CCSEA GUIDLINES

Introduction

Committee for the control and supervision of experiments on animals (CCSEA)

Function :

1. Registration of establishments conducting animal experimentation or breeding of animals for this purpose.
2. Selection and appointment of nominees in the Institutional Animal Ethics Committees of registered establishments.
3. Approval of Animal House Facilities on the basis of reports of inspections conducted by CPCSEA.
4. Permission for conducting experiments involving use of animals.
5. Recommendation for import of animals for use in experiments.

Goal

- The goal of these guidelines is to promote the human care of animal used in biomedical and behavioral research and testing.
- To avoid unnecessary pain before, during and after experiment .
- To provide guideline for -

1. Housing ,care ,breeding and maintenance
2. Source of experimental animals
3. Acceptable experimental procedures for anesthesia and euthanasia.

To assure quality maintenance and safety of animals

To promote the humane care of animals

Enhance animal wellbeing, quality in the pursuit of advancement of biological knowledge that is relevant to humans and animals.

Good laboratory practices

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.
- Observed regularly for sign of illness, injury, or abnormal behavior.
- Contagious disease – isolated from healthy animal.

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

- The veterinarian can also help the establishment in designing appropriate policies and procedures for ancillary aspects of veterinary care, such as use of appropriate methods to prevent and control diseases (e.g. vaccination and other prophylaxis, disease monitoring and surveillance, quarantine and isolation), operative and post-operative care, diagnosis and treatment of diseases as well as injuries. reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

QUARANTINE

- Separation of newly received animals from those already in the facility until the health and possibly the microbial status of newly received animal have been determine.
- A minimum duration of quarantine for small animal-1 week and for larger animal-6 week.
- An effective quarantine minimizes the chance for introduction of pathogens into an established colony.

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

Non-human primates

Effective quarantine limit exposure of humans to zoonotic infections

SURVEILLANCE

- All animals should be observed for signs of illness, injury, or abnormal behaviour by animal house staff (possibly daily).
- 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 (MTB), the group should be kept intact and isolated during the process of diagnosis, treatment, and control.

DIAGNOSIS AND TREATMENT

Haematological data of commonly used laboratory animals.

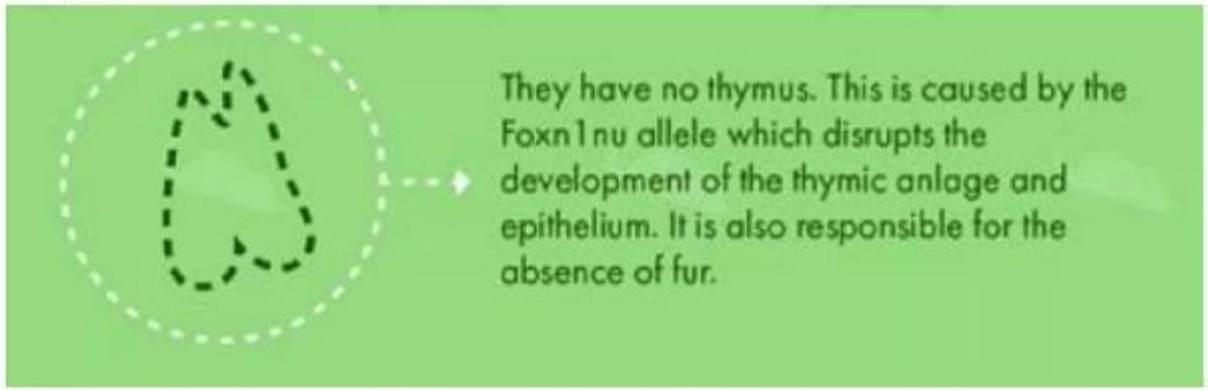
	Mouse	Rat	Hamster	G.pig	Rabbit	Cat	Dog (Beagle)	Monkey (Rhesus)
RBC(x10v /mm³)	7-12.5	7-10	6-10	4.5-7	4-7	5-10	5.5-8.5	3.56-6.96
PCV(%)	39-49	36-18	36-55	37-48	36-48	30-15	37-55	26-48
Hb (g/dl)	10.2-16.6	11-18	10-16	11-15	10-15.5	8-15	12-18	8.8-16.5
WBC(X10³/mm³)	6-15	6-17	3-11	7-18	9-11	5.5-19.5	6-17	2.5-26.7
Neutrophils(%)	10-40	9-34	10-42	28-44	20-75*	35-75	60-70	5-88
Lymphocytes(%)	55-95	65-85	50-95	39-72	30-85	20-55	12-30	8-92
Eosinophils(%)	0-4	0-6	0-4.5	1-5	0-4	2-12	2-10	0-14
Monocytes(%)	0.1-3.5	0-5	0-3	3-12	1-4	1-4	3-10	0-11
Basophils(%)	0-0.3	0-1.5	0-1	0-3	2-7	rare	rare	0-6
Platelets(X10³/mm³)	160-410	500-1300	200-500	250-850	250-656	300-700	200-900	109-597

DIAGNOSIS AND TREATMENT

Biochemical data of commonly used laboratory animals.

