DETERMINATION OF LD50

Wrood S. Alkhfajy, MS.
Laboratory of Toxicology
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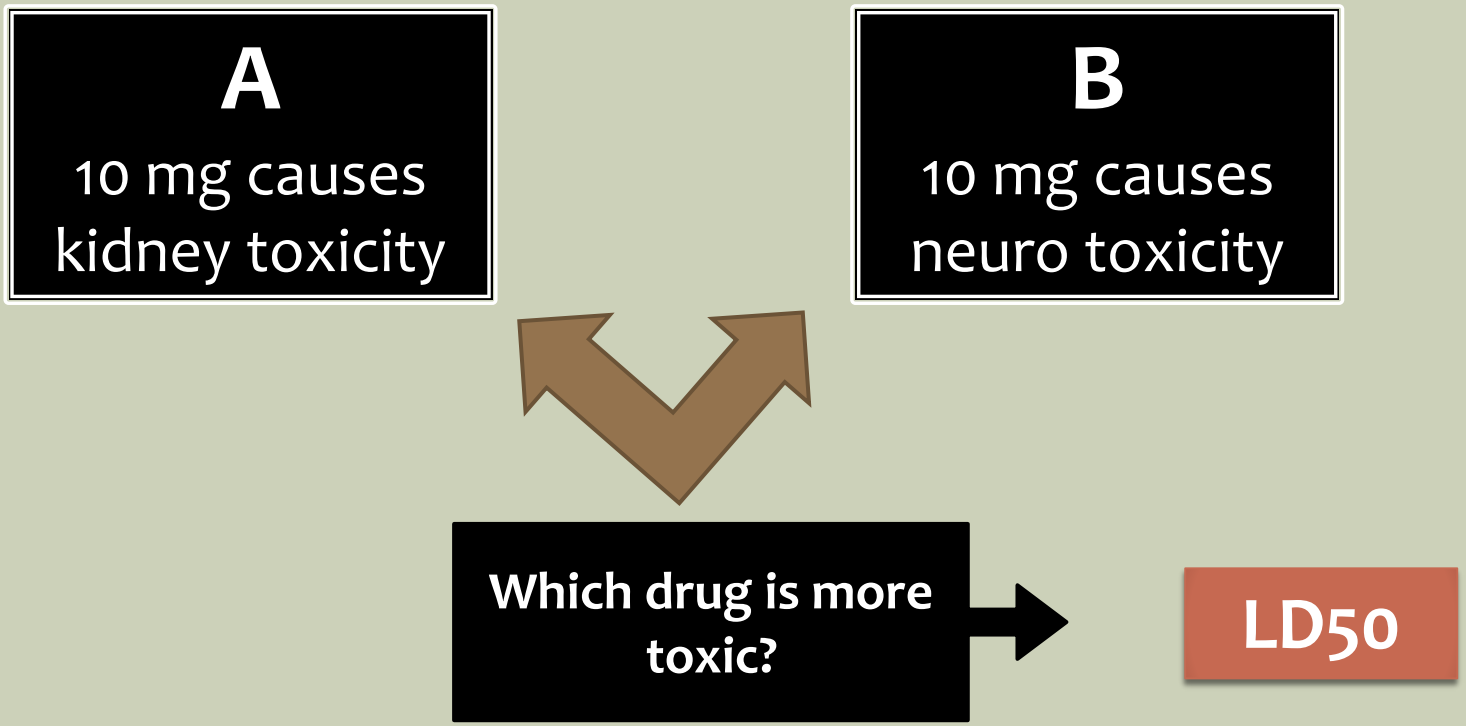
LETHAL DOSE 50 (LD50)

- The amount of a toxic agent (as a poison, virus, or radiation) 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
- Compare the toxic potency or intensity of different chemicals
- A measure of acute toxicity

LETHAL DOSE 50 (LD50)



SIGNS RECORDED DURING ACUTE TOXICITY STUDIES

- These are increased motor activity, anaesthesia, tremors, arching and rolling, clonic convulsions, tonic extension, lacrimation, **Straub reaction**, salivation, muscle spasm, **writhing**, hyperesthesia, **loss of righting reflex**, depression, ataxia, stimulation, sedation, blanching, hypnosis, cyanosis and analgesia

DIFFERENT METHODS FOR THE DETERMINATION OF LD50

- Graphical method, arithmetical method and statistical approach
  - For research purpose, the most widely used method is Litchfield and Wilcoxson
  - For routine practical class work; Reed-Muench, Miller-Tainter and Karber's Method
- Arithmetical method: Karber method
- Graphical method: Miller and Tainter

