laboratory animals husbandry

تربية الحيوانات المختبرية

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defines "laboratory animals" as any live or dead dog ,cat , guinea **pig , hamster , rabbit , or any other warm blooded animal , which is** being used or is intended for use for research, testing, or teaching .this term excludes :birds , rats , mice for use in research . For accreditation purposes, the definition is not limited by the type of housing enclosure (e.g., cage, pen, paddock, pasture, tank, raceway, etc.). International recognizes that the biomedical or agricultural research, testing or teaching objectives as well as the health and welfare of the animals will dictate. the housing and care for farm animals should meet the standards that prevail on a high-quality, well-managed farm. determine which standard(s) applies best with regard to the care and welfare of agricultural animals, based on a performance approach in the context of the requirements of the study and the species used. The definition of a research facility was expanded to include those institutions using covered live animals and not just dogs and cats. These facilities were required to file an annual report.(1)

Occupational health & safety program

An occupational health and safety program must be part of the overall animal care and use program. The basic elements of a program include hazard identification and risk assessment, personnel training and protection, written procedures and policies regarding hazard use and monitoring, and medical evaluation and preventive medicine.

The extent and level of participation of personnel in the program should be based on the hazards posed by the animals and materials used; on the exposure intensity, duration, and frequency; on the susceptibility of the personnel; and on the history of occupational illness and injury in the particular workplace. A health history evaluation is advisable before work assignment to assess potential risks for individual employees. Periodic medical evaluations and appropriate immunization schedules are advisable for some risk categories. Immunization of animal care personnel against tetanus is important. (2)

A.1 Animal Handling

When handling animals, always remember to approach them in a **confident** and **relaxed** manner. Animals should be handled as **regularly** as possible to help reduce stress and to allow the animals to get used to you.
It is important to undergo **training** if you are going to restrain an animal for a procedure, as some techniques require a lot of practice and you may make a mistake if you are unfamiliar with the methods whilst trying to perform a procedure.(3)

A.1.1 General principles for animal handling

Animals should be approached in a confident and relaxed manner. Animals should be handled regularly to help reduce stress and to calm them down when restraining them for procedures to be performed on them .Most animals have sharp claws and prefer not to be placed on slippery surfaces, so, where possible, use a cage top (for rodents) or a nonslip cover/liner for benches.
With practice, most species of animals are easily restrained and handled.
There is no one correct method of handling or restraining animals ,but the general principle is that it should not cause pain. The methods shown in the species-specific sections are recommended, although some people may feel more comfortable using slightly different ways to restrain the animals, which is also acceptable.(4)

Environment:

Micro environment and Macro environment; The *micro environment* of a terrestrial animal is the physical environment immediately surrounding it; that is, the primary enclosure such as the cage, pen, or stall. It contains all the

resources with which the animals come directly in contact and also provides the limits of the animals' immediate environment. The microenvironment is characterized by many factors, including illumination, noise, vibration, temperature, humidity, and gaseous and particulate composition of the air. The physical environment of the secondary enclosure, such as a room, a barn, or an outdoor habitat, constitutes the *macro environment* . Although the microenvironment and the macro environment are generally related, the microenvironment can be appreciably different and affected by several factors, including the design of the primary enclosure and macro environmental conditions. Evaluation of the microenvironment of small enclosures can be difficult. Available data indicate that temperature, humidity, and concentrations of gases and particulate matter are often higher in the animal microenvironment than in the macro-environment.

Temperature and humidity

Maintenance of body temperature within normal circadian variation is necessary for animal well-being. Animals should be housed within temperature and humidity ranges appropriate for the species, to which they can adapt with minimal stress and physiologic alteration. Environmental temperature and relative humidity can be affected by husbandry and housing design and can differ considerably between primary and secondary enclosures as well as within primary enclosures. Exposure to wide temperature and humidity fluctuations or extremes may result in behavioral, physiologic, and morphologic changes, which might negatively affect animal well-being and research performance as well as outcomes of research protocols.(5)

Ventilation and Air Quality

The primary purpose of ventilation is to provide appropriate air quality and a stable environment. Specifically, ventilation provides an adequate oxygen supply; removes thermal loads caused by the animals, personnel, lights, and equipment; dilutes gaseous and particulate contaminants including allergens and airborne pathogens; adjusts the moisture content and temperature of room air; and, where appropriate, creates air pressure differentials (directional air flow) between adjoining spaces. Direct exposure of animals to air moving at high velocity (drafts) should be avoided as the speed of air to which animals are exposed affects the rate at which heat and moisture are removed from an animal.