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

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



They have no thymus. This is caused by the Foxn1nu allele which disrupts the development of the thymic anlage and epithelium. It is also responsible for the absence of fur.

- Preventive medicine programs - vaccinations, ecto- and endoparasite treatments appropriate to the species and source.
- Transgenic and mutant animals may be particularly susceptible to diseases and may require special protection to ensure their health.
- Systems to prevent spread of disease may include facility design features, containment/isolation equipment, and use of SOPs.
- Training of animal care and research staff is essential to prevent spread of animal diseases.
- Cells, tissues (Xenograft), fluids, and transplantable tumors that are to be used in animals should be monitored for infectious or parasitic agents that may cause disease in animals.

PERSONAL HYGIENE

- It is essential that the animal care staff maintain a high standard of personal cleanliness.
- Clothing suitable for use in the animal facility should be supplied and laundered by the institution.
- It acceptable to use disposable gear such as gloves, masks, head covers, coats, coveralls and shoe covers.
- Person should change clothing as often as is necessary to maintain personal hygiene.
- Personnel should not be permitted to eat, drink, smoke or apply cosmetic in animal rooms.

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.

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- In the event of a decision to sacrifice an animal or termination of an experiment or otherwise an approved method of euthanasia should be adopted.
- 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.
- Before using actual anaesthetics the animals are prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardio-pulmonary system and reduce salivary secretion.
- Atropine is most commonly used anti-cholinergic agent.

- Local or general anaesthesia may be used, depending on the type of surgical procedure.
- 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.
- The animal should remain under veterinary care till it completely recovers from anaesthesia and postoperative stress.

COMMONLY USED ANAESTHETIC AGENTS FOR LABORATORY ANIMALS

Drugs (mg/kg)	Mouse	Rat	Rabbit
KETAMINE HCl	87mg/kg IP once (in combination with xylazine)	87mg/kg IP once (in combination with xylazine)	24-35mg/kg IM
PENTOBAR-BITONE SODIUM	35 IV 40-70mg/kg IP	30-40 mg/kg IV 40-60mg/kg IP	30-40mg/kg IV 40mg/kg IP
THIOPENT-ONE SODIUM	25mg/kg IV 50mg/kg IP	20-40mg/kg IV 40mg/kg IP	20 mg/kg IV
URETHANE	1000mg/kg IP*	1000mg/kg IP*	1000mg/kgIP*

*(prolonged anaesthesia: terminal procedures only)

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

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

Anesthesia for Laboratory Animals:

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

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

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

Euthanasia

- Euthanasia should be resorted to events where an animal is required to be sacrificed to reduce suffering or to limit spread of infections or for termination of an experiment or for other ethical reasons.
- The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety.
- For accepting an euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain.
- The choice of a method will depend on the nature of study, the species of animal to be killed .

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The method should in all cases meet the following requirements:

(a) Death, without causing anxiety, pain or distress with minimum time lag phase.

(b) Minimum physiological and psychological disturbances.

(c) Compatibility with the purpose of study and minimum emotional effect on the operator.

(d) Location should be separate from animal rooms and free from environmental contaminants.

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

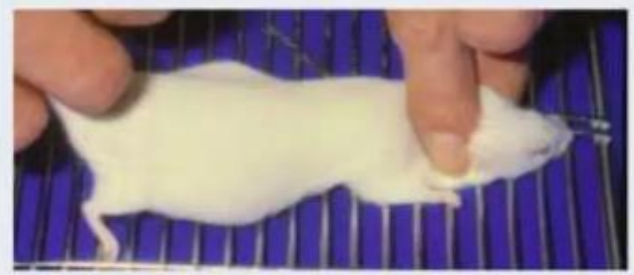
➢ Requirements of method:

I. Death, without causing anxiety, pain or distress with minimum time lag phase.

II. Minimum physiological and psychological disturbances.

III. Compatibility with the purpose of study and minimum emotional effect on the operator.

IV. Location should be separate from animal rooms and free from environmental contaminants.



Used for animals under 200 grams

EUTHANASIA OF LABORATORY ANIMALS

(A – Methods Acceptable NR – Not Recommended)

Species		Mouse	Rat
a) **PHYSICAL METHODS**			
Electrocution		NR	NR
Exsanguination		NR	A
Decapitation (for analysis of stress)		A	A
Cervical dislocation		A	A*
b) **INHALATION OF GASES**			
Carbon Monoxide		A	A
Carbon Dioxide		A	A
Carbon Dioxide plus Chloroform		A	A
Halothane		A	A
c) **DRUG ADMINISTRATION**			
Barbiturate Overdose (route)		A(IP)	A(IP)
Chloral hydrate Overdose (route)		NR	NR
Ketamine Overdose (route)		A(IM/P)	A(IM/IP)
Sodium Pentothol [Overdose (route)]		IP	IP

* Cervical dislocation is not allowed in rats weighing more than 200gms.

IP = Intra Peritoneal, IV= Intra Venous, IM = Intra Muscular