



Ijapc 2018

Volume 8 Issue 2

www.ijapc.com

10/3/2018

Greentree Group



Experimental Toxicology

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ABSTRACT

The amount of pharmacological substances and chemicals being used in today's community have increased manifold. They are present in the food substances, beverages, various medicines other industrial and household products. These pharmacological substances hold threat to the living system when used for a long period of time. Sometimes acute toxicity may also occur when large quantities, capable of developing immediate toxic effect are used.

Experimental Toxicology is the science concerned with identifying and understanding the mechanism of agents adversely affecting the health of humans, other animals and living portion of the environment¹. These experiments are carried out on living organisms in which normal biological process can be studied or in which a spontaneous or induced pathological process can be investigated. Animal testing will always be an emotive subject and some people condemn it for medical research. Such testing is not new but has taken place since about 500B.C. There is currently no other testing how a substance will interact within a complex living body (as opposed to a cell culture). For a research to be accurate, it is important that the animals used for testing are kept in healthy and happy atmosphere. Most researchers support the 3R concept. Refinement- making the experience as painless as possible, Reduction- develop techniques to reduce the number of animals used and Replacement- use alternative methods where ever possible.

KEYWORDS

Experimental toxicology, Acute toxicity, 3R



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Received 20/01/18 Accepted 20/02/18 Published 10/03/18



INTRODUCTION

Biomedical research hires animals to understand how our body works, to find cures and treatment of diseases, to test new drugs for safety and to evaluate medical procedures before they are used on people. Although animals appear very different from us, their physiology resembles to ours in many ways. Research using animals improves the lives of millions of people each year by giving clues to various diseases, its cure, prevention and therapeutic doses of new drugs.

Before conducting any toxicological testing in animals, the study should be approved by the Institute Animals Ethical Committee or the protocol should satisfy the guidelines of the local governing body. People for the Ethical Treatment of Animals (PETA) reported that the National Centre for Laboratory Animal Sciences (NCLAS) in Hyderabad, supplies approximately 50,000 animals to laboratories every year and to 175 institutions in India including pharmaceutical companies and educational institutions.

IDEAL ANIMAL MODEL²:

An animal model is a living organism in which normal biological process can be studied or in which a spontaneous or induced pathological process

can be investigated. To be effective, the process being modeled should resemble closely the analogous process in humans. It should have:

- 1-Visible circulatory system and vital process
- 2-Similarity
- 3- Extrapolating ability.
- 4- Reproducibility.
- 5-Availability.
- 6- Tractability.
- 7- Size and fecundity lifetime.
- 8- Keeping
- 9- Inexpensive

ANIMALS USED FOR EXPERIMENTATION:

In India, among rodent group of animals eg. mice, rat, guinea pig, rabbit is predominantly used.(Fig-1)

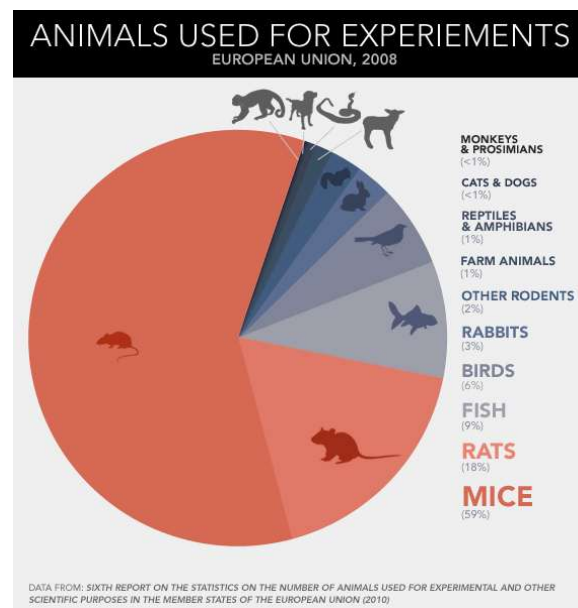


Fig 1 Animals used in experimentation



REGULATORY MECHANISMS IN INDIA³:

- Institute Animal Ethics Committee (IAEC).
- Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA).
- Drug and Cosmetic Act (1940).
- Dept. of Animal Husbandry, Dairies and Fisheries, Ministry of Agriculture, New Delhi-2001.
- Dept of Biotechnology.
- Prevention to Cruelty to Animal Act-1960.

