

**Instruction Hours / week: L: 4 T: 0 P: 0    Marks: Internal: 40 External: 60 Total: 100**

**End Semester Exam: 3 Hours**

### **SCOPE**

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

### **OBJECTIVES**

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**

Modern methods of care, management breeding and maintenance of Laboratory animals – rabbit.

### **UNIT – II**

Modern methods of care, management breeding and maintenance of Laboratory animals – mice.

### **UNIT – III**

Modern methods of care, management breeding and maintenance of Laboratory animals – rat.

### **UNIT – IV**

Modern methods of care, management breeding and maintenance of Laboratory animals– guinea pig.  
Specific pathogen free animal – gnotobiotic animal

### **UNIT – V**

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

### **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<sup>nd</sup> ed.). American College of Laboratory Animal Medicine. eds. Suckow, weisbroth and Franklin. Elsevier, (2006).
5. *Handbook on Genetically Standardized Mice*. (6<sup>th</sup> ed.). Ed. Joanne Curren, The Jackson Laboratory, Bar Harbor, Maine, (2009).
6. *Laboratory Animal Medicine*, (2<sup>nd</sup> 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<sup>rd</sup> 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<sup>nd</sup> ed.). McGraw-Hill Inc, NY.
4. Roy, D.N., and Brown, A.W.A., (2003). *Entomology – Medical and Veterinary*. (1<sup>st</sup> ed.). Part – I, Biotech Books, New Delhi.
5. Warren, D. M. (2002). *Small Animal Care and Management*. (2<sup>nd</sup> ed.). Delmar – Thomson Learning, Columbia, NY.
6. Yadav, M. (2004). *Applied Entomology*. (1<sup>st</sup> ed.). Discovery Publishing House, New Delhi.



**KARPAGAM ACADEMY OF HIGHER EDUCATION**  
**DEPARTMENT OF MICROBIOLOGY**

(Deemed University Established Under Section 3 of UGC Act, 1956)

Eachanari PO, Coimbatore -641 021, India.

**II –M.Sc Microbiology (Batch 2016-2018)**

**Lecture Plan**

**Unit - I**

S. No	Duration	Topic	Reference
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 housing in pens, design of pens environment advantages and disadvantages	R1: 7 to 9 R2: 15 to 17
4.	1	Rabbit care management, breeding in pens and cages	R1: 20 to 23
5.	1	Cage design and environment	R1:24 to 26
6.	1	Environment enrichment for rabbits in pens and cages	R1: 26 to 30
7.	1	Factors affecting the growing methods	
8.	1	Unit Revision	
<b>Total Hours</b>			<b>8</b>

R1: Lynette Chaveet al.,(2003). Animal research review panel (ARRP) guidelines -18: Guidelines for the housing of rabbits in scientific institutions.

R2: Code of practice for the housing and care of laboratory mice, rat, guinepigs, and rabbits. Issued by the ministry of agriculture, The state of Victoria, (2004),Australia.

W1: Guide for the care and use of lab animals. NRC(USA) and Canadian council. [www.CCAC.ca](http://www.CCAC.ca).

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### **Unit- I**

#### **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 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 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 Section 1.5). 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.

#### **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

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states: Species-specific behavioural 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 behavioural 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 minimise 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 sterilised 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 Behaviour Relevant to Housing, Principles**

All laboratory rabbits derive from the European rabbit (*Oryctolagus cuniculus*). The behaviour 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 behaviours when together include lying near each other, grooming each other and nuzzling. Social interaction is therefore important for rabbits. The natural active behaviours 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 behaviours such as hopping and rearing upwards (freedom of movement) 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 4.4.22 (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

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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 behaviours such as grooming penmates, lying together and playing. The behavioural 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 behaviourally 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 labour. 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 stockpersonship in caring for and monitoring rabbits is required. Thus animal carers need to be proficient in recognising all the different aspects of rabbit behaviour. 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 behaviour, for no obvious reason. Fighting and aggressive behaviours 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

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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 behaviour. The minimum space provided should allow each rabbit to carry out its normal behaviour, including a wide range of locomotory behaviours, 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

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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 <sup>2</sup> ) (*ensuring a CLEAR area of 2m <sup>2</sup> 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 behaviour 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 behaviours. 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 behaviour. 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’ behaviour patterns by altering the olfactory field. There is evidence that rabbits avoid sawdust and woodshavings.

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 is that it may not be as moisture absorbent as other bedding materials such as woodshavings.

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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 woodshavings.

### **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 behaviour 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).

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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 behaviour 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 behaviour 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

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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 behaviours 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 behaviour. 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

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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 behaviours 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 behaviours are difficult to assess (because it is difficult or impossible for rabbits to carry out these behaviours) and changes in food and water intake are often the only early indicator of illness.

### **Recommendations**

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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 behaviour 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.

### **Pasteurella multocida (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

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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 mucopurulent 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 week or more.

### **Recommendations**

Rabbits introduced into group housing should be free of *Pasteurella multocida*, 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 behaviour, 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 behaviour 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 behaviour. 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

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and barriers between each nest to obscure the view of adjacent nests (and thus limit aggressive behaviour)

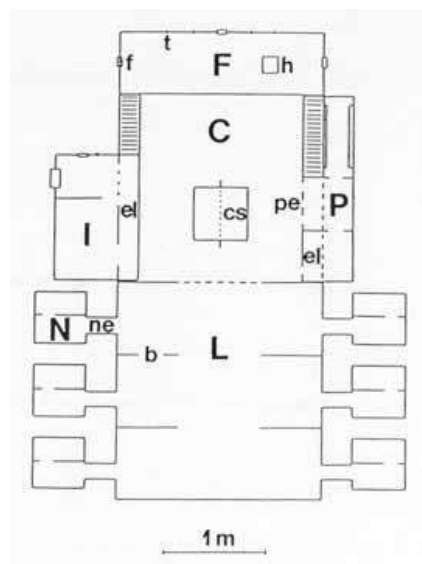
\* A pup area with small entrance passages accessible to rabbits up until about weaning age (about 18- 39 days)

\* 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

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The advantages of housing rabbits singly in cages include:

- (i) Food and water intake can be controlled and monitored.
- (ii) Faeces and urine excretion can be quantified and monitored.
- (iii) Rabbits can be identified without the need to mark them.
- (iv) If strict precautions are taken, the spread of infection may be controlled more easily than group housed rabbits.
- (v) It is easier to maintain a cleaner environment.

#### **Disadvantages of Single Housing in Cages- Principles**

The disadvantages of housing rabbits singly in cages include:

- (i) 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.
- (ii) The behavioural repertoire of singly caged rabbits is severely restricted, largely as a result of social deprivation.
- (iii) Singly housed rabbits in cages carry out a variety of stereotypic behaviours 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 behaviours 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 behaviours are the result of frustration, anxiety, and boredom related to a barren, confined environment. These behaviours are rarely if ever seen in rabbits housed in groups in pens.
- (iv) 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 behaviour may relate to a chronic lack of stimulation and/or to the fact that they have no way of escaping and hiding.
- (v) It is difficult to provide environmental enrichment in cages because of space restrictions.
- (vi) Frequent cleaning and sterilisation may disturb the olfactory environment of the rabbit.
- (vii) Ventilation may be poor in solid sided cages.
- (viii) 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.
- (ix) Cages are expensive to buy and costly and time consuming to clean.
- (x) Singly housed rabbits in cages may be psychologically and physiologically abnormal.

The use of such animals in research may have an influence on experimental results.

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

- (i) Cages are usually constructed from metal (stainless steel or aluminium) or plastic.
- (ii) 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**

- (i) Wire / grid floors are associated with a high incidence of sore hocks, especially in heavier rabbits.

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(ii) 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**

- (i) 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.
- (ii) 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 behaviour 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 behaviours. A suitable height to allow a New Zealand White rabbit to carry out such behaviours 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 include sawdust, 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 woodshavings are used 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 1-2 ppm and not allowed to exceed 10 ppm.

### **Environmental Enrichment for Rabbits in Pens- Principles**

Environmental enrichment has been defined as “any measure which promotes expression of natural, species specific behaviours and a decrease in, if not disappearance of, abnormal behaviours”. 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 behavioural needs (Clause 4.4.22), the implementation of strategies to provide 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 behaviour. 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 behaviour and gives singly housed rabbits an alternative occupation. Providing rabbits with objects to manipulate triggers species-typical behaviours, reduces stereotypical behaviours 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 1.2 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 60 cm to accommodate

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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 behaviours 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 behavioural 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°C, 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°C 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.<sup>5</sup> 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**

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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**

Pens and cages should have labels attached to them that provide the following information:

- \* Rabbit identification
- \* Name, location and contact numbers of the Chief Investigator / Teacher and (if applicable) other investigators / teachers using the rabbits
- \* Name, location and contact numbers of staff associated with the housing and care of the rabbits
- \* Name and approval number of protocol in which rabbits are being used
- \* Age (date of birth) of rabbits
- \* 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.

## UNIT 1

### Question

Stress in rabbit leads to \_\_\_\_\_.

Common diseases in rabbit is caused by \_\_\_\_\_.

Common bacterial infection in rabbit is caused by \_\_\_\_\_.

Myomatosis is commonly encountered in \_\_\_\_\_.

Viral haemorrhagic disease is often seen with \_\_\_\_\_.

Breeding is enhanced by \_\_\_\_\_ in rabbits.

\_\_\_\_\_ rabbits should be selected for breeding.

