ANIMAL CARE AND USE PROGRAM
STANDARD OPERATING PROCEDURE NUMBER 04

Housing, Husbandry and Environmental Enrichment Guidelines for Laboratory Animals

PURPOSE

The purpose of this standard operating procedure is to provide guidelines with respect to the housing and husbandry of laboratory animals used in research and teaching at UMass Boston. The quality of housing and husbandry has a major impact on laboratory animal health and welfare. The caging or housing system is an important factor in the physical environment of laboratory animals and not only influences the well-being of the animals but also acts as an experimental variable.

INFORMATION

Federal guidelines for use of vertebrate animals in research contain specific provisions for basic husbandry. *The Guide for the Care and Use of Laboratory Animals* states: "A good management program provides the environment, housing, and care that permit animals to grow, mature, reproduce, and maintain good health; provides for their well-being; and minimizes variations that can affect research results." Proper diet and a stress-free, sanitary environment are some of the greatest tools in preventing the development or transmission of disease. The following guidelines are used in developing our husbandry procedures. They are adapted from requirements in the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*.

Further considerations for appropriate physical and social environment, housing, space, and management noted include: species, strain, and breed, individual characteristics, (sex, age, size, behavior, experiences, and health), group (social) housing, enrichment, project goals and duration, degree of invasiveness, presence of hazards.

It should be noted that for social species the Guide states "It is desireable that social animals be housed in groups; however, when they must be housed alone, other forms of enrichment should be provided to compensate for the absence of other animals, such as safe and positive interaction with the care staff and enrichment of the structural environment." In addition to exemptions required by study objectives, other exceptions to this policy might include single housing for pregnant mice or rats, incompatible animals, animals during the immediate post-surgical recovery period or animals isolated for medical reasons by the attending Veterinarian.

It is therefore a requirement of the institution that we base our animal management program on these and other factors. This document is prepared to assist the institutional faculty and staff in designing the most appropriate housing, care, and enrichment program for the species they are using in research, teaching, or testing.

ANIMAL HOUSING ROOMS

Animal Housing Standards

All animals housed for research, teaching or testing purposes at UMass Boston must be housed in the Animal Care Facility as listed in the institution's PHS Assurance. Each animal housing room maintains a single species at any given time unless special housing arrangements have been made for compatible species.

Changes from these standards are not permitted except as approved by the IACUC or prescribed by the attending Veterinarian.

All animal housing rooms must be checked daily, including weekends and holidays, foul weather notwithstanding.

- This check should include:
- Monitoring room conditions
- Monitoring for health problems
- Monitoring food and water levels
- Monitoring for proper cage conditions

Problems should be evaluated based on the potential for adverse effects on the animals and remedied as soon as possible.

Documentation of daily checks should be provided in the form of a room log or check-list.

General Room Conditions

Animal rooms must be kept clean, quiet, and uncluttered. The use of procedure rooms is encouraged whenever possible. Research procedures which are permitted within the room include injections, blood collection, examinations and other noninvasive techniques. Procedures which are not permitted in the animal room include surgery, euthanasia and necropsy.

Lighting

Light levels should be adequate for the animal to perform normal behaviors and for the animal care giver to perform their duties. Diffuse lighting in the range of 130-325 lux are normal.

The light cycle should be appropriate for the biology of the animal, if consistent with experimental goals. A diurnal 12 hour light cycle is standard. Reversal of the cycle or alteration of the cycle may be desirable depending on the experiment but justification for such alterations should be included in the research protocol and approved by the IACUC. Light cycles should be changed gradually, not abruptly.

Ventilation

Room ventilation must be adequate to provide oxygen and remove chemical, biological, and heat waste. The standard rate is 10-15 air changes per hour. Lower levels may be acceptable if animal density in the room is low. Fresh air is desirable, although recycled air may be provided if it has been conditioned to remove excess heat, carbon dioxide, moisture, odors, or biological or chemical hazards.

Room ventilation should normally be adjusted to maintain room pressure negative to the corridor to protect adjacent personnel areas, or positive to the corridor if the room is serving as an isolation room.

Ventilation ducts and filters should be cleaned at least monthly.

Temperature

Temperature in rooms should be maintained in a range suitable for the species of animal and the animals should be protected from abrupt changes. Temperature ranges for many animals are specified in the Animal Welfare Act.

