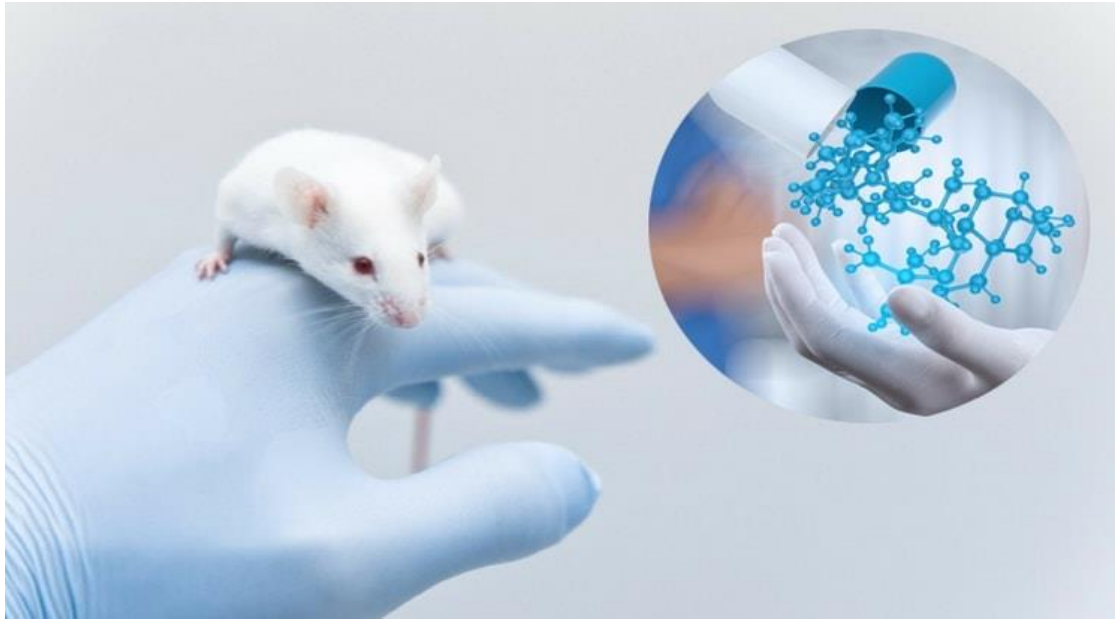
PRE CLINICAL TOXICOLOGICAL STUDIES



Presented by :
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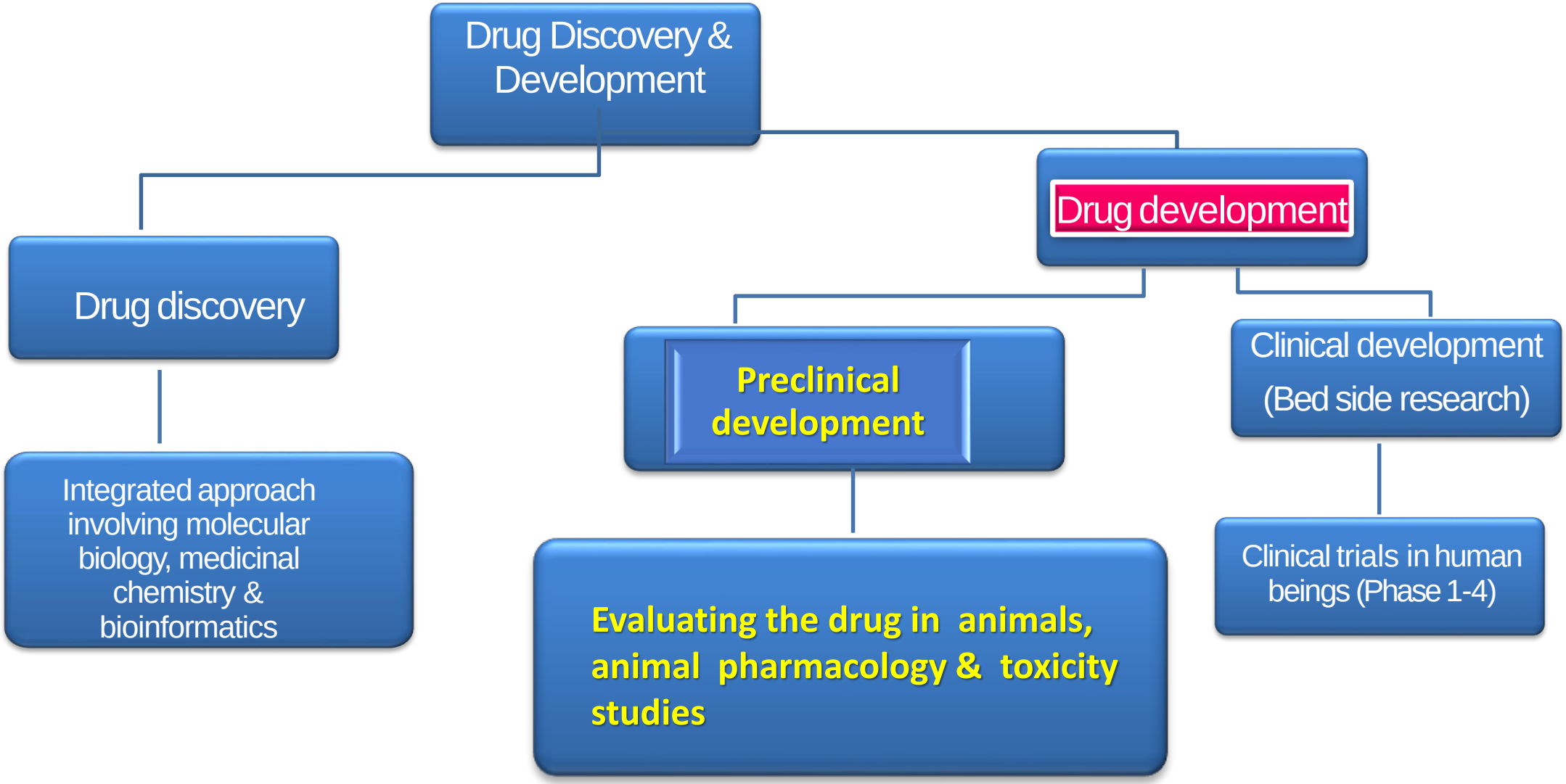
OVERVIEW

