# GUIDELINES

# FOR CARE

# AND USE OF

# ANIMALS

# IN SCIENTIFIC

# RESEARCH

INDIAN NATIONAL
SCIENCE ACADEMY
NEW DELHI

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# FOR CARE AND USE OF ANIMALS IN SCIENTIFIC RESEARCH

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# INDIAN NATIONAL SCIENCE ACADEMY

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# FOREWORD

*Laboratory animals form the life-line of biomedical research and play a very vital role in the drug development programmes. There is an ever-growing need for better laboratory animal facilities for biomedical research, production of antisera, vaccines and novel drugs, especially to combat the dreadful AIDS and other diseases. The need to maintain the animals under standard animal husbandry conditions, to handle them gently and humanely, and limit unnecessary usage by strictly enforcing ethical considerations demands utmost attention. Finding no comprehensive document available in the country to guide the burgeoning biomedical research community, the Academy had deliberated on this issue way-back in 1992 and came out with a document titled 'Guidelines for Care and Use of animals in Scientific Resarch'. Recently, a Committee was constituted under the Chairmanship of Prof. P. N. Tandon to consider the various issues involved in the ethical use of Animal Experimentation, and the Committee besides other recommendations thought it necessary to revise these "Guidelines". Ideally, the housing and environmental conditions for experimental animal facility should conform to these Guidelines. However, the Committee recognized that animal facilities, particularly those attached to hospitals for day-to-day diagnostic purposes may not at present be able to meet these standards because of financial constraints. I would like to thank all members of the Committee for this commendable work and I hope the scientific community will find these guidelines useful.*

*G. Mehtra*

*President*
*Indian National Science Academy*

*Dated : 18 October, 1999*

# INTRODUCTION

The use of laboratory Animals in Scientific Research has been a subject of debate for over a century. Though the animals were first used in research in second century AD their systematic use in research began about 100 years ago, when vaccines for polio and rabies came up for production. Since then, the animals have been used in research investigations and production of biologicals and have played an important role in unfolding vital information about the human and animal life processes. This has helped in the advancement of medicine, development of drugs, diagnostics and production of biologicals for alleviating sufferings of both human and animals.

It must be emphasized that use of animals in research is inevitable and cannot be abandoned in the interest of human and animal welfare. In-vitro alternate methods cannot replace animal experimentation totally, but can work only as adjuncts and reduce the number of animals in some cases. However, efforts to develop in vitro models should continuously be made.

The scientists are deeply concerned about the rational and humane use of animals in research. Ethics committees are functional in many institutes. They are concerned about avoiding unnecessary pain or suffering or injury to animals during holding, experimentation and post-experimental period by monitoring and improving their housing, environment, feeding and veterinary care. The Government of India has authorized the National Accreditation Board of Testing and Calibration Laboratories (NABL), promoted by the Department of Science and Technology, to provide accredition services to laboratories covering a wide range of subjects including biological and clinical laboratories. The NABL is a full member of the International Laboratory Accreditation Cooperation and the Asia Pacific Laboratory Cooperation. Such accreditation of animal facilities would demonstrate their commitment to responsible animal care and use and good science since such an accreditation is an indicator of an institution's ability to comply with its assurances.

In India, the need to develop guidelines for the use of animals in research has been discussed at various forums. Unfortunately no standard document was available for reference till 1992 when the Indian National Science Academy developed the guidelines for use of animals in scientific research. Considering the knowledge generated internationally over the years and the guidelines of WHO, NIH associated NRC, USA and European Union, the INSA guidelines have been updated.

## (a) The Need

The biomedical scientists generally work to unfold the complicated processes of life and to provide new measures for the health and welfare of the society i.e. the humans, the animals and the environment. There is, therefore, need to provide them certain degree of freedom and adequate facilities to use animals wherever necessary. It is evident that certain life processes can not be investigated without involving whole animal system. The in-vitro alternatives can only provide limited information. These cannot totally replace the animals in experiments. This is why the use of animals continues to be mandatory to meet the statutory regulatory requirements. At the same time, it is an obligation of the scientists t ensure that the experiments conducted on animals are rational and unavoidable, and no unnecessary pain or injury is inflicted on them and they are maintained in

best possible environmental conditions. It is, therefore, necessary to have well-defined guidelines which will safeguard the pursuit of knowledge, the interest of society and the welfare of animals.

**(b) The Objectives**

To provide guidelines for

1. housing, care, breeding and maintenance of experimental animals to keep them in physical comfort and good health and to permit them to grow, reproduce and behave normally;
2. sources of experimental animals of known genetic, health and nutritional status;
3. development of training facilities for scientists, technicians and other supportive staff for the care of animals and their use in experiments;
4. acceptable experimental techniques and procedures for anesthesia and euthanasia;
5. developing alternate in-vitro systems to replace animal experiments;
6. the constitution of institutional ethics committees, their functions and the legal and ethical obligations to ensure minimal and ethical use of animals.

**© Current Status**

The INSA guidelines are the only ones available at the National level and adopted by well established institutions in India for care and use of their laboratory animals. However, there is need to adopt these in all the research animal facilities. It is therefore essential that these guidelines are accepted as the national guidelines.

## 2. SOURCES OF EXPERIMENTAL ANIMALS

Animals for experiments should be procured by scientists from recognized animal facilities. The animals trapped from the wild, e.g. the monkeys, feral dogs and cats are also used in research as they are readily available and less expensive compared to colony bred animals. These wild and feral animals are generally quarantined and stabilized in animal facility before use in experiments. The health and genetic status of these animals are not known and therefore a careful screening during quarantine is necessary. The wild and feral animals should be acquired after due clearance from Institutional Animal Ethics Committees and through certified suppliers.

The only authentic source of getting right type of animals for research should be from recognized scientific animal facilities where the animal colonies of known genetic and health status are available. Such animals only can provide reliable results. The scientists should therefore insist upon getting defined animals through organized colonies eliminating unscrupulous traders, which not only supply poor stock of animals but also maintain these animals under most unethical and unhygienic conditions. A list of Scientific Institutions which maintain recognized animal strains is given in Annexure-1. A directory of animal species and strains available with each of these institutions should be prepared and circulated. A list of ‘Physiological Norms of Commonly Used Laboratory Animals’ and ‘Reproductive Data of Commonly Used Laboratory Animals’ is given in Annexure 2 and 3 respectively.

