
بسم الله الرحمن الرحيم
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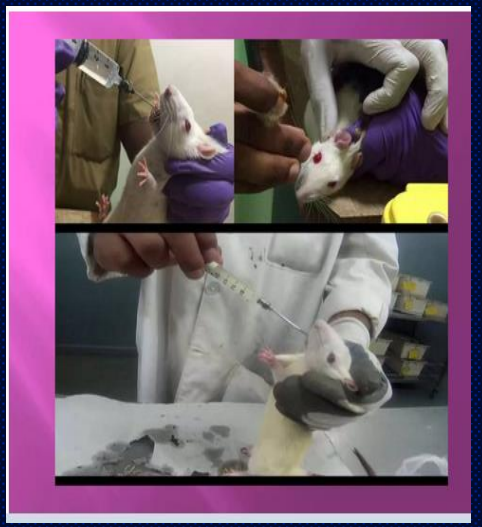
تجویز مواد و داروها ونمونه برداری در حیوانات آزمایشگاهی



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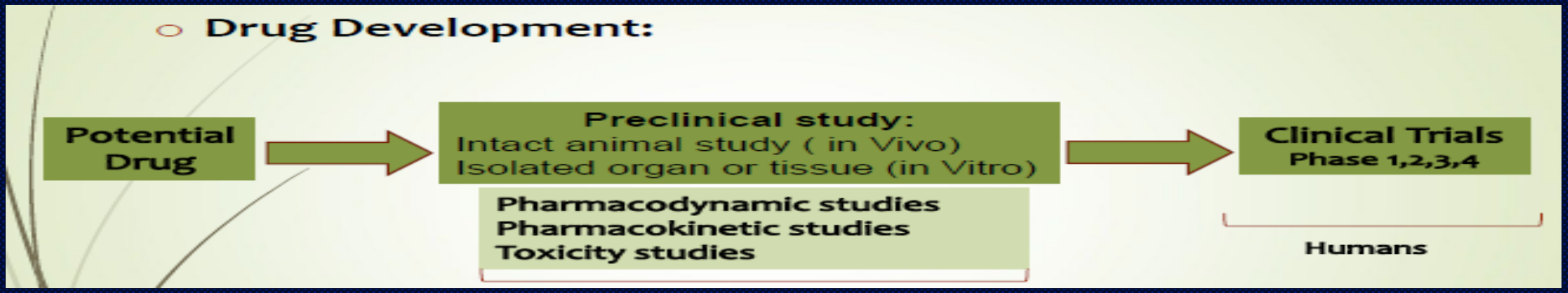
1/31/2023

Over 70% of animals used in research are rats and mice



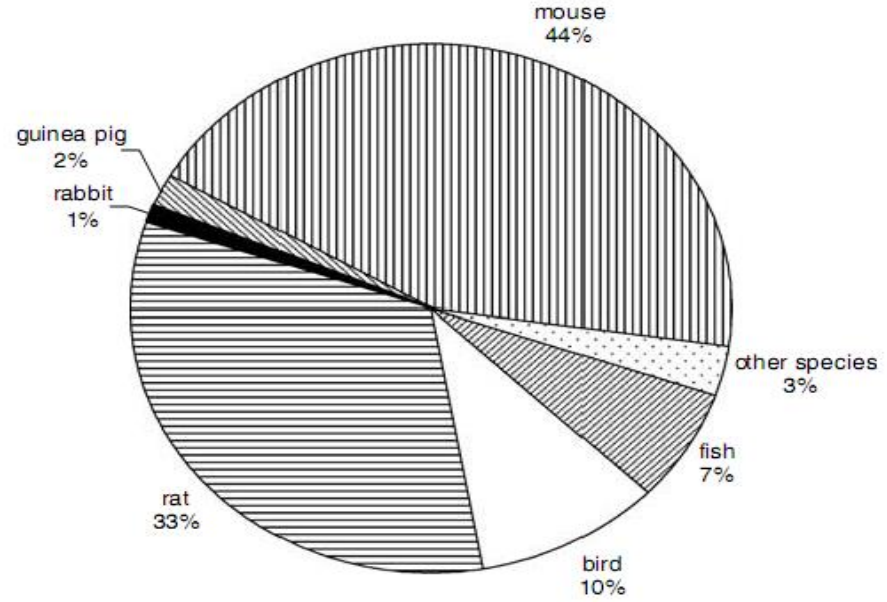


Figure 3 Distribution of vertebrate animal species used for research, testing and education.