- Introduction
- Goals of Preclinical Studies
- Why animals are used in research?
- Species selection
- Types of toxicity studies
- Summary


Drug Discovery & Development
Drug discovery
Integrated approach involving molecular biology, medicinal chemistry & bioinformatics
Drug development
Preclinical development
Evaluating the drug in animals, animal pharmacology & toxicity studies
Clinical development (Bed side research)
Clinical trials in human beings (Phase 1-4)

INTRODUCTION

- **Toxicology** - Study of poisons & concerned with adverse effects of xenobiotics

- Toxicological screening is very important for development of new drugs & for extension of therapeutic potential of existing molecules

- US-FDA - it is essential to screen new molecules for pharmacological activity and toxicity potential in animals

INTRODUCTION…

- Toxicity tests

  - mostly used to examine **specific adverse events** or specific end points such as cancer, cardiotoxicity and skin/eye irritation

  - also helps **to calculate the No Observed Adverse Effect Level (NOAEL)** and no observed effect level (NOEL) which is helpful for clinical studies.

  - carried out with **minimum 3 doses : low, medium & high**, in experimental animals and the toxic effect compared with data from a controlled group of animals.

WHY TO DO TOXICOLOGY TESTING?

Need to prove new drugs are safe:

✓Before clinical trials

✓Before first administration to humans

Preclinical toxicology studies

Studies done to evaluate safety of a candidate drug (New Chemical Entity/ New Biological Entity) in in-vivo or in-vitro test systems to ascertain its safety for human consumption

GOALS OF PRECLINICAL STUDIES

- Identify initial safe dose
- Identify target organs for toxicity
- Study of such toxicity whether reversible
- Identify safe parameters for clinical monitoring

WHY ANIMALS ARE USED IN RESEARCH?

- Very limited number of studies can be done on humans
- Physiology/ anatomy can be matched to human

- Susceptible to the same diseases that affects human
- Short life span allows animals to be studies throughout their life

- Allows controlled experiments

WHY ANIMALS ARE USED IN RESEARCH?