Contents	Method Karber [7]	Method of Miller and Tainter [7]	Method of Lorke [8]
No. of rodents used	More than necessary	More than necessary	Appropriate
Expenditure	High	High	Average
Accuracy of results	Inaccurate	Inaccurate	Doubtful

DIFFERENT METHODS FOR THE DETERMINATION OF LD50

- For calculating LD50 by any one method:
  - Find out the least tolerated (smallest) dose (100% mortality) and most tolerated (highest) dose (0% mortality) by hit and trial method
  - Once these two doses are determined, select at least 5 doses in between them, and observe mortality due to these doses
  - Apply correction factor to 0% and 100% mortality group [for 0% dead = 100 (0.25/n) and for 100% dead = 100x (n-0.25/n), where n = number of death]
  - The percentage mortality values are converted to probit values by reading the corresponding probit units from the probit table
  - Plot the probit value against log doses and read LD50 value as the dose that corresponds to probit

Probit Values

- Bliss proposed transforming the percentage-killed into a "probability unit" (or "probit")
- Defined as arbitrarily as equal to 0 for 0.0001 and 10 for 0.9999)
- Probit table aids other researchers to convert their kill percentages to his probit, which they could then plot against the logarithm of the dose and thereby, it was hoped, obtain a more or less straight line
- Such a so-called probit model is still important in toxicology, as well as other fields

GRAPHICAL METHOD OF MILLER-TAINTER

- The Miller-Tainter method is the standard use in getting LD50
- The dose is plotted against the probit value. Based on the graph, the LD50 will be estimated
- The experiment demonstrates the determination of LD50 of neostigmine on the experimental animals and its comparison to the standard LD50 of neostigmine
  - Neostigmine (0.1 mg/kg, 0.2 mg/kg, 0.4mg/kg, 0.8 mg/kg,1.6 mg/kg, 3.2 mg/kg) i.p
  - 0.5 mg/ml

8 - TOXICOLOGICAL INFORMATION

Oral LD_{50} Mouse: 7500 ug/kg;
SC LD_{50} Mouse: 0.54 mg/kg;
IV LD_{50} Rat: 0.315 mg/kg;
IM LD_{50} Rat: 0.423 mg/kg

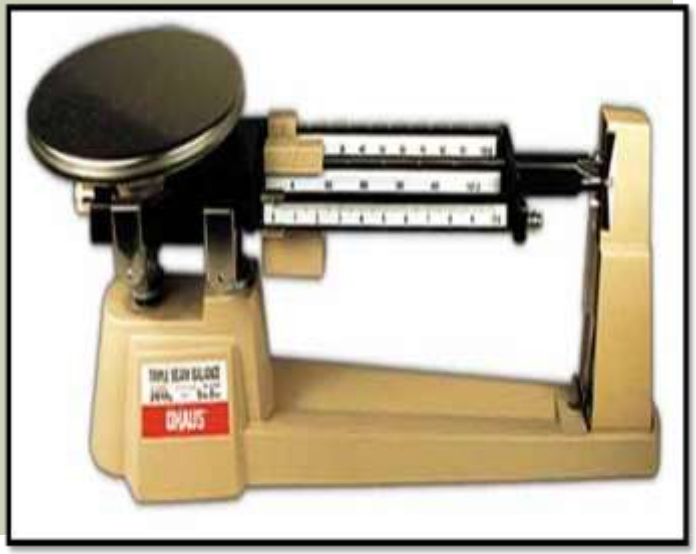
IV LD_{50} Mouse: 0.3 mg/kg;
IM LD_{50} Mouse: 0.395 mg/kg;
SC LD_{50} Rat: 0.445 mg/kg;