Environment, housing, And Managements:

able to exhaust these systems directly into the building's exhaust system to reduce heat load and macro environmental contamination. Static isolation caging (without forced ventilation), such as that used in some types of rodent housing, restricts ventilation (Keller et al. 1989). To compensate, it may be necessary to adjust husbandry practices, including sanitation and cage

change frequency, selection of contact bedding, placement of cages in a secondary enclosure, animal densities in cages, and/or decrease in macro environmental relative humidity to improve the microenvironment and heat dissipation.

Illumination:

Light can affect the physiology, morphology, and behavior of various animals Potential photo stressors include inappropriate photoperiod, photo intensity, and spectral quality of the light .Numerous factors can affect animals' needs for light and should be considered when an appropriate illumination level is being established for an animal holding room. These include light intensity and wavelength as well as the duration of the animal's current and prior exposure to light, and the animal's pigmentation, circadian rhythm, body temperature, hormonal status, age, species, sex, and stock or strain.(6)

Noise and Vibration

Noise produced by animals and animal care activities is inherent in the operation of an animal facility and noise control should be considered in facility design and operation. Assessment of the potential effects of noise on an animal warrants consideration of the intensity, frequency, rapidity of onset, duration, and vibration potential of the sound and the hearing range, noise exposure history, and sound effect susceptibility of the species, stock, or strain. Similarly, occupational exposure to animal or animal care practices that generate noise may be of concern for personnel and, if of sufficient intensity, may warrant hearing protection .Separation of human and animal areas minimizes disturbances to both human and animal occupants of the facility.(7)

Terrestrial Management

behavioral and Social Management

Activity Animal Activity typically implies motor activity but also includes cognitive activity and social interaction. Animals' natural behavior and activity profile should be considered during evaluation of suitable housing or behavioral assessment. Animals maintained in a laboratory environment are generally restricted in their activities compared to free-ranging animals. Forced activity for reasons other than attempts to meet therapeutic or approved protocol objectives should be avoided. High levels of repetitive, unvarying behavior (stereotypies, compulsive behaviors) may reflect disruptions of normal behavioral control mechanisms due to housing conditions or management practices .Dogs, cats, rabbits, and many other animals benefit from positive human interaction.

Husbandry:

Food Animals should be fed palatable, uncontaminated diets that meet their nutritional and behavioral needs at least daily, or according to their particular requirements, unless the protocol in which they are being used requires otherwise. these publications consider issues of quality assurance, freedom from chemical or microbial contaminants and natural toxicants in feedstuffs, bioavailability of nutrients in feeds, and palatability. There are several types of diets classified by the degree of refinement of their ingredients. *Natural-ingredient diets* are formulated with agricultural products and byproducts and are commercially available for all species commonly used in the laboratory. Although not a significant factor in most instances, the nutrient composition of ingredients varies, and natural ingredients may contain low levels of naturally occurring or artificial contaminants . Contaminants such as pesticide residues, heavy metals, toxins, carcinogens, and phytoestrogens may be at levels that induce few or no health squeal yet may have subtle effects on experimental results. *Certified diets* that have been assayed for contaminants are commercially available for use in select studies, such as preclinical toxicology, conducted in compliance with FDA Good Laboratory Practice standards . *Purified diets* are refined such that each ingredient contains a single nutrient or nutrient class; they have less nutrient concentration variability and the potential for chemical contamination is lower. *Chemically defined diets* contain the most elemental ingredients available, such as individual amino acids and specific sugars . The latter two types of diet are more likely to be used for specific types of studies in rodents but are not commonly used because of cost, lower palatability, and a reduced shelf life. Animal colony managers should be judicious when purchasing, transporting, storing, and handling food to minimize the introduction of diseases, parasites, potential disease vectors (e.g., insects and other vermin), and chemical contaminants in animal colonies. Purchasers are encouraged to consider manufacturers' and suppliers' procedures and practices (e.g., storage, vermin control, and handling) for protecting and ensuring diet quality.(8)

