Semester - III

LABORATORY ANIMAL CARE

18MBP305B **4H –4C**

Instruction Hours / Week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 3 Hours

COURSE OBJECTIVES

Aimed to provide training on various methods of handling, concerning the care and use of laboratory animals.

COURSE OUTCOME (CO'S)

Laboratory animal care provides the proper handling and care for various species of animals used in research, testing, and in education. It extensively deals with the amended act on the Animal Welfare and the concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress.

UNIT – I

General introduction - responsibilities of institution and chief investigators, Aspects of rabbit behavior relevant to housing, Rabbit Group housing in pens, advantages and disadvantages, Pens, design of pens environment, Rabbit care management – Regrouping, catching and identification in pens and cages, Rabbit care management – food, water, health and breeding in pens and cages, Cage design and environment, Environment enrichment for rabbits in pens and cages, Ethical guidelines for use of animals in research.

UNIT – II

Introduction-behavior, anatomic and physiological features of mice in lab, Husbandry-Housing, nutrition and breeding requirements and management of lab mice, occupational health and zoonotic diseases, treatment of disease in mice, regulatory agencies and complaines associates with management of lab mice, Restraining and sample collection methods from lab mice, Physical, examination of mice for disease conditions, anesthesia and analgesia -mice, Euthanasia in veterinary care.

UNIT – III

Introduction to anatomical and physiological features of laboratory rat, major color groups and varieties of rats, regulatory management housing of laboratory rats-equipment, feed formulation, ailments & disease management of laboratory rats, disease management and ailments of laboratory rats , restraining and sample collection in lab rats, anesthesia and analgesia of lab rats, breeding of laboratory rats.

UNIT – IV

Introduction – history and classification of guinea pigs, varieties and characteristics of guinea pigs used in labs, characteristics and behaviors of the guinea pig used in labs, housing, nutrition and feeding of guinea pigs, care and handling of guinea pigs in lab, zoonoses of guinea pigs, reproduction and breeding managements in guinea pigs –gnotobiotic animals.

UNIT – V

Various routes of inoculation in mice & rats, various routes of inoculation in mice & rats,

handling and routes of inoculation in rabbits, guinea pigs, laboratory use of animals –role in microbiology, antibody production in animals, disposal of animal house wastes, safety measures in animal house.

SUGGESTED READINGS

TEXT BOOKS

1. *The IACUC Handbook*, 2nd ed., eds. Silverman, Murthy, Suckow. CRC Press, (2006).
2. *Anesthesia and Analgesia in Laboratory Animals*. American College of Laboratory Animal Medicine, second ed.)., eds. Richard Fish, Peggy Danneman, Marilyn Brown, and Alicia Karas. Academic Press, (2008.
3. *The Mouse in Biomedical Research*, second ed.)., eds. James G. Fox, Muriel T. Davisson, Fred W. Quimby, Stephen W. Barthold, Christian E. Newcomer and Abigail L. Smith. Elsevier, (2007.
4. *The Laboratory Rat*, (2^{nd} ed.). American College of Laboratory Animal Medicine. eds. Suckow, weisbroth and Franklin. Elsevier, (2006).
5. *Handbook on Genetically Standardized Mice.* (6^{th} ed.). Ed. Joanne Currer, The Jackson Laboratory, Bar Harbor, Maine, (2009).
6. *Laboratory Animal Medicine*, (2^{nd} ed.). American College of Laboratory Animal Medicine, eds. Fox, Anderson, Lowe, Quimby. Academic Press, (2002).
7. Percy, D.H., and Barthold, S.W., (2007). *Pathology of Laboratory* Rodents and Rabbits, (3^{rd} ed.). Blackwell Publishing Company.

REFERENCES

1. Nalinasundari, M.S., and Santhi, R., (2006). *Entomology*. MJP Publishers, Chennai.
2. Pelczar, Jr. M.J., Chan, E.C.S., and Kreig, N.R., (1993). *Microbiology* McGraw-Hill Inc. New York.
3. Prescott, M., Harley, J.P., and Klein, D.A., (1993). *Microbiology*, (2^{nd} ed.). McGraw-Hill Inc, NY.
4. Roy, D.N., and Brown, A.W.A., (2003). *Entomology – Medical and Veterinary*. (1^{st} ed.). Part – I, Biotech Books, New Delhi.
5. Warren, D. M. (2002). *Small Animal Care and Management*. (2^{nd} ed.). Delmar – Thomson Learning, Columbia, NY.
6. Yadav, M. (2004). *Applied Entomology*. (1^{st} ed.). Discovery Publishing House, New Delhi.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University)
(Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.
(For the candidates admitted from 2017 onwards)

DEPARTMENT OF MICROBIOLOGY

SUBJECT NAME: LABORATORY ANIMAL CARE **SUB.CODE:18MBP305B**
SEMESTER: III **CLASS: II M.Sc (MB)**

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	General introduction - responsibilities of institution and chief investigators.	R1:3 to 6 R2:10 to 39
2	1	Aspects of rabbit behaviour relevant to housing	R1: 6 to 7
3	1	Rabbit Group housing in pens, advantages and disadvantages	R1: 7 to 9 R2: 15 to 17
4	1	Pens, design of pens environment	R1: 10 to 12
5	1	Rabbit care management – Regrouping, catching and identification in pens and cages	R1: 20 to 23
6	1	Rabbit care management – food, water, health and breeding in pens and cages	R1: 20 to 23
7	1	Cage design and environment	R1:24 to 26
8	1	Environment enrichment for rabbits in pens and cages	R1: 26 to 30
9	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit 1=09		
		UNIT-II	
1	1	Introduction-Behaviour, anatomic and physiological features of mice in lab.	T1: 10 to 20
2	1	Husbandry-Housing, nutrition and breeding requirements and management of lab mice.	T1: 44 to 50
3	1	Occupational health and zoonotic diseases, treatment of disease in mice	T1: 41 to 49 T1: 97 to 101
4	1	Regulatory agencies and complaines associates with management of lab mice	T1: 44 to 50
5	1	Restraining and sample collection methods from lab mice	T1: 120 to 134 W1,J1

6	1	Physical examination of mice for disease conditions.	R2: 40 to 44
7	1	Anaesthesia and analgesia -mice	T1: 102 to 119
8	1	Euthanasia in veterinary care	R2: 50-51
9	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit II=09		
		UNIT-III	
1	1	Introduction to anatomical and physiological features of laboratory rat	R2: 10 to 25
2	1	Major colour groups and varieties of rats.	R2: 44 to 55
3	1	Regulatory management Housing of laboratory rats-equipment	R2: 128 to 138 T1: 200 to 201
4	1	Feed formulation, ailments & disease management of laboratory rats	R2:122 to 123; R2:165 to 174
5	1	Disease management and ailments of laboratory rats	R2: 47 to 51 T1: 102 to 103
6	1	Restraining and sample collection in lab rats	T1: 102 to 134
7	1	Anaesthesia and Analgesia of lab rats	R2: 102 to 119
8	1	Breeding of laboratory rats	T1: 202 to 203
9	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Uni III=09		
		UNIT-IV	
1	1	Introduction – history and classification of guinea pigs	R2: 45 to 48
2	1	Varieties and characteristics of guinea pigs used in labs	R2: 102 to 113 T1: 216 to 218
3	1	Characteristics and behaviours of the guinea pig used in labs	R2: 102 to 113 T1: 216 to 216
4	1	Characteristics and behaviours of the guinea pig used in labs	R2: 102 to 113 T1: 216 to 216
5	1	Housing, nutrition and feeding of guinea pigs	T1: 218 to 222
6	1	Care and handling of guinea pigs in lab	T1: 217 to 218
7	1	Zoonoses of guinea pigs	T1: 220 to 221
8	1	Reproduction and breeding managements in guinea pigs –gnotobiotic animals	T1: 220 to 222

9	1	Recapitulation and discussion of question	
	Total No of Hours Planned For Unit IV=09		
		UNIT-V	
1	1	Various routes of inoculation in mice & rats	T1: 9 to 23
2	1	Various routes of inoculation in mice & rats	T1: 9 to 23
3	1	Handling and routes of inoculation in rabbits, guinea pigs	T1: 2 to 8
4	1	Laboratory use of animals –role in Microbiology	T1: 23 to 32
5	1	Antibody production in animals	R2: 120 to 135
6	1	Disposal of animal house wastes	R2: 393 to 396
7	1	Disposal of animal house wastes	R2: 50 to 56
8	1	Safety measures in animal house	R2: 31 to 35
9	1	Recapitulation and discussion of question	
10	1	Old question paper discussion (Last Five years)	
11	1	Old question paper discussion (Last Five years)	
12	1	Old question paper discussion (Last Five years)	
	Total No of Hours Planned for unit V=12		

SUGGESTED READINGS:

TEXT BOOK

1. Dean M. Warrens, Small animal care and management, 2nd edition, 2002. Thomas learning, Columbia, USA. Fritz, H.Kayser, Kurl-A Bienz, Johaanereckert,

REFERENCE

1. Lynette Chaveet al.,(2003). Animal research review panel (ARRP) guidelines -18: Guidelines for the housing of rabbits in scientific institutions.
2. Code of practice for the housing and care of lab mice, rat, guinea pigs, and rabbits. Issued by the ministry of agriculture, the state of Victoria,(2004),Australia.

WEBSITES

1. The Jackson laboratory, 600 Main street, Barharbour, ME.www.jax.org.:

JOURNALS

1. Baker,D.G., Natural pathogens of laboratory mice, rat and rabbit and their effects on research.Clin.Microbiol.,Rev,11,231,1988.

UNIT-I
SYLLABUS

Modern methods of care, management breeding and maintenance of laboratory animal - Rabbit

Introduction

These guidelines are intended for use by people involved in the housing and care of rabbits in scientific institutions. The guidelines are not intended to be a complete manual on rabbit care and management but rather to provide some key guiding principles on best practice standards in rabbit housing. The guidelines will be revised from time to time to take account of advances in the understanding of rabbit physiology and behavior, technological advances, and changes in community attitudes and expectations about the welfare of animals. The guidelines are based on principles regarding the care and management of rabbits taken from scientific literature. These principles are detailed throughout the document, as are recommendations for the care and management of rabbits which are derived from these principles. In some areas, conclusions to be drawn from the available literature are not entirely clear, and in such areas recommendations are extrapolated from information available and practices in rabbit care and management current at the time of writing.

The principles outlined in the document address requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (as outlined below in Section1.5). The requirements of the Code of Practice include that animals held for scientific purposes should have their species-specific behavioral and physical needs met, whilst at the same time ensuring that the animals can adequately be monitored and are protected from disease and taking into account the requirements of the research for which the animals are being used.

Responsibilities of Institutions

Institutions using rabbits for scientific purposes are responsible for responding effectively to recommendations of the institution's Animal Ethics Committee to ensure that facilities for the housing and care of rabbits are appropriate to the maintenance of well-being and health of the rabbits.

Responsibilities of Chief Investigators

The chief investigator/teacher (person in charge of a research/teaching project) has direct and ultimate responsibility for all matters related to the welfare of rabbits under his or her control, which includes their housing and care. (As per the principle contained in Clause of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes). The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to rabbit care and management. (As per the principle contained in Clause of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes)

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

Principles

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states: Species-specific behavioral requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy and contact with others of the same species; provision of single housing for animals where it is appropriate for the species and if necessary for the purpose of the study, eg., during recovery from surgery or collection of samples; species-specific environmental requirements such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations; the need to provide ready access to food and water; the need to clean the pen, cage or container; protection from spread of pests and disease; requirements of the study; and the need to observe animals readily.

Pens, cages and containers must: be constructed of durable, impervious materials; be kept clean; be maintained in good repair; be escape-proof; protect the animals from climatic extremes; not cause injury to animals; be large enough to ensure the animal's well-being; and be compatible with the behavioral needs of the species. The population density of animals within cages, pens or containers and the placement of these in rooms must be such that acceptable social and environmental conditions for the species can be maintained. Where it is necessary to individually house animals of a species that is normally kept in a social group, the conditions should be managed so as to minimize the impact of social isolation. Animals should be housed in these circumstances for the minimum time necessary.

Bedding and litter must be provided if appropriate for the species, and should be comfortable, absorbent, safe, non-toxic, able to be sterilized if needed, and suitable for the particular scientific or educational aims. Pregnant animals must be provided with nesting materials where appropriate. The Animal Ethics Committee and relevant investigators or teachers should be informed in advance of planned changes to these conditions, since these may affect the welfare of animals and the results of the scientific and teaching activities.

Aspects of Rabbit Behavior Relevant to Housing, Principles

All laboratory rabbits derive from the European rabbit (Oryctolagus cuniculus). The behavior of laboratory rabbits is very similar to that of wild rabbits. Wild rabbits are naturally gregarious and live in breeding groups. Within each group there is a linear dominance hierarchy for each sex. Social rank within each group is established by physical fighting or confrontations (eg chasing) between individuals. Studies have shown that, given the opportunity, laboratory rabbits prefer to be together, spending about 79% of their time in each other's company. Amicable behaviors when together include lying near each other, grooming each other and nuzzling. Social interaction is therefore important for rabbits. The natural active behaviors of rabbits include foraging, hopping, running, chasing, playing, grooming, sitting up with ears erect, rearing, leaping and digging. Periods of rest lasting several hours alternate with periods of activity.

Recommendations

To meet the requirements of the Code of Practice (ie., to provide accommodation that meets the species-specific needs of rabbits), housing should be provided which allows rabbits the opportunity for social interaction, the opportunity to carry out normal behaviors such as hopping and rearing upwards (freedom of movement) and the opportunity to rest and withdraw from each other.

The Code of Practice recognizes that there may be circumstances where the requirements of experimental procedures will preclude meeting some species-specific needs (Clause 4.4.22 (ii)). Housing in these situations should still meet the physiological and psychological needs of rabbits as closely as possible.

Definitions

Pen Enclosure for housing rabbits that allows for freedom of movement by rabbits and allows for the provision of a variety of environmental enrichment strategies. Cage Fully enclosed container for housing rabbits which, because of its size, restricts freedom of movement by rabbits and limits the provision of environmental enrichment strategies. Cages are usually constructed from metal or plastic, with solid or mesh sides.

Group Housing in Pens: Advantages of Group Housing in Pens Principles

The advantages of housing rabbits in groups in pens include: Housing in pens provides increased space which allows rabbits freedom of movement to carry out normal activities such as hopping, stretching out, sitting up with ears erect, rearing, and leaping. Physical and psychological well-being is assisted by the opportunity to exercise and explore a complex environment. Rabbits are social animals which benefit from the company of others. Housing rabbits in groups in pens allows for social interaction

and behaviors such as grooming pen mates, lying together and playing. The behavioral repertoire of group housed rabbits is more varied compared to that of singly housed rabbits. Pens provide a greater opportunity than cages for the environment to be enriched and made more behaviorally stimulating (for example by the addition of ledges for climbing on and areas for retreating into).

It is reported that the costs of setting up pens and maintaining rabbits in pens is less than for buying cages and maintaining rabbits in cages. Cost savings may be made in the areas of bedding, cleaning agents, maintenance, energy and labor. Health advantages include that sore hocks (pododermatitis) and gastrointestinal hair balls (trichobezoars) are rare or non-existent in penned animals. The incidence of "snuffles" may also be reduced, possibly because of better ventilation than solid walled cages, although an increased incidence of sneezing has been observed. Thinning of the bones of the femur and spine because of inactivity may be seen in caged rabbits. This can result in a loss of ability to move normally, fractures, spinal distortions and discomfort from trapping nerves in the spine. There is no evidence of such thinning of the bones, with its attendant complications, occurring in rabbits in pens.

Disadvantages of Group Housing in Pens, Principles

The disadvantages of housing rabbits in groups in pens include: A high level of stock personship in caring for and monitoring rabbits is required. Thus animal carers need to be proficient in recognising all the different aspects of rabbit behavior. This is so that both health and social interaction can effectively be monitored. Fighting and bullying may occur, especially in mature males. The grouping of rabbits may become unstable – there may be difficulties in reintroducing a rabbit that has been removed for even a short time. In addition, stable groups may become unstable, resulting in aggressive behavior, for no obvious reason. Fighting and aggressive behaviors may result in severe injuries and stress responses in subordinate animals.

Rabbits may be difficult to catch in pens. Identification of individual rabbits may be more difficult. Bedding disposal may cause a problem because of its volume.Space may be wasted, especially vertical height.There is the potential for disease spread. However this has been reported not to be a problem in practice.It has been argued that rabbits in pens may be subject to more variables which may affect the interpretation of experimental results. This, however, needs to be weighed against the effects of obtaining data from rabbits, singly housed in cages, which may be physiologically and psychologically abnormal.

Recommendations

Rabbits should be housed in groups in pens. Rabbits that cannot be housed in groups (eg intact males or for experimental reasons) should be housed in pens with olfactory, visual and, if possible, physical contact with adjacent rabbits. Rabbits should only be housed in cages with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such housing. In such cases, cages should be enriched by methods as described in this document (such as pair housing in double cages). Lack of space or facilities for pens should not be considered sufficient justification for the use of such cages.

Rabbits should not be housed singly in conventional (unenriched) cages except in exceptional circumstances and with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such housing. Such circumstances may, for example, relate to research into the effects of such caging on rabbits. Lack of space or facilities for pens should not be considered sufficient justification for the use of conventional cages.

Pen Design and Environment, Pen Construction, Costs of Pens

Principles

The cost of group housing in floor pens is likely to be considerably less than single housing in cages. Savings may be made in areas including cage washers (running, maintenance and servicing),

chemicals (descalers and disinfectants) and staff time for cleaning (although some of the staff time saved will need to be spent monitoring and handling rabbits in pens).

Materials for Pens, Principles

A variety of materials may be used, depending on the design of the pen. For example, the pen may occupy a whole room or be adapted from plastic bins made for other purposes or from pens for larger animals. Materials for pen walls may be solid (for example plastic) or be open weave (for example wire mesh) and opaque or transparent / translucent. Opaque solid walls between pens have the advantage of providing additional areas for shelter/privacy, but the disadvantages, as with all solid walls, of restricting air flow and of restricting vision of surroundings (including of rabbits in adjacent pens and people approaching).

Recommendations

Materials used for pen construction should be safe and non-toxic for rabbits. Materials used for pen construction should be easy to clean, water resistant and strong. For the front of the pen, material that allows rabbits to see out (for example wire mesh) is preferable to opaque material, as this allows rabbits to see who is approaching, which helps to eliminate fear responses.

Floor Area of Pens, Principles

The age and activity level of rabbits is more important in determining space requirements than the weight or size of rabbits. For example, young rabbits need more space than adults for play behavior. The minimum space provided should allow each rabbit to carry out its normal behavior, including a wide range of locomotory behaviors, such as hopping, leaping, playing, exploring and stretching out. It has been suggested that the minimum space provided should allow rabbits to complete 3 hops in one direction. Adult New Zealand White rabbits have been measured travelling 1.5 – 2.0 metres in three "normal" hops.

In addition to meeting minimum space requirements for movement, space should be provided to allow the provision of structural complexity and environmental enrichment in pens. For example, additional space should be provided to accommodate objects such as boxes and pipes, which provide rabbits with retreat / hiding areas.

Recommendations

A minimum **CLEAR** area of 2.0 square metres should be provided. Table 1 .A minimum length in one direction of 2.0 metres should be provided. Table 1. An overall minimum floor area of 0.75 – 0.8 square metres per rabbit should be provided for groups of up to 6 rabbits (with an absolute minimum floor area to be provided as in 3.1.3.1 and 3.1.3.2 above). For groups of more than 6 rabbits, space should be allocated at approximately 0.25 square metres per additional rabbit. For a group of 6 – 8 rabbits a minimum floor area of 4.5 – 5.0 square metres should be provided.

Table 1: Summary of space requirements

No. of rabbits	Minimum space (m^2) (*ensuring a CLEAR area of $2m^2$ and a minimum length in one direction of 2m)
1	2.00
2	2.00
3	2.25 - 2.40
4	3.00 - 3.20
5	3.75 - 4.00
6	4.50 - 4.80
7	4.75 - 5.05

8	5.00 - 5.30

Height of Pens, Principles

Normal behavior of rabbits includes rearing up on their hind legs and sitting up erect with their ears pricked. Such erect stances allow rabbits to investigate sights and sounds. The minimum height provided should allow rabbits to carry out these behaviors. Rabbits commonly sit on top of objects (for example ledges and nest boxes), viewing the environment from a raised area, and the minimum height provided should allow for this behavior. Rabbits are capable of jumping very high, and of using objects within a pen as "launching pads" to jump out of pens.

Recommendations

If the pen is covered, a minimum height of 100cm should be provided. The minimum height should enable rabbits to sit on top of raised objects. If the pen is uncovered, a minimum height of 1.25 metres should be provided. The proximity and height of objects near the edges of pens should be taken into account in making a pen secure from escape.

Bedding in Pens, Principles

Bedding ensures a clean, dry, comfortable lying area. Ideally bedding should be dust free, free of microbial or parasitic contamination, non-toxic, ammonia binding, non-traumatic and moisture absorbent. In addition it is desirable for it to be cheap, readily available and easy to use and dispose of. The smell of bedding is important – some woods have resinous aromatic oils which affect rabbits' behavior patterns by altering the olfactory field. There is evidence that rabbits avoid sawdust and wood shavings.

Straw has the advantage of doubling as a means of environmental enrichment as rabbits play with, nibble, manipulate and burrow in straw. It has the theoretical disadvantage of being a potential source of microbial disease due to contamination. To overcome this, straw can be autoclaved, however, this is expensive and creates practical difficulties. It is reported that, in practice, the introduction of disease has not been a problem. A drawback of straw it that it may not be as moisture absorbent as other bedding materials such as wood shavings. Shredded paper has the advantages that it is clean, dust free and odourless. However, it has poor absorbency qualities. Rabbits have been shown to choose shredded computer paper over bare concrete floors, sawdust or wood shavings.

Recommendations

Bedding should be provided. Straw is the bedding of choice and should be supplied to a depth of at least 5cm. Shredded paper is the next bedding of choice. It should be supplied to a depth of at least 2cm. Because of its poor absorbency qualities, it is best to put shredded paper on top of multiple layers of newspaper. Sawdust and wood shavings may be used. They should be supplied to a depth of at least 2cm. If these materials are used they should not smell strongly and should be made from non-resinated (softwood) timber. Autoclaving may remove odours that offend rabbits. To increase the moisture absorbent properties of bedding, while supplying material that can be manipulated and played with, a layer of wood shavings or sawdust may be used under straw.

Cleaning of Pens, Principles

In pens stake out a toilet area - they urinate and defaecate in one corner, although faecal pellets may be scattered with the movement of rabbits in the pen. A balance needs to be struck between the human perception of the need for cleanliness and the level of disturbance to rabbits. A sense of smell appears to be very important to rabbits – social interactions related to sex, age, reproductive status, individual identity, hierarchy and mother/young relationships may be communicated via the olfactory environment. Scent signals arise from urine and secretions from specialised scent glands (chin, inguinal, anal and Harderian glands). It has been suggested that urination and scent marking carried out by rabbits in clean cages are

attempts to restore an "optimum odour field".

Recommendations

Frequency of cleaning will be influenced by factors including the type and depth of bedding, stocking rates and the efficiency of air exchange in decreasing levels of noxious gases. Cleaning the toilet area (for example 3 or 4 times a week) is usually required in between completely changing the pen bedding. Two weeks is the longest recommended interval between complete changes of bedding. If a system of shredded paper over layers of newspaper is used, the newspaper needs to be changed at least twice weekly. A pen should ideally be washed and disinfected (with an odourless disinfectant) at least every month. Pens and rooms should be completely cleaned every time a room is cleared and new stock brought in. The floor should be washed and an odourless disinfectant used.

Rabbit Care and Management in Pens and Cages - Management of Rabbits in Groups, Principles

The success of group housing rests largely with the skill and enthusiasm of the animal carers. Carers must be able to monitor and assess rabbit behavior and implement management strategies accordingly. Some breeds are more aggressive than others. Dutch rabbits are usually more aggressive than New Zealand White rabbits, and are generally unsuitable for group housing. Lop rabbits are usually more docile than New Zealand Whites. However, a stable group of rabbits cannot be guaranteed by selecting less aggressive breeds. The occurrence of aggression, which rabbits will be involved in aggressive encounters, and the severity and outcome of such encounters, can be difficult to predict.It is best to establish groups of rabbits when they are young (around the time of weaning), and at least before they reach puberty (which may begin as early as 2.5 months of age).Ideally littermates should be used, although separate litters of similar age can be grouped. Large weight or age differences can result in aggression. All-female groups are more likely to be stable. Sexes should not be mixed (apart from breeding groups).

Males can be housed in groups until they reach 3 – 4 months of age.However, mature male rabbits fight and this fighting can result in severe injuries. The lack of space in pens for rabbits to escape aggressive encounters makes it impossible to keep mature male rabbits in groups in pens. After about 3 – 4 months of age, housing intact males together in the expectation that they will be separated before fighting starts is unsafe for the rabbits, as the outbreak of severe aggressive encounters is difficult to predict and may occur without warning. Castration has been shown to be a viable alternative for housing males together. Castration should be carried out at about 10 weeks of age – i.e., before aggressive behavior is shown. Some individuals may be highly aggressive. In such cases it may be necessary to remove the dominant or subordinate rabbit temporarily or permanently. It is important that rabbits are provided with sufficient space and objects to assist them to escape and hide from their aggressors. Objects such as boxes, pipes, ledges and vertical barriers provide means for hiding and escape. Sufficient objects should be provided to eliminate competition for such items. Housing more than 6 – 8 rabbits together creates difficulties in developing and maintaining a stable hierarchical system.

Limiting groups to this number also assists in monitoring animals for signs of bullying and ill health. It is very important to keep the composition of a group stable. Where individual rabbits need to temporarily be separated from a group, they should be housed so that visual contact the individual and the group can be maintained. This helps to ensure that they will be readily recognised and accepted as familiar members of the group when returned. In a group where a rabbit has been removed (for example for a scientific procedure) and then returned, rabbits should be closely monitored for signs of aggression (for example, chasing, fighting, fight wounds).

Recommendations

For monitoring the behavior of rabbits in pens and implementing rabbit management strategies, animal carers should have suitable qualifications and experience. Their skill and enthusiasm are important

factors in the successful housing of rabbits in groups in pens. Wherever possible, rabbits in groups in pens should be of similar age and weight, be the same sex (preferably female), and be litter mates or mixed together at an early age (around weaning). Mature (uncastrated) male rabbits should not be housed together. Sufficient space and objects (such as boxes, pipes, ledges and vertical barriers) should be provided to assist rabbits to escape and hide from aggressors. Sufficient objects should also be provided to eliminate competition for these items. The number of rabbits in a group should be limited to 6-8 (with the exception of breeding groups) where individual rabbits need temporarily to be separated from a group, they should be housed so that visual contact between the individual and the group can be maintained, to assist with reintroduction into the group.

Regrouping / Establishing Groups from Caged Rabbits, Principles

It is possible to regroup rabbits, but this requires particular care and intensive monitoring. It is important that rabbits are placed in a fresh neutral area to avoid home territory for any animals. Additional measures include:

- Providing hiding places and breaking up clear areas so that rabbits can escape from each other;
- Placing wire partitions between pens to allow familiarisation between rabbits before they are mixed;
- Scattering faecal pellets and urine soaked litter from each rabbit in the new pen;
- Scattering food in the new pen to encourage foraging;
- Having the usual carer handle rabbits together in small groups before mixing.

A method for regrouping rabbits using sedation has been described by Love 91. In this method rabbits are sedated (fentanyl and droperidol) and placed in a small holding cage, making sure pen mates are not next to each other. The important part of this process is that rabbits chin and rub against each other as they are recovering from sedation, thus spreading recognisable smells. Rabbits are then placed in a pen not previously used by any of the rabbits. Rabbits may display behaviors such as chasing but usually settle within 24 hours. Moving rabbits from cage housing to pens also presents particular problems. Rabbits that have been housed in cages for 6 months or more are prone to fighting (due to lack of social experience) and bone fractures due to osteoporosis. Methods to assist in group housing such animals in pens include providing increasing daily periods of exercise individually and then in small groups, then housing in the group pen during the day and individually in cages at night and finally housing in the group pen. Even with such methods, signs of aggression need to be looked for. Groups may become unstable and may require the removal of individuals.

Recommendations

Particular care should be exercised in regrouping rabbits or in moving rabbits from cage housing to group housing in pens.

Catching / Handling of Rabbits in Pens and Cages, Principles

Because pens provide room to escape, catching rabbits in pens may be difficult. Anticipation of what is to happen once caught plays a major role in a rabbit's behavior. Therefore steps should be taken to reduce the stress of procedures and to catch and handle rabbits without conducting other procedures. Animal carers should also spend time with the rabbits, which will facilitate catching. Rabbits show a reduction in fearfulness to handlers after repeated approach and handling. The use of handling and approach programmes may help reduce general emotional reactivity (not just fear of humans) and strengthen the human-animal bond. A quiet approach should be taken. Rabbits will usually retreat to a darkened hiding place from where they may be picked up. In one study, rabbits were trained to accept a procedure (oral administration of an antibiotic solution) without restraint when given positive reinforcement (drug delivered in a sugar coated syringe after a period of training by administering a sugar solution).

Recommendations

To assist in catching rabbits, a quiet approach should be taken. To assist in catching rabbits, a darkened retreat area from which rabbits may be picked up should be provided. For example, provide plastic pipes of about 20 – 25cm diameter, which can be upended to catch rabbits when they enter them. It is important that rabbits are caught and handled on a regular basis (ideally daily and at least weekly) to facilitate ease of catching and to reduce fearfulness. Such catching and handling should be carried out in addition to catching and handling rabbits in order to conduct procedures. The training and rewarding of rabbits using positive reinforcement or "treats" should be considered when performing procedures on rabbits. This is likely to reduce the stress on rabbits and increase their co-operation.

Identification of Rabbits in Pens and Cages, Principles

Clause 5.6.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states: The method of identification of individual animals must be that which causes the least distress within the context of the research proposal and the least interference with the normal functioning of the animal. Ideally methods of identification should not be painful, not cause adverse reactions, not be uncomfortable and not likely to catch or tear out.

Recommendations

The least invasive method of identification should be used that is compatible with the use of rabbits in the institution. Coat colour and pattern is useful for the identification of individuals in some breeds of rabbits. Dyes including permanent wool dyes, agricultural sheep sprays, fuchsin, acriflavine or gentian violet may be used. These need to be reapplied at intervals, as the dye fades or the rabbit moults. Xylene free permanent markers may be used on ears and fur. Generally these need to be refreshed every 3 weeks. The use of these markers on the inside of the ears is effective and different colours can be used for coding. Fur clipping may be used but needs to be carried out frequently. Microchips and ear tattoos may be used for permanent identification. Note there is some transitory pain associated with applying these forms of identification.

Microchips can be inserted at 6 weeks of age. Anaesthesia or sedation and analgesia should be used in applying tattoos. Leg rings, fitted over the hock at about 6 weeks of age may be used. However, they need to be checked frequently while rabbits are growing (at least weekly) to ensure they do not become too tight. Studs or ear tags should not be used as they may tear out, they may cause infections leading to the growth of fibrous tissue as a chronic inflammatory response, and they are painful to insert. Collars should not be used as they may be chewed off by other rabbits, they may drop off and rabbits may catch their legs in them. Ear notching must not be used.

Food and Water for Rabbits in Pens and Cages-Principles

Dried pelleted diets have the advantages of being standardised with little variation. However, these diets are monotonous and their repetitive presentation without supplements (such as hay and green feed) may compromise the well-being of rabbits. Scattering food reduces boredom by encouraging rabbits to forage. This reduces the amount of time available for fighting and helps to prevent obesity. High fibre feed such as hay helps prevent diarrhoea and trichobezoars (hairballs). A diet with around 18 – 25% crude fibre is suggested. Hay also provides something to manipulate and play with.

Recommendations

A nutritionally adequate diet should be provided for rabbits. It is recommended that more than one source of food and water be provided to reduce the possibility of aggressive competition. Dried pelleted diets should be fed at around the amount of 60 – 80 g/kg/day depending on factors such as the age of rabbits and their opportunities for exercise. To relieve boredom, more than one formulation may be fed. Hay should be fed on a daily basis. For variety, pelleted food should be supplemented with items such as

fruit and vegetables, corn, barley, oats and soya beans. Fruit and vegetables should be washed to reduce the risk of introduction of disease. Food may be spread out to encourage rabbits to forage. Food may be scattered or placed in small piles around the pen. Fouled or uneaten food should be removed daily. If a restricted diet is required to be fed, it should be provided in the late afternoon (which has been shown to reduce the frequency of abnormal behaviors in caged rabbits). A plentiful supply of clean water should always be available. Various systems to supply water may be used. If automatic watering systems or water bottles are used, care should be taken to avoid leakage and overflows. Open water systems (such as chicken water hoppers) should be raised up to prevent water being contaminated with bedding and faeces.

Monitoring of Rabbits in Pens and Cages- Principles

A high standard of animal care is crucial for the success of housing rabbits in groups in pens. Rabbits must be monitored both for health and for social interactions within the group. In cages normal behaviors are difficult to assess (because it is difficult or impossible for rabbits to carry out these behaviors) and changes in food and water intake are often the only early indicator of illness.

Recommendations

Rabbits should be monitored by observation at least daily. Weekly health checks of all rabbits should be carried out by animal carers (in addition to daily observation. Animal carers should be aware of the normal behavior of rabbits and of the individuals within a group and observe for deviations from normal. In particular, subordinate rabbits should be monitored for signs of bullying (which may, for example, result in fight wounds or denial of access to food or water).Rabbits that give cause for concern (either excessively aggressive or timid) may need to be removed from a group. To ensure that individuals can be adequately monitored, group sizes should not exceed 6 - 8 rabbits.

Health of Rabbits in Pens and Cages- Principles

Evidence indicates that infectious disease spread in rabbits housed in groups in pens is no more of a problem than for animals housed singly in cages, providing high standards of care and monitoring are maintained. Possible routes for disease spread include direct contact and shared food and water containers. Unless people exercise extreme care going between singly caged rabbits and unless the ventilation is carefully controlled and directed, the spread of infection could occur equally under single housed or group penned systems.

Coccidiosis

Outbreaks of primary coccidiosis are not commonly observed and many grouped rabbits are not fed coccidiostat supplements because of their potential effects on experimental procedures. It is thought that the combination of high standards of care and low stress enables an immunity to develop.

Trichobezoars (hairballs)

Hairballs and perforated gastrointestinal tract ulcers are rare in penned rabbits. The increased incidence in caged rabbits is thought to be due to factors including lack of exercise, lack of roughage and pathological grooming due to isolation and boredom.

Pododermatitis (sore hocks)

Sore hocks are rare or non-existent in penned rabbits. Certain types of cage flooring (for example grids) are linked with the occurrence of sore hocks.

Pasteurellamultocida (snuffles)

The incidence of snuffles may be reduced in penned rabbits, possibly because of better ventilation in pens compared to solid walled cages. (In one study an increased incidence of sneezing was observed in rabbits in pens, but the cause of this was not confirmed).

Fighting injuries

Fighting injuries may occur in group housed rabbits. In stable groups these are mostly confined to

minor skin abrasions and occasional abscesses.Where conflicts are violent, severe and even fatal injuries can occur.

Bone thinning

Thinning of the bones of the femur and spine, because of inactivity, may be seen in caged rabbits. The bone thinning will occur within 4-6 weeks of confining rabbits in cages (Drescher B perscomm). This can result in a loss of ability to move normally, fractures, spinal distortions and discomfort from the trapping of nerves in the spine. There is no evidence of such thinning of the bones, with its attendant complications, occurring in rabbits in pens. The bone thinning is reversible once rabbits are placed in pens which allow normal movement (Drescher B perscomm).

Calicivirus

Calicivirus causes signs ranging from transient apathy to death with widespread haemorrhages. It is transmitted by direct contact between rabbits, or by transportation of the virus by clothing, other objects, people and animals. Insects such as flies and mosquitoes also act as vectors for the disease.

Myxomatosis

Myxomatosis is caused by a myxoma (pox) virus and results in characteristic signs including swelling and closure of the eyelids and a thick mucopurrulent eye discharge and subcutaneous swellings especially around the face and ears. A progressive loss of condition usually results in death in 11 – 18 days. It is spread by direct contact or insects (fleas and mosquitoes).

Ear mites (Psoroptescuniculi)

Ear mites cause intense local irritation, head shaking and scratching. They result in a yellowish brown exudate accumulating within the ear canal. They are spread by direct contact and indirectly through environmental contamination. Adult mites can survive off the host for a weekor more.

Recommendations

Rabbits introduced into group housing should be free of Pasteurellamultocida, ear mites and coccidia. A health monitoring programme should be instituted and health checks of individual rabbits should be carried out at least weekly. Health checks should include looking for signs of malocclusion, overgrown claws, fight wounds (especially underbelly wounds), sore hocks, ear mites, diarrhoea and snuffles. Weekly weighing should be carried out. Rabbits should be vaccinated against Calicivirus and protected from insects which may act as vectors for Calicivirus. Rabbits should be protected from insects such as mosquitoes which may act as vectors for myxomatosis.

Breeding of Rabbits in Pens and Cages, Principles

Problems that occur in breeding systems which house rabbits in cages include very limited freedom of movement, stereotypies, restlessness, disturbed sexual behavior, disturbed nursing and cannibalism.Breeding groups of 2 - 5 females, one male and their offspring until weaning can successfully be managed in pens.Nest quality can affect the survival of young especially in the first two weeks of life. Does have been found to build better nests if provided with soft nesting materials of natural fibre (such as cashmere or camel hair). Such materials promote a better microclimate (temperature and humidity) within the nest and may be more comfortable for young rabbits. Fibre content of the diet of the doe also has a strong influence on nest quality, with inadequate fibre having a detrimental effect. Normal behavior of female rabbits is to visit their nestlings once a day for nursing, open the nest entrance each time, and close it again afterwards.

The handling of young rabbits helps to reduce fearfulness towards humans and general emotional reactivity, as well as increasing “open field” activity and exploratory behavior.Handling in the first week of life and at a time associated with nursing may be especially effective in reducing fearfulness towards humans. The effect of this handling appears to be long-lasting.Males will mate with females

immediately after they have given birth. There are differing opinions on the effect of such breeding and the subsequent rapid succession of litters on the welfare of the female breeders.

Recommendations

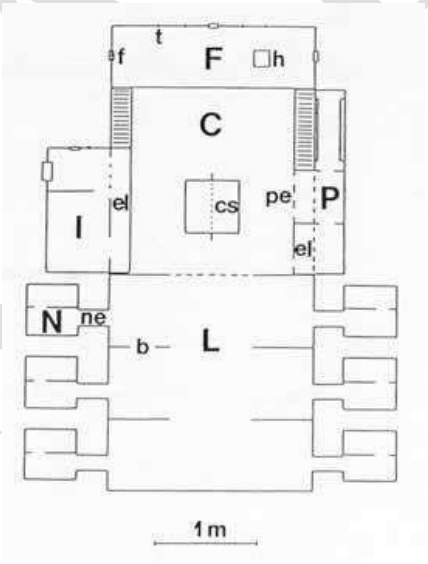
For group housing breeding rabbits in pens the following are recommended for inclusion in the pen design:

- A central area where rabbits can rest
- A feeding area with several sources of food and water
- A nesting area with separate nesting boxes with tunnel-like entrances (which substitute for breeding burrows dug in the earth and allow closing of the nest by the female), straw bedding and barriers between each nest to obscure the view of adjacent nests (and thus limit aggressive behavior)
- A pup area with small entrance passages accessible to rabbits up until about weaning age (about 18-39 days)
- An isolation area (which is needed to separate the male from time to time and for sick females) which allows sight and olfactory contact with rabbits in the main pen.

One nesting box per female should be provided to enable each female to nest alone.

Nest boxes of about 38cm x 25cm and 20cm high are recommended (females may urinate in boxes if they are too large).Females should be provided with straw or other suitable material such as hay or shredded paper to spread in their nesting place.Females should be able to withdraw from their young by retreating to a separate compartment or raised area, and / or by closing the nest after nursing. For a breeding group of 4-5 females, one male and their offspring, a floor area of 9 square metres is recommended.The handling of young rabbits is recommended to reduce fearfulness.

If immediate post-partum mating is carried out, it should be ensured that the doe is in a suitable condition to sustain her pregnancy without jeopardising her health.



C	central area	b	Blind
F	feeding area	cs	central structure
I	Isolation cage	el	raised resting space
L	bedded nesting area	f	pellet feeder
N	nesting box	h	hay rack

P	pup area	ne	tunnel-like nest entrance
		pe	entrance to pup area
		t	Drinker

Figure 1 Rabbit housing system for breeding groups

(Reproduced with kind permission from: Stauffacher (1992) Group housing and enrichment cages for breeding, fattening and laboratory rabbits. Animal Welfare 1: Figure 1 page 114; Publisher: UFAW, The Old School, Brewhouse Hill, Wheathampstead, Herts AL4 8AN, UK)

Single Housing in Cages, Advantages of Single Housing in Cages, Principles

The advantages of housing rabbits singly in cages include:

1. Food and water intake can be controlled and monitored.
2. Faeces and urine excretion can be quantified and monitored.
3. Rabbits can be identified without the need to mark them.
4. If strict precautions are taken, the spread of infection may be controlled more easily than group housed rabbits.
5. It is easier to maintain a cleaner environment.