Nest box is made up of \_\_\_\_\_ in rabbit housing.

Nest box should contain more \_\_\_\_\_ for the rabbit to undergo gestation.

Young one of rabbits are suckled \_\_\_\_\_ minutes per day.

Assurance of security to rabbits is indicated by \_\_\_\_\_.

Cleaning the latrines and faeces in rabbit cages is done in \_\_\_\_\_ days.

Over cleaning of rabbits cage lead to \_\_\_\_\_.

Group housing reduces \_\_\_\_\_ in rabbits.

Rabbit produce copious turbid urine with dark red color due to \_\_\_\_\_.

Fibre requirement of rabbit in feed is \_\_\_\_\_.

Protein requirement of rabbit in feed is \_\_\_\_\_.

Daily need of high energy feed of rabbit is \_\_\_\_\_.

\_\_\_\_\_ is mandatory in for the welfare of the rabbit

Normal quantity of water consumed by rabbit is \_\_\_\_\_ per day/100g body weight.

Rabbits are \_\_\_\_\_.

Rabbits are social animals which are able to utilize \_\_\_\_\_ environment.

Aggressive behaviour is NOT observed in \_\_\_\_\_ rabbits.

If rabbits in a group are separated for a long time \_\_\_\_\_ results

Nowadays \_\_\_\_\_ is used as a safer way to detect the lab rabbits.

Pasteurella is a \_\_\_\_\_ pathogen.

Sudden dietary changes to rabbits lead to \_\_\_\_\_.

Mucoid enteropathy is a common disease associated with \_\_\_\_\_ deficiency.

For sedating rabbit \_\_\_\_\_ is provide

Muscle relaxation in rabbit is achieved by the administration of \_\_\_\_\_.

Rabbits can hear ultra sound at \_\_\_\_\_ Hz.

Poor ventilation in rabbit cage lead to \_\_\_\_\_.

Air change in rabbit cages are done at \_\_\_\_\_ cycles per day.

\_\_\_\_\_ indicates normal behaviour of rabbits in open environment

Ammonia from rabbit urine cause \_\_\_\_\_.

High side open containment used for housing rabbit is called \_\_\_\_\_.

Rabbits communicate with \_\_\_\_\_.

\_\_\_\_\_ is provide to enhance the environmental condition of rabbit housing.

Sore hocks in rabbits are caused by \_\_\_\_\_.

Grid floors are generally provide if the rabbits are housed in \_\_\_\_\_.

Pododermatitis is the infection of rabbit \_\_\_\_\_.

The largest used stock variety of rabbits belong to \_\_\_\_\_.

\_\_\_\_\_ aids in easy identification of rabbits.

\_\_\_\_\_ is not recommended for the use in rabbit identification because of discomfort.

Rabbits cages are designed with features to mainly provide \_\_\_\_\_.

Space in rabbit housing is essential to prevent \_\_\_\_\_.

Lagomorpha is the order to which \_\_\_\_\_ belongs.

\_\_\_\_\_ is an alternative housing method of separating aggressive rabbits in a group.

Housing individual rabbits in a space restricted cage leads to \_\_\_\_\_.

Ambient temperature for rabbit is \_\_\_\_\_ °C.

Female rabbits breeding during \_\_\_\_\_ months.

During breeding rabbits have no \_\_\_\_\_.

Handling rabbits by holding the ears lead to \_\_\_\_\_.

Stoic acceptance is the response of rabbit to \_\_\_\_\_.

Rabbit under pain is recognized by \_\_\_\_\_ property.

Rabbits should never be lifted by \_\_\_\_\_.

Rabbits are \_\_\_\_\_.

The laboratory rabbits is scientifically called as \_\_\_\_\_.

**Laboratory Animal Care**  
**16MBPU303**  
**Department of Microbiology**  
**Karpagam Academy of Higher Education**

Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6
limb damage	ear lesion	heart failure	ocular discharge		
bacteria	protozoa	virus	intestinal helminths		
P. aeruginosa	E. coli	V. cholerae	P. multocida		
mice	rat	guniea pig	rabbit		
thining of hind limb	respiratory illness	lesion	death		
day cycle	night cycle	dim light	bright light		
acitve	inactive	docile attitude	aggressive		
plastic	cardboard	steel	paper		
air	water	light	bedding		
1	5	10	45		
smell	noise	hiding place	same species grouping		
5	7	14	10		
decreased fear	decrease in animal con	decrease in body weig	decrease in activity		
body weight	stereotypy	calmness	size		
ammonia	porphyrin	vitamin	RBC		
22%	5%	10%	1%		
5%	12%	25%	16%		
1g	16g	5g	0.3		
protein	carbohydrate	gut flora	lipids		
10 ml	1 ml	5 ml	20 ml		
dirunal	docile	semiactive	nocturnal		
complex	simple	1D	3D		
pregnant female	breeding adult male	weaning pup	pubescent female		
competence among group mate	Fighting	inactive social partici	incompetence		
colored collar	ear tags	micro chips	limb collar		
gut	liver	lungs	urinary		
diarrhoea	death	gut inflammation	inactivity		
protein	fibre	carbohydrate	lipids		
asprin	medetomidine	flucytocine	rifampin		
asprin	medetomidine	xylazine	rifampin		
6	16	20	25		
suffocation	asphyzation	respiratory illness	inactivity		
5	9	15	35		
cornered hiding	climbing	sleeping	strecthing		
erythema	sedation	inactivity	respiratory disease		
cages	pens	Open housing	in house cages		
ultrasound	high pitch sound	olfactory cues	mild vocals		
hay	corn cobs	chew sticks	pumise stones		
urine	faeces	mites	allergen		
pens	transport vehicles	cages	inhouse cages		

ear	hind limb	fore limb	abdomen
America	India	Newzealand	Australia
eyes	fur coat thickness	fur color	size
tattoo	leg collar	ear tag	paw stain
locomotion	nesting space	breeding chamber	central area
bone thinning	aggressiveness	inactivity	disease
Mouse	Rabbits	Hamster	Rats
pens	single housed cage	space restricted cage	pair housing
agressiveness	bone hypoplasia	inactivity	death
10	20	30	40
4	5	6	7
gestation	phermones	Appetite	oestrous cycle
ear damage	neuronal damage	spinal damage	limb pain
food	light	pain	disease
low activity	low food intake	sleeplessness	running
holding hind limbs	ears	fore limb	dorsal body
coprophagic	necrophagic	autophagic	heterophagic
Orycotolagus cuniculus	Orycotolagus canis	Orycotolagus albinus	Orycotolagus cunlingus

## Answer

ocular discharge

virus

*P. multocida*

rabbit

death

day cycle

docile attitude

cardboard

bedding

10

same species grouping

14

decrease in animal confidence

stereotypy

porphyrin

22%

12%

5g

gut flora

10 ml

nocturnal

3D

weaning pups

incompetence

microchips

lungs

diarrhoea

fibre

medetomidine

xylazine

16

respiratory illness

15

stretching

respiratory disease

pens

olfactory cues

chew sticks

urine

cages

hind limb

New zealand

fur color

leg collar

locomotion

bone thinning

Rabbits

pair housing

bone hypoplasia

20

4

oestrous cycle

spinal damage

pain

low food intake

ears

coprophagic

*Oryctolagus cuniculus*



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**II –M.Sc Microbiology (Batch 2016-2018)**

**Lecture Plan**

**Unit - II**

S. No	Duration	Topic	Reference
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	Restraining and sample collection methods from lab mice	T1: 120 to 134 W1,J1
5.	1	Physical examination of mice for disease conditions.	R1: 40 to 44
6.	1	Anaesthesia and analgesia -mice	T1: 102 to 119
7.	1	Euthanasia in veterinary care	R1: 50-51
8.	1	Unit Revision	T1:102 to 119
<b>Total Hours</b>			<b>8</b>

T1: The laboratory mouse, 2<sup>nd</sup> edition. American College of Laboratory animal medicine eds, Suckow,Danneman and Brayton, 2001, CRC press LLC, USA.

R1: 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.

W1: The Jackson laboratory, 600 Main street, Barharbour,ME.[www.jax.org](http://www.jax.org).

J1: Baker,D.G., Natural pathogens of laboratory mice, rat and rabbit and their effects on research.Clin.Microbiol.,Rev,11,231,1988.

