GUIDELINES SERIES-II

GENERAL GUIDELINES FOR SAFETY/TOXICITY EVALUATION OF AYURVEDIC FORMULATIONS

CENTRAL COUNCIL FOR RESEARCH IN AYURVEDIC SCIENCES
Ministry of AYUSH, Government of India
New Delhi

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Other Related Guidelines :

Volume-I : General Guidelines for Drug Development of Ayurvedic Formulations
Volume - III : General Guidelines for Clinical Evaluation of Ayurvedic Interventions

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PROLOGUE

R&D in the field of AYUSH system in different areas such as drug development including quality assurance, pre-clinical safety evaluation and clinical research are being conducted at different levels such as Research Council under AYUSH, Academic institutions (both AYUSH and non AYUSH institutes such as Medical Colleges, Universities etc.), other Research organization such as ICMR, CSIR etc. Further, research support is also being extended through grant under EMR vide Ministry of AYUSH, DST, DBT, ICMR etc. in the area of traditional medicine.

Lot of research is being conducted at different levels as above in the field of AYUSH adopting different guidelines, methods and protocols and ending up research outcomes with low or poor translational value. Only few of them have led to clinical trial and marketing level. This may be attributed to lack of awareness regarding AYUSH strategies for R&D and provisions of Drug & Cosmetic Act related to AYUSH.

In spite of availability of several guidelines such as GCP guidelines for ASU drugs, ICMR guidelines for bio medical research for human participants, GCP guidelines published by CDSCO Ministry of Health and Family Welfare, WHO guidelines for traditional medical research etc., there is no single comprehensive guidline to conduct research in AYUSH sector is available.

This might be one among the major reasons that has led to R&D in AYUSH sector with diverse approaches with low translational value.

In view of this, it becomes imperative to develop directives on research practices for various components of AYUSH research sectors for uniform adoption across all stake holders such as research councils, academic institutes, funding agencies engaged in AYUSH research.

CCRAS developed three comprehensive and concise Guidelines focusing on drug development (Standardization and quality assurance), safety/toxicity and clinical evaluation for ready reference of stakeholders. These Guidelines encompassed research practices generally to be adopted and followed by researchers in the field of AYUSH system such as research organizations, academic institutions and researchers seeking grant from EMR/IMR schemes of different funding agencies for research on AYUSH system, and would help the researchers while designing and formulating the proposals and also planning academic/ industrial research in the field of AYUSH systems. The users may refer to other two documents for having an overall idea concerning drug development and R & D in this field.

BACKGROUND

Quests for healthy and long life are perhaps as old as human existence and efforts are unremitting to address the challenges and triumph over the bottlenecks across this journey. Ayurveda –the science of life, evolved as a comprehensive system of healthcare systematically through scientific experimentations of high order backed by sound and reproducible evidence base and stood the test of the time. Several strategies and road maps are being drawn to carry forward merits of this science so as to meet the current day heath needs and mainstream its core strengths alongside through research & development in this country and across the globe. The fundamental aspects of holistic systems needs adequate positioning, while designing clinical trials to examine the safety and efficacy of Ayurveda. Furthermore, the other challenges and issues related to quality, safety, dosage forms/delivery system, diverse concepts and complex approaches in trial design, diagnosis and therapy, outcomes of clinical efficacy and drug interactions also pose certain limitations in research. A systems approach may be adopted to validate the therapies with integration of principles of Ayurveda and bio-medicine without losing the vital fundamentals of both systems. Such an approach with well designed research plans could possibly facilitate to generate tangible evidence.

The roots of experimentation for ensuring safety of food and drugs have been in vogue since ancient times. There are evidences that Ayurveda is well versed with the preclinical testing i.e. *Vishanna/Virudhanna Pareeksha* conducted on animals. *Sushruta* mentioned the animal experiments like testing the food/drug by giving it to the animal like birds (pigeon, peacock), animals (rabbit, monkey) in order to establish the safety and toxicity. *Charak Samhita* elaborates the importance of safety of a medicine by defining that medicine is safe and effective, if it alleviates the disease and does not produce any concomitant bad effect. In Ayurveda, plants, animal products and minerals based medicines are used for healthcare and treatment of various disease conditions.

It was known to ancient Ayurvedic scholars that metals, minerals and some plants are toxic and harmful to the body and therefore, it was advocated to process them properly so as to render them therapeutically safe. Ayurveda pharmaceutics strongly recommend various other safety aspects, which are known for their contemporary relevance, like Good Agricultural Practices (GAP), Good Field Collection Practices (GFCP) of medicinal plants and Good Manufacturing Practices (GMP) for preparation of quality assured drugs. Factors like place, soil, season and time for collection of natural drugs also play a significant role in the quality, strength and purity of drugs.

With tremendous expansion in the use of natural products, globally, safety and efficacy as well as quality control of ASU drugs have become pivotal concern for both health authorities and public in general.

Nonclinical evaluation of drug safety usually consists of standard animal toxicology studies. Animal toxicity studies need to be carried out to assess the potential undesirable pharmaco-dynamic effects of drugs on physiological functions in relation to exposure within the therapeutic range.

The development of a drug is a stepwise process involving an evaluation of both animal and human efficacy and safety information. The goals of the nonclinical safety evaluation generally include characterization of toxic effects with respect to target organs, dose dependence, relationship to exposure, and, when appropriate, potential reversibility. This information is used to estimate an initial safe starting dose and dose range for the human trials and to identify parameters for clinical monitoring for potential adverse effects.

Before conducting any toxicological testing in animals, the study should be approved by the Institute Animal Ethics Committee (IAEC) or the protocol should satisfy the guidelines of the local governing body. In India, the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines should be followed for the maintenance of experimental animals.

However, Ayurvedic drugs are prescribed considering various aspects viz. *Anupana*, Dose, Dosage, Age etc. which may play pivotal role in their therapeutic potential. Further, it is pertinent to say that some drugs are used after processing such as *Shodhna, Marana.* Therefore, safety/ toxicity studies of such drugs may only be conducted adopting similar Ayurvedic procedures. Hence, there is a need for fundamentally different approach for toxicological studies that need to be adopted for ASU drugs.

Owing to the importance of safe use of ASU drugs, Ministry of AYUSH has issued guidelines for licensing of these drugs vide Gazette notification Rule 158B, dated 10th august, 2010 wherein, the safety requirements for different categories of ASU drugs are described.

Despite existence of several guidelines such as General Methodologies & Research evaluation Traditional Medicines, OECD guidelines, Schedule Y of Drug and Cosmetic Act etc., GCP Guidelines for ASU Medicines, there is a need to evolve a comprehensive guideline to address system specific issues for conducting safety/toxicity studies of ASU drugs. The present document would certainly serve as a ready reference for researchers engaged in Research and Drug development in ASU systems.

LIST OF ABBREVIATIONS

LD_{50} : Median Lethal Dose
NOEL: No Observed Effect Level
NOAEL: No Observed Adverse Effect Level
OECD: Organization of Economic Cooperation and Development
CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals
IAEC: Institutional Animal Ethics Committee
GLP: Good Laboratory Practice
ABHF: Animal Breeding and Housing Facility
WBC: White blood cell count
RBC: Red blood cell count
HGB: Hemoglobin concentration
HCT: Hematocrit
MCV: Mean corpuscular volume
MCH: Mean corpuscular hemoglobin
MCHC : Mean corpuscular hemoglobin concentration
CHCM: Cellular hemoglobin concentration mean
CH: Cellular hemoglobin
HDW: Hemoglobin concentration distribution width
RDW: Red cell distribution width
PLT: Platelet count
MPV: Mean platelet volume
DLC: Differential leucocytes count
NAD: No abnormalities detected
XALB: Albumin
TBIL: Total Bilirubin
CHOL: Cholesterol
CREA: Creatinine
GLU : Glucose
TP: Total Protein
TGL : Triglycerides
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALP: Alkaline Phosphatase
GGT : Gamma glutamyl transferase
CPK : Creatinine phosphokinase
LDH: Lactate dehydogenase
COA : Certificates of Analysis
M: E : Myeloid: Erythroid ratio

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Design of study and Formulation of Clinical protocols (7) (considering (As per current guidelines and adopting Classical methodology.) Finalization with task force of experts

Bulk preparation of quality assured Drug for clinical trial , packing labeling etc. as per need at

DRUG DEVELOPMENT PHASES (2-8)

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Botanical identification/ Pharmacogonostic/Chemical studies of ingredients. (3) (based on available guidelines and classical methodology.)

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Animal Studies for biological activity/ efficacy (specific/mechanism of action activity for clinical co relation) **(6)**

Execution of Clinical Trial (8)

Approval of IEC/IRB and CTRI registration
Trial conduct
Trial monitoring
Trial coordination
Data analysis
Publication

Note: IPR Protection and issues of filing of patent to be addressed at suitable stage.

Table 1: Research criteria for evaluating the safety/toxicity of ASU drugs

S. No.	Category	Ingredient(s)	Indication(s)	Requirement of Non-clinical Safety data	Requirement of Non-clinical Efficacy Data
(1)	(2)	(3)	(4)	(5)	(6)
Classical ASU drugs as defined under Section 3 (a) of the Drugs and Cosmetics Act, 1940					
1.	1.1 Ayurvedic, Siddha and Unani drugs given in 158B as referred in Section 3(a) of Drugs and Cosmetics Act, 1940	As per text	As per text	Not Required	Not Required
	1.2 Any change in dosage form of ASU Drugs as described in Section 3(a) of Drugs and Cosmetics Act, 1940	As per text	As per text	Not Required	Not Required
	1.3 ASU Drugs referred in section 3(a) of Drugs and Cosmetics Act, 1940 to be used for new indication*	As per text	New	Not Required	Required
Patent or Proprietary Drugs as defined under section 3(h) of the Drugs and Cosmetics Act, 1940					
2.	2.1 Patent or Proprietary Drugs as defined under section 3(h) of Drugs and Cosmetics Act, 1940 containing crude drugs /Aqueous Extract(s) / Hydro-Alcoholic Extract(s).	As per text	Textual rationale	Not required	Not required

	2.2 Patent or Proprietary Drugs as defined under section 3(h) containing **other than** Aqueous and Hydro-alcoholic extract(s) / any other solvent based extract(s)*	As specified	As specified / claimed	**Required:** **For Oral preparations*-** 1. Single dose toxicity test (Acute toxicity) in mice and rats. 2. Repeated-dose Systemic Toxicity Studies (long term toxicity studies in rats. 3. Reproductive and Developmental Toxicity Studies 4. Genotoxicity 5. Carcinogenicity *metal associated toxicity in case of any metal/mineral as one of the ingredient **For topical preparations-** a) Dermal toxicity study. b) Photo-allergy or dermal photo-toxicity. c) Allergenicity / Hypersensitivity in guinea pigs	Required
	2.4 Patent or Proprietary Drugs as defined under section 3(h) containing any of the ingredients of Schedule E (1) of the D&C Act, 1940	As per text	Indication as claimed / specified	**For oral preparations-** 1. Single dose toxicity test (Acute toxicity) in mice and rats. 2. Repeated-dose Systemic Toxicity Studies (long term	**Required**

		toxicity studies) in two species one rodent(rat) and one non-rodent Rabbit/dog. 3.Reproductive and Developmental Toxicity Studies 4. Genotoxicity 5. Carcinogenicity *metal associated toxicity in case of any metal/mineral as one of the ingredient **For Topical preparations-** a) Dermal toxicity study. b) Photo-allergy or dermal photo-toxicity. c) Allergenicity / Hypersensitivity in guinea pigs	

2.1 SINGLE-DOSE ACUTE TOXICITY STUDY

SINGLE-DOSE TOXICITY STUDIES (ACUTE TOXICITY): Acute toxicity studies aim to determine toxic manifestations of the test substance that occur when animals are exposed to one or more doses within a 24-hour period.