A range of 68° F to 74° F is standard for mammals. Temperature and humidity conditions for each species housed should be posted in each animal room. Temperature and humidity conditions must be monitored closely and recorded daily. When temperature and/or humidity exceeds thresholds published in the *Guide,* Physical Plant must be contacted immediately. Back-up systems must always be available in the event of a primary HVAC system failure.

Ectotherms and birds may have different needs. Animals adapted to outdoor environments may also have different needs.

Noise

Animals may detect different sound frequencies than do human beings. Therefore, noise in animal rooms should be minimized whenever possible.

Normally, playing music is not advisable in animal rooms, although it may be necessary under some circumstances to provide enrichment or "white noise" (to mask a different noise).

Quiet species should not be housed with noisy species.

Noise from mechanical equipment in adjacent areas should be avoided to preclude unnecessary stress on the animals. Some species may experience reproductive problems when exposed to excessive noise.

Sanitation

Room surfaces should be constructed of material that is easily sanitized.

Floors, counters and sinks should be cleaned sanitized daily.

Other room surfaces, including cage racks, food storage containers, etc. should be sanitized monthly.

CAGING AND OTHER HOUSING CONTAINERS

Species Standard Housing and Enrichment

PI must opt-out of these requirements on the animal protocol by providing scientific justification and receiving IACUC approval.

Amphibians/reptiles

- Group housed if possible in easily sanitizable and secured enclosures with a safe heating device located in such a way to allow animal to thermoregulate appropriately for the species.
- Substrate that is easily sanitizable and appropriate for the species. Fresh water (dechlorinated for amphibious species) and nutritionally complete feed appropriate for the species.
- Hiding tubes boxes and perches of appropriate size.
- Food items as treats, not to constitute a substantial portion of the standard diet.

Xenopus

- Xenopus are a prey species, therefore it is highly important to provide a form of shelter in which the animal can retreat and hide.
- Group housed in water tanks or tubs with PVC pipe or a floating device that is removable and sanitizable. Objects placed on the surface of the water must still allow frogs to easily access the surface of the water to breathe. Opaque tanks are preferable to completely transparent tanks, however, at least one clear side must be provided to readily observe the animals.
- De-chlorinated water temp appropriate for species flushed daily post feeding, and free of significant debris. Nutritionally complete commercially available food, appropriate for the species.
- Tank dimensions must allow adequate space for a suitable volume of water per animal.
- Avoid housing animals of disproportionate sizes together to prevent predation.

Birds

- Group housed if possible in smooth wire cages adequate for the species. Flight cages if possible or alternate enrichment made available.
- Perches adequate to prevent foot disease.
- Fresh water available at all times.
- Food nutritionally complete for the species.
- Access to materials for maintaining crop.

Mice

- Group housing whenever possible.
- Provision of *Nestlets*, sterilized paper, shepherd shacks or plastic enclosures.
- All singly housed mice should be provided with two or more enrichment options, one of which must be second choice bedding material or hiding device.
- ACP approved polystyrene, polycarbonate or other high temp solid bottom plastic cage fitted with a functioning filter-top.
- Cages may be connected to racks providing filtered air directly to the cage.
- Commercially available paper, wood chip or corn cob type bedding.
- Wire bar lid or frame.
- Water bottle or lixit for automatic watering.
- ACP approved feed in a feed hopper or wire bar lid.

Rats

- Group housing whenever possible.
- Provide hiding devices (e.g. PVC tube) or may be given a resting platform.
- Provision of second bedding material (non-treated paper towels, Envirodri®) may be considered.
- For gnawing, nylon balls or bones or sterilized wood blocks.
- All singly housed rats must be provided with two or more enrichment options, one of which must be a gnawing device.

Hamsters

- Group house if possible; although mature females may be aggressive in groups (in this case, pair housing might be preferable).
- Provision of tunnel/hiding device (PVC or Y-tube).
- Provision of second bedding material (e.g. non-treated paper towels, Enviro-dri®, Neslet® may be considered)
- For gnawing, nylon balls or bones or sterilized wood blocks.
- ACP approved polystyrene, polycarbonate or other high temp solid bottom plastic cage fitted with a functioning filter-top.
- Cages may be connected to racks providing filtered air directly to the cage.
- Commercially available paper, wood chip or Corn Cob type bedding.
- Wire bar lid or frame.
- Water bottle or lixit for automatic watering.