## 3. LABORATORY ANIMAL HUSBANDRY AND MANAGEMENT

### (i) Housing and Environment

Laboratory animals are very sensitive to their living conditions. It is important that they are housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds. The building, cages and environment of animal rooms are the major factors which affect the quality of animals.

In planning an animal facility the space should be well divided for various activities. The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores (8-10%), washing (8-10%), office and staff (8-10%), machine rooms (4-5%) quarantine and corridors (12-15%).

The cages should be made of suitable metal (stainless steel, galvanized iron sheet/rods) or synthetic material (polypropylene/polycarbonate). They should be of suitable size for each species of animal and should have adequate arrangement for feeding and watering. They must be free from crevices, corners and sharp edges for easy cleaning and to avoid injury. The bedding should be of right material and sterilized before use. Common bedding materials used in India are paddy husk, saw dust, paper currings, dry grass and crushed corn cobs.

The environment of animal room (Macro-environment) and animal cage (Micro-environment) is an important factor on which the production and experimental efficiency of the animal depends. Since animals are very sensitive to environmental changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided. The recommended environmental requirements for animal rooms, for different species are given in Annexure-4.

A constant room temperature is essential, because variation in room temperature causes change in food and water intake. A charge in temperature of 4°C can cause 10-fold alteration in biological responses. The temperature also affects fertility and lactation. Coupled with high humidity the increase in temperature causes ammonia built up. If the ventilation is not proper the high ammonia concentration causes respiratory irritation to both animals and attendants, predisposing them to infection by lowering their resistance. An effective ventilation system with 10-12 air changes per hour of 100% fresh air must be provided for animal rooms.

Light and sound are other important factors. The light intensity, the wave length and the photo cycle affect the health and behaviour of the animals. Sudden and sharp sounds in the animal rooms disturb the health and behaviour of animals and may give rise to ear damage, hypertension, cannibalism, etc.

### (ii) Breeding and Genetics

For initiating a colony, the breeding stock must be procured from a reliable/accredited source, ensuring that genetic make-up and health status of animals is known. In case of an inbred strain, the characters of the strain with their gene distribution and the number of inbred generation must be known for further propagation. The health status should indicate their origin, e.g. conventional,

specific pathogen free or transgenic gnotobiotic or knock-out stock. The known nutritional status and feeding habits of the stock are also of advantage.

The animal colonies may be inbred or outbred. In case of inbred colonies the number of generation of brother x sister mating and latest genetic monitoring parameters for various markers should be known. Mutations or genetic contamination can be detected by using screening methods, such as histocompatability (skin grafting), biochemical markers, coat color studies, mandibular biometry or immunological studies. Phenotypic visual characters may also sometime provide clue of genetic contamination (Annexure-5).

**(iii) Transgenic/Knock-out Animals**

Transgenic animals are those animals into whose germ line foreign gene(s) have been engineered, whereas knock-out animals are those whose specific gene(s) have been disrupted leading to loss of function. These animals can be bred to establish transgenic animal strains. Transgenic animals are used to study the biological functions of specific genes, to develop animal models for diseases of humans or animals, to produce therapeutic products, vaccines and for biological screening, etc. These can be either developed in the laboratory or procured fpr R&D purpose from scientific/academic institution or commercial firms, generally from abroad.

Those laboratories developing transgenic animals should pay special attention to the following points :-

1. Photoperiod is a critical regulator of reproductive behaviour of many species of animals. Inadvertent light exposure during the dark cycle should be minimized or avoided. A time controlled lighting system should be used to ensure regular diurnal cycle.
2. Embryo transfer has to be carried out using anesthetics.
3. Pseudopregnant females which receive embryos should be kept in separate rooms where there is no disturbance.
4. Bedding, feed, water or cage should not be changed for about 3-4 days after embryo transfer as at this stage there is high risk of embryo resorption and termination of pregnancy.
5. The bedding changes and handling of the female should be carried out by skilled caretaker till delivery and weaning is over.
6. A high protein diet should be given to the lactating mother.

Maintenance : Housing, feeding, ventilation, lighting, sanitation and routine management practices for such animals are similar to those for the other animals of the species as given in the guidelines. However, special care has to be taken with transgenic/gene knock-out animals where the animals can become susceptible to diseases or where special conditions of maintenance are required due to the altered metabolic activities. The transgenic and knock out animals carry additional genes or lack genes compared to the wild population of the species, and therefore to avoid the spread of the genes in wild population, neither they should inadvertently cross breed with other animals or be released in the wild. Special care should be taken to maintain those animals which have been genetically manipulated to produce models for diseases of humans or other animals. The transgenic and knock-out animals should be maintained in clean room environment or in animal isolators.

Disposal : The transgenic and knock-out animals should be first euthanasised and then disposed off as prescribed elsewhere in the guidelines. A record of disposal and the manner of disposal should be kept as a matter of routine.

The transgenic and knock-out animals need greater level of monitoring than other animals as transgenes might have unexpected effect on the phenotype and its interaction with the environment.

**(iv) Nutrition and Feeding**

The results of an experiment are likely to be influenced by co-existence of nutritional deficiencies and imbalance. It is, therefore, essential that laboratory animals are maintained on a balanced diet based on nutritional requirements of each species. Special care is needed on nutritional elements, ingredients used in diet, and feeding practices. A balanced diet should contain protein, carbohydrates, fat, minerals, vitamins, roughage and water in required proportions for each species of animal. These requirements for commonly used species are given in Annexure-6.

Only quality ingredients should be used in a diet and they should be free from dust, moulds, fungi and other contaminants. Each animal must get required quantity of feed, based on animal maintenance and production requirements.

The feed should be palatable so that it is consumed in adequate quantity by the animals. Any undesirable odor always causes under consumption resulting in nutritional deficiency in the animals.

No drug, hormone or antibiotic should be added in the feed as these are likely to disturb the normal metabolism of the animals and produce biased results.

The ingredients and the prepared feed must be stored and handled carefully so as to avoid any contamination. The food must be of right consistency and should be presented to animals in proper type of hoppers to avoid wastage. In some cases the feed may be divided in 2-3 meals during the day.

Pelleted feeds balanced for different species of animals are now available commercially. These are easy to procure and use without wastage. However one has to be careful on quality of the feed from batch to batch. It should be obligatory on manufacturer to mark each bag with the type of food, date of manufacture, the batch number, the ingredients used and chemical composition. Random chemical analysis must be carried to for major nutrients to monitor the quality of food from time to time.