2.1.1 Animal species: These studies should be carried out in two rodent species, mice and rats

2.1.2 Sex: Both, males and females (nulliparous and non pregnant) should be used

2.1.3 Number of animals: Each group should consist of at least six animals per sex.

2.1.4 Dose levels and route of administration: The limit of 2gm/kg or at least 10 times of the intended clinical therapeutic dose whichever is less, using the same route as recommended for human.

2.1.5 Frequency of administration: The test substance should be administered in one or more doses during a 24-hour period.

2.1.6 Study observations: Toxic signs and the severity, onset, progression and reversibility of the signs and mortality, if any, for 14 days after administration of test compound.

Additional observations will be necessary if the animals continue to display signs of toxicity. Observations should include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress should be humanely killed. When animals are killed for humane reasons or found dead, the time of death should be recorded as precisely as possible. Autopsy of any animal which dies during study period has to be done. If necessary, a histopathological examination should be conducted on any organ/tissue showing macroscopic changes at autopsy.

2.1.7 Result:

- Body weight/body weight changes;
- Tabulation of response data and dose level for each animal (i.e., animals showing signs of toxicity including nature, severity, duration of effects, and mortality);
- Individual weights of animals at the day of dosing, in weekly intervals thereafter, and at the time of death or sacrifice ;
- Time course of onset of signs of toxicity and whether these were reversible for each animal;
- Necropsy findings and any histopathological findings for each animal, if available;
- Maximum tolerated dose may be calculated.
- Statistical treatment of results (description of computer routine used and spreadsheet tabulation of calculations).

3. REPEATED-DOSE ORAL TOXICITY STUDY

3.1 Animal species: These studies should be carried out in two mammalian species of which one should be a non-rodent.

3.2 Sex: Males and females should be used.

3.3 Number of animals: In case of rodents, each group should consist of atleast ten males and ten females. In case of non-rodents, each group should consist of at least three males and three females.

3.4 Route of administration: It should be same as recommended for human.

3.5 Dose levels: The study is required to be done with three dose levels. High dose level should produce observable toxicity (to be selected on the basis of Maximum Tolerated dose calculated in Single-Dose Acute Toxicity Study)while low dose levels should not cause observable toxicity. Within this dose levels the addition of at least one more dose may enhance the possibility of observing a dose response relationship. In addition, vehicle control group should be included.

3.6 Period of exposure: The duration of exposure to study drug should be as per the intended therapeutic duration.

S. No.	Duration of proposed human administration	Period of exposure in toxicity study
1.	Single dose	2 weeks
2.	More than 2 weeks-less than 4 weeks	4 weeks
3.	More than 4 weeks-less than 12 weeks	12 weeks
4.	More than 12 weeks-less than 24 weeks	24 weeks
5.	More than 24 weeks	Same as that of expected period of use of the trial drug

3.7 Recovery phase: In order to investigate the recovery phase from toxic changes, 50% of animals in each group are allowed to live for, atleast for 15 days or varying length of time after cessation period of administration of the test substance, should be examined.

3.8 Observations: The following observations at various intervals should be recorded in any toxicity tests and the same has to be reported in the final report.

3.8.1 General signs: General signs should include monitoring of Home Cage Activity viz., Behavior, Convulsions, Biting, Locomotor activity, Tremors, Eye Prominence, Hair Coat, Lacrimation, Salivation, Respiration Character & Rate, Fecal excretion, Urine output at frequent intervals at least twice in a week.

3.8.2 Body weight: Recording of body weight at least once a week before the start of drug administration should be done. Once the test material administration is started body weights have to be monitored weekly once for first three months followed by every four weeks in case of experimental duration is more than three months.

3.8.3 Food intake: Food intake should be measured at least once a week before the start of drug administration. Once the process of test material administration is initiated, Food

intake is recorded weekly once for first three months followed by every four weeks in case of experimental duration is more than three months.

3.8.4 Clinical Chemistry: The clinical chemistry profile in urine and blood samples should be performed at various intervals during experimental period.

i. Qualitative Urinalysis include monitoring of color, glucose, protein, bile pigment, urobilinogen, occult blood etc., in a fixed number of animals from each group before and at end of exposure phase to test compound in randomly collected urine samples.

ii. Blood Biochemistry include estimation of glucose, lipid profile, liver and kidney function tests along with electrolytes in all group of animals within 48 hours of last exposure to test material and autopsy . In addition, the above parameters must be estimated in post exposure (recovery) group of animals before termination of experiment.

Clinical Chemistry		
1.	Urine qualitative	Appearance, Colour, Glucose, Bilirubin, Ketone, Specific Gravity, Blood, pH, Protein, Urobilinogen, Nitrites &Leucocytes
2.	General parameters	Plasma Glucose, Serum Albumin/Globulin, Total Proteins
3.	Liver function tests	Serum-Alkaline Phosphatase, Bilirubin, SGOT,SGPT,AST
4.	Renal function tests	Blood Urea, Serum Creatinine
5.	Lipid profile	Serum-Cholesterol, HDL, Triglyceride
6.	Electrolytes	Serum-Na^{+}, K^{+}, Ca^{++}.

3.8.5 Haematological examination: Blood samples should be taken from all groups of animals within 48 hours of last exposure to test material and before autopsy to monitor hemoglobin, complete blood picture, differential count etc. In addition, the below mentioned parameters must be estimated in post exposure (recovery) group of animals before termination of the experiment.

Clinical Haematology				
Hemoglobin (Hb/HGB)	Total Red Blood Cell (RBC) count	Hematocrit (HCT)	Reticulocyte count	Total White Blood Cell (WBC) count
Differential White Blood Cell (WBC) COUNT	Platelet Count (PLT)	Terminal bone marrow examination	ESR (Non-Rodents only)	General Blood picture: A special mention of abnormal and immature cells should be made
Coagulation Parameters (Non-rodents only): Bleeding Time, Coagulation Time, Prothrombin Time, Activated Partial Thromboplastin Time.				

3.8.6 Other Function tests: If appropriate, ECG, visual, and auditory tests should be performed. For rodents, ophthalmological examination should be performed on a fixed number of animals from each group at least once during the administration period; for non-rodents, examination should be performed on all animals before the start of drug administration and at least once during the period of administration.

3.8.7 Necropsy and histopathological examination: A macroscopic examination of organs and tissues in all group animals within 48 hours of last exposure to test material should be performed. The organ weights must be measured wherever possible. The full histopathological examinations should be performed in an attempt to identify the severity or degree of the changes in all major and targeted organs. Similarly in all post exposed group of animals gross necropsy and histopathological examination should be conducted. Animals found dead during the examination should be autopsied as soon as possible in an attempt to identify the cause of death and the nature (severity or degree) of the toxic changes present.

Necropsy & histopathological examination			
Brain*: Cerebrum Cerebellum, and Midbrain	(Spinal Cord)	Eye	(Middle Ear)
Thyroid	(Parathyroid)	Spleen*	Thymus
Adrenal*	(Pancreas)	(Trachea)	Lung*
Heart*	Aorta	Oesophagus	Stomach
Duodenum	Jejunum	Terminal ileum	Colon
(Rectum)	Liver*	Kidney*	Urinary bladder
Epididymis	Testis*	Ovary	Uterus*
Skin	Mammary gland	Mesenteric lymph node	Skeletal muscle
* Organs marked with an asterisk should be weighed. () Organs listed in parenthesis should be examined if indicated by the nature of the drug or observed effects.			

3.9 Estimation of Metals: Heavy metal estimation will be carried out in blood and tissue homogenate in case the ASU drug contains any metal/mineral as one of the ingredient. The details are as follows:

3.9.1 Arsenic Estimation: Tissue and blood arsenic concentration were measured by the method of Stahr, (1977).

Procedure: 1.0 g of tissue or 1.0 ml of whole blood was weighed and digested with concentrated nitric acid in microwave sample preparation system. After completion of digestion, the contents were transferred to a 10 ml of volumetric flask and the digestion flask were rinsed with distilled water and poured this water into volumetric flask to make up the volume to10 ml. Arsenic concentrations were measured at 193.7nm using graphite furnace of atomic absorption spectrophotometer. The instrument was set up for maximum sensitivity and a mixture of air-acetylene was used as a fuel. A mixture

of Palladium chloride ($PdCl_2$) and Magnesium nitrate ($MgNO_3$) was used as a matrix modifier. The standards were prepared for each biological matrix from arsenic solution (Merck, Darmstadt, Germany). The recovery based on the standards carried through the same digestion procedure and were 86.5%±5 for blood, 90.8%±8 for liver kidney and brain. For evaluation of the accuracy of the analytical results standard Indian Reference Material (BND-301) supplied by National Physical Laboratory, New Delhi, India) were analyzed together with the samples.

3.9.1 Trace metal (Zinc and Copper etc) Estimation: Zinc and copper contents were measured by the method of Parker *et al.*, (1967).