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**UNIT 2**

Question	Opt 1	Opt 2
Harem system of breeding involves mating of one male with _____	2	6
Stress recognition in rabbit is done by observing _____.	agressiveness	failure to ea
Secondary infection in mice is caused by _____.	S. typhi	P. aeruginos
Blood level of mice is _____ x10 <sup>6</sup> /mm <sup>3</sup>	2-5	15-18
The maximum respiratory rate of mice is _____ min	80	120
The natural life span of a mice is _____ years.	1.5	10
_____ is used as a preferred surgical anaesthesia for lab anima	fentanyl	ketamine
_____ is an anaesthetic used as inhalaing agent for lab animals	fentanyl	barbiturates
Morphine is used as _____ in lab animals.	immunostimulant	antibiotic
Mice are grown in cages made of _____.	mild steel	transparent
The term 'ad libitum' refers to _____ in feeding of animals.	optimum	insufficient
Mice will consume _____ grams of pelleted diet daily.	1	5
_____ affects the food requirements of mice.	size	Activity
Water is supplied to lab rabbits via _____.	bottles	syringes
Lack of water intake in mice leads to _____.	death	dehydration
Daily requirement of water for lab mice is _____ ml.	1-2	20-40
The temperature requiment for mice is _____ °C.	5	25
Female mice reach adolescences at _____ week.	3	6
The reprocutive cycle for female mice is _____ days.	10	25
In mice gestation last _____ days.	21	60
_____ is one among the factors considered as the principle for	Aggressive killing	Health and :
_____ act governs the safety and well being of laboratory mice	Animals Act for Scientific Procedu	Animals Act
According to European Commission Directive for protection of vertebrate	eduaction and training	certification
Anually _____ million lab mice are approximately used in Indi 3		10
In animal welfare _____ approach encompasses a number of etl	non-consequentialist	philosopher
Project licence holders take responsibility for the direction of _____	practial skills	animal hand
_____ act governs the safety and well being of laboratory persc	Health & Safety work Act 1979	Health and :
Work place regulation 1992 does NOT deals with _____.	control of temperature	control of p
The marking CE in the employee uniform refers to _____.	clothing and equipment	clothing anc
COSHH stands for _____.	Control of Substances Handled to I	Control of S
Mice born with an innate absence of immunity is called _____	hybrid mice	filial mice
Food for mice is provided in the form of _____.	semi solid pellets	hard pellets
One of the respiratory behaviour associated with rabbits in lab is _____	Sneezing	Nuzzling
_____ space provides proper movement area for rabbits in cag	blind	open
The nesting area of rabbit cages are designed with _____ entra	wide	narrow
Identification of lab rabbits is NOT done using _____.	fur dyes	steel collar
The weaning time for rabbits is _____ weeks.	1	2
FDA prohibits the the use of _____ as analgesic to rabbits.	Flunixin	Ibuprofen
Rat belongs to the sub-family _____.	Cricetinae	Gerbillinae
The commnly used laboratory rat is of _____ origin.	Indian	European

_____ is a common infection occurring in lab animals	Salmonella	Yeast
_____ viral infection leads to haemorrhagic fever in rabbits	Rota	Polyoma
Diseases that are transmitted from animals to humans is termed as _____	zoonoses	biologicals
_____ infection is transmitted from mice to humans.	Shigella sp.	Leptospira
The scientific name of laboratory mice is _____.	Mus murida	Mus chinch
_____ act as mediators in communication between mice.	urine	eyes
Mice exist in a territory as _____.	colony	group
Foreign male mice will provoke aggression from other male mice, this phenomenon is called _____.	multitude	Bruce
Mice bedding consist of _____.	steel racks	plastic racks
_____ is a stereotypic behaviour in caged mice.	barbering	hopping
Exposure of polypropylene to lab mice leads to _____.	death	metabolic disorders
_____ is NOT a major health hazard to animal handlers.	Strain in handling	Allergy
_____ is an allergic symptom developed by lab animal handlers.	Skin rash	Occupation: Allergy
Allergy in animal handling usually belong to _____ hypersensitivity.	I	II
_____ is an Ig G mediated allergic reaction occurring in mice handlers.	Conjunctivitis	Urticaria
Which of the following is the main mice allergen?	hair	urine
Which of the following is the main mice allergen?	fur	feces
The test used to assess the employees for detecting allergy in animal labs is _____.	RIA	CFT
Control of allergens in animal house is made possible by _____.	air filtration	use of surgical masks
Mice whose immunity is reduced by genetic modification is called _____.	Inbred mice	hybrid mice

Opt 3	Opt 4	Opt 5	Opt 6	Answer
11	20			6
inactivity	loss of weight			loss of weight
S. dysentria	P. pneumotropica			P. pneumotropica
7-12.5	10-20			7-12.5
180	110			120
12	5			1.5
diazepam	medeomidine			medeomidine
Doxapram	Isoflurane			Iso flurane
analgesic	anaesthetic			analgesic
opaque pla	iron			opaque plastics
half empty	satisfactory			satisfactory
25	100			5
Metabolism	Pregnancy			Pregnancy
taps	cans			bottles
infection	loss of appetite			dehydration
100-200	6-7			6-7
37	40			25
12	16			6
5	7			5
10	15			21
Surgery	Selective feeding			Health and Safety
Animals Ac	Animals Act for Scientific Procedure			Animals Act for Scientific Procedure 1986
certification	ethical knowledge on animal handling			education and training
15	5			3
scientific	consequentialist			consequentialist
fabric estab	mangement system			animal handling work
Health and :	Health and safety work act 2001			Health and safety work act 1974
control of :	control of ventilation			control of personal habbits
certification	common number and employee no			clothing and equipment
Containmer	Control of Health and Hazard			Control of Substances Hazardous to Health
out bred mi	nude mice			nude mice
mini pellets	powder			hard pellet
Sniffing	Scratching			Nuzzling
centre	common			blind
elevated na	curved			elevated narrow
limb cuff	skin tag			limb cuff
3	4			3
pethidine	pentobarbitone			pentobarbitone
Murinae	Caviidae			Murinae
American	Norwegian			Norwegian

Leptospira E. coli  
Hantaa Orthomyxo  
pathologica verterinary  
T. gondii henselae  
Mus muscu Mus melidus  
ultrasonic s phermones  
family flock  
Waller Yaslow  
wood chips hard steel mesh  
Flashing Morphing  
behavioral agressiveness  
Infection Injury  
Staphylococ Mite infection  
III IV  
Rhinitis Extrincic allergic alveolitis  
feces salaiva  
urine salaiva  
ELISA Wheal and Felix  
open ventil air-fed visors  
knockout n lab mice

Salmonella  
Hantaan  
zoonoses  
Leptospira  
Mus musculus  
phermones  
colony  
Bruce  
wood chips  
Barbering  
behavioural changes  
Strain in handling  
Occupational asthma  
I  
Extrincic allergic alveolitis  
urine  
fur  
ELISA  
air filteration  
knock out mice



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**Lecture Plan**

**Unit - III**

S. No	Duration	Topic	Reference
1.	1	Introduction to anatomical and physiological features of laboratory rat	R1: 10 to 25
2.	1	Major colour groups and varieties of rats.	R1: 44 to 55
3.	1	Regulatory management Housing of laboratory rats-equipment	R1: 128 to 138 T2: 200 to 201
4.	1	Feed formulation, ailments & disease management of laboratory rats	R1:122 to 123; R2:165 to 174
5.	1	Restraining and sample collection in lab rats	T2: 102 to 134
6.	1	Anaesthesia and Analgesia of lab rats	R1: 102 to 119
7.	1	Breeding of laboratory rats	T2: 202 to 203
8.	1	Unit Revision	
Total Hours			8

T1: Dean M. Warrens, Small animal care and management, 2<sup>nd</sup> edition, 2002. Thomas learning, Columbia, USA.

R1: 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.

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### **Unit-III**

#### **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 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.

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,

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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 rat care and management. (As per the principle 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

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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, *Rattus norvegicus* which lives mainly in burrow systems in the ground (Koolhas 1999). Both wild and domestic 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 Gisell 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 Beteray, 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

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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 Area Principles**

(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

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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 ( $1620\text{ cm}^2$  versus  $540\text{ cm}^2$ ) whether tested singly or in the presence of 4 cage mates. In a paper by Scharmann 1991, the adequacy of a cage sized  $900\text{ cm}^2$  to house 4 rats of 200-250gm was compared with a cage sized  $1,800\text{ cm}^2$ . 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 ( $1088\text{ cm}^2$ ) versus smaller ( $432\text{ cm}^2$ ) 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  $2,400\text{ cm}^2$ ) (Klinger and Kemble 1985). In addition juvenile rats have been shown to be more sensitive than older rats to limitations on space (cages  $408 - 780\text{ cm}^2$  versus  $1080 - 2160\text{ cm}^2$ ) 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).

**Dr. V. USHA RANI**

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Department of Microbiology

(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<sup>2</sup> floor area) for the smallest females and 50 (D) x 30 (W) x 30 (H) cm (1,500cm<sup>2</sup> 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<sup>2</sup> and preferably 1,800cm<sup>2</sup>. 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<sup>2</sup>.

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<sup>2</sup>.

### **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 (Blom 1993).

(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)

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## **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

**Dr. V. USHA RANI**

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Department of Microbiology

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,

**Dr. V. USHA RANI**

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Department of Microbiology

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 from birth (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 strain 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

**Dr. V. USHA RANI**

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Department of Microbiology

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

**Dr. V. USHA RANI**

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Department of Microbiology

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

**Dr. V. USHA RANI**

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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<sup>2</sup> 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

**Dr. V. USHA RANI**

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Department of Microbiology

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<sup>2</sup> 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<sup>2</sup> 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";

**Dr. V. USHA RANI**

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Department of Microbiology

- \* 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).

**Dr. V. USHA RANI**

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Department of Microbiology

(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 Bayliss 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; Vadieli, 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.

**Dr. V. USHA RANI**

Assistant Professor  
Department of Microbiology

(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 fetuses (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 cages 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).

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Department of Microbiology

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, Stodulski et al 1995; Duke, Zammit and Lawson 2001). These effects need to be taken into account when scheduling experimental procedures.

A study by Abou-Ismaïl 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).

**Dr. V. USHA RANI**

Assistant Professor  
Department of Microbiology

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 Valiaho 1995). 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 Valiaho 1995). 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 animal environments (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

**Dr. V. USHA RANI**

Assistant Professor  
Department of Microbiology

preferences for complex environments (Denny 1975). A method has been described for creating a physically complex environment in a standard size (1875cm<sup>2</sup>) 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;

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

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

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

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),

**Dr. V. USHA RANI**  
Assistant Professor  
Department of Microbiology

- \* 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; Tucker 1987) 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

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

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 (Koolhaas 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-Ismaïl 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

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

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 health 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 levels well. 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.