Some older devices used in laboratory animal husbandry :

Polypropylene Mouse Box
The first replacement for the metal mouse cage was produced by North Kent Plastics Limited in the late 60's, following some work with the Laboratory Animals Centre on the original mould. The M1 type with a flat top and space for a bottle and food hopper soon gave way to the M2 with a cut away front so that the animals could be observed more easily. This spawned many generations of improved, lighter

enclosures for small laboratory animals which were easier to keep clean and environmentally more controlled; a major contribution to laboratory animal husbandry.

Photograph from the Laboratory Animals Centre's Gnotobiology Unit in 1973(9)

Buprenorphine

Buprenorphine was at the forefront of clinical analgesia in laboratory animal science: its duration of action and relatively limited adverse effects meant that for the first time effective post-surgical analgesia was possible in rodents. Its rapid uptake within the lab animal community was a credit to the profession and demonstrated the commitment of those involved to provide the best possible care for their animals. *Buprenorphine hydrochloride was first marketed in the 1980s by Reckitt & Colman (now Reckitt Benckiser) as an analgesic, generally available as Temgesic.*

The Individually Ventilated Cage (IVC)

Good laboratory animal science dictates that animals should be disease-free and of high health status, since existing pathogens can alter an animal's response and affect the outcome of research, and that high standards of animal welfare are maintained. Animals with altered immune status are particularly vulnerable to pathogens and are increasingly used in medical research. The development of IVCs, in which each cage is effectively a separate barrier unit preventing the transmission of pathogens between cages, allowed susceptible strains to be maintained in higher health, resulting in fewer numbers bred and used and significant refinement in their condition and welfare.


IVC cage and rack(10)

The natural behavior of rabbits:

Rabbits spend most of the diurnal period underground where they tend to congregate in groups, snuggling together and grooming each other. They venture out in the evening to roam and forage, keeping distinct social distances and appearing less tolerant of one another than during daylight.
Rabbits are gregarious animals living. Dominance-subordination relationships are fairly stable and fighting is generally a rare event. Low ranking rabbits are relatively restricted in their movements and spend more time alone and concealed than high ranking group members.

Management

The caging should be washed at least once a week. Regular cleaning and sanitizing of racks, equipment and room surfaces should also be regularly undertaken. Excreta trays should be cleaned as often as necessary to minimize ammonia buildup in the rooms. Disposal of dirty bedding and cleaning of excreta trays should not be done inside the animal room. Fresh food and water should be provided daily. All animals should be observed at least once daily, with food and water consumption and nature of excrements being noted. Sick or dead animals should be removed immediately**.**

BIOLOGY: The rabbit's life expectancy in the laboratory or breeding colony will rarely

exceed four to five years, although under natural conditions they may, particularly in the case of males, The dental formula of the adult rabbit is: Incisors 2/1, canines 0/0, premolars 3/2, molars 3/3. Average body temperature is 39.5oC (range 38.5-40oC) which usually fluctuates considerably with excitement caused by handling, even when other signs of disturbance are not obvious.

Nutrition: Rabbits are herbivorous and may, under normal conditions, be very adequately maintained on any one of the numerous complete pelleted rations widely available commercially. Fresh water should be provided daily and *adlibitum*. Hay supplements may be provided .Reinjection of feces (coprophagy) is a normal practice amongst rabbits and is important, indeed essential, to maintain adequate nutrition and normal intestinal physiology.