Procedure: 1.0 g of tissue or 1 ml of whole blood was weighed and digested with concentrated nitric acid in microwave sample preparation system (CEM, MDS 2100, USA). After complete digestion, the contents were transferred to a 10 ml of volumetric flask and the digestion flask were rinsed with distilled water and poured this water into volumetric flask to make up the volume to 10 ml. Zinc and copper were read at 213.9, 324.8 nm wavelength respectively using hollow cathode lamp using atomic absorption spectrophotometer (Perkin Elmer, Model AAnalyst 100, USA). The instrument was set up for maximum sensitivity and a mixture of air acetylene was used as a fuel. The standards were prepared for each biological matrix from zinc, copper solution (Merck, Darmstadt, Germany) and carried through the digestion procedure with the tissue specimens. The recovery was 98 %, 89 % for zinc and copper.

3.9.2 Gold and Mercury Estimation: The samples were blot dried prior to digestion. Analysis was carried out as per AOAC (1998) guidelines. For non-volatile heavy metals (Au) weighed tissue sample was digested with digestion mixture containing conc.HNO3:HClO4 (6:1) under controlled temperature in a fume chamber until white residue was obtained. The residue was dissolved and made up to 10ml with 0.1 N HNO3 in a volumetric flask. The solutions were then analyzed on atomic absorption spectrophotometer .All the samples were processed in triplicates. For volatile-heavy metal (Hg), weighed amount of tissue sample was digested in 10 ml conc.H2SO4 overnight. The digested samples were incubated in water bath at 70° C for 1 hr. Samples were then titrated with aq. KMnO4 in ice bath. After the solution reached room temperature, 1 ml of hydroxyl ammonium chloride (20%) was added to reduce KMnO4. This solution was filtered, volume made up to 100 ml with deionised water, analyzed on an atomic absorption spectrophotometer with Vapour Generation Assembly. Mixed working standard (1 and 10 µg/ml) solutions were freshly prepared by diluting the stock solutions of 1000 µg/ml (Merck India). Blanks and spiked samples were also processed and analyzed simultaneously. For estimating Hg in blood, 1ml of anti coagulated whole blood was mixed with 3ml of 5:2:1 mixture of 70% nitric acid, 70% perchloric acid and 98% sulfuric acid in 10 ml graduated test tube. The digestion mixture was warmed to 40°C for 60 minutes and heated at 90°C for at least 60 minutes with frequent mixing until the brown fumes of oxides of nitrogen dissipated and remaining liquid turns golden yellow in color. Volume was made up to 5 ml with deionised ultra pure water. Analysis was done on an atomic absorption spectrophotometer with Vapour Generation Assembly.

3.9.3 Lead Estimation: Lead was estimated by method of Dhanlakshmi et al. (2013). 1.0 g of tissue or 1 ml of whole blood was digested (wet ashing method) with 10 ml of HNO3 (65%) and 2ml of H2O2 (30%) in acid prewashed Teflon vessels. After standing overnight, samples were digested using microwave lab stations with the following program: 250 W, 1min; 0 W, 1 min; 250 W, 6 min; 400 W, 5 mi n; 600 W, 5 min. After sufficient cooling, samples were moved to Teflon vessels and diluted to 25 ml with distilled-deionized water. Analytical blanks were prepared with each batch of digestion set. Inductively coupled plasma- optical emission spectrometry (ICP-OES) was used for estimating the heavy metals. The sample was prepared in a solution form so that it could be easily aspirated through the nebulizer and the concentration of the elements to be determined was provided between 0.1 to 0.5 absorbance units, about 2ml of solution was utilized for each measurement. Lead concentration was read at 220.353 nm wavelength. The working standards were analyzed at the beginning and end of a run and periodically during longer runs. According to the absorbance, the concentration was measured directly, when the sample was well within the linear working range.

4. REPRODUCTION AND DEVELOPMENTAL TOXICITY STUDIES

These studies need to be carried out for all ASU drugs defined under section 3 (h) of the Act containing crude drugs/Aqueous and Hydro-alcoholic extracts(s) (without textual rationale or not as per text)/ any other solvent based extract(s), ingredients of Schedule E (1) of the Rules, proposed to be studied or used in women of child bearing age. The species and strains for these studies should be selected so as to obtain information on any adverse events, if any, on reproductive and developmental function. The preference of animals are mice or rats, and second species, if required, is rabbit.

These studies have to be conducted in the same species and strains and will be referred as Segment I (Female Fertility Study), Segment II (Teratogenicity Study) and Segment III (Perinatal study).

4.1 Segment I. (Female Fertility Study): In this study, test drug should be administered before mating. The drug treatment should continue during mating and subsequently, during the gestation period.

4.1.1 Animals: At least one species of animal of both sexes such as rats or mice should be used.

4.1.2 Number of animals: In the case of rats or mice, each group should consist of at least 20 males and 20 females.

4.1.3 Route of administration: The route of administration ordinarily will be same as the intended clinical route of administration.

4.1.4 Dose levels: The study is required to be done with three dose (intended therapeutic, average, and high) levels of test material, in addition to following control groups.

i. Negative control group of animals should normally receive vehicles or emulsifiers alone which are intended to make formulation.
ii. Positive control group of animals should receive a substance known to have potent reproductive and developmental toxicity,
iii. Comparative control group is desirable. The comparative group of animals should receive a drug with a similar chemical structure or pharmacological effects as the tested drug.

4.1.5 Period of Exposure: When rats or mice are used, males at least 40 days of age should be dosed daily for 60 days or more before mating, and administration should be continued until successful copulation. Sexually mature females should be dosed daily for at least 14 days before mating, during mating and after successful copulation until the beginning of organogenesis.

4.1.6 Observations:

i. During the experimental period, mortality should be recorded, general signs noted and body weights and food intake should be measured.
ii. A treated male and a treated female should be housed together and observed daily for confirmation of successful copulation.
iii. The mating period between the male and female pairs should be about two weeks. If necessary, a treated male and a non-treated female, or a treated female and a

non-treated male should be housed together and observed daily for confirmation of successful copulation.

iv. After successful copulation, females should be autopsied at term, and examined for the number of corpora lutea, successful pregnancies and mortality of fetuses. Additionally, a gross examination of the organs and tissues for all dams should be made.

v. Males used for mating and females without successful copulation should be autopsied at an appropriate time, and gross observation on organs and tissues should be made.

4.2 Segment II (Teratogenicity Study): In this study, the test drug should be administered throughout the period of organogenesis.

4.2.1 Animals: Females of at least one species of rodent and a non-rodent such as rabbits should be used.

4.2.2 Number of animals: Each group should consist of at least 30 animals for rats or mice and at least 12 animals for rabbits.

4.2.3 Route of administration: The route of administration should ordinarily be that expected clinically.

4.2.4 Dose levels: The study is required to be done with three dose (intended Therapeutic, Average, and High) levels of test material, in addition to following control groups.

i. Negative Control group of animals should normally receive vehicles or emulsifiers alone which are intended to make formulation.

ii. Positive control group of animals should receive a substance known to have potent reproductive and developmental toxicity,

iii. Comparative control group is desirable. The comparative group of animals should receive a drug with a similar chemical structure or pharmacological effects as the tested drug.

4.2.5 Observations:

i. During the experimental period, mortality, general signs, body weights and food intake should be measured for all dams.

ii. In the case of rodents such as rats or mice, approximately 2/3 of the dams in each group, and in the case of non-rodents such as rabbits, all the dams in each group should be autopsied at term. They should be examined for successful pregnancy and mortality of fetuses. Observation parameters should include: (Dams) signs of intoxication, effect on body weight, effect on food intake, examination of uterus, ovaries and uterine contents, number of corpora lutea, implantation sites, resorptions (if any); and for the fetuses, the total number, gender, body length, weight and gross/visceral/ skeletal abnormalities, if any. Gross observations on organs and tissues should be made for dams.

iii. For rats or mice, etc., the remaining approximately 1/3 of the dams should be allowed to deliver their offspring. Dams should be examined for abnormality on delivery.

iv. Litter size, mortality, sex and external changes of neonates should be examined, and body weights should be measured.

v. Offspring should be examined for growth and development, appearance of specific signs, reproductive performance, etc. Growth and development should be recorded and morphological, functional and behavioural examinations should be made. Reproductive performance of the offspring, that is, the ability to establish pregnancy, should be examined. If necessary, observation for a longer period should be made.

vi. At an appropriate time, autopsy and gross observation of the organs and tissues of treated dams should be made. If necessary, an examination of the second litters should be done.

4.3 Segment III. (Perinatal study): Study on administration of the test substance during the perinatal and lactation periods

4.3.1 Animals: At least one species of female animals such as rats or mice should be used. Species should be selected from among those used in the study of administration of the test substance during organogenesis specified in the segment II study.

4.3.2 Number of animals: Each group should consist of at least 20 animals for rats or mice.

4.3.3 Route of administration: The route of administration should be the expected clinical route as a rule.

4.3.4 Dose levels: The study is required to be done with three dose (intended Therapeutic, Average, and High) levels of test material, in addition to following control groups.

i. Negative Control group of animals should normally receive vehicles or emulsifiers alone which are intend to make formulation.

ii. Positive control group of animals should receive a substance known to have potent reproductive and developmental toxicity,

iii. Comparative control group is desirable. The comparative group of animals should receive a drug with a similar chemical structure or pharmacological effects as the tested drug.

4.3.5 Observation:

i. During the experimental period, all the dams in each group should be examined for mortality and general signs and body weights and food intake should be measured.

ii. All the dams in each group should be allowed to deliver and nurse their offspring. Dams should be examined for abnormality on delivery.

iii. Litter size, mortality, sex and external changes of neonates should be examined, and body weights should be measured.

iv. Offspring should be examined for growth and development, appearance of specific signs, reproductive performance, etc. For observation of growth and development, morphological, functional and behavioral examinations should be made. Reproductive performance of offspring should be examined on the basis of establishment of pregnancy. If necessary, observation for a longer period should be made.

v. At an appropriate time, autopsy and gross observations on organs and tissues should be made on treated dams. If necessary, an examination of the second litters should be done.

