December, 1982: First release
January 1999: Second release

TRIMMED SPEARMAN-KARBER ESTIMATION OF LC50 VALUES

The program SPEARMAN estimates LC50 values using the Trimmed Spearman-Karber Method. (See Hamilton, M.A., R.C. Russo, and R.V. Thurston "Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays," Environ. Sci. Technol., 1977, 11(7), pp. 714-719; Correction: 1978, 12(4), pp. 417 for a discussion of this method.)

This program originated at Montana State University, and has been modified by programmers at ERL-Duluth.

To run the program, see the following example. A brief explanation of the program is given with the example. A more detailed explanation follows.

TRIMMED SPEARMAN-KARBER METHOD
MONTANA STATE UNIVERSITY

NOTE: Responses and key strokes that can be typed by the user are underlined with the underscore character (_) and/or enclosed by the angle bracket characters (<>).

C:\LC50>SPEARMAN

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

> Can create a plot & summary file. File can be printed for a summary or it can be used to obtain a plot of LC50's over duration

WOULD YOU LIKE TO CREATE A PLOT AND SUMMARY FILE(Y/N)? Y
NAME OF PLOT AND SUMMARY FILE TO BE CREATED: TEST1.DAT
ENTER DATE OF TEST: 8-6-1999 ← Up to 10 alphanumeric characters
ENTER TEST NUMBER: 23-A-1 ← Up to 8 alphanumeric characters
WHAT IS TO ESTIMATED:
(L) LC50
OR (E) EC50 ? L
ENTER SPECIES NAME: FATHEAD MINNOW
ENTER CHEMICAL NAME: KELTHANE
ENTER UNITS FOR CONCENTRATION OF CHEMICAL: MG/L
ENTER THE NUMBER OF CONCENTRATIONS: 4
ENTER THE 4 CONCENTRATIONS (IN INCREASING ORDER):
1.0,2.1,4.0,7.9 ← If equal numbers, can enter just the number/concentration.
ARE THE NUMBER OF INDIVIDUALS AT EACH CONCENTRATION EQUAL(Y/N)? Y
ENTER THE NUMBER OF INDIVIDUALS AT EACH CONCENTRATION: 10
ENTER UNITS FOR DURATION OF EXPERIMENT(HOURS,DAYS,ETC.): HOURS
ENTER DURATION OF TEST: 48
ENTER THE NUMBER OF MORTALITIES AT EACH CONCENTRATION:
3 1 8 9
WOULD YOU LIKE THE AUTOMATIC TRIM CALCULATION(Y/N)? Y ← If yes, the minimum required trim is used. If no, the user inputs the trim. This question is asked only once for a test number.

DATE: 8-6-1999 TEST NUMBER: 23-A-1 DURATION: 48 HOURS
CHEMICAL: KELTHANE SPECIES: FATHEAD MINNOW

RAW DATA:

CONCENTRATION (MG/L)	1.00	2.10	4.00	7.90
NUMBER EXPOSED:	10	10	10	10
MORTALITIES:	3	1	8	9
SPEARMAN-KARBER TRIM:		20.00%		

← This is the minimum required trim since the automatic trim option was chosen.

SPEARMAN-KARBER ESTIMATES:	LC50:	2.90
	95% LOWER CONFIDENCE:	2.39
	95% UPPER CONFIDENCE:	3.51

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION. ← Notes about certain peculiarities are written below the results. See the following discussion for details concerning this note.

If yes, a copy will be sent to the printer, after the user exits the program.

WOULD YOU LIKE TO HAVE A COPY SENT TO THE PRINTER(Y/N)? N
WOULD YOU LIKE TO SAVE THESE RESULTS IN THE PLOT/SUMMARY FILE(Y/N)? Y
WOULD YOU LIKE TO CONTINUE (Y/N)? Y
WOULD YOU LIKE TO ANALYZE THE SAME EXPERIMENT AT A DIFFERENT DURATION (Y/N)? Y

If no, the program will exit at this point.

If yes, the program will ask for input starting with "Enter duration of test," so only duration, mortalities, & trim change. If no, program will go back to the beginning with the creation of a plot summary file.

Since the above was yes, start with duration.

