EXPERIMENT 11-12

DETERMINATION OF ACUTE TOXICITY (LD_{50})

A) General information:

There are three main values used as a measure of acute toxicity. These are;

I- Lethal Dose$_{50}$: LD50 is the abbreviation for lethal dose. It is an acute toxicity measure of solid or liquid chemical substances that enter the organism by all other means except the respiratory tract. Under certain conditions, it is the amount of a chemical required to cause death in 50% of the experimental animals in a group with a single administration. Its unit is mg / kg.

II- Lethal Concentration$_{50}$: LC_{50} is the abbreviation for lethal concentration. It is a measure of the acute toxicity of the chemical substances in the gas form which act by entering the organism through inhalation. In certain conditions, the concentration of the chemical substance in the respiratory air that kills 50% of the experimental animals in a group by respiration. Its unit is ppm or mg / m3 of air.

III- Minimal Lethal Dose: MLD is the abbreviation for minimal lethal dose. It is the least amount of chemical that kills an animal.

Particular emphasis about the LD50 assay will be given here.

Acute toxicity determination must comply with certain conditions in order to reach valid conclusions. We can list them as follows:

- **Type of experimental animal:** The most commonly used animal species in the determination of acute toxicity are; mouse, rat (rat), guinea pig and dogs. The toxicity of a chemical substance is related to its metabolism in the organism. Differences in metabolism between species cause toxicity to differ from species. There is a need to repeat acute toxicity determination in a second species in order to avoid misunderstandings due to species-specific tenderness and endurance.
- **Characteristics of experimental animals:** The gender, age, weight and other characteristics of the experimental animals are important for the determination of acute toxicity.

  Dose groups should be made from 50% female, 50% male as much as possible and pregnant animals should not be used.

  The animals should be young or mature according to the situation, and there should not be big differences between their weights.

  In addition, the animals must be healthy and no drugs have been administered before.
- **Number of experimental animals:** To reach a healthy statistical result, there must be a sufficient number of animals in each dose group. This number is generally 8 or 10 for each dose group.
- **Test conditions:** The chemical compound to be tested is first made to be delivered to the experimental animal by means of solution, suspension or emulsion according to its nature and route of administration. The solution, suspension or emulsion should not have toxic adjuvants, and should not affect the experiment in any way.

  The rate of delivery and volume must be within certain limits.

  Experimental animals should be prepared before the experiment, for example if the substance to be tested is given orally, the animals should be starved the day before, but should be able to drink as much water as they want.

Acute toxicity can be determined by any of the routes of administration (per-os, ip, iv, sc). Naturally, the route of administration used should also be shown with the values found. For example, cocaine hydrochloride LD50 is shown as 26 mg / kg (iv mouse).

To determine dose groups in the LD50 assay, a group of animals are given different doses to give an idea of the toxicity of the substance to be tested. Subsequently, dose groups are determined according to these approximate values.

EVALUATION OF RESULTS

The results of an LD50 assay run in accordance with the conditions indicated by cocaine hydrochloride are shown below.

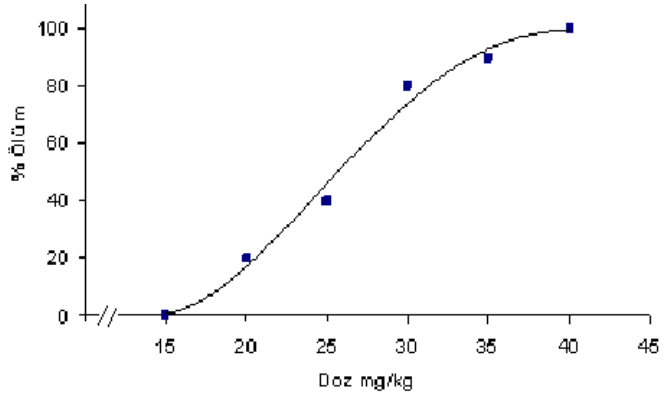
Experimental animal: Mouse
Route of administration: iv

Group	Number of animals in group	Doses(mg/kg)	Death number	% Death
A	10	15	0	0
B	10	20	2	20
C	10	25	4	40
D	10	30	6	80
E	10	35	8	90
F	10	40	10	100

In this experiment, including six dose group and 10 in each dose group, a total of 60 test animals were used.

Different methods can be used to evaluate the results. The most common methods are discussed below.

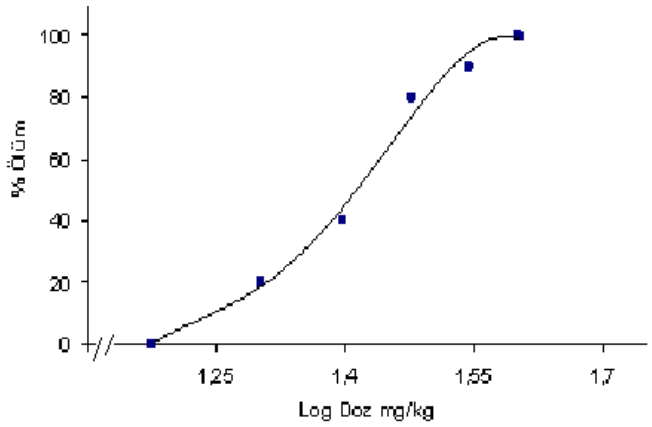
I. Traven method: On a graph paper, the characteristic sigmoidal curve is obtained if the doses are processed in the x-axis and the corresponding percentages of the deaths are processed in the y-axis. In the graph, the LD50 value is found by finding the x-axis counterpart of the 50% mortality rate.