4.3.6 Analysis of results:

i. The results obtained should be presented in the form of tables and figures with discussion of the results. For presentation, summary tables which give an overview of the results of all groups should be prepared. In addition, appendix tables which provide data for individual animals in each group should be prepared for reference.

ii. For statistical analysis of the data obtained before weaning, it is desirable that the litter, instead of the individual fetus or offspring, serve as the unit for analysis.

iii. The discussion should address the no-effect dose level of the test substance concerned with the reproduction of the parent animals and development of the next generation. It is desirable to compare the reproductive and developmental toxicity with that of similar drugs.

5. SPECIAL TOXICITY TESTS

Test substance (described in ASU in 158-B as referred in Section 3(a), 3(h), and any of the ingredients of Schedule E (1) of Drugs and Cosmetics Rules, 1945 intended to be administered for chronic illnesses or otherwise over a long period of time (six months to one year) may be necessary to detect genotoxicity (early tumorigenic) effects and induce carcinogenicity.

5.1 Genotoxicity: Genotoxicity tests are conducted *in-vitro* and *in-vivo* to detect compounds which induce genetic damage directly or indirectly. These tests should enable hazard identification with respect to damage to DNA and its fixation. Genotoxicity data are not required before Phase I and II trials. But these studies should be completed before applying for Phase III trials. The following are the *in-vitro* and *in-vivo* studies generally expected to be conducted:

i. A test for gene mutation in bacteria.
ii. An *in-vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells or an in vitro mouse lymphoma tk assay.
iii. An *in- vivo* test for chromosomal damage using rodent hematopoietic cells.

5.1.1 Test Methods: Ames' Test (Reverse mutation assay in Salmonella): S. typhimurium tester strains such as TA98, TA100, TA102, TA1535, TA97 or Escherichia coli WP2 uvrA or Escherichia coli WP2 uvrA (pKM101) should be used.

i. *In-vitro* exposure (with and without metabolic activation, S9 mix) should be done at a minimum of 5 log dose levels. "Solvent" and "positive" control should be used. Positive control may include 9-aminoacridine, 2-nitrofluorine, sodium azide and mitomycin C, respectively, in the tester strains mentioned above. Each set should consist of at least three replicates. A 2.5 fold (or more) increase in number of revertants in comparison to spontaneous revertants would be considered positive.
ii. *In-vitro* cytogenetic assay: The desired level of toxicity for in vitro cytogenetic tests using cell lines should be greater than 50% reduction in cell number or culture confluency. For lymphocyte cultures, an inhibition of mitotic index by greater than 50% is considered sufficient. It should be performed in CHO cells or on human lymphocytein culture. In-vitro exposure (with and without metabolic activation, S9 mix) should be done using a minimum of 3 log doses. "Solvent" and "positive" control should be included. A positive control like Cyclophosphamide with metabolic activation and Mitomycin C for without metabolic activation should be used to give a reproducible and detectable increase clastogenic effect over the background which demonstrates the sensitivity of the test system. Each set should consist of at least three replicates. Increased number of aberrations in metaphase chromosomes should be used as the criteria for evaluation.
iii. *In-vivo* micronucleus assay: One rodent species (preferably mouse) is needed. Route of administration of test substance should be the same as intended for humans. Five animals per sex per dose groups should be used. Atleast three dose levels, plus "solvent" and "positive" control should be tested. A positive control

like mitomycin C or cyclophosphamide should be used. Dosing should be done on day 1 and 2 of study followed by sacrifice of animals 6 hours after the last injection. Bone marrow from both the femora should be taken out, flushed with fetal bovine serum (20 min.), pelletted and smeared on glass slides. Giemsa-MayGruenwald staining should be done and increased number of micronuclei in polychromatic erythrocytes (minimum 1000) should be used as the evaluation criteria.

iv. *In-vivo* cytogenetic assay: One rodent species (preferably rat) is to be used. Route of administration of test substance should be the same as intended for humans. Five animals/sex/dose groups should be used. At least three dose levels, plus "solvent" and "positive" control should be tested. Positive control may include cyclophosphamide. Dosing should be done on day 1 followed by intra-peritoneal colchicine administration at 22 hours. Animals should be sacrificed 2 hours after colchicine administration. Bone marrow from both the femora should be taken out, flushed with hypotonic saline (20 min.), pelletted and resuspended in Carnoy's fluid. Once again the cells should be pelletted and dropped on clean glass slides with a Pasteur pipette. Giemsa staining should be done and increased number of aberrations in metaphase chromosomes (minimum 100) should be used as the evaluation criteria.

5.2 Carcinogenicity Test: Carcinogenicity studies should be done in a rodent species (preferably rat). Mouse may be employed only with proper scientific justification. The selected strain of animals should not have a very high or very low incidence of spontaneous tumors. This test includes Preliminary and full scale carcinogenicity study.

5.2.1 Preliminary Test: This study is performed to determine the dose levels for the full-scale carcinogenicity study. In case sufficient reliable data is available, the following studies may be omitted.

i. Single dose toxicity studies are performed in small number of animals in order to determine the highest dose to be used in the repeated dose studies.

ii. Repeated dose toxicity studies are performed in order to determine highest dose to be used in the full- scale carcinogenicity study.

5.2.1.1 Animals: At least two species of animals of both sexes should be used. It is desirable to initiate normal animals of same age but no more than six weeks.

5.2.1.2 Number of animals: In case of rodents, each group should consist of at least ten males and ten females. In case of non-rodents, each group should consist of at least three males and three females.

5.2.1.3 Route of administration: The route of administration should be same as intended for human.

5.2.1.4 Dose levels: The study is required to be done with three dose levels with vehicle control groups.

5.2.1.5 **Period of Exposure:** The duration of exposure to study drug should be for 90 days. In case the drug has delayed or cumulative effect exposure should be for longer period.

5.2.1.6 **Observations:** The animals in each group, general signs should be monitored daily along with recording of body weight and food intake at least once a week.

5.2.1.7 **Necropsy & Histopathological Examination:** The organs and tissues of animals found dead during the examination should be autopsied as soon as possible in an attempt to identify the cause of death and the nature (severity or degree) of the toxic changes followed by histopathological examination.

5.2.1.8 **Study Results:** The dose in the preliminary carcinogenicity study that inhibits body weight gain less than 10% in comparison with the control and causes neither death due to toxic effects nor remarkable changes in general signs and laboratory examination findings of animals. This is the highest dose to be used in full-scale carcinogenicity study.

5.2.2 **Full - Scale Study:** The study can be conducted with the highest dose obtained in preliminary investigation.

5.2.2.1 **Animals**: At least two species of animals of both sexes should be used. It is desirable to initiate normal animals of same age but no more than six weeks.

5.2.2.2 **Number of animals:** Each group should consist of at least fifty males and fifty females.

5.2.2.3 **Route of administration:** The route of administration should be same as intended for human.

5.2.2.4 **Dose levels:** The study is required to be done with three dose levels with untreated and vehicle control group.

5.2.2.5 **Period of Exposure:** The duration of exposure to study drug should be for 24 months for rats and 18 months for mice.

5.2.2.6 **Observations:**

i. For all the animals in each group, general signs should be monitored daily along with recording of body weight and food intake at least once a week.

ii. **Blood Biochemistry**; include estimation of glucose, lipid profile, liver and kidney function tests along with electrolytes (Table-3) in all group of animals in the initial stage of experiment and before autopsy.

iii. **Haematological examination:** Blood samples should be taken from all groups of animals to monitor hemoglobin, complete blood picture, differential count etc. (Table-4) at initial stage of experiment and before autopsy.

iv. **Necropsy & Histopathological Examination:** A macroscopic examination of organs and tissues (Table-5) in all groups of animals within 48 hours of last exposure to test materials should be performed. The organ weights must be measured wherever possible. The full histopathological examinations should be performed in an attempt to identify the severity or degree of the changes in all major and targeted organs. Similarly in all post exposed group of animals, gross necropsy and histopathological examination be conducted. Animals found dead

during the examination should be autopsied as soon as possible in an attempt to identify the cause of death and the nature (severity or degree) of the toxic changes present. The organs and tissues of animals found dead during the examination should be autopsied as soon as possible in an attempt to identify the cause of death and the nature (severity or degree) of the toxic changes followed by histopathological examination.

5.2.2.7 Study Results: A test substance is considered to be positive for carcinogenicity when any of the following types of response has been observed in the carcinogenicity study:

i. Development of tumours of a type not seen in the control group.
ii. Development of tumours seen with greater frequency in the test group, compared with the control group.
iii. Greater varieties of organs and tissues are involved in tumour development in the test group, compared with the control group.
iv. Earlier development of tumours in the test group, though in the absence of any significant difference in the incidence of tumours between the test group and the control group.

5.3 Local Toxicity Test for Topical preparations: Local Toxicity Investigations of the test substance are required for the following:

1). ASU drugs as referred to in the section 3(h) of the D&C Act containing crude drugs or Aqueous / Hydro-alcoholic extracts without textual rationale or not as per text;
2). ASU drugs containing other solvent based extracts;
3). ASU drugs containing ingredients of Schedule- E (1) of the D&C Rules, 1945.

Application(s) of the test substance to an appropriate site (e.g., skin or vaginal mucous membrane) as topical/dermatological preparations are required for local toxicity investigations.

5.3.1 Preparation of Test Formulations:

i. Solid preparations: To be prepared by wetting the preparations with water or a suitable solvent to provide a uniform application.
ii. Semi-solid preparations: To be tested as undiluted preparations.
iii. Liquid preparation: To be tested as undiluted preparations. However, an aerosol agent can be diluted if necessary.

5.3.2 Dermal toxicity study: The study should be done in rabbit and rat. Daily topical (dermal) application of test substance in its clinical dosage form should be done. Test material should be applied on shaved skin covering not less than 10% of the total body surface area. Porous gauze dressing should be used to hold liquid material in place. Formulations with different concentrations (at least 3) of test substance, several fold higher than the clinical dosage form should be used. Period of application may vary from 7 to 90 days depending on the clinical duration of use. Where skin irritation is grossly visible in the initial studies, a recovery group should be included in the subsequent repeated-dose study. Local signs (erythema, oedema and Eschar

formation) as well as histological examination of sites of application should be used for evaluation of results.

