MGMARI – IAEC – SOP

PRC

2223/PO/ReRc/S/23/CCSEA

SRI BALAJI VIDYAPEETH

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MAHATMA GANDHI MEDICAL PRECLINICAL RESEARCH CENTRE (MGMPRC)			
STANDARD OPERATING PROCEDURE			
Title:	**Institutional Animal Ethics Committee**		
SOP No.:	**MGMPRC/AHF/039**		
Revision No.:	**00**		
	Name	**Signature**	**Date**
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1. Purpose

Institutional Animals Ethics Committee means a body comprising of a group of persons recognized and registered by the Committee for the purpose of control and supervision of experiments on animals performed in an establishment which is constituted and operated in accordance with procedures specified for the purpose by the Committee as defined in "Breeding of and Experiments on Animals (Control arid Supervision) rules, 1998".

2. Scope

This standard operating procedure outlines the objectives, responsibilities, duty and functions and system to be followed in all the meetings by Institutional Animal Ethics Committee of Mahatma Gandhi Medical Advanced Research Institute (MGMARI).

3. Objective

The objective of this SOP is mainly for the effective functioning of the Institutional Animal Ethics Committee (IAEC) so that a quality and consistent ethical review mechanism for research on animals will be in place for all proposals dealt by the Committee as prescribed by the Committee for Control and Supervision of Experiments on Animals (CCSEA) under PCA Act 1960 and Breeding and Experimentation Rules 1998.

4. Responsibility

IAEC members — MGMARI

5. Procedure

5.1 Composition of IAEC

1. Institutional Animals Ethics committee of establishment should comprise eight members.

2. CCSEA constitutes the IAEC on receipt of five (5) scientist names from the institute/establishment.
    - A biological scientist
    - Two scientist from different biological disciplines
    - A "veterinarian involved in the care of animal
    - Scientist in charge of animals facility of the establishment concerned

3. Chairperson of the Committee and Member Secretary would be nominated by the Institution/Establishment from amongst the eight members.

4. In addition, CCSEA nominates three external members
    - A scientist from, outside the institute

- A non-scientific socially aware member and
- A Main nominee of CCSEA with a provision of a Link nominee for CCSEA nominee

5. Specialist may be co-opted while reviewing special project using hazardous agents such as radio-active substance and deadly microorganisms.

6. The duration of IAEC is for a period of 5 years and is required to be reconstituted at the time of renewal of registration, at least with the replacement of 50% of existing members. Renewal process has to be submitted to CCSEA before 40 days of renewal expiry date.

7. However, changes of members shall be made by submitting justification to CCSEA and their approval through online.

8. The primary duty of IAEC is to work for achievement of the objectives as mentioned above.
    - IAEC will review and approve all types of research proposals involving small animal experimentation before the start of the study.
    - IAEC will monitor the research throughout the study and after completion of study through periodic reports and visit to animal house and laboratory where the experiments are conducted.
    - The committee will ensure compliance with all regulatory requirements, applicable rules, guidelines and laws

9. All members should maintain absolute confidentiality of all discussions during the meeting and sign a confidentiality form.

10. Conflict of interest should be declared by members of the IAEC.

5.2 Quorum requirement

1. Minimum of 6 members are required to compose a quorum. All decisions should be taken in meetings and not by circulation of project proposals.

2. Presence of CCSEA nominee and socially aware member's is a must. Link nominee can attend in case main nominee conveys his unavailability in writing to the Chairman IAEC and at least in one meeting in a calendar year and update him /her about the activities of IAEC.

5.3 General Requirements

1. As per CCSEA instruction, CCTV camera shall be installed in the animal house facility to ensure that the animals are being looked after and maintained as per

guidelines of CCSEA. The recorded footage of CCTV camera should be reviewed once in a fortnight and observations recorded and the document to be available at the time of inspection for Nominee / representative of CCSEA

2. Establishment should have a permanent /full time Veterinarian for ensuring proper care and wellbeing of laboratory animals.

3. Health check-up of animal attendants shall also be ensured regularly and the health records retained in the facility.

4. As per CCSEA, experiments to be conducted at the facility, with Do's and Don'ts as follows:

Do's

- Under' the supervision of a person duly qualified in that behalf, that is, Degree or Diploma holders in Veterinary Science or Medicine or Laboratory Animal Science of a University and under the responsibility of the person performing the experiment
- Experiments shall he permitted by an under the supervision of a person duly qualified in that behalf, that is, Degree or Diploma holders in Veterinary Science or Medicine or Laboratory Animal Science of a University of an Institution recognized by the Government
- Experiments involving operations are performed with due care and humanity under the influence of some anaesthetic of sufficient to prevent the animals feeling pain
- Animals which, in the course of experiments under the influence of anaesthetics, are so injured that their recovery would involve serious suffering, are ordinarily euthanized while still insensible
- Animals intended for the performance of experiments are properly looked after both before and after experiments.
- Suitable records are maintained with respect to experiments performed on animals

Don'ts

- Experiments on animals are avoided in medical schools, hospitals, colleges and the like, if other teaching devices such as books, models, films and the like, may equally suffice;
- Experiments are not performed merely for the purpose of acquiring manual skill.
- 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, mice, rats etc;

5.4 Conduct of the Meeting

1. The meeting of the IAEC should be four times a year or three months once intervals as applicable.
2. Additional meetings may be held, if there are reasons to do expedited review
3. In addition, establishment where more than 40 proposals are conducted annually should mandatorily convene the IAEC meeting once in three months to ensure proper scrutiny of research protocols, proper functioning of IAEC and checking the status of research proposal and health status of laboratory animals.
4. The Chairperson will conduct all meetings of the IAEC. If for reasons beyond control, the Cliairperson is not available, or lias conflict of interest an alternate Chairperson will be elected from the members by the members present, who will conduct the meeting.
5. The Member Secretary is responsible for organizing the meetings, maintaining the records and communicating with all concemed. He/she will prepare the minutes of the meetings and get it approved by the Chairman before communicating to the researchers with the approval of the appropriate authority.
6. IAEC meetings will be organized by member secretary based on the number of proposals available (First cum first serve basis, minimum 10) with the due concurrence of all members.
7. Meeting date notifications will be sent via circular through the university portal.
8. Attendance of members and investigator need to be documented and maintained for each meeting
9. A copy of minutes is required to be sent to Member Secretary CCSEA withing 15 days of the meeting, otherwise, the meeting will not be considered valid.

5.5 Participation by Investigators / experts in IAEC.

1. IAEC may call upon subject experts who may provide special review of selected research protocols, if need be. They are required to give their specialized views but do not take part in the decision making process which will be made by the members of the IAEC. Investigators whose proposals are to be discussed can also be called to present their case to the IAEC.
2. Investigators whose proposals are to be discussed can also be called to present their case to the IAEC.
3. Specialist may be co-opted while reviewing special project using hazardous agents such as radio-active substance and deadly microorganisms. They may provide special review of selected research protocols, if required. They are required to give their specialized views but do not take part in the decision making process which will be made by the members of the IAEC.

5.6 Upper age limit for the Nominees of CCSEA:

The upper age limit for the Nominees of CCSEA is 65 years. However, the nominees who are already working in the IAECs and are above 65 years of age will be allowed to be continued till the end of their tenure in the present IAECs.

5.7 Application procedures

1. All proposals should be submitted in the prescribed application form (Form B which as Part A and Part B). All relevant documents with checklist should be enclosed with application form by researcher. Documents are available in MGMPRC website under "Committee — IAEC – Forms".
2. Required number of copies of the proposal along with the application and documents in prescribed format duly signed by the Researcher /Principal Investigator (PI) and Co- investigators/ collaborators should be submitted to IAEC with declaration of Head of the Department. Soft copy of proposal shall be submitted via email - mgmari@sbvu.ac.in
3. PhD students shall submit the proposal with Guide declaration and provide the details of registration and name co-guide as applicable in Form B.
4. Proposal submitted for the purpose of provisional clearance for funding agency or PhD clearance, it should be mentioned appropriately by researcher.
5. Proposals submitted for the projects funded from government agency or any other source, photocopy of the sanctioned letter received need to be submitted along with proposal.
6. In case of emergency, project proposals may be circulated to all the members either hard / soft copy by member secretary and chairman discretion.