Disadvantages of Single Housing in Cages- Principles

The disadvantages of housing rabbits singly in cages include:

1. Space restrictions in cages mean that rabbits cannot carry out normal activities such as hopping, lying stretched out, sitting up with ears erect, rearing and leaping.
2. The behavioral repertoire of singly caged rabbits is severely restricted, largely as a result of social deprivation.
3. Singly housed rabbits in cages carry out a variety of stereotypic behaviors such as bar gnawing, hopping back and forth, excessive grooming, fur eating, playing with the water nipple, pawing in the corners, head swaying and vertical sliding of the nose between the bars. Additional behaviors that have been observed include sitting in a hunched position for long periods and sitting with the head lowered in a corner. Rabbits also exhibit "restlessness" in cages – a high frequency of changing from one activity to another. It is likely that such behaviors are the result of frustration, anxiety, and boredom related to a barren, confined environment. These behaviors are rarely if ever seen in rabbits housed in groups in pens.
4. Singly caged rabbits tend to over react to relatively small changes in the environment. Such changes are likely to be a source of stress to the rabbits and they may become nervous and take fright or become aggressive. Such behavior may relate to a chronic lack of stimulation and/or to the fact that they have no way of escaping and hiding.
5. It is difficult to provide environmental enrichment in cages because of space restrictions.
6. Frequent cleaning and sterilisation may disturb the olfactory environment of the rabbit.
7. Ventilation may be poor in solid sided cages.
8. Health problems which are relatively common in caged rabbits and rare or unseen in penned rabbits include trichobezoars (hairballs) and gastrointestinal ulcers, pododermatitis (sore hocks), obesity and osteoporosis which may lead to a loss of capacity for normal locomotion, bone fractures and spinal distortion.
9. Cages are expensive to buy and costly and time consuming to clean.
10. Singly housed rabbits in cages may be psychologically and physiologically abnormal.
11. The use of such animals in research may have an influence on experimental results.

Cage Design and Environment, Cage Construction, Materials for Cages, Principles

1. Cages are usually constructed from metal (stainless steel or aluminium) or plastic.
2. Wire cages have the advantages that rabbits can see all around them and they are quieter than solid metal when rabbits move around. They may also allow rabbits in adjacent cages to communicate, not only by sight but by odours and urine spraying. Wire cages also provide good ventilation. However, such cages provide no darkened hiding or retreat area.

Recommendations

Cages may be constructed from materials including metal or plastic. Open sided (eg wire) cages are recommended, although a hiding / retreat area should be provided in such cages.

Flooring for Cages- Principles

1. Wire / grid floors are associated with a high incidence of sore hocks, especially in heavier rabbits.
2. Dimple floors are probably the best for the comfort and health of rabbits. Rabbits lie stretched out on plastic flooring and it is probably more comfortable than metal and subjects rabbits to less heat loss. However, plastic flooring may also be more slippery than metal, which may make it difficult for rabbits to move around within the cage. (Rose M 2001 pers com)

Recommendations

Plastic or metal dimple flooring should be used in cages.

Floor Area of Cages- Principles

1. Ideally cages should allow rabbits to stretch fully in all directions in the cage (length, width and diagonal). An adult New Zealand White rabbit is approximately 80cm long when fully stretched out. To allow freedom to stretch out in any direction, a floor area of 0.6 square metres (0.8 metres x 0.8 metres) would be required.
2. Larger cages may be difficult to manage (for example to catch rabbits and to clean).

Recommendations

Minimum cage floor dimensions of 0.8 meters width x 0.8 metres depth (0.64 square metres) should be provided. A minimum clear space in one direction of 0.8 metres should be provided. Additional clear space and larger caging is recommended to allow for the provision of additional environmental enrichment.

Height of Cages, Principles

Normal behavior of rabbits includes rearing up on their hind legs and sitting up erect with their ears pricked. Such erect stances allow rabbits to investigate sights and sounds. The minimum height provided should allow rabbits to carry out these behaviors. A suitable height to allow a New Zealand White rabbit to carry out such behaviors would be 75cm. However, in practical terms it may be difficult to make and use such cages. A cage height of 60cm would at least allow New Zealand White rabbits to sit up with their ears pricked.

Recommendations

Ideally the cage height should allow rabbits to rear up erect on their hind legs with their ears pricked (75cm for a New Zealand White rabbit). Although ideally cage height should be greater, a minimum cage height of 60cm should be provided.

Cage Trays, Principles

Most cages have trays underneath which catch urine and faeces. The contents of the tray are important because odours will diffuse up into the cages. Materials that are used in trays includesawdust, woodshavings, corrugated paper, pelleted paper, paper sprinkled with absorbent powders, preformed cardboard trays and absorbent pads.Systems in which urine and faeces are drawn under the cages of other rabbits (eg winding paper towels) may be stressful to rabbits because of the odours and may cause reproductive inhibition.

Recommendations

Materials that may be used in cage trays for catching urine and faeces include sawdust, woodshavings, corrugated paper, paper sprinkled with absorbent powders, pelleted paper, preformed cardboard trays and absorbent pads. If sawdust or wood shavings are use they should not smell strongly and should be made from non-resinated (softwood) timber. Autoclaving may remove odours that offend rabbits.

Cleaning of Cages, Principles

A balance needs to be struck between the human perception for the need for cleanliness and the level of disturbance to rabbits.

Recommendations

It is recommended that trays be cleaned and tray material be replaced three times a week.It is recommended that cages be cleaned every one to two weeks. Concentrations of ammonia should be monitored at the level of the cages and should ideally be lower than 1-2ppm and not allowed to exceed 10ppm.

Environmental Enrichment for Rabbits in Pens- Principles

Environmental enrichment has been defined as "any measure which promotes expression of natural, species specific behaviors and a decrease in, if not disappearance of, abnormal behaviors". It should be aimed not just at preventing suffering but at having a positive effect on the physical and psychological well-being of the rabbit. When techniques are used in an effort to provide environmental enrichment for rabbits it is important that the success of the techniques, in terms of improving the rabbits' welfare, is evaluated. In accordance with the requirement of the Code of Practice that housing should take into account species-specific behavioral needs (Clause 4.4.22), the implementation of strategies toprovide environmental enrichment for rabbits should be regarded as a fundamental requisite of rabbit care and management. Environmental enrichment strategies will extend over all facets of rabbit housing from pen design to food provision, opportunities for social contact and the provision of objects for manipulation. The recommendations listed below are therefore in addition to other recommendations throughout the document which contribute to enrichment of a rabbit's environment. Rabbits like to sit on elevated areas such as ledges and nest boxes. It is important for rabbits to be able to hide and to get away from each other. Rabbits play with and chew hay, straw and various objects.

Recommendations

Rabbits should be provided with objects such as ledges, boxes, PVC pipes and bales of straw which will create elevated areas for sitting on and darkened areas for hiding in. Sufficient objects should be provided to allow use by all rabbits and eliminate competition for use of the objects. Raised areas for lying on should be large enough to hold more than one rabbit as rabbits like lying together. Rabbits should be provided with hay and / or straw that can be used for chewing, digging, hiding and building nests. A variety of food should be provided. (See section 4.5 Food and Water) Food may be spread out in the bedding to encourage foraging behavior. Rabbits should be provided with objects to manipulate and gnaw such as wooden sticks, branches with leaves and small cardboard boxes.

Environmental Enrichment for Rabbits in Pens.

The provision of hay reduces abnormal behavior and gives singly housed rabbits an alternative occupation. Providing rabbits with objects to manipulate triggers species-typical behaviors, reduces stereotypical behaviors and results in increased activity by rabbits.

Recommendations

Rabbits housed in cages should be provided with environmental enrichment. Caged rabbits should be housed in compatible pairs wherever possible. This will usually require the use of double cages. Cages may, for example, be joined by a PVC pipe which also acts as a hiding / resting area. The floor space

provided for pair housed rabbits should be greater than that provided for single caged rabbits and at least metres x 0.8 metres. Caged rabbits should have visual and olfactory contact with other rabbits. If rabbits are held in solid sided cages, they should be placed so that the rabbits can view other rabbits. Rabbits should be provided with ledges and / or nest boxes which will create an elevated area for sitting on and a darkened area for hiding in. To increase the available area for rabbits, nest boxes can be attached to the outside of the cage.

Increasing the height of part or all of the cage to accommodate ledges and boxes will increase the available vertical space for rabbits. Ideally ledges should be placed 20 – 30 cm from the floor of the cage and the overall cage height should be 60cm to accommodate this. Rabbits should be provided with hay on a daily basis. To reduce wastage and the need for more frequent cage cleaning, this can be provided, for example, in a hay rack or in a plastic bottle. In the latter, rabbits will spend time manipulating the bottle to get the hay out.Rabbits should be provided with objects to manipulate and gnaw such as wooden sticks, branches with leaves and small cardboard boxes. To provide variety objects may, for example, be hung from the top of the cage. A variety of food should be provided. (See Section 4.5 Food and Water)If a restricted diet is required to be fed, it should be provided in the late afternoon (which has been shown to reduce the frequency of abnormal behaviors in caged rabbits).Rabbits should be taken out of cages on a regular basis for handling / petting and exercise to relieve boredom. Allowing rabbits periods of access to floor pens will assist in providing the opportunity for exercise.

Environmental Variables in Pens and Cages, General, Principles

A variety of environmental factors, including light, temperature, humidity, air quality and sound may impact on the behavioral responses and health of rabbits. The design, construction and management of rabbit pens and cages will largely determine how these factors will impact on the rabbits.

Light, Principles

In the wild, rabbits are nocturnal, and forage mainly at dusk and dawn.There is uncertainty as to whether rabbits in the laboratory are diurnal, nocturnal or crepuscular. It appears that external noise or scheduled feeding during the day can turn laboratory rabbits into predominantly diurnal animals.It may be desirable, particularly when working with albino rabbits, to maintain a low light level except when brighter light is required for working in the room.There is debate about whether it is desirable to create an artificial dawn and dusk period.

Recommendations

A regular light / dark cycle should be provided, and a 12/12 light/dark cycle is recommended.If rabbits are to be used for breeding, a 14 to 16 hour light period is recommended.

Temperature, Principles

Low temperatures are fairly well tolerated by rabbits but heat and draft are not well tolerated. Temperatures of above 30^0C, combined with high relative humidity, can cause heat stress which may result in infertility and mortality.Air temperature in the pen or cage is influenced not only by the design of the enclosure but also by air distribution, ventilation rate, the position of the enclosure within the air flow pattern and its proximity to other enclosures.

Recommendations

A temperature range for rabbit housing of 15 – 21^0C is recommended.

Humidity, Recommendations

A relative humidity for rabbit housing of 45 – 65% is recommended.

Air Quality, Principles

The effective ventilation of rabbit enclosures is a critical consideration in the management of environmental factors. The adequacy of air exchange in the rabbits' immediate environment of the pen or

cage will affect temperature, humidity and air quality. The placement of air inlets and outlets in a room and the rate of air exchange will affect the pattern and efficiency of air distribution. The number of air changes per hour that are needed will in part be determined by the cleaning routine and stocking density of rabbits.[5] Air changes are less important than creating an efficient air flow to keep ammonia levels within the rabbits' immediate environment of the pen or cage at an acceptable level.

Recommendations

A ventilation rate of 15 – 20 air changes per hour is recommended. Lower rates of air change may be acceptable if the cleaning routine is frequent and of a high standard and the stocking density is low. The number of air changes per hour that are needed will be influenced by the air flow patterns at the level of the pen or cage. Concentrations of ammonia should ideally be lower than 1-2ppm and not allowed to exceed 10ppm.

Sound, Principles

Rabbits are sensitive to high sound frequencies which cannot be detected by humans (ultrasound). Ultrasound can be produced by common laboratory equipment such as temperature regulating devices, cage cleaning equipment, vacuum hoses as well as by running water.The effect on rabbits of sound levels of elevated intensity is unclear. Some research has shown levels of 112 decibels to be stressful, whereas another study did not demonstrate effects with sound levels of 96 decibels.

Recommendations

Sources of ultrasound should be considered when assessing sound levels that rabbits are exposed to. The effect of background radio noise to alleviate the effects of ultrasound and loud noises is unclear. If a radio is used, the volume should be kept low.

Records, Pen / Cage Labels, Requirements

1. Pens and cages should have labels attached to them that provide the following information:
    a. Rabbit identification
    b. Name, location and contact numbers of the Chief Investigator / Teacher and (if applicable) other investigators / teachers using the rabbits
    c. Name, location and contact numbers of staff associated with the housing and care of the rabbits
    d. Name and approval number of protocol in which rabbits are being used
    e. Age (date of birth) of rabbits
    f. Date of entry of rabbits into the pen or cage.

Requirements

To assist in monitoring the management of rabbit breeding colonies, regular reports must be provided to the Animal Ethics Committee, for review, on the fertility, fecundity, morbidity and mortality of all rabbit breeding colonies. The frequency of such reports should be at least 6 monthly and more often if deemed necessary by the AEC.

II –M.Sc Microbiology (Batch 2018-2020)

Possible Questions

Unit – I

Two Marks

1. What is animal welfare?
2. Define animal rights?
3. Define three area of animal experimentation?
4. Maintenance of rabbits in cages
5. Name the regulatory bodies involved in animal housing

Eight Marks

1. Write short notes on the maintenance of rabbits in pen
2. Draw the general schematics of housing rabbits in cages with proper lable.
3. Distinguish between the anatomical characteristics of rabbits used in the laboratory.
4. Write short notes on the safety aspects of animal house while handling rabbits.
5. Discuss on the methods of health maintenance done during the care of rabbits
6. How are rabbits bread for the animal house and experimental purposes? Discuss.
7. Explain in detail on the role of different regulatory bodies involved in the animal housing of rabbits.
8. Write short notes on the maintenance of rabbits in cages
9. Discuss on the processing of feed for rabbits.
10. Write short note on the method of cage selection and bedding of rabbits.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB **COURSE NAME: LABORATORY ANIMAL CARE**

COURSE CODE: 18MBP305B **UNIT: I** **BATCH-2018-2020**

S.No	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Stress in rabbit leads to _____.	limb damage	ear lesion	heart failure	ocular discharge	ocular discharge
2	Common diseases in rabbit is caused by _____.	bacteria	protozoa	virus	intestinal helminths	virus
3	Common bacterial infection is rabbit is caused by_____.	P. aeruginosa	E. coli	V. cholerae	P. multocida	P. multocida
4	Myomatosis is commonly encountered in_____.	mice	rat	guniea pig	rabbit	rabbit
5	Viral haemorrhagic disease is often seen with_____.	thining of hind limb	respiratory illness	lesion	death	death
6	Breeding is enhanced by _____in rabbits.	day cycle	night cycle	dim light	bright light	day cycle
7	_____rabbits should be selected for breeding.	acitve	inactive	docile attitude	aggressive	docile attitude
8	Nest box is made up of _____in rabbit housing.	plastic	cardboard	steel	paper	cardboard
9	Nest box should contain more_____for the rabbit to undergo gestation.	air	water	light	bedding	bedding
10	Young one of rabbits are suckled _____minutes per day.	1	5	10	45	10
11	Assurance of security to rabbits is indicated by_____.	smell	noise	hiding place	same species grouping	same species grouping
12	Cleaning the latrines and faeces in rabbit cages is done in_____days.	5	7	14	10	14
13	Over cleaing of rabbits cage lead to _____.	decreased fear	decrease in animal confidence	decrease in body weight	decrease in activity	decrease in animal confidence
14	Group housing reduces_____in rabbits.	body weight	stereotypy	calmness	size	stereoptypy

15	Rabbit produce copious turbid urine with dark red color due to _____.	ammonia	porphyrin	vitamin	RBC	porphyrin
16	Fibre requirement of rabbit in feed is_____.	22%	5%	10%	1%	22%
17	Protein requirement of rabbit in feed is_____.	5%	12%	25%	16%	12%
18	Daily need of high energy feed of rabbit is_____.	1g	16g	5g	0.3	5g
19	_____is mandatory in for the wellfare of the rabbit	protein	carbohydrate	gut flora	lipids	gut flora
20	Normal quantity of water consumed by rabbit is_____per day/100g body weight.	10 ml	1 ml	5 ml	20 ml	10 ml
21	Rabbits are_____.	dirunal	docile	semiactive	nocturnal	nocturnal
22	Rabbits are social animals which are able to utilize_____environment.	complex	simple	1D	3D	3D
23	Aggressive behaviour is NOT observed in_____rabbits.	pregnant female	breeding adult male	weaning pup	pubescent female	weaning pups
24	If rabbits in a group are seperated for a long time_____results	competence among group mates	Fighting	inactive social participation	incompetence	incompetence
25	Nowadays_____is used as a safer way to detect the lab rabbits.	colored collar	ear tags	micro chips	limb collar	microchips
26	Pasterella is a _____ pathogen.	gut	liver	lungs	urinary	lungs
27	Suddent dietary changes to rabbits lead to_____.	diarrhoea	death	gut inflammation	inactivity	diarrhoea
28	Mucoid enteropathy is a common disease associated with	protein	fibre	carbohydrate	lipids	fibre

	_____deficiency.					
29	For sedating rabbit_____is provide	asprin	medetomidine	flucytocine	rifampin	mdetomidine
30	Muscle relaxation in rabbit is achieved by the administration of _____.	asprin	medetomidine	xylazine	rifampin	xylazine
31	Rabbits can hear ultra sound at _____Hz.	6	16	20	25	16
32	Poor vetilation in rabbit cage lead to _____.	suffocation	asphyzation	respiratory illness	inactivity	respiratory illness
33	Air change in rabbit cages are done at _____cycles per day.	5	9	15	35	15
34	_____indicates normal behaviour of rabbits in open environment	cornered hiding	climbing	sleeping	strecthing	stretching
35	Ammonia from rabbit urine cause_____.	erythema	sedation	inactivity	respiratory disease	respiratory disease
36	High side open containment used for housing rabbit is called _____.	cages	pens	Open housing	in house cages	pens
37	Rabbits communicate with_____.	ultrasound	high pitch sound	olfactory cues	mild vocals	olfactory cues
38	_____is provide to enhace the environmental condition of rabbit housing.	hay	corn cobs	chew sticks	pumise stones	chew sticks
39	Sore hocks in rabbits are caused by _____.	urine	faeces	mites	allergen	urine
40	Grid floors are generally provide if the rabbits are housed in_____.	pens	transport vehicles	cages	inhouse cages	cages
41	Pododermatitis is the infection of rabbit_____.	ear	hind limb	fore limb	abdomen	hind limb

42	The largest used stock variety of rabbits belong to_____.	America	India	Newzealand	Australia	New zealand
43	_____aids in easy identification of rabbits.	eyes	fur coat thickness	fur color	size	fur color
44	_____is not recommeded for the use in rabbit identification because of discomfort.	tattoo	leg collar	ear tag	paw stain	leg collar
45	Rabbits cages are designed with features to mainly provide_____.	locomotion	nesting space	breeding chamber	central area	locomotion
46	Space in rabit housing is essential to prevent_____.	bone thining	aggressiveness	inactivity	disease	bone thining
47	Lagomorpha is the order to which _____belongs.	Mouse	Rabbits	Hamster	Rats	Rabbits
48	_____is an alternative housing method of separating aggressive rabbits in a group.	pens	single housed cage	space restricted cage	pair housing	pair housing
49	Housing individual rabbits in a space restricted cage leads to _____.	agressiveness	bone hypoplasia	inactivity	death	bone hypoplasia
50	Ambient temperature for rabbit is _____℃.	10	20	30	40	20
51	Female rabbits breeding during _____months.	4	5	6	7	4
52	During breeding rabbits have no_____.	gestation	phermones	Appetite	oestrous cycle	oestrous cycle
53	Handling rabbits by holding the ears lead to_____.	ear damage	neuronal damage	spinal damage	limb pain	spinal damage
54	Stoic acceptance is the response of rabbit to_____.	food	light	pain	disease	pain
55	Rabbit under pain is recognized by _____property.	low activity	low food intake	sleeplessness	running	low food intake

56	Rabbits should never be lifted by_____.	holding hind limbs	ears	fore limb	dorsal body	ears
57	Rabbits are_____.	coprophagic	necrophagic	autophagic	heterophagic	coprophagic
58	The laboratory rabbits is scientifically called as_____.	Orycotolagus cuniculus	Orycotolagus canis	Orycotolagus albinus	Orycotolagus cunlingus	Orycotolagus cuniculus

UNIT-II
SYLLABUS

Modern methods of care, management breeding and maintenance of laboratory animal - Mice

General Introduction

The **laboratory mouse** is a small mammal of the order Rodentia which is bred and used for scientific research. Laboratory mice are usually of the species *Mus musculus*. They are the most commonly used mammalian research model and are used for research in genetics, psychology, medicine and other scientific disciplines. Mice belong to the Euarchontoglires clade, which includes humans. This close relationship, the associated high homology with humans, their ease of maintenance and handling, and their high reproduction rate, make mice particularly suitable models for human-oriented research. The laboratory mouse genome has been sequenced and many mouse genes have human homologues.

These guidelines are intended for use by people involved in the housing and care of mice in scientific institutions. The guidelines are not intended to be a complete manual on mouse care and management but rather to provide some key guiding principles on best practice standards in mouse housing. The guidelines will be revised from time to time to take account of advances in the understanding of murine physiology and behaviour, technological advances, and changes in community attitudes and expectations about the welfare of animals.

The recent explosion of scientific studies on the subject of the housing of mice has facilitated the development of evidence-based guidelines4. The housing of mice in particular has been targeted as mice used for scientific purposes spend the majority if not all of their existence in laboratory housing. The nature of that housing therefore has the potential to significantly impact upon the welfare of all laboratory mice. The number of mice used in laboratories or maintained in animal facilities is likely to increase, as the use of genetically modified, transgenic and knockout mice to understand gene function has resulted in an increase in the number of animals used in scientific procedures. The implementation of housing guidelines will therefore have a broad impact.

Under the Australian Code Of Practice For The Care And Use Of Animals For Scientific Purposes (see below section 1.3 Responsibilities of Chief Investigators/Teachers), investigators and teachers who use animals for scientific purposes have personal responsibility for all matters regarding the welfare of these animals, and are obliged to treat animals with respect and consider their welfare when planning or conducting projects. The Code of Practice is underpinned by the principals of replacement of animals with other methods, reduction of the number of animals used and refinement of techniques used to reduce adverse impact on animals.

It is in the interest of investigators and teachers to promote improved animal welfare. Improved animal welfare may translate into improved research outcomes, as pain, suffering and distress in mice can lead to physiological and behavioural changes that may confound experimental data9. To minimise confounding variables, investigators should strive to maintain a stable physiological and behavioural baseline. This necessitates a familiarity with behaviour and biology of experimental species and strain on the part of investigators. Furthermore, investigators and teachers must be aware of the potential impact of husbandry and environmental variables on experimental animals. While the guidelines focus on the welfare of mice, it is implicit that conditions contributing to meeting the physiological and behavioural needs of mice will also contribute to the quality of scientific outcomes through provision of the optimum stable environment for the maintenance and care of the animals. The guidelines contain many examples of the physiological and

behavioural responses of mice associated with variables in housing and hence the effects of these variables on mice as research subjects. The guidelines are based on principles regarding the care and management of mice taken from scientific literature. These principles are detailed throughout the document, as are recommendations for the care and management of mice which are derived from these principles. In some areas, conclusions to be drawn from the available literature are not entirely clear, and in such areas recommendations are extrapolated from information available and practices in mouse care and management current at the time of writing.

The principles outlined in the document address requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (as outlined below in Section 1.4). The requirements of the Code of Practice include that animals held for scientific purposes should have their species-specific behavioural and physical needs met, whilst at the same time ensuring that the animals can adequately be monitored and are protected from disease, and taking into account the requirements of the research for which the animals are being used. (vi) The guidelines outline the requirements for housing to meet the physiological and behavioural needs of mice. Where mice are physiologically or behaviourally abnormal, for example post surgery, acute pain models, or disease models such as diabetics and Parkinsonian mice, modification of housing to meet their specific needs may be required.

Responsibilities of Institutions- Recommendations

Institutions using mice for scientific purposes are responsible for responding effectively to recommendations of the institution's Animal Ethics Committee to ensure that facilities for the housing and care of mice are appropriate to the maintenance of well-being and health of the mice.

Responsibilities of Chief Investigators

Recommendations

The chief investigator/teacher (person in charge of a research/teaching project) has direct and ultimate responsibility for all matters related to the welfare of mice under his or her control, which includes their housing and care. (As per the principle contained in Clause 3.1.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to mouse care and management - Personnel training and competencies should be documented. (As per the principle contained in Clause 3.1.3 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

Principles

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states:4.4.19 Animal accommodation should be designed and managed to meet species-specific needs. Pens, cages and containers should ensure animal wellbeing and comfort. Variations to these requirements as part of a project must receive prior AEC approval. The following factors should be taken into account:

1. species-specific behavioural requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy, contact with others of the same species, and environmental enrichment;
2. provision of single housing for animals when appropriate for the species and if necessary for the purpose of the project (for example, during recovery from surgery or collection of samples);

3. species-specific environmental requirements, such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations;
4. the need to provide ready access to food and water;
5. the need to clean the pen, cage or container;
6. protection from spread of pests and disease;
7. requirements of the project; and
8. the need to observe the animals readily.

Pens, cages and containers must:

1. be constructed of safe, durable, materials;
2. be kept clean;
3. be maintained in good repair;
4. be secure and escape-proof;
5. protect animals from climatic extremes;
6. not cause injury to animals;
7. be large enough for the species and the number of animals held; and
8. be compatible with the behavioural needs of the species.

The number of animals in cages, pens or containers and the placement of these should enable social and environmental conditions for the species to be maintained. Where it is necessary to individually house animals of a species that normally exists in social groups, the impact and time of social isolation should be kept to a minimum.

Bedding and litter must be provided if appropriate to the species and should be comfortable, absorbent, safe, non-toxic, able to be sterilised if needed, and suitable for the particular scientific or educational aims. Pregnant animals must be provided with nesting materials, where appropriate.

The AEC, investigators and teachers should be consulted in advance of planned changes to these conditions, since these may affect both the welfare of animals and results of the scientific and teaching activities.

Aspects of mice Behaviour Relevant to Housing

Principles

Mice are physiologically and behaviourally distinct from rats, from which they diverged over 17.5 million years ago10. The laboratory mouse (Order Rodentia, family Muridae, subfamily Murinae, genus *Mus*, subgenus *Mus*, species *musculus*) is descended from the house mouse of North America and Europe, *Mus musculus*.

The genome of laboratory mice is derived from *M. musculus* and *M. domesticus* subspecies. It is believed that fancy mice strains from Europe and East Asia contributed to the genome of common laboratory strains including C57BL/6, BALB/c and DBA. Mice used in biomedical research range from captive wild individuals to strains bred hundreds or even thousands of generations in a laboratory setting, often with spontaneous or deliberately induced genetic alterations. Most laboratory strains originate from pet dealers who became suppliers of laboratory mice.

Mice are social animals. In the wild they live in groups which vary extensively in size. The social patterns and behaviour of wild mice by necessity differ significantly from those of laboratory mice16. Social organisation of wild mice is dynamic and dependent on environmental variables including resource availability and shelter. Complex environments may support a higher density of mice than open areas. Commensal or house mouse territories with stable and abundant food supplies may house up to 10mice/m^2. The extended family unit, known as a deme, may consist of a single dominant male, several subordinate

males and breeding females. Feral or dispersed (non-commensal) mouse populations are typically less dense, and less stable.

From birth to approximately 14 to 21 days of age, pups are dependent on their mother for warmth, food and toileting16. While pups begin to explore beyond the nest at around three to four weeks of age, they tend to remain in the nest until they reach sexual maturity (at around 5 to 6 weeks, although this may be as late as 12 weeks depending on genotype and environmental factors16)

Dispersing mice seek out a protected site in which they can build a nest and establish territorial boundaries. Territory size varies, depending on environmental factors including food availability and population density16. Where a concentrated food source is available, territory size may range from 2-6m2, while feral or non-commensal mice may have a home range of up to 80,000m2 16.

House mice can be polygamous but may pair-bond16. If environmental factors are favourable (ample food and nesting material), a reproductive female can produce up to ten litters a year16. Gestation lasts from 18 to 21 days, with the female building a nest in the days preceding parturition16. During this time females may exhibit aggression towards non-reproductive mice, although pregnant and/or lactating female mice are known to form communal nests with close relatives, and may share nursing duties21. Mothers are known to destroy their own litters (infanticide), a behaviour which may be attributed to disturbances, overcrowding, dietary restriction or other environmental factors16. Adult males are highly infanticidal, although less commonly to their own pups16. Adult males exhibit varying degrees of tolerance to one another22. Murine sensory input is dominated by olfactory, auditory and tactile cues, many of which are beyond the range of human sensation16. The implication is that aspects of the laboratory environment which are highly relevant to mice may be unnoticed by investigators and laboratory personnel23.

Olfactory cues are the primary means of communication between mice24. Mice employ a main olfactory system to detect airborne volatile scents, in additional to the vomeronasal system which detects pheromones24. They signal individual identity via expression of major urinary proteins predominantly in the high mass fraction of their urine25, 26. Urine is deposited in streaks and spots in and around the territory27, with the dominant owner marking more frequently than subordinates24. Dominant males refresh their own marks, and may enter neighbouring territories to over-mark the urine of a competitor16. Aside from urine, mice have other sources of secretions which may act as olfactory cues, including salivaryglands, plantar glands and the preputial gland28. Via odours, mice can recognise kin relationships29, the social status of male mice30 and the oestrus status of female mice31. Mice use olfaction and olfactory cues to assess territorial boundaries, detect food, identify one another, and to evaluate sexual and social status16, 25. Mice commonly sniff the ano-genital region of cohabitants and prospective sexual partners32. Scent impacts a wide range of behaviour including competitive and territorial aggression between males24 28, 33. In addition, pheromones can prime or inhibit reproduction24, 34. For example, male odours induce oestrus and synchronise oestrus in females (the Whitten effect)35 while unfamiliar male odours can prevent the establishment of pregnancy in females (the Bruce effect)36. Inbreeding of mice inhibits their ability to discriminate via olfactory cues because individuals are almost genetically identical37. This may alter competitive/aggressive behaviour24, 37 and therefore experimental outcomes. Olfactory cues should be taken into consideration when devising a cleaning protocol, as inadvertent disruption of chemical signals during cleaning may result in outbreaks of aggression19, 24, 28 (see Section 4.7 Cleaning). In addition, unfamiliar odours (such as those associated with humans) may cause stress responses in mice. (viii) Mice have a well developed sense of hearing, and can hear sounds from 2300Hz (23kHz) to over 85000Hz (85.5kHz)38. Mice produce ultrasonic (above 20kHz) vocalisations during non-aggressive interactions39 that are inaudible to the unaided human ear.

The function of these vocalisations is yet to be established, but in the laboratory setting they occur more frequently in mice housed in socially and environmentally enriched cages39, suggesting that they may be a useful indicator of affect or emotion. The ears of mouse pups are closed for up to 10 days postnatally40, yet they emit ultrasonic vocalisations when separated from the doe, reliably stimulating the mother to retrieve them39, 41, 42. Ultrasonic vocalisations in pups may be context specific42, 43. For example, distinctive vocalisations were associated with isolation outside the nest, jostling for the doe's nipples, being handled roughly by adults or the immediate postpartum period43. It is possible that mice use ultrasonic calls for the purpose of echolocation and judging distances in the darkness, as may be the case in rats16, 44, but this is yet to be established. Differences in ultrasound vocalisation rate and acoustic structures have been observed between different strains of mice41. Some strains of laboratory mice are genetically predisposed to auditory dysfunction and hearing loss (see section 4.6 Sound and Vibration).

Mice have dichromatic colour vision, similar to red-green colour blindness in humans45. They have a retinal mechanism which is maximally sensitive to ultraviolet light. In humans with normal vision, UV is blocked by the cornea, so artificial lighting has been designed to emit little UV. When housed in laboratories without these UV wavelengths, mice may have distorted or altered colour perception23.

As with all small mammals, risk of predation is an important factor influencing activity and movement patterns of mice. Mice exhibit thigmotaxis, a tendency to maintain contact with vertical surfaces such as walls, particularly when exploring a new area46, 47. When faced with a real or perceived threat, mice may retreat or freeze. Retreating mice have a tendency to run away as well as upwards32. In captive animals this often results in the animal landing on the bars of the cage if shelter is not available. Muscle fasiculations or convulsive behaviour may be noted. Alternatively, mice may crouch in one spot. The adoption of a full submissive posture, in which the animal lies on its back, has been reported32.

Mice are primarily crepuscular or nocturnal16, 48, however they may alter their activity patterns depending on food availability and due to light cycle and activities in the laboratory 16.

Mice are omnivorous, but are known to prefer foods high in fat and protein, and will eat meat and live insects16. They may acquire most of the water they need from their food16. Mice eat up to 20 per cent of their body weight daily, consumed in small, frequent portions. This occurs mostly during the dark phase16. Normal behaviours of mice include eating, drinking, urinating, defecating, foraging, exploring, gnawing, hiding, climbing, playing, nesting and digging and engaging in a range of social activities.49, 50.

House mice exhibit developmental plasticity, with aspects of the early environment impacting on adult phenotype16. For example maternal stress during gestation can delay post-natal development; prenatal stress can induce masculinisation of female pups and feminisation of male pups (which impacts in turn on reproductive performance and aggression in later life); and low food availability during gestation can reduce weaning weight and increase aggression16. Quality and quantity of maternal care can affect weaning weight, onset of sexual maturity and corticosterone responses to stress in later life16. Thus the development of mice varies between sites, depending on local environmental factors. This plasticity may account for differences in phenotype between laboratory mice obtained from different facilities16. Some inbred strains display behavioural complexes which are believed to reflect functional adaptations to particular habitats. Thus BALB/c mice, which are adapted to living on the surface, display exploratory behaviour, whereas C57BL/6J mice are traditionally hole-dwellers, with a tendency to dig13. This may influence the way each strain interacts with a particular environment.

Recommendations

To meet the requirements of the Code of Practice (ie to provide accommodation that meets the species-specific needs of mice), housing should be provided which allows rat the opportunity for social

interaction, the opportunity to carry out normal behaviours and the opportunity to rest and withdraw from each other.

The Code of Practice recognises that there may be circumstances where the requirements of experimental procedures will preclude meeting some species-specific needs (Clause 4.4.19). Housing in these situations should still meet the physiological and psychological needs of rat as closely as possible.

Cage Design

Living area

Principles

(i) The living area for mice is three dimensional, comprised of the floor area as well as the vertical space. These parameters need to be considered together, rather than in isolation, including the postulates that the quality of the space is more important than the quantity but there is a minimum quantity that is necessary to ensure quality. Despite numerous experimental studies, there is no consensus on minimal or optimal cage space for mice69. The Council of Europe revised housing guidelines in 2006 to regulate for increased minimum space of laboratory mice70. The rationale behind the change was to allow the incorporation of environmental enrichment to facilitate expression of species-typical behaviour. At the same time, several US-based groups published studies suggesting that less space may be advantageous for mice69, 71-73.

(ii) There is no single definition of crowding or excessive population density for mice18. Given that free-living male mice establish territories ranging from 1m2 to 80,000m2 16, it is possible that even housing mice in the same room as other mice in separate cages within visual, auditory and/or olfactory range may be experienced as crowding18 (see Section 3.1 The Social Environment for further information on population density). Thus if a mouse shows the same behaviour when allowed a floor area of 56.25cm2 and 225cm2, it is possible that both cage areas are too large or too small or acceptable to the mouse69.

(iii) In addition to three dimensional space, the shape of the living area needs to be taken into account in determining optimal living area. (iv) The living area for mice must allow them to satisfy their basic physiological and behavioural needs including the ability to eat, drink, play, rest, groom, forage for food, explore, gnaw, hide, reproduce, engage in a range of social activities, urinate and defaecate49. If given the opportunity, mice tend to compartmentalise their living areas for these different activities, for example feeding, resting, urination and defaecation28, 50, 74, 75. These divisions allow mice to control and predict their environment, including light levels and temperature50. An example of a cage that promotes compartmentalisation is the Cambridge cage76.

(v) The design, construction and management of a mouse's immediate enclosure will determine to a large extent how environmental factors, such as temperature, light levels, humidity and air quality impact on the mouse77.

(vi) Living area or cage size affects feeding and energy expenditure of mice, at least in wild species. Thus wild-type *Peromyscus californicus* housed in smaller cages (L29cm x W19cm x H13cm: 7163cm3) had lower daily energy expenditure and lower food intake than their counterparts housed in larger (L48cm x W27cm x H20cm: 25,920cm3) cages78. In contrast, energy expenditure and food intake of smaller *Peromyscus eremicus* mice were not affected by cage size. Thus the impact of living area varies with species and may also vary with strain of laboratory mice.

(vii) When attempting to determine optimal living area, different studies assess different parameters including cage microenvironment (ammonia, relative humidity, temperature, noise, airflow), reproduction (fertility, litter size), physical parameters (growth, survival), mouse physiology (stress hormones, immune function) behaviour and preference tests69. Recommendations are frequently based on the weight of individual mice and number of animals per cage79. Many studies which assess living area requirements for

mice do not address potential confounding variables such as group size, strain effects, sex, age of mice and stage in breeding cycle, type of space (vertical vs horizontal) or enrichment methods79. Due to the complexity and variability of experimental design, it is difficult to extrapolate optimal cage size and population density.

(viii) The cage lid, insofar as it allows mice to climb, is an important consideration in determining living area (see Section 2.3 Cage Height and Cage Lid)

Recommendations

The living area for mice must allow them to satisfy their basic physiological and behavioural needs including the ability to eat, drink, urinate, defecate, forage, explore, gnaw, hide, climb, play, nest, dig and engage in a range of social activities.

The living area should be constructed and arranged in such a way to allow mice to compartmentalise their space, so that different areas can be used for urination, defecation, eating and resting.

Cage floor area

Principles

(i) There is no consensus in the scientific literature about the minimum cage floor area or maximum stocking density for housing laboratory mice. Different strains may have significantly different space requirements, which may be altered by in-cage furnishings or enrichment items. Table 1 provides a summary of some of the major studies evaluating cage floor area in different strains of mice.

(ii) As discussed in Section 2.1 Living Area, the living area should be large enough to allow mice to compartmentalise their space. At the same time, ages with large quantities of open, empty space without hiding places should be avoided as these may be stressful to mice.

(iii) In terms of physical movements, mice should be able to turn freely without twisting their heads and bodies, walk at least a few steps, stand on their hind limbs and stretch up. They should also have room to shelter and rest. The floor area should ensure that no part of a mouse's body is unavoidably distorted by contact with the cage in any of the postures that mice may adopt. However, this does not imply that a larger cage is necessarily better. Mice exhibit thigmotaxis, and may therefore not respond to an increase in living area in the same way as other species8

(iv) A number of studies have challenged the generosity of cage floor area recommendations published in overseas guidelines, on the grounds that they appear to be based on current practice rather than evidence. In addition, there is evidence that increased cage size may be associated with increased mortality, in particular due to fighting between male mice. However, investigators should keep in mind that most studies confound effects of cage floor area with effects of group size when reviewing the literature. Furthermore, many do not factor in the presence of nesting and bedding material and an in-cage shelter.

(v) In-cage shelters (see section 2.9 In-Cage Shelters) are desirable additions to mouse housing, however dimensions of the floor area must be sufficient to accommodate such furnishings without negatively impacting on mouse behaviour by reducing floor space or restricting access to areas of the cage. Negative effects of increased floor area may be offset by the provision of cage furnishings, dividers or other structural elements. One study reports increased aggression between males with a cage shelter460.

(vi) A 2008 survey of animal units in the United Kingdom found that the floor area per mouse ranged from 22cm2 to 960cm2 94. In the majority of cases (95.9 per cent), mice were housed in cages which allowed for 60cm2 per mouse. Only one per cent of cages allowed for less than 30cm2 per mouse, which was acceptable only for short term housing of recently weaned animals.

(vii) The Cambridge cage, one of the few examples of an environment designed to allow mice to compartmentalise their living area, measured 27cmx22cm, yielding a total floor area of 594cm2 76. (viii)

Optimal cage floor area and housing density will facilitate normal behaviour and support physiologically normal mice, but it is impossible to determine exactly what that will be based on the current literature alone. Given significant strain variation, a single set of recommendations is unlikely to be appropriate79.
(ix) Factors other than floor area may influence how mice use floor space – for example, brightly lit open space is more likely to be avoided95.
(x) The recommendations below represent a best estimation, based on the scientific literature on minimum cage size (see Table 1), as well as literature on the needs of mice. The recommendations are consistent with the dimensions of common commercially available mouse cages in Australia at the time of publication.

Recommendations

As a guide, enclosures should allow for a minimum floor area of 250cm2 for a single housed mouse, a minimum floor area of 500cm2 for two mice and ensuring a minimum floor area of 60cm2 per additional adult mouse when mice are housed in larger groups.
As a guide, a breeding pair or female with pups requires a minimum total cage floor area of 500cm2, with an additional 100cm2 for each additional adult female.
To reduce anxiety and aggression, larger cages should be designed in such a way as to avoid large open spaces. Because of the wide variation in conclusions drawn from studies designed to determine optimum cage floor area, it is necessary for researchers to assess whether a particular strain is coping with a particular living area. Parameters assessed may include tendency to perform normal behaviours, aggressive encounters or fight wounds, weight changes, incidence of illness, reproductive performance, use of space, use of enrichment and amount of thigmotaxis observed.

