



# TOXICOLOGY STUDIES

Toxicology Lab.  
4th Stage / 2nd semester  
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**Done By:**

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# Importance of Toxicology Studies:

Why pre-clinical toxicology studies are required before administered to humans?

- Toxicological screening is very important for the development of new drugs and extension of the therapeutic potential of existing molecules
- Benefit to risk ratio can be calculated
- Prediction of therapeutic index/ safety window (median lethal dose/median effective dose)
- Toxic effect in species will predict adverse effects in man
- Risk assessment can be made by comparison of toxic doses in test species with predicted therapeutic dose in man

# Types of Toxicology Studies:

## 1. Systemic toxicity studies:

- *Acute toxicity (single dose exposure)*
- *Sub-acute & chronic toxicities (repeated exposure)*

## 2. Reproductive toxicity studies:

- *Male fertility*
- *Female reproductive and developmental studies*

## 3. Local toxicity studies

## 4. Hypersensitivity studies

## 5. Genotoxicity studies

## 6. Carcinogenicity studies

# 1- systemic toxicity studies:

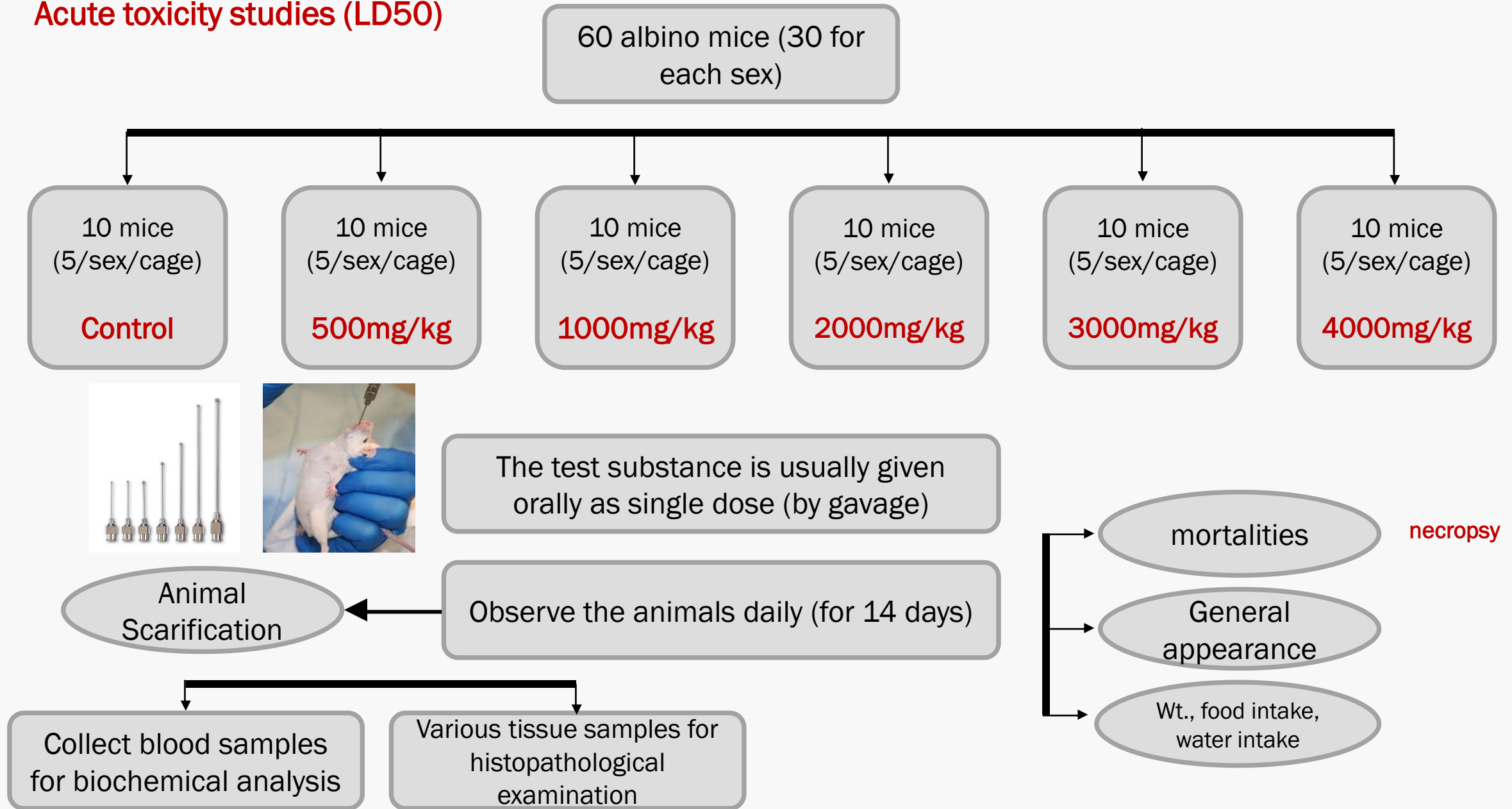
## A. Acute toxicity study (single exposure):