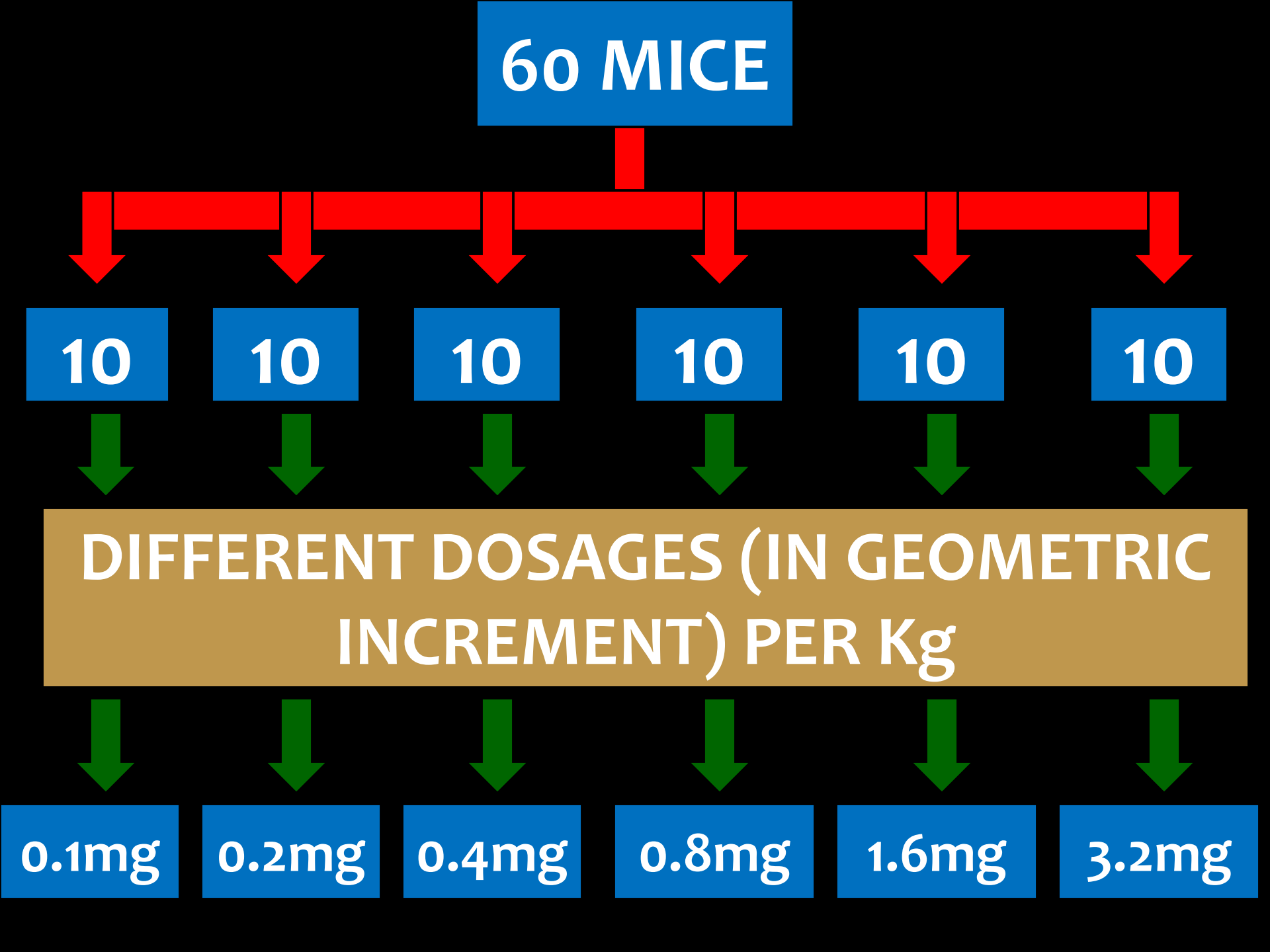
OBJECTIVES

- **General Objective:**
  - To determine the Median Lethal Dose of neostigmine introduced on mice intraperitoneally
- **Specific Objectives:**
  - To determine the number of deaths per dose of neostigmine one hour after intraperitoneal administration
  - To determine the LD50 of neostigmine using the Miller-Tainter method
  - To compare the experimentally determined LD50 to the theoretical standard of LD50

MATERIALS AND METHODS

- 60 male mice, approximately same weight
- Neostigmine 0.5 mg/ml injections




60 MICE
10
10
10
10
10
10
DIFFERENT DOSAGES (IN GEOMETRIC INCREMENT) PER Kg
0.1mg
0.2mg
0.4mg
0.8mg
1.6mg
3.2mg

MATERIALS AND METHODS

- The number of dead mice, within an hour was counted (% dead)
- Abnormal behaviors, tremors and seizures were observed and noted
- The rationale for using the geometric dosage sequencing is that this is more cost-efficient and allows experimenters to see the effect without sacrificing too many animals