- ACP approved feed in a feed hopper or wire bar lid.
- All singly housed hamsters must be provided with two or more enrichment options, one of which must be a gnawing device.

Guinea Pigs

- Group house if possible.
- ACP approved Polystyrene, polycarbonate or other high temp plastic cage fitted with a functioning filter-top.
- Cages may be connected to racks providing filtered air directly to the cage or, standard designed, ACP approved guinea pig rack units.
- Commercially available paper, wood chip or Corn Cob type bedding.
- Wire bar lid or frame.
- Water bottle or lixit for automatic watering.
- ACP approved feed in a feed hopper or wire bar lid as appropriate.
- Provision of *Nestlets*, burrowing materials, such as sterilized paper, shepherd shacks or plastic enclosures.

Rabbits

- Group house or social contact if possible.
- Easily sanitizable wire bottom rabbit caging of appropriate size for the species.
- Social contact if possible.
- Water bottle or lixit for automatic watering.
- Nutritionally complete pelleted food per weight daily.
- Toys such as **PVC** sections may be placed in or attached to the cage.
- Small portions of timothy hay or other grass hay, offered up to three times per week but not enough to offset normal diet.
- Treat provisioning small portions of fruit, vegetables, alfalfa.

Identification

All animals must be identified according to their protocol number, the name and phone number of the investigator, species, strain, sex, age and source.

Rodents and rabbits may be identified to the cage level.

Identification is normally provided by a cage card, with additional marking of individual animals when needed. Groups of animals may be identified using one posting if all information is identical.

Cage Space Requirements

A major consideration is the provision of enough space to allow an animal to make normal postural adjustments and have normal intra-specific interactions (including avoidance).

Cage space requirements are specified by law, and must be strictly adhered to.

Generally, a standard "shoebox" type cage can hold 4-5 adult mice (5 if they are less than 25 g) and a rat shoebox cage 2-3 adult rats (3 if less than 500 g). For other species cages should be measured and the animals weighed to determine if requirements are being met.

The ultimate measure of whether or not cage space is adequate is the condition of the animal. If animal waste is excessive, or if significant aggression is occurring, the cage is too crowded.

For any cage in which cage density requirements are exceeded, the Animal Care Facility Manager (ACFM) will mark the cage with a yellow "Action Required" card that lists a date (generally within the next 48 hours) by which the cage density problem must be corrected. The ACFM will contact the PI by email and copy both the IACUC Chair and compliance manager.

- Cages that exceed the densities listed in this policy are considered out of compliance when the date on the yellow "Action Required" card has expired.
- ACFM is not required to contact the investigator after the expiration date and is authorized to correct the cage and assess a service charge. ACFM will correct the situation by removing a male, or separating litters.
- The ACFM will inform the IACUC of repeated or ongoing problems with compliance with this policy.
- The investigator is responsible for contacting the IACUC if they cannot separate animals or otherwise comply with the policy.
- When investigators are correcting cages, correction may be achieved in several ways. For example: Removing the adult male from a trio breeding cage with 11 pups would satisfy compliance. In some cases, separating mothers with their pups will achieve compliance.

Breeder cages must legibly indicate the date of birth for all litters. This information must be provided by the research staff It is the investigator's responsibility to ensure that mouse cage densities are maintained within this policy. A limited extension of the 48 hour grace period may be allowed for cards placed the day before a weekend. In cases of gross overcrowding, the ACFM is authorized to correct the overcrowded cage without prior notification of the PI.

Investigators should not rely on the placement of yellow cards to manage their colonies!

The IACUC will consider exceptions to this policy on a case-by-case basis if the investigator provides scientific justification with documentation. The exception will not be in effect until the PI has received written notification from the IACUC.

Type of Caging

Solid bottom caging with bedding is the preferred type of caging for all rodents. Animals over 600 g must have this type, unless a grate is necessary for scientific reasons.

Breeding animals must have a solid floor at least in a nest box, bedding and additional nesting material.

Immunodeficient animals, or animals to be maintained specific-pathogen free or gnotobiotic may require housing that has been sterilized, usually by autoclaving.

Some animals may require housing in filter top caging to prevent transmission of diseases into or out of the cage. These cages must be handled in protective hoods using sterilants for anything that will touch the interior of the cage.

Bedding Material

Bedding material should be clean, dry, dust-free, absorbent, non-toxic and preferably soft.

Typical bedding used for rodents include hardwood chip, ground corncob, shredded paper or paper matting. **Aromatic softwood (e.g. cedar) bedding material is not to be used.**

Bedding must be changed when it is visibly wet.

Species-specific Behavioral Considerations

Animals should be group housed with same-sex conspecifics whenever possible.

Group housed animals must be assessed for compatibility and separated if there is significant aggression. If animals are excluding others from food or water, additional feeders and water bottles must be provided.

Environmental Enrichment. Enrichment is a dynamic process in which changes are made to husbandry practices or the animals' standard physical environment for the purpose of promoting expression of the species appropriate behavior and activities, while minimizing stress-inducing behaviors. All animals housed at UMass Boston will be provided different forms of environmental enrichment.

Enrichment can include group housing or other opportunities to socialize such as visual, tactile or olfactory contact with other animals, human interaction, exercise opportunities, nesting material, digging or chewing substrates, food enrichment or other activities that result in a positive psychological state for the animals.

Environmental enrichment for mice includes polycarbonate tubes, polycarbonate igloos and nesting material.

Environmental enrichment for rats and hamsters include polycarbonate rectangular tube and pair or group housing. The ability to pair or group house rats and hamsters is determined by their weight and compatibility. Only those rats and hamsters housed individually will receive an

enrichment device. Pair or group housing meets the requirements of environmental enrichment for these animals.

In some cases, environmental enrichment may not be appropriate for a research study. In those instances, written rationale for withholding environmental enrichment must be provided by the investigator in the research protocol. In such cases, animal cages will be clearly marked with a "NO ENRICHMENT" tag.

Sanitation

Cages and waste pans should be sanitized weekly, or more often if required.

Cages may be sanitized in a commercial cage washer with a soap wash and a high temperature (180° F) rinse. Rinse water temperatures for automatic cage washers should be monitored via the gauge on the machine at least daily and with a thermometer or heat sensitive indicator at lease weekly.

The efficacy of sanitation procedures at should be monitored at least quarterly.

- General procedures include bacteriological culture of equipment and rinse water (for an automatic cage washer).
- Any cultures positive for non-spore forming organisms should be repeated. If subsequent testing is still positive then cage washing procedures must be assessed and modified.

Autoclave efficacy is monitored daily using temperature/pressure indicators and quarterly using *Bacillus* spore strips. Any positive cultures should be repeated. If subsequent testing is positive, the autoclave must be serviced.

Cages may be hand washed with detergent, rinsed in water, then dipped in a sanitizing agent (1/2 oz bleach per gallon of water) and allowed to dry.

NUTRITION AND HYDRATION

Food

Animal food must supply all required *nutrients* unless the requirements of the study preclude it. Normally, animals should be fed *commercially available complete diets* appropriate for their physiologic status.

Growing animals, lactating animals, and animals experiencing physiologic stress such as surgical recovery, sepsis or hypothermia may require additional energy, protein or other nutrients.

- Animals with digestive abnormalities may require more highly digestible food, or may require more fiber.
- Animals with renal insufficiency may require lower dietary protein levels.
- Animals with blood glucose abnormalities may require additional fiber in their diet.
- Animals with pancreatic insufficiency will require enzymatic supplementation.

Rodents normally are fed a *pelleted chow*, which helps to wear down continuously erupting teeth. If powdered diets are to be fed a chewing substrate may be necessary, or tooth growth must be monitored.

Animals should be fed amounts of food to provide at least their *maintenance requirements*. The National Research Council publishes nutritional requirements for most animals. If *food is restricted* for more than 3 hours for neonates, rabbits or rodents for experimental reasons, it must be approved by the IACUC and objective, written criteria for assessing the animals' body condition must be provided. If restricted for medical reasons, the decision should be made based on consultation with the Veterinarian.

Quality Control

Commercially prepared food should only be used if it is within 6 months of its milling date (usually printed on the bottom of the bag). Diets with labile supplements (such as Vitamin C in nonhuman primate or guinea pig chow) have a shorter expiration time.

Specially formulated diets may not have an expiration date. Generally these should be refrigerated and used within a year of manufacture.

Quality control or nutritional analysis data should be obtained from the food manufacturer. If diets are prepared in-house, nutritional analysis should be performed following mixing and again at a later time point to determine the amount of degradation over time.

Food should be stored in sealed, sanitizable containers. The type of food and expiration date should be marked on the container. Containers should be cleaned out weekly and sanitized monthly.

Sterilized food should be stored in sterilized containers and the date of sterilization marked on the container.

Food should not be stored adjacent to animal waste containers or chemicals.

Water

For most purposes tap water from a potable water faucet is adequate for research animals.

For experimental reasons, animals may have special water requirements, such as a need for deionized water, for sterilized water, or for water treated with medications.

Generally, animals should have drinking water available at all times.

If water is restricted for more than 5 hours for rodents or rabbits for experimental reasons it must be approved by the IACUC and objective, written criteria for assessing the animals' hydration must be provided. If restricted for medical reasons the decision should be made based on consultation with the Veterinarian.

Quality Control

For potable tap water, quality control beyond that which the municipality provides is not usually necessary.

Special water needs may require additional monitoring. Water may need to be analyzed for chemicals or cultured for microorganisms.

HEALTH MONITORING

Animal health status must be monitored at least once daily, including weekends and holidays, foul weather notwithstanding.

Animals with specific health problems, animals recovering from anesthesia, or animals on studies that have the potential for rapidly changing the animals' condition (e.g. infectious disease, tumor induction, toxicity) may require more frequent monitoring.

Clinical Signs of Disease

Clinical signs of disease can be extremely variable depending on the species of animal and the condition being monitored.

Changes in behavior, food or water consumption, fecal or urine output, reduction in grooming behavior, aggression, muscular rigidity, reaction to handling can be nonspecific signs of distress or disease.

More specific signs or objective measurements of organ dysfunction should be monitored if indicated by the animal's condition or the expected impact of the experiment.

Veterinary Care

All animals used for research, testing or teaching at the University of Massachusetts Boston must have the attending veterinarian listed on the protocol.

Veterinary care must be available on holidays and weekends as well as during normal work hours.

REFERENCES

Animal Welfare Act
Guide for the Care and Use of Laboratory Animals
UC Davis IACUC Operating Procedures for Environmental Enrichment

Housing Requirements for Mice

This policy follows the space recommendations of the *Guide*. Requirements are outlined in the tables below.

- **Small Mouse Cage**

Available floor space: 70 sq. inches

WEIGHT	#OF ANIMALS PER CAGE	FLOOR AREA PER ANIMAL
< 10 grams	11	6 sq. in. space required
10-15 grams	8	8 sq. in. space required
15-25 grams	5	12 sq. in. space required
>25 grams	4	15 sq. in. space required

- **Tecniplast Ventilated Mouse Cage** Available floor space: 78-82 sq. inches

WEIGHT	#OF ANIMALS PER CAGE	FLOOR AREA PER ANIMAL
< 10 grams	13	6 sq. in. space required
10-15 grams	9	8 sq. in. space required
15-25 grams	6	12 sq. in. space required
> 25 grams	5	15 sq. in space required

* *Guide for Care and Use of Laboratory Animals*, ILAR NRC publication

Housing Requirements for Rats

In accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals*, the UNC-CH program abides by the following housing standards for rats:

- ***Regular Plastic Rat Cage***

Material:	Clear or yellow polycarbonate
Size:	19”w x 10.5” l x 8” h
Available floor space per cage:	143 sq. in.
Height requirement:	7”

WEIGHT	**# OF ANIMALS** *PER CAGE*	**FLOOR AREA PER ANIMAL**
< 100 grams	**8**	17 sq. in. space required
100-200 grams	**6**	23 sq. in. space required
200-300 grams	**4**	29 sq. in. space required
300-400 grams	**3**	40 sq. in. space required
400-500 grams	**2**	60 sq. in. space required
> 500 grams	**2**	70 sq. in. space required

- ***Large Plastic Rat Cage***

Material:	Clear or yellow polycarbonate
Size:	22” w x 12” l x 8” h
Available floor space per cage:	202 sq. in.
Height requirement	7”

WEIGHT	**# OF ANIMALS** *PER CAGE*	**FLOOR AREA PER ANIMAL**
< 100 grams	**11**	17 sq. in. space required
100-200 grams	**8**	23 sq. in. space required
200-300 grams	**6**	29 sq. in. space required
300-400 grams	**5**	40 sq. in. space required
400-500 grams	**3**	60 sq. in. space required
> 500 grams	**2**	70 sq. in. space required