- *Definition: toxicity produced by any chemical substance which is administered as single dose over a period of time not exceeding 24 hr*
- ***Aim of acute toxicity study:***
  - Determination of LD50 and Therapeutic Index (TI)
  - Provide information for possible target organ toxicity and further delayed toxicity
  - Provide information for the design and dose selection for prolonged studies
  - Provide valuable information to the clinician for the prediction, diagnosis and treatment for acute over dose of chemicals
  - Classification and labeling of chemicals for regulatory purposes

– ***Criteria required for this test:***

- Animals used are either rodents (mice or rats) or non-rodents (dogs, cats, monkeys, etc...)
- Each groups must contain both genders (male & female) of the selected animal species (eg: 5/sex/dose)
- Single high dose of the test substance is administered for all the animals during a period of 24 hr
- Route of administration for test chemicals is (oral) or IV (if possible and the chemical is pure)
- Duration of the study is 14 days

# Acute toxicity studies (LD50)



**B. Repeated dose exposure (sub-acute and chronic toxicities):**

**- Sub-acute toxicity studies:**

***Aim:***

- Designed to determine the organs affected by different dose levels
- Determine the maximum tolerable dose (MTD) and the nature of toxicity

***Criteria required for this test:***

- Animals/ rodents and non-rodent species are used (5/sex/dose)
- Doses are generally selected on the basis of information obtained in acute toxicity studies using both LD50 and the slope of the dose response curve.
- Route of administration/ the test chemical is given orally or by other routes (if possible)
- Duration of exposure is usually 14 – 28 days
- The test chemical is administered on daily bases for 14 days
- The study is performed at 4 dose levels (high, intermediate, low, and vehicle)

- Sub-chronic & chronic toxicity studies:

***Aim:***

- To evaluate the cumulative toxicity of chemicals
- To assess carcinogenic potentials

