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A BASIC Program for Estimating LD_{50} Values Using the IBM-PC®

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In a recent publication, Trevors and Lusty (1985) described a BASIC program designed to operate on an Apple II plus microcomputer, for calculating the LD_{50} value, probit transformation, 95% confidence limits, and chi-square test for goodness of fit using quantal response data.

This program has now been modified to operate on an IBM-PC microcomputer and is presented here. Complete details of the program can be found in the original publication of Trevors and Lusty (1985).

MATERIALS AND METHODS

The program listing for the LD_{50} program is presented in Table 1. First the user must boot the system using the IBM-PC DOS Version 2.1. Then load the BASIC operating system by simply typing BASIC. To operate the LD_{50} program, load (F3 key) the program by typing in the file name you have assigned to the LD_{50} program. The program is run by providing the run command (F2 key). The program is user friendly, and prompts the operator for concentrations used, number of bioassay organisms, and the corresponding mortality or percent mortality.

The program can also provide a hard-copy output to a printer by using the print screen command on the IBM-PC keyboard. An example of the data analyzed using the program is presented in Table 2. This data was adapted from an example presented in a text by Buck et al. (1973), who also provide an excellent description and examples of dose-response relationships.

RESULTS AND DISCUSSION

In the example used by Buck et al. (1973) the LD_{50} value was estimated graphically (using probit paper) to be 135 mg/kg. The microcomputer program gave a calculated value of 133.8118, indicating that the methods are extremely close. The small difference could in fact be due to the limits of reading the estimated value from the graph paper. The microcomputer program also calculates the 95% confidence limits (see Table 2) and

estimates the chi-square (X^2) value. The critical value for X^2 was calculated to be 5.94 using the microcomputer program. For 2 degrees of freedom at the 95% level, the chi-square value obtained from a set of standard math tables (Petrie 1978) was 5.99. This slight difference is probably due to the method of computing the table value. It is therefore suggested that a set of standard math tables be used in situations where the table value of chi-square and the calculated value are very close.

With new advances in microcomputer technology and software, large amounts of data can be analyzed and stored in a relatively short period of time. Since LD_{50} values are commonly used in toxicological research, microcomputer programs may offer some advantages over traditional methods.

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Trevors J T, Lusty C W (1985) A BASIC microcomputer program for calculating LD_{50} values. Water Air Soil Pollut 24: 431-442.