ENTER DURATION OF TEST: 96
ENTER THE NUMBER OF NORTALITIES AT EACH CONCENTRATION:
4 10 10 10

DATE: 8-6-1999 TEST NUMBER: 23-A-1 DURATION: 96 HOURS
CHEMICAL: KELTHANE SPECIES: FATHEAD MINNOW

RAW DATA:

CONCENTRATION (MG/L)	1.00	2.10	4.00	7.90
NUMBER EXPOSED:	10	10	10	10
MORTALITIES:	4	10	10	10
SPEARMAN-KARBER TRIM		40.00%		

SPEARMAN-KARBER ESTIMATES: LC50: 1.13
95% CONFIDENCE LIMITS
ARE NOT RELIABLE.

See following discussion for details.

WOULD YOU LIKE TO HAVE A COPY SENT TO THE PRINTER (Y/N)? N
WOULD YOU LIKE TO SAVE THESE RESULTS IN THE PLOT/SUMMARY FILE (Y/N)? Y
WOULD YOU LIKE TO CONTINUE (Y/N)? Y
WOULD YOU LIKE TO ANALYZE THE SAME EXPERIMENT AT A DIFFERENT DURATION (Y/N)? N

Continuing, but with a different experiment, so must go back to the beginning.

WOULD YOU LIKE TO CREATE A PLOT AND SUMMARY FILE(Y/N)? N

No file is created.

ENTER DATE OF TEST: 7/22/1999
ENTER TEST NUMBER: 3
WHAT IS TO ESTIMATED:
(L) LC50
OR (E) EC50 ? E
ENTER SPECIES NAME: DAPHNIA MAGNA
ENTER CHEMICAL NAME: MERCURY
ENTER UNITS FOR CONCENTRATION OF CHEMICAL: PPM
ENTER THE NUMBER OF CONCENTRATIONS: 5
ENTER THE 5 CONCENTRATIONS (IN INCREASING ORDER):
1 2 4 8 16
ARE THE NUMBER OF INDIVIDUALS AT EACH CONCENTRATION EQUAL(Y/N)? N
ENTER THE NUMBER OF INDIVIDUALS AT EACH CONCENTRATION:
10 10 10 9 10

Number of individuals per concentration is not equal so individual values must be input.

ENTER UNITS FOR DURATION OF EXPERIMENT(HOURS,DAYS,ETC.): HOURS
ENTER DURATION OF TEST: 96
ENTER THE NUMBER OF MORTALITIES AT EACH CONCENTRATION:
2 4 9 10 10
ERROR IN NUMBER OF MORTALITIES (NUMBER OF MORTALITIES CANNOT BE GREATER THAN NUMBER OF INDIVIDUALS).
ENTER THE NUMBER OF MORTALITIES AT EACH CONCENTRATION:
2 4 9 9 10
WOULD YOU LIKE THE AUTOMATIC TRIM CALCULATION(Y/N)? N
ENTER THE % TRIM REQUESTED: 10

> Several checks are made on the input and opportunities are given to correct errors. For example, here the fourth mortality was given as 10, but there are only 9 individuals. Re-entry is requested.

> Automatic trim is not requested so a trim value must be input.

DATE: 7/22/1999 TEST NUMBER: 3 DURATION: 96 HOURS
CHEMICAL: MERCURY SPECIES: DAPHNIA MAGNA

RAW DATA:

CONCENTRATION (PPM)	1.00	.00	4.00	8.00	16.00
NUMBER EXPOSED:	10	10	10	9	10
MORTALITIES:	2	4	9	9	10
SPEARMAN-KARBER TRIM:		20.00%			

SPEARMAN-KARBER ESTIMATES:	EC50:	2.14
	95% LOWER CONFIDENCE:	1.46
	95% UPPER CONFIDENCE:	3.15

NOTE: REQUESTED TRIM OF 10.00% IS TOO SMALL.
CALCULATED TRIM OF 20.00% WAS USED.

> See following discussion for details.