5.8 Review procedures:

1. The process of review proposals will be done as follows
   - Proposal will be sent to members at least 15 days in advance.
   - Decisions will be taken by consensus after discussions.
   - Negative view points should be recorded in the minutes. In case consensus is not reached, the case should be referred to CCSEA.
   - Researchers will be invited to make presentation and offer clarifications.
2. Independent consultants/Experts will be invited to offer their opinion on specific research proposals if needed.
3. The decisions will be recorded as minutes and Chairperson's approval will be received along with signature of all the IAEC members present.
4. Decisions will be made only in meetings where quorum is complete.
5. Decision may be to approve, reject or revise the proposals. Specific suggestions for modifications and reasons for rejection should be given.
6. Modified proposals may be reviewed by an expedited review through identified members

5.9 Decision-making

1. Members will discuss the various issues before arriving at a consensus decision.
2. A member should withdraw from the meeting during the decision procedure concerning an application where a conflict of interest arises and this should be indicated to the chairperson prior to the review of the application and recorded in the minutes.
3. Decisions will be made only in meetings where quorum is complete.
4. Only members can make the decision. The experts / investigators / invitees will only offer their opinions.
5. Decision may be to approve, reject or revise the proposals. Specific suggestions for modifications and reasons for rejection should bc given.
6. In cases of conditional decisions, clear suggestions for revision and the procedure for having the application re-reviewed should be specified.
7. Modified proposals may be reviewed by an expedited review through identified members.
8. Procedures for appeal by the researchers should be clearly defined.

5.10 Communicating the decision

1. Decision will be communicated by the Member Secretary in writing.
2. Suggestions for modifications, if any, should be sent by IAEC to researcher.
3. IAEC Approval certificates each proposal/ experiments will be issued by IAEC with chairperson and CCSEA nominee approval.
4. For PhD students who appear for provisional ethical clearance, member secretary will directly send the approval clearance letter to controller of examination.
5. Reasons for rejection should be informed to the researchers.
6. The schedule / plan of on-going review by the IAEC should be communicated to the PI.
7. IAEC approval for experiments will be valid for one year from the date of approval. For extension of period, investigator has to seek the permission from IAEC

5.11 Follow up procedures

1. After experiments are approved by IAEC, the investigators have to follow up the following procedures:
2. Investigators have to submit the experiment initiation form to Veterinarian to initiate the experiment as per approved proposal
3. Once experiment completed, Form D shall be submitted by the investigator for the usage and return of animals as applicable.
4. Reports should be submitted at prescribed intervals for review as applicable or Final Experiment completion report should be submitted at the end of study as applicable.

5. All Serious Adverse Events (SAE's) and the interventions undertaken should be intimated.
6. Protocol deviation, if any, should be informed with adequate justifications.
7. Any amendment to the protocol should be resubmitted to IAEC for renewed approval.

8. Premature termination of study should be notified with reasons along with summary of the data obtained so far.
9. Change of investigators / sites should be informed and approval of IAEC should be taken.

6. Record keeping and Archiving

- Curriculum Vitae (CV) of all members of IAEC including training programs in animal ethics attended.
- Copy of all study protocols with enclosed documents, progress reports.
- Minutes of all meetings duly signed by the Chairperson and the members.
- Copy of all existing relevant national and international guidelines on animal ethics and laws along with amendments.
- Copy of all correspondence with members, researchers and other regulatory bodies.
- Record of import of animals with species, source, quantity, usage etc.
- Record of all Contract research, if conducted at the institute.
- All documents should be archived for a period of 5 years.

7. Updating IAEC members

- All relevant new guidelines and amendments to the Rules and Act should be brought to the attention of the members.
- Members should be encouraged to attend national and international training programs / workshops / conferences in research ethics for maintaining quality in ethical review and be aware of the latest developments in this area.

8. Reporting to CCSEA

- IAEC is required to send a copy of minutes of IAEC meeting to CCSEA within 15 days.
- Inspection report of animal house with photographs by IAEC members is required to be sent once in a calendar year by7 nominee. If action is required, the facility must provide ATR within 30days

9. IAEC related Formats

- Bio data and Consent form for Members of IAEC
- Form B — Application for permission for Animal Experiments

- Form C- Record of Animals bred / acquired: (to be maintained by the Breeder/Establishment)
- Form D - Record of Animals Acquired and Experiments performed: (to be maintained by the Investigator and submitted to Animal House)
- Expriment Completion Report
- IAEC Application processing fee

10. Reference

- CCSEA Standard Operating Procedure (SOP) for IAEC under PCA Act 1960 dnd Breeding and Experimentation Rules 1998
- CCSEA Compendium -2018
- F.No. 25/28/2017-CCSEA - Instruction for animal house facilities registered with CCSEA.
- www.CCSEA.nic.in

CCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY - 2015

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

1. GOAL

The goal of these Guidelines is to promote the humane care of animals used in biomedical and behavioural 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.

2. VETERINARY CARE

a) 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.

b) 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.

c) The veterinarian can also help the establishment in designing appropriate policies and procedures for ancillary aspects of veterinary care, such as use of appropriate methods to prevent and control diseases (e.g. vaccination and other prophylaxis, disease monitoring and surveillance, quarantine and isolation), operative and post-operative care, diagnosis and treatment of diseases as well as injuries. 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.

3. ANIMAL PROCUREMENT

a) All animals (like cattle, buffalo, sheep, goat, pigs, equine etc.) must be acquired lawfully as per the CCSEA guidelines. Small animals and dogs can be procured from registered breeders. Large animals can be procured from farm, farmers or as per guidance of wild life department, as is done in case of macaques. Cats can be bred for their use. Rodents can be imported from abroad after necessary licence from Director General of Foreign trade (DGFT) is obtained for import.

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

c) 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.

4. QUARANTINE, STABILIZATION AND SEPARATION

a) 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. The duration at quarantine in small lab animals is from one week to one month and large animals allowed up to 6 weeks (cat,

dog, monkey, etc). However, duration of quarantine can be increased depending on type of infection / suspected infection noticed in the animals.

b) Effective quarantine procedures should be used for non-human primates to help limit exposure of humans to zoonotic infections. The period varies from 2 to 3 months depending on the reaction of TB testing. Any macaque found positive for TB for at least two times and shows signs of weight loss or ill health should be euthanized as is practiced internationally to prevent spreading of TB to workers and other macaques.

c) 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.

d) Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiological and behavioural changes due to interspecies conflict.

e) 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 can be used as suitable alternatives.

f) In some instances, it shall be acceptable to house different species in the same room, for example, if two species have a similar pathogenic status and are behaviourally compatible. Separate set of personnel should be identified for taking care of these infected (sick) animals and other workers should be restricted from entering in to the facilities unless otherwise required and after handling these animals they should not be handling any other animals in the facilities

5. SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

a) All animals should be observed for signs of illness, injury, or abnormal behaviour 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 & 2).**

b) Post-mortem examination 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. Animals suffering from contagious diseases like Tuberculosis etc. must be euthanized as is practiced internationally to prevent its spread to other animals and often animal handlers.

c) The isolation, quarantine and stabilization programs for newly arrived animals are necessary to provide time to assess their health status, allow them to recover from the stress of shipment and an opportunity to adapt to their new environment. The extent of these programs depends on several factors, including species and source of the animals as well as their intended use. For some animals, such as rodents obtained from reliable sources for which health status is known, visual inspection on arrival may suffice. For species such as nonhuman primates, farm animals, wild animals, dogs, cats and non-specific pathogen free rabbits and rodents, appropriate quarantine and isolation procedures must be employed.

d) Preventive medicine programs such as vaccinations, ecto- and endoparasite treatments and other disease control measures should be initiated according to currently acceptable veterinary medical practices appropriate to the particular species and source. Only animals of defined health status should be used in research and testing unless a specific, naturally occurring or induced disease state is being studied. Systems should be established to protect animals within the institution from exposure to diseases.

e) Transgenic and mutant animals may be particularly susceptible to diseases and may require special protection to ensure their health. Systems to prevent spread of disease may include facility design features, containment/isolation equipment, and use of standard operating procedures. Training of animal care and research staff is essential to prevent spread of animal diseases.

f) Disease surveillance is a major responsibility of the veterinarian and should include routine monitoring of colony animals for the presence of parasitic and microbiological agents that may cause overt or unapparent disease. Additionally, cells, tissues, fluids, and transplantable tumors that are to be used in animals should be monitored for infectious or parasitic agents that may cause disease in animals. The type and intensity of monitoring necessary will depend upon professional veterinary judgment and the species, source, use and number of animals housed and used in the facility.

g) Diagnostic laboratory services must be available and used as appropriate. Laboratory services should include necropsy, histopathology, microbiology, clinical pathology, serology, and parasitology as well as other routine or specialized laboratory procedures, as needed. It is not necessary that all of these services be available within the animal facility (Facilities from other laboratories with appropriate capabilities may be used).

h) Animals with infectious / contagious disease must be isolated from others by placing them in isolation units or separate rooms appropriate for the containment of the agents of concern. In certain circumstances, when an entire group of animals is known or suspected to be exposed or infected, it may be appropriate to keep the group intact during the time necessary for diagnosis and treatment, for taking other control measures, or for completion of a project.

i) The veterinarian must have authority to use appropriate treatment or control measures, including euthanasia in consultation with at least one more additional veterinarian if required, following diagnosis of an animal disease or injury. If possible, the veterinarian should discuss the situation with the principal investigator to determine a course of action consistent with experimental goals. However, if the principal investigator is not available, or if agreement cannot be reached, the veterinarian must have authority to act to protect the health and wellbeing of the institutional animal colony and workers.