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

- (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 31 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

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

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

**Dr. V. USHA RANI**

Assistant Professor

Department of Microbiology

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°C (Gordon, Lee, Chen et al 1991); normothermia is maintained in ambient temperatures between 10°C to 30°C, 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°C 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°C, while during the active nocturnal period they select temperatures between 17-25°C (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°C where it is twice that seen at 23°C (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°C 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°C 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

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Department of Microbiology

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°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°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°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°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).

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Department of Microbiology

(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 (Broderick, 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

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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

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Department of Microbiology

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, Stodulski 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).

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Department of Microbiology

(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) ARRPP Guideline 16: Supervision of Animal Supply by Animal Ethics Committees

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UNIT 3

Question	Opt 1
An example of inhalant anaesthetics for rat is _____.	propofol
Long duration analgesia is _____.	Flunixin
Adult male rat weighs _____ g.	200
Respiratory rate of rats ranges _____ rate/min.	50-100
Oestrouc cycle in female rats lasts _____ days.	4-5
_____ is NOT suitable for nesting rats.	Wood shavi
Rats that are deficient in pigmentation is called as _____.	white rat
Sprague-Dawley rat strains are an example of _____.	Inbred rat
Lewis rat is an example for _____.	Inbred rat
The rat feed pellets are sterilized by _____.	autoclave
Rats are grown in cages with _____ objects.	1D
The gelatinous material attached to vagina of female rat after copulkation is _____.	vaginal plug
Removing pregnant female rats during partuirition leads to _____.	corporal bel
Guinepigs originated from _____.	India
Commonly used variety of guinepigs in lab is _____.	long haired
Rats have prominent _____ teeths.	molar
Bright light in the rat cages lead to _____.	agressivene
Young rats have _____ to regulate the body temperature.	fur
Rats have _____ digits on hind limbs	3
_____ is essential for healthy respiration of rats in cages.	porous vent
Rat diet should contain protein level of _____.	5%
Suitable feed per 100g of rat is _____.	10 g
Water is supplied to lab rats via _____.	tubes
Immunocompromised rats need _____ water	ozonized
Optimal temperature for rats in laboratory is _____ °C .	12
Low humidity in rat laboratory results in _____.	dehydration
Light cycle for lab rats is _____ h.	12/12
Ilumination for the rat cages are maintained at the range of _____ lux.	500-600
Air change in rat cages are done at _____ cycles per day.	2
Puberty occurs in rat during _____ days.	2
Scientific name of commonly used lab rat is _____.	Rattus mexi
Which among the following is an example of out bred rat strain?	Wistar
Which amont the following the special character of a rat?	hermaphord
Thigmomatic behaviour of rat refers to _____.	climb stone
Rats are usually kept in cages made up of _____.	wood
Stocking density of rats per cage is _____.	2
Rats behaviour to eat the feces is termed as _____.	autophagic
Food pellets for caged rats are kept in _____.	containers
Usual recovery time for a rat from anaesthetic condition is _____ min.	2
The dosage of anaeasthetic administered to rat is _____ µg.	300

Normal RBC count of rat is _____ x 10 <sup>6</sup> /mm <sup>3</sup>	7-10
Example of the surgical anaesthesia used in rat is _____.	furanone
Barbiturates are used in mice under surgery during _____.	start of surg
Mating in rat is confirmed by the presence of _____ in the breeding females	blood
In rat the gestation lasts for _____ days.	10
Weaning of rats occurs in _____ days.	50
Breeding one male rat with many female rats is called _____ metho	Monogamoi
Handling of rat is favoured by holding the animal by its _____.	mid regions
The porpyrin secretion from eyes migrates to lower part of eyes and causes _____.	chromodacr
Rats are more susceptible to _____ disease.	bacterial
Sendai virus infects rats specifically during _____ conditions	stress
_____ is NOT a causative agent of disease in rats.	Hantaan vir
_____ is used as anaesthetic in rats.	ketamine
In rat acute pain and restrain is identified by _____.	deep vocali
Stress recognition in rat is done by observing _____.	reduced slee
Modified tear gland of rat is called _____.	lacrymal gla
_____ is the NOT a common behaviour of rats in la	running
_____ lubricates the eye of a rat.	tears
Cages to contain the rats should have a default flooring made up of _____.	wire mesh
Solid bottomed cages for rats should containg _____ bedding.	wood shavi

## Animal Care

13

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Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
Iso flurane	Doxapram	ketamine			Iso flurane
ketamide	doxapram	xylazine			flunixin
400	260	500			500
70-115	80-115	90-115			70-115
6-10	20-30	1-2			4-5
Papers	Fur	Ground corn cobs			Ground corn cobs
albino	wistar	wild			albino
outbred rats	albino	hybrid			outbred rats
outbred rats	albino	hybrid			inbred rats
boiling	evaporation	drying			boiling
2D	3D	Plain			3D
oestrus plug	tissue plug	vaginal fluid			vaginal plug
aggressiveness	enhanced nursing	weaning			enhanced milk production
Europe	Africa	South America			South America
short haired	whorled hair	hairless			short haired
pre-molar	wisdom	canine			canine
climbing ability	retinal damage	lesion on eye			retinal damage
thick skin	epidermal pigmentation	brown fat			brown fat
4	5	2			5
air hole on cage	filtered air	open wired lid			filtered air generator
27%	65%	89%			27%
60g	80g	240 g			5g
cans	sippers	jugs			sippers
filtered	chlorinated	acidified			chlorinated
25	40	31			25
inactivity of	tail ring formation	wrinkled mouth			tail ring formation
8/16	5/19	20/4			12/12
200-400	100-200	50-150			200-400
9	15	3			15
65	20	10			65
Rattus norvegicus	Rattus norvegicus	Rattus indicus			Rattus norvegicus
BALB5	Lewis	cBALB65			Wistar
nocturnal	diurnal	chemo sensitive			nocturnal
climb on PVC	ability to hold	jumping			ability to hold to trees
steel	plastic	iron			plastic
6	10	20			6
coprophagic	Mesophagic	prophagic			coprophagic
cans	hoppers	cage lid			hoppers
60	15	7			15
150	20	60			300

15-20	30-65	100-120
ketamine	xylazine	saffan
mid surgery	in between	post surgery
inactivity	copulatory j	swelling of hind limbs
23	40	90
21	15	5
Multi gamo	Harem	parturition
hind limb	head	fore limb
disacryorrh	swelling	balck ring
viral	fungi	protozoa
immuno coi	knockout	mutated
Sendai viru	Pneumonia	Hepatitis virus
ketamine +	ketamine +	flucosine
crouching u	docile attitu	struggling to feed
climbing	nuzzling	crouching
bulbular coi	Harderian g	lubian gland
hopping	climbing	nuzzling
saliva while	sweat	porphyrin rich secretion
smooth plas	rough alloy	ceramic
plant materi	chopped wc	poly urethane sponge

7-10
xylazine
post surgery
copulatory plug
23
21
Harem
hind limb
chromodacryorrhoea
virus
stress
Hepatitis virus
ketamine + medetomidine
deep vocalization
reduced sleep pattern
Harderian gland
nuzzling
porphyrin rich secretion
wire mesh
wood shavings



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**II –M.Sc Microbiology (Batch 2016-2018)**

**Lecture Plan**

**Unit - IV**

S. No	Duration	Topic	Reference
1.	1	Introduction – history and classification of guinea pigs	R1: 45 to 48
2.	1	Varieties and characteristics of guinea pigs used in labs	R1: 102 to 113 T1: 216 to 218
3.	1	Housing, nutrition and feeding of guinea pigs	T1: 218 to 222
4.	1	Care and handling of guinea pigs in lab	T1: 217 to 218
5.	1	Zoonoses of guinea pigs	T1: 220 to 221
6.	1	Reproduction and breeding managements in guinea pigs – gnotobiotic animals	T1: 220 to 222
7.	1	Video presentation of small animal care	W1
8.	1	Unit Revision	
<b>Total Hours</b>			<b>8</b>

T1: Dean M. Warrens, Small animal care and management, 2<sup>nd</sup> edition, 2002. Thomas learning, Columbia, USA.

R1: 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.

W1: The Jackson laboratory, 600 Main street, Barharbour,ME.[www.jax.org](http://www.jax.org).

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### **Unit-IV**

#### **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,

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Department of Microbiology

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**

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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 Caesarian rederivation 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.

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## 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, inappetence 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

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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 xylazine s.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-mortality 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 gnotobiotic 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.

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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 kept 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 affect 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 countries in 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 sterile 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 history the 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.

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– 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 33-35°C and the humidity kept at 50 per cent or higher. The hand rearing of new young like mice is difficult. So we can feed 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 person 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

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of the room space is required. The room temperature should be at least 18°C 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 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 be 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 be around 50 per cent and ranges between of 40-70 per cent (Clough, 1987). Food and bedding stores should be clean, dry, vermin 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.

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**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 21°C. 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 surface of the walls and floors of the breeding unit and samples of the filtered water supply and of the 'dunk' tank fluid are taken 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.

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– Death records.

