

CPCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY

Committee for the Purpose of Control and Supervision on Experiments on Animals

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Good Laboratory Practices (GLP) for animal facilities is intended to assure quality maintenance and safety of animals used in laboratory studies while conducting biomedical and behavioral research and testing of products.

GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioral research and testing with the basic objective of providing specifications that will enhance animal well-being, quality in the pursuit of advancement of biological knowledge that is relevant to humans and animals.

VETERINARY CARE

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine.

Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour, and well-being is conveyed to the attending veterinarian.

The veterinarian can also contribute to the establishment of appropriate policies and procedures for ancillary aspects of veterinary care, such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

ANIMAL PROCUREMENT

All animals must be acquired lawfully as per the CPCSEA guidelines.

A health surveillance program for screening incoming animals should be carried out to assess animal quality. Methods of transportation should also be taken into account (**Annexure – 4**).

Each consignment of animals should be inspected for compliance with procurement specifications, and the animals should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

QUARANTINE, STABILIZATION AND SEPARATION

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. A minimum duration of quarantine for small lab animals is one week and larger animals is 6 weeks (cat, dog and monkey)

Effective quarantine procedures should be used for non-human primates to help limit exposure of humans to zoonotic infections.

Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic and nutritional stabilization before their use. The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals.

Physical separation of animals by species is recommended to prevent interspecies disease

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transmission and to eliminate anxiety and possible physiological and behavioral changes due to interspecies conflict.

Such separation is usually accomplished by housing different species in separate rooms; however, cubicles, laminar-flow units, cages that have filtered air or separate ventilation, and isolators shall be suitable alternatives.

In some instances, it shall be acceptable to house different species in the same room, for example, if two species have a similar pathogen status and are behaviorally compatible.

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

All animals should be observed for signs of illness, injury, or abnormal behavior by animal house staff. As a rule, this should occur daily, but more-frequent observations might be warranted, such as during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis (**Annexure 1 and 2**).

Unexpected deaths and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious agent (*e.g. Mycobacterium tuberculosis* in non-human primates), the group should be kept intact and isolated during the process of diagnosis, treatment, and control. Diagnostic clinical laboratory may be made available.

ANIMAL CARE AND TECHNICAL PERSONNEL

Animal care programs require technical and husbandry support. Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training to ensure effective implementation of the program (**Annexure – 7**).

PERSONAL HYGIENE

It is essential that the animal care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided *e.g.* showers, change of uniforms, footwears *etc.*

Clothing suitable for use in the animal facility should be supplied and laundered by the institution. A commercial laundering service is acceptable in many situations; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. In some circumstances, it is acceptable to use disposable wear such as gloves, masks, head covers, coats, coveralls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility.

Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics in animal rooms. A separate area or room should be made available for these purposes.

ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

Institutions should have policies governing experimentation with hazardous agents. Institutional Biosafety Committee whose members are knowledgeable about hazardous agents are in place in most of the higher level education, research institutes and in many pharmaceutical industries for safety issues. This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions (**Annexure – 8**).

Since the use of animals in such studies requires special consideration, the procedures and the facilities to be used must be reviewed by both the Institutional Biosafety Committee and Institutional Animal Ethics Committee (IAEC).

MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

Multiple surgical procedures on a single animal for any testing or experiment are not to be practiced unless specified in a protocol only approved by the IAEC.

DURATIONS OF EXPERIMENTS

No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

PHYSICAL RESTRAINT

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

Prolonged restraint of any animal, including the chairing of non-human primates, should be avoided unless essential to research objectives. Less restrictive systems, such as the tether system or the pole and collar system, should be used when compatible with research objectives.

The following are important guidelines for the use of restraint equipments:

Restraint devices cannot be used simply as a convenience in handling or managing animals.

The period of restraint should be the minimum required to accomplish the research objectives.

Animals to be placed in restraint devices should be given training to adapt to the equipment.

Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if lesions or illness associated with restraint are observed. The presence of lesions, illness, or severe behavioral change should be dealt with by the temporary or permanent removal of the animal from restraint.

PHYSICAL PLANT

The physical condition and design of animal facility determine, to a great extent, the efficiency and economy of their operation. The design and size of an animal facility depend on the scope of institutional research activities, animals to be housed, physical relationship to the rest of the institution, and geographic location. A well-planned, properly maintained facility is an important element in good animal care.

PHYSICAL RELATIONSHIP OF ANIMAL FACILITIES TO LABORATORIES

Good animal husbandry and human comfort and health protection require separation of animal facilities from personnel areas such as offices, conference rooms, and most laboratories.

➤ Laboratory animals are very sensitive to their living conditions. It is important that they shall be 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.

➤ This separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should make it possible to place animal housing areas adjacent to or near laboratories, but separated from them by barriers such as entry locks, corridors, or floors.