6. ANIMAL CARE AND TECHNICAL PERSONNEL

a) 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).**

7. PERSONAL HYGIENE

a) 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 with

appropriate Personnel Protective Equipment (PPE) e.g. showers, change of uniforms, footwear etc.

b) 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. It is acceptable to use disposable 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.

c) Washing and showering facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics and perfumes in animal rooms. They should finish the work with animals as early as possible and sit somewhere else, outside and not in the animal rooms / areas. A separate area or room should be made available for these purposes.

8. ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

a) Institutions should have policies governing experimentation with hazardous agents. Institutional Bio-safety 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 taking care of safety issues. This committee shall also examine the proposal on animal experiments involving hazardous agents in addition to its existing functions **(Annexure - 8).**

b) Since the use of animals in such studies requires special considerations, the procedures and the facilities to be used must be reviewed by both the Institutional Bio-safety committee and Institutional Animal Ethics Committee (IAEC). Disposing of tissues and fluids from such used animals must also be appropriately governed as per the laid in practices of the institution / biosafety regulation.

9. MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

a) 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.

b) Individual animals should not be used in more than one experiment, either in the same or different projects, without the express approval of the IAEC. However, it is noted that appropriate re-use of animals may reduce the total number of animals used in a project, result in better design of experiments, and reduce stress or avoid pain to additional animals. Animals that are used in more than one experiment should be permitted to recover fully from the first experiment before the subsequent experiment is performed. Certification of attending veterinarian is however, required before subjecting animal to the second experiment.

10. DURATIONS OF EXPERIMENTS

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

11. PHYSICAL RESTRAINT

a) 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.

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

c) Following points should be considered during handling and restraining animals:-

i. Animals should be handled by competent individuals trained in methods that cause minimal distress and injury (for example, a person with a publication to his/her credit and post/experience in relevant techniques for handling animals is preferable).

ii. The use of restraint devices is sometimes essential for the welfare of the animal and safety of the handler. Restraint devices should be used to the minimum

iii. Extent, for the minimum period required to accomplish the purpose of the experiment and be appropriate for the animal.

iv. Tranquilisers or anaesthetics may initially be used to aid restraint but they may prolong recovery from the procedure. When these agents have been used, recovery of the animals should be closely monitored.

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

i. 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, prior to initiation of the experimentation.

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

12. LOCATION OF ANIMAL FACILITIES TO LABORATORIESLOCATION OF ANIMAL FACILITIES TO LABORATORIES

Good animal husbandry and human comfort and health protection require physical separation of animal facilities from personnel areas such as offices, break room, training and education room.

- 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.

- While 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)**

13. FUNCTIONAL AREAS

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

- Ensure separation of species or isolation of individual projects when necessary;
- Receive, quarantine, and isolate animals; and
- Provide for animal housing.

b) 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 or
- 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 equipment and supplies,
- An autoclave for equipment
- Food, and bedding; and separate areas
- For holding soiled and cleaned equipment
- An area for repairing cages and equipment
- An area to store wastes prior to incineration or removal

14. PHYSICAL FACILITIES

The physical condition and design of animal facility determine, to a great extent, the efficiency and economy of this 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.

a) Housing facility should be compatible with the needs of the species to be housed.
b) Housing Facilities should be designed and operated to facilitate control of environmental factors to exclude vermin and limit contamination associated with the housing of animals, delivery of food, water, bedding, and the entry of people and other animals.
c) Housing Facilities should be maintained in good repair. Walls and floors should be constructed of durable materials with surfaces that can be cleaned and disinfected readily.

d) Housing Facilities should be kept clean and tidy and operated to achieve maximum possible hygiene.
e) There should be a pest control programme to monitor and control vermin.
f) There should be adequate and appropriate storage areas for food, bedding and equipment.
g) Deodorants designed to mask animal odours should not be used in Housing Facilities as they may expose animals to volatile compounds which can alter metabolic processes. In addition, deodorants must not be used as a substitute for good cage and equipment cleaning practices and good ventilation.
h) Cleaning practices should be monitored on a regular basis to ensure effective hygiene and sanitation. This may include visual inspection, monitoring water temperatures and microbiological testing of surfaces after cleaning.
i) There should be proper water supply and drainage.
j) There should be adequate contingency plans to cover such emergencies as flooding and fire, or the breakdown of lighting, heating, cooling or ventilation.
k) In the interest of disease prevention and general animal welfare, access to the Housing Facilities by unauthorised persons should be restricted

I. **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.

II. **Corridor(s)** should be wide enough to facilitate the movement of personnel as well as equipments and should be kept clean.

III. **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.

IV. Animal Room Doors

Doors should not be rust and should be 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.

V. 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 to have visual access to natural environment.

VI. Floors

Floors should be or either monolithic or epoxy smooth, moisture proof, nonabsorbent, skidproof, resistant to wear, acid, solvents and adverse effects of detergents/ disinfectants. They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints.

VII. 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. At the inlet and outlets of the drains should be fitted with wire mesh guard to prevent wild rodent entry

VIII. Walls & 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, disinfectants and the impact of water under high pressure. Materials used for construction of roof should cater needs of local climatic condition to provide comfort to the animals.

IX. 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.

X. Facilities For Sanitizing Equipment And Supplies

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

XI. Experimental Area

All experimental procedures in small animals should be carried out in a separate area away from the place where animals are housed. Aseptic surgery for large animals should include separate functional areas for surgical support, like a preparation area, the operating theatre room or rooms, and an area for post operative care and for treatment of animals.

15. 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 keeping in view of the variations in the number of room occupants the range should be within or approximately between 18 to 29°C (64.4 to 84.2°F) all times.

The relative humidity should be under control 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 appropriate methods e.g. sprinklers should be adopted for cooling open enclosures of large animals.

b) Ventilation

In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures. Heating, ventilation, and air-conditioning systems should be designed with 12-15 air cycles per hour 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 with sufficient number of power points lighting system be installed provide adequate illumination for people to work in the animal rooms and a lowered intensity of light for the animals. Fluorescent lights are efficient and less than 400 lux is preferable for rodent facilities.

A time-controlled lighting system should be used if possible 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 the animal facility. Concrete walls are more effective than metal or plaster walls because their density reduces sound transmission. Preferably less than 85 dB is desirable for rodents and non human primates.

16. ANIMAL HUSBANDRY

i. Caging of Housing System

a) 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 variable

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 access to food and water;
- Provide adequate ventilation
- Meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation, and reproduction;
- Keep the animals dry and clean, consistent with species requirements
- Facilitate research while maintaining good health of the animals**.**

b) They should be constructed of sturdy, durable materials and designed to minimize crossinfection 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.**

c) 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.

d) 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. International guidelines can be referred from time to time to improve caging facilities.

ii. Sheltered or Outdoor Housing

a) When animals are maintained in outdoor runs, pens, or other large enclosures, there must be protection from extremes in temperature or other harsh whether conditions an adequate protective and escape mechanism for submissive animals especially in monkeys by way of providing indoor portion of run. Shelter should have sufficient ventilation, and should be designed to prevent accumulation of waste materials and excessive moisture.

b) 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.

c) Ground-level surfaces of outdoor housing facilities can be cemented or covered with absorbent bedding, sand, gravel, grass, or similar material that can be removed or replaced when that is needed to ensure appropriate sanitation. Accumulation of animal waste and stagnant water should be avoided by, for example, using contoured or drained surface. Other surfaces should be able to withstand the elements and be easily maintained.

d) In case of open runs of macaques, it is obvious in our country (unlike others) to have outside animals and local macaques frequenting the colony pens and runs increasing risk of contracting contagious diseases. Hence, it is advisable to cover such open pens with additional layers of materials (double fencing) to separate outside animals physically from the animals belonging to the colony. Initiation of such practices may reduce spread of infectious diseases like TB etc. found more frequently in Indian colonies of macaques in various establishments and thought to be unavoidable.

17. SOCIAL ENVIRONMENT

a) The social environment includes all interactions among individuals of a group or among those able to communicate. The effects of social environment in caged animals vary with the species.
b) In selecting a suitable social environment, attention should be given whether the animals are naturally territorial or communal and accordingly they should be housed single or in groups.
c) 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.
d) Population density can affect reproduction, metabolism, immune responses, and behaviour. 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 behavioural and physiological functions.
e) Non-human primates should have a run for free ranging activities:

18. ACTIVITY

a) Provision should be made for animals with specialized locomotor pattern to express their natural habitat, especially when the animals are held for long periods. e.g., artificial trees, ropes, bars, and perches are appropriate for non-human primates.
b) 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.