Table 1. Program listing

```
5     CLS
10    REM LD50 PROGRAM
20    REM
30    REM THIS PROGRAM CALCULATES A
40    REM LETHAL DOSE FOR 50 PERCENT OF
50    REM SUBJECTS BASED UPON EXPERIMENTAL
60    REM DATA ENTERED BY THE USER.
70    REM THE SOLUTION TECHNIQUE
80    REM IS ESSENTIALLY THAT PROPOSED
90    REM BY J.J. HUBERT IN HIS BOOK `BIOASSAY', (CHAPTER 4)
100   REM
110   REM SEE CHAPTER FIVE OF THE BOOK
120   REM
130   REM PROGRAM AUTHOR - J.T. TREVORS
140   REM
150   REM
200   REM
210   REM **************************
220   REM VERSION 1.1 -MARCH 15/85
223   REM **************************
230   REM
240   REM
300   REM
```

```
500   CLS: PRINT : PRINT "WELCOME TO THE LD50 CALCULATION PROGRAM"
510   PRINT : PRINT "THIS PROGRAM CALCULATES LD50 CONCENTRATION"
520   PRINT "BASED UPON DATA SUPPLIED BY THE USER."
540   PRINT : PRINT "YOU WILL BE PROMPTED FOR DATA INPUT"
550   PRINT "SIMPLY ANSWER THE QUESTIONS"
560   PRINT : PRINT "PRESS THE RETURN KEY TO CONTINUE"
570   INPUT A$
571   CLS : PRINT "DO YOU WANT DISK STORAGE OF INPUT DATA (Y/N)
572   INPUT X$
573   IF X$ < > "Y" THEN GOTO 578
574   PRINT "ENTER THE FILENAME YOU DESIRE": INPUT X$
575   PRINT "ENTER THE DRIVE NUMBER": INPUT Z$
576   DOS$ = CHR$ (4) :Y$ = "OPEN" + X$ + ",D" + Z$
577   PRINT DOS$;Y$
578   CLS : PRINT "DO YOU WANT HARD-COPY OUTPUT (Y/N)":INPUT T$
580   CLS : PRINT : PRINT "HOW MANY DATA SETS ARE TO BE ENTERED"
590   INPUT NU
595   DIM D(NU),N(NU),R(NU),P(NU),X(NU),Y(NU),YE(NU),Z1(NU),
      Z2(NU),Z3(NU),XT(100)
600   GA = 0
603    DIM Z(NU)
605    DIM PE(NU)
610    FOR I = 1 TO NU
620    CLS : PRINT :PRINT "ENTER DOSE"
630    INPUT D(I)
640    PRINT : PRINT "ENTER THE NUMBER TREATED AT DOSE";D(I)
645    INPUT N(I)
650    REM
660    PRINT : PRINT "ENTER THE NUMBER OF RESPONSES AT DOSE"
670    PRINT D(I)
680    INPUT R(I)
683    NEXT I
685    REM THE NEXT STATEMENT PREVENTS AN ERROR FOR OUT-OF-
       RANGE LOG FUNCTION
686    REM
689    FOR I = 1 TO NU
690    IF D(I) = 0 THEN PRINT "DOSE OF ZERO NOT ALLOWED,
       ABORTING...": STOP
691    NEXT I
692   LF = 1:IC = 0:TE =D(1)
694    IF TE >= 10 AND TE < 100 THEN GOTO 710
695   TE = D(1)
696   IC = IC + 1
697    IF TE > 100 THEN TE = TE * 10 ^ (- IC):LF = 0
698    IF TE < 10 THEN TE = TE * 10 ^ IC:LF = 1
699    IF TE >= 10 AND TE < 100 THEN GOTO 710
700    GOTO 695
710    IF LF = 0 THEN IC = -IC
712    FOR I = 1 TO NU
714   X(I) = LOG (D(I) * 10 ^ (IC)) / LOG (10)
715   P(I) = R(I) / N(I)
716   NEXT I
721   GOSUB 5900
722   REM
724   REM
```

```
726   REM
730   REM THIS IS THE END OF THE DATA INPUT SEQUENCE, NOW THE
      WORK BEGINS...
732   REM
734   REM
736   REM
740   CLS : PRINT: PRINT "ESTIMATING VALUES FOR PROBIT LINE ..."