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UNIT 4

Question	Opt 1
water supply to the guinea pigs are given by _____.	can
The dietary requirement of water for guineapigs on daily basis is _____ ml.	10
Feed is provided to guineapig in the form of _____.	pellet
Fibre requirement of guineapig feed is _____.	16%
Crude protein requirement of guineapig feed is _____ %	20
Guineapig does not synthesize _____ in its body.	Vitamin C
Water content is provided to guinea pigs by _____ feed	Soy
Excessive supplementation of guinea pigs lead to _____.	abdominal distention
The food requirement of the guineapigs per day is _____ g.	10
Optimal temperature for guinea pigs in laboratory is _____ °C .	26
The light cycle for guineapigs in lab is _____ hours.	9
Peruvian variety of guineapigs are characterized by _____.	long hair
Guineapigs are naturally _____.	diurnal
Providing bold-holes within the pen of guineapigs reduce _____.	trampling
The normal behaviour of guinea pigs includes _____.	running
_____ type of hierarchy is observed in guineapigs.	vertical
Male domination in guineapigs is maintained using _____.	ear
Guineapigs like to be housed in _____.	groups
Commonly used housing method for guinea pigs is _____.	cages
_____ is NOT given to guinea pigs as bedding during breeding.	fine wood shavings
Minimal dose of hypnorm given for surgical anaesthesia is _____.	1.5 ml
The scientific name of guinea pig is _____.	Cavia indica
Aspirin is used as _____ in guineapigs.	sedative
Diclofenac is used to _____ in lab guineapigs	reduce pain
The analgesic phenylbutazone is administered via _____ route.	subcutaneous
The combination of anaesthetics are given _____ mins before the surgery.	10
Which among the following is NOT an example of injectable general anaesthesia?	Fentanyl
_____ method is used to assess the pain during guinea pig handling.	pain scoring
Poor ventilation in guineapig cage lead to _____.	ammonia production
Guinea pigs are highly _____ animals.	alert
Guineapigs are commonly infected by _____.	fungi
Primary symptom of bacterial infection in guinea pigs is _____.	lesion
Guineapigs act as carrier for _____.	flu virus
The most prevalent viral infection in guineapigs is caused by _____.	flavi virus
_____ is common in anaesthesia of guineapig.	death

_____ causes obstruction in airway of guinea pigs.	atropine
_____ is used as a sedative for guinea pigs.	hypnorm
The sedative hypnorm is a mixture of _____ .	fentanyl + r
Which of the following is an example of injectible general anaesthesia?	chloroform
Respiratory rate of guinea pig ranges _____ rate/min.	42-104
Birth weight of a guinea pig is _____ g.	70
Normal RBC level of guinea pig is _____ $\times 10^6/\text{mm}^3$	2
_____ percentage of basophil in guinea pigs indicates an allergy.	0.5
Anaesthetics used for guinea pigs are chosen based on _____ .	volume
Natural life span of guinea pig is _____ years	2
Female guinea pigs reach puberty in _____ weeks.	2
Male guinea pigs reach puberty in _____ weeks.	7-10
Mating should be done when the female is _____ g.	100
Mating should be done when the male is _____ g.	250
Male guinea pigs are commonly called as _____ .	pigs
The average weight gain of a young guinea pig should be _____ g	3.5
Restraining of guinea pigs should be done by hold the animal in _____ .	anterior
Loud vocalization in guinea pigs occurs due to _____ .	mild pain
Any unusual sign of acceptance towards handling in guinea pigs indicates _____ .	loss of health
Common dietary need of guinea pigs in comparison to primates is _____ .	more water
The vagina of female guinea pig is covered by _____ .	epithelial
Gestation in guinea pig lasts around _____ days.	30
During the last week of gestation the pubic symphysis separates due to action of _____ hormone	contractin
Maximum age for the female guinea pig to breed is _____ months.	5
Weaning of guinea pigs occur at _____ days	21

## Animal Care

13

## Department of Microbiology

## Academy of Higher Education

Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
dropper	automated s	jug			automated system
20	50	60			10 ml
powder	Raw	milled			pellet
2%	30%	45%			16%
10	5	2			20
salaiva	Phermones	calcium			Vitamin C
Corn cake	Grounut ca	cabbages			cabbages
inactivity	Severe bow	malnutrition			abdominal discomfort
20	30	40			10
36	10	15			26
15	16	30			15
short hair	hairless	whorled hair			long hair
crepuscular	docile	inactive			crepuscular

climbing	nuzzling	Scratching			tamplng
hopping	climbing	resist arrest			resist arrest
horizontal	Stable	unstable			stable
mouth	Tail	olfactory lobes			olfactory lobes
colonies	single	coupled			groups
pens	steel cages	floor pens			pens
Papers	Sponges	cotton			fine wood shavings
5 ml	10 ml	0.5 ml			0.5 ml
Cavia porce	Cavia roder	Cavia cavie			Cavia porcellus
anaesthetic	antibiotic	analgesic			analgesic
reduce infla	reduce weig	reduce irritation			reduce pain
intravenous	Oral	intra muscular			oral
20	30	40			30
Ketamine	Isoflurane	Barbiturates			Isoflurane
square chec	Dunnett	Tukey			Pain scoring
respiratory i	Asphyxatio	death			respiratory illness
ferocious	Timid	agile			alert
virus	Protozoa	bacteria			bacteria
asphyxiation	Abscess	haemorrhage			abscess
simian virus	hanta virus	LCM virus			LCM virus
sendai virus	Simian viru	myxoma virus			sendai virus
resistance to	post-anesth	paralysis			post-anaesthetic complications

flucytosine	rifampin	doxycycline	atropine
atropine	rifampin	doxycycline	hypnorm
fentanyl +fl	fentanyl + a	fentanyl + morphine	fentanyl + fluanisone
ethylene	atropine	hypnorm	hypnorm
50-110	100-150	120-180	42-104
150	200	300	70
10	7	16	7
10	3	5	10
strain	surgical nec	metabolic activity of the animal	surgical need
3	10	8	8
4	5	6	6
8-9	9-11	1-3	7-10
250	300	400	400
650	450	300	650
mare	Boar	flock	boar
1	3	10	3.5
posterior	Lateral	dorsal	anterior
tansient pai	Genralized	acute pain	transient pain
low aggress	Death	anaesthetization	loss of health
requirement	High moist	cooked feed	requirement of vitamin C
squamous	enothellial	mesanchymal	epithellial
72	110	90	72
nor-epinepr	Relaxin	meladunin	relaxin
10	15	20	20
11	13	18	21



**KARPAGAM ACADEMY OF HIGHER EDUCATION**  
**DEPARTMENT OF MICROBIOLOGY**

(Deemed University Established Under Section 3 of UGC Act, 1956)

Eachanari PO, Coimbatore -641 021, India.

**II –M.Sc Microbiology (Batch 2015-2017)**

**Lecture Plan**

**Unit - V**

S. No	Duration	Topic	Reference
1.	1	Various routes of inoculation in mice & rats	T1: 9 to 23
2.	1	Handling and routes of inoculation in rabbits, guinea pigs	T1: 2 to 8
3.	1	Laboratory use of animals –role in Microbiology	T1: 23 to 32
4.	1	Antibody production in animals	R1: 120 to 135
5.	1	Disposal of animal house wastes	R1: 393 to 396
6.	1	Disposal of animal house wastes	R1: 50 to 56
7.	1	Safety measures in animal house	R1: 31 to 35
8.	1	Unit Revision	
<b>Total Hours</b>			<b>8</b>

T1: Dean M. Warrens, Small animal care and management, 2<sup>nd</sup> edition, 2002. Thomas learning, Columbia, USA.

R1: 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.

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## **KARPAGAM ACADEMY OF HIGHER EDUCATION DEPARTMENT OF MICROBIOLOGY**

(Deemed University Established Under Section 3 of UGC Act, 1956)

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### **Unit-V**

#### **Handling Laboratory Animals**

Good animal handling techniques will reduce the risk of injury from bites and scratches, 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, and are insoluble unless they are activated by enzymes during passage through the stomach and 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 specific carrier exists to transport them across the gut wall into the blood. Basic or alkaline compounds are likely to be fully ionised in the acid environment of

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the stomach, and will be poorly absorbed. However, once in the small intestine, the higher pH will reduce the level of ionisation and increase the absorption. The reverse is true for acidic 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 some special cases however. For example, compounds will only enter the central nervous system (CNS) if they can cross the blood-brain barrier. Lipid soluble compounds will accumulate in fatty tissues, even if the blood supply is poor. This phenomenon can be used to ensure rapid elimination of lipid soluble drugs from the CNS by redistribution to fat (see thiopentone in anaesthetics, Chapter 7). Drugs will also tend to accumulate at 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 will enter the fetus. Some compounds may accumulate in the fetus and some may accumulate in specific tissues, such as the kidney. Compounds will generally be secreted into milk if they can cross the lipid membrane in the mammary gland. Milk is slightly more acidic than blood, so basic compounds tend to accumulate in milk because they ionise after having crossed the lipid barrier and cannot then return to the blood. These factors that affect the absorption and distribution of administered compounds define the pharmacokinetics of the compound, and should be studied prior to administration to 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 infusion given 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 maintenance requirement for fluid is approximately 40 ml/kg per 24 h in normal animals and care should be taken not to exceed this unless there are deficiencies to replace.