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

19. FOOD

a) Animals should be fed with palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise.
b) Feeders should allow easy access, while avoiding contamination by urine and faeces
c) Food should be provided in sufficient amounts to ensure normal growth in immature animals and to maintain normal body weight, reproduction, and lactation in adults.
d) Food should contain adequate nutrition, with proper formulation and preparation; and ensure free from chemical and microbial contaminants; bio-availability of nutrients should be at par with the nutritional requirements of the animal. The animal feed should contain

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

e) Laboratory animal diets should not be manufactured or stored in facilities used earlier for farm feeds or any products containing additives such as rodenticides, insecticides, hormones, antibiotics, fumigants, or other potential toxicants.
f) Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals.
g) Precautions should be taken if perishable items such as meats, fruits, and vegetables are fed, because these are potential sources of microbiological and chemical contamination and can also lead to variation in the amount of nutrients consumed.
h) Diet should ideally be free from heavy metals (e.g., Lead, Arsenic, Cadmium, Nickel, Mercury), naturally occurring toxins and other contaminants. Exposure to extremes of relative humidity, unsanitary conditions, light, oxygen, and insects hasten the deterioration of food.
i) Meats, fruits, vegetables, and other perishable items should be refrigerated if required to be stored. Unused, open food should be stored in vermin proof conditions to minimize contamination and to avoid potential spread of disease causing agents.
j) Food hoppers should not be transferred from room to room unless cleaned and properly sanitized.

20. BEDDING

a) Bedding should be absorbent, free from toxic chemicals or other substances that cause irritation, 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.

b) Bedding should be removed and replaced periodically with fresh materials as often as necessary to keep the animals clean and dry. The frequency is a matter of professional judgement of animal care personnel in consultation with the investigation depending on the number of animals and size of cages. In general it is ideal to change the bedding twice a week or whenever requires.

c) 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 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.

d) Nesting materials for newly delivered pups should be provided wherever needed (e.g. Paper cuttings, tissue paper, cotton etc.)

21. WATER

a) Animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their requirements. Periodic monitoring of microbial contamination in water is necessary.
b) Watering devices, such as drinking nozzles and automatic waterers should be examined routinely to ensure their proper operation. Sometimes it is necessary to train animals to drink water from automatic watering devices.
c) Animals should receive appropriate, uncontaminated and nutritionally adequate food according to accepted requirements for the species. The food should be in sufficient quantity and of appropriate composition to maintain normal growth of immature animals, normal weight of adult animals or provide for the requirements of pregnancy or lactation.

d) When animals are fed in groups, there should be sufficient trough space or feeding points to cater to the number and size of animals that eat together at one time so as to avoid undesirable competition for food, especially if feed is restricted.
e) Uneaten perishable food should be removed promptly unless contrary to the eating habits or needs of the species. Any alteration to dietary regimes should be gradual

22. SANITATION AND CLEANLINESS

a) Sanitation is an essential activity in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be properly cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful agents of contamination.
b) Cleaning utensils, such as mops, pails, and brooms, should not be transported between animal rooms.
c) Where animal waste is removed by hosing 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.
d) 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. Generally this can be achieved by washing solid bottom rodent cages and accessories once or twice a week and cages, racks at least monthly.
e) Wire - bottom cages other than rodent cages 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 82.2ºC (180ºF) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms.
f) 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.
g) Rabbits and some rodents, such as guinea pigs, mice and hamsters, produce urine with high concentration of proteins ammonia 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.
h) Water bottles, sipper nozzles stoppers, and other watering equipment should be washed and then sanitized by rinsing with water of at least 82.2ºC (180ºF) or appropriated chemicals agents (e.g. Sodium Hyperchlorite) to destroy pathogenic organisms, if bottles are washed by hand, mechanized 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.
i) 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, feed and bedding is also essential besides 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.
j) Deodorants or chemical agents other than germicidal agents should not be used to mask animal odours. Such products are not a substitute for good sanitation.

23. ASSESSING THE EFFECTIVENESS OF SANITATION

a) Sanitation practices should be monitored appropriately to ensure effectiveness of the process and materials being cleaned; it can include visual inspection of the materials, monitoring of water temperatures, or microbiologic monitoring.
b) The intensity of animal odours particularly that of ammonia should not be used as the sole means of assessing the effectiveness of the sanitation program.

c) A decision to change the frequency of such bedding changes or cage washing should be based on factors such as the concentration of ammonia, appearance of the cage, condition of the bedding and number and size of the animals housed in the cage.
d) Autoclaving : Chemical Indicator - batch wise assessment; Biological indicator – Periodical assessment

24. WASTE DISPOSAL

a) Wastes should be removed regularly and frequently. All waste should be collected and disposed off 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 Public Health and Pollution Control Board regulations.
b) Waste containers 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 disinfection, deco.

25. PEST CONTROL

Adaptation of Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in an animal home environment. Best results can be achieved by giving contracts to people/firm specialized in pest control.

26. EMERGENCY, WEEKEND AND HOLIDAY CARE

There should be an institutional policy to care animals 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 responsible persons 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 near telephone. 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.

27. RECORD KEEPING

It is essential that animal House should maintain 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 and animals
- All SOPs relevant to experiments, care, breeding and management of animals
- Breeding, stock, purchase and sales records
- Minutes of institutional Animals Ethics Committee Meetings
- Records of experiments conducted with the number of animals used (copy of Form D)Mortality,
- Post-mortem Record, wherever required.
- Clinical record of sick animals.
- Training record of staff involved in animal activities
- Water, feed and bedding materials analysis report
- Health monitoring Records.
- Rehabilitation Records, wherever required.

28. STANDARD OPERATING PROCEDURES (SOPs) / Guidelines

The Institute should maintain SOPs describing procedures / methods adapted with regard to Animal Husbandry, maintenance, breeding, animal house activities microbial testing and experimentation.

A SOP should contain the following items:

- Name of the Author
- Title of the SOP
- Date of approval
- Reference of previous SOP on the same subject and date (Issue number and Date)
- Location and distribution of SOP's with sign of each recipient.
- Objectives
- Detailed information of the instruments used in relation with animals with methodology
- (Model no., Serial no., Date of commissioning, etc)
- 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

29. PERSONNEL AND TRAINING

a) 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.

b) The staff must be provided with all required protective clothing (face masks, head covers**,** aprons, gloves, gumboots, other footwear etc.) 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 persons working in animal house should not eat, drink, smoke in animal room and have all required vaccination, particularly against Tetanus and other zoonotic diseases.

c) 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).**

30. TRANSPORT OF LABORATORY ANIMALS

a) 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, mode of transport, containers, animal density in cages, food and water during transit, protection from transit infections, injuries and stress.

b) 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 movement and protection from possible injuries. Sometimes injuries can be avoided by reducing space but parallely decreasing time of transportation. 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 fluid 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)**

31. ANAESTHESIA AND EUTHANASIA

a) The investigators should ensure that the procedures, which are considered painful, are conducted under appropriate anaesthesia as recommended for each species of animals.
b) 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 procedure. If at any stage during the experiment the investigator feels that he has to abandon the experiment or he has inflicted irrepairable injury, the animal should be humanely sacrificed. Neuromuscular blocking agents must not be used without adequate general anaesthesia **(Annexure - 5).**
c) In the event of a decision to sacrifice an animal or 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 of all the animals, that have been euthanised, should be maintained.

I. Anaesthesia:

a) 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.
b) Before using actual anaesthetics the animals are prepared for anaesthesia by overnight 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.
c) 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 an expert supervision for regional infiltration of surgical site, nerve blocks and for epidural and spinal anaesthesia.
d) A number of general anaesthetic agents are used in the form of 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 while using an anaesthetic. Side - effects such as excess 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.

II. Euthanasia

Euthanasia should be resorted to events where an animal is required to be sacrificed to reduce suffering or to limit spread of infections or for termination of an experiment or for other 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 a procedure of euthanasia.

32. 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. Interaction amongst people working in animal house should be organised once in a while to discuss ethical issues favouring wellbeing of animals.

33. 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, etc. These can be either developed in the laboratory or procured for R&D purpose from registered scientific/academic institutions or commercial firms, generally from abroad with approval from appropriate authorities.

34. 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.

35. DISPOSAL

The transgenic and knockout animals should be first euthanized and then disposed off as described elsewhere in the guidelines. A record of disposal and the manner of disposal should be kept as a matter of routine.

