

Aquatic Toxicology Lab 6

Acute Toxicity Test Data Analysis

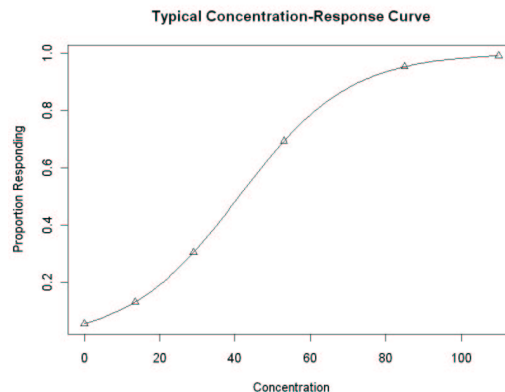
1. Data Analysis Background
2. Analyze *Pimephales promelas* and *Ceriodaphnia dubia* data sets
3. Programs for data analysis can be found at
http://www.ias.unt.edu/~waller/aquatic_toxicology/laboratory/data_analysis

1. Introduction

1. Objective: Identify discharges of toxic effluents in acutely toxic amounts.
2. Two Methods:
 1. LC50 (Concentration Lethal to 50% of organisms tested) – Graphical, Spearman-Kärber, Trimmed Spearman-Kärber, and Probit methods.
 2. NOAEC (No Observable Adverse Effect Concentration) - Hypothesis testing
3. Independence of observations is an important assumption. Randomization of placement of organisms in the test containers is used to ensure that bias will not be introduced into the test.
4. Outliers
 1. inconsistent or questionable data point skewing the data. Outliers should be noted and explained. Data sets can be analyzed with and without the outlier.

2. LC50

1. Concentration-Response
 1. Sigmoidal organismal response when displayed as a cumulative distribution
 2. We want to calculate the concentration where the proportion responding equals a predetermined value, usually 50%.



2. 4 different methods recommended by EPA depending on the shape of the response distribution.
 1. Test acceptability criteria must be met for all methods
 1. 90% survival in controls
 1. 2 mortalities allowed in control based on n of 20
 2. See the handout for a flow chart to guide us through selection of the appropriate method
 3. Graphical
 1. Mathematical method of last resort. If your data do not meet the assumptions of the

previous three tests you can graph the data and interpolate the LC50. The data are graphed on log normal axes placing the percent mortality on the x axis and the log₁₀ of the concentrations on the y axis. It will not give you a 95% confidence limit. The only assumption is:

1. The observed percent mortalities bracket 50%.

4. Spearman-Karber

1. non-parametric assuming

1. The smoothed, adjusted proportion mortality of the lowest effluent concentration must be zero.
2. The smoothed, adjusted proportion mortality of the highest effluent concentration must be one.
3. To calculate 95% confidence limits one effluent concentration must be between zero and one (partial kill).

5. Trimmed Spearman-Karber

1. modified version of the Spearman-Karber non-parametric test. EPA recommends use of this method if the assumptions for the Probit and Spearman-Karber methods are not met. It assumes:

1. The smoothed, adjusted proportion mortalities must bracket 0.5
2. To calculate 95% confidence limits one or more of the smoothed adjusted proportion mortalities must be between zero and one.

2. Calculated using EPA TSK program

6. Probit

1. Probit method should be used if the data meet the following assumptions:

1. Normally distributed log₁₀ response
2. Observed proportion mortalities must bracket 0.5.
3. To calculate 95% confidence limits two or more of the observed proportion mortalities must be between zero and one (partial kill) to obtain confidence limits.

2. Calculated using EPA Probit program

3. Two adjustments are often made on data:

1. When data are not proportion mortalities are not monotonically non-decreasing with concentration, the data are smoothed by averaging the adjacent non-monotonically non-decreasing values.
2. When there are mortalities in the control, the data are adjusted using Abbott's formula.