➤ 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, washing, office and staff, machine rooms, quarantine and corridors. The environment of animal room (macro-environment) and animal cage (microenvironment) are factors 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 space requirements for animal rooms, for different species are given in **(Annexure – 3)**.

FUNCTIONAL AREAS

The size and nature of a facility will determine whether areas for separate service functions are possible or necessary. Sufficient animal area is required to:

- ensure separation of species or isolation of individual projects when necessary
- receive, quarantine, and isolate animals and
- provide for animal housing.

In facilities that are small, maintain few animals or maintain animals under special conditions (e.g., facilities exclusively used for housing germfree colonies or animals in runs and pens) some functional areas listed below could be unnecessary or included

in a multipurpose area. Professional judgement must be exercised when developing a practical system for animal care.

- Specialized laboratories
- Individual areas contiguous with or near animal housing areas for such activities as surgery, intensive care, necropsy, radiography, preparation of special diets, experimental manipulation, treatment, and diagnostic laboratory procedures
- Containment facilities or
- Equipment, if hazardous biological, physical, or chemical agents are to be used
- Receiving and storage areas for food, bedding
- Pharmaceuticals and biologics and supplies
- Space for administration, supervision and direction of the facility
- Showers, sinks, lockers and toilets for personnel
- An area for washing and sterilization of equipment and supplies,
- An autoclave for equipment
- Food and bedding and separate areas
- For holding soiled and unclean equipment
- An area for repairing cages and equipment
- An area to store wastes prior to incineration or removal

PHYSICAL FACILITIES

- (a) **Building materials** should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.
- (b) **Corridor(s)** should be wide enough to facilitate the movement of personnel as well as equipments and should be kept clean.
- (c) **Utilities** such as water lines, drain pipes and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

(d) Animal room doors

Doors should be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

(e) Exterior windows

Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate source of light and ventilation. In primate rooms, windows can be provided.

(f) Floors

Floors should be smooth, moisture proof, nonabsorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants.

They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints.

A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment.

(g) Drains

Floor drains are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces.

(h) Walls and ceilings

Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners.

Surface materials should be capable of withstanding scrubbing with detergents and disinfectants and the impact of water under high pressure.

(i) Storage areas

Separate storage areas should be designed for feed, bedding, cages and materials not in use.

Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste.

(j) Facilities for sanitizing equipment and supplies

An area for sanitizing cages and ancillary equipment is essential with adequate water supply

(k) Experimental area

All experimental procedures in small animals should be carried out in a separate area away from the place where animals are housed. For larger animal functional areas for aseptic surgery should include a separate surgical support area, a preparation area, the operating room or rooms, and an area for intensive care and supportive treatment of animals.

ENVIRONMENT

(a) Temperature and humidity control

Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions or differences in the number and kind of room occupants. Ideally, capability should be provided to allow variations within the range of approximately 18 to 29°C (64.4 to 84.2°F), which includes the temperature ranges usually recommended for common laboratory animals.

The relative humidity should be controllable within the range of 30% to 70% throughout the year. For larger animals a comfortable zone (18 to 37°C) should be maintained during extreme summer by appropriate methods for cooling.

(b) Ventilation

In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures.

Heating, ventilating, and air-conditioning systems should be designed so that operation can be continued with a standby system. The animal facility and human occupancy areas should be ventilated separately.

(c) Power and lighting

The electrical system should be safe and provide appropriate lighting and a sufficient number of power outlets. It is suggested that a lighting system be installed that provides adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals.

Fluorescent lights are efficient and available in a variety of acceptable fixtures.

A time-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required. Emergency power should be available in the event of power failure.

(d) Noise control

The facility should be provided with noise free environment. Noise control is an important consideration in designing an animal facility. Concrete walls are more effective than metal or plaster walls in containing noise because their density reduces sound transmission.

ANIMAL HUSBANDRY

(a) Caging or housing system

The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal well being, meet research requirements, and minimize experimental variables.

The housing system should:

- provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species; **(Annexure – 3)**
- provide a comfortable environment
- provide an escape proof enclosure that confines animal safety
- provide easy acces to food and water;
- provide adequate ventilation
- meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation and reproduction

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- keep the animals dry and clean, consistent with species requirements
- facilitate research while maintaining good health of the animals.

They should be constructed of sturdy, durable materials and designed to minimize cross-infection between adjoining units. Polypropylene, polycarbonate and stainless steel cages should be used to house small lab animals, Monkeys should be housed in cages made of steel or painted mild steel and for other animals such as sheep, horses, the details can be seen in Annexure – 3.

To simplify servicing and sanitation, cages should have smooth, impervious surfaces that neither attract nor retain dirt and a minimum number of ledges, angles, and corners in which dirt or water can accumulate.

The design should allow inspection of cage occupants without disturbing them. Feeding and watering devices should be easily accessible for filling, changing, cleaning and servicing.

Cages, runs and pens must be kept in good condition to prevent injuries to animals, promote physical comfort, and facilitate sanitation and servicing. Particular attention must be given to eliminate sharp edges and broken wires, keeping cage floors in good condition.