***Criteria required for this test:***

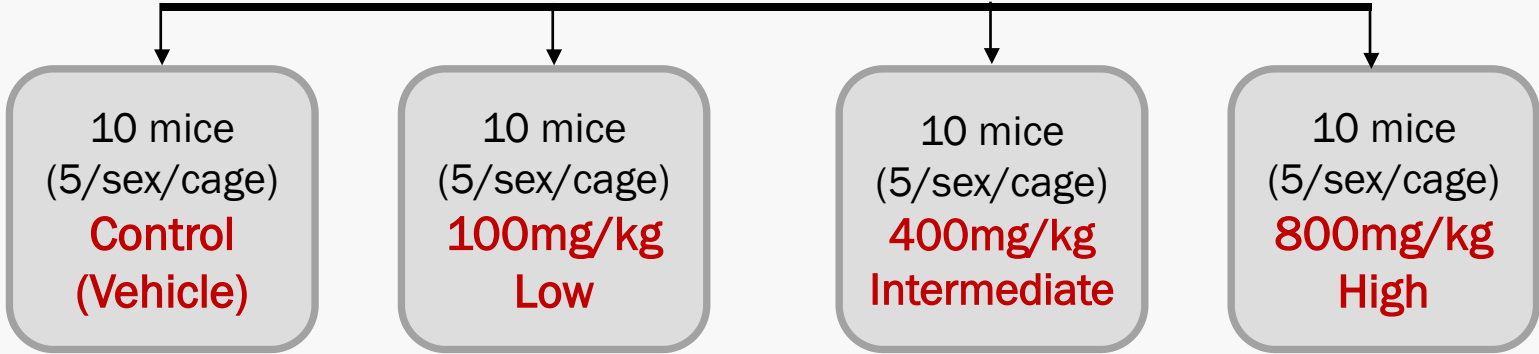
- Animals/ rodents and non-rodent species are used (5/sex/dose) or more
- Doses are generally selected on the basis of information obtained in acute toxicity studies using both LD50 and the slope of the dose response curve.
- Route of administration/ the test chemical is given orally or by other routes (if possible)
- Duration of exposure is usually (1 – 3 months) for sub-chronic toxicity, (6 – 24 months) for chronic toxicity
- The test chemical is administered on daily bases till the end of the study
- The study is performed at 4 dose levels (high, intermediate, low, and vehicle)



# Repeated Dose Exposure:

40 albino mice or rats  
(20 for each sex)

- 1- sub-acute toxicity
- 2- sub-chronic toxicity
- 3- chronic toxicity



The test substance is usually given orally on daily bases (by gavage)

Observe the animals daily or weekly till the end of the study

Animal Scarification

- mortalities
- General appearance
- Wt., food intake, water intake

necropsy

necropsy

Collect blood samples for biochemical analysis

Various tissue samples for histopathological examination

### ***For single exposure (acute toxicity):***

- Animals must be observed daily for 14 days after administration of the test substance
- **Parameters that must be observed are:**
  - *mortalities, (most important)*
  - *general appearance of the animal,*
  - *weight,*
  - *food intake,*
  - *any abnormal clinical signs)*
- Gross **necropsy** must be performed on all animals (those found dead or sacrificed at the end of the experiment)
- At the end of the study, different tissue specimen are collected for histological examination, blood samples are also collected for biochemical analysis

### ***For Repeated exposure:***

- Animals must be observed daily for the first 14 days, then once weekly
- Observations are recorded for each animal individually for clinical signs of toxicity
- **Mortalities, body weight, food consumption, water intake, urine examination, and stool examination are recorded weekly**
- Blood samples collected monthly and at the end of the study for hematology and biochemical analysis
- Animals found dead during the examination should be examined as soon as possible in an attempt to identify the cause of death and severity of toxic changes present
- At the end of the study, surviving animals are sacrificed and different organs and tissues are harvested for histopathological examination

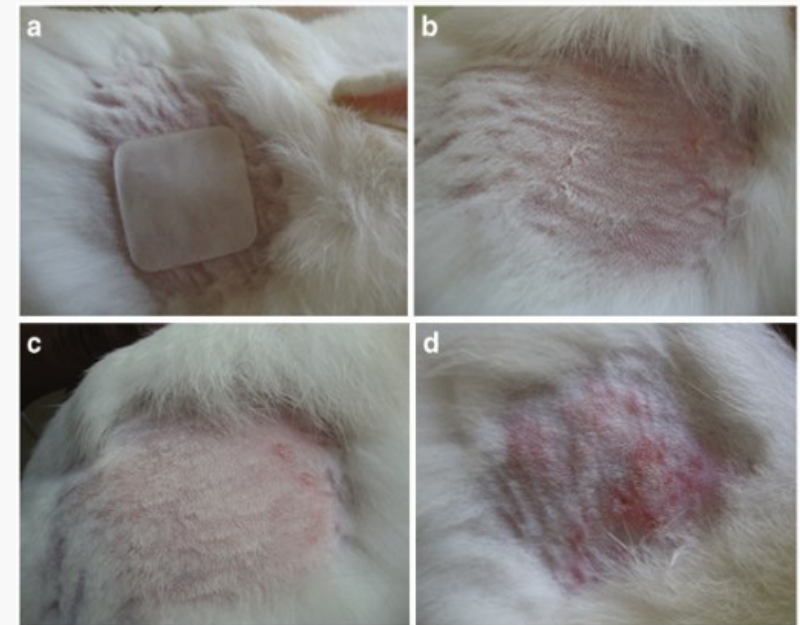
## 2- Reproductive Toxicity Studies:

