



# ACUTE TOXICITY STUDY

## DETERMINATION OF LD50

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# Type of toxicity

They can be divided according to the dose and period of exposure

**1- Acute toxicity**: sudden violent syndrome caused by single large dose of toxicant with high mortality and severe toxic symptoms.

**2- Sub acute toxicity**: repeated large toxic doses for a period less than one month, with severe toxic symptoms and some mortality.

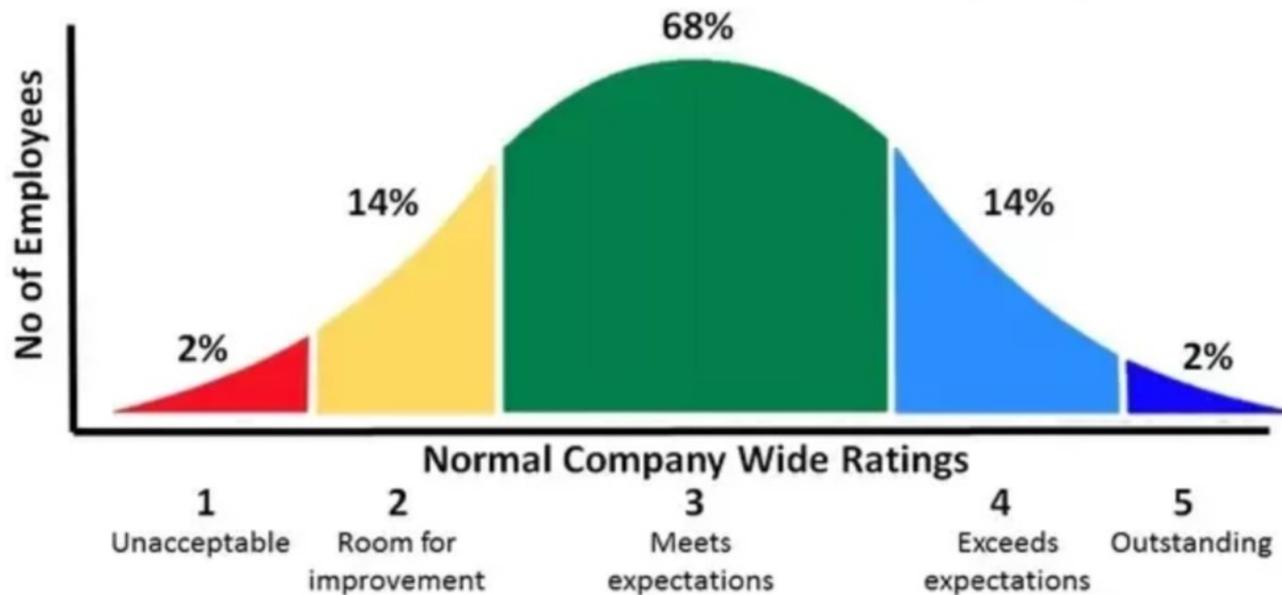
**3- Sub chronic toxicity**: repeated moderate to low toxic doses for a period less than three months with moderate toxic symptoms.

**4- Chronic toxicity**: Long term condition by repeated small doses for a period more than three months with or without any toxicity symptoms, it is used to study carcinogenicity and accumulation.

## Performance Review 1 to 5 Ratings Bell Distribution Curve

Close

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# LD50 : MEDIAN LETHAL DOSE

- ❖ LD 50 is the amount of a material, given all at once, which causes the death of 50% of a group of test animals.
- ❖ The LD 50 is one way to measure the short-term poisoning (acute toxicity) of a material.

- It is an index to determination of medicine and poison's virulence. The lower LD 50 dose, the more toxic.
- It is usually expressed as the amount of chemical administered (e.g. in mg) per 100 gm (for smaller animals) or per kg ( for bigger test subjects) of the body weight of the test animal.
- The LD 50 can be found for any route of entry or administration **but dermal & oral** administration methods are the most common.

# ED50 :

- ED 50 : Is a medical term that stands for "**effective dose**"
- The 50 stands for 50 percent, which is the amount of people who experience a positive therapeutic effect of the treatment in order to be deemed to be effective.
- ED 50 is occasionally used to describe the effectiveness of drugs, most often it's **used in radiology** to describe the effects of radiation treatment.

# DRUG VARIABILITY & TOXICITY ASSESMENT:

- ED 50 : Effective dose for 50% of the subject.
- LD 50 : Lethal Dose for 50% of subject.
- The Therapeutic Index :  $TI = LD_{50} / ED_{50}$
- **No drug is 100% safe.**

# Significance:

1. To compare the toxic potency or intensity of different chemicals.
2. One way is to carry out lethality testing by measuring how much of a chemical is required to cause death.
3. This type of test is also referred to as a “QUANTAL” test because it measures an effect that “OCCURS” or “DOES NOT OCCUR”.

4. As an aid in **developing emergency** procedures in case of a major spill or accident.
5. To help develop guidelines for the use of *appropriate safety clothing and equipment*.
6. For the development of transportation regulations.
7. As an aid in establishing occupational exposure limits.
8. As a part of the information in Material Safety Data Sheets.

- 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.

- **Two most common scales are used:**

1. Hodge and Sterner Scale
2. Gosselin, Smith and Hodge Scale

# Hodge and Sterner Scale:

Toxicity Rating	Commonly used term	LD50 (Rat , Oral)
1	Extremely Toxic	Less than 1 mg/Kg
2	Highly Toxic	1-50 mg/Kg
3	Moderately Toxic	50-500 mg/Kg
4	Slightly Toxic	500-5000 mg/Kg
5	Practically Non Toxic	>5000 mg/Kg

# Gosselin, Smith and Hodge Scale

<b>Toxicity Rating or Class</b>	<b>Dose</b>	<b>For 70-kg Person (150 lbs)</b>
6 Super Toxic	Less than 5 mg/kg	1 grain (a taste - less than 7 drops)
5 Extremely Toxic	5-50 mg/kg	4 ml (between 7 drops and 1 tsp)
4 Very Toxic	50-500 mg/kg	30 ml (between 1 tsp and 1 fl ounce)
3 Moderately Toxic	0.5-5 g/kg	30-600 ml (between 1 fl oz and 1 pint)
2 Slightly Toxic	5-15 g/kg	600-1200 ml (between 1 pint to 1 quart)
1 Practically Non-Toxic	Above 15 g/kg	More than 1200 ml (more than 1 quart)

## COMMON STEPS PERFORMED IN ALL TESTS:

1. The test substance must administered in **graduated doses** to several groups of experimental animals.
2. **Two species** must select- one rodent & other non-rodent, because species differ in their response to toxic agents. Ruminant
3. The substance used in toxicity tests should be as **pure** as the material eventually to be given to humans.

4. The volume of dose administered depends on the size of the test animal. **In rodents** it should not exceed 1ml/100g body wt. and max. of 50 mg/kg.

5. The LD50 value depends on the route of administration. Usually the value are found to increase with the following sequence of routes:

**intravenous** < **intraperitoneal** < **subcutaneous** < **oral**

# What is the difference between a toxic and a non-toxic substance?

- **Toxic substance** is any liquid, solid or gas, which when introduced into the water supply creates, or may create a danger to health and well being of the consumer. An example is Arsenic, Chlorine and snake venom.

- A non-toxic substance is any substance that may create a non-health hazard but its aesthetically objectionable.
- For example, foodstuff, such as **sugar, dog and cat food and the hormones in birth control pills.**

# **METHODS TO DETERMINE LD 50:**

- Different methods used to determine LD 50 are as follows:
  1. Karber's method.
  2. Fixed dose method.
  3. Reed- Muench method.
  4. Miller & Tainter method.
  5. Lorke method.
  6. **Up & down method.**

## UP & DOWN METHOD :

- also called as **staircase** method.
- it occur in two way:
  - A- Main Test.
  - B- Limit Test.

## UP & DOWN method occur by two way:

### 1. MAIN TEST :

- This test must be performed In those situations where there is little or no information about material toxicity, or in which the test material is expected to be **toxic**.
- A single ordered dose progression in which animals are dosed , one at a time , at a minimum of 48-hour intervals.
- The first animal receives a dose a step below the level of the **best estimate of the LD50** .

- If the animal survives, the dose for the next animal is increased by a factor of (3.2 times) the original dose ; if it dies, the dose for the next animal is decreased by a similar dose progression.

## 2. LIMIT TEST :

The limit test is primarily used in situation where the experiment has information indicating that test material is likely to be **non-toxic** . **Such as food additive.**

is a serial test that uses a maximum of 5 animals. A test dose of 5 g or ml /kg used, after overnight fasting for rats.

- Test animals should be observed closely for up to 14 days; symptoms of toxicity and recovery should be noted.
- Gross and histopathological examination of the test animals at the end of the study may help identify toxic effects on target organs.
- If no animals die as a result of this dose, there is no need to test higher dosages.
- The acute toxicity of the compound can then be expressed as being greater than 5 gm (or ml)/kg body weight of the test animal.

- This method is called the "limit test." In general, 5 gm or 5 ml of the test substance/kg body weight is the practical upper limit for the amount of test material that can be administered in one oral gavage dose to a rodent

## Lethal Dose Table

**Lethal dose (LD<sub>50</sub>)** is the amount of an ingested substance that kills 50 percent of a test sample. It is expressed in mg/kg, or milligrams of substance per kilogram of body weight.

Common name	Toxin	Lethal doses	Description	Toxic response
Aspirin	Acetyl- salicylic acid $C_9H_8O_4$	LD <sub>50</sub> 200 mg/kg (rat, oral)	Odorless white crystal	Gastric distress, confusion, psychosis, stupor, ringing in ears, drowsiness, hyperventilation
Table salt	Sodium chloride NaCl	LD <sub>50</sub> 3 g/kg (rat, oral) 12357 mg/kg (human, oral)	White cubic crystal	Eye irritant, elevated blood pressure
Bleach (fumes)	Chlorine $Cl_2$	LD <sub>50</sub> 850 mg/kg (rat, inhaled)	Greenish colored gas, amber liquid, pungent odor	Corrosive to eyes, skin, respiratory tract, nausea, vomiting, pulmonary edema
Helium	Helium He	Not established	Odorless colorless gas	Dizziness, nausea, simple asphyxiant
Lorchel mushroom	Gyromitrin $C_4H_8N_2O$	LD <sub>50</sub> 200 mg/kg (rat, oral)		Nausea, vomiting, severe liver damage, coma, convulsions
Arsenic	Arsenic, arsenic trioxide As, $As_4O_6$	LD <sub>50</sub> 15mg/kg (rat, oral)	Grey, metallic crystals	Acute- irritates the yes, skin, respiratory tract, and nausea. Chronic convulsions, tissue legions, hemorrhage, kidney impairment
Sugar	Glucose $C_6H_{12}O_6$	LD <sub>50</sub> 30 g/kg (rat, oral)	Sweet white powder	Depressed activity, gastrointestinal disturbances. If diabetic- heart disease, blindness, nerve damage, kidney damage
Iron tablets	Iron sulfate $FeSO_4$	~5 adult tablets toxic for a 3 year old	Grayish white powder	Nausea, vomiting, diarrhea, black stool, liver damage, coma
Lead	Lead Pb	Lowest published dose 450 (human, oral)	Bluish or silvery solid	Acute –headache, insomnia, joint pain. Chronic-anemia, kidney disease, reproductive and developmental toxin.
Snake venom	$\alpha$ -bungarotoxin $C_{338}H_{529}N_{97}O_{105}S_{11}$	Not available	Large protein molecule	Paralysis, suffocation, loss of consciousness, seizures, hemorrhaging into tissues
Cola	Caffeine $C_8H_{10}N_4O_2$	LD <sub>50</sub> 140 mg/kg (dog, oral)	White odorless powder or crystals	Acute renal failure, nausea, psychosis, hemorrhage, increased pulse, convulsions
Alcohol	Ethanol $C_2H_6O$	LD <sub>50</sub> 7060 mg/kg (rat, oral)	Colorless liquid, pleasant odor	Nausea, headache, vomiting, dizziness, nervous system depression, confusion, loss of consciousness
Vitamin A	Retinol $C_{20}H_{30}O$	LD <sub>50</sub> 2000 mg/kg (rat, oral)	Yellow crystals, orange solid	Convulsions, unconsciousness, reproductive toxin