RESULTS

- Apply correction factor to 0% and 100% mortality group [for 0% dead = 2.5% and for 100% dead = 97.5%]
- % Response conversion to Probit Units by the Probit Transformation Table
- Convert Doses to log Dose (log10 dose = x)
- The probit value and the percentage of deaths against log dose are plotted

Table 1 – Transformation of Percentage to Probits

%	0	1	2	3	4	5	6	7	8	9
0		2.67	2.95	3.12	3.25	3.36	3.45	3.52	2.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.00	4.12
20	4.16	4.19	4.23	4.26	4.29	4.33	4.36	4.39	4.42	4.45
30	4.48	4.50	4.53	4.56	4.59	4.61	4.64	4.67	4.69	4.72
40	4.75	4.75	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.97
50	5.00	5.03	5.05	5.08	5.10	5.13	5.15	5.18	5.20	5.23
60	5.25	5.28	5.31	5.33	5.36	5.39	5.41	5.44	5.47	5.50
70	5.52	5.55	5.58	5.61	5.64	5.67	5.71	5.74	5.77	5.81
80	5.84	5.84	5.92	5.95	5.99	6.04	6.08	6.18	6.18	6.23
90	6.28	6.34	5.41	6.48	6.55	6.64	6.75	6.88	7.05	7.33
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
99	7.33	7.37	7.41	7.46	7.51	7.58	7.65	7.75	7.88	8.09