Clean, chlorinated water should be available to the animals ad lib.

**(v) Hygience and Disease Control**

The building for housing the animals should be provided with barriers to control the entry of contamination into the building through men, material and wild animals. Strict barriers should be

provided to avoid the entry of wild rodents, birds, insects and pests. Visitors and service staff should be allowed entry with care and when necessary.

On the exit side an efficient monitoring service should be established to monitor the prevalence of any infection in the colony. A regular medical check up of the staff, postmortem of dead and sacrificed animals and screening of waste material of the rooms are essential.

**(vi) Personnel and Training**

The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility.

The staff must be provided with all required protective clothing (masks, aprons, gloves, gumboots, etc.) while working in animal rooms. Facilities should be provided for change over with lockers, wash basins, toilets and bathrooms to maintain personal hygiene. It is also important that a regular medical check-up is arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the animals. He should ensure that persons working in animal house don't eat, drink, smoke in animal room and have all required vaccination, particularly against tetanus and other zoonoses.

Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning of cages and importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine check up of the cages.

At national level suitable training programmes should be organized by the National Centres to provide training in care, breeding, management, handling of animals for the staff working in animal breeding and holding units. Orientation training programmes should also be initiated for the investigators working in different areas to acquaint themselves with various experimental techniques. Such a course should address the undermentioned topics :

a) biology and husbandry of laboratory animals
b) genetic make-up
c) microbiology and diseases
d) health-hazards in the animal house
e) anesthesia, analysis and experimental procedures
f) alternatives to animal use
g) ethical aspects and legislation

The training courses and workshops may also be organized for the senior level biological scientists to evoke awareness among them about the use of animals in research, alternatives available and the ethical and legal provisions in regard to use of animals. This is particularly important care and management for the supervisory staff and veterinarians exists in the country.

The national level training programmes of following type are essentially required.

| | Training level | Qualification | Duration | Course contents |
|---|---|---|---|---|
| 1. | Technician level | Matriculate | 6-12 weeks | Basics |
| 2. | Supervisory level | Graduate | 12-24 weeks | Comprehensive |
| 3. | Scientist level | Veterinary or medical Graduate/ Post-Graduate in Natural Sciences | 8-12 weeks | Specialised |

**(vii) Records and Evaluation**

Good quality animals are those which are free from disease. Animal of a specified strain should also have all the characteristics of that strain, i.e. they should be genetically anthesised. The results of regular monitoring of parameters of genetic purity must be scrupulously recorded.

Proper record-keeping is extremely important and vital for an animal facility. The forms should be simple but complete and preferably computer compatible. Too exhaustive and unnecessary recording should be avoided as these are not useful. Records of breeding and experimentation and deaths of all experimental animals at various stages are essential.

Receipt and issue of food and other stores should be recorded. Log books of various machines such as incinerator, boilers, air-conditioning plant should be maintained. Monthly and annual reports of the activities should be prepared and reviewed for evaluation of work and future planning.

**(viii) Experimentation and Veterinary Care**

The experimental animal units should generally be looked after by qualified investigators. These units must have adequate housing and technical facilities for experiment and post-operative care. The equipment provided in the experimental unit should be appropriate for the needs of the experiments. No technique should be used which may cause avoidable discomfort to the animals. The post-operative holding rooms and cages should be comfortable and such animals should remain under the care and supervision of an experienced scientist or a qualified veterinarian.

The person actually incharge of animal facility should preferably be a veterinarian or a person qualified in laboratory animal science. In any case an experienced veterinarian must be readily available in an animal holding for health care, monitoring, diagnosis and treatment of diseases and injuries. A veterinarian could also be helpful to investigators in animal anaesthesia and surgery.

## 4. TRANSPORT OF LABORATORY ANIMALS

The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are, the mode of transport, the containers, the

animal density in cages, food and water during transit, protection from transit infections, injuries and stress.

The mode of transport of animals depends on the distance, seasonal and climatic conditions and the species of animals. Animals can be transported by road, rail or air taking into consideration above factors. In any case the transport stress should be avoided and the containers should be of an appropriate size so as to enable these animals to have a comfortable, free movement and protection from possible injuries. The food and water should be provided in suitable containers or in suitable form so as to ensure that they get adequate food and more particularly water during transit. The transport containers (cages or crates) should be of appropriate size and only a permissible number of animals should only be accommodated in each container to avoid overcrowding and infighting. Requirements of space in transport cages for each species of animals are given in Annexure-7.

## 5. ANAESTHESIA AND EUTHANASIA

The scientists should ensure that the procedures which are considered painful are conducted under appropriate anaesthesia as recommended for each species of animals (Annexure-8). It must also be ensured that the anaesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the experiment. If at any stage during the experiment the investigator feels that he has to abandon the experiment or he has inflicted irreparable injury, the animal should be sacrificed by overdose of anaesthetic. Neuromuscular blocking agents must not be used without adequate general anaesthesia.

In the event of a decision to sacrifice an animal on termination of an experiment or otherwise an approved method of euthanasia (Annexure-9) should be adopted and the investigator must ensure that the animal is clinically dead before it is sent for disposal.

### (a) Anaesthesia

Unless contrary to the achievement of the results of study sedatives, analgesics and anaesthetics should be used to control pain or distress under experiment. Anaesthetic agents generally affect cardiovascular, respiratory and thermo-regulatory mechanism in addition to central nervous system.

Before using actual anaesthetic the animal is prepared for anaesthesia by over night fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardio-pulmonary system and reduce salivary secretion. Atropine is most commonly used anti-cholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure. Anaesthetic agents used for common species of laboratory animals and their doses are given in Annexure-8.

Local Anaesthesia : Local anaesthetics are used to block the nerve supply to a limited area and are used only for minor and rapid procedures. This should be carried out under expert supervision for regional infiltration of a surgical site, nerve blocks and for epidural and spinal anaesthesia.

General Anaesthesia : A number of agents are used as inhalants. General anaesthetics are also used in the form of intravenous or intra-muscular injections such as barbiturates. Species characteristics and variation must be kept in mind where using an anaesthetic. Side-effects such as excessive

salivation, convulsions, excitement and disorientation should be suitably prevented and controlled. The animal should remain under veterinary care till it completely recovers from anaesthesia and post operative stress.