(b) Sheltered or outdoor housing

When animals are maintained in outdoor runs, pens, or other large enclosures, there must be protection from extremes in temperature or other harsh weather conditions and adequate protective and escape mechanism for submissive animals, as in case of monkeys by way of an indoor portion of a run, should be provided.

Shelter should be accessible to all animals, have sufficient ventilation, and be designed to prevent build up of waste materials and excessive moisture.

Houses, dens, boxes, shelves, perches, and other furnishings should be constructed in a manner and made of materials that allow cleaning or replacement in accordance with generally accepted husbandry practices when the furnishings are soiled or worn out.

Ground-level surfaces of outdoor housing facilities can be covered with absorbent bedding, sand, gravel,

grass, or similar material that can be removed or replaced when needed to ensure appropriate sanitation.

Buildup of animal waste and stagnant water should be avoided for example, by using contoured or drained surface. Other surfaces should be able to withstand the elements and be easily maintained.

(c) Social environment

The social environment includes all interactions among individuals of a group or among those able to communicate. The effects of social environment on caged animals vary with the species and experience of the animals.

In selecting a suitable social environment, attention should be given to whether the animals are naturally territorial or communal and whether they will be housed singly or in groups.

When appropriate, group housing should be considered for communal animals. In grouping animals, it is important to take into account population density and ability to disperse; initial familiarity among animals; and age, sex, and social rank.

Population density can affect reproduction, metabolism, immune responses, and behavior. Group composition should be held as stable as possible, particularly for canine, non-human primates, and other highly social mammals, because mixing of groups or introducing new members can alter behavioral and physiological functions.

Non-human primates should have a run for free ranging activities.

ACTIVITY

Provision should be made for animals with specialized locomotor pattern to express these patterns, especially when the animals are held for long periods. For e.g., ropes, bars, and perches are appropriate for branching non-human primates.

Cages are often used for short-term (up to 3 months) housing of dogs and may be necessary for postsurgical care, isolation of sick dogs, and metabolic studies.

Pens, runs, or other out-of-cage space provide more opportunity for exercise, and their use is encouraged when holding dogs for long periods.

FOOD

Animals should be fed palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise.

Feeders should allow easy access to food, while avoiding contamination by urine and feces.

Food should be available in amounts sufficient to ensure normal growth in immature animals and maintenance of normal body weight, reproduction, and lactation in adults.

Food should contain adequate nutrition, including formulation and preparation; freedom from chemical and microbial contaminants; bio-availability of nutrients should be at par with the nutritional requirement of the animal.

Laboratory animal diets should not be manufactured or stored in facilities used for farm feeds or any products containing additives such as rodenticides, insecticides, hormones, antibiotics, fumigants, or other potential toxicants.

Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals.

Precautions should be taken if perishable items such as meats, fruits, and vegetables are fed, because these are potential sources of biological and chemical contamination and can also lead to variation in the amount of nutrients consumed.

Diet should be free from heavy metals (e.g., lead, arsenic, cadmium, nickel, mercury), naturally occurring toxins and other contaminants.

Exposure to extremes in relative humidity, unsanitary conditions, light, oxygen, and insects hasten the deterioration of food.

Meats, fruits, vegetables, and other perishable items should be refrigerated if required to be stored. Unused, open food should be stored in vermin – proof condition to minimize contamination and to avoid potential spread of disease agents.

Food hoppers should not be transferred from room to room unless cleaned and sanitized.

The animal feed should contain moisture, crude fibre,

crude protein, essential vitamins, minerals crude fat and carbohydrate for providing appropriate nutrition.

BEDDING

Bedding should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes.

Bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry. The frequency is a matter of professional judgement of the animal care personnel in consultation with the investigator depending on the number of animals and size of cages. However it is ideal to change the bedding twice a week.

The desirable criteria for rodent contact bedding is ammonia binding, sterilizable, deleterious products not formed as a result of sterilization, easily stored, non-desiccating to the animal, uncontaminated, unlikely to be chewed or mouthed, non-toxic, non-malodorous, nestable, disposable by incineration, readily available, remains chemically stable during use, manifests batch uniformity, optimizes normal animal behaviour, non-deleterious to cage-washers, non-injurious and non-hazardous to personnel, non-nutritious and non-palatable.

Nesting materials for newly delivered pups wherever can be provided (e.g. paper, tissue paper and cotton).

WATER

Ordinarily animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their particular requirements. Periodic monitoring of microbial contamination in water is necessary.

Watering devices, such as drinking tubes and automatic waterers if used should be examined routinely to ensure their proper operation. Sometimes it is necessary to train animals to use automatic watering devices.

It is better to replace water bottles than to refill them, however, if bottles are refilled, care should be taken that each bottle is replaced on the cage which it was removed.

SANITATION AND CLEANLINESS

Sanitation is essential in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful contamination.

