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TABLE 3.2 Recommended Minimum Space for Commonly Used Laboratory Rodents Housed in Groups*

| Animals | Weight, g | Floor Area/Animal,[a] in.$^2$ (cm$^2$) | Height,[b] in. (cm) | Comments |
|---|---|---|---|---|
| Mice in groups[c] | <10 | 6 (38.7) | 5 (12.7) | Larger animals may require more space to meet the performance standards. |
| | Up to 15 | 8 (51.6) | 5 (12.7) | |
| | Up to 25 | 12 (77.4) | 5 (12.7) | |
| | >25 | ≥15 (≥96.7) | 5 (12.7) | |
| Female + litter | | 51 (330) (recommended space for the housing group) | 5 (12.7) | Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d] |
| Rats in groups[c] | <100 | 17 (109.6) | 7 (17.8) | Larger animals may require more space to meet the performance standards. |
| | Up to 200 | 23 (148.35) | 7 (17.8) | |
| | Up to 300 | 29 (187.05) | 7 (17.8) | |
| | Up to 400 | 40 (258.0) | 7 (17.8) | |
| | Up to 500 | 60 (387.0) | 7 (17.8) | |
| | >500 | ≥70 (≥451.5) | 7 (17.8) | |
| Female + litter | | 124 (800) (recommended space for the housing group) | 7 (17.8) | Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.[d] |
| Hamsters[c] | <60 | 10 (64.5) | 6 (15.2) | Larger animals may require more space to meet the performance standards. |
| | Up to 80 | 13 (83.8) | 6 (15.2) | |
| | Up to 100 | 16 (103.2) | 6 (15.2) | |
| | >100 | ≥19 (≥122.5) | 6 (15.2) | |
| Guinea pigs[c] | Up to 350 | 60 (387.0) | 7 (17.8) | Larger animals may require more space to meet the performance standards. |
| | >350 | ≥101 (≥651.5) | 7 (17.8) | |

*The interpretation of this table should take into consideration the performance indices described in the text beginning on page 55.

[a]Singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.

[b]From cage floor to cage top.

[c]Consideration should be given to the growth characteristics of the stock or strain as well as the sex of the animal. Weight gain may be sufficiently rapid that it may be preferable to provide greater space in anticipation of the animal's future size. In addition, juvenile rodents are highly active and show increased play behavior.

[d]Other considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.

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et al. 2008), while cotton nestlets may lead to conjunctivitis (Bazille et al. 2001). Bedding can also influence mucosal immunity (Sanford et al. 2002) and endocytosis (Buddaraju and Van Dyke 2003).

Softwood beddings have been used, but the use of untreated softwood shavings and chips is contraindicated for some protocols because they can affect metabolism (Vesell 1967; Vesell et al. 1973, 1976). Cedar shavings are not recommended because they emit aromatic hydrocarbons that induce hepatic microsomal enzymes and cytotoxicity (Torronen et al. 1989; Weichbrod et al. 1986, 1988) and have been reported to increase the incidence of cancer (Jacobs and Dieter 1978; Vlahakis 1977). Prior treatment with high heat (kiln drying or autoclaving) may, depending on the material and the concentration of aromatic hydrocarbon constituents, reduce the concentration of volatile organic compounds, but the amounts remaining may be sufficient to affect specific protocols (Cunliffe-Beamer et al. 1981; Nevalainen and Vartiainen 1996).

The purchase of bedding products should take into consideration vendors' manufacturing, monitoring, and storage methods. Bedding may be contaminated with toxins and other substances, bacteria, fungi, and vermin. It should be transported and stored off the floor on pallets, racks, or carts in a fashion consistent with maintenance of quality and avoidance of contamination. Bags should be stored sufficiently away from walls to facilitate cleaning. During autoclaving, bedding can absorb moisture and as a result lose absorbency and support the growth of microorganisms. Therefore, appropriate drying times and storage conditions should be used or, alternatively, gamma-irradiated materials if sterile bedding is indicated.

Bedding should be used in amounts sufficient to keep animals dry between cage changes, and, in the case of small laboratory animals, it should be kept from coming into contact with sipper tubes as such contact could cause leakage of water into the cage.

