

Toxicity Test and Probit Analysis



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Main steps for toxicity test

- Using **gross dilution series** to find the effective dose range.
- Use the effective dose range to carry out toxicity test.
- Run probit analysis to detect the dose-probit regression equation, LD_{50} (or LC_{50}), and the fiducial limits.

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Finding the effective ranges of toxicants

- Using **gross dilution series** for the preliminary test (0, 0.0000001%, 0.000001%, 0.00001%, 0.0001%, 0.001%, 0.01%, etc.) to find the effective range.
- If there is no mortality in 0.000001% and 100% mortality in 0.001%, we then use **finer series** (0, 0.00001%, 0.00002%, 0.00005%, 0.0001%, 0.0002%) for mortality test.
- 20 insects at least for each dose.

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Probit-MSChart can be used on Windows and Mac system

- Probit-MSChart has been used on Windows XP, 7, 8, 10. In most cases, there will be no problem.
- If you use Chinese or non-ASCII codes for the folder names, you may encounter some problems. But you can always find a solution to solve the problems. Only in extreme cases, you may have to re-install your operating system.
- On Mac computer, you have to use Windows operating system to run Probit-MSChart.

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Three ways to run Probit-MSChart

1. Extract **Probit-MSChart-exe.rar**, run Probit-MSChart.exe (or similar) administrator.
2. Extract **Probit-MSChart.rar**, run setup.exe. Then run Probit as a Windows program.
3. Extract Probit-MSChart.rar, run the program directly from the Support folder. (Run as administrator).

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1. Probit-MSChart-exe.rar

1. If you download **Probit-MSChart-exe.rar** and **extract it to a new folder**, there is only one exe file (Probit-MSChart.exe, Probit.exe, or a file with similar name). Just click it to run. You don't need to setup the program.
2. **If you face problems, please click the right mouse key and try to run Probit.exe "as administer".**

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If you cannot run Probit-MSChart.exe and you see following message (or similar),



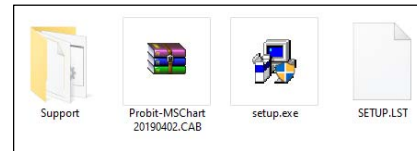
then you have to use **Probit-MSChart.rar** to setup the program.

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2. Setup Probit-MSChart

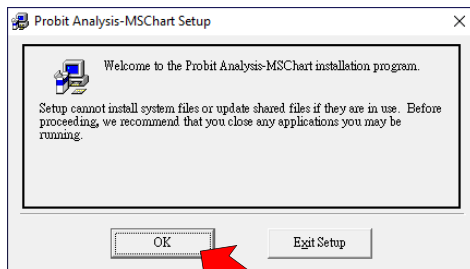
Extract Probit-MSChart.rar to a new folder on Desktop or C: drive. You will find the folder "Support" and three files: Setup.exe, Setup.LST, and Probit-MSChart.CAB (or similar).



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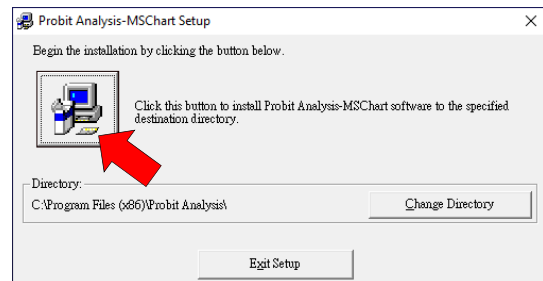
Double click on setup.exe and follow the red arrow



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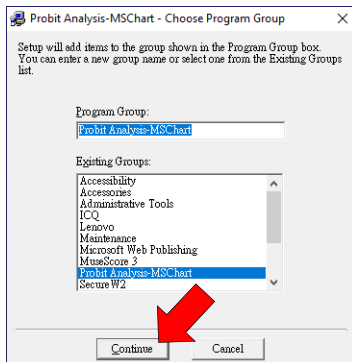
Click this big button to install



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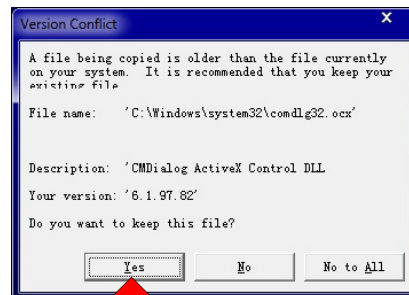
Click on Continue



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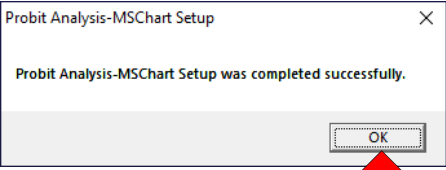
If you see this, just click Yes.