Cleaning utensils, such as mops, pails, and brooms, should not be transported between animal rooms.

Where animal waste is removed by hosting or flushing, this should be done at least twice a day. Animals should be kept dry during such procedures. For larger animals, such as dogs, cats, and non-human primates, soiled litter material should be removed twice daily.

Cages should be sanitized before animals are placed in them. Animal cages, racks, and accessory equipments, such as feeders and watering devices, should be washed and sanitized frequently to keep them clean and contamination free. Ordinarily this can be achieved by washing solid bottom rodent cages and accessories once or twice a week and cages, racks at least monthly.

Wire – bottom rodent cages for all other animals should be washed at least every 2 weeks. It is good practice to have extra cages available at all times so that a systematic cage-washing schedule can be maintained. Cages can be disinfected by rinsing at a temperature of 82.2°C (180°F) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms.

Disinfection can also be accomplished with appropriate chemicals; equipments should be rinsed free of chemicals prior to use. Periodic microbiologic monitoring is useful to determine the efficacy of disinfection or sterilization procedures.

Rabbits and some rodents, such as guinea pigs and hamsters, produce urine with high concentration of proteins and minerals. Minerals and organic compounds in the urine from these animals often adhere to cage surfaces and necessitate treatment with acid solutions before washing.

Water bottles, sipper tubes, stoppers, and other watering equipment should be washed and then sanitized by rinsing with water of at least 82.2°C (180°F) or appropriate chemicals agents (e.g.

hyperchlorite) to destroy pathogenic organisms, if bottles are washed by hand, powered rotating brushes at the washing sink are useful, and provision should be made for dipping or soaking the water bottles in detergents and disinfectant solutions. A two-compartment sink or tub is adequate for this purpose.

Some means for sterilizing equipments and supplies, such as an autoclave or gas sterilizer, is essential when pathogenic organisms are present. Routine sterilization of cages, food and bedding is not considered essential if care is taken to use clean materials from reliable sources. Where hazardous biological, chemical, or physical agents are used, a system of equipment monitoring might be appropriate.

Deodorizers or chemical agents other than germicidal should not be used to mask animal odors. Such products are not a substitute for good sanitation.

ASSESSING THE EFFECTIVENESS OF SANITATION

Monitoring of sanitation practices should be appropriate to the process and materials being cleaned; it can include visual inspection of the materials, monitoring of water temperatures, or microbiologic monitoring.

The intensity of animal odors, particularly that of ammonia, should not be used as the sole means of assessing the effectiveness of the sanitation program.

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

WASTE DISPOSAL

Wastes should be removed regularly and frequently. All waste should be collected and disposed of in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Incinerators should be in compliance with all central, state, and local regulations.

Waste cans containing animal tissues, carcasses, and hazardous wastes should be lined with leak-proof, disposable liners. If wastes must be stored before removal, the waste storage area should be separated

from other storage facilities and free of flies, cockroaches, rodents, and other vermin. Cold storage might be necessary to prevent decomposition of biological wastes. Hazardous wastes should be rendered safe by sterilization, contamination, or other appropriate means before they are removed from an animal facility for disposal.

PEST CONTROL

Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in an animal environment.

EMERGENCY, WEEKEND AND HOLIDAY CARE

Animals should be cared for by qualified personnel every day, including weekends and holidays, to safeguards their well-being including emergency veterinary care. In the event of an emergency, institutional security personnel and fire or police officials should be able to reach people responsible for the animals. That can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animals facilities or by placing them in the security department or telephone center. A disaster plan that takes into account both personnel and animals should be prepared as part of the overall safety plan for the animal facility.

RECORD KEEPING

The animal house should maintain the following records:

- Animal house plans, which includes typical floor plan, all fixtures *etc.*
- Animal house staff record-both technical and non-technical
- Health record of staff/ animals
- All standard operating procedures (SOPs) relevant to the animals
- Breeding, stock, purchase and sales records
- Minutes of institute Animals Ethics Committee Meetings
- Records of experiments conducted with the number of animals used (copy of Form D)
- Death Record
- Clinical record of sick animals

- Training record of staff involved in animal activities
- Water analysis report

STANDARD OPERATING PROCEDURES (SOPs) / GUIDELINES

The Institute shall maintain SOPs describing procedures / methods adapted with regard to animal husbandry, maintenance, breeding, animal house microbial analysis and experimentation records.

A SOP should contain the following items:

- Name of the Author
- Title of the SOP
- Date of preparation
- Reference of previous SOP on the same subject and date (Issue no and Date)
- Location and distribution of SOPs with sign of each recipient
- Objectives
- Detailed information of the instruments used in relation with animals with methodology (Model no., Serial no. and Date of commissioning)
- The name of the manufacturer of the reagents and the methodology of the analysis pertaining to animals
- Normal value of all parameters
- Hazard identification and risk assessment

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 and gumboots and other footwear) while working in animal rooms. Facilities should be provided for change over with lockers, wash basin, toilets and bathrooms to maintain personal hygiene. It is also important 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. The animal house

in-charge should ensure that persons working in animal house do not eat, drink, smoke in animal room and have all required vaccination, particularly against tetanus and other zoonotic diseases.