5.3.3 Photo-allergy or dermal photo-toxicity: It should be tested by Armstrong/ Harber Test in guinea pig. This test should be done if the drug or a metabolite is related to an agent causing photosensitivity or the nature of action suggests such a potential (e.g., drugs to be used in treatment of leucoderma). Pretest in 8 animals should screen 4 concentrations (patch application for 2 hours ±15 min.) with and without UV exposure (10 J/cm2). Observations recorded at 24 and 48 hours should be used to ascertain highest non-irritant dose. Main test should be performed with 10 test animals and 5 controls. Induction with the dose selected from pretest should use 0.3 ml/patch for 2 hour ±15 min. followed by 10 J/cm2 of UV exposure. This should be repeated on day 0, 2,4,7,9 and 11 of the test. Animals should be challenged with the same concentration of test substance between day 20 to 24 of the test with a similar 2-hour application followed by exposure to 10 J/cm2 of UV light. Examination and grading of erythema and oedema formation at the challenge sites should be done 24 and 48 hours after the challenge. A positive control like musk ambrett or psoralin should be used.

5.3.4 Vaginal Toxicity Test: Study is to be done in rabbit or dog. Test substance should be applied topically (vaginal mucosa) in the form of pessary, cream or ointment. Six to ten animals per dose group should be taken. Higher concentrations or several daily applications of test substance should be done to achieve multiples of daily human dose. The minimum duration of drug treatment is 7 days (more according to clinical use), Subject to a maximum of 30 days. Observation parameters should include swelling, closure of introitus and histopathology of vaginal wall.

5.3.5 Rectal Tolerance Test: For all preparations meant for rectal administration this test may be performed in rabbits or dogs. Six to ten animals per dose group should be taken. Formulation in volume comparable to human dose (or the maximum possible volume) should be applied once or several times daily, per rectally, to achieve administration of multiples of daily human dose. The minimum duration of application is 7 days (more according to clinical use), Subject to a maximum of 30 days. Size of suppositories may be smaller, but the drug content should be several fold higher than the proposed human dose. Observation parameters should include clinical signs (sliding on backside), signs of pain, blood and/or mucus in faeces, condition of anal region/sphincter, gross and (if required) histological examination of rectal mucosa.

5.3.6 Other local toxicity tests: Other local toxicity tests may be conducted if the herbal medicine is intended for such use eg. Inhalation toxicity tests, Ocular toxicity tests etc.,

5.3.7 Allergenicity/ Hypersensitivity: Standard tests include guinea pig maximization test (GPMT) and local lymph node assay (LLNA) in mouse. Any one of the two may be done.

5.3.7.1 Guinea Pig Maximization Test: The test is to be performed in two steps; first, determination of maximum non-irritant and minimum irritant doses, and second, the main test. The initial study will also have two components. To determine the intradermal induction dose, 4 dose levels should be tested by the same route in a

batch of 4 male and 4 female animals (2 of each sex should be given Freund's adjuvant). The minimum irritant dose should be used for induction. Similarly, a topical minimum irritant dose should be determined for challenge. This should be established in 2 males and 2 females. A minimum of 6 male and 6 female animals per group should be used in the main study. One test and one control group should be used. It is preferable to have one more positive control group. Intradermal induction (day 1) coupled with topical challenge (day 21) should be done. If there is no response, re-challenge should be done 7-30 days after the primary challenge. Erythema and oedema (individual animal scores as well as maximization grading) should be used as evaluation criteria.

5.3.7.2 Local Lymph Node Assay: Mice used in this test should be of the same sex, either only males or only females. Drug treatment is to be given on ear skin. Three graded doses, the highest being maximum non-irritant dose plus vehicle control should be used. A minimum of 6 mice per group should be used. Test material should be applied on ear skin on three consecutive days and on day 5, the draining auricular lymph nodes should be dissected out 5 hours after i.v. 3H-thymidine or bromo-deoxy-uridine (BrdU). Increase in 3H-thymidine or BrdU incorporation should be used as the criterion for evaluation of results.

6. REFERENCES:

1. Drugs and Cosmetics Act, 1940 & Drugs & Cosmetics Rules 1945.
2. WHO guidelines for traditional system of medicine, 2000/ revised 2014.
3. WHO Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines, 1993.
4. Handbook Non-Clinical Safety Testing - UNICEF/UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR).
5. Operational guidance: Information needed to support clinical trials of herbal products UNICEF/UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR).
6. US Food and Drug Administration's Guidance for Industry Botanical Drug Products Published on June 2004.

7. SUGGESTIVE READINGS:

1. European Medicines Agency's CPMP (CPMP/SWP/1042/99 Rev 1) guideline on "Guideline on Repeated dose toxicity", adopted on 01st September 2010.
2. OECD Guideline for the Testing of Chemicals No. 407, "Repeated Dose 28-day Oral Toxicity Study in Rodents" adopted on 3rd October, 2008.
3. OECD Guideline for the Testing of Chemicals No. 408, "Repeated Dose 90-day Oral Toxicity Study in Rodents" adopted on 21st September, 1998.
4. OECD Guideline for the Testing of Chemicals No. 425, "Acute Oral Toxicity – Up-and-Down Procedure (UDP)" adopted on 3rd October, 2008.
5. OECD Guideline for the Testing of Chemicals No. 420, "Acute Oral Toxicity – Fixed Dose Procedure" adopted on 17th December, 2001.
6. OECD Guideline for the Testing of Chemicals No. 423, "Acute Oral Toxicity – Acute Toxic Class Method" adopted on 17th December, 2001.
7. OECD Guideline for the Testing of Chemicals No. 414, "Prenatal Development Toxicity Study" adopted on 22nd January, 2001.
8. OECD Guideline for the Testing of Chemicals No. 415, "One-Generation Reproduction Toxicity Study" adopted on 26th May, 1983.
9. OECD Guideline for the Testing of Chemicals No. 416, "Two-Generation Reproduction Toxicity" adopted on 22nd January, 2001.

8. GLOSSARY

1. **Dose:** The amount of test substance administered. The dose is expressed as weight of test substance per unit body weight of test animal per day (e.g. mg/kg body weight/day), or as a constant dietary concentration.
2. **Dosage:** It is a general term comprising of dose, its frequency and the duration of dosing.
3. **Evident toxicity:** It is a general term describing clear signs of toxicity following administration of test substance. These should be sufficient for hazard assessment and should be such that an increase in the dose administered can be expected to result in the development of severe toxic signs and probable mortality.
4. **NOAEL:** It is the abbreviation for no-observed-adverse-effect level. This is the highest dose level where no adverse treatment-related findings are observed due to treatment.
5. **Developmental toxicology:** The study of adverse effects on the developing organism that may result from exposure prior to conception, during prenatal development, or postnatally to the time of sexual maturation. The major manifestations of developmental toxicity include 1) death of the organism, 2) structural abnormality, 3) altered growth, and 4) functional deficiency.
6. **Adverse effect:** Any treatment-related alteration from baseline that diminishes an organism's ability to survive, reproduce or adapt to the environment.
7. **Implantation (nidation):** Attachment of the blastocyst to the epithelial lining of the uterus, including its penetration through the uterine epithelium, and its embedding in the endometrium.
8. **Acute oral toxicity**: It refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.
9. **LD_{50} (median lethal dose):** It is a statistically derived single dose of a substance that can be expected to cause death in 50 per cent of animals. The LD_{50} value is expressed in terms of weight of test substance per unit weight of test animal (mg/kg).

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स्वास्थ्य और परिवार कल्याण मंत्रालय

[आयुर्वेद, योग व प्राकृतिक चिकित्सा, यूनानी, सिद्ध एवं होम्योपैथी विभाग (आयुष)]

अधिसूचना

नई दिल्ली, 10 अगस्त, 2010

सा.का.नि. 663(अ).—जबकि औषधि और प्रसाधन सामग्री नियमावली, 1945 में और संशोधन करने के लिए प्रस्तावित नियमों के मसौदे को उक्त अधिसूचना संबंधी सरकारी राजपत्र की सार्वजनिक की गई प्रतियों की तारीख से पैंतालीस दिनों की अवधि की समाप्ति से पूर्व उससे प्रभावित होने वाले सभी व्यक्तियों के आक्षेपों और सुझावों को आमंत्रित करते हुए स्वास्थ्य और परिवार कल्याण मंत्रालय में भारत सरकार की अधिसूचना सं. सा.का.नि. 377(अ), तारीख 3 मई, 2010 के द्वारा भारत के राजपत्र, असाधारण में प्रकाशित किया गया था;

और जबकि, उक्त राजपत्र को 4 मई, 2010 को सार्वजनिक किया गया था;

और जबकि, उक्त मसौदा नियमावली पर लोगों से प्राप्त आक्षेपों और सुझावों पर केंद्र सरकार द्वारा विचार किया गया है;

अब, अतएव, केंद्र सरकार औषधि और प्रसाधन सामग्री अधिनियम, 1940 (1940 का 23) की धारा 33-ङ द्वारा प्रदत्त शक्तियों का प्रयोग करते हुए औषधि और प्रसाधन सामग्री नियमावली, 1945 में निम्नवत् और संशोधन करती है, अर्थात् :—

नियमावली

1. (1) इन नियमों का नाम औषधि और प्रसाधन सामग्री (छठा संशोधन) नियम, 2010 है ।

(2) ये राजपत्र में प्रकाशन की तारीख से प्रवृत्त होंगे ।

2. औषधि और प्रसाधन सामग्री नियमावली, 1945 में (इसके पश्चात् उक्त नियम के रूप में उल्लिखित), नियम 158-क के पश्चात् निम्नलिखित नियम रखे जाएंगे, अर्थात् :

158 (ख) आयुर्वेद, सिद्ध या यूनानी औषधियों के संबंध में अनुज्ञा पत्र जारी करने हेतु दिशा निर्देश ।

1. (क) धारा 3 (क) के अंतर्गत आयुर्वेद, सिद्ध, यूनानी औषधियां :

आयुर्वेद, सिद्ध या यूनानी औषधियों में आंतरिक अथवा बाह्य उपभोगार्थ अथवा रोग के निदान, उपचार, न्यूनीकरण अथवा निवारण अथवा मानवों अथवा पशुओं में विद्यमान विकारों तथा औषधि और प्रसाधन सामग्री अधिनियम, 1940 की पहली अनुसूची में यथा विनिर्दिष्ट आयुर्वेदिक, सिद्ध और यूनानी (तिब्ब) चिकित्सा पद्धतियों की प्राधिकृत पुस्तकों में उल्लिखित फार्मूले के अनुसार अनन्य रूप से विनिर्मित सभी औषधियाँ शामिल होती हैं ।