Table 1: Studies evaluating cage floor area (listed alphabetically)

Author	Strain	Sex	No. mice per cage	Cage dimensions, total floor area or floor area per mouse	Parameters measured	Key results	Conclusion/ Comments
Benhar E[81]	C57BL/6JWn and SWR	M,F	Breeding pairs with litters of 9	29cm x 14cm (406cm^2) or 29cm x 17.5cm (507.5cm^2)	Number of litters, number of weaned mice	C57BL/6JWn mice in the larger cage produced 19 per cent fewer weaned mice. SWR mice in the larger cage produced 15 per cent fewer weaned mice.	Increased cage floor area may reduce reproductive performance.
Davidson LP et al[82]	Cr:SW	M,F	Breeding pairs with litters of ten	429cm^2, 505cm^2 or 729cm^2	Open field, light-dark exploration, elevated plus maze, weaning weight, locomotor skills of pups	No differences in weaning weight between cage size. Mice reared in 505 and 729cm^2 cages explored a significantly larger area; mice in 505cm^2 cages spent more time in the centre than those in the larger cages; failed to establish consistent link between decreased floor space and increased anxiety like behaviour. No consistent association between available floor space and development of locomotor skills in pups.	Increased cage floor area may be associated with increased anxiety.
Forsyth NY et al[83]	C57BL/6NCrl, Crl:CD-1, BALB/cAnNCrl	F	4	15.2cm x 15.9cm (58cm^2/mouse); 15.2cm x 26cm (96.8cm^2/mouse); 43.2cm x 20.3cm (219.4cm^2/mouse)	Organ weight, white blood cell counts	C57BL/6 and CD-1 mice had the lowest total white cell counts in medium cages; BALB/c mice had lowest total white cell counts in small cages. All strains had the highest total white cell counts in the large cages. Cage size did not affect body or organ weight.	Increased cage floor area may be stressful for mice, with some strains more vulnerable than others.
Fullwood et al[84]	C57BL/6	M	3	32.2cm^2/mouse; 64.5cm^2/mouse; 96.8cm^2/mouse; 129cm2/mouse	Body weight, food and water consumption, immunological parameters, mortality,	Cage size did not influence body weight. Mice in smaller cages consumed or wasted more food and water than those in larger cages.	The findings are difficult to interpret as increased plasma glucocorticoid

					adrenal weight, plasma glucocorticoid concentrations	Mice in the smallest cages had greater lymphocyte proliferation, but mice given 64.5cm^2 each had greater natural killer cytotoxicity than those given greater or less space. Mortality increased as more space was provided. In the larger cages mortality was due to bite wounds. In contrast, adrenal weights and plasma glucocorticoid concentrations were progressively greater with less space.	concentrations and adrenal weights are typically considered indicators of stress.
McGlone JJ et al[80]	BALB/cJ	M, F	3 litter mates of same sex	32.2cm^2/mouse; 96.8cm^2/mouse; 129.0cm^2/mouse	Growth rates, mortality, weight, food and water consumption, immunologic parameters, grooming, behavioural parameters	Increased weight gain, sitting behaviours, grooming behaviours and T-lymphocyte proliferative response in females in smallest cages; no mortalities of mice in smallest cages. Necropsy of mice which died in larger cages revealed emaciation, barbering and bite wounds suggesting increased aggression.	Reduced cage floor area (32.2cm^2/mouse) did not adversely affect behaviour, health, immunocompetence or performance in this strain.
McMahon K et al[85]	C57BL/6	F	1 female with pups	20.3cm x 40.2cm (816.06cm^2 total cage size) vs 15.2cm x 25.4cm (386.08cm^2 total cage)	Reproductive performance, microenvironment	Mice housed in larger cages had higher birth rates (9.8pups/female) than those in smaller cages (7.2pups/female). Larger cages had lower ammonia (17ppm) than smaller cages (24ppm), as measured on day of cage change.	Increased cage floor area may increase reproductive performance. The authors suggest that differences in reproductive performance may be due to differing ammonia levels.
Manosevitz M and Pryor[86]	C57BL/6	M,F	1 female with 4-8 pups	Approx 26.7cm x 16.5cm (440.55cm^2) vs 75.6cm x 70.8cm (5352.48cm^2)	Weight, open-field activity and defacation, running wheel activity, exploration, water consumption	Males reared in large cages weighed 12% more than those housed in small cages at 38 days. Animals reared in large cages were 16% more active in an open field. Those reared in small cages defecated 2.2 times more, and had lower water consumption.	Increased cage floor area may be associated with increased body weight, exploration and water consumption. Cage size confounded with cage texture (wire mesh vs plexiglass) and environmental enrichment.
O'Malley J et al[87]	ICR	F	1 female with 5-16 pups or 1 female with litter culled to 6 pups	419.25cm^2 total cage floor area	Faecal corticosterone levels; growth; weaning weights; reproductive performance of progeny.	Growth rates of pups from culled litters (smaller litters) was significantly greater, however when corrected for litter size to account for competition to nurse, growth rate did not differ between pups from intact versus culled litters. Corticosterone levels did not differ significantly between groups nor did reproductive performance of progeny.	Reduced cage floor space per pup is not stressful
Peters A and Festing M[88]	BALB/c, MF1	M,F	6, 10, 35, 36	33cm x 15cm (495cm^2) or 45cm x 28cm (1260cm^2); 33cm^2/mouse; 55cm^2/mouse; 27cm^2/mouse; 37cm^2/mouse	Aggressive encounters, mortality, weight, growth rate, adrenal weight	BALB/c mice gained more weight and had significantly smaller adrenal weights in higher density housing (groups of 35 vs 26).	Reduced cage floor area may reduce anxiety and aggression. Cage size confounded with population density. The difference between 27cm^2 and 37cm^2 may be too small to reveal any adverse effects[69].
Sherwin CM[89]	CB57	F	4	37cm x 21cm (777cm^2) + additional space	Preference for additional space	Mice worked to gain access to additional space, despite increasing costs.	Mice did not show a preference for a particular amount of

				(29x11cm or 319cm^2; 37x21cm or 777cm^2; 50x32cm or 1600cm^2)			additional space over another, thus additional space was an important resource but quantity was not. Findings may indicate true preference for additional space for its own sake. May also be a refuge from other mice or territorial monitoring.
Sherwin CM[90]	TO	M	1	27cm x 10cm (270cm^2) with a range of additional space available (196cm^2 to 1600cm^2)	Preference for additional space	Mice worked to gain access to additional space, despite increasing costs.	As above.
Sherwin, CM[91]	C57BL/6	F	4	Enriched cage 50cm x 32cm (1600cm^2) + additional space 37cm x 21cm (777cm^2)	Preference for additional space	Mice worked to gain access to an empty cage despite being housed in an enriched cage containing cagemates, food, water, nesting material, shelter, cardboard tube, chew sticks and running wheel	As above.
Smith A et al[71]	C57BL/6J	M,F	4-20	Cage size 333cm^2 or 728cm^2 with 20.6cm^2 per mouse – 77.4cm^2 per mouse	Injury, hair loss, aggressive behaviour, survival, body weight, food and water consumption, cage microenvironment, urine testosterone concentration	Ammonia concentrations exceeded limits at 20.6cm^2 although mice had microscopically normal nasal passages and eyeballs. All parameters within normal limits when mice housed at 36.1cm^2 or above.	Reduced cage floor area not associated with adverse effects.
Smith A et al[72]	BALB/cJ, NOD/LtJ, FVB/NJ	M,F	4-20	Cage size 333cm^2 or 728cm^2 with 36.1cm^2 per mouse – 83.2cm^2 per mouse	Injury, hair loss, aggressive behaviour, survival, body weight, food and water consumption, cage microenvironment, urine testosterone concentration	FVB/NJ displayed early onset aggression with reduced floor space; no apparent deleterious effects on BALB/cJ or NOD/LtJ strains.	Reduced cage floor area not associated with adverse effects in 2/3 strains, but associated with increased aggression in one strain. Early onset aggression may be an age effect as investigators were unable to source sufficient numbers of 3 week old mice so ages ranged from 3-5 weeks; alternatively this strain may be highly sensitive to variation in cage floor area.
Van Loo PLP et al[92]	BALB/cAnNCrlBr	M	3, 5 and 8	80cm^2/mouse or 125cm^2/mouse	Frequency of attack, latency to attack, urine corticosterone levels, food and water intake, weight, number of wounds, tyrosine hydroxylase, organ weight	Larger cages associated with moderate increase in aggression, with aggression considerably higher in groups of 8 animals compared with groups of 3. Dominant and subordinate mice demonstrated different stress levels.	Increased cage floor area may be associated with increased aggression. Aggression may be increased at lower population densities where available space can be defended. Decreasing floor size may be used as a temporary measure to reduce high levels of aggression in an existing group of male mice, but group size should be kept to 3-5 animals.

Whitaker J et al[93]	C57BL/6Tac	M,F	3 adults plus 1-20 pups	208.3cm^2 or 315cm^2	Litter size, litter survival to weaning age, average pup weight at 7, 14 and 21 days, and number of days between litter births. Male and female performance in elevated plus maze test, open field assay and acoustic startle test before and after an intraperitoneal saline injection.	Cage size had no significant impact on reproductive parameters and inconsistent effects on behaviour in weaned pups.	No significant difference between mice housed in standard cages and cages that are 50 per cent larger. Enrichment provided in this study (nestlet and PVC tunnel in all cages) may have masked effects of cage size on reproduction and behaviour[69].

Cage Height and Cage Lid

Principles

(i) Normal behaviours of mice include standing on their hind limbs and stretching, sitting on their haunches and grooming, and climbing. Where cage design permits, climbing is a regular component of locomotor activity. Buttner96 found that mice invested more time in climbing on the cage lid than locomotion on the ground. Climbing onto an in-cage shelter is also an important locomotor activity in mice

(ii) Evidence suggests that climbing on the cage lid is important for mice, as it may be a positive natural behaviour associated with exploration of the home environment. However, this is controversial, as one survey found a correlation between increased levels of stereotypy and increased levels of climbing94. The authors noted that this is unsurprising as many documented mouse stereotypies, such as gnawing, circling and somersaulting, tend to occur at or on the bars of the cage lid. They conclude that high levels of climbing may form an integral part of certain stereotypies. In the same study, climbing was associated with increased physical injuries, yet it was also associated with a reduction in the incidence of obesity. An earlier study found that wire-gnawing and jumping (both stereotypies) developed from climbing behaviours99. The same authors found that preventing stereotypic wire-gnawing had no significant effects on chronic measures of stress, therefore they concluded that this behaviour did not reduce stress100. However, another study found that C57BL/6 mice that were prevented from climbing on bars (those housed in a cage with a plexiglass lid) from age three to seven weeks exhibited altered fear responses and impaired fear-motivated associative learning in behavioural tests compared with controls101. Females were particularly sensitive to thwarting of lid-climbing behaviour, demonstrating increasing anxiety levels in an elevated plus maze, hyperactivity in an open field, reduced condition freezing and reduced prepulse inhibition. The authors described this as a “complex syndrome of anxiety and psychotic-like symptoms.” Therefore, based on current knowledge, cage height should not be increased to such an extent that it prevents access to bars rom which mice frequently hang, as this would thwart normal behaviour4. Male and female mice were able to reach and climb on bars that were 19cm off the cage floor101.

(iii) Mice housed in individually ventilated cages have limited climbing opportunities compared with mice housed in conventional wire gridtopped cages. While the provision of a wire grid in individually ventilated cages did not appear to compensate for housing effects on spontaneous behaviour, sensorimotor behaviour and fear learning, it did improve response in fear-potentiated startle tests in singly housed B6J males102.

(iv) Where mice are provided with a food-hopper built into the cage lid, they may utilise this to nest beneath.

(v) Information on the height requirements of mouse caging is scarce and many recommendations are based on available products. According to Article A of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123)1, the minimum height of cages in which mice are housed should be 12cm. This is slightly lower than the US National Research Council Guidelines for the Use of Laboratory Animals, which stipulate that cages have a minimum height of 12.7cm73.

Recommendations

The height of cages should allow mice to stand on their hind legs, stretch up fully and climb on the bars of the cage lid. This height does not need to be provided over the entire area of the cage. The cage lid should incorporate a grid section which will allow the animals to climb. The cage height should allow for provision of enrichment.

Where cages are fitted with platforms or in-cage shelters, the distance between the top of the platform or in-cage shelter and the top of the cage should be sufficient to allow mice to climb on top of the platform or in-cage shelter.

While cage height (over part of the cage) should allow for upright standing behaviour, food and water should be accessible at a level that allows mice to sit while eating and drinking. Until further evidence relating to the height of the cage becomes available, it is recommended that mouse cages are a minimum of 12cm high. 2.3.6 The design of the cage lid should facilitate climbing.

Cage shape

Principles

(i) To date there are no studies investigating the impact of cage shape alone in mice. For example, in one study mice preferred the more square-shaped enclosure but this differed from the other enclosures in its height, opacity and the presence of a shelter103. In another study, mice of different strains housed in the square-shaped Cambridge cage produced more young per female than their standard-housed counterparts104. It should be noted, however, that aside from a different cage-shape, the Cambridge cage incorporates other forms of environmental enrichment including shelter material which may be perceived to be more important to mice than the shape of the cage per se.

(ii) Mice exhibit thigmotaxis and may spend much of their time in contact with the wall of a cage. Thigmotaxis is used as a measure of emotionality (anxiety and fear) in mice, and some strains are more thigmotactic than others105. This should be taken into account when selecting cage shape.

Recommendations

There is no clear evidence of preference among mice for a particular cage shape. Evidence indicates the contents of the cage is more important than cage shape.

Until further evidence comes to light the use of rectangular or square shaped cages is appropriate for mice.

Cage materials

Principles

(i) Cages must be constructed from non-toxic, non-absorbable material that can be cleaned (autoclaved). They must be escape and predator proof.

(ii) Ideally, caging material should be resistant to heat and chemicals, inexpensive and durable106. Most mouse cages today are solid tubs made from plastics such as polypropylene (opaque), polycarbonate, polysulphone and polyetherimide (transparent). Other cage materials include polystyrene and p

lyphenylsulfone. Wood is not a suitable material as rodents tend to chew it. Unless coated with an impervious finish, wood also tends to soak up urine and is extremely difficult to clean.

(iii) Recent studies have shown that some synthetic materials release bioactive substances that may affect mice. For example, high temperature polycarbonate (polyphthalate carbonate) cages and water bottles damaged by one-off washing in a harsh alkaline quarternary ammonium detergent released bisphenol A (BPA), an oestrogenic compound that led to increases in meiotic disturbances, including an 8.3 fold increase in aneuploidy and a 20-fold increase in chromosome misalignment in mice 106, 107. In addition, there was an increased frequency of mortality in young (one- to four-month-old) mice during the period of maximal exposure. In the months following exposure, investigators noted an increase in reproductive tract tumours in exposed mice. In this report the detergent caused visible, progressive damage to the cages including change in colour from yellow, becoming initially slightly crazed, before turning opaque, then whitish and rough, and finally sticky and bubbly106. Water bottles were slower to deteriorate, possibly due a protective effect of water. However, visible damage is not an accurate indicator of the amount of BPA leaching from exposed materials107.

(iv) Laboratory mice housed in polycarbonate and polysulfone cages are exposed to BPA via leaching, with exposure levels highest in older cages108. Bisphenol A hydrolyses and leaches from polycarbonate products under heat and alkaline conditions, with the amount of leaching increasing with use. Significant levels of BPA (up to 310μg/L) were leached from used polycarbonate cages placed in water (neutral pH) at room temperature108. In addition, detectable levels of BPA were released from new polycarbonate cages (up to 0.3μg /L) as well as new polysulphone cages (1.5 μg/L), while no BPA was detected in water incubated in glass or used polypropylene cages. Pre-pubescent female CD-1 mice subjected to BPA by being housed in polycarbonate cages had a 16 per cent increase in uterine weight compared with mice housed in used polypropylene cages, although the difference was not statistically significant.

(v) While normal care and use of some synthetic cages can result in leaching of BPA, exposure to a basic detergent, continued use of cages and/or water bottles beyond the manufacturer's recommended shelf life or high concentration of corrosion-controlling amines in autoclave steam are events which may exacerbate cage and/or water bottle damage and increase the risk of BPA leaching106. Cages may also be damaged by banging the plastic against a hard surface (for example when removing soiled, stuck bedding); over-stacking (mouse cages stacked more than 15 high); washing in hard as opposed to soft water; heating or autoclaving cages that contain debris or disinfectant residue; and use of amine corrosion inhibitors in steam sterilisation systems109.

(vi) Cage materials can affect the microclimate by modifying light and heat exchange. Opaque cages have the advantage of filtering out harmful glare and allowing mice to hide from humans. They have the disadvantages of impeding the observation of mice from outside the cage (thereby necessitating more disruption to check mice), restricting mice's vision of activities outside the cage (including humans and other mice), and blocking the passage of light, resulting in different light levels in boxes at different levels on cage racks. Transparent cages have the advantage of allowing observation of mice from outside the cage. They have the disadvantage of not allowing mice to hide as effectively from humans and high light intensities. Heat is well preserved in solid plastic tubs, such as polypropylene and polycarbonate.

(vii) A change in cage materials may affect the breeding performance of mice. For example, the number of young weaned by CBA does transferred from opaque to transparent cages was lower110. These changes may be transient, as the number slightly increased in the second generation. In another study, inbred BALB/cW, DBA/2W, RIII/W, C3H/A, C57BL/W, BN/a and BN/b mice transferred from wooden to plastic cages showed a decrease in productivity for one to two years, followed by a gradual increase111. Q values

(number of young weaned/prenatal days x 100) for most inbred strains were in fact higher at the end of the study period. For this reason it is important to be consistent in the cage materials used throughout a study.
(viii) Cage materials may impact on mouse body composition. For example, male mice transferred from aluminium to other identical metal cages had a body fat percentage of 21.8 per cent at fourteen weeks of age, compared to 13.6 per cent in male siblings transferred into polypropylene cages112. However, the study confounded cage type with cage volume, light penetrance and the presence of aluminium sulphate, each of which independently varied body fat to some extent. In another study, male C3H/HeJ mice housed in polycarbonate cages showed a consistent trend to higher body weights than those kept in stainless-steel wire mesh cages113. This may have been due to variation in temperature between the cages.
(ix) Behaviour and physiology of mice may be affected by cage colour. Female CBA mice consistently showed a significant preference for white cages over black, green and red cages114. The colour of the home cage strongly influenced behaviour, with mice from white home cages having the highest food consumption, lowest body weight and least anxiety (as evaluated in the Elevated Plus Maze test) than those originally housed in black, green and red cages. The colour of the cage that the animals were born in may have influenced their later behaviour (C Sherwin pers. comm.).

Recommendations

Cages should be constructed from non-toxic, non-absorbable material that is easy to clean. Untreated wooden cages should not be used. Cages should be durable, resistant to heat and chemicals, and escape and predator proof.
Worn or damaged cages and/or water containers should be replaced. Leaching of bisphenol A from polycarbonate and polysulphone cages and water containers is likely if these are washed with strongly alkaline detergents or sterilised in the presence of high concentrations of corrosion inhibiting amines in autoclave steam. Exposure of mice to bisphenol A (even at low levels) should be avoided, particularly in reproductive studies.
Colourless, tinted transparent cages or white opaque cages are preferable for mice. Unless required for a study, cage colour should be consistent throughout the facility.
Cages should be handled and maintained to minimise damage. For example, cages should not be hit or banged against hard surfaces or stacked more than 15 cages high. Plastic cages and bottles should be washed in hot (60-66°C), soft water with a manufacturer-recommended detergent solution. All residue must be removed prior to autoclaving as this may be baked onto the cage except where sterilisation is required to ensure decontamination of waste and prevent zoonosis.

2.6 Cage flooring

Principles

(i) When given a choice between bedding material on a solid floor and a wire mesh floor, mice preferred the former115, however preference was affected by ambient temperature75 (see Section 4.3 Temperature). Similarly, when provided with synthetic gauze pads, group-housed male and female B6C3F1 and individually-housed male CD-1 mice in stainless-steel ventilated cages with wire mesh floors preferred to rest on the pads116 117.
(ii) Housing mice on wire mesh floors can be detrimental to their health and well-being. In a 2 year feeding study, significantly fewer BC63F1 mice housed on wire mesh floors survived to the end of the study compared with those housed in solid floored polycarbonate cages, irrespective of diet, sex and whether they were individually housed118.
(iii) While female B6C3F1 mice housed in suspended wire cages with a flooring grid of 2mm round intersecting stainless steel wires with mesh gaps measuring 8mm x 8mm did not show cage associated

differences in clinical signs, body temperature, grasping power of fore and hind-limbs, tail flick latency or motor nerve conduction velocity than their solid-floor housed counterparts, they exhibited a significant decrease in body weight and serum triglycerides than their solid-floor housed counterparts119.

(iv) Housing mice on wire mesh floors is associated with mouse urological syndrome (MUS), a potentially fatal inflammatory condition of the urinary tract. In one study, all entire male AKR/NCrIBR mice housed in suspended wire cages or raised wire floors for a period of sixteen weeks developed MUS, compared with none of their solid-floor housed counterparts120. While the researchers did note that this strain was highly susceptible to MUS, they found that MUS occurred in B6C3FI/CRlBR and NIH Swiss strains, albeit at a lower incidence (6 per cent and 21 per cent respectively) housed in suspended wire cages. None of the mice kept on a solid floor with bedding got MUS.

(v) Neonatal pups may slip through large-spaced mesh (1cm x 1cm)121.

(vi) Because wire mesh floors are open they allow dissipation of heat from the bodies of mice and may thus influence thermoregulation. When given a choice, mice housed on a wire mesh floor chose an ambient temperature of 28°C75. Thus the cage temperature for mice housed on a wire mesh floor may need to be higher than for mice housed on a solid floor (see Section 4.3 Temperature).

Recommendations

Solid floors are recommended for mouse caging. Wire mesh floors should not be used for mouse caging without express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such flooring. In such cases, a solid floor section sufficient to accommodate all of the mice and nesting material should be provided. The size of the mesh gaps should not exceed 8mm x 8mm (See also 3.3 Metabolism Cages).

Bedding

Principles

(i) Mice have a behavioural need to burrow and are highly motivated to do so 122,123. Burrowing behaviour persists even in the presence of a previously built system of burrows or shelters123-125. Furthermore, the number of burrowing bouts increased, rather than decreased, as burrows were constructed123. Young male TO mice preferred to sleep in sawdust than make use of a selection of pre-fabricated shelters (tubes) to sleep in125.

(ii) Deep bedding provides opportunities for digging and burrowing behaviours and mice spend a significant amount of time digging in such bedding when provided126. When digging, mice tend to alternate between digging with their forepaws and kicking back with their hindpaws32

(iii) Bedding material may allow mice to perform selective soiling behaviour. Male TO mice preferred to defaecate on floors covered with sawdust bedding than on a bare plastic floor74.

(iv) Ideally bedding should be non-toxic, free of dust, microbial, parasitic or chemical contaminants, atraumatic, moisture absorbent and ammoniabinding127. In addition it is desirable for bedding to be inexpensive, readily available, easy to store, easy to use and easy to dispose of11.

(v) There is evidence that the size and manipulability of bedding material are the main determinants of selection by mice. When given a choice between ten commercially available bedding products, pregnant ARS Ha (ICR) Swiss mice most commonly chose a combination of materials, with products of wood origin overwhelmingly preferred128. When offered a choice, male ICR mice chose soft bedding that allowed them to hide and build nests129. In another study, male ICR mice preferred cloth bedding over recycled paper, wood shavings and paper130. Female C57BL/6JIco and BALB/cBYJIco mice prefer bedding consisting of relatively large, rough, fibrous particles over sawdust115.

(vi) Pregnant CF-1 mice preferred sawdust bedding over commercial, deodorised cellulose131.

(vii) Bedding may impact on aggression. Lawton *et al* found that adult MF1 Nu-Nu males housed on thick corn cob bedding were less aggressive than those housed on fine grade corn cob, hemp, sawdust or aspen chip132. Aspen chip appeared to increase aggression in this strain.

(viii) Small bedding particles (<300µm) may irritate or damage the airways of mice127. For example, vermiculite bedding was reported to cause histological changes in the lungs of mice, reduced body weight and lead to fewer litters133. Small particles may also irritate and traumatise the vaginal or preputial mucosa115.

(ix) Variations in absorbency of bedding can affect in-cage humidity, temperature and ammonia levels via concentration of urease-producing bacteria which convert urea into ammonia. For example, relative humidity in cages containing male NOD/LrJ mice varied significantly depending on the type of bedding used134. This is a potential source of experimental variability. A comparative study of absorbency of six commonly used bedding materials found that a product derived from corn cob had higher absorbency per unit volume than one made from wood pulp461.

(x) Some bedding materials contain chemical compounds which can impact on mouse physiology and the response of mice to pharmacological agents. These compounds can enter the experimental model via direct ingestion of bedding, inhalation of volatiles or inhalation of dust particles135. Bedding made from hard and softwoods contains organic compounds such as tannins, alkaloids, lignins and resins that may impact on experimental results and even constitute a health hazard to mice and those working with them135. For example, some softwood products derived from Red cedar (*Juniperus virginiana*), Ponderosa pine (*Pinus ponderosa*), White pine (*Pinus strobus*), Scots pine (*Pinus silvestris*) and Douglas fir (*Pseudotsuga sp.*) induced changes in hepatic enzymes involved in drug metabolism in both mice and rats 136-142 resulting in altered drug metabolism and increasing the incidence of spontaneous tumours139. Some of these also altered barbiturate-induced sleep times139, 140, 142-144 and/or demonstrated cytotoxicity145. In addition, the liver-to-body-weight ratio of mice exposed to red cedar bedding was significantly increased compared to mice exposed to the other beddings140. It should be noted that not all strains were affected to the same extent142. Industrial-derived wood contains antifungal and insecticidal agents which are also potentially toxic145.

(xi) Pelkonen and Hanninen146 examined the cytotoxic and enzyme-inducing effects of a variety of types of bedding from different parts of the world, finding a 200-fold variability in the hepatocyte toxicity of commonly used bedding materials. Pine shavings were generally found to be highly cytotoxic (although the least cytotoxic of these was from Australia) (see also 145). Extracts of corn-cob, rice hulls and straw were found to be minimally toxic. Corn-cob extracts were practically devoid of inducers, whereas straw, rice-hulls and sugar cane based beddings had enzyme inducer activity comparable to the hardwoods. The authors recommend avoidance of softwood bedding, concluding that hardwoods were less problematic than softwoods, and grass-based bedding was better still.

(xii) Where wood-derived bedding is utilised, investigators should be familiar with the species of tree from which it is sourced and the manufacturing process, as well as its potential impact on the biological system and experimental outcomes135. This requires knowledge about naturally occurring compounds present in the bedding that may impact on mouse physiology as well as likelihood of treatment-induced compounds in the bedding that may impact on mouse physiology.

(xiii) Paper typically has a low cytotoxicity and inducer activity141, 145. Recycled or bleached paper products including paper towel had a higher cytotoxicity145, 147 and enzyme inducing activity147 than unbleached pulp. Analysis of telephone book strips found that while this bedding was minimally cytotoxic compared to other wood-derived bedding materials, it had quite high enzyme-inducer activity (comparable

to pine)146. The latter may have been due to the presence of ink or the use of polyhalogenated compounds during manufacture.

(xiv) Treatment of bedding may alter properties of bedding including toxicity. Autoclaving bedding material did not alter barbiturate-induced sleep times or liver:body weight ratios140 nor did it appear to impact on the enzymeinduction properties of bedding145. In fact, potentially toxic compounds could form during treatment (eg heat treating or steam sterilisation) of bedding135.

Recommendations

Bedding should be provided in mouse cages and should be present in sufficient quantity to cover the entire floor. The depth of bedding required will vary with the type of bedding used, the number of mice in the cage and frequency of cleaning. Ideally mice should be able to dig, if not burrow. As a guide, the depth of the bedding should be a minimum of 2cm. Bedding should produce a minimal amount of dust and consist of particles that lend themselves to manipulation by mice. To reduce experimental variability, particularly where pharmacological experiments are concerned, the use of a single type of bedding is recommended. Autoclaving of bedding is recommended to reduce the potential for microbial contamination. It should be ensured (for example by consulting the manufacturer) that toxic compounds are not formed during treatment of bedding. Softwood-derived bedding should be avoided. Paper, grass-based or hardwood material should be utilised instead. Vermiculite bedding or other bedding with small particles should not be used due to the potential for irritation of the mucosal membranes and other health problems.

Mouse care and management

The social environment

Principles

(i) Mice are social animals and should, wherever possible, be maintained in stable, harmonious social groups181. Mice have a strong preference for each other's company. When given a preference, male BALB/cAnNCrlBR mice preferred each other's company to individual housing, irrespective of social status or kinship182, 183.

(ii) Strains may differ in their degree of social affiliation. For example, one study found that DBA mice were more likely to stay close to cage-mates than C57 mice184. This impacted on behaviour in open field tests: C57 mice exhibited increased exploratory behaviour when alone, whereas DBA mice showed increased exploratory behaviour when in groups.

(iii) Aggression between male mice is a well-recognised problem in laboratories world wide185 . Aggressive behaviour may be due to offensive, defensive or predatory motivation, competition for resources, or a mixture of these17. Aggression levels vary markedly with strain. For example, outbred Swiss CD-1 mice exhibited higher levels of inter-male aggression, inter-female aggression, maternal aggression and infanticide than other strains186. Environmental and husbandry factors may exacerbate agression185 (see especially Sections 2.10 Cage Dividers, 3.5 Environmental Enrichment, 4.7 Cleaning).

(iv) Isolation may exacerbate aggression. Individual housing of male mice followed by group housing reliably induces aggression in many strains33, 63, 187. For example, individually-housed male DD/S mice changed to group-housing showed an increased tendency to fight, when compared with their permanently group-housed counterparts

(v) Pair-housing of male mice is not recommended, as the subordinate mouse may be frequently exposed to attacks and suffer subsequent stress and injuries181.

(vi) Behaviour exhibited by dominant mice includes attacking, tail rattling, chasing, biting and adopting a side-on offensive posture181. Conversely, subordinate mice exhibit behaviours such as flight, hiding and freezing. Subordinate mice do not initiate attacks. Where conflict leads to injury, it may be necessary to

remove the dominant mouse181. Identification and removal of dominant male Crl:CD-1 mice lead to a 57 per cent reduction in the number of mice reported for clinical signs, euthanasia and death181. When removed dominant mice should be housed in another room as their urine may stimulate aggressive behaviour in dominants housed in the same room181. Nesting material should be supplied as this may partly compensate for deprivation of social contact165.

(vii) For males unable to be housed with other male mice, ovarectomised females may be suitable companions as they do not induce male behavioural change through copulation or courting behaviour189 and production of unwanted progeny can be prevented190. However, the welfare of the male must be weighed against the welfare of the female, who must undergo major abdominal surgery.

(viii) Female mice may exhibit maternal aggression, attacking both defensively and offensively17. Interfemale aggression may also be stimulated by male urinary odour. For example, virgin Swiss albino females individually housed for 24 hours in a cage previously inhabited by a male showed increased levels of attack and mounting of same-sex intruders17.

(ix) Previous social experiences influence aggressive behaviour. Thus Swiss mice that had repeatedly defeated conspecifics showed increased offensive aggression towards intruders than those without positive fighting experience191.

(x) There is evidence that keeping siblings together may reduce aggression and improve well-being. There were no physiological or behavioural differences detected in dominant or subordinate male Swiss CD-1 mice grouped in same-sex sibling groups from birth192. The authors argue that what is stressful for the mice is not group housing in itself, but a lack of familiarity or relatedness with respect to cage-mates. Therefore grouphousing of same-sex siblings from birth may be used to reduce the risk of detrimental aggression between mice. Male and female mice from litters that had been combined with other litters experienced a marked decrease in weight gain in comparison to undisturbed litters, regardless of litter size193.

(xi) The age at which mice are grouped may impact on behaviour and physiology. For example, 26 to 28 day old male Swiss CD-1 mice mixed into groups of five to six animals from different litters exhibited higher levels of aggression, smaller preputial glands and marked reduction of neophobia in a free exploratory paradigm than controls which remained in same-sex littermate groups since weaning, and those grouped after puberty194.

(xii) Brief periods of isolation, such as those that may occur during husbandry or experimental procedures, may not alter dominant/subordinate relationships. For example, dominant/subordinate relationships between pair-housed Swiss male mice remained unchanged following individual housing for periods of 6 to 12 hours191.

(xiii) Optimal population density depends on a number of factors including strain, age, gender, experimental duration, genotype, degree of inbreeding, previous social experience of mice, familiarity of mice with one another, experimental procedures and the order in which animals are tested69. Male BALB/c Crl mice housed in a stainless steel cage with a floor area of 390cm2 had significantly elevated plasma corticosterone levels and decreased initial peripheral lymphocyte count when housed in pairs or groups of eight, compared to those housed in groups of 4195.

(xiv) The level of aggression between male mice can be influenced substantially by group size, and cage size with aggression increasing with group size and a cage size allowing 125cm2 per animal 92.

Aggressive
behaviour in group-housed male BALB/c mice was best prevented by housing the animals in groups of three to five with a cage size allowing 80cm2 per animal.

(xv) Social structure in female laboratory mice depends on the number of animals per cage. Dominant female mice had significantly lower corticosterone plasma levels than subordinates196. This study found that groups of three or five females were much more stable than pairs or groups of four. In groups of three or five, all females had a sufficient number of social contacts. The dominant female was able to reduce her contacts (for example pushing other mice away) to a minimum, while subordinate females stayed in close contact with one another.

(xvi) High population density has been associated with deleterious effects, although some strains may be more vulnerable than others. In one study comparing weight gain, plasma corticosterone, behaviour and immune parameters in female BALB/c and C57BL/6 mice housed at population densities ranging from 2-10 mice per cage (484cm2 total cage floor area, or 48.4-242cm2/mouse), high density housing had more deleterious effects on BALB/c mice197. Thus BALB/c mice housed at ten animals per cage gained less weight, had higher corticosterone levels, spent more time in the outer portion of the open field and had fewer entries into the open field area than those housed at two animals per cage. Furthermore, helper T (CD4+) cells were lower in BALB/c mice housed at ten per cage. C57BL/6 females housed at ten per cage showed less exploratory behaviour than those housed at two per cage, but other parameters were unaffected.

(xvii) Other adverse effects associated with high population density include:

□□Suppressive effect on granuloma formation when compared to individually housed controls (albino male mice, strain not specified; population density five adult mice in a single 17.8cm x 25.4cm cage)198;

□□An increase in plasma cholesterol and lipid levels, and increased severity of fatty lesion (atherosclerosis) development, in female C57BL/6 mice199 (it should be noted that group sizes in the study ranged from one to five mice, but cage size was not specified);

□□Reduced brain weight in C57BL/10 male mice200; □□Tail dermatitis with possible self trauma in male and female C3H/HeJ mice201. The incidence of these lesions was 4 per cent in breeding pairs but 21 per cent in weaned mice housed in groups of 40. Incidence was lowered among weaned mice kept in groups of 40 in large cages with sexes separated, but healing of the lesions occurred when mice kept in groups of 40 were separated into groups of five in smaller cages.

(xviii) A number of published studies in mice indicate that housing them within visual, olfactory and/or auditory contact of predators, including rats, is stressful202, 203:

□□Four strains of mice (BALB/c, C57BL/6, CD-1 and Swiss-Webster) exposed to a rat through a wire screen demonstrated varying degrees of defensive behaviour including freezing and avoidance204.

□□Group-housed BALB/c mice housed in a room containing rats had increased levels of sympathetic neurotransmitters when compared with controls205. In the same study investigators found that there was a greater increase in sympathetic nervous system activity in individually housed mice exposed to rat odour, suggesting the problem was compounded by the stress of isolation.

□□Chronic exposure to auditory and olfactory cues from rats affected both sucrose intake and behaviour in an elevated plus maze in male CD1 mice206. In this particular study, housing mice in the same room as rats caused such a degree of stress that it reduced their sensitivity to a reward (sucrose) and prevented habituation to the elevated plusmaze.

□□In another study, olfactory and visual exposure to rats elicited anxiety responses in male BALB/cByJ and C57BL/6ByJ207.

□□As with other stressors, exposure to rats can alter immune parameters. For example, group housed male CD-1, BALB/cByJ and C57BL/6ByJ mice given visual and olfactory exposure to rats for a fifteen minute period had reduced macrophage activity and natural killer cell

cytotoxicity208.

□□Exposure to rats reliably provoked an increase in urination and defaceation, as well as fear-associated behaviour including startle response and freezing. Similarly, exposure of BALB/c mice to cat odour resulted in fearful behaviour including reduced locomotion, reduced rearing behaviour and moving away from the odour209.

Mice housed in standard (as opposed to enriched) cages also exhibited higher levels of plasma corticosterone following exposure to cat odour. Male ICR mice exposed to cat urine odour for 58 days failed to habituate to the scent and became more aggressive when compared to mice exposed to rabbit urine or water over the same period of time210. One study in female C57BL/6 mice found no long-term changes in physiological parameters when Wistar rats were introduced into the room with the mice however the authors comment that precautions are necessary in drawing conclusions from the results as the stress response in mice to the presence of rats appears to be context dependent and may differ between genders and, or strains464.

Recommendations

Mice are social animals and should, wherever possible, be maintained in stable, harmonious social groups. Groups of mice should be monitored to ensure social stability as well as the detection of behavioural and physiological abnormalities. There are situations, for example studies involving highly aggressive strains, where group housing is not suitable. Pair housing of male mice is not recommended due to a high probability of aggression. Ideally mouse groups should consist of littermates of the same sex. Mice should be grouped with each other before they reach puberty to minimise aggression between unfamiliar individuals. As a guide, the optimal size for a group of adult mice is three to five for females and three for males. However, in determining group size, factors such as differences between individual animals, strain, sex, cage size and experimental design should be taken into account. Therefore the scientific literature should be consulted when determining the optimal housing for particular strains and animals must be monitored. The disruption of established social groups can cause aggression and should be avoided unless absolutely essential. Separation of cage mates should be limited to less than 24 hours. Mixing adult males from different groups in the same cage should be avoided. Where it is necessary to mix unfamiliar adult males, they should be exposed to each other before they are mixed together. This can be achieved by placing the newcomer into an adjoining cage to allow visual, auditory and olfactory contact with the other male. They should also be closely monitored after mixing to check for aggression. Nesting material should be provided to minimise conflict. Following cage cleaning, for sentinel or breeding cages, nesting material should be transferred from the old to the new cage to minimise aggression (see Section 4.7 Cleaning). Mice should not be housed in the same room, or within auditory, olfactory or visual contact, with predatory species including rats and cats and staff should take care not to transfer scents from predatory species into the mouse room.

Handling (neonates)

Principles

(i) Handling neonates produces effects that may persist through the animal's life263. In rats, the most likely mediator for handling effects is increased maternal care (licking and grooming) following the return of stressed pups to the nest264, 265. In a mouse study, maternal care was not affected by neonatal handling in a highly-aggressive strain (NC900), but it was significantly augmented in a low-aggressive strain (NC100)266.

(ii) The effects of neonatal handling can vary between strains and housing systems. In one study, handling involved placing the entire litter in an opaque plastic beaker for 60 seconds once every 48 hours from day

three postpartum until weaning at 21 days266. Handled NC100 mice had reduced corticosterone levels compared with handled NC900 mice and nonhandled controls. Handled mice of both strains showed an up-regulation in dopamine receptors, with the effect increased in group-housed males. DBA/2 pups removed from their nest, put in a container and replaced back in the nest from day 1 to 24 had significantly reduced survival time following intraperitoneal inoculation of leukaemia cells267. However, there were significant differences between handled and non-handled BALB/c pups when a similar protocol was used268.

(iii) Regular handling of neonates may lead to habituation. Male CD-1 mice removed from their cage, weighed and injected with saline from days 2 to 19 of age showed increased latencies in nociception tests at 35 days of age when compared with non-handled controls263. At days 80 and 140, an increase in body weight was noted.

(iv) Handling of mouse pups can influence neural development, immune parameters and behaviour. Neonatal mice removed from their home cage and exposed to clean, unfamiliar bedding in the absence of their mother for 15 minutes daily for the first two weeks of life demonstrated increased exploratory behaviour and less fearfulness compared to control mice exposed to home cage bedding in the absence of their mother269. CD-1 mice handled for fifteen minutes daily from post-natal days 2 to 14 inclusive exhibited increased nerve growth factor levels and did not respond to an anxiolytic drug (chlordiazepoxide) when confronted with acute, novel stress270. Effects may be delayed. For example, C3H/St pups handled daily from birth to weaning showed no differences in splenic-B and T-cell proliferative mitogen responses at day 21, however they exhibited enhanced humoral and cell-mediated immunity as adults271.

(v) Early-weaning may lead to increased anxiety and aggression in mice272. Male and female BALB/c mice weaned early (at 14 days as opposed to 21 days) had higher levels of anxiety when compared with controls weaned at 21 days273. Early weaned males sustained more fight wounds when regrouped after isolation when compared with controls.

Recommendations

Investigators must be aware that handling of neonates can have a long term impact on the welfare of animals that persists throughout their lives. Handling of neonates should only be performed where necessary and must be performed consistently across a subpopulation or population of mice to minimise experimental variability. Where neonates are handled, handling must be performed quietly and gently. Early weaning of mice (prior to 21 days of age) should only be performed with permission from the Animal Ethics Committee.