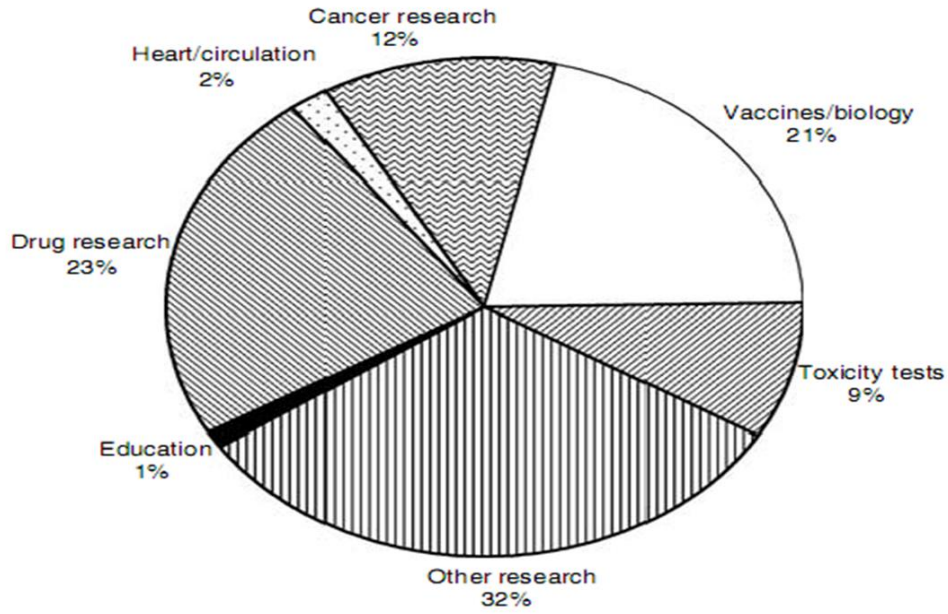


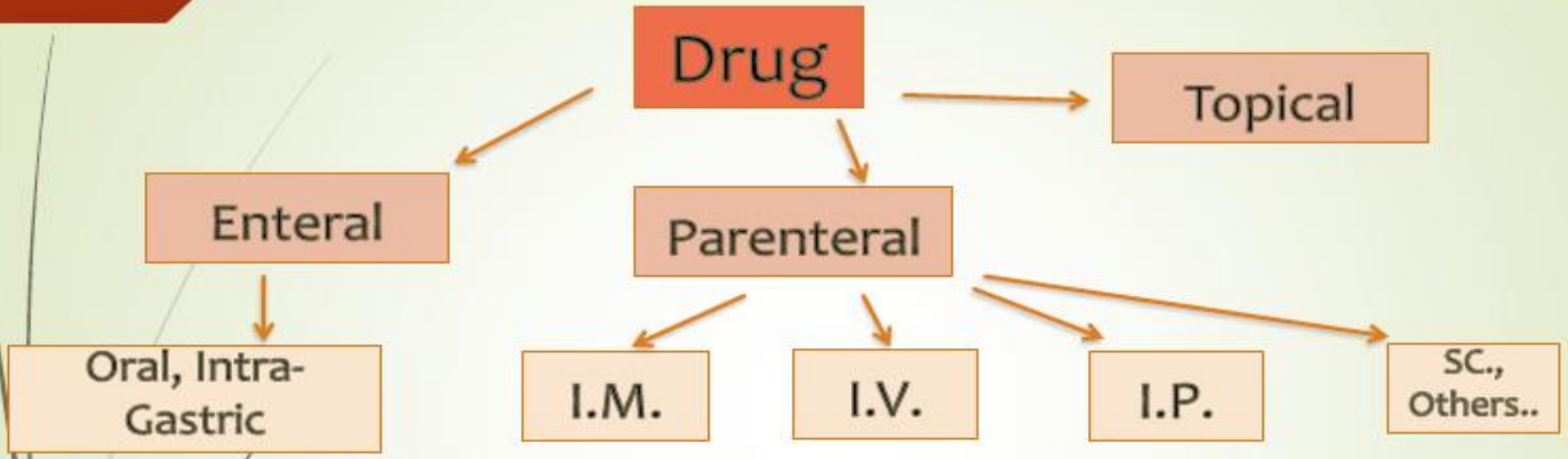
Figure 2 Distribution of the purposes for animal use.

Administration of compounds in Animal lab

- Administration of compounds plays a large part in experimental design using animals

- Before administering any substance (therapeutic or experimental) to an animal subject, one must consider the ***pH, sterility, and chemical nature (odor, taste, mucosal irritability, osmolarity, solubility, light sensitivity, and hazard status)*** of the compound and make appropriate decisions on the dose to be administered, frequency of administration, volume to be administered, the solvent (if necessary), and route of administration

Routes of Drug Administration



Enteral Route of administration

Placement of drug directly into any part of the GIT

It could be Oral, Sublingual ,Intragastric gavage, or Rectal.

1- Oral : Swallowing a drug through mouth, It may be done by adding desired drug to the drinking water or to the food

- The oral route is economical, convenient, relatively safe, and some animals can be trained to cooperate voluntarily, depending on the compound being administered
- This route is not preferable since it inaccurate

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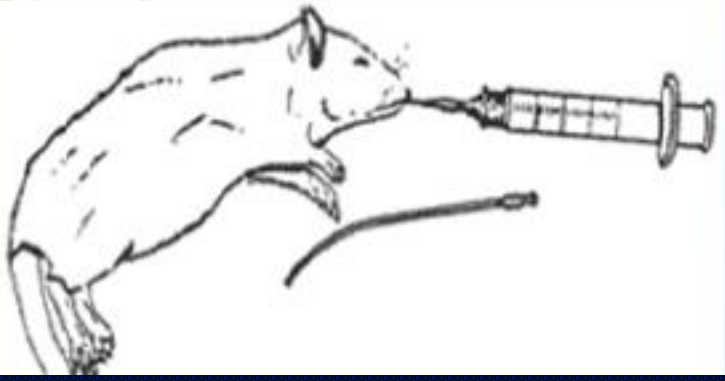
Routes of Drug Administration

Enteral Route of administration

2- Intra-gastric gavage: is the administration of fluids directly into the lower esophageal or stomach.

- Gavage is often used in research settings, instead of mixing substances in water or food, to ensure accurate dosing of animals.
- A small, curved, metal tube, usually with a ball on the end (feeding needle) is often used with small rodents. Entrance may normally be obtained without anesthesia using ordinary hand restraint and the ball prevents trauma to the esophagus and oral cavity.



Procedure for gastric gavage in rodents:

1. Fill the syringe with the appropriate volume of material and attach the needle.
2. Restrain the animal by the scruff. Place the tip or ball of the needle into the animal's mouth. Slide the tip gently past the back of the tongue.
3. The needle should slide easily down the esophagus, if properly placed. **DO NOT FORCE**!!! If any resistance is met, remove the needle and reinsert. **Do not aspirate**. Once the needle is properly placed, administer the material.

- To make sure that the tube is in the esophagus and not in the trachea, dip the end of the tube into a beaker containing water (bubbling indicates wrong position).
- A safe volume to gavage rats and mice is 10 ml gavage solution per kg body weight.

Parenteral routes of administration

- Routes other than Enteral are called Parenteral routes of administration
- Parenteral administration methods typically produce the highest bioavailability of substances because these methods avoid the first-pass effect of hepatic metabolism.