II. Another graphical method used for the same purpose: In this method, the log dose (mg / kg) to the x-axis and the percentage of death to the y-axis are marked. When values are passed to graph;

Log Dose	**% Death**
Log 15: 1,17609	0
Log 20: 1,30103	20

Log 25: 1,39794	40
Log 30: 1,47712	80
Log 35: 1,54407	90
Log 40: 1,60206	100



Finding the LD50 values from this graph is like the Traven method. The value found in the graph must be calculated as the logarithm of the value, and the logarithm of the value should be calculated to find the corresponding dose.

III. Reed-Muench method: It is a graphical method. The difference from the other two methods described is that the % measurements are calculated differently.

Doses mg/kg	Number of deaths in each group	Number of animals alive in each group	Total number of deaths	Total live count	Grand total	Death rate	% Death
15	0	10	0	27	27	0/27	0
20	2	8	2	17	19	2/19	10,5
25	4	6	6	9	15	6/15	40
30	8	2	14	3	17	14/17	82,4
35	9	1	23	1	24	23/24	95,8
40	10	0	33	0	33	33/33	100

The **total death** in the table above shows the sum of death numbers in that dose group and smaller dose groups than that dose group and the sum of **live numbers** in that dose group and in larger dose groups than that dose group. **Grand total** is "Total death + Total live". Which way of thinking is followed by such a calculation? Let's explain this with an example.

Take the 20 mg / kg dose group: In this group, there are 4 deaths and 6 live.

- Animals that survived doses greater than 20 mg/kg were naturally alive if they were in the 20 mg/kg dose group. We can then add the number of animals that survived in the larger dose groups to the number of animals that survived in the 20 mg/kg dose group.
- By the same token, animals that died in dose groups less than 20 mg/kg would die with this dose if they were in the 20 mg/kg dose group. We can then add the number of deaths in the smaller dose groups to the number of deaths in the 20 mg/kg dose group.

With this calculation, the population in each dose group is indirectly increased and more reliable % measurement values are available.

A sigmoid curve is obtained when the resulting % measured values are plotted versus dose or log dose. From this curve, the LD50 is determined as described above. It should not be forgotten that in the above three methods, the sigmoid curve passing through the points found is hand-drawn, but only in certain cases.

IV. Karber-Behrens method: It is among the most commonly used methods. Its formula is:

$$LD_{50} = LD_{100} - \frac{\Sigma(ab)}{n}$$

In this formula;
LD100: 100% mortality dose in the trial,
n: Number of animals in each group,
a: The difference between two consecutive doses,
b: The arithmetic mean of the deaths from two consecutive doses.

If we evaluate our example with this method;

Doses	15	20	25	30	35	40
Death	0	2	4	8	9	10
n	10	10	10	10	10	10
a	5	5	5	5	5	
b	$\frac{0+2}{2}$	$\frac{2+4}{2}$	$\frac{4+8}{2}$	$\frac{8+9}{2}$	$\frac{9+10}{2}$	
ab	5	15	30	42,5	47,5	

$$LD_{50} = 40 - \frac{5+15+30+42{,}5+47{,}5}{10} \Rightarrow LD_{50} = 40 - 14 = 26 \text{ mg/kg}$$

V. Probit-Analysis Method: It is the most valid methods. To be able to use this method, statistical calculations need to be done on a specially prepared logarithmic-Probit graph paper with a regulated Probit-Analysis chart. The logarithmic-Probit graph paper is labeled with the x-axis doses, and the y-axis indicates probit responses from the probit ruler of the % of deaths.

As a result, a line that can be expressed by the equation y = ax + b is obtained, which provides ease of operation. In our practical work, we will first use the first 4 methods.

B) Experimental Procedure:

Drug: Stricnine Sulfate
Experimental animal: Mouse
Route of administration: Intra-peritoneal (ip)
Preliminary to test:

a) By conducting preliminary tests, an approximate toxicity and concomitant determination of dose groups,
b) Preparation of experimental animals,
c) Disassembly and weighing of experimental animals into dose groups,
d) Preparation of Stricnine Sulfate solutions in saline solution at various concentrations, so as not to increase the volume at dose changes, taking into account that the maximal volume to be given by ip route when using mouse as experimental animal is 0.5 mL.

Evaluation of results:

At the end of the first hour and 24 hours, deaths in each dose group will be counted and recorded. The LD50 value according to the number of deaths at the end of the first 24 hours will be determined using the methods described above separately.