Monitoring of mice

Principles

i) Mice are affected by their living conditions, including their physical environment, their social environment and their interaction with humans. When assessing the responses of mice to their living conditions, assessment of physiological and behavioural parameters are useful. Negative trends in these parameters, such as loss of body weight, failure to reproduce and changed behaviour patterns may indicate that mice are distressed and failing to cope with their environment467.

ii) The well-being of prey species including mice can be difficult to assess due to instinctive masking of signs of physical compromise or injury332, 333. In addition the speed with which mice move, their small body size, propensity for burrowing and nocturnal activity compound this difficulty333. Recognition of signs of pain or distress requires sufficient time for observation of an animal or group of animals. As mice are nocturnal the full range of wake-hour behaviours are best observed at night using minimal illumination (see Section 4.2.1 Light intensity). It may be necessary to observe mice in such a way that they are unaware of the presence of an observer, for example by using a camera or recording device334.

iii) Mice should be monitored for signs of pain. As defined by the International Association for the Study of Pain (IASP), pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"335. Pain is a subjective experience that we can detect in animals that exhibit a behavioural response to pain. According to the Federation of European Laboratory Animal Science Associations (FELASA), all mammals including mice may be assumed to perceive and experience pain and remember situations associated with pain sensations336. Alternatively, pain has been defined as "an aversive sensory experience that elicits protective motor actions, results in learned avoidance, and may modify speciesspecific traits of behaviour, including social behaviour.337" Pain is a source of variance in experimental results due to a range of biochemical responses (e.g. neurotransmitter, hormonal) elicited. Animals in pain are therefore often poor research subjects.

iv) Use of cage side assessments of behaviour, appearance and demeanour may be more useful for immediate identification and treatment of pain than retrospective observations such as body weight change. Behavioural changes may give an early indication of pain or that something is wrong with a mouse's well-being. Changes that are subtle and non-specific should not be overlooked333. Signs of pain, discomfort and/or distress in the mouse include but are not limited to: reduction in faecal/urine output; reduction in food/water intake; abnormal gait; vocalisation; rubbing, scratching or chewing at a surgical site or wound; reluctance to move; restlessness; pacing; hunched posture; unusual sleeping position (for example stretched out on one side); social withdrawal; head-pressing; poor grooming/rough hair coat; piloerection; weight loss; increased or laboured respiration (may manifest as open-mouth breathing, pronounced chest movements); porphyrin discharge around eyes and nose336; blood or saliva in bedding; or change in behavioural repertoire246, 330, 333, 338. Other signs include alterations in core body temperature and heart rate; increased faecal glucocorticoid levels; reduced interaction with conspecifics; reduced exploratory and grooming behaviours65; reduced use of nesting material; irritation at injection sites; ptyalism (excessive salivation) and grinding teeth332, 338. Mice experiencing pain may attempt to bite when handled333. Vocalisation may indicate acute pain, but its absence in the face of a painful stimulus should not be interpreted as absence of pain or distress339.

v) Changes in nest building behaviour have been reported to be sensitive early indicators of distress or illness in mice. Thus male HsdHan:NMRI mice not treated with analgesia following exploratory laparotomy damaged their nests, and failed to build proper nests for up to two days following surgery190. In some instances investigators could not identify a nest, or found several fragmentary nests at different locations in the cage. In contrast, mice treated with analgesics built normal nests within the first day and did not engage in nest destroying behaviour. vi) Barbering – defined as the plucking of fur or hiskers from cage mates or oneself340 – is a common form of abnormal repetitive behaviour in mice which may be related to environmental factors such as cage design, cage location, relationships between cage-mates and the presence of other barbers in the cage341. Lesions tend to be larger than those inflicted by aggressive encounters (which may be as small as 1-3mm in diameter); non-pruritic (not-itchy); not inflamed; and with no surrounding scarring or scabbing. Barbered mice may be functionally abnormal as whisker trimming can alter anatomy and function of the barrel cortex, reducing the ability of the mouse to discriminate between textures or control balance, and altering whisking patterns16, 342, 343. While the underlying reasons for barbering are poorly understood342, they may be triggered by husbandry factors. Mice housed in steel cages were 1.82 times more likely to barber than mice housed in plastic cages341, although barbering was more severe overall in plastic cages (3 per cent of body area versus 2.4 per cent in steel cages). Mice housed entirely with siblings were 3.66 times more likely to barber than mice housed entirely with non-siblings. This may signify frustration as mice normally disperse at puberty, the age that barbering

behaviour tends to appear. Some strains are more likely to barber than others340. Provision of environmental enrichment items such as a nesting box, cylinder or manipulable objects reduced the incidence of barbering in one study344.

vii) Stereotypic behaviours are repetitive, unvarying actions with no apparent goal or function100, 345 which may be induced by frustration, attempts to cope and/or central nervous system dysfunction345. They may indicate attempts to cope with past challenges rather than current ones, consequently care is required in interpreting the point of origin and cause344. In mice, stereotypic behaviours include bar mouthing or gnawing, jumping up and down at the cage wall, back-flipping, somersaulting, circling and cage-top twirling346. While one survey found a positive correlation between the incidence of cage climbing and stereotypic behaviour94, climbing on the cage bars and lid are not stereotypic behaviours per se and thwarting this behaviour may lead to anxiety in some strains101. It is estimated that 50 per cent of mice in research and breeding establishments exhibit some form of stereotypic behaviour347. Stereotypic behaviours are often associated with environmental restriction and their incidence may be reduced in an enriched environment348. They are probable indicators of poor welfare. For example, bar chewing may reflect escape attempts and may provide a behavioural indication of the animal's perception of its cage environment349. Some mouse strains are more likely to develop stereotypies than others, with more active strains at a higher risk94, 346. Other risk factors include premature or sudden weaning, lack of shelter and inability to explore cues (for example olfactory cues from adjacent cages) in the surrounding environment346. Over time, stereotypies tend to increase in frequency and duration while becoming increasingly fixed in form and orientation346. Perhaps more of a concern is the fact that these behaviours may persist even in the absence of initiating factors, suggesting changes at a neural level346. Mice exhibiting stereotypic behaviours may therefore be poor research subjects. viii) Investigators should be familiar with strain and/or transgene-mediated health conditions including tumour growth, hair loss, degenerative joint disease, diabetes, respiratory tract disorders and intestinal obstruction so that they can be diagnosed and treated in a timely manner57.

ix) One of the most useful methods of monitoring experimental mice is the adoption of an objective scoring system332, 350, 351, 468. To ensure consistency, score sheets should be filled out by the same staff each time. Scoring parameters should be adjusted to take into account the specific characteristics of a strain, particularly where transgenic mice are concerned352, 469 and particular clinical signs that may be expected during an experiment350. If score sheets are used these should be regularly reviewed to detect subtle changes332.

x) It is important to bear in mind that individual mice vary in their response to pain or stressors, and that this response is influenced by genetic factors, previous experience, age and physiological state353.

xi) The NHMRC has produced Guidelines on the Assessment and Alleviation of Pain and Distress in Research Animals which can aid investigators in developing protocols for assessing, minimising and monitoring pain and distress during studies353.

Recommendations

Welfare monitoring of mice via behavioural observation should be carried out in addition to monitoring for physical health. Investigators should be familiar with strain and/or transgene-mediated health conditions and behavioural problems so that they can be diagnosed and treated in a timely manner. Monitoring should be carried out when a person with whom the mice are familiar is present. It should be ensured that there are sufficient, properly trained staff and resources including staff time to monitor mice effectively. In the monitoring and investigation of health issues (such as growth rate, reproductive performance and disease) the effects of housing conditions should be taken into account. Animal carers

should be familiar with the normal physical appearance and behaviour of mice and of the individuals within a group and note any deviations from the norm, including animals that do not move around the cage normally. Mice that give cause for concern may need to be removed from the group but only if absolutely necessary as aggression may occur subsequently to regrouping. In particular, mice should be monitored for signs of bullying including fight wounds, barbering or loss of body condition secondary to denial of access to food or water. Mice that become sick unexpectedly should be examined and diagnosed by a veterinarian and any animals that die unexpectedly should routinely be submitted for post-mortem and diagnosis. Records and score sheets should be kept and reviewed regularly to detect trends and subtle changes.

Environmental Variables

General

Principles

Good animal husbandry involves maintaining animal health and welfare by meeting physiological as well as behavioural needs. Management of environmental variables such as light, temperature, humidity, air quality and ventilation and noise levels plays a significant role in achieving these ends. If not controlled, environmental variables may confound and compromise experimental data, resulting in the unnecessary use of more animals.

Light

Principles

(i) Light intensity, wavelength and periodicity (light:dark cycles) impact on the behaviour, physiology and reproductive parameters of mammalian species329.

Light intensity and wavelength

Principles

(i) Light intensity can influence the behaviour of mice, as well as progression of eye pathology and reproductive parameters.

(ii) Mice in the wild are typically nocturnal and generally avoid brightly lit areas. Behavioural tests for anxiety, such as open field exploration, the elevated plus maze and light:dark tests are predicated on this aversion of mice to brightly illuminated areas354, 355. Thus in one study, 400 lux illumination in a white cage area was aversive to C57BL/6, DBA2 and albino BKW mice, and inhibited exploratory behaviour356.

(iii) Light intensity decreases with the square of the distance of its source, hence intra-cage illumination is influenced by the position of a cage within a particular room and rack329. Intra-cage light intensity can vary by over 80-fold in transparent plastic cages on racks on shelves (from 3lux at the bottom to 250 lux at the top)357. Even within a single cage light intensity can vary as much as 20-fold (7-140lux), with intra-cage variability lowest in cages farthest from the light source.

(iv) Phototoxic retinopathy (progressive loss of the outer retinal layers associated with excessive exposure to light) can occur in a variety of species, but is most commonly reported in laboratory rodents329. The extent of photoreceptor damage is affected by light intensity, photoperiod duration, temperature, activity levels during the light phase, light levels under which an animal was raised, age, hormone status and albinism329.

(v) Albino mice are particularly sensitive to light-induced photoreceptor degeneration, with some albino strains more susceptible than others. Extremely high light exposure of around 2010 lux for 18-24 months caused retinal atrophy in 20 per cent of exposed BALB/c mice358. In another study, seven different albino strains were exposed to constant fluorescent light at 1265-1430 lux for three weeks prior to histological examination of the eyes. All exhibited photoreceptor degeneration359.

(vi) Studies have shown a relationship between cage shelf-level and retinal atrophy, presumably caused by differences in lighting intensity. In one chronic study, 19.7 per cent of mice on the top shelf of a rack had retinal atrophy when sacrificed at 24 months, as compared to 0.2 per cent of animals on lower shelves. By 33 months, retinal atrophy was present in 30.2 per cent of mice on the top shelf, compared with 12 per cent on the shelf immediately below it and 0.7 per cent on lower shelves358. Light induced complications may be reduced by utilising racks with shaded tops1, or rotating the position of a cage within the rack, shelf and room357.

(vii) Light intensity influenced the oestrus cycle, including duration of vaginal cornification and time periods between vaginal cornification, in outbred albino (LACA) mice360, as well as pigmented C57BL/10 and cogenic albino C57BL/10 mice361.

(viii) Reproductive efficiency of wild mice is reduced under high-intensity lighting. Both laboratory (CF-1) and wild mice bred equally well under a lighting intensity of 10-20lux362. However, at a lighting intensity of 1000lux productivity – especially litter size – of wild mice decreased significantly while that of laboratory mice was not affected. In addition, body weight was depressed in wild mice with increasing light intensity.

(ix) Reproductive efficacy of laboratory mice is reduced under high-intensity lighting. In one study, inbred laboratory mice housed at a cage lighting level of 500lux demonstrated a 50 per cent pre-weaning mortality rate, compared with only 5 per cent losses at a level of five lux363. Brighter illumination was associated with poor maternal behaviour, inadequate nest building and pups being scattered throughout the cage.

(x) Light intensity influenced wall-leaving behaviour in inbred strains of mice, with significant increases in both wall-leaving and cage-crossing behaviour in C57BL/6J, C3H/HeJ and BALB/cJ mice under low illumination (a 25W clear bulb, shielded by a paper towel and suspended 172.7cm above the centre of the open field) as compared to high illumination (a 100W clear bulb suspended 116.8cm above the centre of the field)95. In addition, there was a significant reduction in defecation and urination under low illumination.

Recommendations

Lighting within cages during the light phase should be maintained at a luminance below the threshold of aversion for mice. It is important to keep lighting type intensity and duration constant to avoid experimental variability. For most pigmented strains this is below 60lux and for albino strains it is below 25lux. To enable staff to perform tasks in mouse rooms it may be necessary to increase the lighting to 210lux at working height for the period while workers are in the room. 4.2.1.2 Light intensity can be reduced by using recessed lighting consoles in the ceiling with fluorescent lights of about 25-36 watt and a low spectral intensity (wavelength). This can be achieved by using a low colour number, e.g. colour 33 tubes. Shading should be provided over the top shelves of racks and cages and racks should be positioned in a way that protects mice in the top cages from overhead lights and provides more uniform light levels between cages on different shelves.

Lighting should be diffuse and uniform to avoid glare, heat clusters and fluctuating lighting conditions for individual cages. If halogen lighting is used, a silica glass cover must be interposed between the bulb and mice to minimise genotoxic and carcinogenic effects. If mice are observed during the dark phase red or sodium lamps should be used to minimise any disruption to their nocturnal activities.

Light Cycles

Principles

(i) The circadian clock drives 24 hour variations in a range of physiological and behavioural parameters in mammals, including mice371. For example, processes that regulate growth, metabolic, endocrine, and

immunological parameters in mice are affected by circadian rhythms372, 373. Circadian rhythms are predominantly synchronised by the environmental light:dark cycle374 and the visual perception of light375.

(ii) Exposure to constant light, as may occur with a faulty light clock or timer, may be stressful for mice. Male BALB/cAnNCr1BR mice exposed to continuous light for a week had increased urine corticosterone:creatinine ratios, and demonstrated a shorter latency to their first agonistic encounter when compared with controls376. In addition, these mice had increased weight, despite eating and drinking less than controls. However effects may vary significantly in different strains and even in mice of different gender. Female transgenic growth hormone mice exposed to continuous light over a lifetime grew faster, lived longer and had increased production efficiency than those exposed to a 12:12 light cycle377. Exposure to constant light appeared to reduce pro-viral DNA in male BALB/c-H-2k mice inoculated with murine leukaemia virus378. Constant light delayed onset of sexual maturity, reduced the rate of weight gain and was associated with irregular activity patterns in female ICR/Alb mice when compared with controls379.

(iii) The continual process of renewal of retinal photoreceptors (rods and cones) is influenced by the light:dark cycle470. This may explain why lack of a dark cycle is a causative factor in retinal degeneration of laboratory rodents including mice. (iv) Continuous darkness was associated with an increase in severity of arthritis in DBA/1 mice380, although it was also associated with a decrease in aggression between male sea:ddy mice381.

(v) Changes in light:dark cycles are stressful for mice. For example, male BALB/cJ, CBA/J and C57BL/10J mice subjected to reversal of the light:dark cycle every four days for 76 days then every two days for an additional 54 days had increased circulating plasma corticosterone and decreased barbiturate sleeping time compared to controls382. Lengthening (16:16 L:D)) or shortening (5:5 L:D) the cycle led to increased locomotor activity and corticosterone in male ICR mice374. Expanding cycles beyond a 24 hour period may influence food intake and locomotor activity372. Advancing the onset of the light cycle by eight hours every second day modified the expression of molecular clock genes and genes involved in carcinogenesis and tumour progression, accelerating tumour growth383. In the same study, altering meal times to coincide with the onset of light and darkness helped reduce this effect.

(vi) Mice may require a long period to adapt to changes in light cycles. Male BALB/c, C57BL/6J and CB6 mice subjected to a sudden shift in the light:dark regime (from lights on from 0800 to 2000hrs with half light from 0730-0800 and again at 1930 to 2000hrs (the LD regime) to the reverse, that is, lights on from 2000 to 0800 (the DL regime) demonstrated significant variation in immune parameters between strains, even after five weeks373. Daily mean thymic indices and weights, as well as splenic index and weight, were significantly higher in LD mice than their DL counterparts. In addition, the mean daily number of peritoneal leucocytes was significantly lower in LD mice. CB6 mice kept under DL conditions gained more body weight than CB6 and other (BALB/c and C57BL/6J) LD mice.

(vii) While information about the impact of light contamination during the dark cycle on mice is sparse, rat studies suggest that light leaks can have a profound impact on experimental data. For example, minimal light leaks of 0.2lux during an otherwise uninterrupted dark phase inhibited rat melatonin secretion, increasing the rate of tumour growth and lipid uptake384.

(viii) Flickering light has been shown to be a potent stressor in rats. In one study, exposure to 80 Lux of flickering light for 30 minutes was associated with elevated serum corticosterone and other biochemical markers of stress385. Whilst there are no equivalent studies in mice, studies referenced above indicate that mice are sensitive to changes in light and may therefore experience stress when exposed to flickering light.

Recommendations

A semi-natural light cycle of 12:12 or 10:14 hours light:dark is suggested. Variations in the light:dark cycle to mimic seasonal change could be considered. 4.2.2.3 A change in light cycle should be followed by an acclimatization period before commencing a study.4 Cycles may be disturbed if lighting clocks or timers malfunction. Clocks and timers should be checked regularly. In the event of a disturbance mice should be allowed an additional acclimation/habituation period, as disruption to the light cycle is a source of experimental variability. Care should be taken to prevent light leaks in animal rooms during the dark phase. Lights should be checked for flickering and any flickering rectified. Light intensity should also be monitored

Temperature

Principles

(i) The thermal biology of the laboratory mouse has been extensively investigated386-391. The ambient temperature at which laboratory mice are kept can affect metabolism, cardiovascular function, motor activity, growth and development, body and organ weights, consumption of food and water, haematology and serological parameters, susceptibility to toxins, immunocompetence, reproduction, sleep depth, and behaviour in relation to cohabitants2, 326, 386, 388, 389, 391, 392.

(ii) In-cage temperature is influenced by factors including, but not limited to, cage design and construction, the position of a cage within a rack and a room, the position of the cage within the flow of air, ventilation rate, presence and type of bedding and/or nesting materials and stocking density. For example, heat dissipates rapidly from cages constructed with a wire mesh floor.

(iii) Mice maintain their core body temperature by a range of mechanisms including varying metabolic rate, shivering, non-shivering thermogenesis393, increased physical activity391, grooming (spreading saliva on fur for evaporative heat loss) and thermotropism including huddling with cohabitants225, 388. Thermal preferences may vary between single and group-housed mice390, 393 and may be influenced by sex, current

behaviour and time of day471. Mice may create habitats with a desirable microclimate by burrowing or nesting390.

(iv) Huddling allows group-housed mice to reduce cold stress by t ermoregulating as one larger animal with a smaller surface area, thus less heat loss, than that of the total number of mice393. Even at a housing temperature of 28°C, thermogenic activity of brown adipose tissue was greater in singly housed mice than those housed in pairs or groups of six393. Male MAfSp mice deprived of the opportunity to huddle with cage

II –M.Sc Microbiology (Batch 2018-2020)

Possible Questions

Unit – II

Two Marks

1. What are purpose of animals in research study
2. What are the types of feed for rat in lab
3. What are the nutritional requirements for rat in lab
4. Two points of safety aspects of animals
5. What are different breeds of rat as animal model

Eight Marks

1. Write short notes on the maintenance of rat in pens
2. Draw the general schematics of housing rat in cages with proper lable.
3. Describe the anatomical and social characteristics of rat used in the laboratory.
4. Write short notes on the safety aspects of animal house while handling rat.
5. Discuss on the methods of grouping rat for experimental setups.
6. How are rat bread for the experimental purposes? Discuss.
7. Explain in detail on the types of feed and nutritional requirements for rat in lab.
8. Write short notes on the euthanasia of rat in lab
9. Discuss on application of lab rat with special reference to nude rat.
10. Write short note on the nomenclature of rat strains in lab.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB **COURSE NAME: LABORATORY ANIMAL CARE**

COURSE CODE: 18MBP305B **UNIT: II** **BATCH-2018-2020**

S.No	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Harem system of breeding involves mating of one male with _____females.	2	6	11	20	6
2	Stress recognition in rabbit is done by observing_____.	agressiveness	failure to eat	inactivity	loss of weight	loss of weight
3	Secondary infection in mice is caused by_____.	S. typhi	P. aeruginosa	S. dysentriae	P. pneumotropica	P. pneumotropica
4	Blood level of mice is_____ x10^6/mm^3	2-5	15-18	7-12.5	10-20	7-12.5
5	The maximum respiratory rate of mice is _____min	80	120	180	110	120
6	The natural life span of a mice is_____years.	1.5	10	12	5	1.5
7	_____is used as a preferred surgical anaesthesis for lab animals.	fentanyl	ketamine	diazepam	medeomidine	medeomidine
8	_____is an anaestheic used as inhalaing agent for lab animals	fentanyl	barbiturates	Doxapram	Isoflurane	Iso flurane
9	Morphine is used as_____in lab animals.	immunostimulant	antibiotic	analgesic	anaesthetic	analgesic
10	Mice are grown in cages made of _____.	mild steel	transparent plastic	opaque plastic	iron	opaque plastics
11	The term 'ad libitum' refers to _____in feeding of animals.	optimum	insufficient	half empty	satisfactory	satisfactory
12	Mice will consume _____ grams of pelleted diet daily.	1	5	25	100	5
13	_____affects the food	size	Activity	Metabolism	Pregnancy	Pregnancy

	requirements of mice.					
14	Water is supplied to lab rabbits via_____.	bottles	syringes	taps	cans	bottles
15	Lack of water intake in mice leads to _____.	death	dehydration	infection	loss of appetite	dehydration
16	Daily requirement of water for lab mice is_____ml.	1-2	20-40	100-200	6-7	6-7
17	The temperature requiment for mice is_____℃.	5	25	37	40	25
18	Female mice reach adolescences at _____week.	3	6	12	16	6
19	The reprocutive cycle for female mice is_____days.	10	25	5	7	5
20	In mice gestation last_____ days.	21	60	10	15	21
21	_____is one among the factors considered as the principle for animal care.	Aggressive killing	Health and safety	Surgery	Selective feeding	Health and Safety
22	_____act governs the safety and well being of laboratory mice in UK	Animals Act for Scientific Procedure 1986	Animals Act for Scientific Procedure 1920	Animals Act for Scientific Procedure 1990	Animals Act for Scientific Procedure 1995	Animals Act for Scientific Procedure 1986
23	According to European Commission Directive for protection of vertebrate animals, persons working on animal should have_____.	eduaction and training	certification on health management	certification on surgery	ethical knowledge on animal handling	eduation and training
24	Anually_____ million lab mice are approximately used in India	3	10	15	5	3

25	In animal welfare_____ approach encompasses a number of ethical traditinons, including utilitarianism.	non-consequentialist	philosopher	scientific	consequentialist	consequentialist
26	Project licence holders take responsibility for the direction of _____.	practial skills	animal handling work	fabric establishment	mangement system	animal handling work
27	_____act governs the safety and well being of laboratory personnel from being affected by hazardous materials.	Health & Safety work Act 1979	Health and safety work act 1967	Health and safety work act 1974	Health and safety work act 2001	Health and safety work act 1974
28	Work place regulation 1992 does NOT deals with_____.	control of temperature	control of personal habbits	control of allocated area	control of ventilation	control of personal habbits
29	The marking CE in the employee uniform refers to_____.	clothing and equipment	clothing and embleishment	certification and enrollment	common number and employee no	clothing and equipment
30	COSHH stands for_____.	Control of Substances Handled to Heal	Control of Substances Hazardous to Health	Containment of Solid Hazard Health	Control of Health and Hazard	Control of Substances Hazardous to Health
31	Mice born with an innate absence of immunity is called _____	hybrid mice	filial mice	out bred mice	nude mice	nude mice
32	Food for mice is provided in the form of _____.	semi solid pellets	hard pellets	mini pellets	powder	hard pellet
33	One of the respiratory behaviour associated with rabbits in lab is	Sneezing	Nuzzling	Sniffing	Scratching	Nuzzling

	_____.					
34	_____space provides proper movement area for rabbits in cages	blind	open	centre	common	blind
35	The nesting area of rabbit cages are designed with _____entrance.	wide	narrow	elevated narrow	curved	elevated narrow
36	Identification of lab rabbits is NOT done using_____.	fur dyes	steel collar	limb cuff	skin tag	limb cuff
37	The weaning time for rabbits is _____weeks.	1	2	3	4	3
38	FDA prohibits the the use of _____as analgesic to rabbits.	Flunixin	Ibuprofen	pethidine	pentobarbitone	pentobarbitone
39	Rat belongs to the sub-family _____.	Cricetinae	Gerbillinae	Murinae	Caviidae	Murinae
40	The commnly used laboratory rat is of _____origin.	Indian	European	American	Norvegian	Norvegian
41	_____is a common infection occuring in lab animals	Salmonella	Yeast	Leptospira	E. coli	Salmonella
42	_____viral infection leads to haemorrhagic fever in rabbits	Rota	Polyoma	Hantaa	Orthomyxo	Hantaan
43	Diseases that are transmitted from animals to humans is termed as_____.	zoonoses	biologicals	pathological	verterinary	zoonoses
44	_____infection is transmitted form mice to humans.	Shigella sp.	Leptospira	T. gondii	henselae	Leptospira
45	The scientific name of laboratory mice is_____.	Mus murida	Mus chinchilla	Mus musculus	Mus melidus	Mus musculus

46	_____act as mediators in communication between mice.	urine	eyes	ultrasonic sound	phermones	phermones
47	Mice exist in a territory as _____.	colony	group	family	flock	colony
48	Foreign male mice will provoke agression from other male mice, this phenominon is called_____effect.	multitude	Bruce	Waller	Yaslow	Bruce
49	Mice bedding consist of _____.	steel racks	plastic racks	wood chips	hard steel mesh	wood chips
50	_____is a steriotyphic behavious in caged mice.	barbering	hopping	Flashing	Morphing	Barbering
51	Exposure of polypropylene to lab mice leads to_____.	death	metabolic disorder	behavioral changes	agressiveness	behavioural changes
52	_____is NOT a major health hazard to animal handlers.	Strain in handling	Allergy	Infection	Injury	Strain in handling
53	_____is an allergic symptom developed by lab animal handlers over long term exposure.	Skin rash	Occupational asthma	Staphylococcal infection	Mite infection	Occupational asthma
54	Allegry in animal handling usually belong to _____ hypersensitivity	I	II	III	IV	I
55	_____is an Ig G mediated allegric reaction occuring in mice handlers.	Conjuctivitis	Utricaria	Rhinitis	Extrincic allergic alveolitis	Extrincic allergic alveolitis
56	Which of the following is the main mice allergen?	hair	urine	feces	salaiva	urine

57	Which of the following is the main mice allergen?	fur	feces	urine	salaiva	fur
58	The test used to assess the employers for detecting allergy in animal labs is _____.	RIA	CFT	ELISA	Wheal and Felix	ELISA
59	Control of allergens in animal house is made possible by_____.	air filteration	use of surgical masks	open ventilation	air-fed visors	air filteration
60	Mice whose immunity is reduced by genetic modification is called _____.	Inbred mice	hybrid mice	knockout mice	lab mice	knock out mice

UNIT-III
SYLLABUS

Modern methods of care, management breeding and maintenance of laboratory animal - mice

General Introduction

These guidelines are intended for use by people involved in the housing and care of rats in scientific institutions. The guidelines are not intended to be a complete manual on rat care and management but rather to provide some key guiding principles on good contemporary practice in rat housing. The guidelines will be revised from time to time to take account of advances in the understanding of rat physiology and behaviour, technological advances, and changes in community attitudes and expectations about the welfare of animals.

The guidelines are based on principles regarding the care and management of rats taken from scientific literature. These principles are detailed throughout the document, as are recommendations for the care and management of rats which are derived from these principles. In some areas, conclusions to be drawn from the available literature are not entirely clear, and in such areas recommendations are extrapolated from information available and practices in rat care and management current at the time of writing.

The principles outlined in the document address requirements of the AustralianCode of Practice for the Care and Use of Animals for Scientific Purposes (as outlinedbelow in Section 1.4). The requirements of the Code of Practice include that animals held for scientific purposes should have their species-specific behavioural and physical needs met, whilst at the same time ensuring that the animals can adequately be monitored and are protected from disease, and taking into account the requirements of the research for which the animals are being used.

Whilst the guidelines focus on the welfare of rats, it is implicit that conditions that contribute to meeting rats‟ physiological and behavioural needs will also contribute to the quality of scientific outcomes. The guidelines contain many examples of the physiological and behavioural responses of rats associated with variables in housing and hence the potentially confounding effects of these variables on these animals as research subjects.

The guidelines outline requirements for the housing of normal rats. Where rats are physically or behaviourally abnormal (for example, post-surgery, diabetics, Parkinson models, acute pain models), modifications to housing to meet their needs may be required.

Responsibilities of Institutions- Recommendations

Institutions using rats for scientific purposes are responsible for responding effectively to recommendations of the institution‟s Animal Ethics Committee to ensure that facilities for the housing and care of rats are appropriate to the maintenance of well-being and health of the rats.

Responsibilities of Chief Investigators

Recommendations

The chief investigator/teacher (person in charge of a research/teaching project) has personal responsibility for all matters related to the welfare of rats under his or her control, which includes their housing and care. (As per the principle contained in Clause3.1.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes)

The chief investigator/teacher should ensure that the extent of personnel / staff supervision is compatible with the level of competence of each person and the responsibilities they are given in relation to rat care and management. (As per theprinciple contained in Clause

3.1.2 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes).

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes

Principles

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states:4.4.19 Animal accommodation should be designed and managed to meet species-specific needs. Pens, cages and containers should ensure animal wellbeing and comfort. Variations to these requirements as part of a project must receive prior AEC approval. The following factors should be taken into account:

(i) species-specific behavioural requirements, including the availability and design of space to enable free movement and activity, sleeping, privacy, contact with others of the same species, and environmental enrichment;

(ii) provision of single housing for animals when appropriate for the species and if necessary for the purpose of the project (for example, during recovery from surgery or collection of samples);

(iii)species-specific environmental requirements, such as lighting, temperature, air quality, appropriate day/night cycles and protection from excessive noise and vibrations;

(iv)the need to provide ready access to food and water;

(v)the need to clean the pen, cage or container;

(vi)protection from spread of pests and disease;

(vii) requirements of the project; and

(viii) the need to observe the animals readily.

Pens, cages and containers must:

(i) be constructed of safe, durable, materials;

(ii) be kept clean;

(iii) be maintained in good repair;

(iv)be secure and escape-proof;

(v)protect animals from climatic extremes;

(vi)not cause injury to animals;

(vii) be large enough for the species and the number of animals held; and

(viii) be compatible with the behavioural needs of the species.

The number of animals in cages, pens or containers and the placement of these should enable social and environmental conditions for the species to be maintained. Where it is necessary to individually house animals of a species that normally exists in social groups, the impact and time of social isolation should be kept to a minimum.

Bedding and litter must be provided if appropriate to the species and should be comfortable, absorbent, safe, non-toxic, able to be sterilised if needed, and suitable for the particular scientific or educational aims. Pregnant animals must be provided with nesting materials, where appropriate.

The AEC, investigators and teachers should be consulted in advance of planned changes to these conditions, since these may affect both the welfare of animals and results of the scientific and teaching activities.

Aspects of Rat Behaviour Relevant to Housing

Principles

(i) The most commonly used laboratory rats have evolved from the Norway Rat, Rattusnorvegicuswhich lives mainly in burrow systems in the ground (Koolhas 1999). Both wild

anddomestic rats will create complex, three dimensional burrow environments (Brain 92; Boice 1977).

(ii) Rats are social animals. In the wild they live in colonies, which may consist of hundreds of rats and which have nesting sites and feeding grounds in common (Barnett 76).

(iii) Rats are nocturnal, usually with three activity periods, one at the beginning, one in the middle and one at the end of the night. Feeding for both adults and neonates takes place during these activity periods (Henning and Gisel 1980; Koolhas 1999).

(iv) Rats have highly developed senses of smell, hearing and touch. Rat behaviour and communication is strongly influenced by olfactory cues (Mackay-Sim and Laing 1979; Kikusui, Takigami, Takeuchi and Mori 2001; Koolhas 1999).

(v) Rats emit sonic as well as ultrasonic vocalisations and can hear frequencies at least up to 70kHz (Heffner et al 1994). The use of ultrasound appears to be important for communication and may be used in behaviours such as controlling aggressive encounters, mating and mothering (Sales and Pye 1974; Gamble 1982, Brudzynski and Ociepa 1992).

(vi) Normal behaviours of rats include climbing, walking, standing on their hind legs and stretching upright, burrowing, nesting, gnawing, foraging, grooming (themselves and each other) and retreating (into hiding areas).

(vii) Young rats, and sometimes even older rats, engage in play behaviour which includes leaping, chasing and scuffling (Scharmann 1991). Play behaviour is necessary for the well-being and normal social and sexual development of young rats (Lawlor 2002). One function of play may be to establish stable social relationships (Panksepp 1981).

(viii) Rats under laboratory conditions have been observed to spend about 70 to 75 % of their time resting (Manser, Morris and Broom 1995).

(ix) Rats usually sleep in relatively curled positions (van Betteray, Vossen and Coenen 1991) but have been observed to sleep stretched out at full length with their tails extended (Lawlor 2002). Sleeping positions may be influenced by factors including light, temperature and proximity to walls.

(x) Huddling behaviour of rats (sleeping together in a group) is influenced by needs for thermoregulation but is not solely caused by this. Other sensory stimuli (for example olfactory and/or tactile) appear to play a part in huddling behaviour (Barnett 1976; Sokoloff and Blumberg 2001).

(xi) Rats are highly exploratory and inquisitive (Scharmann 1991; Barnett 1976). Studies have shown that rats will work for stimulation and that they demonstrate preferences for the opportunity to explore (over a blind alley) and for novel visual stimuli (Barnett 1976).

(xii) The normal behaviour of rats when eating is to carry a piece of food by their teeth to a suitable spot where they adopt a squatting posture and hold the food in their forepaws to nibble at it (Lawlor 2002).

(xiii) Gnawing is a behaviour that is necessary not only for the psychological (Beltz, Kennell, Czambel et al 2003) but also for the physiological well being of rats. If not given the opportunity regularly to gnaw, their teeth overgrow, which can make eating and grooming difficult or impossible.

(xiv) Rats exhibit coprophagy – they will ingest 35-65% of their faeces when fed a complete diet. They eat faeces directly from the anus and have been shown to experience growth depression if prevented from doing this (this effect seems to be related to the consumption of fresh faeces, as

eating faecal pellets from the bottom of the cage has no beneficial effect on growth (Newton 1978)). Young rats ingest maternal faeces between 16 and 28 days of age. This significantly decreases after 25 days of age, coinciding with weaning. Young pups deprived of maternal faeces show evidence of malnutrition and abnormal eating behaviours when older (Novakova and Babicky 1989).

(xv) For housing rats, in addition to the normal requirements of rodents for food, water, exercise, shelter and warmth, particular consideration should be given to:

(i) Their nocturnal habits
(ii) Their ultrasonic hearing
(iii) Their response to pheromones
(iv) Their response to isolation
(v) Their exploratory nature
(vi) Their upright-stretching bi-pedal posture
(vii) The effect of group size on social dynamics
(viii) The influence of strain, age, sex and prior experiences on behaviour.

(xvi) Assessments of rat behaviour need to take into account that the behavioural and physiological responses of rats will vary with factors including sex, age, strain, prior experiences and the environments in which they are kept (Galef and Sorge 2000; Brown and Grunberg 1995; Chaouloff, Kulikov, Sarrieau et al 1995; Rebouças and Schmidek 1997; Gomez, Kloet and Armario 1998; Dimitrijevic, Laban, Djuric et al 2001; Hall, Huang, Fong et al 2000; Fernandes, Gonzalez, Wilson and File 1999; Rose 1996).

Recommendations

To meet the requirements of the Code of Practice (ie to provide accommodation that meets the species-specific needs of rats), housing should be provided which allows rats the opportunity for social interaction, the opportunity to carry out normal behaviours and the opportunity to rest and withdraw from each other.

The Code of Practice recognises that there may be circumstances where the requirements of experimental procedures will preclude meeting some species-specific needs (Clause 4.4.19). Housing in these situations should still meet the physiological and psychological needs of rats as closely as possible.

Cage Design Living AreaPrinciples

(i) The living area for rats is three dimensional, comprised of the floor area as well as the vertical space. These parameters need to be considered together, rather than in isolation. In addition to three dimensional space, the shape of the living area and the size of groups of rats (see 3.1 The Social Environment) need to be taken into account in developing optimal living areas.

(ii) The living area for rats must allow them to satisfy their basic physiological and behavioural needs and these include the ability to rest, groom, search for food, explore, gnaw, hide, reproduce and engage in a range of social activities including play (Brain 1995).

(i) Information on the requirements of rats for living space is not conclusive. Rats‟ requirements for height and shape of the living area are reasonably well documented. However, their requirements for floor area are less clear.

(ii) The parameters of cage size, space available per rat (spatial density) and number of rats in the group (social density) are often confounded in housing studies (Hargreaves 2000). There is some evidence to indicate that housing rats at low amounts of space per rat or high group

numbers can influence physiological and behavioural systems and that some of these effects are consistent with a stress response (Hargreaves 2000). However, due to the complexity and variability of experimental design, it is difficult to extrapolate from such studies what should be recommended for rats in terms of cage size and spatial density.

(iii) There is substantial evidence that the shape, size, structure, fittings and the overall design of housing influences biological variables and needs to be taken into account in any experimental design (Clough 1982; Fitzmaurice 1988).

Cage Floor Area- Principles

(i) Determination of minimum floor space requirements for rats may be guided by the basic behavioural and physiological needs of rats. These include eating, drinking, social interaction (including playing and grooming), resting, defaecating and urinating. Rats tend to compartmentalise their living areas for these different activities (Weiss, Ernst and Schick 1982; Novakova and Babicky 1977; Anzaldo, Harrison, Riskowski et al 1994). In terms of physical movements, rats should be able to be able to turn freely without twisting their heads and bodies, walk at least a few steps, stand, stretch upright and play. They also should have room to shelter and rest. The floor area should ensure that no part of a rat"s body is unavoidably distorted by contact with the cage in any of the postures that rats are shown normally to adopt (Lawlor 1987).

(ii) Preference tests for cage size have shown that rats prefer substantially larger cages than standard sizes (Weiss, Ernst and Schick 1982; Patterson-Kane 2002). In the study by Patterson-Kane 2002, it was shown that male and female rats showed a preference for a larger cage (1620 cm^2 versus 540 cm^2) whether tested singly or in the presence of 4 cage mates. In a paper by Scharmann 1991, the adequacy of a cage sized 900cm^2 to house 4 rats of 200-250gm was compared with a cage sized 1,800cm^2. It was concluded that it was doubtful if the smaller cage provided enough space for exercise, and in particular for play.

(iii) Factors other than floor area may influence how rats use a floor space - for example, open space in bright light conditions will be avoided (Matsuo and Tsuji 1988). In cages where the same floor area is available, rats have been shown to prefer cages with vertical partitions, forming an inner chamber, over cages with horizontal partitions (Anzaldo, Harrison, Riskowski et al 1994). In this study, the rats used the inner chamber, created by the vertical partitions, for resting. A study by Foulkes 2004 found that rats did not benefit
from larger (1088cm^2) versus smaller (432cm^2) cages as assessed by stress responses, unless the cages had enrichment items (tube shelter and gnawing / carrying item).

(iv) Guidelines on space requirements commonly relate requirements for space to the weight of the animal (Hackbarth, Bohnet and Tsai 1999). However, other factors need to be taken into consideration such as the stage of the breeding cycle, whether rats are nesting and the age of the rats. A range of behaviours (such as play, sexual activity and sleep) can be affected by the space available (Klinger and Kemble 1985; Saito, Motomura, Taniguchi et al 1996; Kleinlogel 1978). Juvenile rats need space in order to express play behaviour, and play is facilitated by increasing the available space (up to approximately 2,400cm^2) (Klinger and Kemble 1985). In addition juvenile rats have been shown to be more sensitive than older rats to limitations on space (cages 408 – 780cm^2 versus 1080 – 2160cm^2) as assessed by measures of anxiety (Arakawa 2005). Juveniles therefore need more space, relative to their weight, than adult rats.

(v) In addition to body weight and other factors, the body size (for example, length from nose to

tail tip) and shape of rats need to be taken into account in determining their requirements for floor area. The relationship between size and weight varies with factors including age, sex and strain (Lawlor 1987).

(vi) In-cage shelters (see 2.9 In-Cage Shelters) are highly desirable additions to rat housing. The dimensions of the floor area must be sufficient to accommodate such furnishings without negatively impacting on rat behaviours because of reduced floor space or restricted access to areas of the cage. Adding in-cage shelters has the contradictory effect of taking away from the floor space but adding space in the vertical dimension.

(vii) To allow mature rats to adopt species typical stances and carry out behavioural activities, Lawlor 2002 has advocated that a living area needs to measure at least 35 (D) x 25 (W) x 18 (H) cm (875 cm^2 floor area) for the smallest females and 50 (D) x 30 (W) x 30 (H) cm (1,500cm^2 floor area) for the largest males.

Recommendations

As a guide, based on the information from Scharmann 1991, Patterson-Kane 2002 and Koolhaas 1999, the minimum floor area for a group of up to 5 rats of up to 250- 300gm body weight should be 1,500cm^2 and preferably 1,800cm^2. For larger rats, group size should be decreased or cage floor area increased, on the basis that as rats grow, while play behaviour decreases, cage floor area must accommodate other behaviours including social interaction.

As a guide, for a nursing mother and litter (up to weaning at about 21 days), the floor area should be a minimum of 1,500cm^2.

As a guide, for juvenile rats (from weaning to about 50 days), for a maximum group of 12 juveniles, the floor area should be a minimum of 2,000cm^2.

Cage Height- Principles

(i) Part of the normal behavioural repertoire of rats is to stand on their hind legs and stretch upright (Buttner 1993). The base of the tail is used as a stabilising tripod and the forepaws may be rested on a firm surface, allowing the rat to stand on tiptoe (Lawlor 2002). The maximum height achieved by rats during upright standing is about 26 - 30cm (Buttner 1993; Lawlor 2002). In a study by Buttner 1993, rats given additional height up to 30cm used this full height on occasion for upright standing.