**(b) Euthanasia**

Euthanasia means "easy death" and is resorted to in events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly is an atmosphere free from fear or anxiety. For accepting of an euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal and number of animals to be sacrificed. The method should in all cases meet the following requirements :-

(a) Death, without causing anxiety, pain or distress with minimum time lag phase.
(b) Minimum physiological and psychological disturbances.
(c) Compatibility with the purpose of study and minimum emotional effect on the observer and operator.
(d) Location should be separate from animal rooms and free from environmental contaminations.
(e) Method should be reliable, reproducible and safe to the personnel involved.
(f) Simple and economical.

It is recommended that tranquilizers be administered to larger species such as monkeys, dogs and cats before an euthanasia procedure.

A number of euthanasia methods have been recognized humane which could be physical, use of inhalant gases, injectable drugs and general anaesthetics in heavy dose. The methods recognized as appropriate for a commonly used species of animal have been listed in Annexure-9.

## 6. DISPOSAL OF ANIMAL CARCASSES

All animal carcasses whether healthy, infectious or radioactive, must be packed in polythene bags before sending them for disposal. All healthy or infectious animals may be buried deep in the ground covered with lime and disinfectants or burnt in an incinerator. Animals with radioactive material should be packed in double polythene bags and dumped in a special pit meant for this purpose. Details of the pit can be obtained from Bhabha Atomic Research Centre, Mumbai. Strict precautions should be taken to safeguard the health of the personnel handling infectious and radioactive material and in no case these should be brought out in open containers for disposal.

The investigators working with infectious and radioactive material must ensure that the animals are properly disposed off on termination of their experiment and that infection is not transmitted to other animals in the room or to the personnel involved in handling such animals during the experiment and also at the time of disposal. The staff handling such animals for disposal must be apprised of the hazards involved in their job and protective clothing, gloves and mask must be provided to them foro their personal safety.

## 7. LABORATORY ANIMAL ETHICS

All scientists working with laboratory animals must have a deep ethical consideration for the animals they are dealing with. From the ethical point of view it is important that such considerations are taken care at the individual level, at institutional level and finally at the national level.

Individually each investigator has an obligation to abide by all the ethical guidelines laid down in this regard at institutional level. The Head of the Institution, maintaining animals for scientific experiments, should constitute an Animal Ethics Committee for experimentation to ensure that all experiments conducted on animals are rational, do not cause undue pain or suffering to the animals and only minimum number of animals are used. The constitution and terms of reference of the Animal Ethics Committee should be well defined.

An Animal Ethics Committee should include : a senior biological scientist of the Institute, two scientists from different biological disciplines, a veterinarian involved in care of animals, the scientist incharge of animal facility, a scientist from outside the institute, a non-scientific socially aware member and a member or nominee of appropriate regulatory authority of Govt. of India. A specialist may be co-opted while reviewing special projects using hazardous agents such as radioactive substance and deadly micro-organisms etc. The investigator may also be called in for any clarification, if required.

The Animal Ethics Committee has to examine all projects involving use of animals before implementation, to ensure that minimum number of animals is used in the project and the ethical guidelines are strictly adhered to. It will also examine that the scientists and technicians handling animals possess adequate skill to perform the experiment. All animals will be maintained under standard living conditions and experiments will be conducted with care. All invasive experiments will be conducted under proper anaesthesia and on termination of an experiment, the animal will be humanely sacrificed under anaesthesia. Before disposal it must be ensured that the animal is clinically dead.

### (a) Ethical Guidelines for Use of Animals in Scientific Research

1. Animal experiments should be undertaken only after due consideration of their relevance for human or animal health and the advancement of knowledge.
2. The animals selected for an experiment should be of an appropriate species and quality, and minimum number should be used to obtain scientifically and statistically valid results.
3. Investigators and other personnel should treat animals with kindness and should take proper care by avoiding or minimizing discomfort, distress or pain.
4. Investigators should assume that all procedures which would cause pain in human beings may cause pain in other vertebrate species also (although more needs to be known about the perception of pain in animals).
5. Procedures that may cause more than momentary pain or distress should be performed with appropriate sedation, analgesia or anaesthesia in accordance with accepted

veterinary practice. Surgical or other painful procedures should not be performed on unanaesthetized animals.

6. At the end of, or when appropriate during an experiment, the animal that would otherwise suffer severe or chronic pain, distress, discomfort, or disablement that cannot be relieved or repaired should be painlessly killed under anaesthesia.
7. The best possible living condition should be provided to animals used for research purpose. Normally the care of animals should be under the supervision of a veterinarian or a person having adequate experience in laboratory animal care.
8. It is the responsibility of the investigator to ensure that personnel conducting experiment on animals possess appropriate qualifications or experience for conducting the required procedures. Adequate opportunities have to be provided by the institution for inservice training for scientific and technical staff in this respect.
9. In-vitro systems to replace or reduce the number of animals should be used wherever possible.

**(b) In-vitro Systems to Replace Animals**

A number of in vitro systems can be used to reduce/replace animals in experimentation. These systems could be the living or the non-living systems. The living systems are tissue and organ culture, lower animals and microorganisms and human volunteers in restricted cases. The non-living systems could also be used in place of animals in certain areas and these include chemicals, mechanical models, mathematical models, computer simulation, DNA recombinant technology and synthetic substances.

## 8. LEGAL PROVISION

The Prevention of Cruelty to Animal Act of 1960 has provided the constitution of a committee under the Animal Welfare Board to control and supervise experiments on animals. It has also provided inspection of all animal holdings through designated inspectors and the institutions not abiding by the standard requirements can be prosecuted. The relevant sections of the act from Chapter IV are given below :-

**THE PREVENTION OF CRUELTY TO ANIMALS ACT, 1960 (59 OF 1960)**
**As amended upto 30th July, 1982**

**CHAPTER IV**

**EXPERIMENTATION OF ANIMALS**

14. Nothing contained in this Act render unlawful the performance of experiments (including experiments involving operations) on animals for the purpose of advancement by new discovery of physiological knowledge or of knowledge which will be useful for saving or for prolonging life or alleviating suffering or for combating any disease, whether of human beings, animals or plants.