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Setup was successfully



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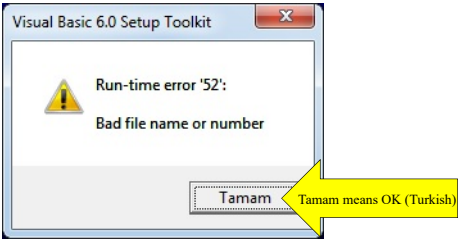
Now you can run Probit Analysis as regular Windows program



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3. Run Probit from the support folder


If you see this message, you can extract the RAR file and put it on the desktop. Then you can run the program from the support folder.



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If you cannot setup at all,

- If you are using Windows XP, you may face trouble and will not be able to setup the program.
- Extract Probit-MSChart.rar to a new folder on your Desktop.
- Open the **Support folder**, find the file **Probit-MSChart.exe**.
- Use right mouse key to click on it.
- Select "Run as administrator".



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If you still cannot run Probit, you may have to register one of OCX file.

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How to register an OCX file

- You can find MSFLXGRD.OCX and other OCX files on the internet. Save them to C:\windows\system32 (or syswow64) folder.
- You need regsvr32.exe and regedit.exe. If you cannot find them in your computer, you can download them from internet.
- Start the command mode: C:>
- Go to C:\Windows\system32
- Run Regsvr32 MSFLXGRD.OCX. If it shows "successfully installed", you can continue.

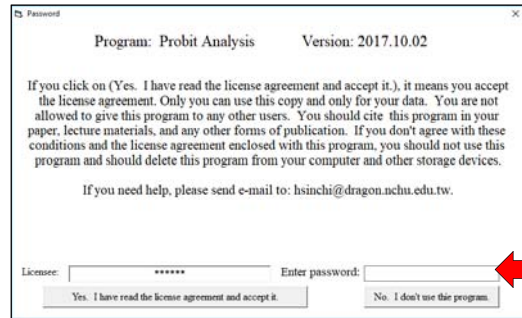
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If you use Probit-MSChart, you must cite following two references

- Chi, H. 2019. Computer program for the probit analysis. <http://140.120.197.173/Ecology/Download/Probit-MSChart.rar>.
- Finney, D. J. 1978. Probit analysis. Cambridge University Press, Cambridge.

Because Probit-MSChart is designed based on Finney (1978), you have to cite both references.

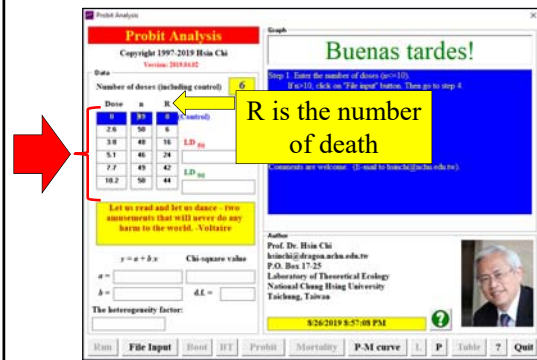
Use **WorldPeace** to run Probit
If you cannot run Probit with this password, please contact Prof. Chi to get a new password.



Enter the number of doses, then press Enter



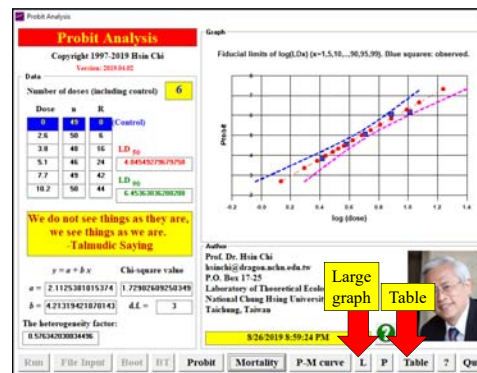
Edit your data, then press Enter



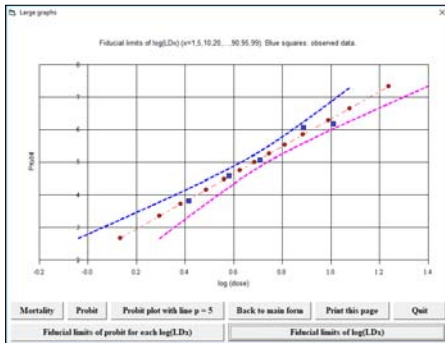
Click Run or press Enter to run



Congratulation!