(ii) Although the ability to rear up is important for rats, they have been shown to prefer a low, dark cage over a high cage, which is most likely related to their desire for shelter. A cage with a variable height may therefore meet rats‟ requirements for shelter and for rearing (Blom1993).

(iii) Although rats stand on their hind legs, they do not do this for prolonged periods (Buttner 1993). In a study by Mihara and Hirano 1998, juvenile rats forced to stand on their hind legs for periods totalling 2 hours per day (to reach for food)) developed bony and cartilaginous damage of the femoral heads.


Rats exhibiting stretching and climbing behaviours. Note that the "low top" cage on the left would not allow the rat to fully stretch upright, whereas the "high top" cage on the right accommodates this behaviour. (Photos courtesy of DarekFiga)

Recommendations

Ideally the height of cages should allow rats to stand on their hind legs and stretch up fully. This height does not need to be provided over the entire area of the cage.

As a guide, for rats weighing 250 - 300gm, a cage height of 22cm over part of the cage should be provided. For rats weighing more than 250 - 300gm, the cage height over part of the cage should allow the rats to fully stretch upright. However, it is recognised that currently available cages (with maximum heights of around 22cm - 24cm) are unlikely to accommodate this.

Where cages are fitted with platforms or in-cage shelters, the distance between the top of the platform or in-cage shelter and the top of the cage should be at least 8cm to allow rats to climb onto the top of the platform or in-cage shelter.

While cage height (over part of the cage) should allow for upright standing behaviour, food and water should be accessible at a level that allows rats (especially juvenile rats) to sit while eating and drinking, to avoid bony and cartilaginous damage.

Cage Shape- Principles

(i) Cage shapes have been investigated in a number of studies and square cage enclosures are abandoned by rats when given the option of occupying a rectangular cage, even if the square and the rectangular cages have the same total floor space. Preferences by rats are for rectangular cages which allow them closer contact with, and presumably security from, walls.

Recommendation

Rectangular rather than square cages should be provided.

Cage Materials-Principles

(i) The design, construction and management of a rat"s immediate enclosure will determine to a large extent how environmental factors, such as temperature, light levels, humidity and air quality impact on the rat.

(ii) Most rat cages today are solid tubs made of plastics such as polypropylene (opaque) or polycarbonate, polysulphone and polyetherimide (transparent), with wire mesh tops (Hargreaves 2000).

(iii) Opaque cages have the advantage of filtering out harmful glare and allowing rats to hide from humans and neighbouring rats. They have the disadvantages of impeding the observation of rats from outside the cage, restricting rats" vision of activities outside the cage (including that of humans and other rats) and blocking the passage of light, resulting in different light levels in boxes at different levels on cage racks.

(iv) Transparent cages have the advantage of allowing observation of rats from outside the cage. They have the disadvantage of not allowing rats to hide from humans and neighbouring rats. Weiss and Taylor 1985 found that rats exhibited a strong preference for a cage where the rear wall was painted black (versus a fully transparent cage).

(v) Heat is well preserved in solid plastic tubs (such as polypropylene, polycarbonate,) .

(vi) Cage tops / lids are usually made of stainless steel mesh. The use of "high top" wire mesh lids creates additional height as well as wall areas through which rats can see outside activities

and neighbouring rats (whilst still allowing retreat behind the opaque walls of the tub) . The use of such "high-top" wire mesh lids creates cage walls that are partially solid and partially open. This facilitates ventilation within the cage.

Recommendations

Rat cages should be made from plastic (for example polypropylene, polycarbonate, polysulphone, polyetherimide) floors and walls ("shoebox" or "tub") with wire mesh tops (unless special purpose cages such as filter top cages or individually ventilated cages are required). Where transparent plastics are used for cage "tubs", particular attention should be paid to providing rats with shelters to allow them to withdraw from light and activities outside their cage. The need to monitor rats by observation (the intensity of which will be dictated by the type of project), and the disturbances to rats that may occur in doing this in opaque cages with opaque shelters, needs to be balanced against preferences for opaque walls by rats. Rat cages should be fitted with "high top" wire mesh lids (on solid sided walls) which enable rats to stretch upright and which facilitate interaction by rats with their surrounding environment (via visual and olfactory inputs). The "high top" area does not need to extend over the entire roof of the cage.

Cage Flooring- Principles

(i) When rats are given a choice between solid or mesh floors, the overwhelming majority of rats will choose solid floors especially when resting. In addition to showing a preference for solid floors in preference tests, rats have been shown to be prepared to make considerable efforts to reach solid floors to rest (lifting up to 83% of their body weight.

(ii) Housing rats on wire mesh floors causes neuropathy of the hind limbs. The changes are seen within one week of rats being exposed to wire mesh floors and result in functional and structural changes to the innervation of the hind feet including increased sensitivity of the feet to touch. The problem is exacerbated the longer a rat is kept on such flooring and the heavier it is. Diabetic rats are more susceptible and may develop neuropathies earlier. Housing rats on wire-mesh floors also interferes with normal sensorimotor gating mechanisms (neurological controls associated with locomotion).

(iii) Rats housed on wire mesh floors with large spaces in the mesh (11mm x 35mm) may develop muscle damage. Rats on such mesh may have to expend effort trying to maintain their balance and keep their feet from slipping through the gaps in the mesh.

(iv) In a study comparing rats housed on wire mesh floors versus those housed on solid floors with bedding, the rats housed on the solid floors were less physically active, which was suggested to be a reflection of their relaxed emotional state. In addition to increased movement, the rats on the wire mesh floor also ate significantly more, with no difference in weight gains, which were postulated to be effects of stress.

(v) Because wire mesh floors are open they allow dissipation of heat from the bodies of rats and may thus influence rats" thermoregulatory responses in order for them to maintain body temperature.

Recommendations

Solid floors should be provided for rat caging. Wire mesh floors should not be used for rat caging unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to use such flooring. In such cases, a solid floor section and nesting material should preferably be provided. The size of the mesh gaps should not exceed 11mm x 11mm.

Bedding- Principles

(i) Ideally bedding should be free of dust, microbial, parasitic, or chemical contaminants, non-traumatic, moisture absorbent and ammonia binding (Potgieter 1993; Kraft 1980). Good bedding material facilitates behavioural thermoregulation. In addition it is desirable for it to be cheap, readily available and easy to use and dispose of.

(ii) Rats have been shown to prefer bedding of larger particle size (wood shavings) over sawdust (Blom et al 1996).

(iii) Deep bedding may provide opportunities for digging/burrowing behaviour.

(iv) Bedding can produce aeroallergens but this problem tends to be confined to conditions of extremely high stocking density (30-60 rats per cage) with materials other than wood-based bedding (Taylor, Gordon and Tee 1994). Bedding also can be a source of airborne bacteria, fungi and endotoxins with the dust being an important factor in the level of air contamination (Kaliste, Linnainmaa, Meklin et al 2004).

(v) The type of bedding (as well as the frequency of bedding changes) is a major factor in influencing the levels of ammonia present in cages (Rose 1996). Materials used for laboratory animal bedding may have ureolytic properties which contribute to elevated ammonia levels. With the exception of some hardwoods, these can be deactivated by autoclaving (Gale and Smith 1981).

(vi) Some bedding materials contain aromatic oils which, being volatile organic compounds, can induce changes in the hepatic enzyme systems involved in drug metabolism (Vesell, (Lang, White et al 1973; Weichbrod, Cisar, Miller et al 1988; Buddaraju and van Dyke 2003) and may result in prolonged action of drugs such as anaesthetics (Ferguson 1966). These effects may last for weeks after rats have been removed from such bedding (Davey, Fawcett, Lee et al 2003) Also, decreased growth and increased mortality rates have been found when rat pups are raised on cedar-wood bedding, possibly due to the aromatic oils (Burkhart and Robinson 1978). Pelkonen and Hanninen 1997 examined the cytotoxic and enzyme inducing effects of a variety of types of bedding from different parts of the world. Pine shavings beddings were generally found to be highly cytotoxic (although the least cytotoxic of these was from Australia). Extracts of corn-cob, rice hulls and straws were found to be practically non-toxic. A paper sample from Australia (telephone book strips) was also non-toxic compared to woods, although it showed high enzyme inducing activity. In a study by Burn et al 2006, aspen chips, although inert compared with pine and other woods were associated with higher sneezing rates, worse interstitial pneumonia and higher weight gain in rats than a compressed paper bedding. The cause of this, although not related to ammonia levels, was not able to be identified (Burn CC perscomm)

Recommendations

Bedding should be provided in rat cages and should be in sufficient quantity to cover the whole floor. The depth of bedding required will vary with factors such as the type of bedding used, the number of rats in the cage and the frequency of cleaning. As a guide, the depth of bedding should be at least a minimum of 2 cm.Ideally bedding should be free of dust, microbial, parasitic, or chemical contaminants, non-traumatic, moisture absorbent and ammonia binding. The properties of bedding provided should also include that its particles can be manipulated and/or that it is suitable for digging / burrowing. In choosing bedding, the potential for bedding types to induce hepatic enzymes needs to be taken into consideration.

Nesting Material- Principles

(i) Rats of most strains, whether wild or captive, build nests routinely. This behaviour has been observed in young and old, male (Jegstrup, Vestergaard, Vach et al 2005) and female rats and is thus not just relevant to adult females or dependent on pregnancy. There is some evidence to suggest that laboratory rats make better use of nesting material, in terms of better nest building and less eating of nesting material, if provided with the material frombirth (Van Loo and Baumans 2004). The provision of nesting material assists rats in manipulating their microenvironment.

(ii)Rats commonly do not build well-shaped nests (Manser, Broom, Overend et al 1998a). However, in a study by Jegstrup, Vestergaard, Vach et al (2005) where in- cage shelters were provided, rats (male) built complex nests within the shelters. In this study, the use of nesting material by the rats varied with the stain of rat, the type of nesting material and the way the nesting material was presented (eg top of cage lid versus within the cage).

(iii) Rats‟ ability to manipulate and move objects is best catered for by providing them with loose, light materials on top of the bedding. Rats show a marked preference for coarse materials for nest building and show a preference for long paper strips (Manser, Broom, Overend et al 1998a; Manser, Broom, Overend et al 1998b), straw (Jegstrup, Vestergaard, Vach et al 2005) or woodwool. Some lighter materials, such as thick paper or wheat husks, offer the additional opportunity for burrowing.

(iv) The use of nesting material is linked to the survival of pups. According to one study, woodwool was superior as a nesting material and led to a significantly higher survival of pups than those raised in paper tissue or without any nesting material (Norris and Adams 1976). However, care should be taken with the type of nesting material provided - for example, pups may suck on cotton wool and subsequently choke; some pulped cotton fibre nesting materials may separate into strands that wind around pups‟ legs.

Recommendations

All rats should be provided with nesting material in addition to bedding material. Nesting material should be loose, manipulable and light enough to be carried. Suitable materials include shredded paper, straw and woodwool. The way in which nesting materials are provided (eg top of cage lid versus within the cage) should take into account stain-specific differences in the use of materials, depending on the site where they are provided.

In-Cage Shelters- Principles

(i) When rats are placed in an outdoor cage with an earthen floor they will dig a burrow almost instantly. Rats, like all rodents, have a strong need for hiding. Rats have been shown to prefer cages with shelters to barren cages. They show more exploratory behaviour and are less fearful to handle when housed in cages with shelters rather than in barren cages.

(ii) Rats‟ preferences for in-cage shelters can be influenced by strain, age, sex and housing conditions. In a study by Galef and Sorge 2000, some male rats used PVC tubes rarely or only at night while juvenile and female rats used them extensively, regardless of the time of day.

(iii) Rats have been shown to choose to be in opaque as opposed to transparent shelters (even when light levels in both shelters are the same, and the same as the light levels outside the shelters), demonstrating a preference for areas where they are not only physically protected but where they cannot be seen.

(iv) In-cage shelters have several functions and can be used for a variety of activities that are part of the rat‟s natural repertoire:

* They allow withdrawal from light.
* They give a choice of microclimates which aid in rats‟ thermoregulation (it is darker, more humid and usually warmer in a shelter).
* They provide a means of escape from aggressive social interactions and offer a degree of control to the rats.
* They better satisfy the thigmotactic (wall hugging) aspects of rat behaviour than one large cage.
* They may provide an additional structure as a climbing platform, enhancing the rats‟ ability to use vertical space.
* They may facilitate the use of nesting material.

(v) Rats, when given a choice, choose housing offering maximum hiding ability. In studies that compared partitions to nest boxes, nest boxes were preferred.

(vi) An enclosed, opaque thermoplastic nest box with a small entrance hole appears to be the preferred option for a shelter but other forms of shelter are possible. For instance, just darkening a wall area by placing some black self-adhesive plastic over the outside of part of the cage (for transparent cages) and placing a roof over the area that has been darkened, has been found to be effective as an area for sheltering. Partitions do not have the same benefits as providing a roofed section but they can assist in allowing an individual to escape from stressful social situations.

(vii) Rats will attempt to chew items, including shelters, placed within their cage. Using materials (such as cardboard or polypropylene) that can be chewed by rats has the advantage of allowing rats to perform this gnawing behaviour but the disadvantage that the shelters will be damaged. In one study, in-cage shelters made from old polypropylene mouse boxes, upturned and with one end cut out, were used. The rats chewed these, but no problems attributable to this were recorded over a period of more than a year.

(viii) Given the opportunity, rats will make use of space in the vertical as well as horizontal plane. In-cage shelters made from slippery materials may prevent rats from making use of the vertical space by making it difficult for them to climb onto the roof of the shelter.

Shelters with flat tops enable rats to use the vertical space to climb and stand on top of the shelters.

(ix) Suitable sizes for in-cage shelters quoted in the literature are: 25cm (D) x 17cm (W) x 12cm (H) (Manser, Broom, Overend et al 1998a), and 33cm (D) x 15cm (W) x 13cm (H) (Townsend 1997).




Rats make use of in-cage shelters (photos courtesy of David Morton)

Recommendations

Rats should be provided with a shelter within their cage. In-cage shelters should ideally have solid, opaque sides and roof that allow withdrawal from the light (and from other rats) and should be constructed so that rats are able to climb onto the roof of the shelter. Where in-cage shelters are made of chewable material, it should be ensured that the material is not toxic to rats. The minimum space between the roof of the shelter and the top of the cage should be 8cm to allow for rats climbing onto the roof of the shelter.

Pens-Principles

(i) Rat pens and enlarged cages that allow for large areas in which rats can move, both in horizontal and vertical planes, have been described (Spangenberg, Augustsson, Dahlborn, Essen-Gustavsson and Cvek 2005; Sorensen, Ottesen and Hansen 2004; Hurst, Barnard, Hare et al 1996). In the study by Spangenberg et al, male rats in groups of 8 in pens measuring 3.15m^2 were shown to have physiological parameters reflecting increased physical fitness (eg increased muscle strength and endurance), over individually housed rats in standard cages. It was concluded that the large pen provided an environment that stimulated physical activity and more varied behaviour. (However, it should be noted that the use of rats housed individually may have introduced confounding variables influencing the findings of this study).

An enlarged cage with shelters, ledges and a ramp, allowing for movement in both horizontal and vertical directions

Recommendations

The use of pens and enlarged cages, with furnishings such as shelters, ledges and ramps, may be considered as a viable alternative to conventional caging for rats.

Rat Care and Management-The Social Environment- Principles

(i) Rats are well adapted to living in groups (Lore and Flannery 1977).

(ii) Social contact with conspecifics is important to the rat (Patterson-Kane, Hunt and Harper 2002, Hurst, Barnard, Nevison et al 1998. Successful group housing is not just a matter of stocking density but of the combination of individuals. (Hurst, Barnard, Tolladay et al 1999).

(iii)Within the cage environment, social interaction is influenced by the space available per rat (spatial density) and the numbers of rats in the group (social density). Crowding can result from a decrease in spatial density and/or an increase in social density.

(iv) Confined environments (inherent in rat caging) have a negative effect on the behaviour of rats

by frustrating social rules of conduct (Hurst, Barnard, Tolladay et al 1999).

(v) The impact of crowding parameters on rats appears to differ between males and females, with one study indicating that males show a greater stress response to spatial crowding, and females show a similar response to spatial and social crowding (and a maximal stress response to single housing) (Brown and Grunberg 1995). Other studies in male rats, where available space was decreased and number of rats per group increased, have shown significant effects on taste preferences, body weight gain and feed and water intake, and a variable effect on the measures of stress responses (Scalera 1992; Chaouloff and Zamfir 1993); however exploratory behaviour was not affected (Chaouloff and Zamfir 1993).

(vi) Lawlor 1990 has stated that group size generally has a greater effect on the well-being of rats than cage size (ie social density versus spatial density). In studies conducted by Lawlor 1987, when the size of the group of rats was 12 or 24 at low spatial densities (low space per rat: 67-133cm^2 per rat), growth and health were impaired and the rats were more nervous to handle (although their general appearance erroneously suggested that they were normal). Rats in groups of 6 at intermediate spatial densities showed similar, but less marked abnormalities. Rats kept from weaning in groups of 3 at high spatial densities (high space per rat: 500-1000cm^2 per rat) did not exhibit these abnormalities. Lawlor concluded that a group of 3 rats, especially if caged together before maturity, can establish and maintain orderly social relationships while a group of 6 or more cannot (Lawlor 1987). Klir et al 1984 looked at the effects of housing male rats in groups of 2, 3, 4, 6 and 8 and concluded that housing rats in groups of 3 or 4 per cage had the least effects on physiological changes. In a study using male rats in groups of 1,2 and 4, Sharp et al 2002 found that stress-like responses were significantly reduced when rats were housed in groups of 4 compared with rats housed alone. Housing the rats in pairs did not always reduce the stress-like responses to the same degree as housing 4 per cage. Patterson-Kane, Hunt and Harper 2004 conducted a study that showed that female rats preferred a group size of 6 (versus 1,2,4 and 12) when tested over 90 minute sessions. Hurst et al 1999 looked at the effect of housing both male and female rats in groups of 1, 3, 5, and 8 on behavioural and pathophysiological indices of stress and found that group size had a limited long term effect on behaviour and did not affect pathophysiological responses. In this study, a high level of individual variation was found, which may be due to the fact that these rats were housed in wire cages

(vii) Lawlor 1987 has stated that in juvenile rats, holding littermates together in groups of up to 12 after weaning until approaching sexual maturity has been found to cause no marked disadvantages (for example in growth and ability to carry out normal behaviours), providing the rats have sufficient space. (See 2.2 Cage Floor Area).

(viii) Peters et al 1981 found group sizes influenced toxicological responses in rats housed in groups of 1, 2 or 3 and showed significant differences between males and females, in that group numbers had a more significant effect on males and isolation had a more significant effect on females.

(ix) Husbandry procedures such as handling can disturb rats‟ abilities to recognise cage mates (social memory). After such procedures, the behaviour of rats should be monitored to ensure that any disturbance does not result in agonistic and potentially injurious behaviour (Burman and Mendl 2004). It has been shown that juvenile rats can successfully recognise familiar cage mates after a period of separation of at least 48 hours. Successful recognition was not demonstrated at 96 hours (Burman and Mendl 2006).

(x) Young rats, and sometimes even older rats, engage in play behaviour which includes leaping, chasing and scuffling (Scharmann 1991). In monitoring rats for "real fighting" versus "play fighting" indicators including the following can be used:

* target of contact – during "play fighting", snout or oral contact is directed at the opponent's nape of the neck, whereas in "real fighting" contact is directed at the opponent's rump;
* hard bites – bite wounds may be sustained in "real fighting";
* raised hairs (piloerection) in "real fighting" (Pellis and Pellis 1987);
* 50 kHz (ultrasonic) vocalisations during play (Portfors 2007).

Recommendations

As a guide, optimal numbers for groups of adult rats is probably up to 4 individuals. In deciding optimal group sizes, factors such as differences between individuals, strain, sex and cage size should be taken into account. Juvenile stock rats may be housed in groups of up to 12 rats (preferably as litter mates) until approaching sexual maturity (around 50 days of age) (Lawlor 1987). Ideally rat groups should be made up of litter mates of the same sex. Rats should be grouped with each other before they reach puberty to avoid or minimise problems of aggression between unfamiliar individuals (Hurst, Barnard, Nevison et al 1999). The disruption of established social groups should be avoided. When removing rats from, and reintroducing them to, cage mates, it should be aimed to keep the period of separation to 48 hours or less (to take advantage of social memory). Where possible, adult males from different groups should not be placed in the same cage. Where it is necessary to mix unfamiliar adult males, they should be exposed to each other before they are mixed together. This can be achieved by placing the newcomer into an adjoining cage with visual and olfactory contact with the other male.

Shelters should be provided within cages to enable rats to hide in case of conflict. Groups of rats should be monitored to ensure social stability as well as the detection of behavioural and physiological abnormalities. In monitoring rats for "real fighting" versus "play fighting" indicators during skirmishing such as the target of contact (rump versus the nape of the neck), hard biting and raised hairs (piloerection) can be used (as well as measurement of the frequency of ultrasonic vocalisations).

Isolation / Individual Housing- Principles

There are many reports in the literature of the effects of "isolation" on behavioural and physiological measures in rats where "isolation stress" is used as a treatment variable. However, in most circumstances, rats are housed individually but are not completely isolated, in that they maintain olfactory, auditory and visual contact with conspecifics (Brain and Benton 1979). Thus, data need to be interpreted carefully, as most reports refer to social rather than physical isolation.

Although the notion of "isolation stress" in the rat has been challenged (Brain and Benton 1979; Holson, Scallet, Ali and Turner 1991), individual housing of rats (ie social isolation), is associated with a range of behavioural and physiological changes, some of which indicate a stress response.

The effects of individual housing will vary with the period of isolation, age, sex and strain and the prior housing history of the individual.

(iv) Some of the effects of individual housing can be ameliorated by ensuring visual, auditory

and olfactory contact with other rats (Hurst, Barnard, Nevison et al 1997; ibid 1998).

(v) The behavioural and physiological effects of individual housing can be reversed when animals are returned to group housing (Hatch, Wiberg, Balazs and Grice 1963; Gentsch, Lichsteiner, Frischknecht et al 1988) or ameliorated by handling (Gardiner and Bennett 1977;Gentsch, Lichsteiner, Frischknecht et al 1988; Holson, Scallet, Ali and Turner 1991; Reboucas and Schmidek 1997).

(vi) Environmental enrichment by provision of toys, reduces baseline levels of ACTH and corticosterone in both male and female rats housed individually, and lowers the ACTH response to a mild stressor in female rats (Belz, Kennell, Czambel et al 2003).

(vii) Reported behavioural and physiological consequences of individual housing in rats include:

* Behavioural changes consistent with social deprivation (such as reduced mobility, increased tail chasing and self-grooming) (Hurst, Barnard, Nevison et al 1997),
* Altered reactivity to a novel environment when compared with group housed animals, but this response is seen only in some aspects of behaviour (Gentsch, Lichsteiner and Feer 1981; Hall, Humby, Wilkinson and Robbins 1997a) and is dependent upon the aversiveness of the test environment eg. light conditions (Hall, Humby, Wilkinson and Robbins 1997b),
* When reared in isolation from weaning, increased aggression when introduced to other rats in an aversive environment (Wongwitdecha and Marsden 1996),
* Modulation of the daily rhythms of hypothalamic catecholamines, their metabolites and circulating hormones (Greco, Gambardella, Sticchi et al 1992; Gambardella, Greco, Sticchi et al 1994),
* Increased levels of circulating corticosterone and prolactin were reported in male rats (Gambardella, Greco, Sticchi et al 1994), but Brown and Grunberg 1995 when comparing individual versus crowded housing conditions, found an increase in corticosterone levels in female but not males when housed individually,
* Increase in blood pressure and heart rate (Carlier, Crine, Yerna and Rorive 1988; Gardiner and Bennett 1977; Lawson, Churchill and Churchill 2000; Sharp, Zammit, Azar and Lawson 2003), myocardial hypertrophy (Carlier, Crine, Yerna and Rorive, 1988) and increased responsiveness to noradrenaline in arterial strips (Parra, Funetes and Alsasua 1994),
* Variations in biochemical (Perez, Canal, Dominguez et al 1997) and immunological (Baldwin, Wilcox and Baylosis 1995) measurements,
* A deficit in sensorimotor gating (Krebs-Thompson, Giracello, Solis and Geyer 2001) which is attenuated by handling, and
* The development of an abnormal gait in animals housed in social isolation from weaning (Roberts, Clarke and Greene 2001).

(viii) There are equivocal results in studies involving measures of stress in response to individual housing and the diversity of test conditions in the different studies is likely to be a major factor contributing to these equivocal findings.

Recommendations

Rats should not be housed individually unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house rats in this way. In such cases, rats should be able to be in visual, auditory and olfactory contact with other rats.

Metabolism Cages- Principles

(i) When metabolism cages are used to house rats individually, the degree of physical isolation is greater than from individual housing in standard cages, in that the design of metabolism cages will restrict their exposure to olfactory, auditory and visual contact with other rats. Further, they will be housed on a wire mesh floor (see section 2.6 Cage Flooring). Thus the potential impact on the well-being of the rats is greater and there are fewer options to ameliorate these effects.
(ii) When rats are housed in metabolism cages there is a decrease in food and water intake, urine output and creatinine clearance (Damon, Eidson, Hobbs and Hahn 1986; Vadiei, Berens and Luke 1990) and an increase in urinary excretion of corticosterone and aldosterone (Gomez-Sanchez and Gomez-Sanchez 1991).
(iii) A minimum acclimatisation period of 4 days is recommended for rats to recover from the effects of being placed in metabolism cages.
(iv) A significant reduction in the kinetics of drug excretion is seen when rats are housed in metabolism cages for 8 days (Brunner, Dipiro and Feldman 1993).
(v) Behavioural and metabolic effects of housing rats in metabolism cages varies with age. In both young (3 months) and older (12 months) rats, there is an initial increase in urinary excretion of norepinephrine which is sustained in the older animals but returns to normal in the young animals (Gil, Aguirre, Lemoine et al 1999).
(vi) Pregnant rats housed in metabolism cages decrease food intake and lose weight, and there is an increase in the incidence of skeletal malformations in the foetuses (Bosque, Domingo and Corbella 1994).
(vii) A study has been reported in which metabolism cages were enriched with an area of solid floor or with an area of solid floor and a nest box. The rats frequently used the enrichment and the enrichment had no significant effects on food and water intake, faeces production or urine creatinine.

Recommendations

Rats should not be housed in metabolism cages unless with the express permission of the Animal Ethics Committee of the institution on the basis of compelling evidence for the need to house rats in this way. In such cases, rats should be able to be in visual, auditory and olfactory contact with other rats as far as possible. Rats should be acclimatised to the metabolism cage before studies commence.
Where metabolism cages have to be used, consideration should be given to enriching the cages (for example with an area of solid floor and a nest box), providing this does not interfere with the study.

Individually Ventilated Cages-Principles

(i) There is limited information in the literature on individually ventilated ages related to the welfare of rats. Possible concerns related to individually ventilated cages include the limitations on size (and hence limitations on the provision of physical and social enrichment), the use of transparent walls, and possible ultrasound from air being forced in and out (Sherwin C perscomm) (see also 4.5 Air Quality and Ventilation and 4.6 Sound and Vibrations).

Recommendations

Principles and recommendations related to housing rats in conventional cages apply to housing rats in individually ventilated cages.

Effects of Handling and Human Activity-Principles

In both animal holding facilities and the laboratory it is inevitable that rats come into

contact with humans either directly when they are handled or indirectly when they are exposed to human activity. In both situations, interactions with humans elicit physiological and behavioural responses which have implications for animal welfare and the validity of data collection (Claassen 1994). Physiological and behavioural changes are seen not only when rats are handled during experimental procedures (Gartner, Buttner, Dohler et al 1980), or in routine animal care, such as cage cleaning (Duke, Zammit and Lawson 2001; Saibaba, Sales, Stodulski et al 1996), but also when animals are moved into a new facility (Dymsza, Miller, Maloney and Foster 1963; Fortmeyer 1974; Landi, Bowman and Campbell 1988), to a different room (File and Peet 1980; Morato and Brandao 1996; Tabata, Kitamura and Nagamatsu 1998) or moved within a room, for example from a cage rack to a work bench (Gartner, Buttner, Dohler et al 1980). Familiarity with the room and their home cage affects measures of locomotor activity (Galani, Duconseille, Bildstein and Cassel 2001), and removing animals from a familiar cage affects drug kinetics (Hashimoto, Kawasaki and Gomita 2000; Sun, Falk, Nguyen and Lau 2000).

Habituation of rats to the testing room and apparatus attenuates hormone responses (File and Peet 1980; Cooper, Mole, Rehnberg et al 1992), and behavioural indicators in pain studies (Milne and Gamble, 1989; Aloisi, Albonetti and Carli 1994). Rats also respond to the level of activity in their surroundings. For example, significant fluctuations in urine and faecal output were found during weekends compared with weekdays in rats housed in metabolic cages (van der Touw, Thrower and Olley 1978) and significant differences in the architecture and permeability of the mesenteric microvasculature can be associated with the general level of human activity in the room where rats are housed (Wilson and Baldwin 1998). Rats communicate their experience of stress by olfactory and aural cues. Procedures or treatments which elicit physical or emotional stress responses in rats will elicit a stress response in non-treated animals held in the same room. For example, control rats placed in the same room will show the same corticosterone response as adjacent animals which have experienced restraint stress (Pitman, Ottenweller and Nantelson 1988). Similar effects have been shown with stress-induced hyperthermia (Kikusui, Takigami, Takeuchi and Mori 2001), increased heart rate (Sharp, Zammit, Azar and Lawson 2003) and immunological challenge (Fernandes 2000). Further, when a rat is introduced into a new room it will show behavioural evidence of stress, changes which are also seen in the non-transported animals in the same room (de Laat, van Tintelen and Beynen 1989). Removing a rat from a cage causes an increase in corticosterone levels in the remaining cage mates (van Bergeijk, van Herck, de Boer et al 1990). This communication of the stress response also results in an effect, related to the order in which rats are removed from their cage, on biological measures (Knott, Hutson and Curzon 1977; Brodin, Rosen, Schott and Brodin 1994). Behavioural and physiological changes are seen in rats for several hours following routine cage cleaning (Saibaba, Sales, Stodulscki et al 1995; Duke, Zammit and Lawson 2001). These effects need to be taken into account when scheduling experimental procedures.

A study by Abou-Ismail et al (in press) compared the effects on rats of husbandry procedures (eg weighing and cage cleaning) carried out during the light phase or the dark phase (in the presence of dim red light) of the light/dark cycle. The results suggested that rats having husbandry procedures carried out during the light phase displayed higher levels of various behavioural, physiological and pathological measures indicative of reduced welfare, such as higher aggression, less sleep, elevated chromodacryorrhoea and lighter thymus glands compared to the „dark phase" rats.

Significant differences in behavioural and physiological responses can be seen depending on whether or not rats are familiar with the people involved in activities (McCall, Lester and Dolan 1969; Gartner, Buttner, Dohler et al 1980; Dobrakovova, Kvetnansky, Oprsalova and Jezova 1993; Thompson, Brannon and Heck 2003, van Driel and Talling 2005). Human interaction with rats by handling has been shown, in some circumstances, where rats are accustomed to such handling, to be rewarding for rats (Davis and Perusse 1988).

Habituation to handling is associated with a significant reduction in the stress response in serum prolactin, corticosterone and ACTH (Yelvington, Weiss and Ratner 1985; Uphouse, Nemeroff, Mason et al 1982) but not norepinephrine, suggesting that habituation involves the hypothalamic pituitary adrenal axis but not the peripheral sympathetic system (Dobrakovova, Kvetnansky, Oprsalova and Jezova 1993). Systematic, gentle handling can be used to habituate rats to handling and routine procedures. Such habituation can ameliorate the effects of stress associated with human interactions. This will have benefits both for the welfare of the rats as well as for reducing the influence of stress responses on experimental results (for example, Corda, Biggio and Gessa, 1980; Shyu, Mordenti, Nightingale et al 1987).

Failure to handle young rats can have varied deleterious effects. Pham et al 1999 state that these effects include: deficits in some learning tasks and exploratory behaviour, hippocampal dysfunction and impaired adrenocortical response when exposed to stressful stimuli. Handling, in individuals unaccustomed to it, may cause temporary or long term interference with rats‟ abilities to recognise cage mates (social memory). Familiarising rats to handling can therefore be used to decrease its disruptive / stressful effects (Burman and Mendl 2004). “Gentling”, or habituation to handling, is a process of allowing rats to explore the human carer and accustoming them to being gently stroked and held.

Cage mates are initially exposed to a human hand (for example while in their home cage, or confined in a bucket) and allowed to sniff and explore it. The rats are touched if they allow it, and the hand may be gently moved under and over the rats. After one to two days the rats can usually be lifted a few inches in one hand and gently stroked. This procedure is repeated for about 5 minutes once or twice daily over about a week (Hirsjärvi and Valiaho1995). As well as reducing fear reactions to handling, gentling has been shown to reduce fear reactions in rats exposed to novel or fear-inducing situations (Hirsjärvi and Valiaho1995). Handling, when coupled with a negative experience, such as an injection, will not result in habituation (Briese and de Quijada 1970; Stewart and Eikelboom 1981), but will lead to rats developing an anticipatory stressor response with associated hyperthermia which has consequences for metabolic studies (York and Regan 1982). It may be possible to train rats to accept a procedure, such as oral administration of drugs, without restraint using positive reinforcement, for example, drugs delivered in chocolate after a period of training (Huang-Brown and Guhad 2002).

Recommendations

Steps should be taken to allow rats to become familiar with the people who will be handling them so as to reduce the stress of handling. This should include the process of “gentling” (whereby rats are allowed to explore their handler and are gently stroked and held). “Gentling” (habituation to handling) of rats should not be linked with (ie neither proceed nor follow) procedures that may cause distress to rats.

Handling rats for routine husbandry should not be linked with (ie neither proceed nor follow) procedures that may cause distress to rats. To reduce the effect of stress responses on rats

and subsequently the effects on data collection, rats should be habituated to their surroundings and to routine procedures.

Handling rats at all times should be done quietly and gently. Experimental procedures should be scheduled taking into account the potential effects on rats of routine husbandry procedures. The training and rewarding of rats using positive reinforcement or "treats" should be considered when performing procedures on rats. This is likely to reduce the stress on rats and increase their co-operation.

Environmental Enrichment- Principles

(i) **"**Environmental enrichment" is a vague term referring to improvements in captive animalenvironments (Newberry 1995). It has been defined as "a concept which describes how the environments of captive animals can be changed for the benefit of the inhabitants" (Young 2003). It has also been defined as "any measure which promotes expression of natural, species specific behaviours and a decrease in, if not disappearance of, abnormal behaviours" (Brinkman 1996, Shomer 2001).

(ii) The aims of environmental enrichment should be not just to prevent suffering, but to have a positive effect on the physical and psychological well-being of the rat (Morton 1993).

(iii) Rats held in "standard" cages are in a relatively barren environment (lacking in complexity) over which they have little control. Rats have been shown to have long term preferences for complex environments (Denny 1975). A method has been described for creating a physically complex environment in a standard size ($1875cm^2$) rat cage. It was shown that access to this enriched cage was highly rewarding for rats (van der Harst, Fermont, Bilstra and Spruijt 2003).

(iv) In a review of enrichment of laboratory caging for rats, Patterson-Kane 2004 concluded that rats demonstrate a high demand for social contact and prefer larger cages and cages with shelters, nesting material and foraging devices. She contended that enrichments such as social contact and shelter should be considered basic husbandry requirements rather than optional improvements.

(v) A study by Joffe et al 1973 showed that rats given a degree of control over their environment (over lighting and food and water delivery) showed less "emotionality", or fearful reactions (as assessed by defaecation), in open field tests, indicating an enhanced ability to deal with novel situations.

(vi) As early as 1947 it was shown that rats exposed to enriched environments were better able to problem solve (in a maze apparatus) (Hebb 1947). Exposing rats to enriched environments results in significant changes to their cerebral anatomy, neurochemistry and behaviour. These changes include an increase in the weight and thickness of the cerebral cortex and significant improvements in problem solving abilities. Based on this kind of evidence, it is proposed that rats raised in enriched environments may be better adapted to environmental variation and hence less reactive to change (Rose 1996). There is evidence of greater effects on learning behaviours when rats are exposed to enriched environments in the pre-weaning rather than the post-weaning phase (Venable, Pinto-Hamuy, Arraztoa et al 1988; Pascual and Figueroa 1996).

(vii) In a study by Patterson-Kane et al 1999, rats housed in enriched environments showed less fearfulness in behavioural tests than rats kept in standard cages or housed singly. It was suggested that keeping rats in cages that make them more sensitive to stressors (standard cages) is equivalent to increasing the stressors, which is detrimental to their welfare. The study also showed that the rats from the enriched environments had improved problem solving abilities, implying that a standard environment may not be sufficiently stimulating for rats" problem

solving abilities to develop optimally. Van der Harst et al 2003b, in a study looking at the effects of standard versus enriched housing on rats‟ sensitivity to rewards, also concluded that rats housed in standard cages are stressed, probably because of an inability to satisfy behavioural needs. Rats have been shown to have significantly lower baseline stress responsive hormones (adrenocorticotrophic hormone and corticosterone) when provided with environmental enrichment (items for nesting and gnawing) compared with barren cages (Belz, Kennell, Czambel et al 2003).

(viii) Elliot and Grunberg 2005 looked at differences between social enrichment and physical enrichment. They found that social enrichment had the greatest effect (over physical and no enrichment) on improving cognitive performance (simple information processing) in both male and female rats. Overall the effect of enrichment (social and physical) on improving cognitive performance appeared to be greater for males than females.

(ix) The effects of environmental enrichment strategies are manifest in rats‟ behavioural and physiological responses These involve, at one end of the scale, reduction of the harmful side-effects of standard caging (such as high levels of corticosterone, weight loss, developmental delays or stereotypy) and at the other end of the scale, adding interest to the rats‟ daily routines and having benefits for the overall health and wellbeing of rats, prolonging their life and usually also leading to them being better research subjects (Rosenzweig, Bennett and Diamond 1972; Diamond, Rosenzweig, Bennett et al 1972; Por, Bennett and Bondy 1982; Widman and Rosellini 1990; Eskola, Lauhikari, Voipio et al 1999; Galef 1999; Benefiel and Greenough 1998, Belz, Kennell, Czambel et al 2003).

(x) It should be noted that the failure to enrich rats‟ environments may, by imposing constraints on behaviour and brain development, result in aberrant or maladaptive brain functions, which has implications for the usefulness of these animals for research, and in particular for behavioural neuroscience (Wubel 2001). In a review by Sherwin 2004, it was concluded that the development and responses of rodents in standard cages were often unrepresentative and idiosyncratic, indicating that data are likely to have reduced external validity. Sherwin suggests that animals from standard (barren) cages may be “abnormal” and therefore may not provide valid baseline data.

(xi) Rats have five important groups of natural behaviours that should be allowed expression:

* social interaction;
* chewing/gnawing;
* locomotion (including climbing, exploring and playing);
* resting/hiding; and
* manipulating, carrying and hoarding food and objects.

(xii) The suitability of items for enrichment should critically be assessed to ensure that the strategies improve, and are not detrimental to, rats‟ welfare. Assessment may include, for example, whether enrichment strategies assist with the expression of any of the above behaviours.

(xiii) Enrichment items need to be assessed for their health risks. Some materials, such as some plastics or galvanised or painted materials, may be dangerous because of their toxicity when chewed.

(xiv) In some of the studies quoted above and that of Zimmermann et al 2001 (which describes the provision of a “near-to-natural” environment), the degree of environmental complexity

provided may not be practical for the day to day management of rats. It is, however, possible to provide opportunities for rats to express particular behaviours within "standard" cages (see for example van der Harst ,Fermont, Bilstra and Spruijt 2003).

(xv) Examples of enrichment items include: Social interaction:

* See 3.1 The Social Environment

Chewing / gnawing:

- Small block of wood drilled with holes (if the block is small enough, it will be chewed into small pieces and disposed of during cage cleaning before it has a chance to become significantly soiled) (Chmiel and Noonan 1996)
- Branches and softwood sticks (eg tongue depressors) (Patterson-Kane, Hunt and Harper 1999; Scharmann 1991; Johnson, Patterson-Kane and Niel 2004)
- "Kong Toys" and "Nylabones" (Belz, Kennell, Czambel et al 2003)
- Locomotion (including climbing, exploring and playing):
- Branches (Patterson-Kane, Hunt and Harper 1999)
- Running wheel (Patterson-Kane, Hunt and Harper 1999)
- Ledges
- Space (in 3 dimensions) (Patterson-Kane, Hunt and Harper 1999; Scharmann 1991)
- Provision (and rotation on a regular basis) of novel objects such as plastic "toys" (Patterson-Kane, Hunt and Harper 1999; Scharmann 1991)
- Resting / hiding:
- Nesting boxes (Patterson-Kane, Hunt and Harper 1999; Patterson-Kane 2003)
- Nesting material (Patterson-Kane, Hunt and Harper 1999)
- Ledges
- Manipulating, carrying and hoarding food and objects:
- Providing food within the cage that can be picked up and held and hoarded (such as sunflower seeds) (Johnson and Patterson-Kane 2003)
- Cellulose paper and straw on the lid of the cage (Scharmann 1991)

Seeds provide a source of food which can be manipulated and hoarded. (Photo courtesy DarekFiga)

Rats exhibiting climbing behaviour in a cage with a "mezzanine" level. (Photo and cage design courtesy of DarekFiga)

Recommendations

Rats should be provided with items to enrich their environment. Items that assist rats to perform each of the five following categories of behaviours should be provided:

* social interaction (see Section 3.1 The Social Environment),
* chewing/gnawing,
* locomotion (including climbing, exploring and playing),
* resting/hiding, and
* Manipulating, carrying and hoarding food and objects.

When techniques are used in an effort to provide environmental enrichment for rats it is important that the success of the techniques, in terms of improving the rats" welfare, is evaluated.

Identification-Principles

(i) Clause 4.7.1 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states in part:

Animals must be identifiable, whether individually or in groups. Where possible, animals should be identified by the attachment of a label to the cage, container, pen, yard or paddock where they are kept. Otherwise, identification of individual animals may require a physical mark such as a tattoo, neckband, individual tag, or electronic numbering device such as a microchip……The method chosen should be the most appropriate for the species and project and result in the least pain and distress to the animal.

(ii) Ideally methods of identification should not be painful, not cause adverse reactions, not be uncomfortable and not likely to catch or tear out.

Recommendations

Where it is necessary to individually identify rats, the least invasive method that is compatible with the use of rats should be used.

Non-toxic dyes and permanent markers may be used on the fur and tail. These methods of identification usually need to be replaced every two weeks. Fur clipping may be used but needs to be carried out frequently.

Subcutaneous microchipping, tattooing and ear notching may be used where permanent identification is necessary. Note there is some transitory pain associated with applying these forms of identification and the use of anaesthesia and/ or analgesia should be considered.

Toe and tail tip amputation are painful procedures and should not be used.

Food and Water-Principles

(i) Rats need to have food and water provided ad libitum. Note that food and water consumption are affected by the social environment (see 3.1 (iv)).
(ii) Food and water should be free of contaminants (Newberne 1975; Lang and Vesell 1976; Tucker1987) and be of a quantity and quality to meet the rat‟s nutritional needs, taking into account the special needs of pregnant, lactating or growing animals (National Research Council 1995). If possible, the types of food provided should be varied.
(iii) Rats are nocturnal feeders (Siegel 1961; Wong and Oace 1981). The normal behaviour of rats when eating is to carry a piece of food by their teeth to a suitable spot where they adopt a squatting posture and hold the food in their forepaws to nibble at it (Lawlor 2002).
(iv) Providing items of food within the cage rather than in fixed dispensers can encourage foraging behaviours and allow rats to adopt normal postures for eating.
(v) Ad lib feeding may result in obesity in rats. In a study by Wrightson and Dickson 1999, food hoppers were modified to reduce the area over which food was available. Food was provided ad lib but rats worked harder for their food, enabling them to burn more calories and be significantly lighter than control rats after a period of eight months. However, in a study by Johnson, Patterson-Kane and Neill 2004, rats preferred a device that enabled foraging over other feeding sources including a limited access hopper. The rats that were able to forage showed reduced aggression and were able to search for and manipulate food, but they had significant gains in body weight. This weight gain was thought to be due to rats gaining access to whole pellets and it was postulated that the effects could be mitigated by providing a lower calorie food.

Recommendations

A nutritionally adequate diet should be provided for rats.

Food and water should be provided ad libitum unless special permission has been obtained to vary this regime from the Animal Ethics Committee of the institution.

Variations in the types of food and how it is presented should be provided (for example, commercial pellets, dried sunflower seeds, corn on the cob, fresh vegetables).

Food items should be provided not only in food hoppers but should also be sprinkled onto the cage floor bedding to add interest, foster foraging behaviour and promote normal postures during feeding. The rat‟s nocturnal feeding patterns should be taken into account in study design, especially when treatments are given in the diet.

Monitoring of Rats- Principles

(i) Rats are affected by their living conditions, including their physical environment, their social environment and their interaction with humans. When assessing the responses of rats to their living conditions, assessment of physiological and behavioural parameters are useful. Negative trends in these parameters, such as loss of body weight, failure to reproduce and changed behaviour patterns may indicate that the rats are distressed and failing to cope with their environment.
(ii) Behavioural observations can provide useful cues and early warnings that something is wrong with a rat‟s state of health and wellbeing. A range of responses may be observed from subtle changes in normal patterns of behaviour to stereotypy (which is a clear sign of a rat‟s inability to cope with its environment). Excessive grooming, aggression or states of fear are examples of behavioural indicators that a rat is distressed. Abnormal behaviour may manifest itself as an increased reactivity to environmental stimuli, leading to panic reactions, or to an increased passivity or state of depression (Koolhas 1999). Persistent and intense gnawing, as well as short-

distance pacing, are typical stereotypic behaviours. The duration and kind of stereotypic behaviour is important when assessing its welfare significance.

(iii) Excessive secretion of porphyrin from the Harderian gland which is behind the eye (chromodacryorrhoea) is an indicator of stress in rats (Canpolat 2003). This can be seen as a red stain around the eyes and muzzle but often as red streaks from the eyes across the back of the head as the animals spread the excess dye by grooming. Mason et al (2004) showed that low level transient Harderian secretions (seen as specks around the nose) can be scored to assess low to moderate levels of stress.

(iv) As rats are nocturnal, the full range of wake-hour behaviours can best be observed at night using minimal light illumination.

(v) Fearfulness can manifest itself in behaviours such as freezing, hesitancy, and long latency in emerging from familiar spaces (Patterson-Kane, Hunt and Harper 1999). Other signs of fearfulness can be intense vocalisations, teeth chattering, fur fluffing, coupled with immediate defecation and urination. Examples of situations that can elicit fearful behaviour include: exposure to an unfamiliar environment, social isolation, exposure to predators and the conduct of painful procedures.

(vi)Abou-Ismail et al 2007 showed that low frequencies of sleep behaviour and low sleep duration in rats (during the light phase of the light/dark cycle) correlated with some indicators of stress (adrenal weight and body weight gain). They postulate that the stress experienced disrupts sleep behaviour and therefore that the monitoring of sleep behaviour may provide a non-invasive indicator of stress and welfare.

(vii) The results of a study by Burman et al 2007 suggested that ultrasonic vocalisation by rats of 22kHz could induce a negative emotional state of increased anxiety in rats hearing the vocalisation, and could therefore be a useful indicator of welfare for rat groups, including both callers and non-calling group mates. Additionally the recording of 50kHz vocalisations (indicating a positive emotional state) could be used to distinguish normal play in juveniles from aggression (Portfors 2007).

(viii) Gnawing and chewing objects other than food is generally considered to be a natural behaviour that can escalate into a stereotypy with chronic stress (Levine and Morley 1982; Reynolds and Kimm 1972). Sorensen et al 2004 have proposed that excessive gnawing may be escape related and may be indicative of frustration related to the rats‟ environment and hence suggestive of reduced welfare.

(ix) Similar to gnawing, grooming serves a number of functions. Increased grooming is seen when rats are stressed, as part of their coping mechanism (van Erp, Kruk, Meelis and Willekens-Bramer 1994; Moyaho and Valencia 2002). In rats which are chronically stressed this excessive grooming can result in hair loss.

(x) As noted in the section on “Effects of Handling and Human Activity” (3.4), rats respond behaviourally and physiologically to unfamiliar people. Consequently, the „setting‟ in which behaviour is monitored and assessed is important so as to be able to critically evaluate the impact of housing conditions on the rat‟s well being.

(xi) The development of objective monitoring systems for animals undergoing research procedures, to assist in the recognition of pain and distress, have been described (Morton and Griffiths 1995, Bate 2003, Roughan and Flecknell 2006).

Recommendations

Welfare monitoring of rats via behavioural observation should be carried out in addition to monitoring for general physical health. Monitoring should be carried out when a person with whom the rats are familiar is present. In the monitoring and investigation of heath issues (such as growth rate, reproductive performance and disease) the effects of housing conditions should be taken into account.

Environmental Variables-General-Principles

(i) The goals of good animal care and management should be to keep animals healthy and comfortable and to meet their physiological and behavioural needs. Management of environmental variables such as temperature, humidity and lighting can play a significant role in achieving these ends. Some adverse factors such as noise, flashes, and vibrations may not easily be identified but their absence will contribute significantly to the well-being of rats.

Light- Principles

(i) Light is an important environmental variable which has the potential to affect the health and behaviour of rats (Schlingmann, De Rijk, Pereboom and Remie 1993a). For the overall well-being of rats and for their basic locomotor needs, it is essential to manage light levelswell. Room illumination, including the light reaching the actual cages, has important effects on rat well being because day-length, light intensity and spectral quality (wavelength) can affect the functioning of the physiological systems and the behaviour of rats (Belhorn 1980).

Light Intensity and Wavelength

(i) Lighting intensity of various levels has been associated with eye pathology and with causing behaviours in rats indicating aversion. Light intensity can influence factors such as how rats use a living space and their sleeping patterns.

(ii) Rats are nocturnal and the photoreceptors in their eyes are adapted to dim lighting conditions between 1- 40 lux (Vandershuren, Niesink, Spruijt et al 1995). Albino rats lack the pigment melanin that normally protects the eye against high light intensity. These strains have greater light aversion than pigmented strains (Matsuo and Tsuji 1988).

(iii) Tests of avoidance behaviour at different light intensities have shown that albino rats avoid light intensities as low as 25 lux and pigmented rats from as low as 60 lux (Schlingmann, De Rijk, Pereboom et al 1993a). The authors concluded, because the rats were motivated to leave a warm nest to avoid these light intensities, that exposure to these intensities caused distress.

(iv) Exposure to bright (high intensity) light (100 - 200 lux) reduces activity levels in most strains of adult rats, thus affecting normal locomotor behaviour (Matsuo and Tsuji 1988).

(v) In juvenile rats, exposure to bright light (572 lux) suppresses play behaviour which is otherwise displayed regularly when they are housed under dim (low intensity) (0.4 - 1 lux) lighting conditions (Vandershuren, Niesink, Spruijt et al 1995).

(vi) Light levels within the cage influence sleep postures and wall contact by rats. Van Betteray, Vossen and Coenen 1991 found that when exposed to bright (high intensity) light (500 – 600 lux), rats sleep curled up into a ball (nose to tail) and in contact with the cage walls. Under low intensity light (6 - 9 lux) they lie with their head extended, rather than tucked under their body. The lower the light intensity, the less they slept against the cage walls. These authors postulated that wall hugging was a sign of fearfulness and that bright light may provoke fear behaviour.

(vii) The level of light to which rats are exposed will be influenced by the position of the rack in the room, relative to the light source, and the position of the cage within a rack (Kupp, Pinto, Rubin and Griffin 1989). Subtle differences in light levels, for example between the top and

bottom levels of a rack, will influence behaviours and can interfere with investigations, such as those looking at the effects of treatments on behaviour (Exner and Clark 1993). Modifications to the top shelf of a rack by providing extra shading helps to shield the lower shelves from light and create a more uniform light level between shelves (Williams and Howell 1983; Schlingmann, De Rijk, Pereboom et al 1993b).

(viii) Rats undergo some degree of biochemical adaptation if light is a fraction brighter than would be expected in the natural environment (Penn and Anderson 1992).

(ix) Light exposure can be associated with retinal pathology. In albino rats, retinal degeneration develops within 13 weeks of exposure to light intensities as low as 60 lux (Stotzer, Weisse, Knappen and Seitz 1970) and, in these conditions, blindness can occur (Weihe 1976). It should be noted that such light intensities are commonly found within cages. However, exposure to higher light levels for a brief period also will cause permanent retinal damage. A study by Williams, Howard and Williams 1985 showed that albino rats exposed to 133 lux for three days had lost 50% of their retinal rod nuclei as compared to the control group maintained at 3l lux.

(x) The retina of the rat is only partially developed at birth, developing even after rats have opened their eyes (Penn and Williams 1985; Kupp, Pinto, Rubin and Griffin 1987; Penn and Anderson 1992). In rats that have been reared under conditions of relatively high light intensities, the development of the rods in the retina is inhibited. This may later lead to the false assumption that these rats are less sensitive to light when, in fact, this is only the result of damage inflicted on the eyes early in the rats‟ lives (Semple-Rowland 1987 as cited in Schlingmann, De Rijk, Pereboom et al 1993a).

(xi) Weihe et al 1969 showed that light intensity significantly affects reproductive performance, specifically the number of litters born, the numbers per litter and weight gain during gestation.

(xii) Although rats should not be exposed to high light intensity, operators in animal rooms need to have enough light to perform visual tasks. Schlingmann et al 1993b concluded that 210 lux at working height is sufficient for the health and performance of technicians, but would be the minimum under which they should be expected for work for any length of time.

(xiii) Flickering light at only 80 lux intensity, and for a period of 30 minutes, has been shown to be a potent stressor on albino rats causing significant biochemical changes, comparable to those reported in rats that were stressed by electric shock treatment (Lalitha, Suthanthirarajan and Namasivayam 1988).

(xiv) Although it is commonly believed that rats cannot see red light, some writers assert that rats can detect red light, because the rat eye can absorb some low intensity red light and it also has a small number of cones which may be red-sensitive (McCormack and Sontag 1980). However, when placed under red light, rats almost immediately show increased (night time) activity. A failure to do this indicates a serious problem (Morton D perscomm, Morton 2000).

(xv) There is evidence to indicate that rats can detect light in the ultraviolet range (Jacobs, Neitz and Deegan 1991, Jacobs, Fenwick and Williams 2001).

Recommendations

Lighting within cages during day hours should be held at lux ranges below thresholds of aversion for rats. For most pigmented rat strains this is below 60 lux and for albino rats below 25 lux. To enable operators in rat rooms to perform visual tasks, it may be necessary to increase light levels (to approximately 210 lux at working height) for the period that the operators are in the rooms.

Light intensity can be reduced by using recessed lighting consoles in the ceiling with fluorescent lights of about 25 to 36 Watt and a low spectral intensity (wavelength) (which can be achieved by using a low colour number (for example colour 33 tubes)).

Shading should be provided over the top shelves of racks to protect rats in the top cages from overhead lights and to provide a more uniform light level between cages on different shelves.

Lighting should be diffuse and uniform to avoid glare, heat clusters and fluctuating lighting conditions for individual cages .

Under bright operating lights the eyes of rats of any strain should be protected to prevent retinal damage.

Lights should be checked for flickering and any flickering rectified.

Light Cycles- Principles

(i) Aspects of rat physiology (for example serum triglyceride levels) show circadian rhythms (Cayen, Givner and Kraml 1972; Henning and Gisel 1980). The light/dark cycle affects the normal circadian rhythm of various parameters (such as body temperature) (Fioretti, Riccardi, Menconi et al 1973).

(ii) The light / dark cycle can also affect behaviour – for example the nocturnal feeding pattern of rats is reversed when light / dark periods are reversed (Wong and Oace 1981).

(iii) Light period protocols are not only important for the proper care of rats but they can markedly influence the outcome of scientific investigations and the interpretation of their results. For example, in a study by Dauchy et al 1997 looking at tumour growth, minimal "light leaks" of only 0.2 lux during an otherwise normal dark phase inhibited host melatonin secretion and increased the rate of tumour growth and lipid uptake and metabolism.

(iv) Setting lighting on a 12 hours light/12 hours dark cycle throughout the year effectively eliminates seasonal fluctuations. There have been few studies looking at the impact of this on biological measures. Ahlers et al 1989 showed that seasonal variations in corticosterone responses were not affected by this lighting schedule. However, changing the light/dark cycle has been shown to alter the pattern of immunological responses in mice (McEachron, Tumas, Blank et al 1995).

(v) The transition between light and dark can be handled by a dimmer which changes the light gradually and is preferable to a sudden turn off of light, because a sudden turn off of light gives no time for physiological and behavioural adjustment to be made. The twilight period can be quite important behaviourally (Allen 1980).

Recommendations

Regular light cycles of 12/12 – 10/14 hours light/dark are suggested. Variations in the light dark cycles to mimic seasonal changes could be considered.

The use of dimmers in rat rooms is suggested to allow the creation of twilight periods between the light and dark cycles.

Temperature- Principles

(i) Variation in temperature is one of the most obvious and important factors in a rat"s environment.

(ii) The thermal biology of the rat has been extensively studied (Gordon 1990). Rats maintain metabolic homeostasis by a range of mechanisms including variation in metabolic rate, altered patterns and kinds of activities such as shivering and huddling and by creating habitats with special thermal characteristics for example, nest building.

(iii) The rat‟s thermo-neutral zone ranges from 27 to 30^0C (Gordon, Lee, Chen et al 1991); normothermia is maintained in ambient temperatures between 10^0C to 30^0C, rats being better able to cope with low temperatures. Vascular responses to heat and cold are seen primarily in the tail, ears and feet with approximately 20% of heat production dissipated through the tail. Fluctuations in blood flow to the feet and tail occur at ambient temperatures within the thermo-neutral zone. During heat stress, rats spread saliva over their bodies to promote evaporative cooling. Shivering normally occurs below 20^OC unless the rat is active.

(iv) Unlike other rodents, such as the mouse and the guinea pig, which select an ambient temperature associated with minimal energy expenditure, rats, when active, choose an ambient temperature which is below their thermo-neutral zone.

(v) As nocturnal animals, rats mainly rest and sleep during the day and are active at night. For the resting period, if given a choice, rats select areas with an ambient temperature of 25-30^0 C, while during the active nocturnal period they select temperatures between 17-25^0C (Clough 1982; Gordon 1990; Gordon 1993). Transition between wakefulness and sleep is sensitive to body temperature with an increase in REM sleep with increased ambient temperature; within their thermo-neutral zone, REM sleep varies significantly being
maximal at 29^0C where it is twice that seen at 23^0C (Gao, Franken, Tobler and Borbely 1995).

(vi) In an extensive study over two generations into the effects of room temperature on reproduction, body and organ weights, food and water intake and haematological and serum biochemical measures, Yamauchi, Fugita, Obara and Ueda 1981 concluded that room temperature between 20-26^OC is optimum for rats, being associated with minimal variation in the measures studied. This range coincides with that of the rat‟s behavioural preferences (Gordon 1990).

(vii) Using measurements of tail skin temperature and behavioural responses, Yoshida and Sugiyama 1981 concluded that the optimal environmental temperature for rats is 26^OC irrespective of whether they were housed in a group or singly.

(viii) Variations in environmental temperature outside the compensatory capacity for rats will affect reproductive performance with decreased litter size, increased embryonic deaths and impaired growth, and cause significant variation in food and water intake and in haematological and biochemical parameters.

(ix) Gestating and lactating dams have reduced thermoregulatory ability (Knecht, Toraason and Wright 1980) and this also is true of pups where homeothermy is achieved at 3 - 4 weeks of age (Clough 1982).

(x) Temperature in the cage is influenced by cage design and construction, the position of the cage within a rack and a room, pattern of air distribution, ventilation rate, the position of the cage within the air flow pattern and its proximity to other cages (Clough and Donnelly 1984; Hirsjarvi and Valiaho 1987).

(xi) Differences between room (macro) and cage (micro) temperatures (Clough and Donnelly 1984) must be taken into account in the management of ambient temperature so as to meet the physiological and behavioural needs of the rat.

(xii) Rats generate a heat load within the cage (Besch and Woods 1977). Consequently, the ambient temperature within a cage will be determined by this heat load, the ambient room temperature, the thermoregulatory properties of the cage materials as well as the effectiveness of heat exchange by ventilation. With wire mesh cages, heat is dissipated rapidly and cage

temperature and room temperature are equilibrated but rats housed in such cages have no protection from variations in room temperature and can be subjected to draughts. In cages with solid sides and floors, the cage temperature will be at least 5^{O}C above ambient room temperature but animals held in these conditions are less susceptible to fluctuations in room temperature. With solid walls and sides, heat dissipation will be influenced by the thermal properties of the cage materials, for example, stainless steel versus plastic (Hirsjarvi and Valiaho 1987). A greater disparity between cage and room temperatures can occur, depending on stocking density and the various positions of cages on a rack.

(xiii) Under laboratory conditions, rats‟ abilities to control their environmental temperature have largely been replaced by external systems under human control. Where possible, strategies which enable rats to regulate or choose their microclimate, such as the provision of bedding and nesting materials and in-cage shelters, should be provided.

Recommendations

A room temperature range for rat housing between 20 - 26^{0}C is recommended.

Significant swings in room temperature should be avoided.

Rats should be provided with nesting materials and in-cage shelters to enable them to regulate the microclimate temperature, particularly for sleeping .

Special attention should be given to those circumstances where the rat‟s thermoregulatory ability is compromised. Cage temperature for pregnant and lactating rats and pups up to 3-4 weeks of age should be at the higher end of the recommended range (24-26^{0}C).

If rats are held in wire bottomed cages without some solid resting area and nesting material, (for example in metabolism cages) the room temperature should be in the range of 24-26^{0}C.

Temperature should be monitored within the cage and at various positions within the room to monitor variation so as to optimally manage the microenvironment.

Humidity- Principles

(i) Ambient relative humidity is important to the health and well-being of laboratory rats as it influences their capacity to thermoregulate (Weihe 1965) as well as playing a role in the transmission of pathogens (Clough 1982). Environmental temperature and humidity act together on the rat‟s thermoregulatory ability (Weihe 1965; Clough 1982).

(ii) There is a greater risk of rats developing ringtail when they are housed at humidity levels below 40% (Flynn 1967). Low humidity (10-12%) also may contribute to the development of middle-ear disease (Lovejoy, McGuirt, Ayres et al 1994).

(iii) High humidity can enhance the proliferation of bacteria and ammonia production in cages (Reeb, Jones, Bearg et al 1998) and thus place animals at greater risk of infection.

Recommendation

A relative humidity at the level of rat cages of 40-70% is recommended.

Air Quality and Ventilation- Principles

(i) Air quality is largely affected by the concentration of micro-organisms, dust particles and noxious gases, in particular ammonia and carbon dioxide. The level of exposure to these contaminants in the rat‟s environment can have a major impact on their health (Clough 1982; Fox 1983) and will be influenced by the relative humidity in which this occurs, the turbulence of air within the cage and the presence or absence of draughts.

(ii) Significant lung pathology has been reported in rats exposed to ammonia levels above 25 ppm, the threshold level for human safety over a 40 hour week (Broderson, Lindsey and

Crawford 1976; Gamble and Clough 1976; Schoeb, Davidson and Lindsey 1982).

(iii) Volatile pollutants from soiled bedding, in concert with ammonia, produce nasal pathology in rats (Bolon, Bonnefoi, Roberts et al 1991; Ischikawa, Matsuoka and Mori 1995).

(iv) The adequacy of air exchange in the rat"s immediate environment affects levels of, and variance in, environmental temperature, humidity and air quality and determines the respective levels of these measures in the macro and micro environments (Clough 1984).

(v) Air exchange is determined by the pattern of air distribution and air velocity. The placement of air inlets and outlets in a room and the rate of air exchange will affect the pattern and efficiency of air exchange. An increased ventilation rate does not necessarily mean better air exchange between cages (White 1990).

(vi) Ventilation systems in animal houses are usually set between 15 and 20 air changes per hour, although there is some debate as to the frequency required (Besch 1980; Clough 1984).

(vii) The distribution and flow of air within an animal room can play a significant role in the distribution of micro-organisms (Teelman&Weihe 1974). High ventilation rates result in greater dispersion of micro-organisms and dust but lower relative humidity and so may decrease the viability of pathogens (Clough 1984).

(viii) When housed in individually ventilated cages, rats showed a preference for conditions where air changes are kept below 80 per hour. Above this level, both heart rate and systemic blood pressure increase (Krohn, Hansen and Dragsted 2003). In this study, air speeds in individually ventilated cages of up to 0.5m/s had no demonstrable effect on the rats.

Recommendations

The number of air changes per hour needs to be adjusted to keep air quality, temperature and humidity at acceptable levels within cages.

Room ventilation rates of about 15-20 air changes per hour may be needed.

For rooms holding individually ventilated cages, usually 5 air changes per hour will be sufficient to maintain room air quality.

For individually ventilated cages, to ensure low levels of ammonia, air changes should be kept at around 50 times per hour (Krohn, Hansen and Dragsted 2003).

Racks should be positioned in a room so as to optimise air exchange and avoid animals being exposed to draughts.

Cleaning regimes should be managed to maintain ammonia levels within a cage below 25 ppm.

Sound and Vibrations- Principles

(i) There are sounds in animal rooms which may have negative effects on rats, including sounds which cannot be detected by the human ear.

(ii) Hearing in rats is acute and extends well into the ultrasonic range (Gamble 1982). In a study by Heffner et al 1994, rats were found to have a hearing range between 0.25 kHz and 70kHz. Rats are extremely sensitive to ultrasound and respond to it even under anaesthesia. The most sensitive range of hearing for rats lies largely in the ultrasonic range (20-40 kHz) (Clough 1982). Heffner et al 1994 demonstrated maximum sensitivity of hearing at 8kHz and between 32 - 38 kHz.

(iii) Vocalisations also reach into the ultrasonic range; rats use the ultrasonic range in communication and in mating (Knutson, Burgdorf and Panksepp 2002). Male rats emit a pulsed ultrasound before they copulate and an ultrasonic post-copulation call (at 22 kHz).

(iv) The same ultrasonic vocalisation (22kHz) is emitted in response to a variety of stressors and

in circumstances where they are stressed, rats housed singly emit more ultrasonic calls than those housed in groups (Brudzynski and Ociepa 1992). Burman et al 2007 summarises the findings of a number of studies that indicate laboratory rats have two distinct types of ultrasonic vocalisation indicating either (mostly) positive emotional states (50kHz) or (mostly) negative emotional states (22kHz). The findings of this study indicate that ultrasonic vocalisation by rats of 22kHz could induce a negative emotional state of increased anxiety in rats hearing the vocalisation, and could therefore be a useful indicator of welfare for rat groups, including both callers and non-calling group mates.

(v) Sound can have a negative effect on behavioural patterns and physiological responses in rats (Gamble 1982) and is used as a stressor in experimental studies, although usually at levels above that experienced in the animal house (above 100 dB –often referred to as „noise stress"). Differences in the magnitude and kind of physiological responses to noise stress have been found between rabbits and rats (Friedman, Byers and Brown 1967; Nayfield and Besch 1981). Examples of the effects of noise stress in rats include:

* Changes in pattern of eating behaviour (Krebs, Machr, Weyers et al 1996),
* Decreased food intake (Nayfield and Besch 1981),
* Weight loss and adrenal hyperfunction (de Boer, Slangen and van der Gugten 1988),
* Significant weight loss in pregnant rats (Kimmel, Cook and Staples 1976),
* Negative effects on cardiac responsiveness (Morvai, Szakmáry, Székely et al 1994; Breschi, Scatizzi, Martinotti et al 1994), and blood pressure (Alario, Gamallo, Villanua et al 1987),
* Exacerbation of collagen-induced arthritis (Rogers, Trentham, McCune et al 1980), and,
* Onset of autoimmune disease in neonates (Dimitrijevic, Laban, von Hoersten et al 1994),
* Changes to the morphology of intestinal mucosa (in rats exposed to 15 minutes of white noise 90dB) daily for 3 weeks (Baldwin, Primeau and Johnson 2006).

(vi) Human activity and laboratory equipment are important sources of sounds which may impact on rats (Milligan, Sales and Khirnykh 1993; Wilson and Baldwin 1998). The proximity of animal housing to construction sites can also have significant, negative effects (Fernandes and File 1993; Dallman, Akana, Bell et al 1999).

(vii) Reducing noise levels in laboratories can be difficult. If possible, equipment that emits ultrasound should not be used in an area where rats are held (for example computers and oscilloscopes). At the very least, such equipment, should be packed in screening material, such as polystyrene foam plates (which often come as packing material) to dampen the sounds (Birke 1988).

(viii) Ultrasound can be measured using equipment such as bat detectors. The monitoring and control of the acoustic environment of rats may require the input of hearing related specialists and an understanding of some of the principles of acoustics and the measurement of sound (Hughes 2007).

(ix) Exposure to music (less than 40 dB between 9.00am and 2.00pm daily) enhanced immune responses in rats (Nunez, Mana, Linarea et al 2002).

(x) Vibrations tend to have similar effects on rats as exposure to noise. Changes in the hippocampus and amygdala have been observed after rats were exposed to noise and vibration (Fernandes and File 1993). Whole body vibration (at 20Hz, 4.0g) can have similar effects as noise stress to various regions of the brain (Nakamura, Moroji, Nagase et al 1994).

(xi) To avoid vibrations, individually ventilated cages need to be checked for vibratory

properties.

Recommendations

- Noise (loud sounds) within the human hearing range as well as in the ultrasonic range should be reduced where possible.
- Computers, or any other equipment likely to emit high frequency ultrasonic signals, should not be used in rooms where rats are housed. If the use of such equipment is unavoidable then measures, such as packing the equipment in polystyrene foam plating, should be taken to dampen ultrasonic noises.
- The effect of background radio sounds to alleviate the effects of ultrasound and loud noises is unclear. If a radio is used, the volume should be kept low.
- Vibrations in rat holding rooms, and especially of cages, should be eliminated.
- Individually ventilated cages should be checked for vibrations.
- Due to the vibrations created, placing motorised equipment on bench tops with cages should be avoided.

Monitoring of Environmental Variables- Principles

(i) Environmental variables of the rat‟s living area require regular monitoring especially at the cage level. Temperature and humidity should be checked daily. Diurnal variation also should be checked where appropriate.

(ii) Temperature, humidity and air quality are affected by the system of air control.

(iii) The monitoring of ventilation in individually ventilated cages is especially important, as ventilation failure can result in death in a relatively short period .

Recommendations

- Rat rooms should have temperature and humidity read-outs in a position where staff can easily see them.
- Sensors should be fitted to monitor and report malfunctions in ventilation, temperature and humidity control on a 24 hour basis, with automatic alarm activation.
- Even if centralised computer systems are used to regulate the general environmental conditions, it is still essential to check these variables regularly at the cage level.

Cleaning- Principles

(i) There have been few systematic studies on the effects of cleaning on rat health and behaviour and not many are recent (Cisar and Jayson 1967).

(ii) Cleaning has two components: handling (see Section 3.4 Effects of Handling and Human Activity) and cleaning of cages.

(iii) Behavioural and physiological changes are seen in rats for several hours following routine cage cleaning (Saibaba, Sale, Stodulscki et al 1995; Duke, Zammit and Lawson 2001). These effects need to be taken into account when scheduling experimental procedures. In a study using male rats, Burn, Peters and Mason 2006 concluded that cage cleaning did not cause stress (according to the parameters measured) in rats (although it resulted in increased levels of play-like “skirmishing” activity). However, in breeding rats, twice weekly cleaning has been shown to cause more cannibalism of pups than weekly or every two week cleaning (Burn CC perscomm).

(iv) In a long term preference test (during dark and light periods), rats showed no preference for their own soiled bedding over clean bedding (Burn CC perscomm).

Recommendations

- The need for changing bedding depends on the kind of bedding used and air quality. The frequency of bedding changes also will be influenced by stocking rates, strains of rats and particular disease conditions, for example, diabetes. As a guide, bedding is commonly replaced about once a week.
- Cleaning regimes should be managed to maintain ammonia levels within a cage below 25 ppm.
- Cleaning of cages should be done in a separate room designated for maintenance and cleaning tasks. The cage washing area should not be located near rat holding rooms to minimise disturbance from the associated activities.
- Rat rooms should have smooth, hard and impervious surfaces throughout with no exposed joints or cracks.
- All surfaces should be washed down periodically to keep them clean.
- Rat holding rooms should not contain floor drains and if they do they should be rodent proof.
- Procedures to reduce the risk of disease spread during cleaning should be developed with particular attention to staff working in contaminated areas and with diseased animals.
- Clean storage space for cages, food and bedding should be provided.

Records- Cage Labels

Recommendations

All cages should have labels attached to them that provide the following information, or cross reference to a central record in the same room containing this information:

* Rat identification (strain, sex, number of rats)
* Age (date of birth) of litters or of individual rats.
* Date of entry into cage.
* Name and approval number of project in which rats are being used.
* Name, location and contact numbers of the chief investigator/teacher and, if applicable, other investigators/teachers using the rats.
* Name, location and contact numbers of staff associated with the housing and care of the rats.
* Treatments / procedures

Breeding Records - Principles

(i) Clause 4.5.8 of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states:

The person-in-charge must maintain adequate records to allow effective management of the breeding stock including the detection of the origin and spread of disease.

Records should include:

(i) the source, care, allocation, movement between locations, use and fate of all animals;
(ii) details of any diseases;
(iii) the fertility, fecundity, morbidity and mortality in breeding colonies; and
(iv) the health status, genetic constitution and physical environment of the animals.
(v) ARRP Guideline 16: Supervision of Animal Supply by Animal Ethics Committees

II –M.Sc Microbiology (Batch 2018-2020)

Possible Questions

Unit – III

Two Marks

1. Types of enriched feed for rats
2. What are the sub groups of lab rat species
3. Dosage of administering anaesthesia of lab rat
4. Define pets
5. Types of groups made in laboratory rats

Eight Marks

1. Write short notes on the care and maintenance of rats in cages.
2. Comment on the general anatomical characteristics of rats.
3. Describe the role of enrichment methods in housing lab rats.
4. Write short notes on the lab ratdiseases and their etiology.
5. Discuss on the methods of grouping and identification of lab rats for experimental setups.
6. Discuss in detail on the sub groups of labs rat‟s species.
7. Explain in detail on the types of feed and nutritional requirements for rat in lab.
8. Write short notes on the method of administering anaesthesia of lab rat.
9. Discuss in detail about the specification for building a rat cage in lab.
10. Write short note on the nomenclature of rats in lab.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB **COURSE NAME: LABORATORY ANIMAL CARE**

COURSE CODE: 18MBP305B **UNIT: III** **BATCH-2018-2020**

S.No	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	An example of inhalant anaesthetics for rat is_____.	propofol	Iso flurane	Doxapram	ketamine	Iso flurane
2	Long duration analgesia is _____.	Flunixin	ketamide	doxapram	xylazine	flunixin
3	Adult male rat weighs _____g.	200	400	260	500	500
4	Respiratory rate of rats ranges _____rate/min.	50-100	70-115	80-115	90-115	70-115
5	Oestrouc cycle in female rats lasts _____days.	4-5	6-10	20-30	1-2	4-5
6	_____is NOT suitable for nesting rats.	Wood shavings	Papers	Fur	Ground corn cobs	Ground corn cobs
7	Rats that are deficient in pigmentation is called as_____.	white rat	albino	wistar	wild	albino
8	Sprague-Dawley rat strains are an example of_____.	Inbred rat	outbred rats	albino	hybrid	outbred rats
9	Lewis rat is an example for _____.	Inbred rat	outbred rats	albino	hybrid	inbred rats
10	The rat feed pellets are sterilized by _____.	autoclave	boiling	evaporation	drying	boiling
11	Rats are grown in cages with _____objects.	1D	2D	3D	Plain	3D
12	The gelatinous material attached to vagina of female rat after copulkation is_____.	vaginal plug	oestrus plug	tissue plug	vaginal fluid	vaginal plug
13	Removing pregnant female rats during	corporal behaviour		enhanced milk	weaning	enhanced milk

	partuirition leads to _____.		aggressiveness	production		production
14	Guinepigs origniated from _____.	India	Europe	Africa	South America	South America
15	Commonly used variety of guinepigs in lab is_____.	long haired	short haired	whorled haire	hairless	short haired
16	Rats have prominent _____teeths.	molar	pre-molar	wisdom	canine	canine
17	Bright light in the rat cages lead to _____.	agressiveness	climbing activity	retinal damage	lesion on eye	retinal damage
18	Young rats have_____to regulate the body temperature.	fur	thick skin	epidermal pores	brown fat	brown fat
19	Rats have_____digits on hind limbs	3	4	5	2	5
20	_____is essential for healthy respiration of rats in cages.	porous ventilation	air hole on cage	filtered air generator	open wired lid	filtered air generator
21	Rat diet should contain protein level of_____.	5%	27%	65%	89%	27%
22	Suitable feed per 100g of rat is _____.	10 g	60g	80g	240 g	5g
23	Water is supplied to lab rats via_____.	tubes	cans	sippers	jugs	sippers
24	Immunocompromised rats need _____ water	ozonized	filtered	chlorianted	acidified	chlorinated
25	Optimal temperature for rats in laboratory is_____℃ .	12	25	40	31	25
26	Low humidity in rat laboratory results	dehydration	inactivity of	tail ring	wrinkled	tail ring formation

	in_____.		mice	formation	mouth	
27	Light cycle for lab rats is _____h.	12/12	8/16	5/19	20/4	12/12
28	Ilumination for the rat cages are maintained at the range of _____lux.	500-600	200-400	100-200	50-150	200-400
29	Air change in rat cages are done at _____cycles per day.	2	9	15	3	15
30	Puberty occurs in rat during _____ days.	2	65	20	10	65
31	Scientifc name of commonly used lab rat is_____.	Rattus mexicanus	Rattus norvegicus	Rattus romulus	Rattus indicus	Rattus norvegicus
32	Which among the following is an example of out bred rat strain?	Wistar	BALB5	Lewis	cBALB65	Wistar
33	Which amont the following the special character of a rat?	hermaphordite	nocturnal	diurnal	chemo sensitive	nocturnal
34	Thigmomatic behaviour of rat refers to_____.	climb stones	climb on PVC pipes	ability to hold to tree	jumping	ability to hold to trees
35	Rats are usually kept in cages made up of_____.	wood	steel	plastic	iron	plastic
36	Stocking density of rats per cage is _____.	2	6	10	20	6
37	Rats behaviour to eat the feces is termed as_____.	autophagic	coprophagic	Mesophagic	prophagic	coprophagic
38	Food pellets for caged rats are kept in _____.	containers	cans	hoppers	cage lid	hoppers
39	Ususal recovery time for a rat from	2	60	15	7	15

	anaesthetic condition is _____min.					
40	The dosage of anaeasthetic administered to rat is _____µg.	300	150	20	60	300
41	Normal RBC count of rat is _____x 10^6/mm^3	7-10	15-20	30-65	100-120	7-10
42	Example of the surgical anaesthesia used in rat is_____.	furanone	ketamine	xylazine	saffan	xylazine
43	Barbiturates are used in mice under surgery during_____.	start of surgery	mid surgery	in between blood transfusion	post surgery	post surgery
44	Mating in rat is confirmed by the presence of_____in the breeding females	blood	inactivity	copulatory plug	swelling of hind limbs	copulatory plug
45	In rat the gestation lasts for _____ days.	10	23	40	90	23
46	Weaning of rats occurs in _____days.	50	21	15	5	21
47	Breeding one male rat with many female rats is called_____ metho	Monogamous	Multi gamous	Harem	partuirition	Harem
48	Handling of rat is favoured by holding the animal by its_____.	mid regions	hind limb	head	fore limb	hind limb
49	The porpyrin secretion from eyes migrates to lower part of eyes and causes_____.	chromodacryorrhoea	disacryorrhoea	swelling	balck ring	chromodacryorrhoea

50	Rats are more susceptible to _____disease.	bacterial	viral	fungi	protozoa	virus
51	Sendai virus infects rats specifically during_____conditions	stress	immuno compromised	knockout	mutated	stress
52	_____is NOT a causative agent of disease in rats.	Hantaan virus	Sendai virus	Pneumonia virus	Hepatitis virus	Hepatitis virus
53	_____is used as anaestic in rats.	ketamine	ketamine + medetomidine	ketamine + para fentanyl	flucosine	ketamine + medetomidine
54	In rat acute pain and restrain is identified by_____.	deep vocalization	crouching under bedding	docile attitude	struggling to feed	deep vocalization
55	Stress recognition in rat is done by observing_____.	reduced sleep pattern	climbing	nuzzling	crouching	reduced sleep pattern
56	Modified tear gland of rat is called _____.	lacrymal gland	bulbular conjuctiva	Harderian gland	lubian gland	Harderian gland
57	_____is the NOT a common behaviour of rats in la	running	hopping	climbing	nuzzling	nuzzling
58	_____lubricates the eye of a rat.	tears	saliva while licking	sweat	porphyrin rich secrection	porphyrin rich secrection
59	Cages to contain the rats should have a default flooring made up of _____.	wire mesh	smooth plastic	rough alloy	ceramic	wire mesh
60	Solid bottomed cages for rats should containg_____bedding.	wood shavings	plant materials	chopped wood logs	poly urethane sponge	wood shavings

UNIT-IV
SYLLABUS

Modern methods of care, management breeding and maintenance of laboratory animal – Guinea pig

General Introduction
Guinea Pig

The guinea pig, Caviaporcellus, is a rodent that originated from South America. There are several different varieties of guinea pig available, including the short hair (English and American varieties), Abyssinian (which have hair in whorls), and Peruvian (which have long hair). Commonly used laboratory stocks are derived from the short-hair variety. The Dunkin–Hartley and Hartley guinea pigs are outbred stocks, and strains 2 and 13 are inbred strains.

Behaviour

Guinea pigs are amenable animals which rarely bite. Naturally they are crepuscular, but in the laboratory they will be active for periods throughout the day and night. When startled, guinea pigs have a tendency either to become immobile or to stampede and vocalise, leading to the risk of trampling young and making capture difficult. Providing bolt-holes and barriers within the pen and frequent handling to habituate the animals will reduce the problem. The approach of a person will cause excitement, and the scatter reaction should be elicited as an attempt is made to capture a guinea pig. The normal behaviour is for the guinea pig to „resist arrest" and vocalise strongly. If this does not occur it may indicate that there is a problem. Group-housed familiar guinea pigs will soon establish a stable hierarchy, which is male dominated and maintained mainly by olfactory cues, but with some barbering and chewing of subordinate males. If unfamiliar males are placed together, fighting will ensue particularly in cramped conditions or if oestrous females are present. Guinea pigs are creatures of habit and become increasingly unable to cope with changes in routine as maturity approaches. If there are any changes in the type of food hopper or water bottle, or in the type of food or water, the guinea pig may be unable to adapt and cease eating and drinking. This is particularly disastrous with pregnant females. Similarly, if there are changes in the type of housing, problems may be encountered.