OECD GUIDELINE⁴:

OECD guidelines are a set of internationally accepted specifications for the testing of chemicals decided on by the Organization for Economic Co-operation and Development(OECD).They were first published in 1981.They are split into five sections.

Section 1- Physical chemical properties.

Section 2- Effects on biotic systems.

Section3-Degradation and accumulation

Section 4-Health effects

Section 5-Other test guidelines

OBJECTIVE OF THE GUIDELINES:

- 1- Identification of hazardous properties of a chemical.
- 2 -Identification of target organs.

3-Characterisation of the dose response relationship

4-Identification of a no-observed-adverse-effect-level (NOAEL)

5-Prediction of chronic toxicity effects at human exposure level.

GUIDELINES OF CPCSEA FOR EXPERIMENTAL ANIMALS⁵

Goals:

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 daily observation of the animals is accomplished by trained personnel.

ANIMAL PROCUREMENT:

A health surveillance program for screening in coming animals is carried out to assess animal quality. The animals procured should be quarantined and stabilized according to procedures appropriate to the species and circumstances.

QUARANTINE, STABILIZATION AND SEPARATION:

Physical separation of animals by species is recommended to prevent interspecies



disease transmission and to eliminate anxiety.

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE :

All the animals should be observed for signs of illness, injury or abnormal behavior by animal house staff. Any illness or distress should be reported to the veterinary medical officer. Animals showing signs of contagious disease are isolated and treated accordingly.

ANIMAL CARE:

It is the sole duty of the concerned institution to take special care of the animals and employ people trained in laboratory animal science.(fig-2,fig-3)



Fig 2 Animal care



Fig 3 Animal care

DURATION OF EXPERIMENTS:

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

HOUSING OF ANIMALS:

In planning an animal facility the space should be well divided for various activities. The animal rooms should occupy around 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 and animal cage are factors on which the production and experimental efficiency of the animal depends.(fig-4,fig-5,fig-6, fig-7)



Fig 4 Animal environment



Fig 5 Animal environment



Fig 6 Animal care



Fig 7 Animal care

TEMPERATURE AND HUMIDITY:

Air conditioning is an effective means of regulating temperature required for laboratory animals. The temperature range usually recommended for common laboratory animals is approximately 18-29 degree centigrade. The relative humidity should be maintained between 30-70% throughout the year.

VENTILATION:

Heating, ventilating and air conditioning systems should be designed so that operation can be carried out in a stand-by system. Separate ventilation facility should be provided for animals and human occupancy.

POWER AND LIGHTING.

The lighting system installed should provide adequate illumination while people are working in the animal rooms and a lowered intensity of light for the animals.

NOISE CONTROL:

The animal house for laboratory experiments should be provided with noise free environment.

ANIMAL HUSBANDRY:

A-CAGING AND HOUSING SYSTEM:

The most important elements in physical and social environment of research animals is caging or housing system (fig-8). It should provide

- Adequate space, permit freedom of movement and should have a resting place according to the species.
- Comfortable environment.
- Adequate ventilation.
- Escape proof enclosure.
- Easy access to food and water.
- Meet the biological needs of the animals.
- Should be dry and clean.



- Facilitate research and maintain good health of the experimental animals.
- Drinking water should be clean and changed every day



Fig 8 Caging of animals

B-SHELTERED OR OUTDOOR HOUSING:

Animals that are kept outdoors or in large enclosures must be protected from the extreme temperature with adequate protection from submissive animals. Build up for animal wastes and stagnation of water should be avoided.(fig-9)



Fig 9 Housing of animals

C-SOCIAL ENVIRONMENT:

The effect of social environment on caged animals varies with the species. Population density can affect reproduction, metabolism, immune response and behavior. Non human

primates should have a run for free ranging activities.