1- **Intravenous (IV)** directly in the vascular system through a vein

2- **Intraperitoneal (IP)** - injected into the abdominal cavity

3- **Intramuscular (IM)** injected into a muscle

4- **Subcutaneous (SC)** injected under the skin

5- **Intradermal (ID)** - injected between the layers of the skin

6- **intracerebral(IC)-** injected into the brain

7-**Epidural** : injected into the epidural space of the spinal cord

8-**Intranasal:** sprayed into the nose for absorption across the nasal mucou

9- **Inhalation:** Inspiration through nose or mouth

10-**Intra-articular:** injection directly into the joint space

Injection site and volume in Rodents

Route	Maximum needle size	Optimal volume	Site
Gavage	Mice: 20 Gauge, (3.8cm) length Rat: 16 Gauge, (7.6cm) length	5 mL/kg (to 20 mL/kg)	intragastric
IV	25	Up to 5 mL/kg	tail or Retro-orbital vein
Sc.	25	Maximum of 5 mL/kg per site	Intrascapular (Scruff), neck, Flank
IM	25-27	Maximum of 0.05 mL/kg per site	caudal thigh , quadriceps muscles
IP	23-25	Maximum of 10 mL/kg	Lower ventral quadrants

Recommended maximum volumes for dosing

	Mouse	Rat	Guinea-pig	Rabbit
Oral	20 ml/kg	20 ml/kg	20 ml/kg	10 ml/kg *
Subcutaneous	20 ml/kg	5 ml/kg	5 ml/kg	1 ml/kg
Intramuscular	0.05 ml total	0.1 ml total	0.1 ml total	0.25 ml/kg/site
Intravenous **	10 ml/kg	5 ml/kg	5 ml/kg	2 ml/kg
Intraperitoneal	20 ml/kg	10 ml/kg	10 ml/kg	4 ml/kg

* for doses by gavage.

** limits quoted are for bolus injection carried out over a relatively short period of time (less than 1 minute).

The limits described are for once daily dosing on a routine basis. Exceptions are certainly possible, but may need special care and supervision.

Acute Lethality Tests (LD50 test)

OBJECTIVES

1. Estimate LD50 or LC50 for comparison
2. Identify target organ of intoxication to predict toxicity effect in human
3. Establish reversibility of toxicity
4. Calculate dose range guiding for further repeated-dose test

COMPONENTS

Acute lethality + Eye irritation + Skin test

Acute Lethality Tests (LD50 test)

METHOD

Route: intended route (e.g. p.o. or parenteral)
Species: 1 rodent + 1 non-rodent
Dose : > 5 level
Observed period: up to 14 days

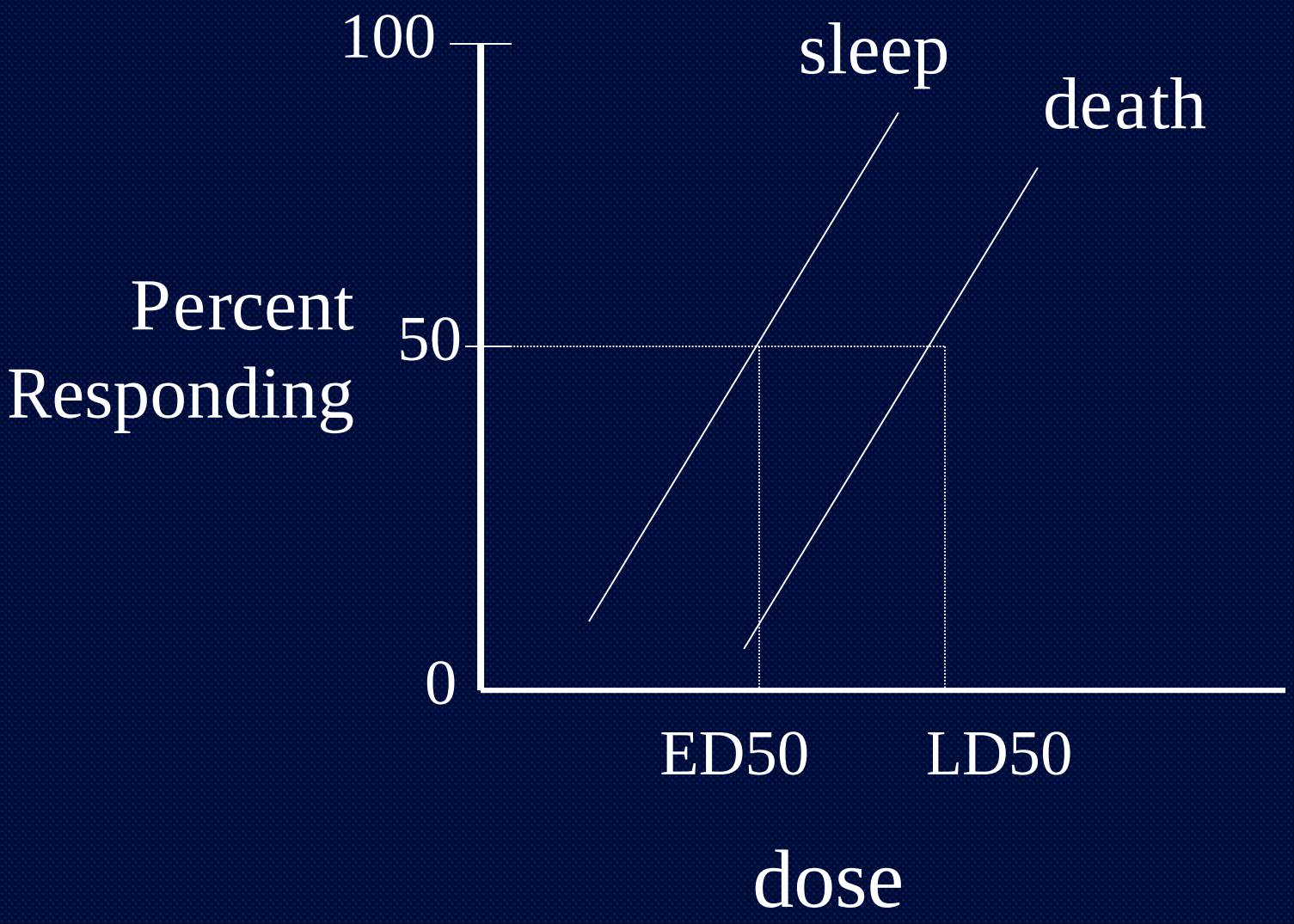
INDICATORS

LD50 $\pm$ 95% confidence interval
Functional toxicity
Histo/pathology, hematology, autopsy, etc.