- Environmental variables can be controlled
- Dosage/route of exposures can be controlled/varied
- Experiments can be replicated

SPECIES SELECTION

- Data in two species is required by the Regulatory authority
- Why two species?
  - Species difference in response
- Rodent (rat) is the most common choice
  - Mouse has poor clinical consistency
- Non-rodent- Dog, non-human primates

TYPES OF TOXICITY STUDIES

1. Systemic toxicity studies
2. Male fertility studies
3. Female reproduction & developmental toxicity studies
4. Local toxicity
5. Allergenicity /hypersensitivity
6. Genotoxicity
7. Carcinogenicity

1. SYSTEMIC TOXICITY STUDIES

a) **Single dose toxicity studies**

b) **Repeated dose toxicity studies**

a) SINGLE DOSE TOXICITY STUDIES

- **Acute toxicity studies**

- **Animals** : Done in 2 rodent species (mice & rats)
  - 5 animals of either sex in each group

- **Route of drug administration** – same as intended route in humans
  - One additional route to ensure systemic absorption

- **Dose** : At least 3 graded dose levels used
  - Max dose used – 2 g/kg or 10 times human dose

- **Treatment** : Given in a single bolus / by continuous infusion / several doses within 24 hours.

SINGLE DOSE TOXICITY STUDIES…

- **Observation period** – 14 days
  - Maximum tolerated dose (MTD), minimum lethal dose (MLD)
  - Target organ of toxicity determined
- Observations
  - General appearance, activity & behaviour, Signs of intoxication
  - Effect on body weight, organ weight
  - Gross pathological changes
  - Histopathology of grossly affected organs
  - Haematological, biochemical & urine analysis

Limitations of Acute Toxicity Testing

- Acute toxicity testing permits the 50% lethal dose (LD 50) of the investigational product to be determined.
- The LD50 was used as an indicator of acute toxicity previously.
- The determination of the LD involves large numbers of animals, and the mortality ratio is high.
- Because of these limitations, modified methods were developed:
  - **The fixed dose procedure (FDP)**
  - **The acute toxic category (ATC) method**
  - **The up-and-down procedure (UDP) method**

The Fixed Dose Procedure (FDP)

- Used to assess the **nonlethal toxicity** rather than the lethal dose.

- The investigational product is administered at fixed dose levels of 5, 50, 500, and 2000 mg/kg

- The experimental animal is observed for a specified period.

The Acute Toxic category (ATC) method

- The ATC method is a sequential procedure in which **three animals of the same sex** are used in each step at any defined dose levels.

- In the ATC screening method, four pre-identified starting doses may be used, and the **test dose should be selected based on the Globally Harmonized Classification system.**

The up-and-down (UDP) method.

- The staircase design.
- Most recommended approach by various regulatory agencies
  - because this method reduces the number of vertebrate animals in research.
- The UDP screening method involves dosing single animals sequentially at 48 hr intervals.
- Female rodents are preferable for UDP testing.

- A dose less than the best-estimate LD dose is selected and administered to an animal, and the animal is observed for 48 h.

- If it survives, the study is continued with a higher dose (twice the original dose); if the animal dies, testing is conducted with a lower dose with another animal of the same sex as the original animal.

- UDP testing is limited to doses up to 2000mg/kg.

b). Repeated dose toxicity studies

- **Animal** : At least two mammalian species (one non-rodent)
- Preceded by dose ranging studies
- **Study duration** depends on
  - Duration, indication & scale of the proposed therapeutic indication)
- **Treatment** : Drug given for 14, 28, 90 & 180 days
- **Route of administration** – same as intended clinical route
- **Dose** : Minimum 3 graded dose groups along with control group

Selection of Dose

Highest dose: produce observable toxicity

Intermediate dose : cause some symptoms but not gross toxicity or death, placed logarithmically between other two doses

Lowest dose_: not cause observable toxicity

Duration of repeated dose toxicity studies	Number of animals required	
	Rodents	Non-rodents
14 – 28 days	6-10/sex/group	2-3/sex/group
> 90 days	15-30/sex/group	4-6/sex/group

- Parameters monitored
  - Body weight, food intake
  - Behaviour & Physiology
  - Biochemical
  - Haematological
  - Gross & histopathological examination of viscera/ organs
- ECG & Fundus examination in non-rodent species

LABORATORY PARAMETERS TO BE MONITORED IN TOXICITY STUDIES

Haematological Parameters:

Haemoglobin	Total Red Blood Cell count	Haematocrit	Reticulocyte
Total White Blood cell count	Differential White Blood cell count	Platelet count	Terminal Bone Marrow Examination
Erythrocyte sedimentation rate (ESR) (Nonrodents 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			