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 cages (**Annexure – 7**).

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 of 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 be accommodated in each container to avoid overcrowding and infighting (**Annexure – 4**).

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.

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. Neuromuscular blocking agents must not be used without adequate general anaesthesia (**Annexure – 5**).

In the event of a decision to sacrifice an animal on termination of an experiment or otherwise, an approved method of euthanasia should be adopted (**Annexure – 6**) and the investigator must ensure that the animal is clinically dead before it is sent for disposal. The data about large animals, which have been euthanised, should be maintained.

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 anaesthetics the animal is prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardio-pulmonary system and reduce salivary secretion. Atropine is the most commonly used anticholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure.

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 surgical site, nerve blocks and for epidural and spinal anaesthesia.

A number of general anaesthetic agents are used in the form of inhalants. General anaesthetics are also used in the form of intravenous or intramuscular injections such as barbiturates. Species characteristics and variation must be kept in mind while 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 postoperative stress.

Euthanasia

Euthanasia is resorted to 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 in an atmosphere free from fear or anxiety. For accepting 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 to be killed (**Annexure – 6**). 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 operator.
- (d) Location should be separate from animal rooms and free from environmental contaminants.

Tranquilizers have to be administered to larger species such as monkeys, dogs and cats before an euthanasia procedure.

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.

TRANSGENIC ANIMALS

Transgenic animals are those animals, into whose germ line foreign gene(s) have been engineered, whereas knockout 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. These can be either developed in the laboratory or produced for R&D purpose from registered scientific/academic institutions or commercial firms, and generally from abroad with approval from appropriate authorities.

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 guidelines. However, special care has to be taken with transgenic/gene knockout animals where the animals can become susceptible to diseases where special conditions of maintenance are required due to the altered metabolic activities. The transgenic and knockout animals carry additional genes or lack genes compared to the wild population. To avoid the spread of the genes in wild population care should be taken to ensure that these are not inadvertently released in the wild to prevent cross breeding with other animals. The transgenic and knockout animals should be maintained in clean room environment or in animal isolators.

DISPOSAL

The transgenic and knockout animals should be first euthanized 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.

BREEDING AND GENETICS

For initiating a colony, the breeding stock must be procured from CPCSEA registered breeders or suppliers ensuring that genetic makeup and health status of animal 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 knockout stock.

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Annexure – 1

Haematological data of commonly used laboratory animals.

	Mouse	Rat	Hamster	G.pig	Rabbit	Cat	Dog (Beagle)	Monkey (Rhesus)
RBC(x10 ⁶ /mm ³)	7-12.5	7-10	6-10	4.5-7	4-7	5-10	5.5-8.5	3.56-6.96
PCV(%)	39-49	36-18	36-55	37-48	36-48	30-15	37-55	26-48
Hb (g/dl)	10.2-16.6	11-18	10-16	11-15	10-15.5	8-15	12-18	8.8-16.5
WBC(X10 ³ /mm ³)	6-15	6-17	3-11	7-18	9-11	5.5-19.5	6-17	2.5-26.7
Neutrophils(%)	10-40	9-34	10-42	28-44	20-75*	35-75	60-70	5-88
Lymphocytes(%)	55-95	65-85	50-95	39-72	30-85	20-55	12-30	8-92
Eosinophils(%)	0-4	0-6	0-4.5	1-5	0-4	2-12	2-10	0-14
Monocytes(%)	0.1-3.5	0-5	0-3	3-12	1-4	1-4	3-10	0-11
Basophils(%)	0-0.3	0-1.5	0-1	0-3	2-7	rare	rare	0-6
Platelets(X10 ³ /mm ³)	160-410	500-1300	200-500	250-850	250-656	300-700	200-900	109-597

* Neutrophils often resemble eosinophils due to granules
 (NOTE- The range of normal values may vary in a laboratory using specific species, strain or substrain of these animals. Any major deviation on higher or lower side may be considered as a condition and not a disease *per se*)

Annexure – 2

Biochemical data of commonly used laboratory animals.

	Mouse	Rat	Hamster	G.pig	Rabbit	Cat	Dog	Monkey
Protein (g/dl)	3.5-7.2	5.6-7.6	4.5-7.5	4.6-6.2	5.4-7.5	6-7.5	6-7.5	4.9-9.3
Albumin (g/dl)	2.5-4.8	2.8-4.8	2.6-4.1	2.1-3.9	2.7-4.6	2.5-4.0	3-4	2.8-5.2
Globulin (g/dl)	0.6	1.8-3	2.7-4.2	1.7-2.6	1.5-2.8	2.5-3.8	2.4-3.7	1.2-5.8
Glucose (mg/dl)	62-175	50-135	60-150	60-125	75-150	81-108	54-99	46-178
Urea nitrogen	12-28	15-21	12-25	9-31.5	17-23.5	3.5-8.0	3.5-7.5	8-40
Creatinine (mg/dl)	0.3-1	0.2-0.8	0.91-0.99	0.6-2.2	0.8-1.8	<180	<120 (nmol/l)	0.1-2.8 (nmol/l)
Bilirubin (mg/dl)	0.1-0.9	0.2-0.55	0.25-0.6	0.3-0.9	0.25-0.74	<4.0	<5.0 (mmol/l)	0.1-2 (nmol/l)
Cholesterol (mg/dl)	26-82	40-130	25-135	20-43	35-53	2-4	4-7 (mmol/l)	108-263 (mmol/l)

The range of normal values may vary in a laboratory using specific species, strain or sub strain of these animals. Any major deviation on higher or side may be considered as a condition and not a disease *per se*.

Annexure – 3A

Minimum floor area recommended for laboratory animals (based on their weight/size and behavioral activity)

Animal	Weight In grams	Floor area/Animal (cm ²)	Cage height (cm ²)	
Mice	<10	38.7	12	
	upto15	51.6		
	upto25	77.4		
	>25	96.7		
Rats	<100	109.6	14	
	upto200	148.3		
	upto300	187.0		
	upto400	258.0		
	upto500	387.0		
	>500	>=451.5		
Hamsters/Gerbils/	>60	64.5	12	
Mastomys/ Cotton rats	upto 80	83.8		
	upto100	103.2		
	>100	122.5		
Guinea pigs	<350	387.0	18	
	>350	>=651.4		
Rabbits	<2000	1.5	14	
	Upto 4000	3.0	14	
	Upto 5400	4.0	14	
	>5400	5.0	14	
	Mother with kids	4.5	0.40	14

Annexure – 3B

Example for calculating the number of mice to be kept per cage, based on floor area recommended for animal according to their weight (size) and size of the cage

Recommended floor Area per animal (cm ²)	38.7	51.6	77.4	96.7
Weight of animals (g)	<10	upto15	upto25	>25
Example I: Cage size 24 x 14 cm <i>i.e.</i> floor area of 336 cm ² maximum number of animals	8	7	4	3*
Example II: Cage size 32.5 x 21 cm <i>i.e.</i> floor area of 682 cm ² maximum number of animals	17	14	9	7

Note: Cage size, specially length and breadth may vary. However, the minimum floor area and cage height recommended for group housing has to be taken into consideration. Thus, the number of animals which can be housed in a particular cage (of different sizes) can be calculated on the basis of a) floor area of the cage, b) recommended floor area per animal and c) weight of animal.

* In case of breeding pairs, three adults (*i.e.* 1 male and 2 females) along with the pups from delivery up to weaning stage are permitted.

Annexure – 3C

Example for calculating the number of rats to be kept per cage, based on floor area recommended per animal according to their weight (size) and size of the cage

Recommended floor area per animal (cm ²)	109.6	148.3	187.0	258.0	387.0	>451.5
Weight of animal (grams)	<100	upto 200	upto 300	upto 400	upto 500	>500
Example: Cage size 32.5 x 21 cm <i>i.e.</i> floor area of 682 cm ² maximum number of animals	6	5	4	3	2	1

Note: Cage size, specially length and breadth may vary. However the minimum floor area and cage height recommended for group housing has to be taken into consideration. Thus, the number of animal which can be housed in a particular cage (of different sizes) can be calculated on the basis of (a) floor area of the cage, (b) recommended floor area per animal and (c) weight of animal.

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Annexure – 3D

Example for calculating the number of Hamster/ Gerbils/ Mastomys/Cotton rats to be kept per cage, based on floor area recommended per animal according to their weight (size) and size of the cage

Recommended floor area per animal (cm ²)	64.5	83.8	103.2	122.5
Weight of animal (g)	<60	upto80	upto100	>100
Example: Cage size 32.5 x 21 cm <i>i.e</i> floor area of 682 cm ² maximum number of animals	10	8	6	5

Note: Cage size, specially length and breadth may vary. However the minimum floor area and cage height recommended for group housing has to be taken into consideration. Thus, the number of animal which can be housed in a particular cage (of different sizes) can be calculated on the basis of a) floor area of the cage, b) recommended floor area pre animal and c) weight of animal.