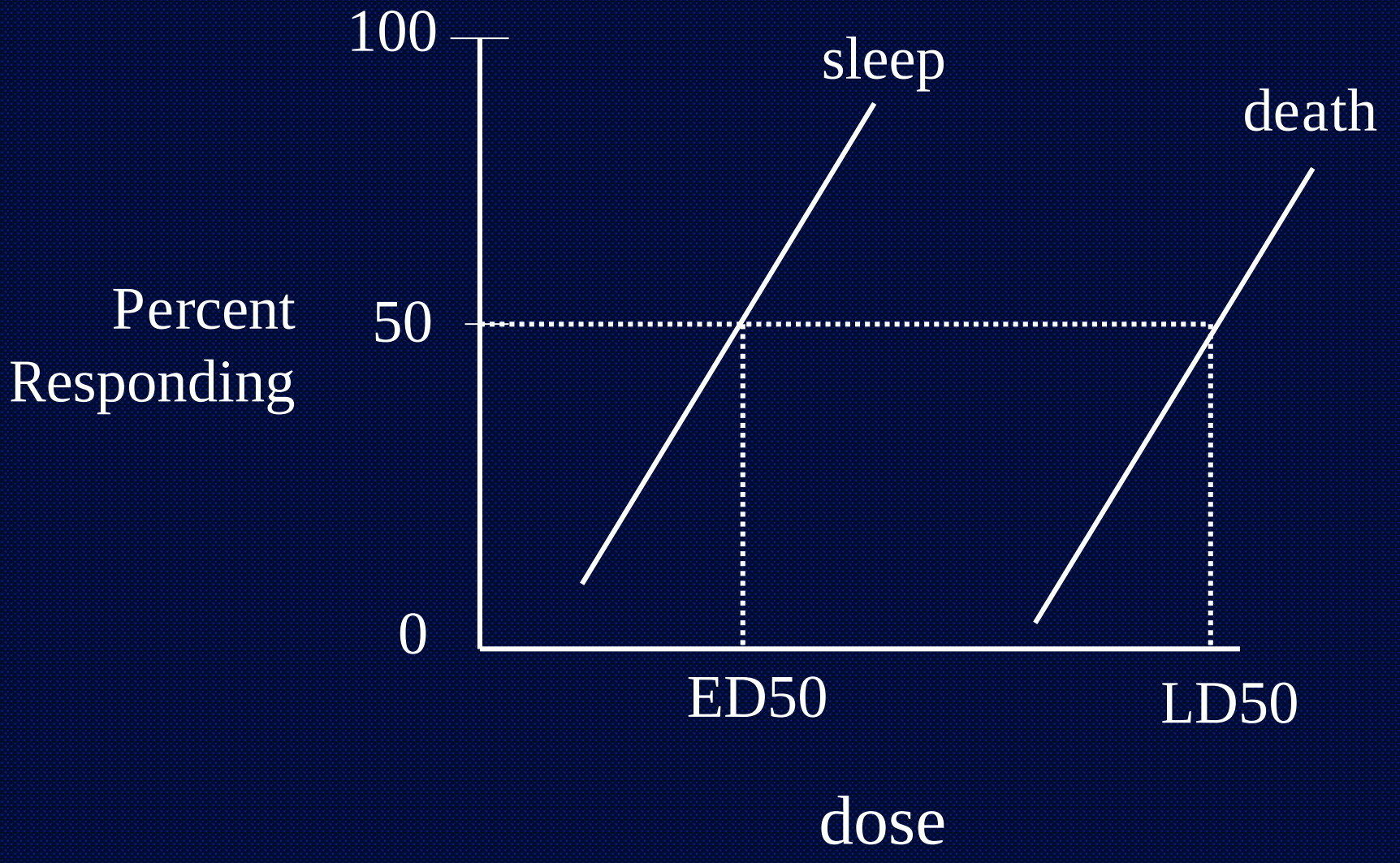
Quantification of drug safety

$$\text{Therapeutic Index} = \frac{\text{TD50 or LD50}}{\text{ED50}}$$

Drug A



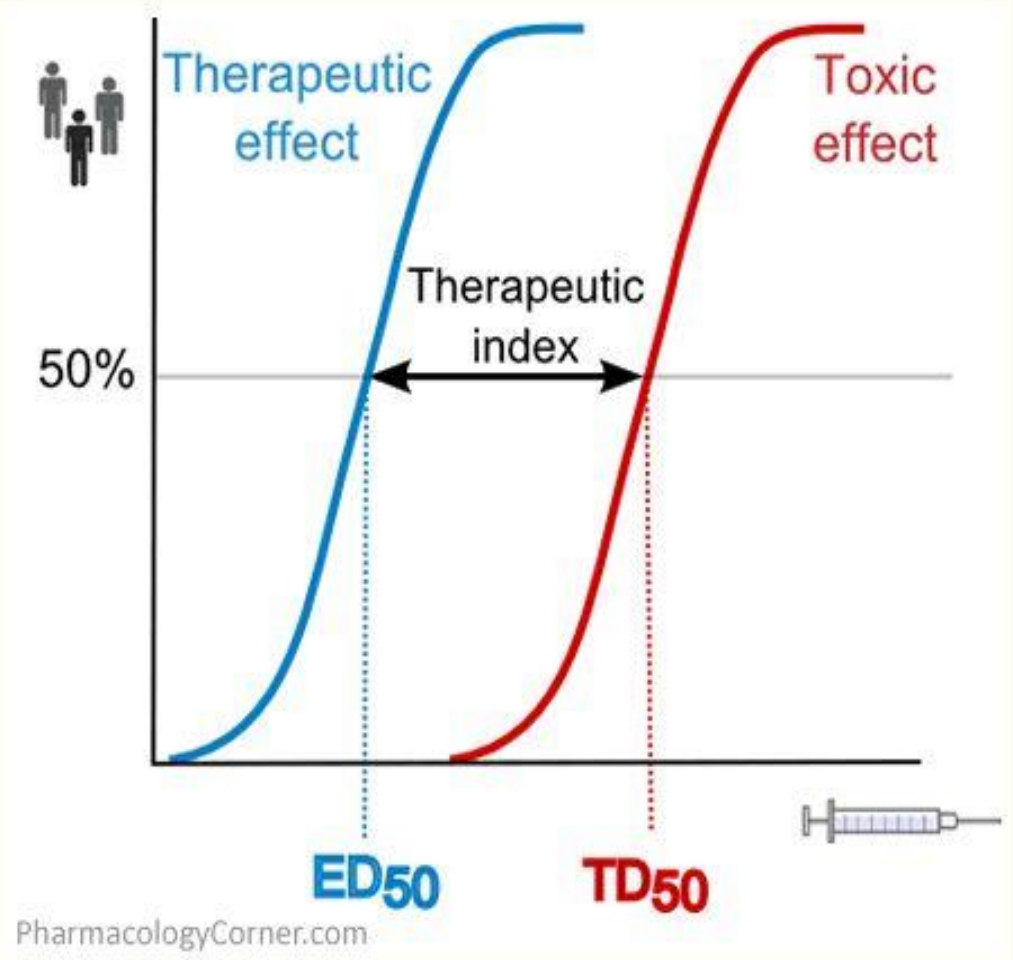
Drug B



Therapeutic Index

- The ratio of the dose that produces the desired therapeutic effect (ED_{50}) to the dose that produces a toxic effect (TD_{50}).

$$\text{Therapeutic index} = \frac{TD_{50}}{ED_{50}}$$



therapeutic index (TI)

- Also may be defined as:

$$\frac{LD_{50}}{ED_{50}}$$

Where LD_{50} is the median lethal dose – the dose of the drug that is lethal to 50% of the animal population tested

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Tutorials for IB Chemistry

Therapeutic index

The therapeutic index is the ratio between the dosage of a drug that causes a toxic (or lethal) effect and the dosage that causes a therapeutic effect.