36. BREEDING AND GENETICS

For initiating a colony, the breeding stock must be procured from established 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

Annexure – 1

HAEMATOLOGICAL DATA OF COMMON LABORATORY ANIMALS

	Mouse	Rat	Hamster	G. Ppig	Rabbit	Cat	Dog (Beagle)	Primate (Rhesus)
RBC(x10 /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 - 48	36 – 55	37 – 48	36 – 48	30 – 45	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 sub strain of these animals. Any major deviation onhigher or lower side may be considered as a condition and not a disease per se)

Annexure – 2

BIOCHEMICAL DATA OF COMMON 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(mg/dl)	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 (n mol/I)	<120 (n mol/I)	0.1 - 2.8
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 (m mol/l)	<5.0 (n mol/l)	0.1 - 2
Cholesterol (mg/dl)	26 - 82	40 - 130	25 - 135	20 - 43	35 - 53	2 - 4 (m mol/l)	4 - 7 (m mol/l)	108 - 263

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 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)
Mouse	<10	38.7	
	upto15	46	
	upto25	74	
	>25	96.7	12
Rat	<100	109.6	
	upto200	148.3	
	upto300	187.0	
	upto400	258.0	
	upto500	387.0	
	>500	>=451.5	14
Hamster /Gerbil/ Mastomy/ Cotton rat	>60	64.5	
	upto 80	83.8	
	upto100	103.2	
	>100	122.5	12
Guinea pig	<350	387.0	
	>350	>=651.4	18

		Floor area (Sq.ft)	(Sq.meter)	Height (inches)
Rabbit	<2000	1.5	0.135	14
	Upto 4000	3.0	0.27	14
	Upto 5400	4.0	0.36	14
	>5400	5.0	0.45	14
	Mother with Pups	4.5	0.40	14

Annexure – 3B

Example for calculating the number of Mice to be kept per cage, based on floor arearecommended 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 (Grams)	<10	upto 15	upto 25	>25
Example I Cage Size 24 x 14 cm i.e. floor area of 336 cm^2 maximum number of animals	9	7	4	3*
Example IICage Size 32.5 x 21 cm i.e floor area of 682.5 cm^2 maximum number of animals	18	13	9	7

Note: Cage size, specially length and breadth may vary. However, the minimum 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 female) 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.e floor area of 682.5 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 pre animal and c) weight of animal.

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 (grams)	<60	upto80	upto100	>100
Example Cage size 32.5 x 21 cm i.e floor area of 682.5 cm^2 maximum number of animals	11	8	7	6

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 below)

Weight Kg)	Floor area		Height(in
	(in Ft^2)	(in Cm^2)	(in Cm)
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

b) The floor area for langurs upto 6 kg weight, 5000 cm^2 and above 6 kg, 6000 - 9000 cm^2 is recommended. The height of the cage in either case remains the same, i.e. 90cm.

c) If the experimental protocol demands individual 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 150sq.ft and height not less than 2 meters for free ranging activities.

ANNEXURE – 4

SPECIFICATIONS FOR TRANSPORT OF LABORATORY ANIMALS BY ROAD, RAIL AND AIR

	Mouse	Rat	Hamster	G. pig	Rabbit	Cat	Dog	Primate
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.Sq.)	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

Cattle, Buffalo, Equine, Sheep, Goat and Pigs may be procured (transported) as per the Transport of Animals (Amendment) Rules, 2009 (Refer: www.awbi.org/awbi-pdf/Transport of Animals (Amendment) Rules, 2009.pdf)

ANNEXURE – 5

Drugs (mg/kg)	Mouse	Rat	Hamster	Guinea pig	Rabbit	Cat	Dog	Primate	Cattle/ Buffalo	Equine
KETAMINE HCl	87mg/kg IP once (in combination with xylazine)	87mg/kg IP once (in combination with xylazine)	200 mg/kg IP once (in combination with xylazine)	40 mg/kg IM, 60 mg/kg IP once (in combination with xylazine)	24-35mg/kg IM	22-33mg/kg IM	11 to 22 mg/kg IM.	5 - 15mg/kg	2 to 2.2mg/kg IV	2.2 - 2.75 mg/kg IV
PENTOBAR-BITONE SODIUM	35 IV 40-70mg/kg IP	30-40 mg/kg IV 40-60mg/kg IP	70-90mg/kg IP	30mg/kg IV 40mg/kg IP	30-40mg/kg IV 40mg/kg IP	25-35 mg/kg IV	10 – 33mg/kg IV	20-30mg/kg IV	5.5-15.4 mg/kg IV	15-18mg/kg IV
THIOPENT-ONE SODIUM	25mg/kg IV 50mg/kg IP	20-40mg/kg IV 40mg/kg IP	20 mg/kg IV 40mg/kg I/P	20 mg/kg IV 55 mg/kg IP	20 mg/kg IV	13.2-26.4mg/kg IV	13.2-29 mg/kg IV	15-20mg/kg IV	5.5-15.4mg/kg IV	6-15.4mg/kg IV
URETHANE	1000mg/kg IP*	1000mg/kg IP*	1500mg/kg IP*	1500mg/kg IP*	1000mg/kgIP*	750mg/kg IV*, 1500mg/kg IP*	1000 mg/kg IP*	-	-	-

COMMONLY USED ANAESTHETIC AGENTS FOR LABORATORY ANIMALS

*(prolonged anaesthesia: terminal procedures only)

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 heartfrom vagal inhibition, given prior to anaesthesia.

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

Anesthesia for Laboratory Animals:

For mice ketamine is used alone intramuscularly. Usually I/M is not recommended in Mice due small muscle mass, and may cause lameness in mice. Also Injection may cause discomfort and local tissue irritation. Ketamine is rarely administered alone due to its poor muscle relaxation. Ketamine has been used in combination with various other anesthetic drugs, but it is most commonly combined with Xylazine or Meditomidine.

The Drugs/dose and route of administration is as follows:-

Drug (mg/Kg)	Mouse	Rat	Rabbit	Hamster	G.Pigs	Cat	Dog	Primate
Ketamine+Xylazine	80mg+10mg i/p	75+10mgi/p	35-40 +5-10mg i/m	200+10 mg/i/p	40+5 mg i/p	20 +1mg i/m	5+1.5 mg i/m	10+0.5mg i/m

ANNEXURE – 6

EUTHANASIA OF LABORATORY ANIMALS

(A – Methods Acceptable NR – Not Recommended)

Species		Mouse	Rat	Hamster	Guinea pig	Rabbit	Cat	Dog	Primate
a) PHYSICAL METHODS									
Electrocution		NR	NR	NR	NR	NR	NR	NR	NR
Exsanguination		NR	A	A	A	A	A	NR	NR
Decapitation (for analysis of stress)		A	A	A	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		A	A	A	A	A	A	NR	NR
Halothane		A	A	A	A	A	A	A	A
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/P)	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

*** Cervical dislocation is not allowed in rats weighing more than 200gms.IP = Intra Peritoneal, IV= Intra Venous, IM = Intra Muscular**

METHODS NOT ACCEPTABLE FOR EUTHANASIA OF ANY SPECIES OF ANIMAL:-

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 (vii) Air Embosium

For Cattle, Equine, Swine, Sheep & Goat, Thiopentone Sodium is given three times the anaesthetic dose for that species.

Equines

Sedation with acepromazine I/M / xylazine I/M followed by Thiopentone sodium I/V or Potassium chloride 10% solution I/V.

Livestock

Sedation with xylazine I/M and ketamine I/V followed by 10% potassium chloride/Thiopentone sodium

Swine

For swine restraining with Gallamine I/M, followed by sedation with xylazine, followed by Potassium chloride/Thiopentone sodium I/V.

NOTE

Potassium chloride 10% solution must be given intravenously at a very fast pace after proper sedation of the animal.

Barbiturates (Thiopentone) at three times the anaesthetic dose can be administered for euthanasia.

Annexure - 7

QUALIFICATIONS & KNOWLEDGE REQUIRED FOR LABORATORY ATTENDANT

a. Certificate of animal handling and welfare from any recognised institution.

b. 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), 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).

c. 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.

d. Handling of animals - precautions while handling animals - common injuries and ailments in animals - litter - weaning - maintenance - record keeping.

e. 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.

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

Annexure – 8 (for reference)

Institutional Biosafety Committee (IBSC)

(a) 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

(b) 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 shouldbe made and may be subjected to inspection on accountability.

The biosafety functions and activity include the following:

(a) 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.

(b) 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.

(c) Tailoring biosafety program to the level of risk assessment.

(d) Training of personnel on bio safety.

(e) 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, at 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.

(f) 3 Adopting emergency plans.

References

Handbook of Laboratory Animal Science, 2nd Edition, Volume 1: Essential Principles and Practices, J. Hau and G. L. Van Hoosier, Jr., CRC Press, 2002.

