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# ❖ BRL BULLETIN ❖

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This edition of the *BRL Bulletin* will discuss the rodent occupancy policy at the University of Illinois at Chicago. As an institution we have adapted our housing practices to more closely align with the space requirements outlined in the 2011 edition of the *Guide for the Care and Use of Laboratory Animals*; thus, a new policy for rodent occupancy was approved by the Animal Care Committee in the summer of 2014. This *Bulletin* is intended as a resource for investigators, providing information on approved housing practices in mice, rats, and guinea pigs, as well as the basis for these policies.

## Introduction

The *Guide for the Care and Use of Laboratory Animals* provides recommendations and requirements for the environment, housing, and management of laboratory animals to ensure animal well-being. According to the *Guide* and UIC policy, social housing is considered the default housing method for mice, rats, and guinea pigs. The *Guide* provides minimum space recommendations for mice, rats, and guinea pigs that are individually and group housed as well as recommendations for breeding females. The goal of the space recommendation is to allow all animals adequate room to express natural postures, make postural adjustments, have access to food, and rest in areas free from urine and feces. These recommendations are the basis for the occupancy guidelines at UIC, with the goal of balancing the benefits of social housing against the detrimental effects of overcrowding.

## Importance of social housing

Mice, rats, and guinea pigs are all social species. This means that they interact highly with members of their own species, and lack of social contact has negative effects on health, welfare, and development. Access to compatible conspecifics allows fulfillment of basic behavioral needs and promotes welfare. Preference testing demonstrates that mice would rather have social contact than enrichment items (Van Loo, 2004). In the laboratory setting male mice form stable dominance hierarchies that are maintained through agonistic behavior of the dominant animal with mice becoming more aggressive as they age (Van Loo, 2001). These hierarchies can be upset by

the introduction of new animals. For this reason, it is beneficial to maintain mice in socially stable groupings. Rodents should only be singly housed if it is scientifically justified in an approved ACC protocol, for veterinary reasons, in the immediate post-operative period, or due to social incompatibility. Non-social housing can lead to altered behavioral and physiologic responses. Singly housed rodents differed significantly from group housed mice in response to tests for antidepressant drug activity (Karolewicz, 2001). In another study individually housed mice showed increased food intake compared to group housed animals leading to increased plasma glucose and triglycerides (Perez, 1997). Further information on social housing and approved management and husbandry exemptions can be found in "UIC Policy: Social Housing of Animals Used in Research, Testing, and Teaching," which is available on the ACC website at <https://research.uic.edu/sites/default/files/0342.pdf>.

## Occupancy for mice

All mice at UIC are maintained in static microisolator caging. The *Guide* lists minimum space requirements based on the weight of the animals with mice less than 25 g requiring 12 in<sup>2</sup> of floor space and mice greater than 25 g requiring 15 in<sup>2</sup> of floor space. At UIC cage occupancy for mice is independent of the weight of the animals in order to facilitate colony management for investigators and husbandry staff, as well as ensure socially stable groupings of mice as they grow. Cages on the West Campus provide 75 in<sup>2</sup> of floor space with a maximum occupancy of 5 weaned non-breeding mice of the same sex. On the East Campus, cages provide 66 in<sup>2</sup> of floor space and the maximum cage occupancy is 4 weaned mice of the same sex. Adult male mice that have not been cohoused since weaning will fight with each other when combined in order to establish a dominance hierarchy. For this reason, mice should be placed into stable groups at weaning. Retired male breeder mice should be singly housed if they must be maintained for further use. Male mice can seriously harm cagemates, causing life-limiting injuries.

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Any cage that contains more than one sex of mice is considered to be a breeding cage. The maximum occupancy for a breeding cage of inbred mice is one adult male, two adult females, and their litters. Inbred strains of mice can be bred in monogamous pairs or in trios of one adult male and two adult females. Configurations exceeding these maximums are at increased risk for overcrowding and aggression. Trio breeding is the most efficient means of colony expansion with the greatest number of pups born in relation to available adult animals and cage space. In addition, this method of breeding more closely approximates the natural behavior of mice. Female mice co-nest in the wild and in the laboratory setting females will preferentially combine litters at a single nesting site and share nursing duties. Some studies have shown that communally reared pups have increased weaning weight and survival (Heiderstadt, 2014). Female mice have a post-partum estrus within 28 hours of giving birth which allows them to become pregnant again immediately when the male remains in the cage. As a result, litters may be born as quickly as 21 days apart. While this is a highly efficient means of producing pups, great care must be taken to wean pups from trio cages in a timely manner. Increasing numbers of pups cause the quality of the cage microenvironment to decline.