FOODS:

Laboratory animal diets should be manufactured, kept clean and enclosed to prevent entry of insects and other animals. It should contain adequate nutrition, including formulation and with the nutritional requirement of the animals. Diet should be free from heavy metals and other contaminants Perishable food items should be refrigerated to minimize contamination.

BEDDING:

The amount of bedding used should be sufficient to keep the animals dry, should be absorbent, free of toxic chemicals and substances that could injure animal. Bedding should be replaced frequently.

WATER:

The laboratory animals should be provided with fresh, portable and uncontaminated drinking water with continuous access.

SANITATION AND CLEANLINESS:

The whole area used for animal experimentation, housing, feeding etc. should be cleaned with appropriate detergents and disinfectants. Cages should be sanitized before animals are placed in it.

WASTE DISPOSAL

Wastes should be removed regularly and frequently. It should be collected carefully



and disposed off in a safe and sanitary manner. Hazardous wastes should be made safe by proper sterilization and are removed from an animal facility for proper disposal.

TRANSPORT OF LAB ANIMALS:

It is important to take utmost care when laboratory animals are transported from one place to another. The mode of transport used, the containers density of animals within the cage, water and food supply, protection from transit infections, injuries and stress should be taken care of.

ANESTHESIA

Appropriate use of anesthesia for full duration of experiment is recommended for each species of animal to ensure that the procedure undertaken is painless.

EUTHANASIA

The process of euthanasia should be carried out painlessly on termination of an experiment or otherwise for ethical reasons.

TYPES OF TOXICITY STUDIES⁶

- 1- Single dose studies/Acute toxicity studies.
- 2- Repeated dose studies/sub acute or chronic studies.
- 3- Local toxicity studies
- 4- Allergency/Hypersensitivity toxicology studies.
- 5- Genotoxicity studies.

6- Carcinogenicity/ Oncogenicity studies.

***In-vivo* STUDIES^{7,11}:**

In in-vivo studies the effect of various biological entities are tested on whole living organism or cells, usually animals, including humans and plants as opposed to a tissue extract or dead organism. Animal testing and clinical trials are major elements of in-vivo research. In-vivo testing is often employed to study the overall effects of an experiment on a living subject. While there are many reasons to believe in-vivo studies have the potential to offer conclusive insights about the nature of medicine and disease.

***In-vitro* STUDIES^{7,12}:**

The technique of performing a given procedure or experiment in a controlled environment outside a living organism is called in-vitro studies. Cells and tissues are removed from their natural environment, thereby eliminating the interaction and protection mechanisms otherwise available from the donor organism. It should also be emphasized that a modification that has occurred on a cellular level during tests does not mean that the whole organism would experience the same effects.

***In silico* STUDIES⁷**

In silico means “performed on a computer or via computer simulation.” Although it



represent relatively new avenue of enquiry, it has begun to be used widely in studies in which predict how drug interact with the body and with pathogens. There is a variety of in silico techniques, but the two that are discussed the most in connection with the Marshall Protocol are 1-Bacterial sequencing techniques and 2-Molecular modeling 3-Whole cell simulation.

ACUTE TOXICITY STUDIES⁸:

They are conducted to evaluate the effect of a single substance. Usually each animal receives a single dose of the test substance in this study design. On rare occasions, repeated dose may be administered, but in any event, all doses are administered within 24 hr or less.

LORKE'S METHOD:

This method has two phases.

Phase 1-

This phase requires 9 animals which are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100, 1000 mg/kg). The animals are then placed under observations for 24 hr to monitor their behaviour as well as if any mortality occurs.

Phase 2-

This phase involves the use of three animals which are distributed in to three groups of one animal each. The animals are

administered higher doses (1600, 2900, 5000 mg/kg) of the test substance and then observed for 24 hrs for behaviour as well as mortality. The lethal dose (LD_{50}) is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where D_0 = highest dose that gave no mortality.

D_{100} = lowest dose that produced mortality.

Proposed new method:

This method is divided into stages whose outcome determines the next stage.

STAGE 1- The initial stage requires four animals which are divided into four groups of one animal each. Then different doses of the test substance are administered to different animals. Then the animals are kept under observation for 1 hr after administration and 10 minutes every 2 hours for 24 hrs. The behaviour, sign of toxicity and mortality is then recorded. With no mortality the test proceeds to the next phase.