- A type of repeated exposure study
- Duration of exposure (1 – 3 months)
- Aims to assess the toxic effect of test chemical on the reproductive system of male and female animal species
- For male animal species/ weight of testis and epididymis is recorded, histology examination, sperm examination for motility and morphological changes
- For female animal species/ fertility and reproductive performance, teratogenicity, embryonic development and growth disturbances



# 3- Local Toxicity Studies:

- A type of repeated exposure studies
- Route of administration/ any route (other than the oral) including:
  - *Dermal*
  - *Vaginal*
  - *Rectal*
  - *Ocular*
  - *Inhalation*
  - *Parenteral (IM, SC, ID)*
- **Observational signs:**
  - *Erythema, swelling, pain and itching*
  - *Mucus secretions, discharge, blood*
  - *Vaginal and rectal tissue histology*
  - *Ocular/ changes in cornea, iris, aqueous humor*
  - *Inhalation/ changes in RR, mucus secretions, lung tissue histology*



# 4- Hypersensitivity Studies:

- A type of acute toxicity (single exposure) study
- **Uses:**
  - *to evaluate the development of erythema, oedema, pain and itching*
  - *Determine the maximum irritant dose, maximum non-irritant dose*
- The test chemical is usually applied on the skin
- Lymph node near the application area are examined regularly for enlargement and swelling
- At the end of the experiment, blood samples are collected, skin tissues and lymph nodes for histopathological examination

# 5- Genotoxicity (Mutagenicity) Studies:

- Aims to detect the tumorigenic effects of certain cases of chronic illness or chronic use of medications
- It depends of the ability of tissue cells to adapt and repair, **specially** the ability of the DNA repair mechanisms to detect any mutations and initiate repair
- Performed by two assays:
  - *In vitro tests:*
    - Test for gene mutations in bacteria
    - Cytogenetic evaluation of chromosomal damage in mammalian cells
  - *In vivo tests:*
    - Chromosome damage in rodents hematopoietic cells

# 6- Carcinogenicity Studies:

- A type of repeated exposure toxicity
- Exposure to a test chemical with a chemical structure that indicates carcinogenic potentials
- Duration of study is usually (18 – 24) months depending on the life span of the animal species
- Observation for any gross morphological changes, then histopathological examination of different tissues and organs

# The Experiment

## Determination of LD50





# Determination of LD50:

- The amount of a toxic agent that is sufficient to kill 50 percent of a population of animals usually within a certain time
- Also called median lethal dose
- Expressed as milligrams of substance per kilogram of body mass (mg/kg)
- A measure of acute toxicity



## *LD<sub>50</sub> Example*



Chemical A: LD<sub>50</sub> = 3.2 mg/kg

Chemical B: LD<sub>50</sub> = 48 mg/kg

Which is more toxic?

■ The purpose of measuring or studying the LD50:

- *to compare the toxic potency or intensity of different chemicals*
- *The LD50 gives a measure of the immediate or acute toxicity of a chemical in the strain, sex, and age group of a particular animal species being tested. (Changing any of these variables (e.g., type animal or age) could result in finding a different LD50 value.) why?*
- *As an aid in developing emergency procedures in case of a major spill or accident.*
- *To help develop guidelines for the use of appropriate safety clothing and equipment*
- *For the development of transportation regulations.*
- *As an aid in establishing occupational exposure limits.*
- *As a part of the information in Safety Data Sheets.*