Large graph



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Table

Table of Result

Regression equation is: $y = 2.1125581015374 + 4.21319421870143 x$
 Chi-squared = 1.72902609250349
 Variance of slope: 0.23095316627551 SE of slope: 0.48057580426993
 log(LD50): 0.685337952294201 SE of log(LD50): 2.20087129317163E-02
 Variance of log(LD50): 4.87028099462502E-04
 LD(50): 4.84549279679758
 Lower 95% fiducial limit of LD(50): 4.28366875390572
 Upper 95% fiducial limit of LD(50): 5.4784232366966
 Probit = 9, mortality = 99.996825%
 LD(Probit 9):
 Lower 95% fiducial limit of LD(Probit 9): 0
 Upper 95% fiducial limit of LD(Probit 9): 0

Dose	log(D)	n	r	M	M'	probit	Expected probit	95% fiducial limits of probit	
0	0	43	0	0	0	3.8243	3.8609	3.4574 4.2644	
1	2.6	0.414973	50	6	12	12	4.5692	4.5503	4.2091 4.8215
2	3.8	0.579793	48	16	33	33	5.0544	5.0937	4.8703 5.317
3	5.1	0.707570	46	24	52	52	6.0676	5.8475	5.5403 6.1547
4	7.7	0.886490	43	42	85	85	6.1751	6.362	5.9445 6.7794
5	10.2	1.088600	50	44	88	88			
6									
7									
8									
9									
10									

Save Mainform Expected values Print Transformation Quit

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Transformation

Transformation

Probit Mortality (%)

1 Transform

Mortality (%) Probit

Transform

Mortality (%) Dose

Transform

Main form Mort-Probit End

Transform bootstrap "corrected mortalities" to probit

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Transformation

Transformation

Probit Mortality (%)

4 Transform 15.86552

Mortality (%) Probit

80 Transform 5.8415

Mortality (%) Dose

50 Transform 4.845

Main form Mort-Probit End

Transform bootstrap "corrected mortalities" to probit

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Expected values

Expected dose at different mortality

Mortality	log(Dose)	Dose	Lower limit (95%)	Upper limit (95%)
1	0.133191557125881	1.35891269898854	0.918922717815477	1.98176880025242
5	0.294921580625728	1.97206661325508	1.47058841361069	2.61826394582852
10	0.381150693536638	2.40519721184775	1.8449427573217	3.0449326901947
20	0.485584521140659	3.0590355113023	2.53387215092245	3.673360871093281
30	0.560871817390591	3.63807641956877	3.11679188148883	4.231685220071
40	0.625217296361546	4.21907549268502	3.69212109583674	4.81191036199717
50	0.685337952294201	4.84549279679758	4.28366875390572	5.4784232366966
60	0.745458608226856	5.56491593585478	4.91287735606268	6.3097679772937
70	0.80980408719781	6.45363036288288	5.62030975039291	7.42953044638156
80	0.885991383138742	7.67522972608096	6.50290234162295	9.09884344282396
90	0.989525211051764	9.76169447999781	7.50923856833858	12.1936634212426
95	1.0757432396267	11.9056812492404	9.16330048888658	15.6094026600764
99	1.23748434746252	17.2776370853646	12.1204290699922	24.9512787501741

Back to Mainform Previous page End

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Save the output file

Table of Result

Regression equation is: $y = 2.1125581015374 + 4.21319421870143 x$
 Chi-squared = 1.72902609250349
 Variance of slope: 0.23095316627551 SE of slope: 0.48057580426993
 log(LD50): 0.685337952294201 SE of log(LD50): 2.20087129317163E-02
 Variance of log(LD50): 4.87028099462502E-04
 LD(50): 4.84549279679758
 Lower 95% fiducial limit of LD(50): 4.28366875390572
 Upper 95% fiducial limit of LD(50): 5.4784232366966
 Probit = 9, mortality = 99.996825%
 LD(Probit 9):
 Lower 95% fiducial limit of LD(Probit 9): 0
 Upper 95% fiducial limit of LD(Probit 9): 0

Description line

Enter the description for your data (Species name, insecticide, method, etc.)