Handbook of Laboratory Animal Science, 2nd Edition, Volume 2: Animal Models, J. Hau and G. L.Van Hoosier, Jr., CRC Press, 2002.

Management of Laboratory Animal Care and Use Programs, M. A. Suckow, F. A. Douglas and R. H.Welchbrod, CRC Press, 2001.

UFAW Handbook on the Care and Management of Laboratory Animals, 2 volumes, T. B. Poole(editor), 7th Edition, Blackwell Science, 1999.

Guide for the Care and Use of Laboratory Animals: National Research Council, National Academy Press, 1996. Guide to the Care and Use of Experimental Animals, Canadian Council onAnimal Care, Volume 1, 2nd Edition, 1993.

Handbook of Facilities Planning, Laboratory Animal Facitilies, T. Ruys (editor), Van Nostrand Reinhold, 1991. Laboratory Animal Medicine, J. G. Fox, B. I. Cohen and F.M. Loew, editors, Academic Press, 1984.

PAIN & DISTRESS

Appendix I of the New Zealand "Good Practice Guide for the Use of Animals in Research, Testing and Teaching" entitled "Pain: Some Concepts and Definitions".

Chapter X of the Canadian Council on Animal Care "Guide to the Care and Use of Experimental Animals", Volume 1, 2nd Edition, 1993.

Recognising and Assessing Pain, Suffering and Distress in Laboratory Animals – A Survey of Current Practice in the UK with Recommendations by Penny Hawkins, Research Animals Department, RSPCA.

EUTHANASIA

Monograph entitled "Euthanasia of Animals used for Scientific Purposes" by the Australian and NewZealand Council for the Care of Animals in Research and Teaching (ANZCCART), 1993.

Chapter XII of the Canadian Council on Animal Care "Guide to the Care and Use of

(a) Experimental Animals", Volume 1, 2nd Edition, 1993. Report of the AVMA Panel on Euthanasia,1993

GUIDELINES ON THE REGULATION OF SCIENTIFIC EXPERIMENTS ON ANIMALS

INTRODUCTION

1.1 Background

The use of animals in scientific research has been an area of concern in India, given the sharp polarization of views between animal welfare activists and the scientific community of the country regarding use of animals. This led to proliferation of litigation, which impeded the pace of research.

In order to eliminate the potential for conflict, it was considered necessary to examine the international norms regarding the use of animals in scientific experiments, update regulations, streamline and simplify procedures, while ensuring ethical use of animals and reducing infliction of pain and stress on animals, during experimentation.

1.2 Process of Evolution of the Guidelines:

Against this backdrop, in 2004, the Ministry of Environment and Forests set out to create a sound and cohesive regulatory framework for the use of animals in experimentation. A consultative Group was set up, to facilitate interaction with a wide spectrum of stakeholders, both within and outside the government, including the scientific community, as also animal welfare activists. To clarify the underlying ethical principles, a professor of Philosophy was also associated in the exercise.

Recognizing the intrinsic worth of animals as sentient beings, the consultative Group enunciated the underlying ethical principles and identified objectives of s scientific experiments which would justify the use of animals in the cause of scientific advancement and promoting human welfare while ensuring humane treatment of such animals.

Deliberations of the Group led to a consensus between hitherto divergent viewpoints. Six brainstorming sessions were held, wherein the principles and practices of utilization and care of animals in testing, research and training were finalized.

The report of the consultative Group was communicated to the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CCSEA) in terms of Section 17 (3) of the Prevention of Cruelty to Animals Act, 1960. The report was accepted by CCSEA in to, in its meeting held on 20 December 2004, and formed the basis of the Breeding of and Experiments on Animals (control and supervision) Amendment rules, 2006. The report has been well received and its impact may be noted from the fact of speedy settlement of pending court cases and absence of any new court case.

However, in order to clarify various aspects regarding the use of experimental animals, there was a perceived need for a comprehensive set of Guidelines that could be used as reference material by the scientific establishments regarding ethical use of animals in scientific experiments. The present Guidelines respond to that need.

1.3 Aim

The aim of these Guidelines is to ensure humane and ethical treatment of animals, while facilitating legitimate scientific research involving experiments on animals.

2. Statutory provisions regarding scientific experiments on animals

Persons engaged in conducting scientific experiments on animals must act in conformity with the provisions of the prevention of Cruelty to Animals Act, 1960, and the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended.

These provisions are enforced by the independent Committee for the Purpose of Control and supervision of Experimentation on animals (CCSEA), a statutory body under the Prevention of Cruelty to Animals Act, 1960, in the Ministry of Environment and Forests.

2.1 Other legal provisions regarding animal experimentation

Compliance is also required with CCSEA Guidelines for Laboratory animal facility.

3. Principles for scientific experiments on animals, relevant changes in Rules and guidelines for specific situations evolved by the Consultative Group accepted by CCSEA

3.1 Ethical principles adopted by CCSEA for use of animals in scientific experiments

Principle 1

"Experiments on animals" (including experiments involving operations on animals) may be carried out for the purposes of advancement by new discovery of physiological knowledge; or of knowledge which is expected to be useful for saving or prolonging human life or alleviating suffering; or for significant gains in the well-being for the people of the country; or for combating any disease, whether of human being, animals or plants.

Principle 2

Animals lowest on the phylogenetic scale (i.e., with the least degree of sentience), which may give scientifically valid results, should be used for any experimental procedure. Experiments should be designed with the minimum number of animals to give statistically valid results at 95% level of confidence. Alternatives not involving animal testing should be given due and full consideration and sound justification provided, if alternative, when available, are not used.

Principle 3

Proper use of animals in experiments and avoidance or minimization (when avoidance is not possible) of pain and suffering inflicted on experimental animals should be an issue of priority for research personnel, and unless the contrary is scientifically established, investigators should process on the basis that procedures that cause pain or suffering in human beings will also cause similar pain or suffering in animals. All scientific procedures adopted with animals that may cause more than momentary or slight pain and/or suffering should be performed with appropriate sedation, analgesia or anaesthesia.

Principle 4

Persons engaged in animal experimentation have a moral responsibility for the welfare of the animals after their use in experiments. Investigators are responsible for the aftercare and/or rehabilitation of animals after experimentation, and may be permitted to euthanize

Animals only in the following situations:

(a) When the animal is paralyzed and is not able to perform its natural functions; it becomes incapable of independent locomotion; and/or can no longer perceive the environment in an intelligible manner.

(b) During the course of experimental procedure the animal has been left with a severe recurring pain and the animal exhibits obvious signs of long term extreme pain and suffering.

(c) In situations where non-termination of the animal experimented upon would be life threatening to human beings or other animals.

Costs of aftercare and/or rehabilitation of animals post-experimentation are to be part of research costs and should be scaled per animal in positive correlation with the level of sentience of the animals.

Principle 5

The living conditions of animals should be appropriate for their species and contribute to their health and comfort. The housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist in a relevant discipline who is trained and experienced in the proper care, handling, and use of the species being maintained or studied. In all circumstances, veterinary care shall be provided as necessary.

3.2.1 CCSEA Guidelines on specific aspects regarding the use of animals in scientific experiments

3.2.1 Need to avoid/minimize pain and suffering inflicted on experimental animals

Proper use of animals in experiments and avoidance or minimization (when avoidance is not possible) of pain and suffering inflicted on experimental animals should be an issue of priority for research personnel, and unless the contrary is scientifically established, investigators should proceed on the basis that procedures that cause pain or suffering in human beings will also cause similar pain or suffering in animals. All scientific procedures adopted with animals that may cause more than momentary or slight pain and/or suffering should be performed with appropriate sedation, analgesia or anaesthesia.

3.2.2 Proper care, handling and use of experimental animals

The living conditions of animals should be appropriate for their species and contribute to their health and comfort. The housing, feeding, and care of all animals used for biomedical purposes must be directed by a veterinarian or other scientist in a relevant discipline who is trained and experienced in the proper care, handling, and use of the species being maintained or studied. In all circumstances, veterinary care shall be provided as necessary.

3.2.3 Agricultural production research

The conventional regulatory framework may not be applied regarding use of experimental animals in agricultural production research. The practitioners would be responsible for self-regulation, based on operational guidelines to be framed by CCSEA.

3.2.4 Powers of the Institutional Animals Ethics Committee (IAEC)

IAEC is not empowered to clear research project proposals that involve experiments on animals higher on the phylogenetic scale than rodents.

3.2.5 Inspection of animal house facilities

Both announced and unannounced visits by duly authorized personnel (only) to inspect the animal house facilities of institutes may be carried out. However, the personnel undertaking inspections may not order either temporary or permanent closure of the animal house facility, or suspension of registration of the animal facility, or impose any other penalty, but must report their finding to the CCSEA for further action.