WOULD YOU LIKE TO HAVE A COPY SENT TO THE PRINTER(Y/N)? N
WOULD YOU LIKE TO CONTINUE (Y/N)? N
RUN COMPLETED

The plot and summary file created by the program SPEARMAN can be used for two purposes. A print-out of the file gives a summary of the experiment, along with the LC50 and 95% confidence intervals for the various durations. See Figure I for an example.

concentration	1.0	2.0	4.0	8.0
number exposed	10	10	10	10
mortality	2	4	9	10

The file is also used to obtain a plot of the LC50 with confidence intervals over time. A plot can be generated using either the Tektronix 4663 plotter, a retrofitted VT100, or a Tektronix 4025 or 4010. To obtain the plot, run SPEARPLOT. The name of the plot/summary file is needed as input. See Figure II for an example of a plot.

The Spearman-Karber estimate of the LC50 has been used for many years, since the early part of the century. However, the Trimmed Spearman-Karber estimate (see Hamilton, et. al., 1977) is a recent modification of the Spearman-Karber estimate.

The following section explains several aspects of the Trimmed Spearman-Karber estimation procedure. The estimation of the LC50, determination of trim, and confidence intervals are among the topics discussed. For further details see the articles listed in the reference section.

First, the basic calculations for estimating the LC50 (Hamilton, et. al., 1977) will be discussed. The following notation will be used:

k	The number of concentrations
n(i)	The number of individuals exposed at concentration i, i=l, ..., k
r(i)	The number of individuals that responded at concentration i, i=l, ..., k
p(i)=r(i)/n(i)	The proportion of individuals that responded at concentration i, i=l, ..., k
x(i)	Natural log of concentration i
m	Mean of the log tolerance distribution, i.e., ln of the LC50

$$\text{Then } m = \sum_{i=1}^{K-1} (p(i) + p(i+1)) \left(\frac{x(i) + x(i+1)}{2}\right)$$

if p(l)=0.0 and p(k)=1.0.

For example, consider the following test:

concentration (mg/1)	0.5	1.0	2.0	4.0	8.0
number exposed	10	10	10	10	10
mortality	0	2	4	9	10

ln concentration	-0.693	0.0	0.693	1.386	2.079
mortality proportion	0.0	0.2	0.4	0.9	1.0

$$m = (0.2 - 0.0)\,\frac{(-0.693 + 0.0)}{2} + (0.4 - 0.2)\,\frac{(0.0 + 0.693)}{2}$$

$$+ (0.9 - 0.4)\,\frac{(0.693 + 1.386)}{2} + (1.0 - 0.9)\,\frac{(1.386 + 2.079)}{2}$$

$$= 0.693$$

LC50 = exp(m) = 2.0

FIGURE 1
EXAMPLE OF A SUMMARY FILE

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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