**Dr. V. USHA RANI**

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**Administration techniques**

Injections should be performed using aseptic techniques, as for blood sampling. It is important to use equipment appropriate for the species (see Tables 9.2a and b). For example, 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 are adequate. For farm animals, needles larger than 21 G may be used. The viscosity of the substance also affects the size of needle used. Thick, viscous liquids may not pass through narrow gauge needles. Intradermal injections are performed with 25–27 G needles. To minimise the distress caused to animals during administration of substances, they must be carefully and expertly handled, and given a sedative or short acting general 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, unless the 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 that care is taken with adjuvants, because if there is an adjuvant reaction in the skin over the respiratory muscles this can cause pain on breathing. In sheep and goats fluid can be given under the skin over the ribs. For pigs, small volumes can be injected into the skin behind the ear, or into the fold between the leg and the abdomen. Pigs have much subcutaneous fat, and injections given elsewhere are likely to enter the fat, where absorption 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 into the muscles of the thigh. Larger volumes or irritant compounds should be injected into the quadriceps group on the front of the thigh. The muscle can be immobilised with one hand while injecting with the other. Injections can be given into the caudal thigh muscles, but as the sciatic nerve runs through these muscles, irritant compounds should not be given here or damage may be caused to the nerve. In rodents, the quadriceps feels like a small peanut on the front of the thigh, and can be immobilised with 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 the spine, and in large animals the gluteal muscles are used. In adult pigs, injections are given 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 caudal thigh 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 can also 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 used in dogs, cats, hamsters and ferrets (see Figure 9.8), and is the method of choice in ruminants and horses. The ear veins are used in guinea pigs and pigs. Fowl can be injected via the brachial vein.

### **Intradermal injections**

Intradermal injections for most species can be given in the same area as subcutaneous injections. 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 of the 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 animal should be held either by an assistant or in one hand on its back, upright, so that it is comfortable and securely supported. The needle is angled at 45° to the skin and no resistance should be encountered to the passage of the needle (Figure 9.5).

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Figure 9.5 Intraperitoneal injection.

### Oral administration

Substances may be given orally by inclusion in the diet or drinking water. These methods have the disadvantages that it is impossible to be sure that the animal has had the entire 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 overeat and thus ingest an overdose of the drug. In mice particularly, adding drugs to the water tends to lead to dehydration, because the mouse avoids drinking it, and this can lead to a rapid deterioration in the condition of the mouse, especially if the compounds have been given for therapeutic reasons. If the watering system is automated, it is impossible to give compounds in this way. To overcome these problems, gastric intubation or gavage may be employed (Figure 9.6). Flexible catheters or stainless steel needles with rounded tips are used. The animal is restrained with its neck extended, and the needle or catheter passed gently down the oesophagus. Care must be taken not to damage the oesophagus, or to put the needle into the trachea. The needle or catheter can usually be observed passing down the oesophagus on the left side of the neck. Damage to the catheter from chewing can be avoided by using an oral speculum, or by using a flexible nasogastric or pharyngostomy tube instead. The animal 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 animal by placing one hand over the top of the head, placing the thumb at the commissure of the 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 forefinger of the other hand, and the middle finger used to open the mouth. The tablet can then be placed onto the tongue as far back as possible, to stimulate the swallowing reflex.

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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 an animal without a marketing authorisation (a product licence) for the indication and species. Exceptions may be made in the absence of a licensed product (the cascade principle). In the UK, prescription of veterinary medicines is primarily the responsibility of registered veterinary surgeons. Personal licence holders under the ASPA who are authorised to perform anaesthesia and surgery in research animals and who do not hold veterinary qualifications must obtain and administer drugs under the direction of a veterinary surgeon. A pharmacist may only supply licensed medicines for use in animals under the direction or prescription of a veterinary surgeon. Medical practitioners may not legally prescribe medicines for use in animals. The cascade for prescribing veterinary medicines where no product for the species or 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 or another 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 by an 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).

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### **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 will specify the requirements. It is good practice to keep a record of all drugs held and used, it is a legal requirement to keep records of purchase and use of all controlled drugs listed in Schedules 1–3 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 stressful components.

- 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 trained to 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, and requires considerable skill. The expertise required to carry this out successfully must be gained first by watching others, then by practising on cadavers or models such 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 which depend on the volume of blood removed (as a percentage of the total), and the speed of withdrawal. The rapid removal of large quantities of blood will cause the animal to go into hypovolaemic shock, and may even cause death. The percentage blood loss required to cause hypovolaemic shock varies with the speed of withdrawal, whether or not fluid is replaced concurrently, and the psychophysiological state of the animal at the time. Chronic slow haemorrhage is tolerated better than acute blood loss, and placid animals tolerate greater losses than nervous ones, again indicating the need for competent handling and training of the animals. Stress responses in the animal result in the release of hormones and other substances to counteract the stress, which can cause anomalous experimental results. It is essential to minimise the stress caused to an animal when removing blood from the humane viewpoint, and also to ensure good scientific practice. The experimental techniques should be refined such that the quantity of blood removed is minimised. This is particularly important in small mammals, such as mice, where the blood volume is small and sample volume is critical. The withdrawal of blood from any vessel requires skill in handling the animal and in manipulating the equipment. The person taking the samples should be fully familiar with the chosen technique, and have all the equipment ready before starting. You are advised to consult the BVA/FRAME/RSPCA/UFAW (1993) working group report on the removal of blood from laboratory mammals and birds.

#### **Quality of samples**

To achieve meaningful results, any samples taken must be of good quality, and be preserved in the best possible manner. If the sampling technique is poor, blood may clot or haemolyse rendering results invalid. To avoid these problems, samples must be taken 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 or scalpel blade. The latter however is not

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best practice, as inadvertent movement can result in the severing of an appendage. If needles are used, the needle should be as large as is practicable for the species. This allows blood to flow faster, reducing the likelihood of clotting, and also causes less damage to the red cells, reducing the possibility of haemolysis. Thought should be given to the desirability of using anticoagulants. Different anticlotting agents 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 serum from clotted blood, which may make heparin a good choice for collecting blood for harvesting antibodies. Sodium heparin is sometimes used if preservation of the white cells is required.
- Potassium EDTA (ethylene diamine tetra-acetic acid): This is used for haematological analyses.
- Oxalate/fluoride: This is used for blood glucose determination. Several other anticoagulants are available, for example, for collecting blood for transfusions or for analysis of clotting factors. After collection into anticoagulant, the blood should be mixed thoroughly by rolling, not by shaking as this can damage the cells 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 a few hours after blood collection. It is advisable to determine the exact requirements of the laboratory protocol prior to sample collection.

### **Venepuncture technique**

#### **Restraint**

When taking samples, the animal should be gently restrained by an experienced handler who is known to the animal. Chemical restraint is generally only required if the technique involves more than a pinprick, for example, for tail tip amputation, or in primates 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 palpating its course, and to have it immobilised, before piercing the skin. The handler may be required to raise the vein, by occluding the venous drainage proximal to the site of venepuncture. 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. The use of a quick release tourniquet greatly facilitates blood sampling in some species and vasodilating 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 clipped to 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, it may 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 such animals as the cat, dog, rabbit, or pig.

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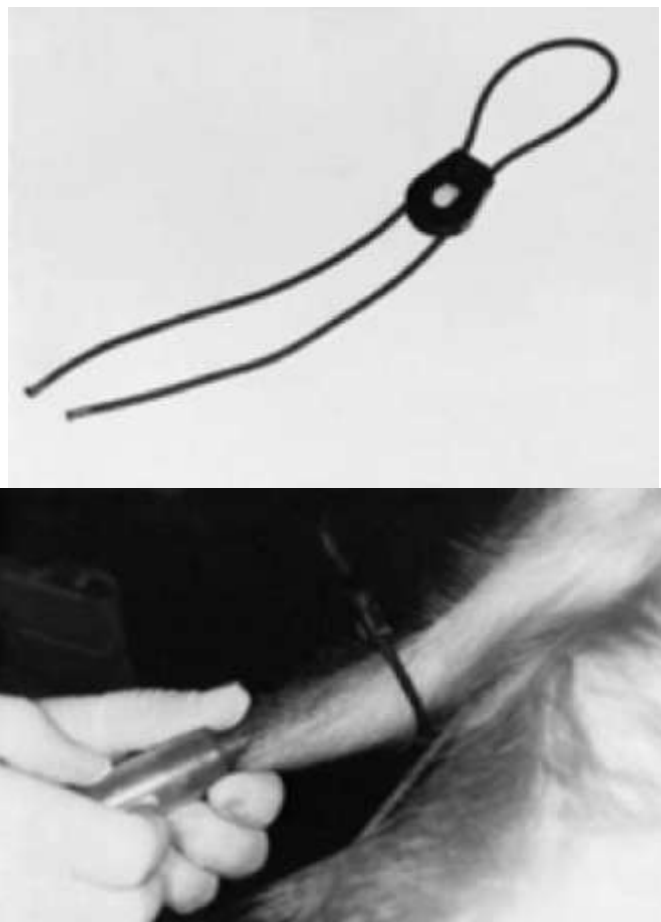


Figure 9.7 (a) Quick release tourniquet and (b) tourniquet in use.