4. Procedures for approval of scientific experiments on animals

4.1 Definition of experiment In terms of Rule 2 (e) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, "Experiments" means any programme or project involving use of animal(s) for the acquisition of knowledge of a biological, physiological, ethological, physical or chemical nature; and includes the use of animals(s) in the production of reagents and products such as antigens and antibodies, routine diagnostics, testing activity and establishment of transgenic stocks, for the purpose of saving or prolonging life or alleviating suffering, or significant gains in the well-being for people of the country or for combating any disease, whether of human beings, animals or plants.

4.2 Experimental animals which are subject to regulation The relative sentience of different species of animals are as follows:

Invertebrates (e.g., cockroaches) <Birds <Rodents <Canines/Felines <Bovine/Equines <Primates (e.g., Rhesus Macaque)<More evolved Primates (e.g., chimpanzee)

Anything higher than invertebrates in terms of level of sentience requires regulation. Thus rats, mice, birds, and farm animals are also subject to regulation.

4.3 Function of CCSEA

All establishments engaged in research and education involving animals, are required to comply with the various guidelines, norms and stipulations set out by CCSEA.

The main functions of CCSEA are:

- Registration of establishments conducting animal experimentation or breeding of animals for this purpose.
- Selection and appointment of nominees in the Institutional Animal Ethics Committees of registered establishments.
- Approval of Animal House Facilities on the basis of reports of inspections conducted by CCSEA.
- Permission for conducting experiments involving use of animals.
- Recommendation for import of animals for use in experiments.
- Action against establishments in case of violation of any legal norm/stipulation.

4.4 Functions of the Institutional Animals Ethics Committee (IAEC) Every establishment constituted and operated in accordance with the procedures specified by CCSEA is required to constitute an Institutional Animals Ethics Committee (IAEC).

In terms of Rule 13 of the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998, as amended, every IAEC shall include a biological scientist, two scientists from different biological disciplines, a veterinarian involved in the care of animals, the scientist in charge of the animal facility of the establishment concerned, a scientist from outside the institute, a non-scientific socially aware member and a representative or nominee of the CCSEA. A specialist may be co-opted while reviewing special projects using hazardous agents such as radioactive substances and deadly micro organisms.

IAEC may approve experiments on animals, up to the phylogenetic level of rodents (e.g. mice, rats and rabbits). However, IAEC is not empowered to clear research project proposals that involve experimentation on animals higher on the phylogenetic scale than rodents. In such cases, IAEC may consider proposals for scientific experiments involving animals above the sentience level of rodents, and forward its recommendations for consideration by CCSEA.

4.5 Registration of establishments

In terms of Rule 3 of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, no establishment shall carry on the business of breeding of animals or trade of animals for the purpose of experiments unless it is registered, In terms of Rule 4 of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, no establishment shall perform any experiment on animals unless it is registered with CCSEA. Every such establishment shall stop performing experiments on animals or breeding of animals for use in experiments, if registration is refused to it by CCSEA.

The establishment involved in breeding of animals and birds for laboratory purpose has to be registered however, the establishments breeding the farm animals and poultry for slaughter purpose shall not be liable for registration with CCSEA.

4.6 Approval of animal house facilities

In terms of Rule 5 of the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998, as amended, approval of animal house facilities by CCSEA is required to be obtained, for premises where experiments are to be conducted.

4.7 Use of animals in experiments

In terms of Rule 9 (bb) of the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998, as amended, animals lowest on the phylogenetic scale which may give scientifically valid results should be first considered for any experimental procedure, and the experiment should be designed with the minimum number of animals to give statistically valid results at 95% degree of confidence.

Replacement alternatives, not involving experiments on animals, should be given due and full consideration and sound justification must be provided, in case alternatives, though available, are not used.

4.8 Procurement of animals

In terms of Rule 10 of the Breeding of and Experiments on Animals (Control and Supervision) Rules 1998, as amended,

(i) an establishment shall acquire animals for experiments from registered breeders only;

(ii) in case of non-availability of animals from registered breeders, the animals may be procured from alternative legal sources;

(iii) in case the animal is procured from alternative legal sources, the same shall be procured after taking written permission from the authority competent under the law for the time being in force, to give such permission; and Replacement alternatives, not involving experiments on animals, should be given due and full consideration, and sound justification must be provided, in case alternatives, though available, are not used.

4.9 Welfare of animals during use in experiments

In terms of Rule 9 (cc) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, personnel using the experimental animals shall be responsible for the welfare of the animals during their use in experiments. The CCSEA Guidelines for Laboratory Animal Facility also spell out the baseline procedures to be followed when using animals in the course of scientific experimentation, including quarantine and animal care.

4.10. Aftercare and rehabilitation of animals after use in scientific experiments

In terms of Rule 9 (cc) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, Investigators shall be responsible for the aftercare and rehabilitation of the animals after experimentation.

Costs of aftercare and rehabilitation of the animals after experimentation shall be made part of research costs and shall be scaled in positive correlation with the level of costs involved in such aftercare and rehabilitation of the animals.

Rehabilitation treatment of an animal after experimentation shall extend till the point the animals is able to resume a normal existence by providing a lump-sum amount as costs for rehabilitation and care of such animal to cover its entire statistical expected life span; and

The establishment undertaking experiments or duly licensed and authorized animal welfare organizations under the control of the Committee may, on payment of lump-sum amount, undertake rehabilitation of animals.

4.11. Situations where euthanasia of animals is permissible

In terms of Rule 9 (cc) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, Investigators shall not euthanize animals except in situations as defined below:

(i) When the animal is paralyzed and is not able to perform its natural functions or it becomes incapable of independent locomotion or it can no longer perceive the environment in an intelligible manner; or

(ii) If during the course of experimental procedure the animal has been left with a recurring pain wherein the animal exhibits obvious signs of pain and suffering; or

(iii) Where the non-termination of the life of the experimental animal will be life threatening to human beings or other animals.

4.12. Suspension/revocation of registration of an establishment by CCSEA

Rule 14 of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended, provides as follows:

(a) The Committee, if it is satisfied with the report of the Member-Secretary or the authorized officer of the Committee (made to it as a result of any inspection or information received or otherwise) that-

(i) the rule made by it are not being complied with by an establishment or breeder; or

(ii) a violation of the directions of the Committee has been committed by any establishment or breeder and the Committee's directions to rectify such violation have not been complied within the period so specified,

The Committee may, by order in writing, suspend or revoke the registration of the establishment or breeder and / or direct the closure of the animal house facility for such a period as may be specified in the order:

Provided that no order under this clause shall be made without giving the establishment or breeder any opportunity of being heard in the matter.

Provided further that no order for suspension or revocation of registration or closure of animal house facility shall be issued in a case of minor violation.

Explanation: For the purpose of this clause, "minor violation" means an act of commission or omission which does not have direct bearing on the health of an animal; which may not lead to adverse health effect or pain or suffering or death of an animal.

APPENDIX

Relevant changes in Rules based on recommendations of the Consultative Group Based on the ethical principals so enunciated, the Consultative Group recommended changes in the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended. These were further deliberated upon, and duly incorporated after the Report of the Consultative Group was accepted *intoto* by CCSEA. The changes in the relevant Rules are summarized as follows:

1. Change in Rule 2 (e) in the Breeding of and Experiments on Animals (Control and Supervision0 Rules 1998, as amended

The definition of experiments has been widened to include the term "significant gains in the well-being of the people of the country", as additional criteria justifying the use of animals in experiment.

2. Insertion of Rule 9 (bb) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended

This addition provides that preference be according to the use of the minimum number of animals, lowest in the phylogenetic scale, which provide for statistically valid results at 95% degree of confidence. Use of replacement/alternatives is encouraged and sound justification is required in case alternatives to use of animals are not used, when available.

3. Insertion of Rule 9 (cc) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended

This provision makes the personnel using animals in experiments responsible for their welfare after use in experimentation, including aftercare and rehabilitation and also makes it mandatory for the costs of aftercare and rehabilitation to be made part of the research costs, as a lump sum provision based on the statistically expected life span of the animals. Rehabilitation may be undertaken by the establishment or by a duly licensed and authorized animal welfare organization.

4. Insertion of Rule 9 (ff) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended

This provides for the specific parameters, which are to be adopted when considering euthanasia of any animal used in scientific experiments. These include impairment of the natural functions of the animal including independent locomotion, when the animal faces recurring pain and suffering, and when the non termination of the life of the experimental animal would be life threatening of humans or other animals.

5. Amendment of Rule 10 (b) of the Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, as amended

This amendment allows the establishment to procure animals from any other legal source in case of non-availability with registered breeders, with suitable documentationto establish legality of the procurement process.

6. Amendment of Rule 10 (e) of the Breeding of and Experiments on Animals(Control and Supervision) Rules, 1998, as amended.