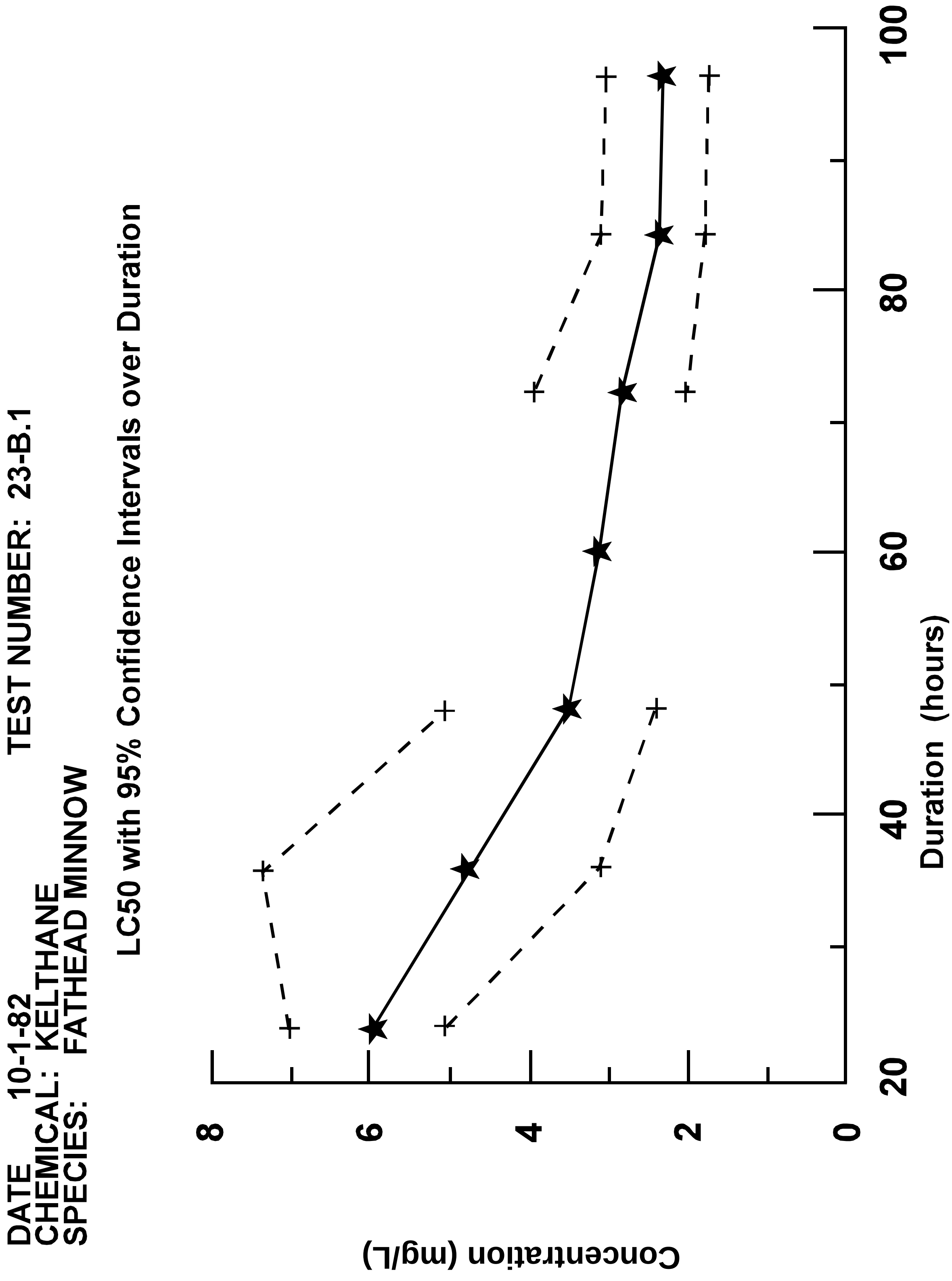
DATE: 10-1-82 TEST NUMBER: 23-B.1
CHEMICAL: KELTHANE SPECIES: FATHEAD MINNOW

RAW DATA:

CONCENTRATION(mg/L)	1.00	2.00	4.00	8.00
NUMBER EXPOSED:	10	10	10	10

DURATION (hours)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
24	5.94	5.04	7.00	20.00
36	4.76	3.10	7.30	20.00
48	3.45	2.36	5.03	20.00
60	3.08			20.00
72	2.74	1.95	3.85	15.00
84	2.25	1.69	3.01	10.00
96	2.18	1.63	2.92	10.00

FIGURE II
EXAMPLE OF A PLOT OF LC50 WITH CONFIDENCE INTERVALS
OVER DURATION



Note that these proportions must be monotonically increasing (i.e., $P(l) \leq p(2) \leq ... \leq p(k)$). If they are not, the proportions must be adjusted before the calculations are performed.

The proportions are adjusted in the following manner. All adjacent proportions that are not in a monotonically increasing sequence are adjusted to the same value. This value is obtained by summing the mortalities over all these concentrations and dividing by the sum of individuals at these concentrations. For example, consider the following experimental results:

concentration (mg/l)	1.1	2.3	4.5	8.8	17.1
number exposed	10	10	9	10	10
mortalities	1	5	4	2	7
mortality proportion	0.10	0.50	0.44	0.20	0.70

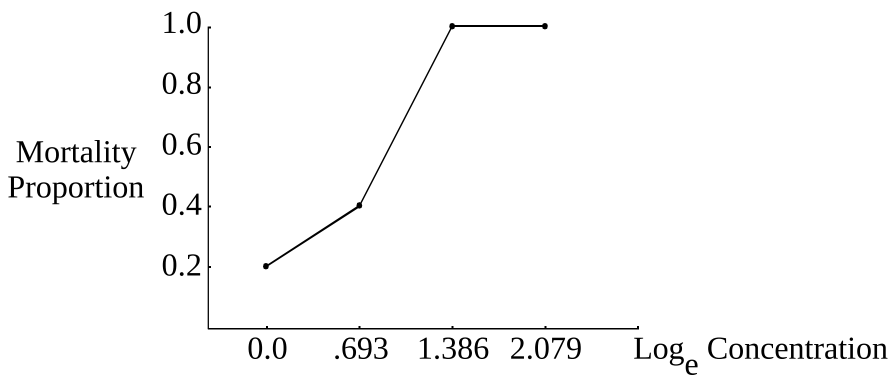
Since $p(2) > p(3) > p(4)$ these proportions must be adjusted. The adjusted proportion for the second through fourth concentrations is (5+4+2)/(10+9+10) = .38.