*Sanitation* Sanitation—the maintenance of environmental conditions conducive to health and well-being—involves bedding change (as appropriate), cleaning, and disinfection. *Cleaning* removes excessive amounts of excrement, dirt, and debris, and *disinfection* reduces or eliminates unacceptable concentrations of microorganisms. The goal of any sanitation program is to maintain sufficiently clean and dry bedding, adequate air quality, and clean cage surfaces and accessories.

The frequency and intensity of cleaning and disinfection should depend on what is necessary to provide a healthy environment for an animal. Methods and frequencies of sanitation will vary with many factors, including the normal physiologic and behavioral characteristics of the animals; the type, physical characteristics, and size of the enclosure; the type, number, size, age, and reproductive status of the animals; the use and type of bedding materials; temperature and relative humidity; the nature of the materials that

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create the need for sanitation; and the rate of soiling of the surfaces of the enclosure. Some housing systems or experimental protocols may require specific husbandry techniques, such as aseptic handling or modification in the frequency of bedding change.

Agents designed to mask animal odors should not be used in animal housing facilities. They cannot substitute for good sanitation practices or for the provision of adequate ventilation, and they expose animals to volatile compounds that might alter basic physiologic and metabolic processes.

*Bedding/Substrate Change* Soiled bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry and to keep pollutants, such as ammonia, at a concentration below levels irritating to mucous membranes. The frequency of bedding change depends on multiple factors, such as species, number, and size of the animals in the primary enclosure; type and size of the enclosure; macro- and microenvironmental temperature, relative humidity, and direct ventilation of the enclosure; urinary and fecal output and the appearance and wetness of bedding; and experimental conditions, such as those of surgery or debilitation, that might limit an animal's movement or access to clean bedding. There is no absolute minimal frequency of bedding changes; the choice is a matter of professional judgment and consultation between the investigator and animal care personnel. It typically varies from daily to weekly. In some instances frequent bedding changes are contraindicated; examples include portions of the pre- or postpartum period, research objectives that will be affected, and species in which scent marking is critical and successful reproduction is pheromone dependent.

*Cleaning and Disinfection of the Microenvironment* The frequency of sanitation of cages, cage racks, and associated equipment (e.g., feeders and watering devices) is governed to some extent by the types of caging and husbandry practices used, including the use of regularly changed contact or noncontact bedding, regular flushing of suspended catch pans, and the use of wire-bottom or perforated-bottom cages. In general, enclosures and accessories, such as tops, should be sanitized at least once every 2 weeks. Solid-bottom caging, bottles, and sipper tubes usually require sanitation at least once a week. Some types of cages and housing systems may require less frequent cleaning or disinfection; such housing may include large cages with very low animal density and frequent bedding changes, cages containing animals in gnotobiotic conditions with frequent bedding changes, individually ventilated cages, and cages used for special situations. Other circumstances, such as filter-topped cages without forced-air ventilation, animals that urinate excessively (e.g., diabetic or renal patients), or densely populated enclosures, may require more frequent sanitation.

The increased use of individually ventilated cages (IVCs) for rodents has led to investigations of the maintenance of a suitable microenvironment with extended cage sanitation intervals and/or increased housing densi-

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ties (Carissimi et al. 2000; Reeb-Whitaker et al. 2001; Schondelmeyer et al. 2006). By design, ventilated caging systems provide direct continuous exchange of air, compared to static caging systems that depend on passive ventilation from the macroenvironment. As noted above, decreased sanitation frequency may be justified if the microenvironment in the cages, under the conditions of use (e.g., cage type and manufacturer, bedding, species, strain, age, sex, density, and experimental considerations), is not compromised (Reeb et al. 1998). Verification of microenvironmental conditions may include measurement of pollutants such as ammonia and $CO_2$, microbiologic load, observation of the animals' behavior and appearance, and the condition of bedding and cage surfaces.