15. (1) If at any time, on the advice of the board, the Central Government is of opinion that it is necessary so to do for the purpose of controlling and supervising experiments on animals, it may, by notification in the official Gazette, constitute a Committee consisting of such number of officials and non-officials, as it may think fit to appoint thereto.

(2) The Central Government shall nominate one of the members of the committee to be its Chairman.

(3) The Committee shall have power to regulate its own procedure in relation to the performance of its duties.

(4) The funds of the committee shall consist of grants made to it from time to time by the Government and of contributions, donations, subscription, bequests, gifts and the like made to it by any person.

15.A(1) The committee may constitute as many sub-committees as it thinks fit for exercising any power or discharging any duty of the committee or for inquiring into or reporting and advising on any matter which the committee may refer.

(2) A sub-committee shall consist exclusively of the members of the committee.

16. Subject to the control of the Central Government, the committee may appoint such number of officers and other employees as may be necessary to enable it to exercise its powers and perform its duties, and may determine the remuneration and other terms and conditions of service of such officers and other employees.

17.(1) It shall be the duty of the committee to take all such measures as may be necessary to ensure that animals are not subjected to unnecessary pain or suffering before, during or after the performances of experiments on them, and for that purpose it may, by notification in the Gazette of India and subject to the condition of previous publication make such rules as it may think fit in relation to the conduct of such experiments.

17.1(A) In particular, and without prejudice to the generality of the foregoing power, such rules may provide for the following matters, namely :

(a) The registration of persons or institutions carrying on experiments on animals.
(b) The reports and other information which shall be forwarded to the committee by persons and institution carrying on experiments on animals.

(2) In particular, and without prejudice to the generality o the foregoing power, rules made by the committee shall be designed to secure the following objects namely :

(a) That in cases where experiments are performed in any institution, the responsibility therefore is placed on the person in charge of the institution and that, in cases where experiments are performed outside an institution by individuals, the individuals are qualified in that behalf and the experiments are performed on their full responsibility.

(b) That experiments are performed with due care and humanity, and that as far possible experiments involving operations are performed under the influence of some anaesthetic agent of sufficient power to prevent the animals feeling pain.
(c) That animals which, in the course of experiments under the influence of anaesthetics, are so injured that their recovery would involve serious suffering, are ordinarily destroyed while still insensible.
(d) That experiments on animal are avoided wherever it is possible to do so, as for example, in medical schools, hospitals, colleges and the like, if other teaching devices such as books, models, films and the like may equally suffice.
(e) That experiments on larger animals are avoided when it is possible to achieve the same results by experiments upon small laboratory animals like guinea-pigs, rabbits, frogs and rats.
(f) That, as far possible, experiments are not performed merely for the purpose of acquiring manual skill.
(g) That animals intended for the performance of experiments are properly looked after both before and after experiments.

(3) In making any rules under this section, the Committee shall be guided by such directions as the Central Government (consistently with the objects for which the Committee is set up) may give to it, and the Central Government is hereby authorized to give such directions.

(4) All rules made by the committee shall be binding on all individuals performing experiments outside institutions and on persons in charge of institutions in which experiments are performed.

18. For the purpose of ensuring that the rules made by it are being complied with, the committee may authorize any of its officers or any other person in writing to inspect any institution or place where experiments are being carried on and report to it as a result of such inspection and any officer or person so authorized may :

(a) enter at any time considered reasonable by him and inspect any institution or place in which experiments on animals are being carried on, and
(b) require any person to produce any record kept by him with respect to experiments on animals.

19. If the Committee is satisfied, on the report of any officer or other person made to it as a result of any inspection under section 18 or otherwise, that the rules made by it under section 17 are not being complied with by any person or institution carrying on experiments on animals, the committee may, after giving an opportunity to the person or institution of being heard in the matter, by order, prohibit the person or institution from carrying on any such experiments either for a specified period or indefinitely, or may allow the person or institution to carry on such experiments subject to such special conditions as the Committee may think fit to impose.

20. If any person :

(a) contravenes any order made by the committee under section 19, or
(b) commits a breach of any condition imposed by the Committee under that section.

He shall be punishable with fine which may extend to two hundred rupees, and when the contravention or breach of condition has taken place in any institution, the person in charge of the institution shall be deemed to the guilty of the offence and shall be punishable accordingly.

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## *Annexure-1*

## LIST OF MAJOR INSTITUTIONS MAINTAINING ANIMAL STRAINS

**(A) National Level Facilities of Laboratory Animals :**

1. National Laboratory Animal Centre, Central Drug Research Institute, Lucknow.
2. National Centre for Laboratory Animal Sciences (NCLAS) National Institute of Nutrition, Hyderabad

**(B) Institutional Laboratory Animal Facilities at :**

(i) Research/Academic Institutions :

All India Institute of Medical Sciences, New Delhi
Cancer Research Institute, Mumbai
Central Food Technology Research Institute, Mysore
Central Research Institute, Kassauli (H.P.)
Centre for Cellular and Molecular Biology, Hyderabad
Haffkine Bio-Pharmaceutical Corporation, Mumbai
Haryana Agricultural University, Hissar
Indian Institute of Science, Bangalore
Indian Veterinary Research Institute, Izatnagar
Institute of Microbial Technology, Chandigarh
Institute for Research in Reproduction, Parel, Mumbai
Indian Institute of Chemical Biology, Calcutta
National Institute of Communicable Diseases, New Delhi
National Institute of Immunology, New Delhi
National Institute of Virology, Pune
Post Graduate Institute of Medical Education and Research, Chandigarh
Regional Research Laboratory, Jammu Tawi
National Centre for Cell Science, Pune

(ii) Industrial Institutions :

Indian Drugs and Pharmaceuticals Ltd., Hyderabad
Indian Drugs & Pharmaceuticals Ltd, Rishikesh
Indian Immunologicals, Hyderabad
Hoechst Pharmaceuticals Ltd, Bombay
Sarabhai Chemicals Research Centre, Baroda
Ranbaxy Research Laboratory, New Delhi
Reddy Research Laboratory, Hyderabad