RESULTS

- Sample Probit Computation

- For the
  20% response

- 20% = 4.16

%	0	1
0		2.67
10	3.72	3.77
20	4.16	4.19
30	4.48	4.50
40	4.75	4.75
50	5.00	5.03

RESULTS

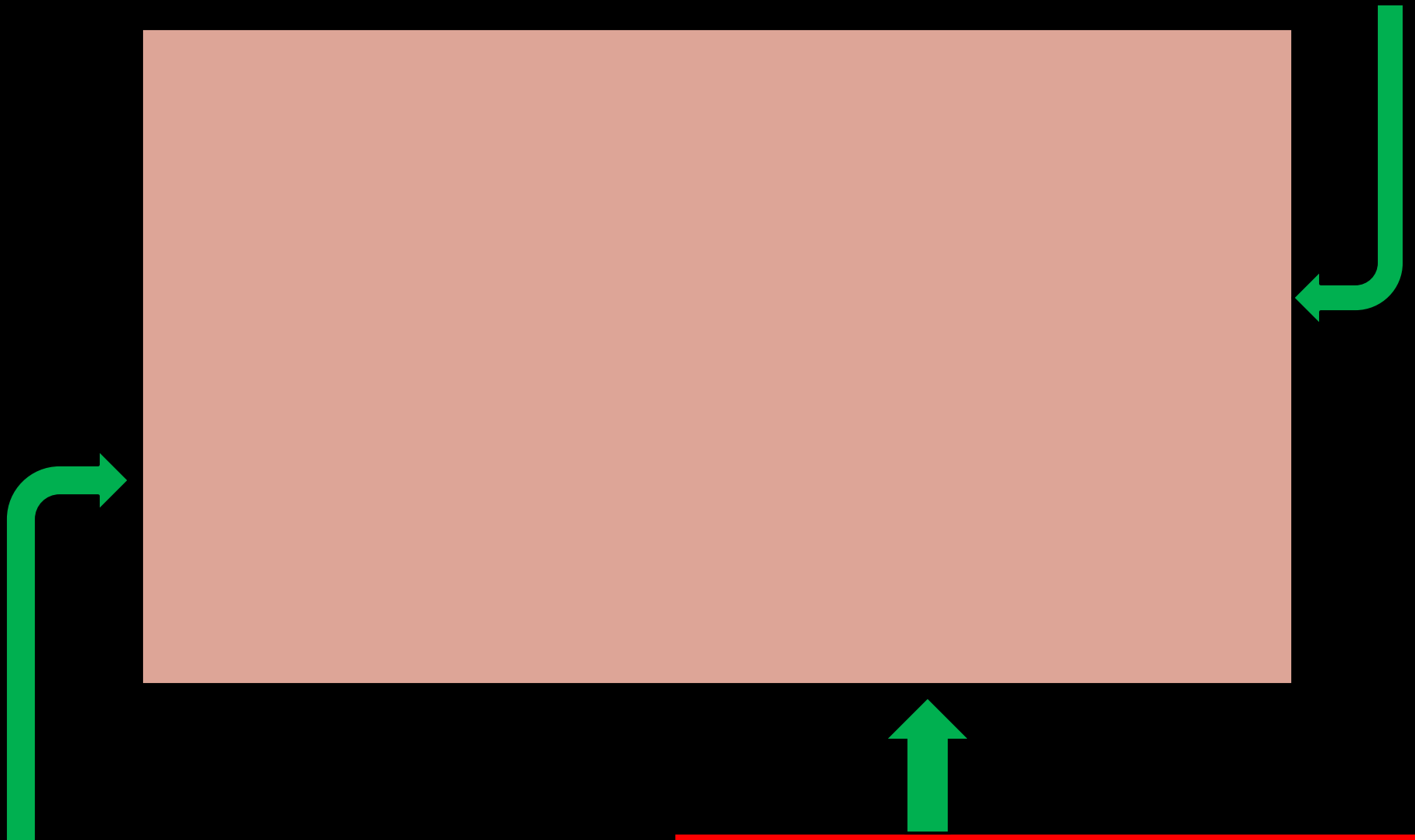
- Sample Probit Computation

- For the 2.5% response
- 2.5% = $\frac{2.95 + 3.12}{2}$ = 3.035

%	0	1	2	3	4
0		2.67	2.95	3.12	3.25
10	3.72	3.77	3.82	3.87	3.92

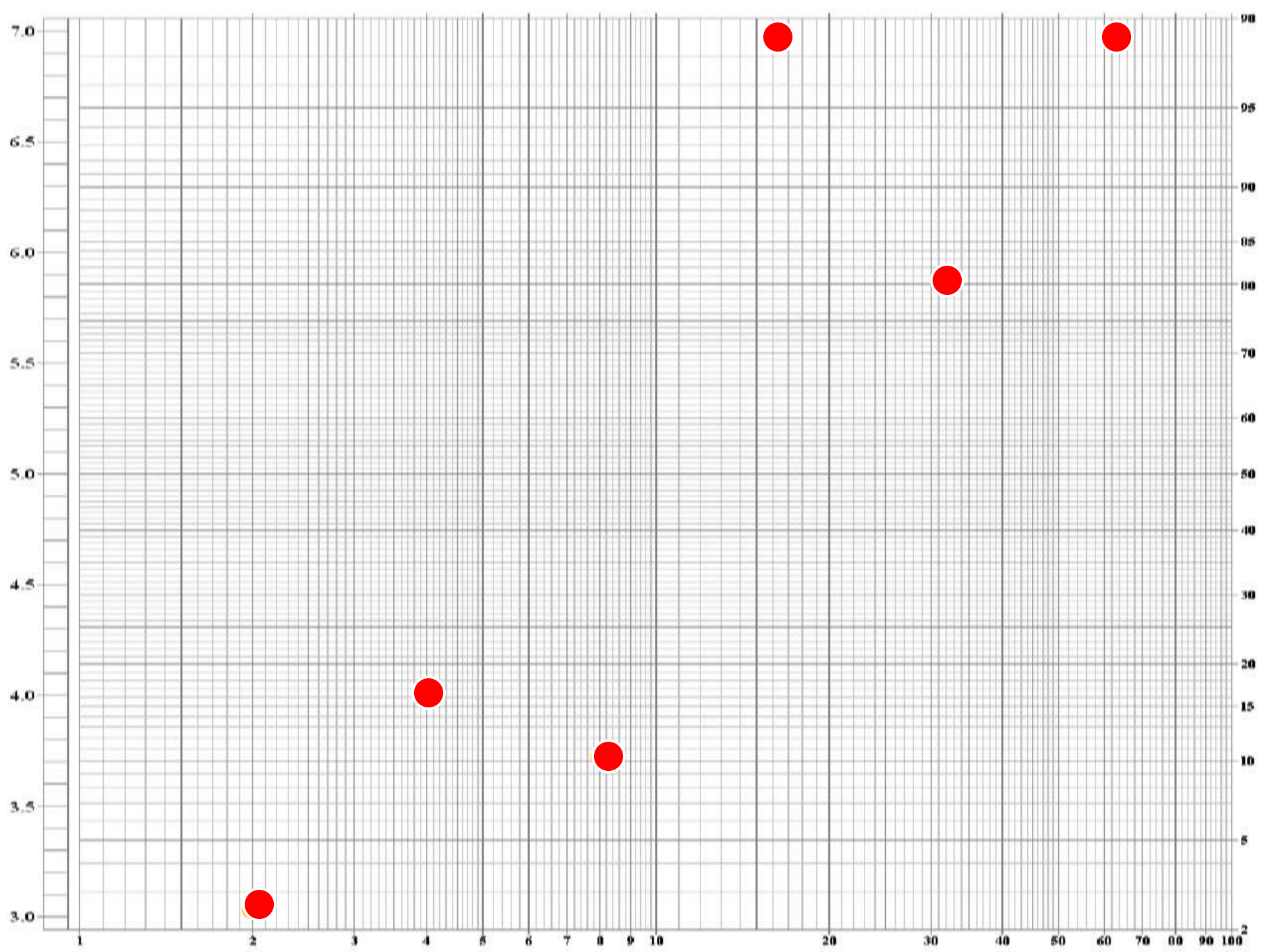