Housing

As gregarious animals, guinea pigs like to be housed in groups. This may be in floor pens, or large plastic or steel cages. Although guinea pigs rarely jump, cages should have sides at least 23 cm high, and more height is required for open-topped floor pens (Figure 12.8). Guinea pigs thrive on solid floors, but are sometimes kept on slats or mesh if accustomed to these types of floor. Mesh floors may also predispose to footpad ulcers and increased stress levels, and are certainly contraindicated with experiments involving joints and feet.Bedding materials provide comfort and a substrate for rooting behaviour. Materials such as wood shavings, paper-based bedding, ground corn cobs or sawdust may be used, together with hay. Fine shavings and sawdust alone may cling to moist areas, such as the perineum, and probably are best not given to breeding

guinea pigs. Larger shavings are better for these animals. Guinea pigs are messy animals and will disperse opaque, creamy coloured urine and faecal pellets throughout the pen. All pens, cages, feeding receptacles and water bottles must be cleaned and disinfected at least weekly. Removal of urine scale may require the use of acidic-cleaning agents.

Figure: Guinea pigs housed in floor pens.

Feeding and water

As guinea pigs are messy, food and water bowls placed on the floor will soon become soiled with bedding, urine and faeces, and should be suspended above the floor or cleaned frequently. There is a tendency to play with drinkers, which leads to messy floors, and bottles quickly become empty. Automated watering systems ensure a constant water supply, but in solid-floored systems, care must be taken to prevent flooding. All watering systems need to be checked and cleaned frequently. Any changes in watering system will upset the routine, and the guinea pig will need help to adapt. The water requirement of a guinea pig is 10 ml/100 g body weight daily.

Guinea pigs are fastidious eaters and will reject unfamiliar food. They require a pelleted, freshly milled complete guinea pig diet, not one designed for any other species. Supplements of hay may be given, but with care as digestive disturbances may result from an excessive amount, or from greens that have not been properly washed. The food requirement is 6 g/100 g body weight daily. However because much of the food is wasted more should be supplied. The food should contain 18–20% crude protein, and 10–16% fibre. Guinea pigs are unable to synthesise vitamin C, and require 5 mg/kg daily normally, and up to 30 mg/kg if pregnant. This can be supplied in the food or water, or by giving cabbage, kale or oranges. Food with added vitamin C must be used within 90 days of manufacture, or the vitamin C will degrade. Coprophagy does occur in the guinea pig, but may not be essential.

Environment

Guinea pigs thrive at temperatures between 18°C and 26°C, with a humidity of 40% and 70%. They should have 12–15 air changes per hour, and 12–15 h light daily.

Breeding

Female guinea pigs reach puberty from 5 to 6 weeks, and males from 8 weeks. The average is 9–10 weeks. Pairing should be done when the female is 400 g (at 2–3 months), and the male 650 g (3–4 months). One boar can be housed with one to ten females. The oestrous cycle of the female lasts 15–17 days, and she is receptive for 6–11 h. The vagina is covered by an epithelial membrane, which is intact except during oestrus and parturition, both of which are signalled by perforation of the membrane. Gestation lasts 59–72 days, depending on litter size. In the last week of gestation, the pubic symphysis separates under the influence of the hormone relaxin, and once the gap reaches 15 mm parturition will take place within 48 h. Females should have their first litter before reaching 7–8 months of age, or the symphysis will be unable to separate sufficiently and dystocia will result. In any case, there is often a high incidence of dystocia and fetal death. Abortions and stillbirths are common.

Female guinea pigs can breed until they are 20 months old. Thereafter, the litter size tends to drop and dystocia is more common. Neonatal guinea pigs are precocious, weighing 60–100 g, and they begin to eat solid food within a few days. Hand rearing is not difficult, making Caesarianrederivation of colonies quite easy. The young are not hungry until 12–24 h after birth, and can then be fed cows‟ milk or soaked guinea pig pellets. If the females are not kept in harem groups, the young may be removed at birth and hand reared, to allow the sow to be mated at the post-partum oestrus. Otherwise, weaning takes place at 180 g (15–28 days), or 21 days (165–240 g). Weaned males intended for breeding need to be weaned late or group housed to allow development of normal adult reproductive behaviour.

Growth

The growth depends largely on the strain of guinea pig. Young guinea pigs should gain 2.5–3.5 g daily up to 60 days.

Handling

Guinea pigs are easily startled, and they will vocalise and try to avoid capture when approached. They should be grasped quickly and smoothly, placing the thumb and fingers of one hand on either side of the shoulders, then lifted and the free hand placed beneath the hindquarters to support the weight. The guinea pig can then be turned over for i.p. injections or sexing. Positioning the thumb under the foreleg and beneath the chin as in the rat will provide additional restraint. Alternatively, one hand may be placed under the thorax and the other under the rear feet. It is particularly important to support pregnant females with two hands.

Recognition of pain and stress

Guinea pigs are alert, apprehensive animals who will try to avoid capture and restraint. Any unusual sign of acceptance indicates the animal is unwell. Loud vocalisation accompanies even minor and transient pain. They often appear sleepy when in pain and rarely show

aggression. They are stoical animals and it can be difficult to assess whether they are in pain from a single glance. A carefully used pain scoring assessment method should be employed.

Common diseases and health monitoring

Relatively few infectious diseases are seen in guinea pigs. Guinea pigs are unique among non-primates in having a dietary need for vitamin C however, and will develop signs of deficiency, if fed diets that are not designed for guinea pigs, which have been stored incorrectly, or fed after the use by date, since vitamin C is labile and will degrade over a period of time. Most infectious diseases seen in guinea pigs are bacterial, with abscesses and non-specific infections most commonly encountered. However, guinea pigs can carry LCM virus (a zoonosis) and Sendai virus, and these two antigens should be included in regular screening programmes.

Figure: Handling the guinea pig.

Drug doses for anaesthesia in the guinea pig

These are probably the most difficult rodents in which to achieve safe and effective general anaesthesia. The response to injectable agents is variable and post-anaesthetic complications, such as respiratory infection, generalised depression, inappetance and digestive disturbances, may frequently be seen. Many of these problems may be avoided by careful selection of anaesthetic agents and high standards of pre-, intra- and postoperative care.Atropine (0.05 mg/kg s.c.) may be given to decrease airway obstruction. To counteract respiratory depression, use doxapram 5–15 mg/kg. The injection of both Hypnorm and ketamine in the guinea pig has been associated with tissue necrosis at the site leading to self-mutilation post-operatively.

Sedation

For sedation alone Hypnorm (fentanyl/fluanisone) at 0.5 ml/kg i.m. can be used, but there will be poor muscle relaxation. Alternatively, use diazepam 2.5 mg/kg i.m. or acepromazine 2.5 mg/kg i.m.(hypotensive), or ketamine (100 mg/kg).

Injectable general anaesthesia

Fentanyl/fluanisone combinations

For surgical anaesthesia lasting about 45 min, combine Hypnorm 0.5–1.0 ml/kg with midazolam 2.5 mg/kg i.p. If continued anaesthesia is required, further doses of Hypnorm may be given at 0.5 ml/kg i.m. every 20–30 min. The fentanyl component can be reversed with naloxone (0.1 mg/kg) or, for continued analgesia with nalbuphine 1 mg/kg i.p. or s.c., or butorphanol 1 mg/kg i.p. or s.c., or buprenorphine 0.05 mg/kg i.p. or 0.01 mg/kg i.v. If combining Hypnorm with diazepam, use 1 ml/kg Hypnorm plus 2.5 mg/kg diazepam. Fentanyl 160 mg/kg and medetomidine 400 g/kg i.p. will give about 20 min anaesthesia in the guinea pig, but it can be of rather variable depth. Nalbuphine (1 mg/kg) and atipamezole (1 mg/kg) can be used for reversal.

Ketamine combinations

Xylazine and ketamine will give about 30 min of surgical anaesthesia (5 mg/kg xylazines.c. plus 40 mg/kg ketamine i.m.). Ketamine may also be combined with diazepam (100 mg/kg ketamine plus 5 mg/kg diazepam i.m.), or with acepromazine (125 mg/kg ketamine plus 5 mg/kg acepromazine), or with medetomidine (40 mg/kg ketamine plus 250g/kg medetomidine). All these combinations give about 30 min anaesthesia with a 2–3 h recovery time. The response to the medetomidine component can be unpredictable in the guinea pig and is reversed with atipamezole (1 mg/kg).

Saffan

A short period of surgical anaesthesia can be induced i.v. (ear vein) with alphaxalone/ alphadolone (Saffan) at the rate of 40 mg/kg. It may also be given i.p.

Barbiturates

Pentobarbitone will induce anaesthesia lasting 15–60 min at 35 mg/kg i.p., but it has a very narrow safety margin and there will be a high-mortaility rate.

Inhaled agents

Isoflurane is the agent of choice. For induction it can be used with an anaesthetic chamber and for maintenance given via a face mask.

Dose of drugs for analgesia in the guinea pig

Opioids

- Buprenorphine: 0.05 mg/kg s.c. lasts about 8 h.
- Pethidine: 10–20 mg/kg s.c. lasts 2–3 h.
- Butorphanol: 0.5 mg/kg s.c.

NSAIDs

- Flunixin: 2.5 mg/kg i.m. or s.c.
- Piroxicam: 5.7 mg/kg orally.
- Diclofenac: 2 mg/kg orally.

- Phenylbutazone: 40 mg/kg orally.
- Aspirin: 85 mg/kg orally.

Specific pathogen free and gnobiotic animals

Specific pathogen free animals are those animals which are free from a group of particular pathogen but these animals are not necessarily free from other organism, which are not specified, in the conducted experiment. Gnotobiotic: A word derived from the Greek "gnotos" and "biota" meaning known flora and fauna. An animal stock or strain derived by aseptic cesarean section (or sterile hatching of eggs) which are reared and continuously maintained with germfree techniques. According to International Committee on Laboratory Animals (ICLA) "Specific Pathogen Free (SPF) animals, which are free of specified micro-organism and parasites but not necessarily free of on the ones which are not specified." Synonyms of SPF animals are – Disease free animals, healthy animals, pathogen free animals, clean animals, caesarian derived animals.

Historically, the concept of gnotobiotic experimentation is credited to Pasteur"s efforts in 1885 Nonhuman primates are important for disease investigation, therapy and vaccination (Schmidt, 1972 and Dormant et al., 1990). Within the past 20 years there has been a widespread interest in the artificial rearing of animal removed from the dam at or near the end of gestation and keep isolated from conventionally reared animals. There are two primary reasons for producing animals under such conditions. The 1st of these is to break the cycle of the some of the infectious disease organism present commonly transmitted from animal to animal. The 2nd is to provide more uniform experimental animal for many phase of basic and applied research by reducing one of the variables diseases. It has been estimated that approximately 20 million animals are being used for testing and are killed annually; about 15 million of them are used to test for medication and five million for products. Different factors may affects experimental animals which results in change in experiments (Melby, 1983; Small, 1983). China has become one of the biggest country using lab animals and highest number of lab animals are found in China. (e.g., specific pathogen free; genetically modified) increasingly used in scientific research-16 million a year, compared to 12 million in the 25 European Union countriesin 2005. People for the ethical treatment of animals (PETA) reported that the National Centre for Laboratory Animal Sciences (NCLAS) in Hyderabad, supplies approximately 50,000 animals to laboratories every year.

Use of specific pathogen free and gnotobiotic animals

These animals are used in certain conditions of body which become severe by secondary complication. Ex. Wound. In India, among rodent group of animals e.g. mice, rat, G. pig, rabbit, mice are predominantly used in most of cases followed by others. SPF animals are very much useful in experiments which are carried out for longer period.

Mouse: Most frequently used. Pharmacology, genetics of mammals, virology, models of human diseases.

Rat: Physiology of cognitive processes, behaviour, models of diabetes.

Rabbit: Serology, insulin quantification, pyrogens quantification.

Guinea-pig: In microbiology and serology, physiology of the auditory system.

Hamster: Genetics.

Frog: Physiology of blood circulation, electrophysiology.

Fish: Molluscs, insects.

These animals are used in studying of defense mechanism of the body. These animals are used in studying the ageing process of individual in animals and human being. In body relationship between different microflora can be study by using the specific pathogen free and gnotobiotic animals. These animals may be used as the steril organs and tissue for different investigations and research. Different diet related researches and its reaction can be estimated by using these animals.

Purchase and techniques for the SPF and gnotobiotic animals

Before the purchasing these animals we should get the important information about these animals. We should select a defined specific pathogen list for the stock. Different diagnostic and detection methods should apply during the purchasing. Previous screening and screening test organization should be cleared. By the use of historythe Surveillance programme and disease history should be carefully monitored.

The principle is that obtain animals from a stage in their life cycle when they are either a minimum number of contamination or not at all.

Caesarian technique: The placenta acts as a very efficient filter and prevents the fetus from becoming infected with most bacteria, virus and parasites. The object is to remove and free from the conventional pathogens.

– Normal parturition is delayed by giving daily injection of progesterone to the mother during the last three days of pregnancy.

– In case of bird fertilized eggs can be passed directly from the outside via the germicidal trap, to the interior where hatch normally.

– The pregnant dam is prepared for surgery by removing the hair from abdomen by shaving.

– In case of small animals like rat and mice cervical dislocation is followed which is quick and humane. For large spp. Halothane/oxygen mixture can be used for anaesthesia. Hysterectomy used for obtaining young from the dam.

– Gravid uterus is put in a sterile plastic box which containing some sterilizing will kill microorganism. The uterus is opened dries the fetuses. The fetal membranes removed, leave the placenta in contact at the umbilicus for a short time while respiration has been initiated.

– Young one usually requires stimulation by gentle with sterile gauze and drying of the nostril.

– Food is given by hand day and night until they are already inside the barrier.

Hand rearing: The animals maintained at a temperature 33-350C and the humidity kept at 50 per cent or higher. The hand rearing of new young like mice is difficult. So we can fed the new born mice with the help of rubber nipple or stomach tube which stimulated to pass urine and faeces.

Foster nursing: The foster nursing of surgically derived pups is possible in a barrier room. We should introduce a number of good foster mother, who should be mated so as to deliver 1-3 day prior to the date of surgery. The new born young should put into warmed receptacle containing fostering mother. After half an hour foster mother is removed from the litter and put with the new young ones.**Care and management**

– Discipline of the whole operation of SPF unit is that of preventing reinfection and invasion of the clean area by pathogen. A persons working inside should be clean because they act as carrier for many diseases.

Staff: A person should be in good health, active, intelligent and well trained. Smoking eating drinking and eating should be prohibited in all working area. Person working with this should be well aware and well understood of the operations aspects of the unit. Personnel are required to take a shower while entering and then don sterilized garments including hair nets, face masks, and jump suits. Personnel facilities include staff and record room, sufficient changing room, decontamination area and first aid.

Boot cleaning and disinfection: Visible organic material may be removed from boots. Boots may be disinfected by soaking in a clean bath of an appropriate disinfectant. It is important to frequently empty, clean, and refill the boot bath to prevent it from being contaminated with organic matter. Disposable boots may be used.

Training for SPF animal care and use: All SPF animal care and use personnel must be trained on SPF protocols. All SPF trained personnel must follow the SPF care and use guidelines at all times.

Veterinary rounds: The veterinary staff makes regular rounds through the facility to observe the animals, their housing conditions and husbandry procedures. All animals in SPF care are observed daily by an animal care staff. Each area of the facility is also assigned a veterinary technician and an area veterinarian.

Decontamination of the room: The room should be properly sealed and left over night after fumigation and thereafter should be properly ventilated. The different methods adopted by animal houses depend upon the facilities available, the cost, the simplicity and efficiency of the procedure. When liquid formalin is used 1 ml of 10 per cent solution for every cubic foot of the room space is required. The room temperature should be at least 180C with relative humidity about 80 per cent. The sterilization of all consumable is very essential Formaldehyde gas may be produced by exothermic reaction. Usually two parts of formalin are added to one part of crystals of potassium permanganate. When liquid formalin are added to one part of crystals of crystals of potassium permanganate.

Animal room: Animal room should be protected against ingress by pets such as wild rodents and insects. Holes created should be sealed. Adequate arrangement should be provided to the receipt or incoming animals. Baker (1979) recommended a noise intensity of 85 db. Animal brought into an animal house should not put at risk animals which are already there. In cage rearing system housing temperature may affected by the nesting materials and type of chosen animals (Woods, 1980 and Corning, 1992). Where surgery is to be performed suitable operation facilities should be provided including separate preparation area for the animals, equipments and staff. There should be post-operative recovery area. The relative humidity of the laboratory animals should around 50 per cent and ranges between of 40-70 per cent (Clough, 1987).Food and bedding stores should be clean dry vermins and insect free. In addition food stores should be cooled and sunless provided with ventilation. Perishable food should be stored in cool room refrigerators and freezers.Floors should be moisture resistant, non-absorbent, impact resistant, and relatively smooth, although textured surfaces may be required in some high-moisture areas and for some species.A vermin free collection area should be provided for waste prior to its disposal. Special arrangement should be made for handling carcasses and radioactive or other hazardous materials.Each animal room is emptied, cleaned and fumigated with formaldehyde and water vapors at least once per year so as to prevent the buildup of bacterial contamination. The

fumigation is carried out by evaporating a mixture of formaldehyde and water to near dryness by boiling. In rat and mouse house half a liter of formalin (40% formaldehyde) and one liter of water is allowed for each 1000 ft.

Inside the SPF unit:Laminar flow hood (LFH)

A unit which provides a sterile work environment by very high efficiency filtration of the air that circulates across the work surface. Room air is taken in through the back of the hood and passed through the HEPA filter. Sterile air moves across the work surface from back to front and is expelled through the sash opening.

MI cage/unit: A housing unit consisting of a polycarbonate shoebox-style bottom, stainless steel wire-bar lid and a polycarbonate top that holds a permeable filter. Additional items also include bedding, water bottle, food, and cage card holder.

Mobile shelf-unit (MSU): Mobile shelving unit and cover used to temporarily store and transport clean micro isolator cage units.

Bath: The polycarbonate cage bottom located in the laminar flow hood which contains the disinfectant solution. If the bath becomes cloudy or excessively soiled with feces or bedding, the solution should be emptied in the sink and box rinsed with tap water.

Introduction of animals: Specific-pathogen-free (SPF) rats and germ-free mice were purchased; the animals inside their delivery box passed into the breeding unit through the „dunk" tank and there allowed to breed.

Dispatch of animals: Weanling animals, other than breeding replacements, are not kept in the rodent breeding unit, but are transferred to a stock room in an experimental unit. A litter is transferred by placing them in a sterile cardboard box which is then sealed into a polythene bag and passed out through the „dunk" tank. The litter then enters the experimental unit through a further „dunk" tank, where the polythene bag is removed and the litter caged. The transport boxes are stored flat and made up as required.

Biosecurity: Good biosecurity begins with personal cleanliness. Showering or washing facilities and supplies should be provided, and personnel should change their clothing as often as necessary to maintain personal hygiene. Personnel should not be permitted to eat, drink, apply cosmetics, or use tobacco in animal facilities. Visitors should be limited as appropriate, and institutions should implement appropriate precautions to protect the safety and well-being of the visitors and the animals. It is essential that the agricultural animal care staff maintain a high standard of biosecurity to protect the animals from pathogenic organisms that can be transferred by humans. Disposable gear such as gloves, masks, coats, coveralls, and shoe covers may be required under some circumstances. Personnel should not leave the work place in protective clothing that has been worn while working with animals.

Ventilation: Ventilation, humidity, temperature, lightening and noise contribute for good science. High level of Ammonia causes the respiratory problems in the rodents (Lindsey et al., 1978). In coming air should be filtered from dust particles, when most sources are also removed. Ultraviolet pathway within the dunk tank can be used. Temperature should be maintained between 10 to 210C. Number of air changes 5 to 15/ hr. provided adequate ventilation. Heat is removed from the exhaust air by means of a heat pump unit incorporated in the extract system and used to heat the water in the breeding unit. Light intensity is very important for the laboratory animals because it may influence the aggression and cannibalism in the animals (Fall, 1974;

Weihe, 1976). Belhorn (1980) suggested a light intensity level of 323 lux (30 fc) for animals care and management practices.

Materials of biological origin: Materials of biological origin such as cells, tissues, serum and cultures will be damaged or destroyed by autoclaving or gas sterilization techniques. Therefore, they must be tested before they are introduced into animals. Please contact Rodent health monitoring.

Stem: Most commonly used method of sterilization carried by means of double autoclave situated in the barrier with one door.

Sterile diets: Diets are autoclaved and ethylene oxide fumigation. Water is decontaminated by acidification, hyper chlorination and/or filter sterilization,. Sometimes nutritive value destroyed but sterilization with ionizing radiation and microwave infrared is healthy.

Screening and control of pathogen: Regular routine sampling of stock for bacteria and parasites is done.

Serological examination: Each SPF room will have serology conducted every 3 months. The serum test will be done annually by a basic panel. The infected animals will be immediately treated or discarded.

Routine screening procedures: Swabs from the surfaceof the walls and floors of the breeding unit and samples of the filtered water supply and of the „dunk" tank fluid aretaken four times at weekly intervals. These are examined for the number and type of organisms present. Salmonella, Mycobacterium, Bordetella, Pasteurella, Mycoplasma and Corynebacterium.

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.

Record keeping: Record keeping is important for-

– Animal house plans which includes typical floor plans, all fixtures etc.
– Breeding stock, purchases and sales records.
– Minutes of institute animals ethics committee meeting.
– Records of sick animals.
– Death records.

II –M.Sc Microbiology (Batch 2018-2020)

Possible Questions

Unit – IV

Two Marks

1. Define gnotobiotic
2. Maintenance of safety aspects in handling animals
3. What are the different guinea pig species used in lab
4. How to maintain cage for guinea pig
5. Standard required feed for guinea pig

Eight Marks

1. Write short notes on the handling of guinea pigs in lab.
2. Comment on the unique characteristics of different guinea pig species used in lab.
3. Describe the housing of guinea pigs in laboratories.
4. Write short notes on the safety aspects to be followed while handling guinea pigs.
5. Discuss on the methods of identification used in guinea pigs.
6. Discuss on the breeding strategies used for guinea pigs in labs.
7. Write shorts notes on the nutritional requirements of guinea pigs.
8. Write short notes on the disease management of guinea pigs in lab.
9. Gnotobiotic animal and their applications.
10. Write short note on the specific pathogen free animals.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB **COURSE NAME: LABORATORY ANIMAL CARE**

COURSE CODE: 18MBP305B **UNIT: IV** **BATCH-2018-2020**

S.No	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	water suplly to the guinea pigs are given by_____.	can	dropper	automated system	jug	automated system
2	The dietary reqirement of water for guineapigs on daily basis is_____ml.	10	20	50	60	10 ml
3	Feed is provided to guineapig in the form of_____.	pellet	powder	Raw	milled	pellet
4	Fibre requirement of guineapig feed is_____.	16%	2%	30%	45%	16%
5	Crude protein requirement of guineapig feed is_____%	20	10	5	2	20
6	Gunieapig does not synthesize _____in its body.	Vitamin C	salaiva	Phermones	calcium	Vitamin C
7	Water content is provided to guinea pigs by_____fee	Soy	Corn cake	Grounut cake	cabbages	cabbages
8	Excessive supplementation of guinea pigs lead to_____.	abodmial distrubances	inactivity	Severe bowel syndrome	malnutrition	abdominal discomfort
9	The food requirement of the guineapigs per day is_____ g.	10	20	30	40	10
10	Optimal temperature for guinea pigs in laboratory is_____℃ .	26	36	10	15	26
11	The light cycle for guineapigs in lab is _____hours.	9	15	16	30	15
12	Peruvian variety of guineapigs are characterized by_____.	long hair	short hair	hairless	whorled hair	long hair

13	Guinepigs are naturally _____.	dirunal	crepuscular	docile	inactive	crepuscular
14	Providing bold-holes within the pen of guineapigs reduce_____.	trampling	climbing	nuzzling	Scratching	tampling
15	The normal behaviour of guinea pigs includes_____.	running	hopping	climbing	resist arrest	resist arrest
16	_____type of hierarchy is observed in guineapigs.	vertical	horizontal	Stable	unstable	stable
17	Male domination in guineapigs is maintained using_____.	ear	mouth	Tail	olfactory lobes	olfactory lobes
18	Guineapigs like to be housed in _____.	groups	colonies	single	coupled	groups
19	Commonly used housing method for guinea pigs is_____.	cages	pens	steel cages	floor pens	pens
20	_____is NOT given to guinea pigs as bedding during breeding.	fine wood shavings	Papers	Sponges	cotton	fine wood shavings
21	Minimal dose of hypnorm given for surgical anaestesis is _____.	1.5 ml	5 ml	10 ml	0.5 ml	0.5 ml
22	The scientific name of guniea pig is _____.	Cavia indicus	Cavia porcellus	Cavia rodentus	Cavia cavie	Cavia porcellus
23	Aspirin is used as _____ in guineapigs.	sedative	anaesthetic	antibiotic	analgesic	analgesic
24	Diclofenac is used to _____in lab guineapigs	reduce pain	reduce inflammation	reduce weight	reduce irritation	reduce pain
25	The analgesic phenylbutazone is	subcutaneous	intravenous	Oral	intra muscular	oral

	admininstered via_____ route.	injection	injection			
26	The combination of anaesthetics are given_____mins before the surgery.	10	20	30	40	30
27	Which among the following is NOT an example of injectable general anaesthesia?	Fentanyl	Ketamine	Isoflurane	Barbiturates	Isoflurane
28	_____method is used to assess the pain during guniea pig handling.	pain scoring	square check	Dunnett	Tukey	Pain scoring
29	Poor vetilation in guineapig cage lead to_____.	ammonia precipitation	respiratory illness	Asphyxation	death	respiratory illness
30	Guinea pigs are highly _____animals.	alert	ferocious	Timid	agile	alert
31	Guineapigs are commonly infected by_____.	fungi	virus	Protozoa	bacteria	bacteria
32	Primary symptom of bacterial infection in guinea pigs is_____.	lesion	asphyxation	Abscess	haemorrhage	abscess
33	Guineapigs act as carrier for _____.	flu virus	simian virus	hanta virus	LCM virus	LCM virus
34	The most prevalent viral infection in guineapigs is caused by _____.	flavi virus	sendai virus	Simian virus	myxoma virus	sendai virus
35	_____is common in anasthesia of guineapig.	death	resistance to anaesthesis	post-anesthetic complications	paralysis	post-anaesthetic

						complications
36	_____causes obstruction in airway of guinea pigs.	atropine	flucytosine	rifampin	doxycylcine	atropine
37	_____ is used as a sedative for gineapigs.	hypnorm	atropine	rifampin	doxycyline	hypnorm
38	The sedative hypnorm is a mixture of _____.	fentanyl + rifampin	fentanyl +fluanisone	fentanyl + atropine	fentanyl + morphine	fentanyl + fluanisone
39	Which of the following is an example of injectible general anaesthesia?	chloroform	ethylene	atropine	hypnorm	hypnorm
40	Respiratory rate of guineapig ranges _____rate/min.	42-104	50-110	100-150	120-180	42-104
41	Birth weight of a guineapigs is _____g.	70	150	200	300	70
42	Normal RBC level of guinea pigs is _____x10^6/mm^3	2	10	7	16	7
43	_____percentage of basophil in guineapigs indicates an allergy.	0.5	10	3	5	10
44	Anaesthetics used for guineapigs are chosen based on_____.	volume	strain	surgical need	metabolic activity of the animal	surgical need
45	Natural life span of guinea pigs is_____years	2	3	10	8	8
46	Female guineapigs reach puberty in _____weeks.	2	4	5	6	6
47	Male guineapigs reach puberty in _____weeks.	7-10	8-9	9-11	1-3	7-10

48	Paring should be done when the female is_____g.	100	250	300	400	400
49	Paring should be done when the male is_____g.	250	650	450	300	650
50	Male guinea pigs are commonly called as_____.	pigs	mare	Boar	flock	boar
51	The average weight gain of a young guinea pig should be_____ g	3.5	1	3	10	3.5
52	Restraining of guinea pigs should be done by hold the animal in _____.	anterior	posterior	Lateral	dorsal	anterior
53	Loud vocalizationin guinea pigs occurs due to_____.	mild pain	tansient pain	Genralized pain	acute pain	transient pain
54	Any unusual sign of acceptance towards handling in guinea pigs indicates_____.	loss of health	low aggressiveness	Death	anaesthetization	loss of health
55	Common dietary need of guineapigs in comparison to primates is_____.	more water need	requirement for Vit C	High moisture	cooked feed	requirement of vitamin C
56	The vagina of female gunieapig is covered by_____.	epithellial	squamous	enothellial	mesanchymal	epithellial
57	Gestation in guinea pig lasts around _____days.	30	72	110	90	72
58	During the last week of gestation the pubic symphysis seperates due to action of_____hormone.	contractin	nor-epineprine	Relaxin	meladunin	relaxin

59	Maximum age for the female guinea pigs to breed is_____ months.	5	10	15	20	20
60	Weaning of guineapigs occur at _____days	21	11	13	18	21

UNIT-V
SYLLABUS

Handling – various routes of inoculation and bleeding. Laboratory use of animals in microbiology antibody production. Disposal of animal house wastes and carcasses.

Handling Laboratory Animals

Good animal handling techniques will reduce the risk of injury from bites andscratches, and will increase the confidence of both the handler and the animal, thus reducing stress to all those involved. All animals will respond in some way to the presence of a human and most species can recognise individuals and will be nervous of strangers. It is, therefore, important for the licensee to establish a friendly relationship with the animal to reduce nervousness on both sides. An animal that is confident and relaxed with its handler will be more co-operative enabling procedures to be carried out more easily. Some aspects of handling will vary according to the species. A brief description of techniques for each species can be found in the remainder of this chapter, but with each it is essential to get advice and assistance from people with previous experience.

Administration of Substances

Introduction to pharmacokinetics

Administration and absorption

Substances may be administered to laboratory animals orally, or by intravenous (i.v.), intradermal (i.d.), intraperitoneal (i.p.) or subcutaneous injection, per rectum, or by injection directly into other body parts such as joints or parts of the gastrointestinal system. The substances may act locally or may act throughout the body or at single target systems after absorption into the bloodstream. The rate at which administered compounds are absorbed into the bloodstream depends on the site of administration (Figure 9.1), the nature of the compound, and the manner in which it is presented. Compounds that are given i.v. reach a high blood concentration immediately. This then tails off as the compound is eliminated, for example, by the liver and kidneys, or redistributed, for example, by absorption into fat. Compounds given by other routes are absorbed at rates depending on the blood flow to the site, and the solubility of the compound in the tissue fluids. Muscles have a good blood supply, so substances administered intramuscularly (i.m.) are absorbed more quickly than those given subcutaneously, as the subcutis has a poorer blood supply. Compounds that are highly soluble in the tissue fluids will be absorbed quickly. Some injectable preparations are designed to have a long duration of action, and the active compound in these is mixed with a carrier of low solubility to slow down absorption into the bloodstream. With orally administered compounds, absorption tends to be slower, and takes place over a longer period.

Compounds that are given orally may be absorbed at various points in the gastrointestinal tract. Substances will only be absorbed across the gut wall if they are lipid soluble. Some compounds are designed to be absorbed locally in the large bowel, andare insoluble unless they are activated by enzymes during passage through the stomachand small intestines. The absorption of other compounds may be affected by pH.Compounds that are ionised are not lipid soluble and will only be absorbed if a specificcarrier exists to transport them across the gut wall into the blood. Basic or alkalinecompounds are likely to be fully ionised in the acid environment of the stomach,and will be poorly absorbed. However, once in the small

intestine, the higher pH willreduce the level of ionisation and increase the absorption. The reverse is true foracidic compounds.

For solids, the particle size affects the rate of absorption, because the surface area relative to volume increases as particle size decreases, presenting more of the compound for solubilisation and absorption. Intestinal passage time will vary depending on the species and on whether or not the animal has been food deprived prior to the administration. Another factor which complicates oral administration is the first pass effect. All substances absorbed in the stomach or intestine, have to pass through the liver before reaching the systemic circulation. This may result in the metabolism of some or all of the compound to active or inactive products, reducing the amount of the original compound which reaches the circulating blood volume. Many compounds induce the liver to produce enzymes, which metabolise them, thereby increasing the first pass effect with repeated dosing. Alternatively, there may be some enterohepatic recycling, in which the compound is conjugated and secreted into bile, and therefore, passed back into the intestine without reaching the systemic circulation.

These factors affect the bioavailability of substances administered orally, which is essentially the difference between the amount of an oral dose which reaches the bloodstream compared with an equivalent i.v. dose. However, even if the bioavailability is high, it does not necessarily mean that the blood concentration reaches the peak seen immediately after an i.v. dose. Many compounds are absorbed very slowly after oral administration, and may never reach high levels in the blood even if the bioavailability is high.

Distribution

Where and how compounds are distributed within the body also depends on the bloodflow to the site and the solubility of the compound in the tissue fluids. There are somespecial cases however. For example, compounds will only enter the central nervoussystem (CNS) if they can cross the blood–brain barrier. Lipid soluble compounds willaccumulate in fatty tissues, even if the blood supply is poor. This phenomenon can beused to ensure rapid elimination of lipid soluble drugs from the CNS by redistributionto fat (see thiopentone in anaesthetics, Chapter 7). Drugs will also tend to accumulateat sites of metabolism or excretion, such as the liver and kidneys. In pregnant animals,the fetus is separated from the mother by the placenta so only certain compounds willenter the fetus. Some compounds may accumulate in the fetus and some may accumulatein specific tissues, such as the kidney. Compounds will generally be secreted intomilk if they can cross the lipid membrane in the mammary gland. Milk is slightly moreacidic than blood, so basic compounds tend to accumulate in milk because they ioniseafter having crossed the lipid barrier and cannot then return to the blood.These factors that affect the absorption and distribution of administered compoundsdefine the pharmacokinetics of the compound, and should be studied prior to administrationto ensure that the compound is being given in the most effective way.

Administration volumes

The volume of any substance given must be as small as is practicable for the procedure,and will be limited ultimately by the size of the animal (Table 9.1). If fluids are to be administered by infusion, the flow rate should be as low as possible, and the infusiongiven over as short a time as possible. However, if too much fluid is given too rapidly,the circulation may become overloaded, causing pulmonary oedema. If the

administration is slower, excess fluid can be cleared by the kidneys. The maintenancerequirement for fluid is approximately 40 ml/kg per 24 h in normal animals and careshould be taken not to exceed this unless there are deficiencies to replace.

Administration techniques

Injections should be performed using aseptic techniques, as for blood sampling. It isimportant to use equipment appropriate for the species (see Tables 9.2a and b). Forexample, injections in small rodents should be given with 25 or 27 G needles. For rabbits,guinea pigs and cats, 23 or 25 G needles are best, and for dogs 21 G needles areadequate. For farm animals, needles larger than 21 G may be used. The viscosity ofthe substance also affects the size of needle used. Thick, viscous liquids may not passthrough narrow gauge needles. Intradermal injections are performed with 25–27Gneedles. To minimise the distress caused to animals during administration of substances,they must be carefully and expertly handled, and given a sedative or short actinggeneral anaesthetic, if required.

Subcutaneous injections

For most species, subcutaneous injections can be given into the scruff of the neck(Figure 9.2). A fold of skin is lifted using the thumb and first two fingers of one hand,and the needle is passed through the skin at the base of the fold parallel to the body, to avoid penetrating deeper tissues. Subcutaneous injections are rarely painful, unlessthe substance being injected causes stinging.

Figure 9.2 Subcutaneous injection.

Figure 9.3 Intramuscular injection.

In rabbits, subcutaneous injections can also be given over the flank, provided thatcare is taken with adjuvants, because if there is an adjuvant reaction in the skin overthe respiratory

muscles this can cause pain on breathing. In sheep and goats fluid canbe given under the skin over the ribs. For pigs, small volumes can be injected into theskin behind the ear, or into the fold between the leg and the abdomen. Pigs have muchsubcutaneous fat, and injections given elsewhere are likely to enter the fat, whereabsorption will be particularly slow due to the poor blood supply.

Intramuscular injections

Intramuscular injections are frequently painful, due to the distension of muscle fibres,which occurs, and therefore, good restraint is required. They are usually given intothe muscles of the thigh. Larger volumes or irritant compounds should be injectedinto the quadriceps group on the front of the thigh. The muscle can be immobilisedwith one hand while injecting with the other. Injections can be given into the caudalthigh muscles, but as the sciatic nerve runs through these muscles, irritant compoundsshould not be given here or damage may be caused to the nerve. In rodents, thequadriceps feels like a small peanut on the front of the thigh, and can be immobilisedwith the thumb and forefinger of one hand while injecting with the other (Figure 9.3).

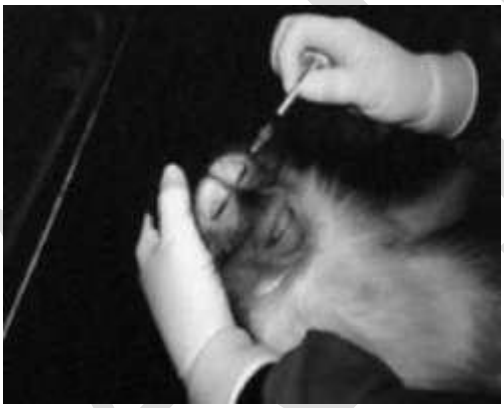


Figure 9.4 Intradermal injection used for tuberculin testing in a macaque.

In dogs and cats, injections can be given with care into the muscles on each side of thespine, and in large animals the gluteal muscles are used. In adult pigs, injections aregiven into the neck muscles, but a long needle is required to penetrate the fat layer.Piglets can be injected by lifting them by one hindleg and injecting into the caudalthigh muscle on that side. Fowl are given i.m. injections into the pectoral muscles.After the injection, the site should be massaged to disperse the dose.

Intravenous injection

Intravenous injections may be given into the cephalic veins of dogs, cats, primates,and ferrets (see Figure 9.7b). In rats and mice, the lateral tail vein is used. Rats canalso be given injections into the dorsal metatarsal vein. The hindlimb is held in extension,and the vein raised by occluding it at the stifle joint. The jugular vein can be usedin dogs, cats, hamsters and ferrets (see Figure 9.8), and is the method of choice inruminants and horses. The ear veins are used in guinea pigs and pigs. Fowl can beinjected via the brachial vein.

Intradermal injections

Intradermal injections for most species can be given in the same area as subcutaneousinjections. For tuberculin testing in primates, the skin of the upper eyelid is often used(Figure 9.4).

Intraperitoneal injections

Intraperitoneal injections in rodents are given in the lower left or right quadrant ofthe abdomen as there are no vital organs in this area. The quadrants are demarcated by the midline and a line perpendicular to it passing through the umbilicus. The animalshould be held either by an assistant or in one hand on its back, upright, so that itis comfortable and securely supported. The needle is angled at 45° to the skin and noresistance should be encountered to the passage of the needle (Figure 9.5).

Figure 9.5 Intraperitoneal injection.

Oral administration

Substances may be given orally by inclusion in the diet or drinking water. These methodshave the disadvantages that it is impossible to be sure that the animal has had theentire dose, and in some species it will lead to the animal refusing to eat and drink.With ad lib feeding, or if there is an increase in metabolic rate, the animal may overeatand thus ingest an overdose of the drug. In mice particularly, adding drugs to thewater tends to lead to dehydration, because the mouse avoids drinking it, and this canlead to a rapid deterioration in the condition of the mouse, especially if the compoundshave been given for therapeutic reasons. If the watering system is automated,it is impossible to give compounds in this way. To overcome these problems, gastricintubation or gavage may be employed (Figure 9.6). Flexible catheters or stainlesssteel needles with rounded tips are used. The animal is restrained with its neckextended, and the needle or catheter passed gently down the oesophagus. Care mustbe taken not to damage the oesophagus, or to put the needle into the trachea. Theneedle or catheter can usually be observed passing down the oesophagus on the leftside of the neck. Damage to the catheter from chewing can be avoided by using anoral speculum, or by using a flexible nasogastric or pharyngostomy tube instead. Theanimal may or may not need to be starved prior to administering the compound,depending on the nature of the compound and the particular project.For dogs and cats, tablets may be administered orally in the conscious animalby placing one hand over the top of the head, placing the thumb at the commissure ofthe lips on one side and the fingers at the other, and tilting the head back. This will cause the mouth to open slightly. The tablet can be held between the thumb and forefingerof the other hand, and the middle finger used to open the mouth. The tablet canthen be placed onto the tongue as far back as possible, to stimulate the swallowingreflex.

Figure 9.6 Gavage in the rat.

Legal Categories And Restrictions Governing The Use Of Drugs Used In Experimental Animals

The Medicines (Restrictions on the Administration of Veterinary Medicinal Products)Regulations 1994 prevent the administration of a veterinary medicine to ananimal without a marketing authorisation (a product licence) for the indication andspecies. Exceptions may be made in the absence of a licensed product (the cascadeprinciple). In the UK, prescription of veterinary medicines is primarily the responsibilityof registered veterinary surgeons. Personal licence holders under the ASPA whoare authorised to perform anaesthesia and surgery in research animals and who donot hold veterinary qualifications must obtain and administer drugs under the directionof a veterinary surgeon. A pharmacist may only supply licensed medicines foruse in animals under the direction or prescription of a veterinary surgeon. Medicalpractitioners may not legally prescribe medicines for use in animals.The cascade for prescribing veterinary medicines where no product for the speciesor indication has a marketing authorisation (off label prescribing) is as follows:

No veterinary-licensed product exists for a condition in a particular species?