Urinalysis Parameters

Colour	Appearance	Specific Gravity	24 hours urinary output
Reaction(pH)	Albumin	Sugar	Acetone
Bile Pigments	Urobilinogen	Occult Blood	
Microscopic examination of urinary sediment			

LABORATORY PARAMETERS TO BE MONITORED IN TOXICITY STUDIES

Blood Biochemical parameters

Glucose	Cholesterol	Triglycerides	High Density Lipoproteins (HDL) cholesterol (Non-rodents only)
Low density lipoproteins (LDL)	Bilirubin	Serum glutamic pyruvic transaminase (SGPT) (Alanine aminotransferase (ALT)	Serum glutamic oxaloacetic transaminase (SGOT)
Cholesterol(Non-rodents only) Aspartate aminotransferase (AST)			
Alkaline Phosphatase (ALP)	GGT (Non-rodents only)	Blood urea Nitrogen	Creatinine
Total proteins	Albumin	Globulin (Calculated values)	Sodium
Potassium	Phosphorus	Calcium	

2. MALE FERTILITY STUDIES

- **Animal** : Done in 1 rodent species (Rat)
  - 6 adult males/ group
- **Dose** : 3 graded dose levels
  - Highest dose level – minimal toxicity in systemic studies
  - A control group should be taken

- **Route of drug administration** – same as intended in clinical route
- **Treatment**
  - Test drug given daily for min 28 days & max 70 days
  - Paired with females of proven fertility for mating (ratio 1:2)
  - Drug treatment of males continued during pairing
  - Pairing continued till vaginal plug (predicting pregnancy) detected or 10 days (whichever earlier)

MALE FERTILITY STUDIES…..

- **Observation**
  - Pregnant females – examined for fertility index after day 13 of gestation
  - Males sacrificed at end of study
  - Testis & epididymis weighed separately
  - Sperms from one epididymis – examined for motility & morphology
  - Other epididymis & both testes – histological examination

3. FEMALE REPRODUCTION & DEVELOPMENTAL TOXICITY STUDIES

- Required for drugs studied/ used in women of child bearing age

- 3 segments
  - Segment I – **Female fertility study**
  - Segment II – **Teratogenicity study**
  - Segment III – **Perinatal study**

- Segment I & III – Albino mice or rats used
- Segment II – Albino mice or rats + rabbits

(A) Female fertility study

- **Animal** : Rodent species (rat preferred)
  - Min 15 animals/sex/group
- **Treatment** : Drug given to both males & females before mating
  - Drug treatment continued during mating & gestation
- **Route of drug administration** – same as intended human route
- **Dose** : 3 graded dose levels
  - Highest dose level – usually MTD

Female fertility study…

- Observation made in dams
  - Body weight, food intake
  - Clinical signs of toxicity
  - **Mating behavior**
  - **Progress of gestation**
  - **Length of gestation, parturition**
  - Post partum health
  - Macroscopic & histopathological exam of organs

(B) Teratogenicity study

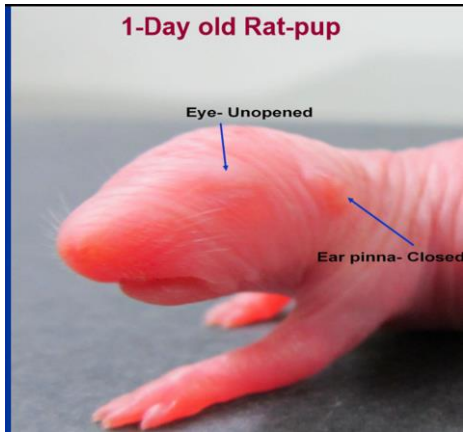
- **Animals** : One rodent (rat) & one non-rodent (rabbit)
  - Minimum 20 pregnant rats/ group
  - Minimum 12 rabbits/ group

- **Treatment** : Drug given throughout organogenesis
- **Dose** : 3 dose levels used along with control
  - Highest dose level : Minimum maternal toxicity

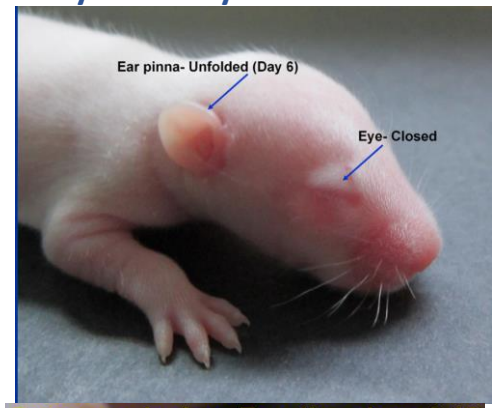
- **Route of drug administration** – same as intended human route
- All foetus examined – skeletal/ visceral abnormalities.