The conventional Spearman-Karber estimate can be used only if there are concentrations for which there are 0% and 100% kills. If this does not occur, the Trimmed Spearman-Karber Method can be used.

Consider the first example given. If the lowest concentration had not been used, the following results would have been obtained:

concentration	1.0	2.0	4.0	8.0
number exposed	10	10	10	10
mortality	2	4	9	10

A plot of these data gives:



A trim value, $\propto$, must be chosen such that:

(i) $0 \leq \propto \leq 0.5$

(ii) $\propto \geq p(1)$

(iii) $\propto \geq 1.0 - p(k)$,

where p(l) and p(k) are the mortality proportions after the proportions have been adjusted to be monotonically increasing.

The minimum required trim is found by taking the minimum value that satisfies all three constraints listed above. For the example, the minimum trim required is 0.2. Any value greater than this minimum value, but not greater than 0.5, can be used for the trim. However, it is usually a good procedure to use the minimum trim required or a trim of 10%. More information is ignored when a large trim is used; when the minimum trim is used, the most information is used. However, for some distributions, the estimates are more accurate if a 10% trim is used (Hamilton, 1980).

The trim is used to cut off the upper and lower 100 ∝ percent of the plot. The data that is then analyzed is the middle 100(1-2∝)% of the tolerance distribution. By adjusting the proportions according to the trim value, the formula given above can be used to obtain the LC50 estimate. Note that if ∝ = 0, there is no trim and the estimate is the same as the conventional Spearman-Karber estimate.

The Trimmed Spearman-Karber estimate of the LC50 is obtainable for most experiments. In order to estimate an LC50 the only requirement is that at least one mortality proportion must be less than or equal to 50% and at least one must be greater than or equal to 50%. That is, it is not possible to calculate the LC50 by the Spearman-Karber Method if the mortality proportions are all less than 50% or are all over 50%.

If the LC50 can be estimated, the 95% confidence intervals may not be reliable. There are two situations when the confidence intervals may not be reliable:

(i) When the trim is 50%

and (ii) let p(i) be the largest mortality proportion that is less than or equal to the trim. Let p(j) be the smallest proportion that is greater than or equal to the quantity (1.0 - ∝). Then if: (a) there are no proportions between p(i) and p(j) and (b) p(i)=0.0 and/or p(j)=1.0 the confidence intervals are generally not reliable.

95% confidence intervals can be calculated, but unless the concentrations are very narrowly spaced, the estimates will not be very reliable.

For example, consider the following results of an experiment:

concentration (mg/l)	1.0	2.0	4.0
number exposed	10	10	10
mortality	3	10	10
mortality proportion	.3	1.0	1.0

A minimum trim of 30% is needed. P(1)=0.3 and P(2)=1.0. Since there are no proportions between p(l) and p(2) and p(2)=1.0, the confidence interval will not be reliable. Note, that if the second proportion were less than 1.0, say 0.9, the confidence interval estimate would be more reliable.

An intuitive explanation of the lack of reliability follows. A plot of the proportions versus log concentration is given in Figure IIIa. A solid line connects the observed values. The first observed concentration at which there is 100% mortality is c(2). However, if other concentrations had been used between c(l) and c(2), 100% mortality may have been observed at lower concentrations than c(2). Any of the dotted lines shown in Figure IIIa would be plausible. As a result, it is difficult to obtain a measure of confidence in the LC50 estimate. The confidence limits will generally be reliable only if the concentrations are very narrowly spaced, so that mortality greater than 0% and less than 100% would be obtained if c(l) were increased slightly and c(2) decreased slightly, respectively.