(ख). धारा 3 (ज) के अंतर्गत पेटेंट अथवा स्वामित्वाधीन औषध

(i) प्रथम अनुसूची में विनिर्दिष्ट आयुर्वेद, सिद्ध अथवा यूनानी तिब्ब चिकित्सा पद्धतियों की प्राधिकृत पुस्तकों में विवरणित सूत्रों में उल्लिखित केवल ऐसे घटकों वाले आयुर्वेद, सिद्ध अथवा यूनानी तिब्ब चिकित्सा पद्धतियों से संबंधित सभी औषध योगों से है। इससे अभिप्रेत यह भी है कि इन औषध योगों में ऐसी औषधि शामिल नहीं होती है जिसे आन्त्रेतर मार्ग द्वारा दिया जाता है। इसके अतिरिक्त इसमें औषधि और प्रसाधन सामग्री अधिनियम 1940 के खंड (क) में यथा विनिर्दिष्ट प्राधिकृत पुस्तकों में उल्लिखित औषध योग भी शामिल होता है।

(ii) औषधि एवं प्रसाधन सामग्री अधिनियम की प्रथम अनुसूची में उल्लिखित और संवर्धनात्मक एवं निवारक स्वास्थ्य के लिए संस्तुत घटकों वाले **खाद्य/पोषक/मृदव्यी/उन्नायुपोत्कल/सापेक्ष स्वास्थ्य संवर्धक औषध योग।**

(iii) औषधि एवं प्रसाधन सामग्री अधिनियम की प्रथम अनुसूची में उल्लिखित और मुखौष, त्वचा, केश और शरीर परिचर्या के लिए संवर्धनात्मक एवं निवारक स्वास्थ्य के लिए संस्तुत घटकों वाले **सौन्दर्य प्रसाधक (हुस्न अफजा)/अजहाग-साधन।**

(iv) जलय अथवा हाइड्रो-अल्कोहल सहित अधिनियम की प्रथम अनुसूची में उल्लिखित पादप से प्राप्त अर्क **औषध यन (औषधीय पादप-अर्क-शुष्क/आर्द्र)**

II. (क). सुरक्षा अध्ययन और प्रभावकारिता के अनुभव अथवा साक्ष्य से संबंधित दशाओं के लिए आयुर्वेद, सिद्ध और यूनानी के बारे में औषधियों के अनुज्ञा पत्र को निर्गत करने की प्रक्रिया अधोलिखित तालिका के स्तंभ (5) और (6) में किए गए उल्लेख के अनुसार होगी :

क्रम संख्या	श्रेणी	घटक	विनिर्देशन	सुरक्षा अध्ययन	प्रभावकारिता का अनुभव/साक्ष्य	
(1)	(2)	(3)	(4)	(5)	(6)	
					प्रकाशित साहित्य	प्रभावकारिता का प्रमाण
1	(क) 3(क) में यथा उल्लिखित 158-ख में दिए गए आयुर्वेद, सिद्ध और यूनानी औषध	ग्रंथ के अनुसार	ग्रंथ के अनुसार	अनापेक्षित	अपेक्षित	अनापेक्षित
2	(ख) औषधि एवं प्रसाधन सामग्री अधिनियम, 1940 की धारा 3 (क) में यथा उल्लिखित आयुर्वेद, सिद्ध और यूनानी की खुराक में कोई अंतर	ग्रंथ के अनुसार	ग्रंथ के अनुसार	अनापेक्षित	अपेक्षित	अनापेक्षित
3.	(ग) नए विनिर्देशन के उपयोगार्थ 3 (क) में उल्लिखित आयुर्वेद, सिद्ध और यूनानी औषध	ग्रंथ के अनुसार	नया	अनापेक्षित	यदि अपेक्षित हो	अपेक्षित

II.ख पेटेंट अथवा स्वामित्वाधीन औषधि के संबंध में अनुज्ञा पत्र निर्गत करने के लिए । सुरक्षा अध्ययन और प्रभावकारिता से संबंधित अनुभव अथवा साक्ष्य निम्नानुसार यथा विनिर्दिष्ट किया जाएगा:-

क्रम संख्या	श्रेणी	घटक	विनिर्देशन	सुरक्षा अध्ययन	प्रभावकारिता का अनुभव/साक्ष्य	
(1)	(2)	(3)	(4)	(5)	(6)	
					प्रकाशित साहित्य	प्रभावकारिता का प्रमाण
1	पेटेंट अथवा स्वामित्वाधीन औषधि	ग्रंथ के अनुसार	ग्रंथ संबंधी प्रासंगिकता	अनापेक्षित	घटकों से संबंधित	*आयुर्वेद, सिद्ध और यूनानी औषधों के लिए संबद्ध नयाचार के अनुसार प्रायोगिक अध्ययन।
2	औषधि एवं प्रसाधन सामग्री अधिनियम, 1940 की अनुसूची ङ (1) के घटकों में से किसी एक के साथ आयुर्वेद, सिद्ध और यूनानी औषध।	ग्रंथ के अनुसार	वर्तमान	अपेक्षित	अपेक्षित	अपेक्षित

(III). वल्य और पोषक औषधियों के संबंध में अनुज्ञा पत्र निर्गत करने हेतु अनुज्ञा पत्र के लिए आवेदन करने वाले व्यक्ति से निम्नलिखित प्रस्तुत करने की अपेक्षा की जाती है:-

(i) प्रथम अनुसूची की पुस्तक में यथा उल्लिखित औषध योग में प्रयुक्त घटकों के ग्रंथ संदर्भ की छाया प्रति ।

(ii) आयुर्वेद, सिद्ध एवं यूनानी औषधियों के औषध योगों के मूल्यांकन हेतु दिशा निदेशों के अनुसार अनुसूची ङ(1) में यथा विनिर्दिष्ट उत्पाद में घटकों में से किसी एक के समाहित होने की स्थिति में, सुरक्षा अध्ययन निष्पादित करना।

(iii) ग्रंथ विनिर्देशनों के लिए सुरक्षा एवं प्रभावकारिता अध्ययन अपेक्षित नहीं है।

(IV). सौंदर्य प्रसाधक (हेन्ना अफजा/अक्षागु शोधन) के संबंध में अनुज्ञा पत्र निर्गत करने हेतु अनुज्ञा पत्र के लिए आवेदन करने वाले व्यक्ति से निम्नलिखित प्रस्तुत करने की अपेक्षा की जाती है:-

(i) प्रथम अनुसूची की पुस्तक में यथा उल्लिखित औषध योग में प्रयुक्त घटकों के ग्रंथ संदर्भ की छाया प्रति प्रस्तुत करना।

(ii) आयुर्वेद, सिद्ध एवं यूनानी औषध योगों के मूल्यांकन हेतु दिशा निदेशों के अनुसार अनुसूची ङ(1) में यथा विनिर्दिष्ट औषध योग में घटकों में से किसी एक के समाहित होने की स्थिति में, सुरक्षा अध्ययन निष्पादित करना।

(iii) ग्रंथ विनिर्देशनों के लिए सुरक्षा एवं प्रभावकारिता अध्ययन अपेक्षित नहीं है।

(V). औषध घन औषधि {औषधीय पादप के अर्क(शुष्क या आर्द्र)} के संबंध में अनुज्ञा पत्र निर्गत करने हेतु ।

क्रम संख्या	श्रेणी	घटक	विनिर्देशन	सुरक्षा अध्ययन	प्रभावकारिता का अनुभव/साक्ष्य	
1	2	3	4	5	6	
					प्रकाशित साहित्य	प्रभावकारिता का सबूत
1.	(क) जलय	ग्रंथ के अनुसार	ग्रंथ के अनुसार	अनापेक्षित	अनापेक्षित	अनापेक्षित
2.	(क1) जलय	ग्रंथ के अनुसार	नए विनिर्देशन	अनापेक्षित	अनापेक्षित	अपेक्षित
3.	(ख) हाइड्रो-एल्कोहल	ग्रंथ के अनुसार	ग्रंथ के अनुसार	अनापेक्षित	यदि अपेक्षित हो	अनापेक्षित
4.	(ख1) हाइड्रो-एल्कोहल	यथा विनिर्दिष्ट	नए विनिर्देशन**	अपेक्षित	यदि अपेक्षित हो	अपेक्षित
5.	हाइड्रो/हाइड्रो-एल्कोहल के अलावा	यथा विनिर्दिष्ट	यथा विनिर्दिष्ट	अपेक्षित तीव्र, जीर्ण, म्यूटाजेनिसिटी तथा टेराटोजेनिसिटी	यदि अपेक्षित हो	अपेक्षित

* मानक मताधार में आयुर्वेद, सिद्ध, यूनानी केंद्रीय अनुसंधान परिषदों तथा अन्य सरकारी /अनुसंधान निकायों द्वारा प्रकाशित अनुपन, प्रकृति व त्रिदोष की अवधारणा भी शामिल होगी।

** नए विनिर्देशन का तात्पर्य औषधि एवं प्रसाधन सामग्री अधिनियम, 1940 की प्रथम अनुसूची पुस्तक में उल्लिखित के अलावा है।

[सं. वैक. 11020/02/2010-डीसीसी (आयुष)]

एस. जलजा, सचिव (आयुष)

पाद टिप्पण : मूल नियम भारत के राजपत्र में अधिसूचना सं. एफ-28-10/45-एच(1), तारीख 21 दिसम्बर, 1945 द्वारा प्रकाशित किए गए थे और पिछली बार संशोधन सं. सा.का.नि. 602(अ), तारीख 19-7-2010 द्वारा किया गया ।

MINISTRY OF HEALTH AND FAMILY WELFARE

[Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH)]

NOTIFICATION

New Delhi, the 10th August, 2010

G.S.R. 663(E).—Whereas the draft of certain rules further to amend the Drugs and Cosmetics Rules, 1945 was published, vide notification of the Government of India in the Ministry of Health and Family Welfare, number G.S.R. 377 (E), dated 3rd May, 2010, in the Gazette of India, Extraordinary, inviting objections and suggestions from persons likely to be affected thereby before the expiry of a period of Forty Five days from the date on which copies of the Official Gazette containing the said notification were made available to the public;

And whereas, the said Gazette was made available to the public on the 4th May, 2010;

And whereas, objections and suggestions received from the public on the said draft rules have been considered by the Central Government;

Now, therefore, in exercise of the powers conferred by section 33-N of the Drugs and Cosmetics Act, 1940 (23 of 1940) the Central Government, hereby makes the following rules further to amend the Drugs and Cosmetics Rules, 1945, namely:-

RULES

1. These rules may be called the Drugs and Cosmetics (6th Amendment) Rules, 2010.

They shall come into force on the date of their publication in the Official Gazette.