(C) Perinatal study

- Done if drug is given to pregnant or nursing mothers <u>for long period</u>
- **Dose** : 3 dose levels along with control group
- **Animals** : 1 rodent (rat) needed. Atleast 4 groups (including control)
  - Min 15 dams/ group
- **Treatment** :
  - Drug given throughout last trimester of pregnancy (from day 15 gestation)
  - Then Dose that causes low fetal loss continued throughout lactation & weaning
- **Observation** : Monitor growth parameters of F2 generation till weaning



Rat pups: Pinna unfolding- days 4- 6 days



4. LOCAL TOXICITY

- Required if intended **route of administration** of the drug is special other than oral
  - Dermal toxicity study
  - Photo-allergy or dermal photo-toxicity
  - Vaginal toxicity test
  - Rectal tolerance test
  - Ocular toxicity studies
  - Inhalational toxicity studies
- Done in 2 species
- 3 dose levels along with control group
- Increasing group size with increase in duration of treatment.

ACUTE TOXICITY TESTING FOR INHALATION

- For Aerosol-like preparations
- Animal acclimatized to Lab conditions (temp 22 ± 2ºC)
- Exposed to test substance min 4 hrs (max 6hrs/day & 5 days/week) & then monitored for 14 days
- Food is withheld during the exposure period
- **Observations** :
  - Tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma, respiratory rate.
  - Mortality during exposure & observation period
  - Dead animals : Histological & pathological changes

ACUTE TOXICITY STUDIES FOR TOPICAL PREPARATIONS

- Eye irritation test & skin irritation test are important for Ophthalmic & Dermal preparations.
- Draize tests are used in rabbits & guinea pigs

Eye Irritation test

- 0.5 ml of a test substance administered to an animal's eyes
- Animal restrained for 4hr.
- Observed for 14 days
Redness, swelling, discharge, ulceration, haemorrhage & blindness.

Skin Irritation test

- 0.5 g of test substance applied to shaved surface of animal's skin for 7-90 days
- Observed for 14 days
Erythema and Edema
- Animal are sacrificed & pathological changes evaluated

Photo-allergy or Dermal Photo-toxicity

- Tested by Armstrong/Harber test in guinea pig
- Done if the drug or its metabolite or the nature of action is related to cause photosensitivity
- Pretesting in 8 animals to screen 4 concentration (patch application for 2hrs ± 15 min) with or without UV exposure
- Observation recorded at 24 and 48 hours
- Highest non-irritant dose is determined

Photo-allergy or Dermal Photo-toxicity (contd.)

- Main test : 10 test animals and 5 controls
- Induction with dose selected from pre-test, use 0.3ml/patch for 2hrs ± 15 min followed by UV exposure (10J/cm2)
- Should be repeated on day 0,2,4,7,9,11
- 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.
- **Observation** : Erythema and oedema formation at the challenge sites , 24 and 48 hours after the challenge.

Vaginal Toxicity Test

- **Animals :**
  - Study is to be done in rabbit or dog.
  - 6 to 10 animals/dose group taken.
- **Treatment** :
  - Test substance should be applied topically (vaginal mucosa) in the form of pessary, cream or ointment.
- **Dose** :
  - Higher concentrations or several daily applications of test substance to achieve multiples of daily human dose
- **Minimum duration** of drug treatment
  - is 7 days (more according to clinical use), subject to a maximum of 30 days
- **Observation** parameters
  - swelling, closure of introitus and histopathology of vaginal walls

Rectal tolerance test

- **Animals :**
  - Performed in rabbits or dogs.
  - 6 to 10 animals per dose group should be taken.
- **Dose :**
  - volume comparable to human dose (or the maximum possible volume)
  - applied once or several times daily, per rectally, to achieve administration of multiples of daily human dose.
  - Size of suppositories may be smaller, but the drug content should be several fold higher than the proposed human dose
- **Duration** : min is 7 days , maximum of 30 days.
- **Observation**
  - clinical signs (sliding on backside), signs of pain, blood or mucus in faeces, condition of anal region or sphincter, gross and (if required) histological examination of rectal mucosa.