## *Annexure-2*

### Physiological Norms of Commonly Used Laboratory Animals

| | **Mouse** | **Rat** | **Hamster** | **G.Pig** | **Rabbit** | **Cat** | **Dog** (Beagle) | **Monkey** (Rhesus) |
|---|---|---|---|---|---|---|---|---|
| **Weight at Birth(grams)** | **1-2** | **4-5** | **2-3** | **80-100** | **40-60** | **100-130** | **400-500** | **460-500** |
| **Age at Weaning (weeks)** | **3** | **3** | **3** | **3** | **8** | **4-6** | **6-8** | **20-24** |
| **Wt. At Weaning (grams)** | **9-12** | **40-50** | **30-40** | **250-300** | **800-900** | **400-700** | **-** | **400-700** |
| **Age at Maturity W=weeks Y=Years** | **6-8w** | **10-12w** | **6-8w** | **16-20w** | **24-32w** | **30-35w** | **1-1.2y** | **4-5y** |
| **Wt. at Maturity** | **18-22g** | **150-200g** | **80-90g** | **250-400g** | **1.5-2.0kg** | **4-6kg** | **15-25kg** | **9-10kg** |
| **Adult Weight** | **25-30g** | **200-300g** | **80-100g** | **400-500g** | **2.0-2.5kg** | **3-5kg** | **12-15kg** | **10-12kg** |
| **Rectal Temp0C(Average)** | **37.4** | **37.5** | **37.6** | **38.6** | **38.7** | **39.5** | **38.6** | **38.4** |
| **Respiratory Rate per minute** | **90-180** | **80-150** | **40-120** | **60-110** | **35-56** | **20-30** | **14-28** | **30-54** |
| **Pulse Rate per minute (Average)** | **600** | **300** | **450** | **150** | **133** | **110** | **95** | **200** |
| **Life span (Years)** | **1.5-2.0** | **2.5-3.0** | **1.5-2.0** | **4-5** | **4-5** | **8-12** | **10-15** | **15-20** |
| **Diploid Chromosome number 2n=** | **40** | **42** | **44** | **64** | **44** | **38** | **78** | **42** |

Source : Bibliography : Ref.Nos.1,2,3,4,7,8,13,15,16,18,20,27,28,30,31

## *Annexure-3*

**Reproductive Date of Commonly Used Laboratory Animals**

| | **Mouse** | **Rat** | **Hamster** | **G.Pig** | **Rabbit** | **Cat** | **Dog** (Beagle) | **Monkey** (Rhesus) |
|---|---|---|---|---|---|---|---|---|
| **Oestrus Cycle(days)** | **4-5** | **4-5** | **4-5** | **16** | | **14** | **Biannual** | **28** |
| **Duration of Oestrus** | **10h** | **13-15h** | **20h** | **6-11h** | **-** | **3-6d** | **14-21d** | **-** |
| **Time of Ovulation h=hour da=day** | **2-3 h after Est.** (spont.) | **8-10 h** (spont.) | **8-10h** (spont.) | **10h** (spont.) | **Induced10-11 h** after mating | **Induced25-26 h** after mating | **1-3 d** (spont.) | **11-14d after on set of menstruation** |
| **Gestation Period (days) average** | **21** | **21** | **16** | **68** | **30** | **63** | **62** | **164** |
| **Litter size** | **6-10** | **8-12** | **5-8** | **1-4** | **4-6** | **3-6** | **4-8** | **1** |
| **Oestrus after parturition** | **Post partum** | **Post partum** | **1-8d** | **Post partum** | **35d** | **4$^{th}$ week or lactation** | **Next neat season** | **After weaning of young ones** |
| **Reproductive Life span (years)** | **1** | **1** | **1** | **3-4** | **2-3** | **6** | **6-8** | **12-15** |
| **Mating System M:F ratio** | **Pair/Trio/** Harem | **Pair/ Harem** | **Pair Harem** | **Harem** | **Hand Mating** | **Harem** | **Pair/ Harem** | **Pair /Harem** |
| **Max. number Of females per male** | **5** | **5** | **5** | **6** | **10** | **6** | **6** | **10** |
| **Mammary Glands (T.A.P.) no. Of pairs** | **3,1,1** Five | **3,1,2** Six | **-,5,1** Six | **-,-,1** One | **1,2,1** Four | **2,2,-** Four | **2,2,1** Five | **1,-,-** One |

A=Abdominal, P=Pelvic, T=Thoracic, h=hours, d=days = Menstrual Cycle

Source : Bibliography : Ref.Nos.1,2,4,7,13,15,16,17,18,19,20,26,27,28,30,31

## *Annexure-4*

**Housing and Environmental Requirements for Commonly used Laboratory Animals**

| | **Mouse** | **Rat** | **Hamster** | **G.Pig** | **Rabbit** | **Cat** | **Dog** (Beagle) | Rhesus | Bonnet | Langur |
|---|---|---|---|---|---|---|---|---|---|---|
| Avg. Adult weight | 25-30g | 200-300g | 80-100g | 400-500g | 2.0-2.5kg | 3-5 kg | 12-15kg | 3.5-8kg | 3.5-8kg | 6-15kg |
| Type of Housing | Cage | Cage | Cage | Cage/Pan | Cage | Cage/Run | Run | Cage | Cage | Cage |
| Floor Area per Animal (sq.cm) | 65-100 | 100-150 | 90-120 | 300-600 | 3700-4600 | 2500-3500 | 7000-12000 | 4000-6000 | 4000-6000 | 6000-9000 |
| Cage height Minimum(cm) | 12 | 14 | 12 | 18 | 36 | 36 | - | 72 | 72 | 90 |
| Room Temp$^{o}$C | 22-24 | 22-24 | 22-24 | 22-24 | 22-24 | Air dried | Air dried | Air dried | Air dried | Air dried |
| Relative Humidity(%) | 45-60 | 50-60 | 45-60 | 45-60 | 45-60 | 45-60 | 45-60 | 45-60 | 45-60 | 45-60 |
| Suitable Bedding Material | Paddy husk saw dust | Paddy husk saw dusk | Paddy husk shredded paper | Paddy husk Saw dust | - | - | - | - | - | - |
| Nesting Material | Paper cutting | Paper cutting | Paper cutting | Wood shavings dry grass | Cotton paper cutting dry grass | Cotton paper cutting | Fine jute paper cutting | - | - | - |
| Ventilation Air changes per hour | 10-12 | 10-12 | 10-12 | 10-12 | 10-12 | Continuous | Continuous | Continuous | Continuous | Continuous |
| Light Intensity (LUX) | 300-400 | 300-400 | 300-400 | 300-400 | 300-400 | 300-400 | 300-400 | 300-400 | 300-400 | 300-400 |
| Photocycle (Light :Dark) | 12:12 | 12:12 | 12:12 | 12:12 | 12:12 | 12:12 | 12:12 | 12:12 | 12:12 | 12:12 |