750   FLAG = 0
770    FOR I = 1 TO NU: GOSUB 10000:Y(I) = Z(I) + 5:  NEXT I
772    REM
774    REM
800    REM ESTIMATE THE VALUES FOR A AND B IN Y=A+BX
805    REM AE AND BE ARE THE AFOREMENTIONED ESTIMATES
807    REM
810    REM
820   AE = 0:BE = 0:S1 = 0:S2 = 0:S3 = 0:S4 = 0
830    FOR I = 1 TO NU
840   S1 = S1 + X(I)
850   S2 = S2 + Y(I)
860   S3 = S3 + X(I) ^ 2
870   S4 = S4 + (X(I) * Y(I))
880    NEXT I
890   BE = (S4 - (S1* S2) / NU) /(S3 - (S1 ^ 2) / NU)
900   AE = (S2 - (BE * S1 )) / NU
902    REM
903    REM
904    REM END OF ESTIMATOR
905    REM
906    REM
907    REM
908    REM ESTIMATE THE LD50
909    REM
910    REM
911   M = (5 - AE) / BE
916    REM
917    REM ESTIMATE COMPLETE
918    REM
919    REM
920    REM TEST THE ADEQUACY OF THE PROBIT LINE
935    REM
937    REM
939    CLS
940    PRINT "NOW TESTING ADEQUACY OF FIT FOR PROBIT LINE"
942    REM
944    REM
945   Z1 = 0:Z2 = 0:Z3 = 0:Z4 = 0
950    FOR I = 1 TO NU
955   FLAG = 1
957   YE(I) = AE + BE * X(I)
960    GOSUB 10000
970   Z1 = N(I) * PE(I)
980   Z2 = R(I) - (N(I) * PE(I))
1000  Z4 = Z4 + ((Z2 ^ 2) / (Z1 * (1 - PE(I))))
1001   NEXT I
1005  ZS = 1.64485
```

```
1010  DF = NU - 2
1015  CH = DF * (1 - 2 / (9 * DF) + (ZS * (2 / (9 * DF )) ^
      (1 / 2))) ^ 3
1030  REM     '
1200  REM
1201  REM
1202  REM END OF ADEQUACY
1203  REM
1204  REM
1210  REM FIND THE CONFIDENCE LIMITS FOR THE TEST
1230  REM
1240  REM
1245  CLS : PRINT :PRINT "FINDING CONFIDENCE LIMITS FOR ANALYSIS."
1250  GOSUB 12000
1260  REM
1270  REM
2000  GOSUB 5000
2010  END
5000  REM
5010  REM
5020  REM     OUTPUT SUBROUTINE
5030  REM
5040  REM
5045  FOR I = 1 TO NU:P(I) = P(I) * 100:NEXT I
5070  CLS
5080  PRINT :PRINT "PROBITS"
5090  PRINT "X                    P                    Y"
5100  FOR I = 1 TO NU: PRINT X(I),P(I),Y(I): NEXT I
5110  PRINT :PRINT "TEST OF FIT DATA"
5120  PRINT "CHI^2 (";DF;" D.F.,95%) IS = ";CH
5130  PRINT "RESULT OF CHI^2 FOR THIS ANALYSIS   IS--> ";Z4
5140  PRINT :PRINT "NOMINAL LD50--> ";LD(2)
5150  PRINT "95% CONFIDENCE LIMITS-- > ";LD(3);",";LD(1)
5155  IF T$ = "Y" THEN GOSUB 5500
5160  RETURN
5500  REM
5510  REM  PAPER OUTPUT ROUTINE
5520  REM
5525  CLS
5530  PRINT "TURN ON PRINTER AND PREPARE FOR OUTPUT"
5535  DOS$ = "
5540   PRINT "ENTER THE TITLE YOU DESIRE"
5545   INPUT A$
5547   LPRINT : PRINT DOS$;"80N"
5550   FOR J = 1 TO 8:  LPRINT : NEXT J
5552   REM
5560  Q = LEN (A$)
5570  Q = INT ((80 -Q) / 2)
5580  S$ = " "
5590   FOR J = 1 TO Q - 1:S$ = S$ + " ": NEXT J
5600   LPRINT S$ + A$
5610   PRINT : PRINT : LPRINT "  INPUT DATA"
5615   PRINT : LPRINT "DOSE", "NO. TESTED", "NO.RESPONSES"
5620   LPRINT : FOR I = 1 TO NU:  LPRINT D(I),N(I),R(I)