Reproduction: Spontaneous ovulation does not occur in the female rabbit (doe). Coitus is normally required to induce ovulation. Because of this, there is no defined estrus cycle in this species. Under optimal conditions the mature doe will remain ready to accept the male (buck) indefinitely, and will have a constant but fluctuating number of ripe (graafian) follicles ready to rupture if coitus occurs. Whether or not the doe is actually ready to mate can be determined by the state of her vulva, which in heat should be enlarged and somewhat red, and by her immediate behavior when placed with a buck.**(11)**

Mice (*Mus musculus*):

Physiologic parameters
Body temperature 36.5 C–38.0C
Heart rate 325–780/min
Respiratory rate 94–163/min
Careful handling and restraint are required to minimize discomfort when injecting any substance into a small animal. Practice should be carried out by first using models or euthanized animals. Always use aseptic techniques. Mice should be picked up by the base of the tail, close to the body. Pregnant animals and young animals (preening) may need to be scooped up with one or both hands. Wearne mice may need to be picked up by the tail, and care should be taken as they are usually very lively and will jump out of the cage at any given opportunity.
Mouse handling and sexing — for removal from caging and transport
1. Grasp the mouse near the base of its tail .
2. Lift the animal out of the cage and place it in new caging or on a firm surface.
3. Do not suspend the mouse by its tail for a prolonged period of time because of stress on the animal. Support its body weight quickly, especially for pregnant animals.
4. Always double-check the sex of the animal with the cage Card.

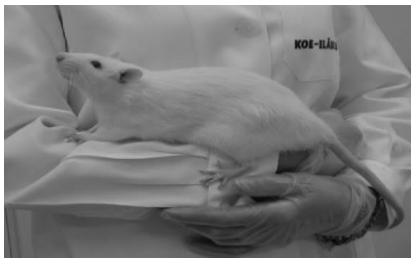
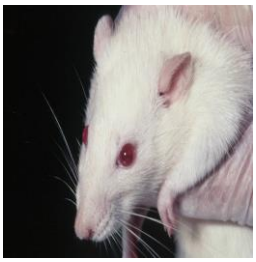
Life span: Mice from short-lived strains can be expected to die between 5 and 16 months of age. Mice from long-lived strains often survive to 24, 30 or even 36 months of age.

Reproduction ;Sexual maturity in mice occurs very early in life and varies with strain and environmental influences. Ovarian follicle development begins at 3 weeks of age and matures by 30 days. Puberty in males occurs up to two weeks later. Female mice are poly-estrous, spontaneous ovulations and cycle every 4-5 day. Factors such as season, diet, genetic background and environmental factors influence the estrous cycle. The cyclicity of estrus and ovulation are controlled by the diurnal rhythm of the photoperiod. Mating, estrus and ovulation most often occur during the dark phase. Light cycles of 12-14 hours light and 12-10 hours dark are necessary to maintain regular estrus cycles. Mating can be detected within 24 hours after copulation by the formation of a waxy vaginal plug (a mixture of sperm and secretions from the seminal vesicles and the coagulating glands of the male). The gestation period is 19-21 days, depending on the strain. Litter size commonly ranges from 1-14 pups, depending on the strain. Pregnant females build nests for giving birth.

HOUSING: Housing systems for laboratory animals have often been designed on the basis of economic and ergonomic aspects (such as equipment, costs, space, workload, ability to observe the animals and to maintain a certain degree of hygiene) with little or no consideration for animal welfare. laboratory mice are usually housed throughout their lives in relatively barren cages and provided with *ad libitum* food, which frequently results in adverse effects on the animal's behavior and physiology and in as shortened life span due to overfeeding and inactivity. Wire-mesh floors are used if experiments require continuous collection of feces and/or urine or elimination of contact between the animal and bedding material. Solid floor cages containing bedding and nesting materials should be used whenever possible. Wood shavings are widely used as bedding material, but fine particles can cause preputial and respiratory disorders. The type of wood can affect physiological parameters in the animal, such as hepatic microsomal enzyme function, Nesting material, such as paper towels, tissues, and wood wool, can provide shade from lighting, the opportunity to regulate the animal's microclimate, a shelter to hide from conspecifics and the ability to control the environment.