## Methods of venepuncture

### Rodents

For most rodents saphenous vein puncture is the most satisfactory site for obtaining small quantities of blood (see Hem et al. 1998). For those rodents with tails, this is a good site for obtaining rather larger quantities of blood. The lateral coccygeal or tail vein may be easily visualised. Warming the tail first increases the blood flow to the site and makes sample collection easier. Blood samples may be obtained by incising the skin over the vein using a sterile lancet and collecting the blood into a plain or anticoagulant coated capillary tube, or allowing it to drip into a container. Scalpel blades are not recommended as they can easily slip causing damage to the tail. Larger samples can be obtained from rats by placing a 23–26 G flexible cannula or butterfly needle into the tail vein and allowing blood to drip into a collection pot or applying gentle suction with a syringe. If a butterfly needle is used, the plastic tube should be shortened to prevent clotting within it. Amputation of the tail tip to obtain a sample of mixed arterial and venous blood can be carried out on rats, mice, and gerbils, but must be done under general anaesthetic (BVA/FRAME/RSPCA/UFAW, 1993), gaseous anaesthesia using isoflurane being ideal for such a procedure. In mice, this does not appear to involve the removal of 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. The vein is raised on one side of the neck by applying pressure at the thoracic inlet, and a needle placed through the skin and into the vein pointing towards the head. For animals with no tails, such as guinea pigs and

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hamsters, it is possible to obtain tiny samples from the ear veins. A technique has been described for cannulating the lateral saphenous vein in the hindlimb of guinea pigs under general anaesthesia for repeated sampling (Nau and Schunck, 1993). For terminal sampling it is acceptable to perform cardiac puncture, which must be done under general anaesthesia. There are many potentially harmful sequelae to this procedure, 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 piercing the left ventricle through the chest wall at the sixth intercostal space, one third of the way up, to obtain arterial blood, or by piercing the right ventricle with the animal on its left side for venous blood. Alternatively, the animal may be placed on its back, and the heart 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 for the animal, and although it has been used for bleeding rats and tail-less animals, it is not recommended for sampling with recovery.

### **Rabbits**

Blood can be collected relatively easily from the marginal ear vein using an over-the-needle cannula or butterfly needle. A peripheral vasodilator may be applied to the 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 it to drip into a pot. After collection, the vasodilator is wiped off and pressure applied until the bleeding ceases. Haemostatic dressings can be applied as discussed earlier in this chapter. Bleeding from the central ear artery is possible, but can result in the formation of large haematoma, 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 be clipped and a drop of blood collected into a capillary tube. For moderate sized samples the saphenous or tail veins can be used, while larger quantities can be collected from the jugular vein. The fur on the neck needs to be well clipped, and the vein raised by placing a thumb over the jugular groove in the thoracic inlet. Blood is collected by inserting 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 penetrating the skin. Pressure on the vein in the thoracic inlet is maintained until the blood has been collected (see Figure 9.8).

### **Primates**

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

### **Dogs and cats**

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

**Dr. V. USHA RANI**

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### **Ruminants and horses**

For sheep, goats, cattle and horses the jugular vein is used. The vein can be visualised by clipping hair or wool from over the jugular groove. The vein is raised by applying firm pressure to the base of the jugular groove. The needle is advanced through the skin up towards the head (Figure 9.11). Cattle can also be bled from the ventral coccygeal 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 needle is inserted in the thoracic inlet, and angled slightly upwards and medially to enter the anterior vena cava.

### **Birds**

The brachial (alar or wing) vein is usually used for blood sampling in birds. It can be visualised as it crosses the elbow by plucking the feathers over the medial surface of the wing. Samples should be removed slowly to prevent the vein collapsing. Pressure is generally not applied after venepuncture in birds as this can promote haematoma formation. The right jugular vein can also be used. It is found between the feathers on the dorsolateral surface of the neck. It can be raised by applying pressure at the base of the neck. Haematoma 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

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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

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

Route	Species				
	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

## 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 daily and 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.

### **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,

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- 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,

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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°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°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 alkaline solution 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

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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 over a 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.

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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

## UNIT 5

### Question

Collection of tissue from live animal for genetic analysis is called \_\_\_\_\_.

\_\_\_\_\_ samples are collected during the stage of oestrous cycle.

Cells consisting primarily of polymorphonuclear lymphocytes (PMNs), with some epithelial cells are called \_\_\_\_\_.

Nucleated and cornified epithelial cells, with some PMNs in early stages are called \_\_\_\_\_.

Cornified epithelial cells predominate, with a few nucleated cells seen in the early stage \_\_\_\_\_.

Cornified epithelial cells predominate, with a some nucleated epithelial cells \_\_\_\_\_.

Physical separation of animals by species is recommended to prevent \_\_\_\_\_.

Helicobacter bilis can infect \_\_\_\_\_.

Subclinical microbial infections occur frequently in \_\_\_\_\_.

Healthy, well-cared-for animals are a prerequisite for \_\_\_\_\_.

Genetically modified microbes are used to \_\_\_\_\_.

Xerograft refers to organ transplant between \_\_\_\_\_.

\_\_\_\_\_ is an example of the GM animal product.

Genetic makeup of the animal is \_\_\_\_\_.

Phenotype of an animal refers to \_\_\_\_\_.

Genetically modified animals with essential genes in chromosome deleted is called \_\_\_\_\_.

In harmful mutant animals \_\_\_\_\_ type of mutation is seen.

Lab animals with point mutations is called \_\_\_\_\_.

A cloned animal is one that has been derived by inserting \_\_\_\_\_ into another animal.

\_\_\_\_\_ is an example for genetically altered animal.

Homozygous refers to \_\_\_\_\_.

\_\_\_\_\_ is known as the unit of specific genes.

Hybrid animals have a mother of one inbred strain and \_\_\_\_\_ father.

\_\_\_\_\_ is a chart used to register the data of animal ancestors.

\_\_\_\_\_ record covers the essential data on the colony size of the lab animal.

\_\_\_\_\_ is a small, isolated animal colony that is used to produce breeding stock for the larger colony.

\_\_\_\_\_ record covers the breeding data of animals.

The size of syringe used to draw blood in animal is called \_\_\_\_\_ mm gauge.

Which of the following is an example of an advanced temperature measurement technique method in animals?

Abdominal palpitation of lab animals are measured in \_\_\_\_\_ side.

Transfer of one cell from an animal in to another animal for the process of cloning is called \_\_\_\_\_.

Embryonic stem cells can be grown in \_\_\_\_\_.

If a gene is inserted into the genotype of wild animal then it is called as \_\_\_\_\_.

The animals that receive the genetic material is called \_\_\_\_\_.

\_\_\_\_\_ cells are enucleated before transfer.

ASPA act stand for \_\_\_\_\_.

Ethyl nitrosurea is a \_\_\_\_\_.

Large scale mutagenic process is done in \_\_\_\_\_.

Efficient mutagenesis and compatibility is seen in \_\_\_\_\_.

Supervolution is a process in which \_\_\_\_\_ with hormone is regulate

\_\_\_\_\_ is used to detect the damage done by infectious agents to the host tissue.

\_\_\_\_\_ technique is used to detect the protozoal infection in animals.

Up to \_\_\_\_\_ % of blood volume can be removed from lab animal.

For repeated sampling of blood \_\_\_\_\_ % of blood is collected

In \_\_\_\_\_ method the animal is manually restrained and small bore pipette is placed at the medial canthus.

\_\_\_\_\_ is a technique used to obtain comparatively large volume of blood

In \_\_\_\_\_ method the tip of the tail is cut to collect blood

The size of syringe used to draw blood from cardiac puncture is \_\_\_\_\_ mm gauge.

Urine is collected from mice by \_\_\_\_\_ restraining.

Feces is collected from lab animals by a process called \_\_\_\_\_.

Which of the following is NOT a method of administering substances to lab animals?

Commonly used method for drawing blood in lab animals is \_\_\_\_\_.

\_\_\_\_\_ is a feature considered while performing injection to animals.

CNS stands for \_\_\_\_\_.

The average volume of the solutions administered to lab mice during intravenous injection is \_\_\_\_\_ per 20 g

The blood for hematological analysis is collected from \_\_\_\_\_.

Heterozygous refers to \_\_\_\_\_.

\_\_\_\_\_ involves the implantation of small telemetric transmitter.

In touch thermometer the range of temperature is \_\_\_\_\_.

\_\_\_\_\_ method is used in the identification of disease in animals.

**Laboratory Animal Care**  
**16MBPU303**  
**Department of Microbiology**  
**Karpagam Academy of Higher Education**

Opt 1	Opt 2	Opt 3	Opt 4	Opt 5	Opt 6	Answer
autopsy	biopsy	Haemorrhic	necrotic tissue			biopsy
tissue biops	rectal swab	vaginal swa	perineal swab			vaginal swab
diestrus	proestrus	estrus	metestrus			diestrus
diestrus	proestrus	estrus	metestrus			proestrus
diestrus	proestrus	estrus	metestrus			estrus
diestrus	proestrus	estrus	metestrus			metestrus
agression	easy sample	prevent dise	prevent anxiety			prevent disease transmission
rat	mice	Rabbit	hamster			rat
rat	mice	rabbit	hamster			rat
science stud	practical ap	Good quali	diagnosis of pathogen virulence			good quality
breeding	export	Studies on	pharmaceutical companies			studies on human research
same specie	same family	Same order	different species			different species
Vaccine	Protein	Milk	Carbohydrate			milk
phenotype	genotype	Allelotype	Auxotype			genotype
body makeu	genetic mak	Immune m	cellular makeup			body makeup
Knockout	transgenic	mutant	filial generation			knockout
spontaneous	point	Frame shift	transgenesis			point mutation
immuno mo	immuno coi	Nude	clone			nude
one cell	two cells	three cells	four cells			one cell
albino rat	wistar rat	Nude mous	shuttle hamster			albino rat
same allele	different all	species spec	species non specific allele			different allele
chromosom	nucleoid	Allele	RNA			allele
inbred strai	outbred stra	Mutated str	identical strains			outbred strain
pedigree	colony man	Colony ma	ancestral record			pedigree chart
colony man	census reco	Pedigree re	ancestral record			census record
filial colony	mother colo	brood colon	foundation colony			foundation colony
prodcution	mother colo	breeding rec	brood record			breeding record
22	10	5	1			22
infrared me	Hg thermon	red oil meas	heat sensory thermometer			infrared thermometer
dorsal	anterio dors	Ventral	posterior			ventral
chromosom	gene walkin	Nuclear tra	molecular morphing			nuclear transfer
host tissue	egg yolk	RPMI-1650	HAT			RPMI-1650
knock up	knock down	knock out	knock in			knock in
donor	recepient	clone	source			recepient
spermatocyt	spleenocyte	Hepatocyte	oocytes			oocytes
Animals Sci	Animal Sale	Animal Sci	Animal Slaughter Project Act			Animal Science Project Act
alkylating a	phosphoryla	Deaminatir	hydrolysing agent			alkylating agent
Drosophila	Rabbits	Sheeps	dog			Drosophila
mice	rat	hamster	guineapig			mice
donor male	recepient m	Donor fem	recepient female			donor female