5625   NEXT I
```

```
5630   PRINT : PRINT : LPRINT "PROBITS"
5640   PRINT : LPRINT "X","Y"
5650   LPRINT : FOR I= 1 TO NU: LPRINT X(I),Y(I):NEXT I
5660   PRINT : LPRINT "ESTIMATED PROBIT LINE"
5670   LPRINT "Y = ";AE;" + ";BE;"X"
5675   LPRINT "CHI^2 (";DF;" D.F.,95%) IS = ";CH
5680   PRINT : LPRINT "RESULTS OF CHI^2 ANALYSIS --> ";Z4
5690   PRINT : LPRINT "NOMINAL LD50 --> ";LD(2)
5700   LPRINT "95% CONFIDENCE LIMITS --> ";LD(3);",";LD(1)
5720   RETURN
5900   REM
5910   REM DISK OUTPUT SUBROUTINE
5920   REM
5930  Y$ = "WRITE " + X$
5940   PRINT DOS$;Y$
5945   PRINT NU
5950   FOR I = 1 TO NU
5960   PRINT D(I);",";N(I);",";R(I)
5670   NEXT I
5980  Y$ = "CLOSE " + X$
5990   PRINT DOS$;Y$
5991   REM
5992   REM
5999   RETURN
9998   REM
10000  REM THIS ROUTINE FINDS THE AREA UNDER `NORMAL' CURVE
10010  REM THE SOLUTION IS A SIMPLE SIMPSON'S APPROXIMATION
10020  REM CERTAIN INTERMEDIATE VALUES ARE STORED IN A LOOK-UP
       TABLE TO INCREASE ACCURACY
10021  REM
10022  REM
10023  REM IF XT'S PREVIOUSLY DEFINED THEN SKIP AROUND
10024  IF GA = 1 GOTO 10068
10025 GA = 1
10030 XT(0) = 1.9095:XT(1) = 2.6732:XT(2) = 2.9458:XT(3)
      = 3.1188:XT(4) = 3.2489:XT(5) = 3.3548
10031   REM
10032  XT(6) = 3.4449:XT(7) = 3.5239:XT(8) = 3.5947:XT(9) =
       3.659:XT(10) = 3.7183
10033   REM
10034  XT(11) = 3.773:XT(12) = 3.8249:XT(13) = 3.8735:XT(14)
       = 3.9196:XT(15) = 3.9636
10035   REM
10036  XT(16) = 4.0056:XT(17) = 4.0459:XT(18) = 4.0847:XT(19) =
       4.1222:XT(20) = 4.15855
10037   REM
10038  XT(21) = 4.1938:XT(22) = 4.228:XT(23) = 4.2614:XT(24) =
       4.294:XT(25) = 4.3258
10039   REM
10040  XT(26) = 4.357:XT(27) = 4.3875:XT(28) = 4.4175:XT(29) =
       4.447:XT(30) = 4.476
10041   REM
10042  XT(31) = 4.5046:XT(32) = 4.5327:XT(33) = 4.5605:XT(34) =
       4.588:XT(35) = 4.6151
10043   REM
```

```
10044  XT(36) = 4.642:XT(37) = 4.6686:XT(38) = 4.695:XT(39) =
       4.7221:XT(40) = 4.7471
10045   REM
10046  XT(41) = 4.7729:XT(42) = 4.7985:XT(43) = 4.824:XT(44) =
       4.8493:XT(45) = 4.8746
10047   REM
10048  XT(46) = 4.8993:XT(47) = 4.9249:XT(48) = 4.95:XT(49) =
       4.975:XT(50) = 5!
10049   REM
10050   REM
10055   FOR K = 51 TO 99
10056  J = 100 - K
10058  XT(K) = (5 - XT(J) + 5: NEXT K
10068   REM
10069   REM FLAG IS SET IF TESTING ADEQUACY OF FIT (AS OPPOSED TO
       ESTIMATING PROBIT)
10070   IF FLAG = 1 THEN GOTO 10199
10075   IF P(I) = 1 THEN Z(I) = 3.5: RETURN
10080   IF P(I) <= .001 THEN X1 = 1.6:A = .003: GOTO 10300
10090   FOR J = 0 TO 99
10095   TT = J / 100
10100   IF P(I) < TT THEN GOTO 10130
10110   NEXT J
10120  XI = XT(99):A= 99 / 100: GOTO 10300
10130  A = (J - 1) / 100:X1 = XT(J - 1): GOTO 10300