To continue the example, consider Figure IIIb, where the mortalities are 30%, 90%, and 100%. Here the line connecting the proportions is much more stable. It is not apparent how the line connecting c(2) and c(3) behaves, but that is not important for estimation, since in this example the trim eliminates that portion of the curve. As a result, the calculated confidence interval around the estimated LC50 will be more reliable than the estimate for the data in Figure IIIb.

FIGURE III

Plots of Proportions Versus Log Concentration

Figure IIIa

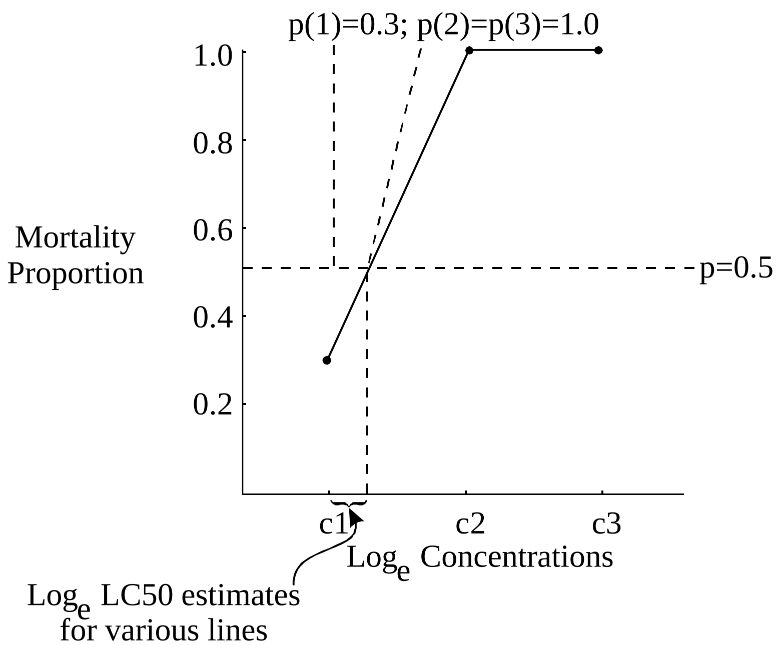
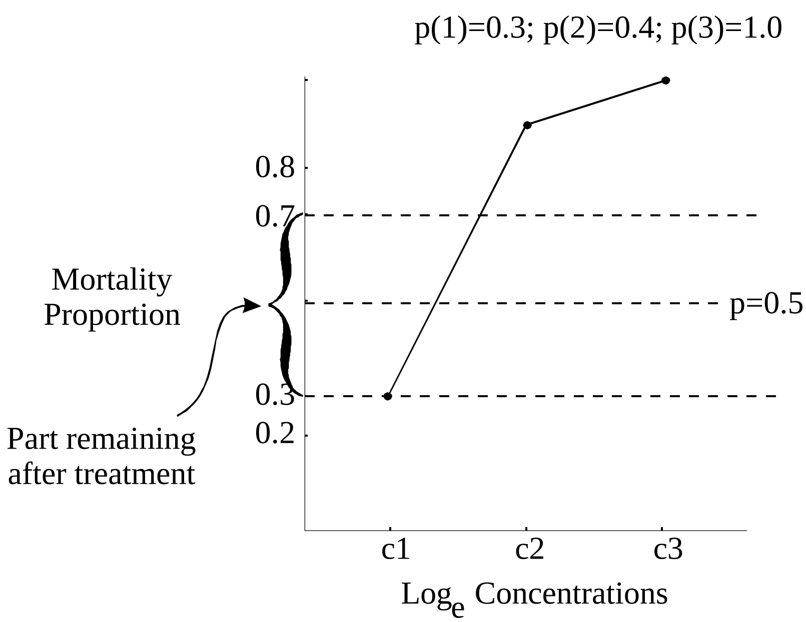


Figure IIIb



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Finney, David J., 1978, Statistical Method in Biological Assay, 3rd Ed., Charles Griffin and Co., London.

Hamilton, Martin A., 1979, "Robust Estimates of the ED50," Journal of The American Statistical Association, 74, pp. 344-354.

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Stephan, C.E., 1977, "Methods for Calculating an LC50," in Aquatic Toxicology and Hazard Evaluation, ASTM STP 634, F.L. Mayer and J.L. Hamelink, Eds., American Society for Testing and Materials, pp. 65-84.