Primary enclosures can be disinfected with chemicals, hot water, or a combination of both.[2] Washing times and conditions and postwashing processing procedures (e.g., sterilization) should be sufficient to reduce levels or eliminate vegetative forms of opportunistic and pathogenic bacteria, adventitious viruses, and other organisms that are presumed to be controllable by the sanitation program. Disinfection from the use of hot water alone is the result of the combined effect of the temperature and the length of time that a given temperature (cumulative heat factor) is applied to the surface of the item. The same cumulative heat factor can be obtained by exposing organisms either to very high temperatures for short periods or to lower temperatures for longer periods (Wardrip et al. 1994, 2000). Effective disinfection can be achieved with wash and rinse water at 143-180°F or more. The traditional 82.2°C (180°F) temperature requirement for rinse water refers to the water in the tank or in the sprayer manifold. Detergents and chemical disinfectants enhance the effectiveness of hot water but should be thoroughly rinsed from surfaces before reuse of the equipment. Their use may be contraindicated for some aquatic species, as residue may be highly deleterious. Mechanical washers (e.g., cage and rack, tunnel, and bottle washers) are recommended for cleaning quantities of caging and movable equipment.

Sanitation of cages and equipment by hand with hot water and detergents or disinfectants can also be effective but requires considerable attention to detail. It is particularly important to ensure that surfaces are rinsed free of residual chemicals and that personnel have appropriate equipment to protect themselves from exposure to hot water or chemical agents used in the process.

Water bottles, sipper tubes, stoppers, feeders, and other small pieces of equipment should be washed with detergents and/or hot water and, where

[2]Rabbits and some rodents, such as guinea pigs and hamsters, produce urine with high concentrations of proteins and minerals. These compounds often adhere to cage surfaces and necessitate treatment with acid solutions before and/or during washing.

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appropriate, chemical agents to destroy microorganisms. Cleaning with ultrasound may be a useful method for small pieces of equipment.

If automated watering systems are used, some mechanism to ensure that microorganisms and debris do not build up in the watering devices is recommended (Meier et al. 2008); the mechanism can be periodic flushing with large volumes of water or appropriate chemical agents followed by a thorough rinsing. Constant recirculation loops that use properly maintained filters, ultraviolet lights, or other devices to disinfect recirculated water are also effective. Attention should be given to the routine sanitation of automatic water delivery valves (i.e., lixits) during primary enclosure cleaning.

Conventional methods of cleaning and disinfection are adequate for most animal care equipment. However, it may be necessary to also sterilize caging and associated equipment to ensure that pathogenic or opportunistic microorganisms are not introduced into specific-pathogen-free or immunocompromised animals, or that experimental biologic hazards are destroyed before cleaning. Sterilizers should be regularly evaluated and monitored to ensure their safety and effectiveness.

For pens or runs, frequent flushing with water and periodic use of detergents or disinfectants are usually appropriate to maintain sufficiently clean surfaces. If animal waste is to be removed by flushing, this will need to be done at least once a day. During flushing, animals should be kept dry. The timing of pen or run cleaning should take into account the normal behavioral and physiologic processes of the animals; for example, the gastrocolic reflex in meal-fed animals results in defecation shortly after food consumption.

*Cleaning and Disinfection of the Macroenvironment* All components of the animal facility, including animal rooms and support spaces (e.g., storage areas, cage-washing facilities, corridors, and procedure rooms) should be regularly cleaned and disinfected as appropriate to the circumstances and at a frequency based on the use of the area and the nature of likely contamination. Vaporized hydrogen peroxide or chlorine dioxide are effective compounds for room decontamination, particularly following completion of studies with highly infectious agents (Krause et al. 2001) or contamination with adventitious microbial agents.

Cleaning implements should be made of materials that resist corrosion and withstand regular sanitation. They should be assigned to specific areas and should not be transported between areas with different risks of contamination without prior disinfection. Worn items should be replaced regularly. The implements should be stored in a neat, organized fashion that facilitates drying and minimizes contamination or harborage of vermin.

*Assessing the Effectiveness of Sanitation* Monitoring of sanitation practices should fit the process and materials being cleaned and may include visual inspection and microbiologic and water temperature monitoring (Compton et al. 2004a,b; Ednie et al. 1998; Parker et al. 2003). The intensity of animal odors, particularly that of ammonia, should not be used as the

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sole means of assessing the effectiveness of the sanitation program. A decision to alter the frequency of cage bedding changes or cage washing should be based on such factors as ammonia concentration, bedding condition, appearance of the cage and animals, and the number and size of animals housed in the cage.