2. In the Drugs and Cosmetics Rules, 1945, (herein after referred to as the said rules), after rule 158-A, the following rules shall be inserted, namely:-

158(B) Guidelines for issue of license with respect to Ayurveda, Siddha or Unani drugs.

1. (A). Ayurveda, Siddha Unani Medicines under section 3 (a):-

Ayurveda, Siddha or Unani drugs includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae described in the authoritative books of Ayurvedic, Siddha and Unani Tibb system of medicine, as specified in the First Schedule;

(B). Patent or Proprietary medicine under section 3(h);

(i) In relation to Ayurvedic, Siddha and Unani Tibb system of medicine of all formulations containing only such ingredients mentioned in the formulae described in the authoritative books of Ayurveda, Siddha or Unani Tibb system of medicines specified in the First Schedule, but does not include a medicine which is administered by parenteral route and also a formulation included in the authoritative books as specified in clause (a);

(ii) **Balya/Poshak/Muqawi/Unavuporutkal/positive health Promoter** formulations having ingredients mentioned in books of First Schedule of the Drugs and Cosmetics Act and recommended for promotional and preventive health.

(iii) **Saundarya Prasadak (Husane afza)/Azhagh-sadhan** formulation having ingredients mentioned in Books of First Schedule of the Drugs and Cosmetics Act and recommended for oral, skin, hair and body care.

(iv) **Aushadh Ghana (Medicinal plant extracts – dry/wet)** extract obtained from plant mentioned in books of First Schedule of the Act including Aqueous or hydro-alcohol.

II.(A) For issue of licence to the medicine with respect to Ayurvedic, Siddha and Unani, the conditions relating to safety study and the experience or evidence of effectiveness shall be such as specified in columns (5) and (6) of The Table given below:-

Serial number	Category	Ingredient (S)	Indication (s)	Safety study	Experience/ Evidence of Effectiveness	
(1)	(2)	(3)	(4)	(5)	(6)	
					Published Literature	Proof of Effectiveness
1.	(A) Ayurveda, Siddha and Unani drugs, given in 158-B as referred in 3(a)	As per text	As per text	Not Required	Required	Not Required
2.	(B) Any change in dosage form of Ayurveda Siddha and Unani drugs as described in section 3(a) of the Drugs and Cosmetics Act, 1940	As per text	As per text	Not Required	Required	Not Required
3.	(C)Ayurveda, Siddha and Unani drugs referred in 3(a) to be used for new indication	As per text	New	Not Required	IF Required	Required

II.B For issue of license with respect to Patent or Proprietary medicine. The condition relating to Safety studies and experience or evidence of effectiveness shall be specified as follows:-

Serial number	Category	Ingredient (S)	Indication (s)	Safety study	Experience/ Evidence of Effectiveness	
(1)	(2)	(3)	(4)	(5)	(6)	
					Published Literature	Proof of Effectiveness
1.	Patent or Proprietary medicine	As per text	Textual rationale	Not Required	Of Ingredients	* Pilot study as per relevant protocol for Ayurveda, Siddha and Unani drugs.

2.	Ayurveda Siddha, Unani drug with any of the ingredients of Schedule E (1) of The Drugs and Cosmetics Act, 1940.	As per text	Existing	Required	Required	Required

(III) For issue of license with respect to Balya and Poshak medicines the person who applied for license is required to submit the following:

(i) Photo-copy of the textual reference of ingredients used in the formulation as mentioned in the book of 1st schedule;

(ii) Conduct safety studies in case the product contains of any of the ingredients as specified in the Schedule E (1), as per the guidelines for evaluation of Ayurveda Siddha and Unani Drugs formulations;

(iii) For textual indications the safety and effectiveness study is not required.

(IV) For issue of license with respect to Saundarya Prasadak (Husane afza/Azhaga Sodhan) the person who applied for license is required to:-

(i) Submit photo-copy of the textual reference of ingredients used in the formulation as mentioned in the book of 1st schedule;

(ii) Conduct safety studies, in case the formulation contains of any of the ingredients as specified in the Schedule E (1), as per the guidelines for evaluation of Ayurveda, Siddha and Unani formulation;

(iii) For textual indications the safety and effectiveness study is not required.

(V) For issue of license with respect to medicine Aushadh Ghana [extract of / medicinal plant (dry or wet).

Sl.no.	Category	Ingredient (S)	Indication (s)	Safety study	Experience/ Evidence of Effectiveness	
1.	2.	3.	4.	5.	6. –	
					Published Literature	Proof of Effectiveness
1.	(A) Aqueous	As per Text	As per Text	Not Required	Not Required	Not Required
2.	(A1). Aqueous	As per Text	New indication	Not Required	Not Required	Required
3.	(B) Hydro-Alcohol	As per Text	As per Text	Not Required	If Required	Not Required
4.	(B1) Hydro-Alcohol	As specified	New Indication**	Required	If Required	Required

5.	Other than Hydro/Hydro –Alcohol	As specified	As specified	Required Acute, Chronic, Mutagenicity and Teratogenicity	If required	Required

* The standard protocol will also include concept of Anupan, Prakriti & Tridosh etc. published by Central Research Councils Ayurveda, Siddha, Unani and other Government/Research Bodies.

** New indication means which is other than mentioned in 1st schedule books of Drugs & Cosmetics Act 1940.

[No. K. 11020/02/2010-DCC (AYUSH)]

S. JALAJA, Secy. (AYUSH)

Foot Note : The Principal rules were published in Official Gazette vide notification No. F. 28-10/45-H(I) dated the 21st December, 1945 and the last amended *vide* No. GSR 602(E), dated 19-7-2010.

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GUIDELINES ON THE REGULATION OF SCIENTIFIC EXPERIMENTS ON ANIMALS

Ministry of Environment & Forests

(Animal Welfare Division)

Government of India

June 2007

CONTENTS

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 (CPCSEA) in terms of Section 17 (3) of the Prevention of Cruelty to Animals Act, 1960. The report was accepted by CPCSEA 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 establishment. 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 (CPCSEA), 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 CPCSEA 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 CPCSEA

3.1 Ethical principles adopted by CPCSEA 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 anesthesia.

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. CPCSEA 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 anesthesia.

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 CPCSEA.

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 CPCSEA 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 CPCSEA

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

The main functions of CPCSEA 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 CPCSEA.
- 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) Everyestablishment constituted and operated in accordance with the

procedures specified by CPCSEA 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 CPCSEA. 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 CPCSEA.

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 CPCSEA. Every such establishment shall stop performing experiments on animals or breeding of animals for use in experiments, if registration is refused to it by CPCSEA.

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 CPCSEA 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 CPCSEA 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 euthanisation 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 wiyth 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 CPCSEA

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 nay 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.

Note: All relevant Rules, Guidelines and minutes of the meetings of CPCSEA are available on the website of the Ministry of Environment and Forests: www.envfor.nic.in.

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 in toto by CPCSEA. 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 undertake n 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 euthanisation 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 pan 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 documentation to 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 (Control and 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 thereunder.

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

The Rule has been amended to allow CPCSEA 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, CPCSEA 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.

CPCSEA GUIDELINES FOR LABORATORY ANIMAL FACILITY - 2005

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 behavioral research and testing of products.

GOAL

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

VETERINARY CARE

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

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

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

ANIMAL PROCUREMENT

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

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

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

QUARANTINE, STABILIZATION AND SEPARATION

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony. The duration at quarantine in

small lab animals from one week to one month and large animals allowed up to 6 weeks (cat, dog, monkey, etc.)

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.

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

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

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

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

Separate set of personnel should be identified for taking care of these animals and other people 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

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

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

Postmortem 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. Diagnostic clinical laboratory may be made available.

ANIMAL CARE AND TECHNICAL PERSONNEL

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

PERSONAL HYGIENE

It is essential that the animal care staff maintain a high standard of personal cleanliness. Facilities and supplies for meeting this obligation should be provided with appropriate Personnel Protective Equipment (PPE) e.g. showers, change of uniforms, footwear etc.

Clothing suitable for use in the animal facility should be supplied and laundered by the institution. A commercial laundering service is acceptable in many situations; however, institutional facilities should be used to decontaminate clothing exposed to potentially hazardous microbial agents or toxic substances. 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.

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 some where else out side and not in the animal rooms / areas. A separate area or room should be made available for these purposes.

ANIMAL EXPERIMENTATION INVOLVING HAZARDOUS AGENTS

Institutions should have policies governing experimentation with hazardous agents. Institutional 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)**.

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).

MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

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

DURATIONS OF EXPERIMENTS

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

PHYSICAL RESTRAINT

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

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

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

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

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

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

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

PHYSICAL 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.

LOCATION 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).**

FUNCTIONAL AREAS

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.

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

PHYSICAL FACILITIES

(a) **Building materials** should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

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

(c) **Utilities** such as water lines, drain pipes, and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

ANIMAL ROOM DOORS

Doors should not be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

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.

FLOORS

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

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

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

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

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.

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.

FACILITIES FOR SANITIZING EQUIPMENT AND SUPPLIES

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

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 & intensive care and for treatment of animals.

ENVIRONMENT

(a) Temperature And Humidity Control

Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions 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.

(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 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.

ANIMAL HUSBANDRY

(a) Caging Or Housing System

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

The housing system should:

- Provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species; **(Annexure – 3)**
- Provide a comfortable environment
- Provide an escape proof enclosure that confines animal safety
- Provide easy 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.

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

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

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

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

SHELTERED OR OUTDOOR HOUSING

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

Shelter should be accessible to all animals, with sufficient ventilation, and should be designed to prevent accumulation of waste materials and excessive moisture.

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

Ground-level surfaces of outdoor housing facilities can be covered with absorbent bedding, sand, gravel, grass, or similar material that can be removed or replaced when 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.

SOCIAL ENVIRONMENT

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.

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.

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

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

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

ACTIVITY

Provision should be made for animals with specialized locomotor pattern to express 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.

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

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

FOOD

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

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

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.

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.

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.