5. Allergenicity/ Hypersensitivity testing

- Guinea pig maximization test or local lymph node assay in mouse (any 1/2)

- **Guinea Pig Maximization Test**
  - Performed in two steps
  - Test & control groups along with positive control
  - **Animal** : Minimum 6 male and 6 female animals per group
  - **Treatment** : Intradermal induction (day 1) coupled with topical challenge (day 21)
  - If no response, re-challenge done 7-30 days after primary challenge
  - **Observation** : Erythema and oedema evaluated

Allergenicity/ Hypersensitivity testing…

- **Local lymph node assay in mice**
  - **Dose** : Three graded doses
  - **Animal** : Minimum 6 mice/ group
  - **Treatment** : Test drug applied on ear skin on 3 consecutive days
  - **Observation** : On day 5, draining auricular lymph nodes dissected out 5 hours after I.V. - H-thymidine or bromo-deoxy-uridine (BrdU)
  - Increase in H-thymidine or BrdU incorporation ⟹ positive test

6. GENOTOXICITY TESTING

- To detect compounds inducing genetic damage
- Standard test battery includes
  - Test for gene mutation in bacteria (Ames' test)
  - In vitro test
    - In vitro cytogenetic test using cell lines
    - In vitro mouse lymphoma tic assay
  - In vivo test for chromosomal damage using rodent hematopoietic cells
    - Micronucleus assay
    - Chromosomal aberrations in rodent bone marrow

7. CARCINOGENICITY STUDIES

- **Indication**
  - Drugs with expected clinical use for more than 6 months
  - Drugs used frequently in an intermittent manner in the treatment of chronic or recurrent conditions
  - Drugs with concern about carcinogenic potential in previous agents in the same class
  - Evidence of preneoplastic lesions in repeated dose toxicity studies
  - Long-term tissue retention of parent compound or metabolite leads to local tissue reactions/ pathophysiological responses
- Done in a rodent species (preferably rat)

Carcinogenicity Studies….

- **No. of animals** : Minimum 50 animals/sex/group
- Drug given 7 days a week
- Usual period of dosing
  - 24 months for rats
  - 18 months for mice
- High dose satellite group
  - 20 animals of each sex

Carcinogenicity Studies….

- **Observation parameters**
  - Signs of intoxication
  - Effect on body weight, food intake
  - Clinical chemistry parameters, hematology parameters, urine analysis
  - Organ weights, gross pathology and detailed histopathology
  - Comprehensive descriptions of benign and malignant tumor development, time of their detection, site, dimensions, histological typing

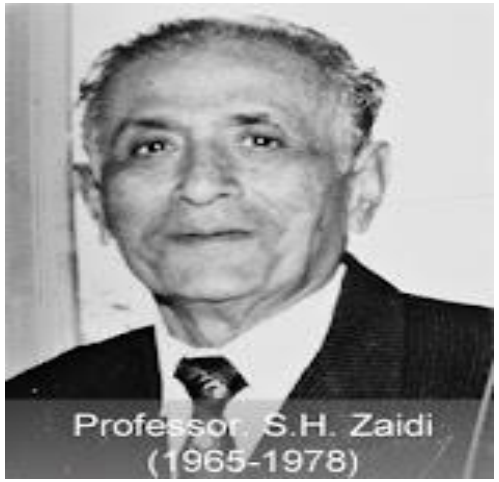
FATHER OF TOXICOLOGY

- **Paracelsus: (1493-1541)** said that

"All things are poisons. It is the 'dose' that differentiates between poison and remedy"

FATHER OF INDIAN TOXICOLOGY

Dr Sibte Hasan Zaidi (1918- 2008)



(Founding Director of INDIAN INSTITUTE OF TOXICOLOGY RESEARCH, Lucknow.)

SUMMARY

- Animal testing is used in pharmaceutical and industrial research to predict human toxicity.

- Rats and mice have been preferred experimental models because of their relatively short life span, their widespread use in pharmacological and toxicological studies

- Toxicity studies provides information on toxic effects of substance, indicate target organs & possibility of accumulation

SUMMARY…

- All individuals performing or assisting in research on animal should have adequate training in animal care & handling so as to misnimize pain and suffering to animal

- It is wasteful of resources (time & money), unethical from an animal welfare point of view and potentially dangerous to humans to perform safety testing in an inappropriate animal model

- Keeping in mind all ethical consideration & protest against experiments on animals by several societies, its better to adopt alternatives of animal experiments whenever possible

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THANKYOU