3. NOAEC

1. Hypothesis Testing

1. Comparison of exposed groups to control

1. Null hypothesis – There is no statistically significant difference
2. Alternative hypothesis – There is a statistically significant difference

2. We will reserve discussion of Hypothesis testing for Chronic test data analysis

4. Following these guidelines, calculate LC50 values for

1. Practice data sets
2. Both fish data sets
3. Your group's *C. dubia* data sets.

Practice Data Sets

Set 1

Concentration	A	D	P
Control	20	0	
6.25%	20	0	
12.50%	16	4	
25.00%	8	12	
50.00%	4	16	
100.00%	0	20	

Set 2

Concentration	A	D	P
Control	19	1	
6.25%	20	0	
12.50%	16	4	
25.00%	0	20	
50.00%	0	20	
100.00%	0	20	

Set 3

Concentration	A	D	P
Control	20	0	
6.25%	20	0	
12.50%	9	11	
25.00%	12	8	
50.00%	0	20	
100.00%	0	20	

Set 4

Concentration	A	D	P
Control	20	0	
6.25%	20	0	
12.50%	0	20	
25.00%	0	20	
50.00%	0	20	
100.00%	0	20	

Set 5

Concentration	A	D	P
Control	20	0	
6.25%	20	0	
12.50%	20	0	
25.00%	20	0	
50.00%	20	0	
100.00%	1	19	

Set 6

Concentration	A	D	P
Control	18	2	
6.25%	19	1	
12.50%	20	0	
25.00%	15	5	
50.00%	3	17	
100.00%	0	20	

P. Promelas 10 day old

Concentration	A	D	P
Control	21	0	
6.25%	20	0	
12.50%	20	0	
25.00%	20	0	
50.00%	20	0	
100.00%	2	20	

P. Promelas 4 day old

Concentration	A	D	P
Control	20	0	
6.25%	20	0	
12.50%	20	0	
25.00%	19	0	
50.00%	18	2	
100.00%	0	20	

P. Promelas Tuesday

Concentration	A	D	P
Control	17	3	
6.25%	18	2	
12.50%	19	1	
25.00%	18	2	
50.00%	17	3	
100.00%	0	20	

Acute Toxicity Test Data Sheet

Discharger _____

Analyst Monday Green

Location _____

Test Start Date/Time 2-19-01 3:45 PM

Date Sample Collected _____

Test End Date/Time 2-21-01 3:25 PM

Organism *Ceriodaphnia dubia*

Water Quality Parameters

	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	pH S.U.	Dissolved Oxygen mg/L	Conductivity µmhos/cm	Temperature °C
100% Effluent						
Control						

Test Data

	24 Hours								48 Hours								Total	
	Rep 1		Rep 2		Rep 3		Rep 4		Rep 1		Rep 2		Rep 3		Rep 4			
Conc.	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
Control	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20	0
3.125%	5	0	5	0	5	0	5	0	5	0	5	0	5	0	4	1	19	1
6.25%	5	0	3	2	3	2	1	4	2	3	2	3	0	5	0	5	4	16
12.5%	1	4	2	3	1	4	1	4	2	3	2	3	2	3	2	3	8	12
25%	2	3	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20
50%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20
100%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20

Notes:

Acute Toxicity Test Data Sheet

Discharger _____

Analyst Monday Red

Location _____

Test Start Date/Time 2-19-01 3:45 PM

Date Sample Collected _____

Test End Date/Time 2-21-01 3:25 PM

Organism *Ceriodaphnia dubia*

Water Quality Parameters

	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	pH S.U.	Dissolved Oxygen mg/L	Conductivity µmhos/cm	Temperature °C
100% Effluent						
Control						

Test Data

	24 Hours								48 Hours								Total		
	Rep 1		Rep 2		Rep 3		Rep 4		Rep 1		Rep 2		Rep 3		Rep 4				
Conc.	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	
Control	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20	0	
3.125%	4	1	5	0	3	1	4	1	1	4	5	0	1	3	2	3	9	10	
6.25%	5	0	3	2	3	0	5	0	4	1	3	2	1	2	2	3	10	8	
12.5%	4	1	5	0	5	0	2	2	3	2	3	2	4	1	1	3	11	8	
25%	3	0	4	1	3	1	4	1	1	2	0	5	0	4	0	6	1	17	
50%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20	
100%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20	

Notes:

Acute Toxicity Test Data Sheet

Discharger _____

Analyst Monday Blue

Location _____

Test Start Date/Time 2-19-01 3:45 PM

Date Sample Collected _____

Test End Date/Time 2-21-01 3:25 PM

Organism *Ceriodaphnia dubia*

Water Quality Parameters

	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	pH S.U.	Dissolved Oxygen mg/L	Conductivity µmhos/cm	Temperature °C
100% Effluent	180	110	8.1	8.1	17000	23
Control	184	105	7.59	8.1	550	23.2