Food: The mouse is an omnivorous animal. The incisors and molars grow continuously and are worn down by mastication. Attention should be paid to malocclusion leading to under-nutrition. Feeding behavior in rodents shows a diurnal pattern with the majority of food consumed during the dark period. Fasting overnight, which is sometimes part of an experimental protocol, might lead to an increase of activity, resulting in unwanted variation in experimental results**.** The food rack should be kept sufficiently full, as it is difficult for the animals to gnaw the food when there are only a few pellets left. Restricted feeding has been shown to be beneficial in the long run in terms of reduced morbidity and mortality. Enrichment related to food, e.g. grain scattered through the bedding, will meet the animal's need for foraging and will prevent boredom.(12)

Rats (*Rattus norvegicus*):


Physiologic parameters
Body temperature 35.9C–37.5C
Heart rate 250–450/min
Respiratory rate 70–115/min

Rat handling and sexing
1. First, assess the rats in their cage for normal behavior. The rats should be alert and inquisitive, and will usually stand on their hind legs and move around the cage exploring their environment.
2. Place your hands into the cage, and gently pet and touch the animals. At this point, be careful of touching their faces and of stressing them. Try to calm them and let them sniff you.
3. With firm but gentle pressure, grasp the rat around the thorax with your thumb and forefinger under each of its front legs.
4. Lift the rat out of the cage and place it in a new cage or on a firm surface.
5. For aggressive rats, pick them up by grasping them by the base of the tail, close to the body.

General biology: the rat is most active in the dark and rests during the light period. In the laboratory, rats have a clear circadian rhythm, which follows the light cycle, corresponding usually to 12/12 hours of light and dark. The most active periods occur at the beginning and end of the dark period. this rhythm is also known to regulate various physiological variables. rats have cones sensitive to visible light and others to ultraviolet, and evidence of dichromatic color vision in the rat has been found in behavioral discrimination tests.

Lifespan: varies between 2-4 years, depending on the strain and sex of the animal, its diet and living conditions.

Husbandry procedures: Husbandry procedures may vary depending on such factors as the type of cages, animal number in cages, feeding and watering systems. In general, the rats are changed once or twice a week into clean cages with a simultaneous change of diet, water and enrichment tools. In the most hygienic systems, caretakers carry a personal protective mask, gloves and clothing to prevent the spread of pathogens. The changes which occur

within the room, like paper towel on the cage lid, white noise, stroboscope light, or room entry caused smaller and shorter responses in rats.
Feeding: content and presentation of the diet should meet the nutritional and behavioral needs of the animal, and uncontaminated drinking water should always be available to all animals. Feed intake can be influenced by the energy content of the diet, environmental factors such as temperature and light cycle, or by other management practices, such as group housing, restrictive feeders and cage design. suitable diet can be chosen to meet situational needs, such as the breeding or maintenance of animals. As rodents, rats gnaw their food, thus the standard pelleted food may be considered to an optimal form for this animal. obesity and ultimately decreased survival, leading to an increased incidence of tumors in older animals.(13)

Guinea Pigs (*Cavia porcellus*)

Guinea pigs have a mild disposition and are generally easy to handle. Care must be taken when approaching guinea pigs, as they are nervous animals and are easily startled. Approach them slowly and gently, and try not to make sudden movements or loud noises.

Physiologic parameters

Body temperature 37.2 C–39.5C
Heart rate 230–380/min
Respiratory rate 42–104/min

Feeding: The food has to be stored under cool, dry conditions and protected against contamination. It should be available *ad libitum*. Guinea pigs should be fed daily at a fixed time. Access to food has to be guaranteed for all animals. Furthermore it is important to ensure the availability of vitamins?(vitamin C, since guinea pigs are susceptible to vitamin C deficiency.). It is also important to feed hay several times per week. Besides fulfilling dietary requirements, hay can also serve as material for play. Furthermore, guinea pigs must be able to regularly engage in gnawing behavior to prevent overgrowth of their incisors. For this purpose hard food pellets, carrots or wooden branches are suitable.
General Housing: Guinea pigs can be kept in either pens or cages. Good experiences have been achieved with ground housing. In this case, bedding (such as wood shavings) - is necessary. However, it should be noted that flagstones can be irreversibly contaminated with urolithic acid. It is also important to provide sufficient space. Overcrowded conditions in cages can lead to endocrine stress reactions and higher frequencies of aggression.(14)