rodents

- Pathogens in pet breeding facilities are common
- Keeping pet rodents poses high risk to laboratory animals thus the health of other animal users

Zoonotic Disease

Prevention of zoonotic diseases

- Do not keep pet rodents or snakes which are fed with live rodents
- Prevention of bites and scratches
 - Familiarize yourself about the animals that you will be working with
 - Wear appropriate protective gear
- Post-injury treatment
 - Thoroughly wash any bite or scratch wounds and report injuries. Rabies exposure is reportable to public health authorities
- Do not eat, drink or apply makeup while handling animals or in animal housing areas
- Keep animal areas clean and disinfect equipment after using it on animals or in animal areas

Protocol related hazards

Biological or chemical hazard

- Identify the use of viral vector expressing oncogenic (or suspected oncogenic) or toxin product, use of infectious agents and tumor cells
- Identify the use of carcinogenic or toxic chemicals
- Take the respective biosafety/chemical safety precaution procedures (if required, consult HSEO or LAF for the agent specific-precaution)
- Notify LAF for the precaution on the animal and soiled cage handling
- Use the appropriate PPE to avoid direct contact with the biological agents
- Note personal hygiene to avoid carrying over of the biological agents

Protocol related hazards

Physical hazards

- Flammable substances
- Sharps
 - Do not recap needles
 - Discard needles into designated sharp box
- Electricity shocks by high voltage equipment

Protocol related hazards

Important notes

- be familiar with the corresponding precaution procedures and
- seek specific training from LAF or your research group before conducting procedures involving potential hazards
- notify LAF for proper animals and cage handling

Part 3

Animal Works in Your Research Laboratory

Researcher's responsibility

Hong Kong Legislation

- [*The Code of Practice for Care and Use of Animals for Experimental Purposes*](#)

*5.2 Investigators have **direct and ultimate responsibility** for all matters related to the welfare of their animals. They must act in accord with all requirements of this Code.*

Animal Works in Your Research Laboratory

Considerations

- Environment in the laboratory is different from a designated animal holding room:
 - Inappropriate air temperature control
 - Lack of a tight light dark cycle
 - High light intensity
 - Noise
 - Not enough fresh air supply to remove allergens
- Inappropriate environment may cause stress to animals and hazards to laboratory personnel (animal wellbeing issues).
- The increase of frequency and duration of keeping animals in the laboratory increases the dispersal of allergens to all personnel in the laboratory (occupational safety issue)

Reason for performing animal procedures in the laboratory

Justify the need of bringing animals to the laboratory which risks animal welfare and allergen hazard to the laboratory personnel.

- Bringing animals away from the animal facility only when performing a particular procedure in the animal facility is impossible, e.g.
 - procedures requiring a bulky equipment being shared with other non-animal users
 - isolation of tissue must be done shortly before further processing, which must be performed in the laboratory
- Multiple treatment on the animals in a single day, without the need of special equipment may be possible in the animal facility

Time arrangement on the animal procedures

- The period when the animals stay in the laboratory should be as short as possible by:
 - Prepare the experimental set up and reagents ahead in the laboratory
 - Remove the animals from the LAF immediately before the procedures

Precaution on transferring the animals

- Cages should be covered by the appropriate filter cover to contain allergens and allow the proper air supply
- Avoid bright light by properly shielding the cages
- Cages should be carried with a stable trolley/cart or manually
- Use air conditioned indoor pathways whenever possible and avoid transportation path with direct sunlight

Temporary keeping of animals in the laboratory

- If required, live animals should be temporarily kept in a place with the following conditions:
 - ambient temperature
 - quiet
 - dim
 - without chemicals around (especially volatile and powder)
 - NO chemical fume cabinet
 - NO oven of any temperature for pups (keep pups warm with the original nest material or finely teased paper tissue)

Note during the animal procedures

- ALL procedures must be performed as described in an Animal Ethic Committee approved *Protocol* and by approved persons listed in the *Protocol*
- If surgery is performed, the same requirement of aseptic procedures must be applied
- If a terminal procedure is performed, the animals are euthanized according to the requirement by the *AVMA Guidelines for the Euthanasia of Animals (2020 Edition)*
- Adult mice are commonly euthanized by cervical dislocation, carbon dioxide, or pentobarbital by injection, while young pups are euthanized by decapitation

Note after procedures

After the procedures

- Record the procedures and the use of animals accordingly
- Reusable cage materials should be doubly wrapped in autoclavable bags and returned to LAF 7J facility entrance D as soon as possible
- Animal carcasses should be doubly bagged and returned to designated freezers as soon as possible

END of General Animal Use Training