(1) use a veterinary medicinal product licensed for use in another animal species oranother condition in the same species;

(2) if no product as described in (1) exists, a human-licensed product; or (3) if no product as described in (2) exists, a product prepared extemporaneously byan authorised person in accordance with a veterinary prescription.

Legal classifications of veterinary medicines

(Medicines Act 1968)
General sales list GSL
Pharmacy medicines P
Pharmacy and merchants list medicines PML
Prescription-only medicines POM
Controlled drugs (Misuse of Drugs
Regulations 1985 amended 2002) CD (Schedules 1–5).

Storage and record keeping

All drugs must be stored appropriately to ensure that they maintain their full activity.Some must be kept in a refrigerator at 2–5°C, some need to be kept away from light,some should not be kept in plastic bottles. The data sheet supplied with the drug willspecify the requirements.It is good practice to keep a record of all drugs held and used, it is a legal requirementto keep records of

purchase and use of all controlled drugs listed in Schedules1–3 of the Misuse of Drugs Regulations.

Removal of Blood

Causes of stress

The removal of blood from an animal is a procedure with three potentially stressfulcomponents.

● Handling and restraining the animal is stressful. To minimise the distress caused,the licensee should be familiar with humane methods of handling and restraint(see Section 2 for species-specific information), and should consider using an appropriate sedative or anaesthetic (see Chapter 7). Many animals can be trainedto accept the handling required to take blood samples, such as cats, dogs, rabbits,pigs and primates (see Figure 15.5), and although this takes time, it is worthwhile.

● Venepuncture causes some minor pain and discomfort, whatever the site, andrequires considerable skill. The expertise required to carry this out successfullymust be gained first by watching others, then by practising on cadavers or modelssuch as the KOKEN rat simulator, and then by carrying out the technique oneself,once a licence has been granted, under direct supervision.

● The removal of blood causes physiological responses, the magnitude of whichdepend on the volume of blood removed (as a percentage of the total), and thespeed of withdrawal. The rapid removal of large quantities of blood will causethe animal to go into hypovolaemic shock, and may even cause death. The percentageblood loss required to cause hypovolaemic shock varies with the speed ofwithdrawal, whether or not fluid is replaced concurrently, and the psychophysiologicalstate of the animal at the time. Chronic slow haemorrhage is toleratedbetter than acute blood loss, and placid animals tolerate greater losses than nervousones, again indicating the need for competent handling and training of theanimals.Stress responses in the animal result in the release of hormones and other substancesto counteract the stress, which can cause anomalous experimental results. It isessential to minimise the stress caused to an animal when removing blood from thehumane viewpoint, and also to ensure good scientific practice. The experimental techniqueshould be refined such that the quantity of blood removed is minimised. This isparticularly important in small mammals, such as mice, where the blood volume issmall and sample volume is critical. The withdrawal of blood from any vessel requiresskill in handling the animal and in manipulating the equipment. The person taking thesamples should be fully familiar with the chosen technique, and have all the equipmentready before starting. You are advised to consult the BVA/FRAME/RSPCA/UFAW (1993) working group report on the removal of blood from laboratorymammals and birds.

Quality of samples

To achieve meaningful results, any samples taken must be of good quality, and be preservedin the best possible manner. If the sampling technique is poor, blood may clotor haemolyse rendering results invalid. To avoid these problems, samples must betaken skillfully, and treated appropriately thereafter.Blood may be collected using syringes and hypodermic or butterfly needles,through indwelling cannulae, with double ended needles and evacuated tubes (e.g.„Vacutainers"), or in very small species by incision of a vein using a sterile lancet orscalpel blade. The latter however is not best practice, as inadvertent movement canresult in the severing of an appendage. If needles are used, the needle should be aslarge as is practicable for the species. This allows blood to flow faster, reducing thelikelihood of clotting, and also causes less

damage to the red cells, reducing thepossibility of haemolysis.Thought should be given to the desirability of using anticoagulants. Different anticlottingagents are suitable for different purposes:

● No anticoagulant: Blood clots, and serum can be removed after centrifugation.

● Lithium heparin: This is the anticoagulant of choice for most biochemical assays.The yield of plasma from heparinised blood is greater than the yield of serumfrom clotted blood, which may make heparin a good choice for collecting bloodfor harvesting antibodies. Sodium heparin is sometimes used if preservation of thewhite cells is required.

● Potassium EDTA (ethylene diamine tetra-acetic acid): This is used for haematologicalanalyses.

● Oxalate/fluoride: This is used for blood glucose determination.Several other anticoagulants are available, for example, for collecting blood fortransfusions or for analysis of clotting factors. After collection into anticoagulant, theblood should be mixed thoroughly by rolling, not by shaking as this can damage thecells and lead to haemolysis.It is preferable for samples to be submitted fresh for analysis. If this is impossible,samples may need to be refrigerated or deep frozen. For some enzyme determinations,degeneration of the enzyme renders analysis useless if performed more than afew hours after blood collection. It is advisable to determine the exact requirementsof the laboratory protocol prior to sample collection.

Venepuncture technique

Restraint

When taking samples, the animal should be gently restrained by an experienced handlerwho is known to the animal. Chemical restraint is generally only required if thetechnique involves more than a pinprick, for example, for tail tip amputation, or inprimates if the animals are not trained or are under quarantine restrictions.

Site and location of the vein

It is important to be certain of the location of the vein, either by visualising it or palpatingits course, and to have it immobilised, before piercing the skin. The handler maybe required to raise the vein, by occluding the venous drainage proximal to the site ofvenepuncture. This must be performed correctly, or withdrawal of blood will be difficult.If unsure of the position of the vein, venepuncture should not be attempted. Theuse of a quick release tourniquet greatly facilitates blood sampling in some species andvasodilating agents may be used in other species (e.g. „Vasolate", IMS) (Figure 9.7).

Preparation of the site

Blood should be collected using an aseptic technique. The area should be clippedto remove hair if necessary, then cleaned. The use of warm water with or without disinfectant will help dilate superficial veins as well as cleansing the skin. After cleansing,the skin can be swabbed with 70% ethanol or disinfectant. In some species, itmay be advantageous to apply local anaesthetic cream (e.g. „EMLA", Astra Pharmaceuticals)to the site 30–60 min before venepuncture to prevent any discomfort in suchanimals as the cat, dog, rabbit, or pig.

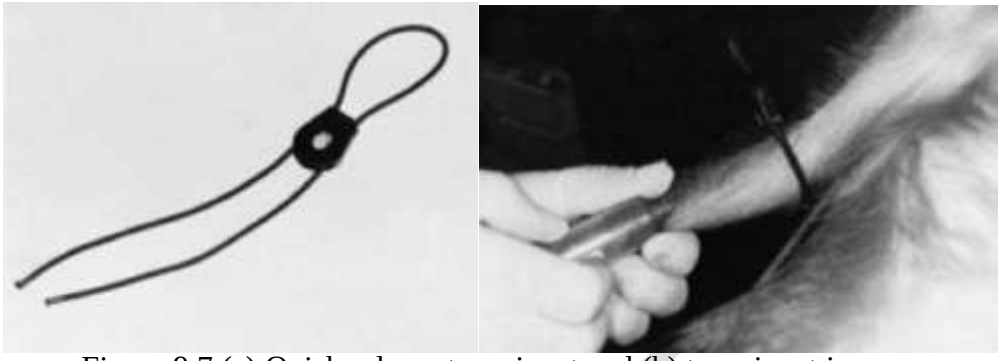


Figure 9.7 (a) Quick release tourniquet and (b) tourniquet in use.

Methods of venepuncture

Rodents

For most rodents saphenous vein puncture is the most satisfactory site for obtainingsmall quantities of blood (see Hem et al. 1998). For those rodents with tails, this is agood site for obtaining rather larger quantities of blood. The lateral coccygeal or tailvein may be easily visualised. Warming the tail first increases the blood flow to the siteand makes sample collection easier. Blood samples may be obtained by incising theskin over the vein using a sterile lancet and collecting the blood into a plain or anticoagulantcoated capillary tube, or allowing it to drip into a container. Scalpel bladesare not recommended as they can easily slip causing damage to the tail. Larger samplescan be obtained from rats by placing a 23–26 G flexible cannula or butterflyneedle into the tail vein and allowing blood to drip into a collection pot or applyinggentle suction with a syringe. If a butterfly needle is used, the plastic tube should beshortened to prevent clotting within it.Amputation of the tail tip to obtain a sample of mixed arterial and venous bloodcan be carried out on rats, mice, and gerbils, but must be done under general anaesthetic(BVA/FRAME/RSPCA/UFAW, 1993), gaseous anaesthesia using isofluranebeing ideal for such a procedure. In mice, this does not appear to involve the removalof any vertebrae, which it does in rats. This should be performed only once in rats,or a maximum of twice in mice.Large samples can be obtained from rats and gerbils via jugular venepuncture. Thevein is raised on one side of the neck by applying pressure at the thoracic inlet, and aneedle placed through the skin and into the vein pointing towards the head.For animals with no tails, such as guinea pigs and hamsters, it is possible to obtaintiny samples from the ear veins. A technique has been described for cannulating thelateral saphenous vein in the hindlimb of guinea pigs under general anaesthesia forrepeated sampling (Nau and Schunck, 1993).For terminal sampling it is acceptable to perform cardiac puncture, which must bedone under general anaesthesia. There are many potentially harmful sequelae to thisprocedure, such as cardiac tamponade, and it should be done under terminal anaesthesia.The heart may be reached by placing the animal on its right side and piercingthe left ventricle through the chest wall at the sixth intercostal space, one third of theway up, to obtain arterial blood, or by piercing the right ventricle with the animal on itsleft side for venous blood. Alternatively, the animal may be placed on its back, and theheart reached by passing the needle under the sternum and through the diaphragm.Jugular venepuncture, although possible, is extremely difficult in these animals.Collection of blood from the orbital venous sinus can have severe consequences forthe

animal, and although it has been used for bleeding rats and tail-less animals, it isnot recommended for sampling with recovery.

Rabbits

Blood can be collected relatively easily from the marginal ear vein using an overthe-needle cannula or butterfly needle. A peripheral vasodilator may be applied tothe skin over the vein, (e.g. „Vasolate", IMS), 5–10 min before blood collection. Once the vein is engorged, the cannula is inserted and blood can be collected by allowing itto drip into a pot. After collection, the vasodilator is wiped off and pressure applieduntil the bleeding ceases. Haemostatic dressings can be applied as discussed earlier inthis chapter. Bleeding from the central ear artery is possible, but can result in the formationof large haematomata, which can cause damage to the ear or even necrosis.

Ferrets

For tiny quantities of blood, for example, for an Aleutian disease test, a toenail can beclipped and a drop of blood collected into a capillary tube. For moderate sized samplesthe saphenous or tail veins can be used, while larger quantities can be collected fromthe jugular vein. The fur on the neck needs to be well clipped, and the vein raised byplacing a thumb over the jugular groove in the thoracic inlet. Blood is collected byinserting a needle up the vein towards the head, or down towards the thoracic inlet.Collection is facilitated by bending the needle to an angle of 30° prior to penetratingthe skin. Pressure on the vein in the thoracic inlet is maintained until the blood hasbeen collected (see Figure 9.8).

Primates

The best method of blood withdrawal in primates is to use the femoral vein, in thegroin. This can be used for Old World Monkeys and New World Monkeys. The needleis inserted in the femoral triangle, slightly medial to the femoral pulse, pointingtowards the head. For larger Old World Monkeys, the cephalic vein on the top of theforeleg below the elbow can be used, as for cats and dogs. The jugular vein can be usedas an alternative route. The micro-capillary tube method can be used to collect smallsamples from the heel of primates without anaesthetic after they have been trainedto accept minimal restraint (see Figure 15.7). Marmosets may also be bled from thecoccygeal vein.

Dogs and cats

These can be bled from the jugular vein. A handler places their right arm over thebody of the animal to hold the forelegs. The elbow is used to hold the body of theanimal to the body of the handler (Figure 9.10). The left hand is placed under the chinto raise the head. The person collecting the blood raises the vein by placing a thumbin the jugular groove at the thoracic inlet. The cephalic vein can also be used.

Ruminants and horses

For sheep, goats, cattle and horses the jugular vein is used. The vein can be visualisedby clipping hair or wool from over the jugular groove. The vein is raised by applyingfirm pressure to the base of the jugular groove. The needle is advanced throughthe skin up towards the head (Figure 9.11). Cattle can also be bled from the ventralcoccygeal vein.

Pigs

These are probably the most difficult animals to bleed. For small volumes in large pigs,an ear vein can be used. For larger volumes, the anterior vena cava is used. A long needleis inserted in the thoracic inlet, and angled slightly upwards and medially to enterthe anterior vena cava.

Birds

The brachial (alar or wing) vein is usually used for blood sampling in birds. It can bevisualised as it crosses the elbow by plucking the feathers over the medial surface ofthe wing. Samples should be removed slowly to prevent the vein collapsing. Pressureis generally not applied after venepuncture in birds as this can promote haematomaformation.The right jugular vein can also be used. It is found between the feathers on the dorsolateralsurface of the neck. It can be raised by applying pressure at the base of theneck. Haematomata rarely form after jugular venepuncture.

Laboratory use of animals in antibody production

Purpose

Rodents and rabbits are often used to produce antibodies for a variety of research objectives. The following guidelines are intended to eliminate or reduce to a minimum, animal discomfort associated with the use of antibody production in laboratory animals.

Choice of Species and Strain

The choice of species and strain must be justified by the investigator in the Animal Use Protocol (AUP). *Ex vivo* methods of antibody production or off-the-shelf commercially available antibodies should always be considered before *in vivo* antibody production. When commercial sources are used, refer to the ACUC guidelines for custom antibody production. For further information on required justification for monoclonal antibody production, please refer to the NIH report on this subject: http://grants.nih.gov/grants/policy/antibodies.pdf.

Immunizing Antigen

The purity and method of preparation of the immunizing antigen is extremely important. The immunizing material must be virtually free of toxic substances (e.g., urea, acetic acid). It should present no risk of pathogenicity or toxicity to the host animal, other animals in the colony, or personnel. If toxic or pathogenic immunogens must be used, they must be justified in the AUP and approved by the Animal Care and Use Committee (ACUC). Proposed arrangements for animal housing, monitoring, and antibody procedures must be fully documented. Investigators must notify the Office of Laboratory Animal Care (OLAC) before animals are inoculated with cells that may harbor transmissible pathogens (human or murine). Please refer to OLAC"s policy "Testing Biologicals Used in Laboratory Rodents" for additional information.

Procedures for Polyclonal Antibody Production

1. Injections

a. Injections for routine antibody production should be administered subcutaneously in two to four sites per animal, generally on the back, away from the spine. Other routes, such as intradermal, intramuscular, intraperitoneal may also be used; however, the intradermal route should not be used in mice and intramuscular injections should not be used in small rodents. Recommended injection volumes and amounts are listed in Table 1; maximum injected volumes are listed in Table 2. The inoculum should be free of extraneous microbial contamination. Always use the smallest inoculum and total volume possible. Filtration (0.2 micron) of the antigen before it is mixed with adjuvant is recommended. Injection sites should be free of debris and disinfected with alcohol, Betadine or chlorhexidine.

b. Intravenous (antigen only), footpad, and popliteal lymph node injections are allowable, but not necessary. Footpad injections in rabbits are not acceptable due to the lack of anatomical structure. Protocols for these types of injections must be justified and approved by the ACUC. The

description should clearly describe the experimental objective, details of the antigen and the entire procedure, including monitoring of the animals after injection. No adjuvant should ever be administered intravenously (IV).

Table 1. Recommended immunization sites and injection volumes for injection of immunogen/depotforming adjuvant.

Route	Species				
	Mice	**Rats**	**Hamsters**	**Guinea pigs**	**Rabbits**
Subcutaneous (SC)	0.1 ml/site 4 sites max	0.1-0.2 ml/site 4 sites max	0.1 ml/site 4 sites max	0.1-0.2 ml/site 4-6 sites max	0.1-0.25 ml/site 8-10 sites max
Intramuscular (IM)	0.05 ml if required: Not recommended	Not recommended	0.05 ml if required: Not recommended	Not recommended	0.25ml/site 2 sites max
Intraperitoneal (IP)	0.1 ml/site maximum	0.25 ml/site maximum	0.25 ml/site maximum	0.25 ml/site Maximum	Not recommended
Intradermal (ID)	Not recommended	Not recommended	Not recommended	Not recommended	0.025 ml/site 5-8 sites

2. **Adjuvants**

The proven safety and efficacy of other adjuvants makes it difficult to justify use of Freund adjuvants. Because of the potential adverse effects of Freund‟s adjuvants, and the availability of other, potentially less harmful adjuvants, the justification for use of Freund‟s adjuvant must be included in the AUP and approved by the ACUC. If Freund adjuvants must be used, the "complete" adjuvant can be used only for the first (priming) immunization. Personnel using complete Freund adjuvant should be particularly careful to avoid accidental self-injection with needle tips, which causes a protracted, painful inflammation at the injection site.

3. **Bleeding**

10% of blood volume can be removed without replacement at one time and repeated every 2 weeks. For recommendations regarding bleeding volumes and methods, please refer to OLAC‟s

guidelines, "Blood Collection Techniques and Limits".

4. **Duration of Experiment**

All animal use protocols for antibody production should clearly state when and how the response will be evaluated (e.g., immunoassay, western blot, immunofluorescence, etc.), and how long the animals will be maintained. Instead of housing rabbits for months or years with an occasional boost and bleeding, it is preferable to raise a good response and perform a terminal bleed if an ongoing need for the antibody is required. Therefore, rabbits should not be maintained longer than 18 months for antibody production when adjuvants are utilized.

5. **Alternative Techniques**

Antibody production in chickens is an alternative in vivo technique for polyclonal antibody production. Antibody production in chickens offers the advantage of providing a non-invasive means to obtain antibody that is recovered from the egg yolk.

Another alternative method in rabbits consists of placing a subcutaneous whiffle ball chamber. Immunizations are made directly into the surgically implanted chamber and antibody-rich fluid is harvested from the chamber. This procedure has been shown to provide an efficient alternative method to ear bleedings for antibody collection. Advantages cited for this technique include greater flexibility in immunogen preparation, minimal discomfort and minimal tissue reaction, ease of immunization and fluid collection, and recovery of large volumes of antibody-rich fluid with low cellularity and absence of lipids. Investigators should contact OLAC Veterinary Staff and submit a Research Service Request for this procedure.

6. **Note about Species Used**

It is also possible to produce substantial amounts of polyclonal antibodies by inducing ascites in mice that have raised antibodies to a particular immunogen. Mice cost much less than rabbits to purchase and maintain, they require much less space, are easier to handle, generally respond to less antigen, and their genetics of immunoresponsiveness offers more options. A high-titer ascites from two to four mice may give the user about as much antibody as all of the serum from a rabbit

In this case, the ascites is induced by intraperitoneal injection of a sarcoma cell line, and the desired antibodies are secreted into the ascitic fluid by the host‟s B-lymphocytes. The sarcoma cells used to generate polyclonal ascites can be stored indefinitely in liquid nitrogen.

Procedures for Monoclonal Antibody Production

The most common reason for ascites production is the growth of hybridoma lines as ascites to obtain large amounts of monoclonal antibodies.

1. **Priming for Monoclonal Ascites Production**

Rodents should be primed once intraperitoneally with 0.2 ml of Pristane 10 to 14 days before hybridoma cells are injected. Use of a larger volume or other adjuvant requires scientific justification and must be approved by the ACUC.

2. **Monitoring**

After inoculation with an ascites-producing tumor line, mice should be observed at least three times per day for the first week, and then daily thereafter including weekends and holidays. The amount of abdominal distention should be monitored, as well as signs of illness and distress. Mice should be weighed dailyand should not gain more than 20% of their baseline body weight before harvesting ascites.

3. **Harvesting Ascites**

a. The ability to judge when and how to harvest ascites and when to euthanize the mice should be learned from experienced personnel. New personnel and students should be trained using anesthetized mice.
b. Ascites fluid should be removed by peritoneal tap before abdominal distention is great enough to cause obvious discomfort or interfere with normal activity.
Unanesthetized mice may be held by properly trained personnel during the procedure or the animal may be anesthetized. The abdominal area should be disinfected with 70% ethanol or Betadine and gently dried before puncture with the needle. Shock due to hypovolemia may be prevented by subcutaneous injection of 2-3mls of warm saline or lactated Ringer‟s solution. When collection is complete, the puncture site should be disinfected again before the animal is returned to its cage. A maximum of three taps per mouse may be performed. Animals should be euthanized immediately following the third tap.
c. Mice that fail to produce ascites within 25 days after hybridoma injection should be euthanized. Mice that form solid tumors should be euthanized if the tumor mass exceeds 10% of the average body weight. Mice that show signs of distress, cachexia (loss of weight), failure to eat and drink, abnormal respiration, or any signs of abdominal hemorrhaging or infection (bloody or cloudy ascites) should be euthanized.

Table 2. Recommended maximum volume of injection used for antigen without adjuvant

	Species				
Route	**Mice**	**Rats**	**Hamsters**	**Guinea Pigs**	**Rabbits**
Subcutaneous (SC)	0.5 ml	1.0 ml	1.0 ml	1.0	1.5 ml
Intramuscular (IM)	0.5ml	0.1 ml	0.1 ml	0.1 ml	0.5 ml
Intraperitoneal (IP)	1 ml	5 ml	2-3 ml	10 ml	5 ml
Intradermal (ID)	Not recommended				0.05 ml
Intravenous (IV)	0.2 ml	0.5 ml	0.3 ml	0.5 ml	0.5 ml

Disposal of animal house wastes and carcasses

When faced with a major animal disease outbreak, along with the need for immediate disease containment, comes a very significant question that requires an urgent decision. This question relates to the method for handling potentially large numbers of carcasses. If stamping out, the most common and successful approach to disease eradication is chosen, then the method of animal carcass disposal for slaughtered animals must also be decided.

Decision-making requires an evaluation of a number of operative parameters within a broad range of disciplines. Examples of these parameters include:

- Impact on the environment,
- The intensity of livestock production and the potential number of animals involved,
- The impact on trade and the economic implications,

- Animal welfare considerations,
- The characteristics of the pathogenic organism,
- Disease control implications,
- The impact on individual producers,
- Financial and logistical considerations,
- The reaction of the public.

Speed of decision-making is critical at the time of such a crisis. To allow for the most appropriate decision, Veterinary Administrations are advised to carefully think through the options, in advance of the event to establish essential linkages, to pre-determine which options are possible for their particular areas, and to evaluate what this implementation would require. In this way, at the time of need, the best balanced choice can be made and implemented in the shortest possible time.As well, this approach permits planning and scheduled investment in equipment in preparation for a disaster which inevitably will come.There are, apart from disease outbreaks, many situations which also demand the same preparation. These situations can take advantage of the same planning strategy. These situations include natural disasters such as flooding or hurricanes which could produce a large number of carcasses, as well as animal contamination by toxic chemical spills, ingestion of contaminated feed, large fires, slaughter for animal welfare reasons such as starvation or humane culling, or deliberate bioterrorism.If you consider for a moment the massive destruction and waste of such large scale slaughter, you come to the inevitable conclusion that there must be an alternative which will permit avoidance of this destruction while affecting the required disease control. Therefore the very best method of dealing with disposal of animal carcasses is to avoid the need to slaughter the animals.To provide you with an example of the factors for consideration, we circulated the questionnaire with the intent of being able to evaluate and discuss the status of the region as a whole and to suggest possible areas for emphasis which could hopefully reduce any possible vulnerabilities.To begin we can consider these factors as general principles, recognizing that primary consideration must be given to disease control and eradication as the most important aspect.

General Principles

1. Speed is of the essence - the earlier the official intervention, the fewer the number of animals that will require disposal,
2. Complete inactivation of the pathogenic agent must be insured,
3. An emergency management plan must be defined in advance and regularly communicated to all levels of the agricultural system,
4. All required legal authorities and links to involved industries must be established in advance,
5. The Veterinary Administrations must assume primary leadership of an animal disease outbreak,
6. Veterinary Administration action must precede uncontrolled animal movement based on unofficial rumours,
7. All potential consequences of an outbreak, especially financial consequence, should be assessed in advance to minimize the negative impact on involved industry sectors,
8. Producers should be assisted to develop an economic understanding and compliance with the principles of disease control,
9. General broad zoning areas can be predetermined and defined in advance for immediate implementation to limit animal movement based on knowledge of normal trade routes of animal

movement,

10. A system of traceability is required to allow immediate trace back of disease,
11. Establish a list of pathogens with methods of transmission, zoonotic potential, environmental resistance, and susceptibility to disinfectants as well as disinfectant availability,
12. Determine the availability of effective vaccines,
13. Technical capabilities should be established at every step for animal slaughter, storage, and disposal including licensing for emergency situations,
14. Environmental assessments should be conducted in advance for suitable burial sites,
15. An information policy should be established in advance to promote an understanding by the public of the approach taken and the rationale for it.

Available Technology

These technologies are presented as a hierarchy based on their reliability for pathogen inactivation.

Rendering

This is a closed system for mechanical and thermal treatment of animal tissues leading to stable, sterilized products, e.g animal fat and dried animal protein. It grinds the tissue and sterilizes it by heat under pressure. The technology exists in fixed facilities and is in normal usage. It produces an effective inactivation of all pathogens with the exception of prions where infectivity is reduced. A medium sized rendering plant could process 12 tonnes per hour of operation. The availability of the capacity should be determined in advance. Such plants can operate within environmental standards.

Incineration

This technology can be applied as:

o fixed, whole-carcass incineration,

o mobile air curtain whole carcass incineration, o municipal incinerators,

o co-incineration.

Fixed whole carcass incineration occurs in an established facility in which whole carcasses or carcass portions can be completely burned and reduced to ash. This process is normally fuelled by natural gas. Effective inactivation of pathogens is produced. Without additional technology, the exhaust emissions are not subjected to environmental control. However these emissions can be subjected to air scrubbing procedures to meet environmental standards. Mobile air curtain whole carcass incineration is a mobile system which can be taken on-site. Whole carcasses can be burned and reduced to ash using wood as a fuel. Because it can be used on site, there is no requirement for transportation of the animal material. It also produces effective inactivation of pathogens and may actually achieve higher temperatures (1000^{o}C).Municipal incinerators are pre-established facilities which are normally used for the burning of household or industrial waste. Although they may not be currently licensed to burn carcasses, use of these facilities allows an expanded capacity for effective inactivation of pathogens.

Co-incineration is a process in which meat and bone meal, carcasses or parts of carcasses are burned in conjunction with other substances, e.g.:

a) hazardous waste incineration,

b) clinical waste incineration,

c) other industrial incinerations such as: o power plants, cement kilns, blast furnaces, coke ovens.

In practice meat and bone meal has been used as a secondary fuel on a large scale in cement kilns and power plants.

Pyre Burning

This is an open system of burning carcasses either on-farm or in collective sites fuelled by additional materials of high energy content. This is a well established procedure that can be conducted on site with no requirement for transportation of the input material. However, this process is contrary to environmental standards for air, water, and soil. It takes an extended period of time and has no verification of pathogen inactivation. In fact, there is a possibility of particulate transmission from incomplete combustion. Further, because the process is open to view, there is a negative reaction and lack of acceptance by the public.

Composting

This is a process of aerobic microbiological decomposition conducted in either open or closed systems. It preferably requires prior grinding of tissues and as well the addition of organic material for microbial maintenance. Additionally, mixing or aeration is required to assure homogeneous decomposition. This simple process, which can be conducted on site at low cost, can achieve temperatures of up to 70^{o}C. It does, however, require a significantly extended period of time. Further it is necessary to insure a constant temperature throughout the material for the total time period and it is difficult to verify the effectiveness of pathogen inactivation.

Mass Burial or Open Farm Burial

This is a system to deposit whole carcasses below ground level and to be covered by soil, with no additional inactivation of pathogens. It is an established procedure which if conducted on site does not require transportation and is used to control the spread of disease. It does however require an environmental assessment because of the potential contamination of groundwater, or of aquifers if leachate is not controlled. Further, it does not inactivate all pathogenic agents.

Licensed Commercial Landfill

This process involves deposition of carcasses in predetermined and environmentally licensed commercial sites. Because the site has been previously licensed, all environmental impacts such as leachate management, gas management, engineered containment, flooding, and aquifers have already been considered. However, the area is open and uncovered for extended periods, there is a potential emission of aerosols, and there is resistance from the public to such an approach.

Mounding

This process is one of mass burial above ground and it has similar considerations to those of mass burial.

Fermentation

This process is a closed system of anaerobic microbiological decomposition which requires prior mechanical and thermal treatment and which results in the production of biogas. This process does not inactivate pathogens, but typically uses non-dried rendered product as the input material.

Technologies under Development

Alkaline Hydrolysis: Alkaline hydrolysis consists of treating carcasses or tissue in an aqueous alkalinesolution at elevated temperatures under pressure. It converts proteins, nucleic acids, and lipids of all cells and tissues into a sterile aqueous solution of small peptides, amino acids, sugars, and soap. What remains are the mineral constituents of the bones and teeth. This

process requires specialized equipment and operates at 150° C for three hours. It completely inactivates pathogens with the exception of prions where infectivity is reduced, and is environmentally responsible.

Biosphere Process: The biosphere process is a bio-refining technology which employs a biolytichydrolyzer, operating under high temperature, steam pressure, and internal agitation in a sealed steel vessel. The process produces hydrolysis of protein and carbohydrate materials, fracturing long chain molecules and yielding sterile, high nutrient fertiliser as an output. It operates at 180° C under 12 atmospheres of pressure for a period of 40 minutes. It inactivates all pathogens and is environmentally sound. Inactivation of prions is still undetermined.

Special Considerations for Prion Diseases

One of the problems in demonstrating the effectiveness of the inactivation of prions is the lack of a simple, rapid and inexpensive test for the presence of the infective agent, especially at low concentrations. The ultimate test is bioassay in a sensitive detector species by an efficient route, but usually this is only relevant in research. Typically this is done using panels of mice bred to be susceptible to particular types of transmissible spongiform encephalopathies (TSEs). However it must be recognized that the mouse to cattle species barrier has been demonstrated to be 500, therefore affecting sensitivity.

Although rendering at 133° C and three bars of pressure for 20 minutes is a defined standard, reductions of infectivity by this technology are in the order of 1:200 - 1:1000. Commercial incinerators have an inactivation rate of one million fold, while burning on pyres has a reduction rate of 90%. (It should be noted that pyres are not suitable for sheep because of the wool and fat.) Alkaline hydrolysis produces a 3-4 log reduction in infectivity overa three hour period. Landfill and deep burial are suggested to have a reduction in infectivity of 98 - 99.8% over three years. Based on this information, rendering, incineration, and alkaline hydrolysis are the most reliable technologies at this time.

The significance of small amounts of infectivity become evident when you consider that experimentally it has been shown that exposure of sensitive species to as little as 1.0, 0.1 or even 0.01 grams of infected nervous tissue can induce infection.Given all of the above, it must be recognized that no process has been demonstrated to be 100% effective in removing TSE infectivity and there will be some residual levels of infectivity remaining after treatment.

Thoughts on alternatives to animal disposal

While addressing the current practicalities of animal disposal technology, it is perhaps also worth while to look ahead a bit and consider alternatives to the present approach of depopulation and animal carcass disposal.The best method of animal disposal is to avoid the need to slaughter the animals permitting them to reach their potential in terms of the reasons for which they were bred. However, above all else, animal disease control must be achieved as a primary consideration.

Stamping out is deeply entrenched in the veterinary organizational culture. It is a tried and true approach that is advocated by the OIE for effective disease eradication. It has been used successfully in numerous animal disease outbreaks and is regarded as the standard.On the other hand, trends are now developing which are introducing factors that are creating pressure for a philosophical change in the approach to animal disease control and ultimately to depopulation and animal carcass disposal.

Such factors include:

- logistical factors - following the developing trend of larger farms with more animals on small geographic areas,
- economic factors - following globalization and international trade considerations in which disease control actions are often market driven,
- societal factors - creating pressures based on public perceptions and ethical issues, e.g. the trend may be for the public to become less tolerant of the potential for the waste of vast amounts of edible protein because of depopulation practices,

- animal welfare consideration - and the public‟s reaction to mass slaughter and carcass disposal,
- Environmental factors - which force higher standards and more extensive environmental assessments to protect the status of the environment.

A summary of the growing trend is that society is rejecting the excessive waste of valuable animal products, the negative environmental and animal welfare outcomes, and the devastating economic impacts on agricultural industries as well as on national economies.

Approaches that can be taken to address this include:

- Prediction - to avoid disease occurrences by pre-emptive trend identification,
- Prevention - of disease or minimization of any disease that occurs (vortex containment concept where the approach is to direct all movement towards the centre of an outbreak),
- Speed of disease detection or control.

Ultimately what will be required is a complete paradigm shift in thinking to a new concept of disease control which incorporates these driving forces and trends into its

II –M.Sc Microbiology (Batch 2018-2020)

Possible Questions

Unit – V

Two Marks

1. Define incineration.
2. Define biohazard
3. Types of inoculation methods followed in animal house
4. Prevention of animal disease
5. Characteristic of animal house

Eight Marks

1. Write short notes on the different types of inoculation used in lab animals.
2. Describe the methods of blood collection from laboratory animals.
3. Discuss on the role of lab animals in Microbiology and their application.
4. Write short notes on the safety measures employed while designing animal house.
5. Discuss in detail on the method of antibody production using lab animals.
6. Give an account on the different types of quarantine methods used during disease condition in lab animals.
7. Write short notes on the drugs used as anaesthesia for lab animals.
8. Discuss methods of disposing animal waste in laboratories.
9. Define biohazard and comment on its importance in the animal house maintenance.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II MSc MB **COURSE NAME: LABORATORY ANIMAL CARE**

COURSE CODE: 18MBP305B **UNIT: V** **BATCH-2018-2020**

S.No	Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
1	Collection of tissue from live animal for genetic analsysis is called_____.	autopsy	biopsy	Haemorrhid tissue	necrotic tissue	biopsy
2	_____samples are collected during the stage of oestrous cycle.	tissue biopsy	rectal swab	vaginal swab	perineal swab	vaginal swab
3	Cells consisting primarily y of polymorphonuclear lymphocytes(PMNs), with some epithelial cells are called _____.	diestrus	proestrus	estrus	metestrus	diestrus
4	Nucleated and cornified epithelial cells, with some PMNs in early stages are called_____.	diestrus	proestrus	estrus	metestrus	proestrus
5	Cornified epithelial cells predominate, with a few nucleated cells seen in the early stage_____.	diestrus	proestrus	estrus	metestrus	estrus
6	Cornified epithelial cells predominate, with a some nucleated epithellial cells_____.	diestrus	proestrus	estrus	metestrus	metestrus
7	Physical separation of animals by species is recommended to prevent_____.	agression	easy sample collection	prevent disease transmission	prevent anxiety	prevent disease transmission
8	Helicobacter bilis can infect_____.	rat	mice	Rabbit	hamster	rat
9	Subclinical microbial infections occur frequently in_____.	rat	mice	rabbit	hamster	rat
10	Healthy, well-cared-for animals are a	science study	practical	Good quality	diagnosis of	good quality

	prerequisite for_____.		application		pathogen virulence	
11	Genetically modified microbes are used to_____.	breeding	export	Studies on human research	pharmaceutical companies	studies on human research
12	Xerotransplantation refers to organ transplant between_____.	same species	same family	Same order	different species	different species
13	_____is an example of the GM animal product.	Vaccine	Protein	Milk	Carbohydrate	milk
14	Genetic makeup of the animal is _____.	phenotype	genotype	Allelotype	Auxotype	genotype
15	Phenotype of an animal refers to _____.	body makeup	genetic makeup	Immune makeup	cellular makeup	body makeup
16	Genetically modified animals with essential genes in chromosome deleted is called_____.	Knockout	transgenic	mutant	filial generation	knockout
17	In harmful mutant animals _____type of mutation is seen.	spontaneous	point	Frame shift	transgenesis	point mutation
18	Lab animals with point mutations is called_____.	immuno modified	immuno compromised	Nude	clone	nude
19	A cloned animal is one that has been derived by inserting _____ into another animal.	one cell	two cells	three cells	four cells	one cell
20	_____is an example for genetically altered animal.	albino rat	wistar rat	Nude mouse	shuttle hamster	albino rat
21	Homozygous refers to_____.	same allelle	different allele	species specific allele	species non specific allelle	different allele
22	_____is known as the unit of	chromosome	nucleoid	Allele	RNA	allele

	specific genes.					
23	Hybrid animals have a mother of one inbred strain and _____ father.	inbred strain	outbred strain	Mutated strain	identical strains	outbred strain
24	_____is a chart used to register the data of animal ancestors.	pedigree	colony management record	Colony maintenance record	ancestral record	pedigree chart
25	_____record covers the essential data on the colony size of the lab animal.	colony management recor	census record	Pedigree record	ancestral record	census record
26	_____is a small, isolated animal colony that is used to produce breeding stock for the larger colony.	filial colony	mother colony	brood colony	foundation colony	foundation colony
27	_____record covers the breeding data of animals.	prodcution record	mother colony	breeding record	brood record	breeding record
28	The size of syringe used to draw blood in animal is called_____mm gauge.	22	10	5	1	22
29	Which of the following is an example of an advanced temperature measurement rechnique method in animals?	infrared measurement	Hg thermometer	red oil measurement	heat sensory thermometer	infrared thermometer
30	Abdorminal palpitation of lab animals are measured in_____side.	dorsal	anterio dorsal	Ventral	posterior	ventral
31	Transfer of one cell from an animal in to another animal for the process of cloning is called_____.	chromosome walking	gene walking	Nuclear transfer	moleular morphing	nuclear transfer
32	Embryionic stem cells can be grown in	host tissue	egg yolk	RPMI-1650	HAT	RPMI-1650

	_____.					
33	If a gene is inserted into the genotype of wild animal then it is called as _____.	knock up	knock down	knock out	knock in	knock in
34	The animals that receive the genetic material is called_____.	donor	recepient	clone	source	receipient
35	_____cells are enucleated before transfer.	spermatocytes	spleenocytes	Hepatocytes	oocytes	oocytes
36	ASPA act stand for_____.	Animals Sciene Project Act	Animal Sale Project Act	Animal Science Project Act	Animal Slaughter Project Act	Animal Science Project Act
37	Ethylnitrosurea is a_____.	alkylating agent	phosphorylating agent	Deaminating agent	hydrolysing agent	alkylating agent
38	Large scale mutagenic process is done in _____.	Drosophila	Rabbits	Sheeps	dog	Drosophila
39	Efficient mutabgenesis and compatibility is seen in_____.	mice	rat	hamster	guineapig	mice
40	Supervolution is a process in which _____with hormone is regulate	donor male	recepient male	Donor female	receipient female	donor female
41	_____is used to detect the damage done by infectious agents to the host tissue.	pathology	serology	Immunology	histopatholoy	histopathology
42	_____technique is used to detect the protozoal infection in animals.	pathology	PCR	microscopy	RIA	PCR
43	Up to_____% of blood	5	1.5	15	2	15

	volume can be removed from lab animal.					
44	For reperated sampling of blood _____% of blood is collecte	5	1	7.5	12	7.5
45	In_____method the animal is manually restrained and small bore pipette is placed ate the medial canthus.	Trail laceration	Cardiac puncture	Retro orbital sinus puncture	decapitation	Retro orbital sinus puncture
46	_____ is a technique used to obtain comparitively large volume of blood	cardiac puncture	trail laceration	Retro orbital sinus puncture	decapitation	decaptation
47	In_____method the tip of the tail is cut to collect bloo	cardiac puncture	trail laceration	Retro orbital sinus puncture	decapitation	tail laceration
48	The size of syringe used to draw blood from cardiac puncture is_____ mm gauge.	20	10	16	24	24
49	Urine is collected from mice by_____restrainting.	hind limb	catheter transfer	abdominal pressure	perineal pressure	hind limb
50	Feces is collected from lab animals by a process called_____.	pad collection	anal milking	rectal prolaption	abdominal pressure	anal milking
51	Which of the following is NOT a method of administering substances to lab animals?	intravenous	intramuscular	intrperitoneal	feed additive	feed additive
52	Commonly used method for drawing blood in lab animals is_____.	venipuncture of fore limb	venipuncture of hind limb	venipuncture of peritoneal cavity	cardiopunture	venipuncture of fore limb
53	_____is a feature condidered while performing injection to animals.	volume of solution	bioavailability of the sample	Size of the particle	metabolic rate of the sample	bioavailability
54	CNS stands for_____.	Central neuron	Central Node	Central	Central soft	central nervous

		system	system	nervous system	system	system
55	The average volume of the solutions administered to lab mice during intravenous injection is_____ per 20 g	10 ml	1 ml	0.2 ml	5 ml	0.2 ml
56	The blood for hematological analysis is collected from_____.	bleeding from portal vein	bleeding from hepatic vein	Bleeding from subcutaneous	bleeding from thoracic vein	bleeding from subcutaneous vein
57	Heterozygous refers to _____.	same allelle	different allele	species specific allele	species non specific allelle	same allele
58	_____ involves the implantation of small telemetric transmitte.	telemetric monitoring	Hg thermometer	Touch monitoring	thermocouple monitoring	thermocouple
59	In touch thermometer the range of temperature is_____.	36.5	38	40	45	36.5
60	_____method is used in the identification of disease in animals.	DNA fingerprinting	VNTR sequencing	ELISA	RIA	ELISA