STAGE 2- This stage involves three animals, divided into three groups of one animal each and again different doses of the test substance are administered, higher than the doses used in stage-1. The animals are then observed for 1 hr after administration and periodically for 24 hrs. Behaviour, signs of toxicity and mortality is then recorded. If



no mortality occurs the test proceeds to the next stage-3.

STAGE 3- Procedure followed in the above stages is repeated with a highest dose of 5000 mg/kg of the test substance administered to different animals, observation is done for 1 hr after administration and 10 minutes every 2 hrs for next 24 hrs. Behaviour, signs of toxicity and mortality is then recorded. This is the final stage of testing and where no mortality is recorded at this stage, the LD₅₀ of the test substance is said to be greater than 5000 mg/kg and hence higher degree of safety.

CONFIRMATORY TEST: When mortality occurs at a given dose, a confirmatory test is carried out to actually validate that the test substance was the cause of mortality. This test involves the administration of the test substance that caused mortality to two animals. The observation is done for 1 hr after administration of the test substance and 10 minutes for every 2 hrs for 24 hrs. Where at least a single animal out of the two die, it serves as a confirmation and validation of the test result.

The main test did not show any mortality at 5000 mg/kg of the test dose a confirmatory test can be done by administering 5000 mg/kg to 2 animals. Observation should be

done for 1 hr after administration and 10 minute every 2 hrs for 24 hrs. Recording of no mortality should be a confirmation of test result.

Calculation of LD₅₀

$$LD_{50}=(M_0 +M_1)\div 2.$$

Where M₀=highest dose of test substance that gave no mortality

M₁=lowest dose of test substance that gave mortality.

SUB ACUTE TOXICITY:

To determine the toxicity after repeated administration of test material is performed at 4 dose levels viz: high, mid, low and vehicle. The compounds are administered daily for 14 days via one of the route for administration (po, id, sc ,im).The animals are then observed individually after dosing periodically during the 24 hrs ,and daily thereafter for 14 days.

OBSERVATIONS:

Observations are recorded systematically for each animal for clinical signs, body weight, food, consumption and water intake is measured once a week.

CHRONIC TOXICITY^{9,10}

It is the development of adverse effects as a result of long term exposure to a toxicant. It takes generally 6 months to 1 yr in duration with regard to chronic toxicity testing .The ICH S4 A guidance (20) recommends 6



month chronic toxicity studies in rodents and 9 months in non rodents. For rodents at least 40 animals (20 males and 20 females) should be used at each dose level concurrent control group. For non rodents a smaller number of animals but at least 4 per sex per group is taken. At least 3 dose levels should be used in addition to concurrent control group. Frequency of exposure is normally daily. A careful clinical examination should be made at least once each day. Clinical signs including neurological and ocular changes, as well as mortality should be recorded for all animals. Time of onset and progression of toxic conditions is recorded. Body weight of all the animals is recorded once a week during first 13 weeks.

PROCEDURE FOLLOWED FOR CHRONIC TOXICITY.

Species	Rodent and Non rodent(Rat and Dog)
Age	Young adults
No: of animals	Rodents-20/sex and in Non rodents- 4/sex
Dosage	3 dose level recommended, includes a toxic level and NOAEL
Exposure time	Generally 6-12 months

CONCLUSION

Experimental toxicology provides new means to evaluate complex biological system and the impact of chemicals on

living systems. Specifically, experimental toxicology is applied in the analysis of chemical hazards, access effect to chemicals in early life exposure to chemicals, analyze compounds, modes of action, screen for potential toxic responses, refine exposure, assessment and analyze biologic effect of combined exposures. Application of experimental toxicology can improve understanding and minimize adverse effects to chemicals on humans, animals and living portion of the environment.

It is now important to develop new methods for testing toxicity, which if adapted, should produce more accurate and reproducible results using few animals. Only through the humane use of animals in research, can we hope to continue to improve the lives of both humans and animals. We all owe them a depth of gratitude for the part they play in saving the lives of millions of people around the world.



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