pathology	serology	Immunolog	histopathology	histopathology
pathology	PCR	microscopy	RIA	PCR
5	1.5	15	2	15
5	1	7.5	12	7.5
Trail lacera	Cardiac pun	Retro orbita	decapitation	Retro orbital sinus puncture
cardiac pun	trail lacerati	Retro orbit	decapitation	decaptation
cardiac pun	trail lacerati	Retro orbit	decapitation	tail laceration
20	10	16	24	24
hind limb	catheter trar	abdominal p	perineal pressure	hind limb
pad collecti	anal milking	rectal prolap	abdominal pressure	anal milking
intravenous	intramuscul	intrperitone	feed additive	feed additive
venipunctur	venipunctur	venipunctur	cardiopuncture	venipuncture of fore limb
volume of s	bioavailabil	Size of the	metabolic rate of the sample	bioavailability
Central neu	Central Noc	Central ner	Central soft system	central nervous system
10 ml	1 ml	0.2 ml	5 ml	0.2 ml
bleeding frc	bleeding frc	Bleeding fr	bleeding from thoracic vein	bleeding from subcutaneous vein
same allele	different all	species spec	species non specific allele	same allele
telemetric n	Hg thermon	Touch moni	thermocouple monitoring	thermocouple
36.5	38	40	45	36.5
DNA finger	VNTR sequ	ELISA	RIA	ELISA

Reg. No. \_\_\_\_\_

[16MBP305B]

**KARPAGAM UNIVERSITY**

(Established Under Section 3 of UGC Act, 1956)

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

**M. Sc., DEGREE INTERNAL EXAMINATION July – 2017**

**MICROBIOLOGY**

**LABORATORY ANIMAL CARE**

**Time: 2 hours**

**Maximum:50 marks**

**Date:**

**Part - A (20 X 1 = 20 Marks)**

1. \_\_\_\_\_ is one among the factors considered as the principle for animal care.
  - a. Aggressive killing
  - b. Health and safety
  - c. Surgery
  - d. Selective feeding
2. The largest used stock variety of rabbits belong to \_\_\_\_\_.
  - a. America
  - b. India
  - c. New Zealand
  - d. Australia
3. Rabbits are \_\_\_\_\_.
  - a. diurnal
  - b. docile
  - c. semi-active
  - d. nocturnal
4. Space in rabbit housing is essential to prevent \_\_\_\_\_.
  - a. bone thinning
  - b. aggressiveness
  - c. inactivity
  - d. disease
5. \_\_\_\_\_ is an alternative housing method of separating aggressive rabbits in a group.
  - a. pens
  - b. single housed cage
  - c. spaced
  - d. caged pair housing
6. Scientific name of commonly used lab rat is \_\_\_\_\_.
  - a. Rattus mexicanus
  - b. Rattus norvegicus
  - c. Rattus romulus
  - d. Rattus indicus
7. Thigmotaxis behaviour of rat refers to \_\_\_\_\_.
  - a. climb stones
  - b. climb on PVC pipes
  - c. ability to hold to tree
  - d. jumping.
8. Cages to contain the rats should have a default flooring made up of \_\_\_\_\_.
  - a. wire mesh
  - b. smooth plastic
  - c. rough alloy
  - d. ceramic.
9. Exposure of polypropylene to lab mice leads to \_\_\_\_\_.
  - a. death
  - b. metabolic disorder
  - c. behavioral changes
  - d. aggressiveness
10. Which of the following is the main mice allergen?
  - a. hair
  - b. urine
  - c. feces
  - d. saliva
11. Light cycle for lab rats is \_\_\_\_\_ h
  - a. 12/12
  - b. 8/16
  - c. 5/19
  - d. 20/4

- Part - B Answer all the questions (3 X 2 = 6 Marks)**

- Part - C Answer all the questions (3 X 8 = 24 Marks)**

- b. Define euthanasia and discuss on the disease maintenance of lab rat.

Reg. No. \_\_\_\_\_

[16MBP305B]

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**M. Sc., DEGREE INTERNAL EXAMINATION September – 2017**

**MICROBIOLOGY**

**LABORATORY ANIMAL CARE**

**Time: 2 hours**

**Maximum:50 marks**

**Date:**

**Part - A (20 X 1 = 20 Marks)**

1. The normal behaviour of guinea pigs includes \_\_\_\_\_.  
a. Running                      b. Hopping                      c. Climbing                      d. Resist arrest
2. Genetically modified microbes are used to \_\_\_\_\_.  
a. Breeding                      b. Export                      c. Studies on human research                      d. Pharmaceutical companies
3. Ethyl-nitros-urea is a \_\_\_\_\_.  
a. Alkylating agent                      b. Phosphorylating agent                      c. Deamination agent                      d. Hydrolyzing agent
4. Large scale mutagenic process is done in \_\_\_\_\_.  
a. Drosophila                      b. Rabbits                      c. Sheeps                      d. Dog
5. Efficient mutagenesis and compatibility is seen in \_\_\_\_\_.  
a. Mice                      b. Rat                      c. Hamster                      d. Guinea pig
6. Superovulation is a process in which \_\_\_\_\_ with hormone is regulated.  
a. Donor male                      b. Recipient male                      c. Donor female                      d. Recipient female
7. If a gene is inserted into the genotype of wild animal then it is called as \_\_\_\_\_.  
a. Knock up                      b. Knock down                      c. Knock out                      d. Knock in
8. The animals that receive the genetic material is called \_\_\_\_\_.  
a. Donor                      b. Recipient                      c. Clone                      d. Source
9. \_\_\_\_\_ cells are enucleated before transfer.  
a. Spermatocytes                      b. Spleenocytes                      c. Hepatocytes                      d. Oocytes
10. Lab animals with point mutations is called \_\_\_\_\_.  
a. Immuno modified                      b. Immuno compromised                      c. Nude                      d. Clone
11. The dietary requirement of water for guineapigs on daily basis is \_\_\_\_\_ ml.  
a. 10                      b. 20                      c. 50                      d. 60
12. Feed is provided to guineapig in the form of \_\_\_\_\_.  
a. Pellet                      b. Powder                      c. Raw                      d. Milled
13. A cloned animal is one that has been derived by inserting \_\_\_\_\_ into another animal.  
a. One cell                      b. Two cells                      c. Three cells                      d. Four cells
14. \_\_\_\_\_ is an example for genetically altered animal.  
a. Albino rat                      b. Wistar rat                      c. Nude mouse                      d. Shuttle hamster
15. Transfer of one cell from an animal in to another animal for the process of cloning is called \_\_\_\_\_.  
a. Chromosome walking                      b. Gene walking                      c. Nuclear transfer                      d. Molecular morphing
16. Embryonic stem cells can be grown in \_\_\_\_\_.  
a. Host tissue                      b. Egg yolk                      c. RPMI-1650                      d. HAT
17. Water content is provided to guinea pigs by \_\_\_\_\_ feed.  
a. Soy                      b. Corn cake                      c. Groundnut cake                      d. Cabbages
18. If a gene is inserted into the genotype of wild animal then it is called as \_\_\_\_\_.  
a. Knock up                      b. Knock down                      c. Knock out                      d. Knock in
19. The animals that receive the genetic material is called \_\_\_\_\_.  
a. Donor                      b. Recipient                      c. Clone                      d. Source
20. -----cells are enucleated before transfer.  
a. Spermatocytes                      b. Spleenocytes                      c. Hepatocytes                      d. Oocytes

**Part - B Answer all the questions (3 X 2 = 6 Marks)**

- 21. Importance of record keeping
- 22. what are the types practiced in administering substance
- 23. Write any 4 parameters in disposing animal waste

**Part - C Answer all the questions (3 X 8 = 24 Marks)**

- 24. a. Comment on disease management in guinea pigs.

**or**

- b. Write short notes on the environmental enrichment for guinea pigs in laboratory

- 25. a. Discuss about the handling method of in lab animals during sample collection.

**or**

- b. Define incineration and comment on the waste disposal methods animal house.

- 26. a. How are antibodies produced from lab animals? Discuss.

**or**

- b. Define analgesic and anaesthetics and their role in animal handling