Annexure – 3E

Minimum floor area and height recommended for monkeys (rhesus and bonnet) based on their weight (size) and behavioral activity (for langurs, the recommended space is in the foot note)

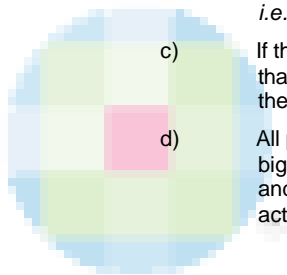
Weight (in kg)	Floor area		Height (cm)
	ft ²	cm ²	
Upto 1	1.6	1440	50
Upto 3	3.0	2700	72
Upto 10-12	4.3	3870	72
Upto 12-15	6.0	5400	72
Upto 15-25	8.0	7200	90

Note: a) The height of the cage should be sufficient for the animals to stand erect with their feet on the floor, whereas the minimum height of the cage for langurs has to be 90 cm as mentioned in INSA guidelines.

b) The floor area for langurs upto 6 kg weight, 5000 cm² and above 6 kg, 6000-9000 cm² is recommended. The height of the cage in either case remains the same, *i.e.* 90 cm as mentioned in INSA guidelines.

c) If the experimental protocol demands caging for more than 6 months, animals should be provided with double the floor space mentioned above.

d) All primate facilities should have one or more runs as big as possible with minimum floor space of 150 sq.ft and height not less than 2 meters for free ranging activities.



Annexure – 3F

Recommended space for cats, dogs and birds

Animals	Weight, kg ^a	Floor area/animal, ft ^{2b}	Height inches
Cats	<4	3.0	24
	>4	>4.0	24
Dogs	<15	8.0	-
	Up to 30	12.0	-
	>30	>24.0	-
Pigeons	-	0.8	-
Chicken	<0.25	0.25	-
	Up to 0.5	0.50	-
	Up to 1.5	1.00	-
	Up to 3.0	2.00	-
	>3.0	>3.00	-

Annexure – 3G

Recommended space for commonly used farm animals

Animals/enclosure	Weight (kg ^a)	Floor Area/Animal ft ^{2b}	Height (ft)
Sheep and goats	<25	10.0	8
	Up to 50	15.0	
	>50	20.0	
2-5	<25	8.5	
	Up to 50	12.5	
	>50	17.0	
>5	<25	7.5	
	Up to 50	11.3	
	>50	15.0	
Swine	Up to 25	12.0	8
	Up to 50	15.0	
	Up to 100	24.0	
	Up to 200	48.0	
	>200	>60.0	
2-5	<25	6.0	
	Up to 50	10.0	
	Up to 100	20.0	
	Up to 200	40.0	
	>200	>52.0	
>5	<25	6.0	
	Up to 50	9.0	
	Up to 100	18.0	
	Up to 200	36.0	
	>200	>48.0	
Cattle 1	<75	24.0	8
	Up to 200	48.0	
	Up to 350	72.0	
	Up to 500	96.0	
	Up to 650	124.0	
	>650	>144.0	
	>650	>120.0	
2 –5	<75	20.0	
	Up to 200	40.0	
	Up to 350	60.0	
	Up to 500	80.0	
	Up to 650	105.0	
	>650	>120.0	
>5	<75	18.0	
	Up to 200	36.0	
	Up to 350	54.0	
	Up to 500	72.0	
	Up to 650	93.0	
	>650	>108.0	
Horses/ponies		144.0	
1 – 4	<200	72.0	8
>4/pen	>200	60.0	10
		>72.0	

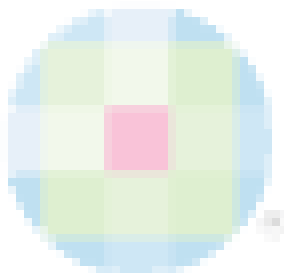
^aTo convert kilograms to pounds multiply by 2.2; ^bTo convert square feet to square meters multiply by 0.09
Larger animals might require more space.

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Annexure – 4

Requirements for transport of laboratory animals by road, rail and air.

	Mouse	Rat	Hamster	G. pig	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 box	Metal Cardboard, Synthetic material	Metal Cardboard, Synthetic material	Metal Cardboard, Synthetic material	Metal Cardboard, Synthetic material	Metal Cardboard, Synthetic material	Metal	Metal	Bamboo / wood / metal
Space per Animal (cm ²)	20-25	80-100	80-100	160-180	1000-1200	1400-1500	3000	2000-4000
Minimum height of box (cm)	12	14	12	15	30	40	50	48



Annexure- 5

Commonly used anaesthetic drugs for laboratory animals.

Drugs (mg/kg)	Mouse	Rat	Hamster	G. pig	Rabbit	Cat	Dog	Monkey
Ketamine HCl	22-24 i/m	22-24 i/m	-	22-24	22-24	30 i/m	30 i/m	15-40
Pentobarbitone sodium	35 i/v	25 i/v	35 i/v	30 i/v	30 i/v	25 i/v	20-30 i/v	35 i/v
" "	50 i/p	50 i/p	-	40 i/p	40 i/p	-	-	-
Thiopentone sodium	25 i/v	20 i/v	20 i/v	20 i/v				
" "	50 i/p	40 i/p	40 i/p	55 i/p	20 i/v	25 i/v	25 i/v	25 i/v60 i/p
Urethane	-	0.75 i/p	-	1.5 i/p	1.0 i/p, i/v	1.25 i/v	1.00 i/v	1.0 i/v
" "						1.50 i/p		

Atropine: Dose 0.02 – 0.05 mg/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

Annexure - 6

Euthanasia of laboratory animals.