## *Annexure-5*

## GENETIC MONITORING

Genetic monitoring of the animals is necessary particularly among the inbred and special strains to ensure genetic homogenicity of desired characters in a particular strain. The inbred strains particularly need such monitoring at regular time interval to ensure freedom from genetic contamination. The different methods, used in detecting the status of inbred strains for their homogenicity are:

1. Histocompatibility or Skin grafting : It is a very convenient and reliable method for routine testing of all inbred strain against genetic contamination. Skin grafts exchanged among members of the same inbred strain or F1 hybrid or from either parent to an F1 hybrid should be accepted.
2. Electrophoresis or Biochemical Markers : Inbred strains differ at many genetic loci. Some of these loci code for proteins and enzymes that may be identified by a number of biochemical techniques, the most important of which is electrophoresis. Typically, the samples of body fluids or organ homogenates are electrophorosed on starch cellulose acetate or polyacrilamide gels with specified buffer systems and under controlled conditions. After an appropriate time the gels are stained.
3. Immunological Markers : Inbred strains differ in the alloantigens that they carry and these immunological markers can be used effectively in routine genetic quality control. For immunological markers, there are two basic methods for demonstrating allo-antibodies. The first is the haemagglutination technique in which the erythrocytes are the test cells. The second is the cytotoxic test in which the lymphocytes are typically the test cells.
4. Coat Colour Studies : The coat colour genes carried by most inbred strains of mice and many inbred strains of rats are known. Some pigmented strains have an unusual or unique coat colour, which may be sufficient both to type the strain and to serve as an indicator in the event of genetic contamination. A cross with virtually any other strain would result in offspring that would not have the parental coat colour, and the occurrence of coat colour variants would immediately suggest genetic contamination.
5. Mandibular biometry : Strain differences in the morphology of the skeleton have been known for many years. One method of genetic quality control using skeleton morphology is based on mandible shape. The technique is highly sensitive. All strains seem to differ in mandible shape and even closely related sublines can be distinguished, by measuring different parameters on the mandibles of 10-15 random animal samples taken from the group.

## *Annexure-6*

**Nutritional Requirements of Common Laboratory Animals**

| | Mouse | Rat | Hamster | Guineapig | Rabbit | Cat | Dog | Monkey |
|---|---|---|---|---|---|---|---|---|
| Protein(%) | 18.00 | 12.00 | 15.00 | 18.00 | 17.00 | 30.00 | 20.00 | 15.00 |
| Fat(%) | 5.00 | - | 5.00 | 1.00 | 2.00 | 9.00 | 4.50 | - |
| Linoleic acid(%) | 0.30 | 0.60 | - | - | - | 1.00 | 0.90 | 1.00 |
| Fiber(%) | 5.00 | - | - | 10.00 | 10-12 | - | - | - |
| Digestible energy (Kcal) | 3000 | 3800 | 4200 | 3000 | 2500 | - | - | 100 |
| **Vitamins** | | | | | | | | |
| A(1U/kg) 15000 | 500 | 4000 | 3636 | 23333 | 580 | 25000 | 4500 | 10000- |
| D(1U/kg) | 150 | 1000(e) | 2484 | 1000 | - | 1000 | 450 | 2000 |
| E (1U/kg) | 20 | 30(f) | 3 | 50 | 40 | 120 | 45 | 50 |
| K1(1U/kg) | 3000 | 50(g) | 4000 | 2500 | - | - | - | t |
| C(mg/kg) | t | t | t | 200 | t | t | t | 100 |
| Biotin(mg/kg) | 0.20 | t | 0.60 | 0.30 | - | - | 0.90 | 0.10 |
| Choline(mg/kg) | 600 | 1000 | 2000 | 1000 | 1200 | 3000 | 1100 | - |
| Folic acid(mg/kg) | 0.50 | 1.00 | 2.00 | 4.00 | - | 1.00 | 0.16 | 0.20 |
| Niacin(mg/kg) | 10.00 | 20.00 | 90.00 | 10.00 | 180.00 | 4.00 | 10.30 | 50.00 |
| Pantothenic acid | 10.00 | 8.00 | 40.00 | 20.00 | - | 5.00 | 9.00 | 15.00 |
| Riboflavin | 7.00 | 3.00 | 15.00 | 3.00 | - | 4.00 | 2.00 | 5.00 |
| Thaimine | 5.00 | 4.00 | 20.00 | 2.00 | - | 5.50 | 0.90 | - |
| VitaminB6 | 1.00 | 6.00 | 6.00 | 3.00 | 39.00 | 4.00 | 0.90 | 2.50 |
| VitaminB12 (mg/kg) | 10.00 | 50.00 | 10.00 | 10.00 | - | - | 20.00 | t |
| **Minerals** | | | | | | | | |
| Calcium(%) | 0.40 | 0.50 | 0.60 | 0.8 | 0.75 | 1.00 | 1.00 | 0.50 |
| Chloride(%) | t | 0.50 | - | - | 0.30 | 1.00 | 1.00 | 0.2-0.5 |
| Magnesium(%) | 0.05 | 0.04 | 0.06 | 0.10-0.30 | 0.04 | 0.054 | 0.036 | 0.15 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phosphorus(%) | 0.40 | 0.40 | 0.30 | 0.40-0.70 | 0.50 | 0.80 | 0.80 | 0.40 |
| Potassium(%) | 0.20 | 0.36 | 0.61 | 0.50-1.40 | 0.60 | 0.60 | 0.50 | 0.80 |
| Sodium(%) | t | 0.05 | 0.15 | - | 0.20 | - | 1.00 | 0.20-0.40 |
| Sulphur(%) | - | 0.03 | - | - | - | - | - | - |
| Copper(mg/kg) | 4.50 | 5.00 | 1.60 | 6.00 | 3.00 | 7.20 | 6.50 | - |
| Iodine(mg/kg) | 0.25 | 0.15 | 1.60 | 1.00 | 0.20 | 0.54 | 1.39 | 2.00 |
| Iron | 25.00 | 35.00 | 140.00 | 50.00 | 100.00 | 65.00 | 54.00 | 180.00 |
| Manganese (mg/kg) | 45.00 | 50.00 | 3.65 | 40.00 | 8.50 | 5.00 | 4.50 | 40.00 |
| Selenium(mg/kg) | t | 0.10 | 0.10 | 0.10 | - | 0.15 | 0.10 | - |
| Zinc(mg/kg) | 30.00 | 12.00 | 9.20 | 20.00 | 1.00 | 54.00 | 45.00 | 10.00 |
| **L-Amino Acids** | | | | | | | | |
| Arginese(%) | 0.30 | 0.60 | 0.76 | - | 0.60 | - | - | - |
| Histidine(%) | 0.20 | 0.30 | 0.40 | - | 0.30 | - | - | - |
| Isoleucine(%) | 0.40 | 0.50 | 0.89 | - | 0.60 | - | - | - |
| Leucine(%) | 0.70 | 0.80 | 1.39 | - | 1.10 | - | - | - |
| Lysine(%) | 0.40 | 0.70 | 1.20 | - | 0.65 | - | - | - |
| Methionine(%) | 0.50 | 0.60 | 0.32 | - | 0.60 | - | - | - |
| **Phenylalanine+** | | | | | | | | |
| Tyrosine(%) | 0.40 | 0.80 | 0.83 | - | 1.10 | - | - | - |
| Niasine(%) | 0.50 | 0.80 | 0.83 | - | 0.20 | - | - | - |
| Tryptophan(%) | 0.10 | 0.15 | 0.34 | - | 0.20 | - | - | - |
| Water Consumption (ml/day) | 3-7 | 20-45 | 8-12 | 12-15 | 80 –100 | 100-200 | 25-35 | 350-950 |
| Food Consumption (g/per day) | 3-6 | 10-20 | 7-15 | 20-35 | 75-100 | 110-225 | 250-1200 | 350-950 |