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

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

Diet should be free from heavy metals (e.g., Lead, Arsenic, Cadmium, Nickel, Mercury), naturally occurring toxins and other contaminants.

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

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

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

BEDDING

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.

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.

The desirable criteria for rodent contact bedding is ammonia binding, sterillizable, 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.

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

WATER

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

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.

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

SANITATION AND CLEANLINESS

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.

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

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.

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.

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 82.2C (180°F) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms.

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

Rabbits and some rodents, such as guinea pigs, 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.

Water bottles, sipper nozzles stoppers, and other watering equipment should be washed and then sanitized by rinsing with water of at least 82.2°C (180°F) 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.

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.

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

ASSESSING THE EFFECTIVENESS OF SANITATION

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.

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

A decision to 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.

Autoclaving : Chemical Indicator - batch wise assessment; Biological indicator - Periodical assessment.

WASTE DISPOSAL

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.

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 sterilization, decontamination, or other appropriate means before they are disposed off from an animal facility.

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.

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 telephone center. A disaster plan that takes into account both personnel and animals should be prepared as part of the overall safety plan for the animal facility.

RECORD KEEPING

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, Postmortem Record
- 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

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 no 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

PERSONNEL AND TRAINING

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

The staff must be provided with all required protective clothing (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 animal house in-charge should ensure that persons working in animal house don't eat, drink, smoke in animal room and have all required vaccination, particularly against Tetanus and other zoonotic diseases.

Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning of cages and importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine check up of cages **(Annexure - 7)**.

TRANSPORT OF LABORATORY ANIMALS

The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are, mode of transport, containers, animal density in cages, food and water during transit, protection from transit infections, injuries and stress.

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

only a permissible number of animals should only be accommodated in each container to avoid overcrowding and infighting **(Annexure - 4)**

ANAESTHESIA AND EUTHANASIA

The investigators should ensure that the procedures, which are considered painful, are conducted under appropriate anaesthesia as recommended for each species of animals.

It must also be ensured that the anaesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the 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)**.

In the event of a decision to sacrifice an animal or termination of an experiment or other wise 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.

Anaesthesia

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

Before using actual anaesthetics the animals are prepared for anaesthesia by over night fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardio-pulmonary system and reduce salivary secretion. Atropine is most commonly used anti-cholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure.

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.

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.

Euthanasia

Euthanasia is resorted to events where an animal is required to be sacrificed or termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting an euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal to be killed **(Annexure - 6)**. The method should in all cases meet the following requirements:

(a) Death, without causing anxiety, pain or distress with minimum time lag phase.
(b) Minimum physiological and psychological disturbances.
(c) Compatibility with the purpose of study and minimum emotional effect on the operator.
(d) Location should be separate from animal rooms and free from environmental contaminants.

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

LABORATORY ANIMAL ETHICS

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

TRANSGENIC ANIMALS

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

MAINTENANCE

Housing, feeding, ventilation, lighting, sanitation and routine management practices for such animals are similar to those for the other animals of the species as given in guidelines. However, special care has to be taken with transgenic/gene knockout animals where the animals can become susceptible to diseases where special conditions of maintenance are required due to the altered metabolic activities. The

transgenic and knockout animals carry additional genes or lack genes compared to the wild population. To avoid the spread of the genes in wild population care should be taken to ensure that these are not inadvertently released in the wild to prevent cross breeding with other animals. The transgenic and knockout animals should be maintained in clean room environment or in animal isolators.

DISPOSAL

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

BREEDING AND GENETICS

For initiating a colony, the breeding stock must be procured from CPCSEA registered breeders or suppliers ensuring that genetic makeup and health status of animal is known. In case of an inbred strain, the characters of the strain with their gene distribution and the number of inbred generation must be known for further propagation. The health status should indicate their origin, e.g. conventional, specific pathogen free or transgenic, gnotobiotic or knockout stock.

Annexure – 1

HAEMATOLOGICAL DATA OF COMMON LABORATORY ANIMALS

	Mouse	Rat	Hamster	G. Ppig	Rabbit	Cat	Dog (Beagle)	Primate (Rhesus)
RBC(x10 /mm³)	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(X10³/mm³)	6 - 15	6 - 17	3 - 11	7 - 18	9 - 11	5.5 - 19.5	6 - 17	2.5 - 26.7
Neutrophlis(%)	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(X10³/mm³)	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 on higher 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		Height
		(Sq.ft)	(Sq.meter)	(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 Pubs	4.5	0.40	14

Annexure – 3B

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

Recommended floor Area per animal (Cm^2)	38.7	51.6	77.4	96.7
Weight of animals (Grams)	<10	upto15	upto25	>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 II Cage 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 (in Kg)	Floor area (cm) Ft^2	Cm^2	Height
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 - 3F

Recommended Space for Cats, Dogs and Birds

Animals	Weight, kg[a]	Floor area/animal, ft²[b]	Height in inches
Cat	<4	3.0	24
	<4	>4.0	24
Dog	<15	8.0	-
	Up to 30	12.0	-
	>30	>24.0	-
Pigeon	-	0.8	-
Chicken	<0.25	0.25	-
	Up to 0.5	0.50	-
	Up to 1.5	1.00	-
	Up to 3.0	2.00	-
	>3.0	>3.00	-

b To convert square feet to square meters Multiply with 0.09

Annexure - 3G

Recommended Space for Commonly Used Farm Animals

Animals/Enclosure	Weight kg[a]	Floor Area/Animal ft² b	Height(ft.)
Sheep and Goats			8
	<25	10.0	
	Up to 50	15.0	
	>50	20.0	
2-5	<25	8.5	
	Up to 50	12.5	
	>50	17.0	
>5	<25	7.5	
	Up to 50	11.3	
	>50	15.0	
Swine			8
	Up to 25	12.0	
	Up to 50	15.0	
	Up to 100	24.0	
	Up to 200	48.0	
	>200	>60.0	
2-5	<25	6.0	
	Up to 50	10.0	
	Up to 100	20.0	
	Up to 200	40.0	
	>200	>52.0	
>5	<25	6.0	
	Up to 50	9.0	
	Up to 100	18.0	
	Up to 200	36.0	
	>200	>48.0	

Animals/Enclosure	Weight. Kg[a]	Floor Area/Animal, ft²b	Height (ft)
Cattle			8
1	<75	24.0	
	Up to 200	48.0	
	Up to 350	72.0	
	Up to 500	96.0	
	Up to 650	124.0	
	>650	>144.0	
2 –5	<75	20.0	
	Up to 200	40.0	
	Up to 350	60.0	
	Up to 500	80.0	
	Up to 650	105.0	
	>650	>120.0	
>5	<75	18.0	
	Up to 200	36.0	
	Up to 350	54.0	
	Up to 500	72.0	
	Up to 650	93.0	
	>650	>108.0	
Horses	144.0	10	
Ponies		8	
1 - 4	72.0		
>4/pen	<200	60.0	
	>200	>72.0	

[a]To convert kilograms to pounds. Multiply with 2.2
b To convert square feet to square meters. Multiply with 0.09
Larger animals might require more space to meet performance Stan

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

ANNEXURE – 5

COMMONLY USED ANAESTHETIC AGENTS FOR LABORATORY ANIMALS

Drugs (mg/kg)	Mouse	Rat	Hamster	Guinea pig	Rabbit	Cat	Dog	Primate
KTEAMINE Hcl	22 - 24 i/m	22 - 24 i/m	-	22 - 24 i/m	22 - 24 i/m	30 i/m	30 i/m	15 - 40
PENTOBAR-BITONE SODIUM	35 i/v 50 i/p	25 i/v 50 i/p	35 i/v -	30 i/v 40 i/p	30 i/v 40 i/p	25 i/v -	20 - 30 i/v -	35 i/v -
THIOPENT-ONE SODIUM	25 i/v 50 i/p	20 i/v 40 i/p	20 i/v 40 i/p	20 i/v 55 i/p	20 i/v	25 i/v	25 i/v	25 i/v 60 i/p
URETHANE	-	0.75 i/p	-	1.5 i/p	1.0 i/p, i/v	1.25 i/v 1.50 i/p	1.00 i/v	1.0 i/v

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

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

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
Helothane	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

Methods Not Acceptable for any species of animals **IP =**
intraperitioneal

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

IV = Intravenous

IM = Intramuscular

Annexure - 7

QUALIFICATIONS & KNOWLEDGE REQUIRED FOR LABORATORY ATTENDANT

Basic Education: 10^{th} standard

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

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

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

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

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

Annexure – 8

Institutional Biosafety Committee (IBSC)

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

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

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

The biosafety functions and activity include the following:

(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.

Expert involved in development of Guidelines and consultative process

Working group

1. Vd. (Prof.) Kartar Singh Dhiman
   Director General | Central Council for Research in Ayurvedic Sciences | New Delhi
2. Dr. N. Srikanth
   Deputy Director General | Central Council for Research in Ayurvedic Sciences | New Delhi
3. Dr. Sudesh N. Gaidhani
   Assistant Director (Pharmacology) | Central Council for Research in Ayurvedic Sciences | New Delhi

Members of National Consultation

1. Dr. S.J.S. Flora
   Director | National Institute of Pharmaceutical Education and Research | Raebareli | Uttar Pradesh
2. Dr. S.K. Maulik
   Professor (Pharmacology) | All India Institute of Medical Sciences | New Delhi
3. Dr. Smrati Bhadauria
   Senior Scientist (Toxicology) | CSIR-Central Drug Research Institute | Lucknow | Uttar Pradesh
4. Dr. B. Dinesh Kumar
   Scientist- F & HOD Toxicology Division | National Institute of Nutrition | Hyderabad

Technical Support

1. Mrs. Simmy Kawatra
   Senior Research Fellow (Pharmacology) | Central Council for Research in Ayurvedic Sciences | New Delhi
2. Dr. Manish Kumar Gautam
   Senior Research Fellow (Pharmacology) | Central Council for Research in Ayurvedic Sciences | New Delhi

CENTRAL COUNCIL FOR RESEARCH IN AYURVEDI

Ministry of AYUSH (Government of India)
Jawahar Lai Nehru Bhartiya Chikitsa Evam Homeopathy Anusar
No. 61-65, Institutional Area, Opp. "D" Block, Janakpuri, New D
Tele.: 91-11-28524457, Fax: 91-11-28520748
Email : dg-ccras@nic.in, Website : www.ccras.nic.i