10199  Z(I) = YE(I): IF Z(I) > 7.7482 THEN A = .997:X1 =
       7.7482: GOTO 10290
10200   IF Z(I) <= 1.9 THEN X1 = 1.6:A = .003: GOTO 10290
10210   FOR J = 0 TO 99
10220   IF Z(I) <= XT(J) THEN GOTO 10250
10230   NEXT J
10240  X1 = XT(99):A = 99 / 100: GOTO 10290
10250  X1 = XT(J-1):A = (J - 1) / 100
10260   REM
10270   REM INTEGRATION
10280   REM
10290   IF FLAG = 1 THEN GOTO 10700
10300  DX = .001:X1 = X1 -5
10310  SR = 1 / SQR (2 * 3.1416)
10320  YI = SR * EXP ( - (X1 ^ 2) / 2)
10330  XI = X1 + DX
10340  Y2 = SR * EXP ( -(X1 ^ 2) / 2)
10350  A = A + (1 / 2) * ((Y1 + Y2) * DX)
10370   IF A < P(I) THEN GOTO 10320
10670  Z(I) = X1
10675   GOTO 10999
10700  DX = .001:X1 = X1 - 5: IF Z(I) > 8! THEN PE(I) = .999999:
       RETURN
10705  Z(I) = Z(I) - 5
10710  SR = 1 / SQR (2 * 3.1416)
10720  YI = SR * EXP ( - (XI ^ 2) / 2)
10730  X1 = X1 + DX
10740  Y2 = SR * EXP ( -(X1 ^ 2 ) /2)
10750  A = A + ((1 / 2) * ((Y1 + Y2) * DX))
10760   IF X1 < Z(I) THEN GOTO 10720
```

```
10900  PE(I) = A
10999   RETURN
12000   REM
12010   REM
12020   REM THIS SUBROUTINE EMPLOYS A WEIGHTING TECHNIQUE TO FIND
        THE CONFIDENCE
12021   REM LIMITS FOR THE LD50 TEST
12030   REM THE METHOD EMPLOYED IS EXPLAINED IN `BIOASSAY'
        (PAGE 57)
12040   REM
12050   REM
12100  T1 = 0:T2 = 0:T3 = 0:T4 = 0
12110   FOR I = 1 TO NU
12115  PX = P(I): IF PX = 0 THEN PX = .000001
12116   IF PX = 1 THEN PX = .999999
12120  W(I) = EXP ( - ((Y(I) - 5) ^ 2) / 2)
12121  W(I) = (W(I) / SQR (2 * 3.1416)) ^2
12122  W(I) = W(I) / (PX * (1 - PX))
12130  NW(I) = W(I) * N(I)
12140  WX(I) = NW(I) * X(I)
12150  XW(I) = NW(I) * X(I) ^2
12160  T1 = T1 + N(I)
12170  T2 = T2 + NW(I)
12180  T3 = T3 + WX(I)
12190  T4 = T4 + XW(I)
12200   NEXT I
12210  XB = T3 / T2
12220  BX = T4 - ((T3 ^ 2) / T2)
12225  LD = M
12230  SM = (1 / BE) * SQR ((1 / T2) + (((LD - XB) ^ 2) / BX))
12240  MM(2) = M
12250  MM(1) = M +(1.96 * SM):MM(3) = M - (1.96 *SM)
12260  LD(1) = (10 ^ MM(1)) * 10 ^ ( - IC)
12270  LD(2) = (10 ^ MM(2)) * 10 ^ ( - IC)
12280  LD(3) = (10 ^ MM(3)) * 10 ^ ( - IC)
12999   RETURN
```

Table 2. Sample calculation using LD_{50} program.

Dose	No. Treated	% Mortality
50	100%	2.5
100	100%	20
200	100%	90
400	100%	97.5

```
100% = 10 bioassay organisms
PROBITS
   X                P                Y
1.69897          2.5              3.039798
2                20               4.15955
2.30103          90               6.2827
2.60206          97.5             6.961204
TEST OF FIT DATA
CHI^2 (2  d.f., 95%) IS = 5.936855
RESULT OF CHI^2 FOR THIS ANALYSIS IS -->  11.30116
```

NOMINAL LD_{50} --> 133.8118
95% CONFIDENCE LIMITS --> 121.9529, 146.8239

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