Due to the increased potential for overcrowding, outbred stocks of mice may only be bred in pairs. The maximum occupancy for a breeding cage of outbred mice is one adult male, one adult female, and their litter. Outbred mice are much hardier than their inbred counterparts. They have shorter intervals between litters, produce more pups per litter, and have larger, faster growing pups. For this reason, cages with more than one breeding female may have animals that cover more than 50% of the floor space, a violation of the ACC occupancy policy, in the cage long before the pups reach weaning age. Mouse biomass rapidly increases in breeding cages of outbred mice, making the timely weaning of litters of paramount importance. Crosses of mice of mixed backgrounds may also display the reproductive vigor seen in outbred stocks. If cages with breeding trios of mixed background routinely become overcrowded, it is recommended that monogamous breeding be used.

All mouse pups should be weaned between 21 and 28 days of age. By 21 days pups can eat solid food (although some will still nurse at this point) and are able to thermoregulate independently. Weaning at 21 days is appropriate for all outbred mice. Some inbred strains may develop more slowly and benefit from

later weaning. Weaning before 21 days or later than 28 days should only be done in consultation with a BRL veterinarian.

### Occupancy for rats

There are two sizes of static microisolator cages available for rats at the BRL. Standard rat cages provide 132 in<sup>2</sup> of floor space while extra-large cages provide 214 in<sup>2</sup> of floor space. According to the *Guide*, rats less than 400 g require 40 in<sup>2</sup> of floor space, rats of 400-500 g require 60 in<sup>2</sup> of floor space, and rats of greater than 500 g require greater than 70 in<sup>2</sup> of floor space. Occupancy guidelines for rats at UIC meet the space requirements provided in the *Guide*. For non-breeding cages the UIC occupancy guideline allows three rats less than 400 g, two 400-500 g rats, or one rat greater than 500 g to be housed in a standard cage. The extra-large rat cages allow larger rats to be cohoused. Three 400-500 g rats or two rats greater than 500 g can be cohoused. According to the *Guide*, three rats greater than 500 g can be cohoused in an extra-large rat cage, but this leads to decreased environmental quality and more frequent cage changing. As such it may only be done to maintain a previously stable group under the supervision of a BRL veterinarian. To avoid this situation all rats should be pair housed upon arrival at the BRL unless an exemption from social housing has been approved by the ACC.

Maximum occupancy is based on the weight of the heaviest rat in a given cage. For this reason it may be necessary to weigh rats intermittently as they grow, since increasing weight requires increased space. If an investigator notes that an animal has increased in weight to the point that extra space is required, the veterinary staff should be informed to facilitate the appropriate cage change. The veterinary staff may also weigh rats independently to ensure that animals are maintained in correctly sized enclosures. Rats are highly social and should always be cohoused if possible. Increasing cage size is preferential to breaking up socially stable groupings of animals.

For breeding animals a female and her litter may be kept in a standard cage, or a male with a female and her litter may be kept in an extra-large rat cage. If a breeding female is housed in a standard rat cage, the male must be removed before the female gives birth. If a male and female rat are cohoused for breeding for an extended period of time and one of the animals weighs more than 500 g, the pair should be housed in an extra-large cage for the duration of

cohabitation. Rat pups mature earlier than mice, and should be weaned between 19 and 21 days of age. Retired breeding males, or breeding males not being bred to a female, should be individually housed to prevent fighting. However, unlike male mice, unfamiliar non-breeding male rats can be cohoused and derive great benefit from social housing.

### **Occupancy for guinea pigs**

There are two types of cages available for guinea pigs at the BRL. Guinea pigs may be maintained in standard rat cages or extra-large ventilated cages that provide 214 in<sup>2</sup> of floor space. The *Guide* occupancy guidelines for guinea pigs are 60 in<sup>2</sup> of floor space for guinea pigs weighing up to 350 g and 101 in<sup>2</sup> of floor space for animals that weigh more than 350 g. Guinea pigs should only be housed in single-sex configurations to prevent breeding. The maximum occupancy for guinea pig cages is based on the weight of the heaviest animal in the cage.

Standard rat cages can be used to house up to two guinea pigs weighing less than 350 g or a single guinea pig weighing more than 350 g. Extra-large rat cages or ventilated guinea pig cages can be used to house up to three guinea pigs weighing less than 350 g or two guinea pigs weighing more than 350 g. Unless animals will be maintained for a very short period of time, it is recommended that no more than two guinea pigs be cohoused to avoid breaking up stable groups as the animals grow. Guinea pigs should be maintained with compatible conspecifics whenever possible.

### **Impacts of overcrowding**

Appropriate housing density is necessary to maintain both animal welfare and the scientific validity of data gained from animals. While the species discussed above are all social animals that derive significant welfare benefits from social housing, maintaining animals at densities greater than those in UIC's "Rodent Occupancy Policy" can be detrimental to the animals' health and well-being with wide ranging impact on physiologic, morphologic, and behavioral characteristics. It also creates non-experimental variables that can have lifelong impact. Overcrowding significantly alters the microenvironment of the cage. The most direct impact of overcrowding can be observed in air quality. Increased density of animals raises temperature, humidity, carbon dioxide, and ammonia levels. While there are no rodent standards for ammonia exposure, the OSHA standard of 25 ppm for human exposure limit is commonly used. This is an imperfect approximation; the OSHA standard

applies to an eight-hour exposure period, while laboratory rodents are exposed to ammonia concentrations over periods of days to weeks. A seven-day exposure to approximately 10x the OSHA maximum led to marked nasal pathology in mice, and seven days of exposure to levels approaching 25 ppm led to evidence of airway insult in mice housed on some types of bedding (Ferrecchia, 2014). Increased nasal pathology in breeding mice compared to non-breeding counterparts has been observed, suggesting effects due to increased ammonia levels from the presence of pups (DiVincenti, 2012). Changes observed due to increased density are not limited to the respiratory system. Mice in overcrowded conditions have increased adrenal gland weight and fecal cortisol (Nicholson, 2009, Whittaker 2012), which are indicative of stress. Seminal vesical and testicular size is decreased in males housed at higher densities due to androgenic depression (Whittaker, 2012). There are also behavioral changes associated with increasing density. Outbred mice in breeding trios show an increase in the frequency of press postures, where the mouse is in an upright position with the ventral body surface flattened against the cage wall. This behavior is thought to be a means of escaping contact with pups as pups grow larger and as cage floor space decreases (Gaskill, 2015). Some studies have shown that pups exhibit decreased play behavior as cage size decreases (Gaskill, 2015). Mice housed at higher densities also show increased anxiety behavior such as reduced exposure to open space in an open field test and increased startle response (Nicholson, 2009, Whittaker, 2012).

### **Maintenance of occupancy standards**

The ACC considers colony management to be the responsibility of the investigator, which includes maintaining occupancy standards and separating overcrowded cages in a timely manner. All investigators should ensure that the individuals directly responsible for rodent colony management are familiar with UIC's "Rodent Occupancy Policy." This policy is available on the ACC website at <https://research.uic.edu/sites/default/files/0342.pdf>. Non-breeding cages of mice are at low risk for overcrowding. These cages should be monitored at least once per week by investigative staff. However, the dynamic nature of breeding cages necessitates more frequent monitoring. Careful monitoring will allow accurate approximation of birthdates, timely weaning of pups, and evaluation of the success and efficiency of a breeding unit.

A cage is considered overcrowded if it contains more than five adult mice of a single sex; more than one male, two females, and their litters for inbred mice; or more than one male, one female and their litter for outbred mice. Cages are also considered overcrowded if the mass of mice covers more than 50% of the area of the floor of the cage, regardless of the age of the mice, as it impedes the ability of the animals to make normal postural adjustments and negatively impacts the microenvironment of the cage. These cages should be separated either by weaning pups of appropriate age, or separating the mice, taking care to ensure that all pups below weaning age remain with a lactating female. A BRL veterinarian may be consulted if needed.

Any mice older than 28 days of age are treated as adults. Delayed weaning can lead to juvenile females becoming pregnant before they are weaned, and producing litters in cages that already contain more than 3 adult mice. This causes further overcrowding, may disrupt planned breeding schemes, and leads to production of unnecessary animals requiring euthanasia.

Overcrowded cages may be noted by BRL husbandry or veterinary staff and are designated with a yellow card placed behind the cage card. The investigator is notified by the veterinary staff and given a timeline in which to separate the cage. More severely overcrowded cages may have an abbreviated time frame for separation out of regard for the animals' welfare. Investigators should ensure that the BRL veterinary staff has appropriate contact information for the individuals responsible for colony management to facilitate dealing with these issues promptly. Should an investigator fail to separate overcrowded cages in a timely manner, the BRL staff will separate the cage and the investigator will be charged for the service. The charge for separation of overcrowded cage is \$20 for each new cage created. If there are questions regarding the separation of overcrowded cages, investigators should contact the BRL veterinary staff. The BRL is not responsible for any issues that might arise during the cage separation process when cages are separated by BRL staff.

For further guidance on rodent occupancy, colony maintenance, or husbandry practices, please contact a member of the BRL veterinary staff.

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## ANNOUNCEMENTS

*Welcome Aboard*—The staff of the BRL would like to welcome Dr. Michael Eichner, a Florida native and a University of Florida graduate, who started our postdoctoral training program in laboratory animal medicine in July. Even after a visit to UIC in February, Dr. Eichner decided to join our team at UIC. Make sure you get a warm coat for the upcoming winter – you will need it!