PLOT

% RESPONSE



PROBIT UNITS

LOG DOSE


7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
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95
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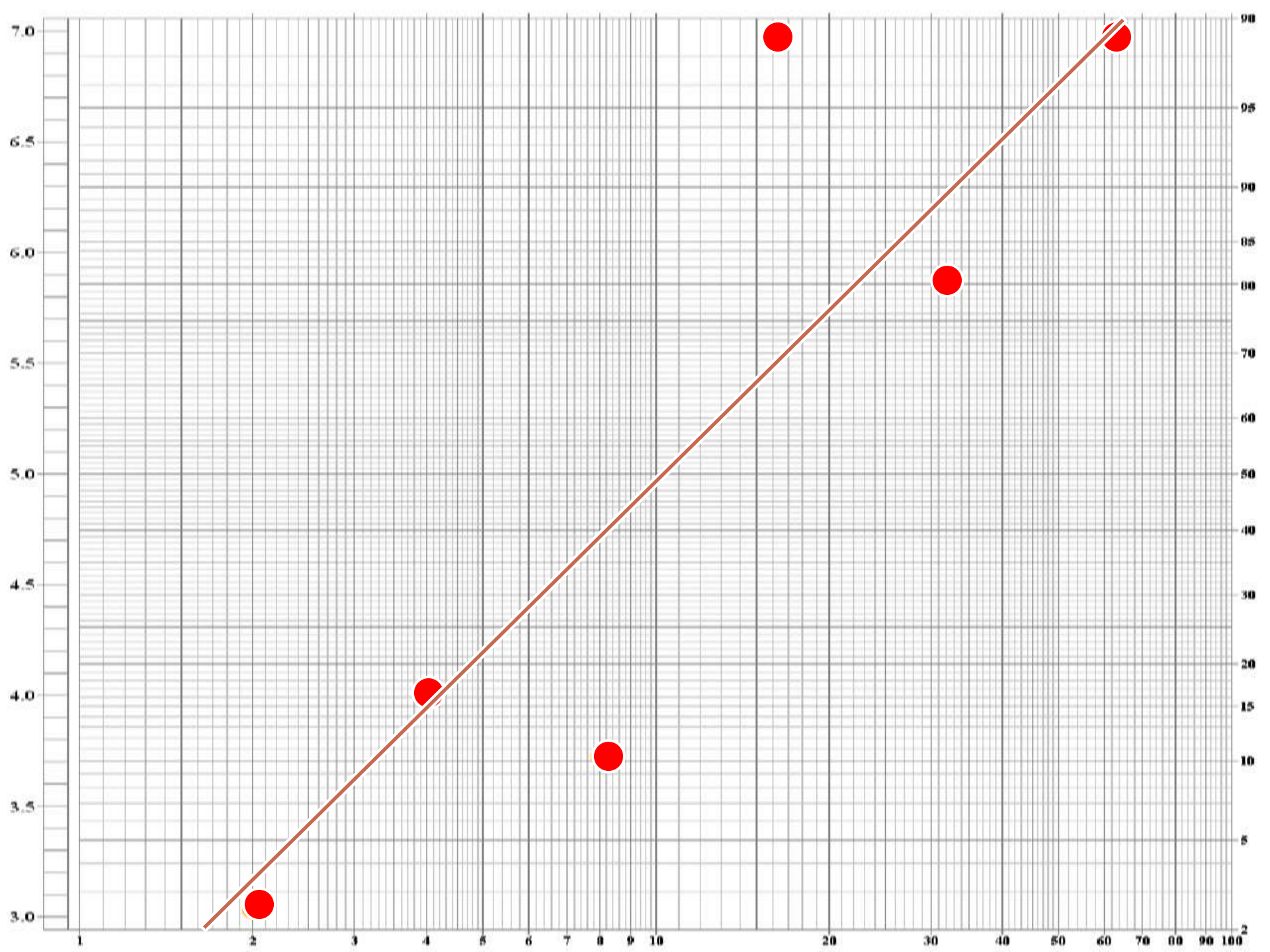
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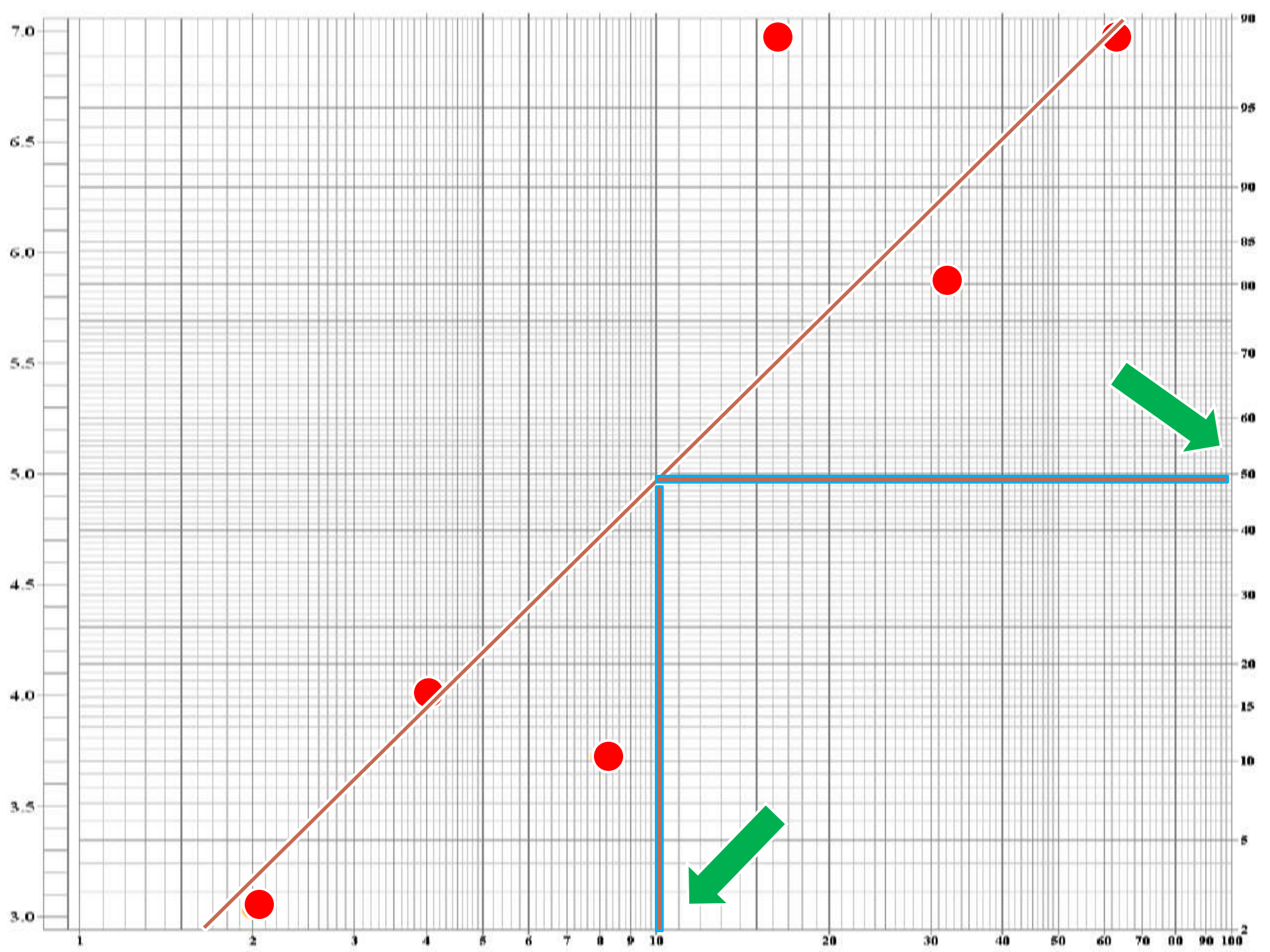
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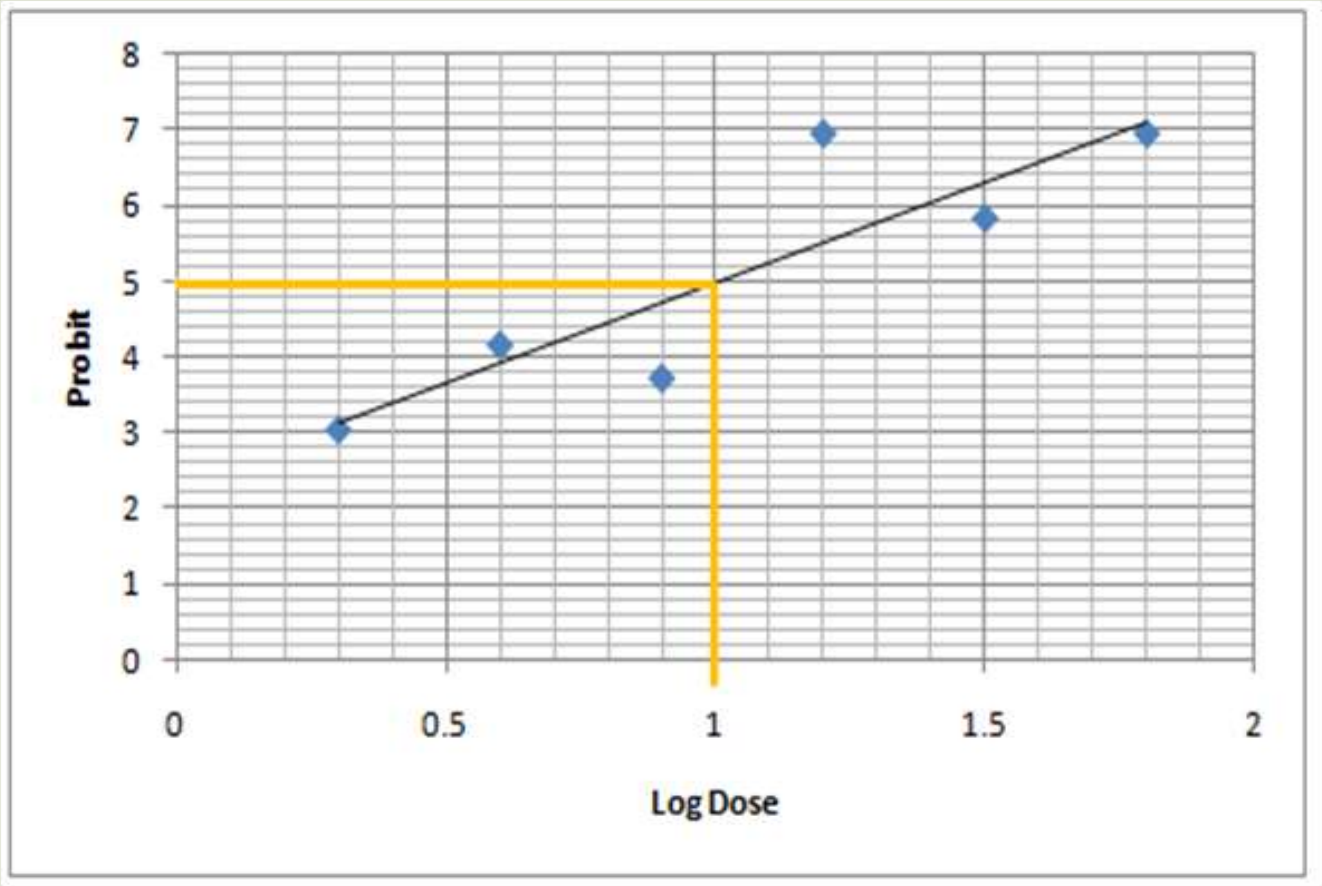
LINEAR REGRESSION



RESULTS

- From the regression of the probit-log dose line, the log dose was extrapolated corresponding to probit units of 5
- The extrapolated dose corresponds to the median lethal log dose, and the antilog of this log dose value would be the LD50 value
- The dose corresponding to 50% or probit 5 was taken as LD50

LIMITATIONS OF LD50

- The LD50 gives a measure of the immediate or acute toxicity
- Results may vary greatly
- LD50 is not tested on humans
- All relation to humans are only a guess
- The LD50 test is neither reliable nor useful Because the human lethal dose is difficult to be predicted from animal studies

REPORT

- Introduction, Aim
- Materials and Methods
- Results
- Discussion