- = Not known/No data available

Source : Bibliography : Ref.Nos.5,21,22,23,24,25,27,28,29,30

## *Annexure 7*

### Requirements for Transport of Laboratory Animals by Road, Rail and Air

### Nutritional Requirements of Common Laboratory Animals

| | **Mouse** | **Rat** | **Hamster** | **Guineapig** | **Rabbit** | **Cat** | **Dog** | **Monkey** |
|---|---|---|---|---|---|---|---|---|
| Maximum No. of Animals per Cage | 25 | 25 | 25 | 12 | 2 | 1 or 2 | 1 or 2 | 1 |
| Material used In Transport | Metal Cardboard Synthetic Material | Metal Cardboard Synthetic Material | Metal Cardboard Synthetic Material | Metal Cardboard Synthetic Material | Metal Cardboard Synthetic Material | Metal | Metal | Metal |
| Space per Animal(sq.) | 20-25 | 80-100 | 80-100 | 160-180 | 1000-1200 | 1400-1500 | 3000 | 4000 |
| Minimum height Of Box(cm) | 12 | 14 | 12 | 15 | 30 | 40 | 50 | 48 |

Source : Bibliography Ref.Nos.9,10,11,12,27,30

## *Annexure-8*

**Commonly used Anaesthetic Drugs for Laboratory Animals**

| Drugs (mg/kg) | Mouse | Rat | Hamster | Guineapig | Rabbit | Cat | Dog | Monkey |
|---|---|---|---|---|---|---|---|---|
| Ketamine Hcl | 22-24i/m | 22-24i/m | - | 22-24 | 22-24 | 30i/m | 30/im | 15-40 |
| Pentobarbitone Sodium | 35i/v 50i/p | 25i/v 50i/p | 35i/p | 30-i/v 40i/p | 30i/v 40i/v | 25i/v | 20-30i/v | 35i/v |
| Thiopentone | 25i/v 50i/p | 20i/v 40i/p | 20i/v 40/p | 20i/v 55i/p | 20i/v | 25i/v | 25i/v | 25i/v 60i/p |
| Urethane | - | 0.75i/p | - | 1.5i/p | 1.0i/p,i/v | 1.25i/v 1.50i/p | 1.00i/v | 1.0i/v |

Atropine : Dose 0.02-0.05mg/kg for all species by s/c or i/m or i/v routes used to reduce salivary and bronchial secretions and protect heart from vagal inhibition, given prior to anaesthesia

I/m= intramuscular, i/v = intravenous, i/p = intraperitoneal, s/c = subcutaneous

Source : Bibliography : Ref.Nos.3,14,27,30

## *Annexure-9*

## Euthanasia of Laboratory Animals

**A**. Methods acceptable for species of animals indicated **NR**= Not Recommended

| Species | Mouse | Rat | Hamster | Guinea Pig | Rabbit | Cat | Dog | Monkey |
|---|---|---|---|---|---|---|---|---|
| **a)Physical Methods** | | | | | | | | |
| Electrocution | NR | NR | NR | NR | NR | NR | A | NR |
| Exsanguination | NR | A | NR | A | A | A | A | A |
| Decapitation | A | A | A | NR | A | NR | NR | A |
| Cervical Dislocation | A | A | A | A | A | NR | NR | A |
| **b) Inhalation of Gases** | | | | | | | | |
| Carbon Monoxide | A | A | A | A | A | A | A | A |
| Carbon Dioxide | A | A | A | A | A | A | NR | NR |
| Carbon Dioxide + Chloroform | A | A | A | A | A | A | NR | NR |
| **c) Drug Administration** | | | | | | | | |
| Barbiturate Overdose(route) | A(1P) | A(1P) | A(1P) | A(1P) | A(IV,1P) | A(IV,1P) | A(IV,1P) | A(IV,1P) |
| Chloral hydrate Overdose(route) | NR | NR | NR | NR | A(IV) | A(IV) | A(IV) | A(IV) |
| Ketamine Overdose(route) | A(1M) | A(1M) | A(1M) | A(1M) | A(1M) | A(1M) | A(1M) | A(1M) |

**B. Methods not acceptable for any species of animals**

a) PHYSCIAL METHODS :

(i) Decompression (ii) Stunning

b) INHALATION OF GASES

(i) Nitrogen Flushing (ii) Argon Flushing

c) DRUG ADMINISTRATION

(i) Curariform drugs (ii) Nicotine Sulphate (iii) Magnesium Sulphate (iv) Potassium chloride

(v) Strychnine (vi) Paraquat (vii) Dichlorvos (viii) Air emboism

Source : Bibliography : Ref.Nos.3,27,30