- **Methods used for measuring the LD50:**

- *Graphical method, arithmetical method and statistical approach (limitation for these methods is the number of animals is small):*
  - Karber method
  - Miller and Tainter method
  - Reed-Muench method
- *Other alternative methods for measuring the LD50:*
  - **Fixed Dose Procedure (FDP):** This method does not use death as an endpoint, instead it uses the observation of clear signs of toxicity developed at one of a series of fixed dose levels to estimate the LD50.
  - **Acute Toxic Class method (ATC):** This method does not use death as the only endpoint, it also uses signs of toxicity in its stepwise approach to estimating the LD50.
  - **Up-and-Down Procedure (UDP):** This method does still use death as an endpoint, but doses animals one at a time to see if the dose needs to be put up or down to achieve an estimate of the LD50 therefore giving the minimum number of animals a lethal dose of the test substance.

## Signs recorded during acute toxicity studies:

- motor activity
- Anesthesia
- Tremors
- arching and rolling
- clonic convulsions
- tonic extension
- Lacrimation
- salivation
- Straub reaction
- muscle spasm
- Writhing
- Hyperesthesia
- loss of righting reflex
- Depression
- Sedation & hypnosis
- Blanching
- Ataxia
- Analgesia

# Materials & Methods:

- Insulin syringes
- Sensitive digital balance
- Calculator
- Albino mice (male & female)
- Test substance (to determine the LD50)



# Method (Procedure):

- Put 4 albino mice of both sexes (male and female) in separate cages
- Observe the general appearance and vital signs of the animal
- Weigh each mouse then take the average weight (weight of all test animals is preferably similar)
- Calculate the required dose that must be administered according to body weight
- Administer the test substance by IP route to each mice
- Record the number of dead animals within an hour of the test substance administration
- Observe and record any abnormal behavior, tremor and seizures

## Objective of the experiment:

- To determine the Median Lethal Dose of the test substance introduced on mice intraperitoneally

# Example: Determining LD<sub>50</sub>

Starting population



100 mg/kg exposure



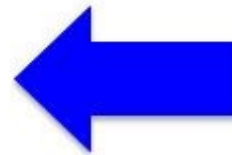
200 mg/kg exposure



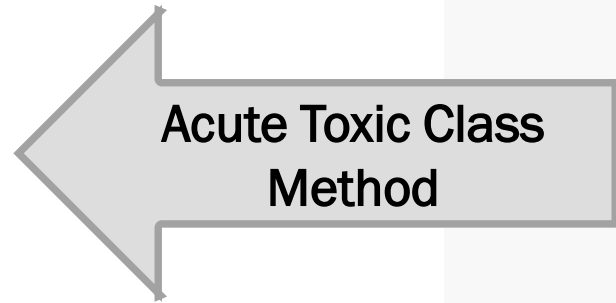
300 mg/kg exposure



400 mg/kg exposure



LD<sub>50</sub>



# Calculating the correct dose:

Test substance: **Phenobarbital** available as 200mg/1ml amp.

Make a (stock sol.) of phenobarbital >>> dilute 200mg in 10ml of DW or NS (final conc. 20mg/ml)

LD50 for phenobarbital = **150mg/kg** route of admin. = IP

Let us assume that the Wt. of the test animal (mouse) = 32.8gm. How to calculate the correct dose that must be administered?

$$\begin{array}{l} 150\text{mg} \quad 1000\text{gm} \\ X \quad 32.8\text{gm} \end{array} \gg \gg X = 4.92\text{mg}$$

**Note:** each 1ml = 100 IU

$$\begin{array}{l} 20\text{mg} \quad 100 \text{ IU} \\ 4.92\text{mg} \quad X \end{array} \gg \gg X = 24.6 \text{ IU (the correct dose administered as IP)}$$



A close-up photograph of a white mouse and its pup resting on a light blue surface. The adult mouse is in the background, and the pup is in the foreground. A small, tan stuffed mouse toy is positioned between them. The text "THANK YOU" is overlaid in the center of the image.

THANK YOU