Test Data

	24 Hours								48 Hours								Total	
	Rep 1		Rep 2		Rep 3		Rep 4		Rep 1		Rep 2		Rep 3		Rep 4			
Conc.	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
Control	5	0	5	0	3	1	4	1	5	0	5	0	3	2	2	3	15	5
3.125%	3	2	5	0	2	3	5	0	2	3	3	2	2	3	4	1	11	9
6.25%	4	1	4	2	5	0	4	1	2	3	3	2	5	0	3	2	13	7
12.5%	5	0	5	0	3	2	3	2	1	4	5	0	5	0	2	3	13	7
25%	2	3	1	4	1	4	2	3	1	4	1	4	1	4	1	4	4	16
50%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20
100%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20

Notes:

Acute Toxicity Test Data Sheet

Discharger _____ Analyst Tuesday Blue
 Location _____ Test Start Date/Time 2-20-02 9:00 PM
 Date Sample Collected _____ Test End Date/Time 2-22-01 5:00 PM
 Organism Ceriodaphnia dubia

Water Quality Parameters

	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	pH S.U.	Dissolved Oxygen mg/L	Conductivity µmhos/cm	Temperature °C
100% Effluent	172	115	8.3	8.3	1728	22
Control	172	100	7.61	8.3	550	22

Test Data

Conc.	24 Hours								48 Hours								Total	
	Rep 1		Rep 2		Rep 3		Rep 4		Rep 1		Rep 2		Rep 3		Rep 4		A	D
	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
Control	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20	0
3.125%	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20	0
6.25%	4	1	5	0	5	0	5	0	3	2	4	1	5	0	5	0	17	3
12.5%	4	1	5	0	5	0	4	1	4	1	3	2	5	0	5	0	13	7
25%	4	1	4	1	5	0	4	1	0	5	2	3	1	4	1	4	4	16
50%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20
100%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20

Notes:

Acute Toxicity Test Data Sheet

Discharger _____

Analyst Tuesday Red

Location _____

Test Start Date/Time 2-20-02 9:00 PM

Date Sample Collected _____

Test End Date/Time 2-22-01 5:00 PM

Organism *Ceriodaphnia dubia*

Water Quality Parameters

	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	pH S.U.	Dissolved Oxygen mg/L	Conductivity µmhos/cm	Temperature °C
100% Effluent						
Control						

Test Data

	24 Hours								48 Hours								Total	
	Rep 1		Rep 2		Rep 3		Rep 4		Rep 1		Rep 2		Rep 3		Rep 4			
Conc.	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
Control	5	0	5	0	5	0	4	1	5	0	5	0	5	0	4	1	19	1
3.125%	6	0	5	0	5	0	5	0	3	3	4	1	1	4	1	4	9	12
6.25%	5	0	4	1	5	0	5	0	3	2	3	2	5	0	4	1	15	5
12.5%	4	0	5	0	5	0	4	1	2	3	4	1	5	0	4	1	15	5
25%	3	2	4	1	0	5	5	0	0	5	0	5	0	5	0	5	0	20
50%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20
100%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20

Notes:

Acute Toxicity Test Data Sheet

Discharger _____

Analyst Tuesday Green

Location _____

Test Start Date/Time 2-20-02 9:00 PM

Date Sample Collected _____

Test End Date/Time 2-22-01 5:00 PM

Organism *Ceriodaphnia dubia*

Water Quality Parameters

	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	pH S.U.	Dissolved Oxygen mg/L	Conductivity µmhos/cm	Temperature °C
100% Effluent	172	105	8.57	4.6	17000	23.1
Control	164	120	8.19	10	550	22.8

Test Data

	24 Hours								48 Hours								Total	
	Rep 1		Rep 2		Rep 3		Rep 4		Rep 1		Rep 2		Rep 3		Rep 4			
Conc.	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D
Control	5	0	5	0	5	0	5	0	3	2	4	1	4	1	5	0	16	4
3.125%	5	0	4	1	5	0	5	0	4	1	4	1	3	2	5	0	16	4
6.25%	5	0	5	0	8	0	5	0	3	2	3	2	2	6	4	1	12	11
12.5%	6	1	6	1	5	0	5	0	5	2	6	1	5	0	5	0	21	3
25%	5	0	4	1	5	0	6	0	3	2	0	3	4	1	5	1	11	7
50%	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	20
100%	3	2	0	5	0	5	0	5	2	3	0	5	0	5	0	5	2	18

Notes: