Cara Menentukan LC50

Sample probit analysis calculations

- Imagine that we had carried out a bioassay using six concentrations of a chemical, measured in milligrams per liter, including a control (0 mg/L)
- Twenty individuals of our bioassay species were exposed, and we observed how many were dead after 48 hours of exposure
- We will calculate a 48-hour LC50 value here.

Conc. (mg/L)	Total No.	No. Dead (48 hours)
0.00	20	1
2.30	20	0
3.00	20	1
3.90	20	4
5.12	20	9
6.96	20	16

Step 1:
Set up a probit calculation table

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit

Step 2:

Enter concentrations and log10-transform them

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit
0.00	-					
2.30	0.362					
3.00	0.477					
3.90	0.591					
5.12	0.709					
6.96	0.843					

* Note there is no log for 0 mg/L (the control) so its value is left blank.

Step 3:

Enter species response data

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit
0.00	-	20	1			
2.30	0.362	20	0			
3.00	0.477	20	1			
3.90	0.591	20	4			
5.12	0.709	20	9			
6.96	0.843	20	16			

Step 4:
Sample probit calculation table

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit
0.00	-	20	1	5		
2.30	0.362	20	0	0		
3.00	0.477	20	1	5		
3.90	0.591	20	4	20		
5.12	0.709	20	9	45		
6.96	0.843	20	16	80		

Percent is calculated by dividing number dead by total number, then multiplying by 100, *rounded to nearest whole number.*

Control mortality

- The purpose of the control (i.e., 0 mg/L) is to determine whether any bioassay organisms died due to factors *other than* exposure to our test chemical.
- In this example, there was 5% control mortality (1 out of 20) so we have to "correct" observed mortalities in the other treatments for it, using Abbott's formula.

Abbott's formula for control mortality

$$\text{Corrected mortality (\%)} = \frac{M_{obs} - M_{control}}{100 - M_{control}} \times 100$$

For example, if control mortality is 1 out of 20 (i.e. 5%), and observed treatment mortality was 10 in 20 (i.e. 50%), the Abbott correction would be as follows:

Corrected = 100 x (50 – 5) / (100 – 5)
= 100 x 45 / 95 = 47%

Step 5:
Mortalities corrected for control value

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit
0.00	-	20	1	5	-	
2.30	0.362	20	0	0	0	
3.00	0.477	20	1	5	0	
3.90	0.591	20	4	20	16	
5.12	0.709	20	9	45	42	
6.96	0.843	20	16	80	79	

Round all corrected values to nearest whole number.

Probit transformation

- If we graphed the corrected percent mortality data versus the log10 of concentration, we would ***not*** get a straight line.
- The purpose of the probit transformation is to straighten the line so we can estimate LC50 more easily.

Probit table

	0	1	2	3	4	5	6	7	8	9
0		2.67	2.95	3.12	3.25	3.35	3.44	3.52	3.59	3.66
10	3.72	3.77	3.82	3.87	3.92	3.96	4.01	4.05	4.08	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	4.62	4.64	4.67	4.70	4.72
40	4.75	4.77	4.80	4.82	4.85	4.87	4.90	4.92	4.95	4.98
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	5.38	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	5.88	5.92	5.95	5.99	6.04	6.08	6.13	6.18	6.23
90	6.28	6.34	6.41	6.48	6.56	6.65	6.75	6.88	7.05	7.33

For example, the probit value of 47% is 4.92.

Note there is no probit value for 0% or 100%.

Step 6:

Completed probit table

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit
0.00	-	20	1	5	-	-
2.30	0.362	20	0	0	0	-
3.00	0.477	20	1	5	0	-
3.90	0.591	20	4	20	16	4.01
5.12	0.709	20	9	45	42	4.80
6.96	0.843	20	16	80	79	5.81

Step 7:

Use only values for which we have probits

Conc. (mg/L)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Corrected % Mortal.	Probit
0.00						
2.30	0.					
3.00	0.					
3.90	0.591	20	4	20	16	4.01
5.12	0.709	20	9	45	42	4.80
6.96	0.843	20	16	80	79	5.81

Only three concentrations of our test chemical have corresponding probit values so these are the ones we use in calculating LC50.

Step 8:
Calculating LC50

- Regress Log_{10} concentration on probit value then, using the regression formula, estimate the Log10 concentration associated with a probit value of 5 (the probit of 50%)

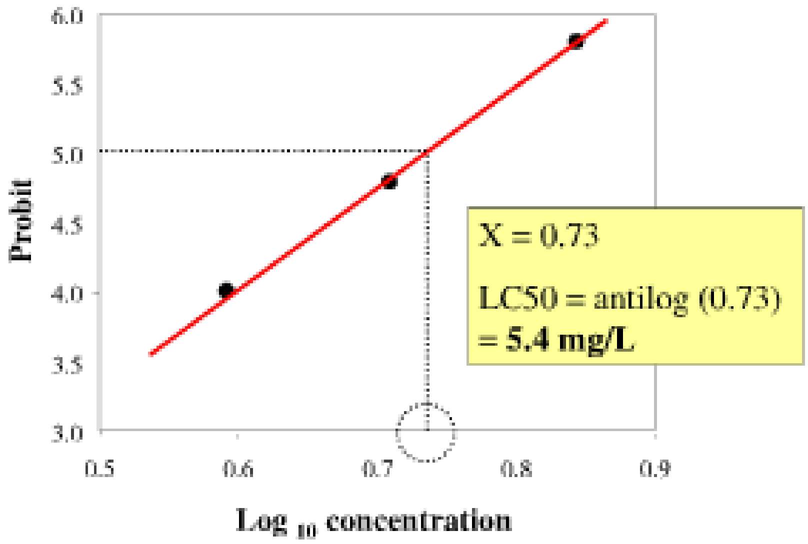
OR

- Graph probit values (Y-axis) against Log_{10} concentration (X-axis) and draw a straight line of best line through plotted points, then use this line to estimate the Log10 concentration associated with a probit of 5

LC50: regression method

- $Y = -0.235 + 7.15X$
  $r = 1.00$
  Y = probit value X = Log_{10} concentration
- $5 = -0.235 + 7.15X$
- $X = 0.732$
- LC50 = antilog (0.732) = **5.40 mg/L**

LC50: graphical method



Step 9:

Finally, check your work!

- Note that we obtained essentially the same answer regardless whether we used the mathematical or graphical method.
- Check the answer to see if it "makes sense" with the original raw data.
- We see that 5.12 mg/L killed 9 of 20 organisms (slightly less than 50%) so an LC50 of 5.4 mg/L seems reasonable.

Conc. (mg/L)	Total No.	No. Dead (48 hours)
0.00	20	1
2.30	20	0
3.00	20	1
3.90	20	4
5.12	20	9
6.96	20	16