(A – Methods Acceptable for species of animals indicated NR – Not Recommended)

Species	Mouse	Rat	Hamster	G. pig	Rabbit	Cat	Dog	Monkey
a) Physical methods								
Electrocution	NR	NR	NR	NR	NR	NR	NR	NR
Exsanguination	A	A	A	A	A	A	NR	NR
Decapitation (for analysis of stress)	A	A	NR	NR	NR	NR	NR	NR
Cervical dislocation	A	A	A	NR	NR	NR	NR	NR
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 plus chloroform/halothane	A	A	A	A	A	A	NR	NR
c) Drug administration								
Barbiturate overdose (route)	A(IP)	A(IP)	A(IP)	A(IP)	A(IV, IP)	A(IV, IP)	A(IV, IP)	A(IV, IP)
Chloral hydrate overdose (route)	NR	NR	NR	NR	A(IV)	A(IV)	A(IV)	A(IV)
Ketamine overdose (route)	A(IM/IP)	A(IM/IP)	A(IM/IP)	A(IM/IP)	A(IM/IV)	A(IM/IV)	A(IM/IV)	A(IM/IV)
Sodium pentothol [overdose (route)]	IP	IP	IP	IP	IV	IV	IV	IV

Methods Not Acceptable for any species of animals

a) Physical 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 Embolism

IP = intraperitoneal
IV = Intravenous
IM = Intramuscular

Annexure – 7

Certificate course for laboratory attendant (Basic education: 8th standard)

Introduction - Definition of plants and animals – types of animals – animals without back bones (invertebrates) and those with back bones (chordates/vertebrates) – animals that live in water (aquatic), air (aenar), land (terrestrial) – wild animals and domesticated animals – poisonous and non-poisonous animals – laboratory bred and non-laboratory bred animals – diurnal and nocturnal animals (suitable and relevant Indian examples to be given).

Animals rooms – animals chambers/cages – sizes of animal chambers general dimensions for monkey and rat cages stocking density – need for light (LD cycles), air water and feed – cleaning animal chambers, animal runs, aquana and animal rooms – frequency of feeding – frequency of cleaning.

Handling of animals – precautions while handling animals – common injuries and ailments in animals – litters – weaning – maintenance – record keeping.

Personal hygiene – need to use apron, gloves, mask, handling of detergents and other cleaning substances – zoonoses – need of safety handling – antidotes for specific poisons if handling poisonous animals like venomous snakes – first aid.

Emergency situations: escaping animals – use of fire extinguishers – emergency lamps – sirens.

Annexure – 8

Institutional Biosafety Committee (IBSC)

Institutional Biosafety Committee (IBSC) is to be constituted in all centers engaged in genetic engineering research and production activities. The committee will constitute the following.

- i. Head of the institution or his nominee
- ii. 3 or more scientists engaged in DNA work or molecular biology with an outside expert in the relevant discipline.
- iii. A member with medical qualification-Biosafety officer (in case of work with pathogenic agents/large scale used.)
- iv. One member nominated by DBT

The Institutional Biosafety Committee shall be the point for interaction within institution for implementation of the guidelines. Any research project which is likely to have biohazard potential (as envisaged by the guidelines) during the execution stage or which involve the production of either micro-organisms or biologically active molecules that might cause biohazard should be notified to ISBC. ISBC will allow genetic engineering activity on classified organisms only at places where such work should be performed as per guidelines. Provision of suitable safe storage facility of donor, vectors, recipients and other materials involved in experimental work should be made and may be subjected to inspection on accountability.

The biosafety functions and activity include the following:

- I. Registration of Biosafety Committee membership composition with RCGM and submission of report.
ISBC will provide half yearly reports on the ongoing projects to RCGM regarding the observance of the safety guidelines on accidents, risks and on deviations if any. A computerized Central Registry for collation of periodic reports on approved projects will be setup with RCGM to monitor compliance on safeguards as stipulated in the guidelines.
- II. Review and clearance of project proposals falling under restricted category that meets the requirements under the guidelines.
IBSC would make efforts to issue clearance certificates quickly on receiving the research proposals from investigators.
- III. Tailoring biosafety program to the level of risk assessment
- IV. Training of personnel on biosafety
- V. Instituting health monitoring program for laboratory personnel
Complete medical check up of personnel working in projects involving work with potentially dangerous microorganism should be done prior to starting such projects. Follow up medical check ups including pathological test should be done periodically, annually for scientific workers involved in such projects. Their medical record should be accessible to the RCGM. It will provide half yearly reports on the ongoing projects to RCGM regarding the observance of the safety guidelines on accidents, risks and on deviations if any.
- VI. Adopting emergency plans.

So far biosafety committee have been already set up in 124 institutions. The other institutions will be asked to take similar action.

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