This provision allows the establishment to import genetically defined animals with the permission of DGFT, in case such animals are not available with registered breeders or other legal sources within the country. The condition of non-availability will not apply to genetically defined or laboratory bred rats and mice.

7. Amendment of Rule 12 in the Breeding of and Experiments on Animals (Controland Supervision) Rules, 1998, as amended

This Rule has been amended to allow establishments to undertake contract research as per the provisions of the PCA Act 1960 and the rules made there under.

8. Amendment of Rule 14 in the Breeding of and Experiments on Animals (Controland Supervision) Rules, 1998, as amended

The Rule has been amended to allow CCSEA to take action against an establishment or breeder, based on the report of the Member Secretary or authorized officer, regarding any violations of the rules, or of directions of the Committee. In case of a major violation, CCSEA may by written orders, suspend or revoke the registration of the establishment and / or order closure of the animal house facility, after giving the establishment or breeder an opportunity of being heard in the matter.

Study Requisition Form

☐Collaboration ☐Industry ☐Academia ☐ SBV

Study Title	:					
Principle Investigator	:					
Department/ Organisation	:		Mail id Contact no	: :		
Documents attached	:	☐Letter from Head/Organisation			☐IAEC* - Certificate and proposal	
		☐IHEC* - Certificate and proposal			☐ Detailed Proposal	

**Study will be initiated only after the approval of the ethical committee*

Study Details

Toxicity			
In vivo Studies Rodents	:	Administration Route	☐Oral ☐ Dermal ☐Eye
		Study Type	☐Acute ☐ Sub-acute ☐Sub-Chronic ☐Chronic
		Biochemistry	☐Blood ☐ Urine ☐ Plasma ☐ Serum ☐ Saliva
		Pathology	☐Histopathology ☐Immunohistochemistry ☐Special staining specify:
In vitro Studies	:	Mammalian	☐ Chromosomal Aberration Test ☐ Micronucleus Test
		Microbial	☐ AMES Test
Ecotoxicity Studies	:	Zebrafish	☐ Fish Toxicity ☐ Fish Embryo Toxicity
Efficacy			
In vivo Studies	:	Species: Strain:	Study:
In vitro Studies	:	Cells/cell line:	Study:
Others			

Signature and seal
Date:

(For Office Use Only)

S.No	Study Initiation Date#	Study Completion Date	Other Experimental Details	MGMPRC staff assigned	Amount to be charged#

#*subject to MGMARI norms*

Signature and seal
Date:

Form B (per rule 8(a)* for Submission of Research Protocol (s)

Application for Permission for Animal Experiments

Application to be submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC)

Section -I

1.	Name and address of establishment	Mahatma Gandhi Medical Advanced Research Institute (MGMARI), SBV Campus, Pillaiyarkuppam, Pondy-Cuddalore Main road, Pondicherry – 607402
2.	Registration number and date of registration.	2223/PO/ReRc/S/23/CCSEA & 11.12.2023
3.	Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in parts B & C	CCSEA approved vendor
4.	Place where the animals are presently kept (or proposed to be kept).	Mahatma Gandhi Medical Preclinical Research Centre (MGMPRC), Mahatma Gandhi Medical Advanced Research Institute (MGMARI), SBV Campus, Pillaiyarkuppam, Pondy-Cuddalore Main road, Pondicherry – 607402
5.	Place where the experiment is to be performed (Please provide CPCSEA Reg. Number)	Mahatma Gandhi Medical Preclinical Research Centre (MGMPRC), Mahatma Gandhi Medical Advanced Research Institute (MGMARI), SBV Campus, Pillaiyarkuppam, Pondy-Cuddalore Main road, Pondicherry – 607402
6.	Date and Duration of experiment.	
7.	Type of research involved (Basic Research / Educational/ Regulatory/ Contract Research)	

Signature

Date:
Place:

Name and Designation of Investigator

Section -II

Protocol form for research proposals to be submitted to the Institutional Animal Ethics Committee/ CPCSEA, for new experiments or extensions of ongoing experiments using animals.

1. Project / Dissertation / Thesis Title:

2. Principal Investigator / Research Guide / Advisor: Principal Investigator
   a) Name :
   b) Designation :
   c) Department :
   d) Telephone No :
   e) E-mail Id :
   f) Experience in Lab animal experimentation :
3. List of all individuals authorized to conduct procedures under this proposal.

4. Funding Source / Proposed Funding Source with complete address (Please attachthe proof)
5. Duration of the animal experiment.
   a. Date of initiation (Proposed) –
   b. Date of completion (Proposed) –
6. Describe details of study plan to justify the use of animals (Enclose Annexure)
7. Animals required
   a) Species and Strain :
   b) Age and Weight :
   c) Gender :
   d) Number to be used (Year-wise breakups and total figures needed to begiven in tabular form)
   e) Number of days each animal will be housed:
8. Rationale for animal usage
   a) Why is animal usage necessary for these studies?
   b) Whether similar study has been conducted on *in vitro* models? If yes,describe the leading points to justify the requirement of animal experiment.

c) Why are the particular species selected?

d) Why is the estimated number of animals essential?

e) Are similar experiments conducted in the past in your establishment?

f) If yes, justify why new experiment is required?

g) Have similar experiments been conducted by any other organization insame or other *in vivo* models? If yes, enclose the reference.

9. Describe the procedures in detail:

   a) Describe all invasive and potentially stressful non-invasive procedures thatanimals will be subjected to in the course of the experiments)

   b) Furnish details of injections schedule Substances:

      Doses :

      Sites :

      Volumes :

   c) Blood withdrawal Details:

      Volumes :

      Sites :

   d) Radiation (dosage and schedules):

   e) Nature of compound/Broad Classification of drug/NCE (the chemical characteristic details of NCE and its likely reaction to the biological system and characteristic details of invitro study of that NCE have to be submitted by the establishment) :

10. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedures? If yes, justify.

11. Will survival surgery be done?

    If yes, the following to be described

    a) List and describe all surgical procedures (including methods of asepsis)

    b) Names, qualifications and experience levels of personnels involved.

    c) Describe post-operative care

    d) Justify if major survival surgery is to be performed more than once on a single animal.

12. Describe post-experimentation procedures.

    a) Scope for Reuse :

b) Rehabilitation (Name and Address, where the animals are proposed to be rehabilitated):

c) Describe method of Euthanasia:

d) Method of carcass disposal after Euthanasia:

13. Describe animal transportation methods if extra-institutional transport is envisaged.

14. Use of hazardous agents (use of recombinant DNA-based agents or potential human pathogens requires documented approval of the Institutional Biosafety Committee (IBC). For each category, the agents and the biosafety level required, appropriate therapeutic measures and the mode of disposal of contaminated food, animal wastes and carcasses must be identified).

If, your project involved use of any of the below mentioned agent, attach copy of the approval certificates of the respective agencies:

(a) Radionucleotides (AERB)

(b) Microorganisms / Biological infectious Agents (IBSC)

(c) Recombinant DNA (RCGM)

(d) Any other Hazardous Chemical / Drugs

Investigator's Declaration

1. I certify that the research proposal submitted is not unnecessarily duplicative of previously reported research.
2. I certify that, I am qualified and have experience in the experimentation on animals.
3. For procedures listed under item 10, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any procedure described herein which may cause less pain or distress.
4. I will obtain approval from the IAEC/ CPCSEA before initiating anychanges in this study.
5. I certify that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / otherbody).
6. I certify that I will submit appropriate certification of review and concurrence for studies mentioned in point 14.
7. I shall maintain all the records as per format (Form D) and submit to Institutional Animal Ethics Committee (IAEC).
8. I certify that, I will not initiate the study before approval from IAEC/ CPCSEA received in writing. Further, I certify that I will follow the recommendations of IAEC/ CPCSEA.
9. I certify that I will ensure the rehabilitation policies are adopted (wherever required).

Signature

Name of Investigator

Date: ( )

Certificate

This is to certify that the project entitled “ ” has been approved by the IAEC having IAEC approval No……

Authorized by	Name	Signature	Date
Chairman:	…….	……	……..
Member Secretary:	…….	……	……..
Main Nominee of CPCSEA:	…….	……	……..

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

Annexure-I

1. **Aim of the study**:
2. **Objective of the study**
3. **Describe details of study plan to justify the use of animals.**
4. **Experimental Design**
5. **Justifiable Parameters**
6. **Reference**

Form D

Record of Animals Acquired and Experiments performed

1. **Title of the Project:**
2. **Study No:**

Date	No. of animals acquired (specify species, sex and age	Name, Address and registration No. of the Breeder from who acquired with voucher/bill no.	Date and IAEC approval number	Duration Of Experiment	Name and address of the person authorized to conduct the experiment	Signature of the investigator certifying that all conditions specified for such an experiment has been complied.