Toxicity test of DDT

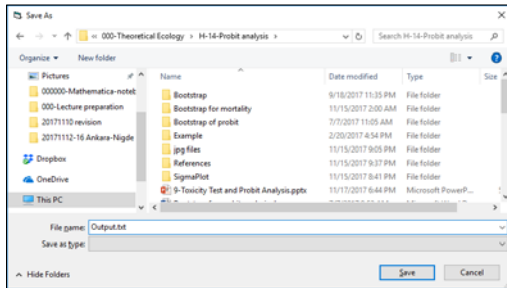
Save your results

Save Mainform Expected values Print Transformation Quit

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Enter a filename for your output



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Output file

Output_2_output.txt - Notepad

File Edit Format View Help

Project : Toxicity test of DDT
Date : 11/18/2017
Time : 11:08:01 AM
Licensee: Prof. Dr. Hsin Chi

Table 1. Results of probit analysis

Dose (d)	Log dose (x)	Sample size (n)	Mortality size dead (t)	Corrected mortality (p)	Probit y'	Expected probit of y (y)	Variance of y	5% fiducial limits (lower) (upper)
2.6	0.41497	50	6	12	12	3.825	3.861	0.0161 3.457 4.264
3.8	0.57978	48	16	33.33	33.33	4.57	4.555	0.007 4.289 4.821
5.1	0.70757	46	24	52.17	52.17	5.054	5.094	0.0049 4.87 5.217
7.7	0.88649	49	42	85.71	85.71	6.068	6.047	0.0093 5.54 6.155
10.2	1.0086	50	44	88	88	6.175	6.362	0.0172 5.945 6.779

Sample size of control group (blank group): 49
Number of death in control group: 0
Mortality in control group: 0

Regression line: $y = 2.1125381015374 + 4.21319421870143 x$
Variance of slope: 0.23095316627551
Standard error of slope: 0.48057586942693
Variance of log(LD50): 4.87028090442592E-04
Degree of freedom (df): 3
Chi-square value: 1.72902409250349

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You can write your data file using notepad and run Probit analysis

```

"Project: Toxicity test of DDT"
"User: Goethe"
"dose, sample size, no. dead"
0,49,0
2.6,50,6
3.8,48,16
5.1,46,24
7.7,49,42
10.2,50,44
    
```

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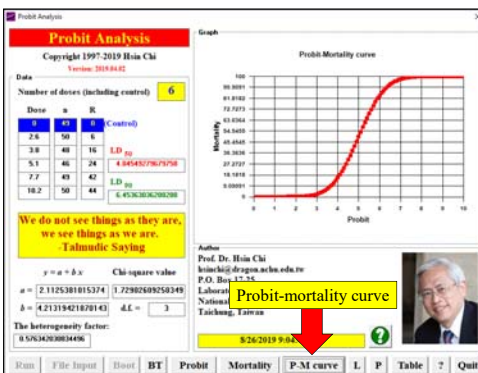
Input your data from a file



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For VIP user only



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Critical thinking

- Do you know what the percent mortality for probits 3, 4, 5, 6, and 7 are?

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Critical thinking

If the probit regression equation is $y = 2.1125 + 4.2132 x$, where y is the probit and x is $\log(\text{dose})$, we can find $\log LD_{50} = 0.6853$. If the standard error of $\log(LD_{50})$ is 0.0220 and $10^{0.0220} = 1.052$, is a statement such as “the LC_{50} is 4.845 ± 1.052 ” justified?

The regression equation is: $y = 2.1125381015374 + 4.21319421870143 x$
 Variance of slope = 0.23095316627551
 SE of slope = 0.480575869426993
 $\log(LD50) = 0.685337952294201$
 SE of $\log(LD50) = 2.20687129317163E-02$
 Variance of $\log(LD50) = 4.87028090462502E-04$
 LD50 = 4.84549279679758
 Lower 95% fiducial limit of LD50 = 4.28366875396572
 Upper 95% fiducial limit of LD50 = 5.47842232366966

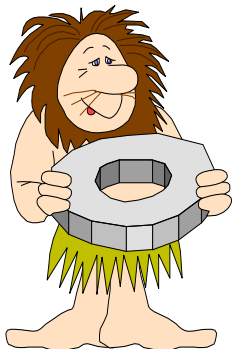
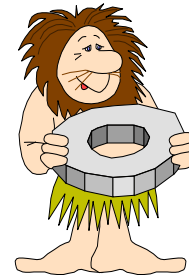
Never write
 LD_{50} as
 1.51 ± 0.45 ppm

Probit analysis

There are many programs have been used for probit analysis. You have to make your own choice.

- Probit analysis
- POLO
- SAS
- SPSS
- Mathematica
- BMDP
-

Critical evaluation:
 The only reliable road to knowledge.



Teşekkür ederim!

سپاسگزارم

謝謝!

ขอบคุณครับ

Děkuji

Danke!

¡Muchas gracias!

Thank you!

ご清聴ありがとうございます
 います!