Mechanical washer function should be evaluated regularly and include examination of mechanical components such as spray arms and moving headers as well as spray nozzles to ensure that they are functioning appropriately. If sanitation is temperature dependent, the use of temperature-sensing devices (e.g., thermometers, probes, or temperature-sensitive indicator strips) is recommended to ensure that the equipment being sanitized is exposed to the desired conditions.

Whether the sanitation process is automated or manual, regular evaluation of sanitation effectiveness is recommended. This can be performed by evaluating processed materials by microbiologic culture or the use of organic material detection systems (e.g., adenosine triphosphate [ATP] bioluminescence) and/or by confirming the removal of artificial soil applied to equipment surfaces before washing.

*Waste Disposal* Conventional, biologic, and hazardous waste should be removed and disposed of regularly and safely (Hill 1999). There are several options for effective waste disposal. Contracts with licensed commercial waste disposal firms usually provide some assurance of regulatory compliance and safety. On-site incineration should comply with all federal, state, and local regulations (Nadelkov 1996).

Adequate numbers of properly labeled waste receptacles should be strategically placed throughout the facility. Waste containers should be leakproof and equipped with tight-fitting lids. It is good practice to use disposable liners and to wash containers and implements regularly. There should be a dedicated waste storage area that can be kept free of insects and other vermin. If cold storage is used to hold material before disposal, a properly labeled, dedicated refrigerator, freezer, or cold room should be used that is readily sanitized.

Hazardous wastes must be rendered safe by sterilization, containment, or other appropriate means before their removal from the facility (DHHS 2009 or most recent edition; NRC 1989, 1995b). Radioactive wastes should be kept in properly labeled containers and their disposal closely coordinated with radiation safety specialists in accord with federal and state regulations; the federal government and most states and municipalities have regulations controlling disposal of hazardous wastes. Compliance with regulations concerning hazardous-agent use (see Chapter 2) and disposal is an institutional responsibility.

Infectious animal carcasses can be incinerated on site or collected by a licensed contractor. Use of chemical digesters (alkaline hydrolysis treat-

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ment) may be considered in some situations (Kaye et al. 1998; Murphy et al. 2009). Procedures for on-site packaging, labeling, transportation, and storage of these wastes should be integrated into occupational health and safety policies (Richmond et al. 2003).

Hazardous wastes that are toxic, carcinogenic, flammable, corrosive, reactive, or otherwise unstable should be placed in properly labeled containers and disposed of as recommended by occupational health and safety specialists. In some circumstances, these wastes can be consolidated or blended. Sharps and glass should be disposed of in a manner that will prevent injury to waste handlers.

*Pest Control* Programs designed to prevent, control, or eliminate the presence of or infestation by pests are essential in an animal environment. A regularly scheduled and documented program of control and monitoring should be implemented. The ideal program prevents the entry of vermin and eliminates their harborage in the facility (Anadon et al. 2009; Easterbrook et al. 2008). For animals in outdoor facilities, consideration should be given to eliminating or minimizing the potential risk associated with pests and predators.

Pesticides can induce toxic effects on research animals and interfere with experimental procedures (Gunasekara et al. 2008). They should be used in animal areas only when necessary and investigators whose animals may be exposed to them should be consulted beforehand. Use of pesticides should be recorded and coordinated with the animal care management staff and be in compliance with federal, state, or local regulations. Whenever possible, nontoxic means of pest control, such as insect growth regulators (Donahue et al. 1989; Garg and Donahue 1989; King and Bennett 1989; Verma 2002) and nontoxic substances (e.g., amorphous silica gel), should be used. If traps are used, methods should be humane; traps that catch pests alive require frequent observation and humane euthanasia after capture (Mason and Littin 2003; Meerburg et al. 2008).

*Emergency, Weekend, and Holiday Care* Animals should be cared for by qualified personnel every day, including weekends and holidays, both to safeguard their well-being and to satisfy research requirements. Emergency veterinary care must be available after work hours, on weekends, and on holidays.

In the event of an emergency, institutional security personnel and fire or police officials should be able to reach people responsible for the animals. Notification can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animal facilities or by placing them in the security department or telephone center. Emergency procedures for handling special facilities or operations should be prominently posted and personnel trained in emergency procedures for these areas. A disaster plan

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