$$\text{TI (humans)} = \frac{TD_{50}}{ED_{50}}$$

$$\text{TI (animals)} = \frac{LD_{50}}{ED_{50}}$$

ED_{50} (median effective dose) is the dose that produces the therapeutic effect in 50% of the population.

TD_{50} / LD_{50} (median toxic/lethal dose) is the dose that is toxic / lethal to 50% of the population.

Classification chemicals on LD50

Practically non toxic LD_{50} **>15 g/kg**

Slightly toxic LD_{50} **5-15 g/kg**

Moderatly toxic LD_{50} **0.5-5 g/kg**

Very (High) toxic LD_{50} **50-500 mg/kg**

Extremelly toxic LD_{50} **5-50 mg/kg**

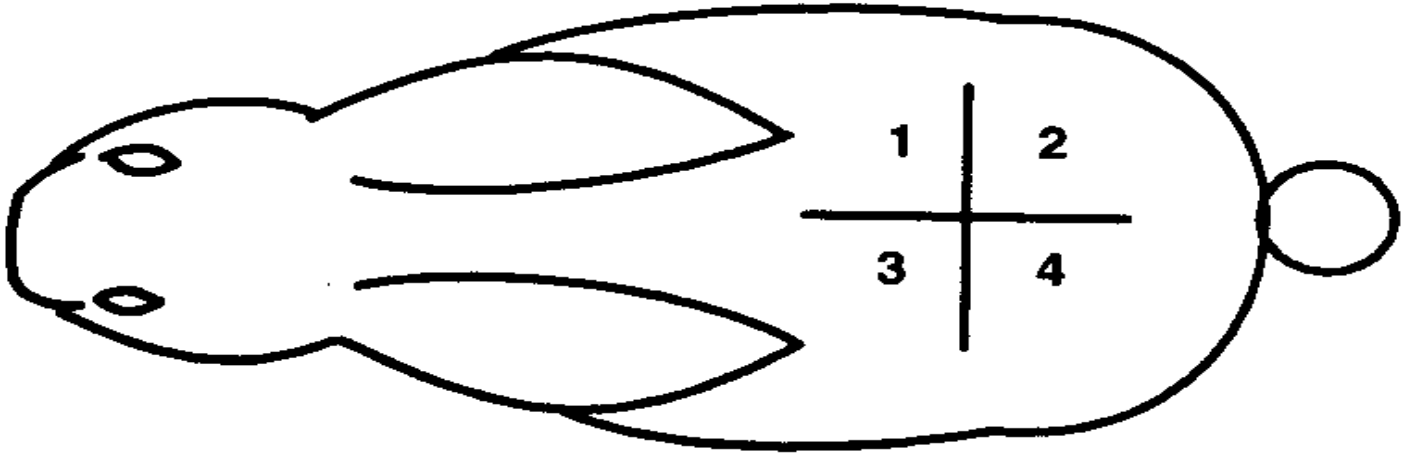
Super toxic LD_{50} **<5 mg/kg**

Skin Irritation Test

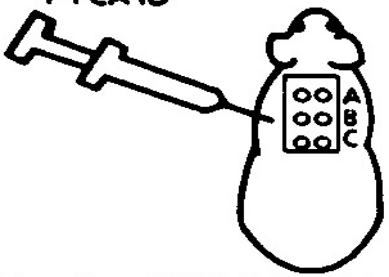
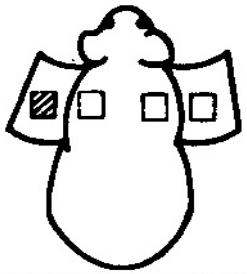
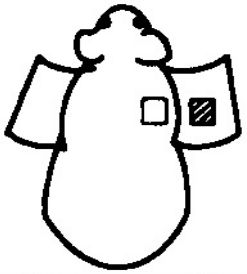
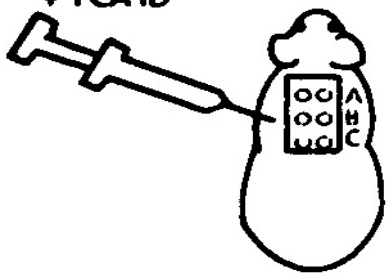
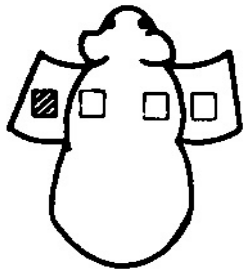
AREAS OF APPLICATION TO RABBIT SKIN

Skin Site:

1 Test Substance
2. Negative Control (Untreated Gauze Patch)
3. Positive Control (1% Sodium Lauryl Sulfate)
4. Vehicle Control

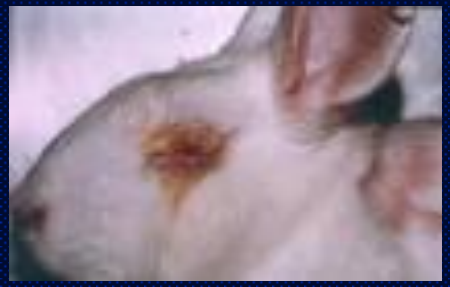


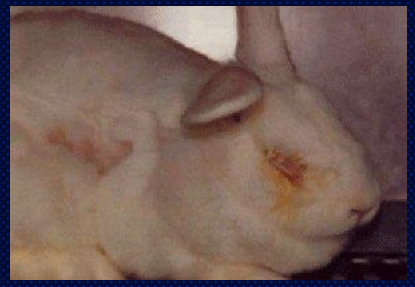
Skin Sensitization Test

Stage	INDUCTION		CHALLENGE	RECHALLENGE
Day	0	7	21	28
TEST GROUP (15)	A. 0.1 ML Substance ID B. 0.1 ml FCA ID C. 0.1 ml Substance + FCA ID 	Closed Patch-48H Application of Substance 	Closed Patch-24H Substance Vehicle 	Closed Patch-24H Vehicle 
TEST GROUP (15)	A. 0.1 ML Vehicle ID B. 0.1 ml FCA ID C. 0.1 ml Vehicle + FCA ID 	Closed Patch-48H Application of Vehicle 	Closed Patch-24H Substance Vehicle 	Closed Patch-24H Substance 

Eye Irritation (Draize) Test

METHOD





Exception of test : pH ≤2 or ≥12

Route: eye
Species: Rabbit (New Zealand White)
Dose : 0.01- 0.1 ml or 100 mg
Control : contralateral eye

Measurement : cornea, iris, conjunctiva

Subchronic Tests

METHOD

Route: intended route

Species: 1 rodent + 1 non-rodent

Dose : > 3 level + control

high dose …… < 10% fatality

……

low dose …… *No apparent toxicity*

Observed period: 14-28 days (animal)/30-90 days (Human)

Chronic Tests

- It involves Sub-lethal concentration and long-term exposure,
- Effect could be anything (biochemical, physiological), but not death.

- Carcinogenicity
- Teratogenicity
- Mutagenicity

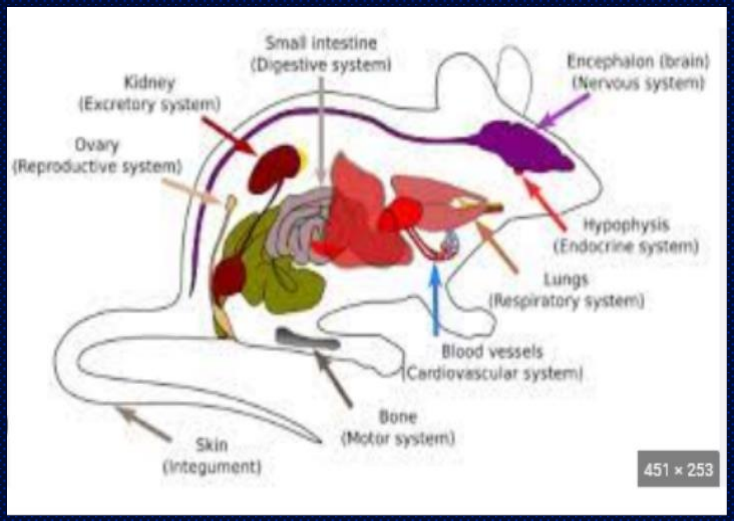
Chronic Tests

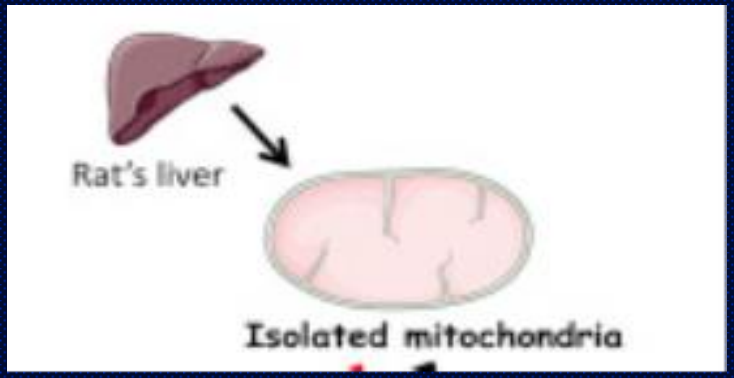
METHOD

Route: intended route
Species: 1 rodent + 1 non-rodent
Dose : > 3 level + control
high dose …….. MTD
then 1/4, 1/8, ……..

Observed period: >90 days to 2 yrs

Sampling in Laboratory Animals





Blood Sampling

- Collection of blood from small laboratory animals is necessary for a wide range of scientific research and there are a number of efficient methods available for that.
- It is important that blood sample collection from experimental animals should be least stressful because stress will affect the outcome of the study.
- Various regulatory agencies and guidelines have restricted the use of animals and the techniques used for blood collection in laboratory animals.

Alternative Blood Collection Methods

Species	Recommended site for blood collection
Mouse*	Tail vein or artery, lateral saphenous vein, facial vein Retroorbital sinus subject to stipulations outlined in this guideline and in the animal use protocol.
Rat*	Tail vein or artery, saphenous vein, lateral saphenous vein, jugular vein Retroorbital sinus subject to stipulations outlined in this guideline and in the animal use protocol.
Rabbit*	Marginal ear vein (small volumes), auricular artery (large volumes)
Guinea pig*	Ear vein, saphenous vein Anterior vena cava collection subject to stipulations outlined in this guideline and in the animal use protocol.
*Cardiac collection subject to stipulations outlined in this guideline and in the animal use	

Blood Collection Volumes

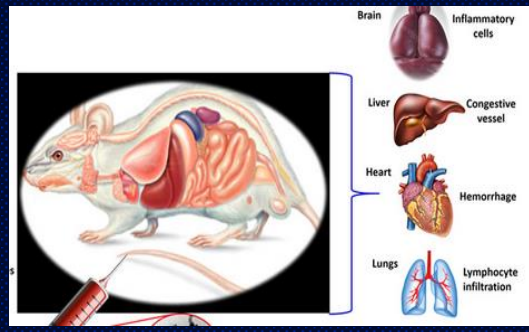
(Serial blood sampling limit vary by species, strain, and frequency of blood collection
(Animal Care and Use Committee (ACUC)

Species	Blood Volume Mean (ml/kg)	Blood Volume Range (ml/kg)	Blood Volume (average)		
			7.5%	10%	15%
Mouse (25 g average weight)	58.6	55-80	110µl	146µl	220µl
Rat (250 g)	64	58-70	1.2 ml	1.6 ml	2.4 ml
Rabbit (4 kg)	56	44-70	17 ml	22 ml	34 ml
Nonhuman primate (NHP; 8 kg)	56	55-75	34 ml	45 ml	67 ml

Tissues Sampling in Animal Lab

For example Rat/Mice tissues isolation

A: Tissue(Liver,kidney,lung, Brain ,Heart,
Pancrase *and….) homogenization*



Tissue homogenate protocol:

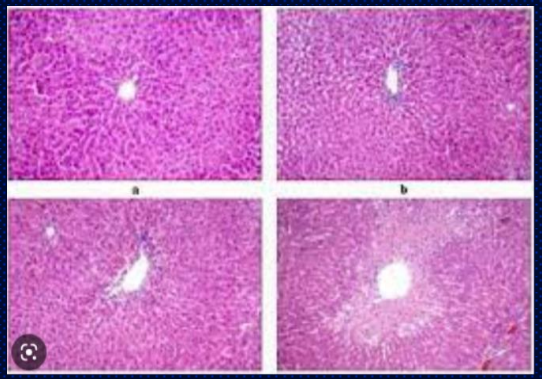
Assay biochemical & molecular parameters

Tissues Sampling in Animal Lab

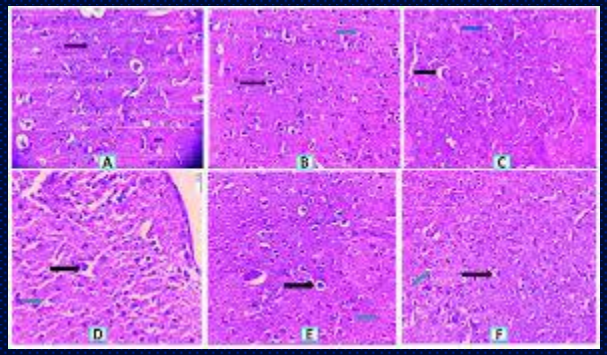
For example Rat/Mice tissues isolation

B: Histological examination: Hematoxylin and eosin (H&E) dyes

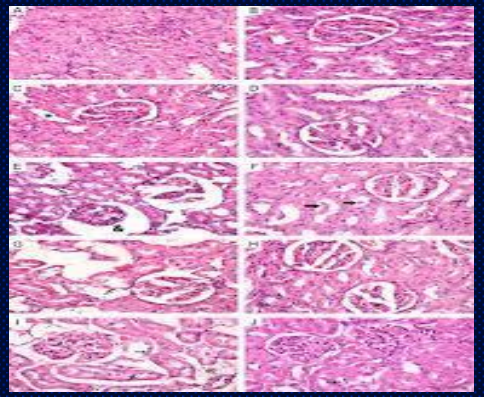
Rat Liver



Rat Brain

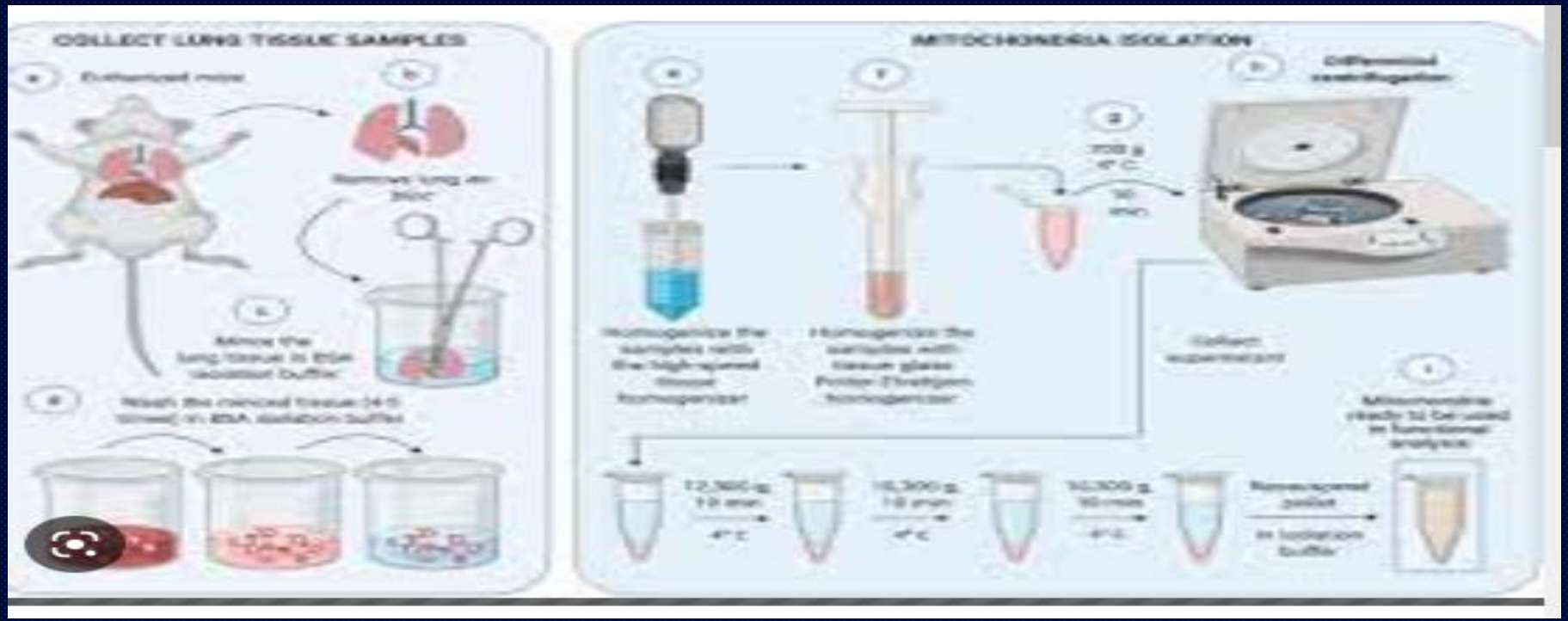


Rat Kidney



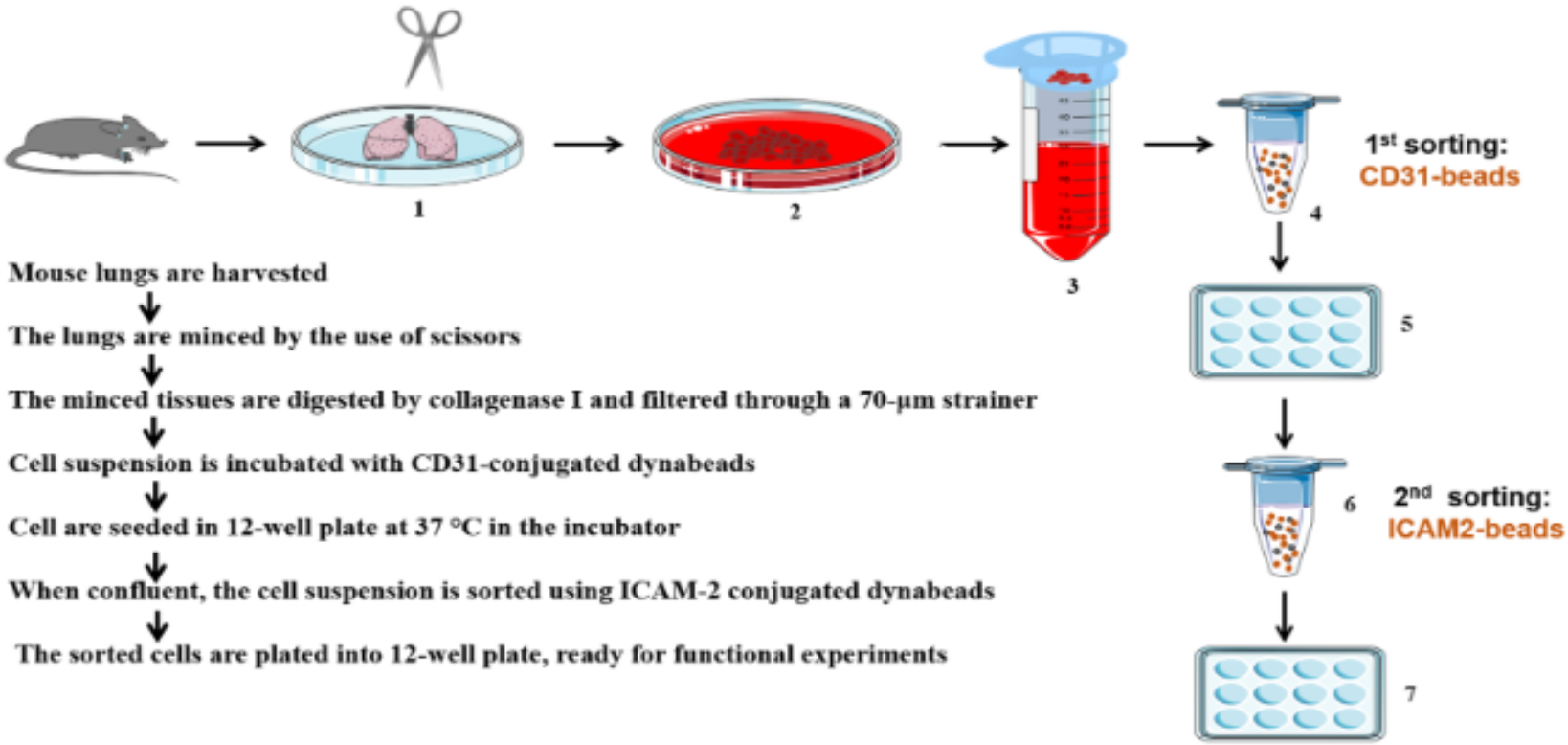
Tissues Organels Sampling in Animal Lab

For example Rat Brain/Lung/ Liver Mitochondrial Isolation



Implication animal tissues in primary cell culture

(Isolating Mouse Lung Endothelial Cells)



Mouse lungs are harvested

↓

The lungs are minced by the use of scissors

↓

The minced tissues are digested by collagenase I and filtered through a 70-µm strainer

↓

Cell suspension is incubated with CD31-conjugated dynabeads

↓

Cell are seeded in 12-well plate at 37 °C in the incubator

↓

When confluent, the cell suspension is sorted using ICAM-2 conjugated dynabeads

↓

The sorted cells are plated into 12-well plate, ready for functional experiments

What Are the Alternatives to Animal Testing?

Alternatives to Animal Testing in the 21 st Century

More than 111 million mice and rats are killed in U.S. laboratories every year.

In vitro methods

Advanced computer-modeling techniques (often referred to as in silico models)

Studies with human volunteers

Animals are not ours

Refrencese

1. IACUC (Institutional Animal Care and Use Committee) protocols
2. Administration of Substances to Laboratory Animals: Routes of Administration
3. GOOD PRACTICE GUIDELINES :Administration of Substances
(Rat, Mouse, Guinea Pig, Rabbit
4. IG048: GUIDELINE ON ADMINISTRATION OF SUBSTANCES TO LABORATORY ANIMALS
5. Intraperitoneal Route of Drug Administration: Should it Be Used
in Experimental Animal Studies?
6. Standard Operating Procedures for Tissue Sampling of Rodents and Other Species
7. IACUC for blood collection in laboratory animals
8. Blood sample collection in small laboratory animals
9. A simple protocol for isolating mouse lung endothelial cells
10. Reducing the stress of drug administration: implications for the 3Rs


BE KIND TO
ANIMALS