RESEARCH ARTICLE

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Detailed Algorithms of Interactive Computer Programs in MATLAB for the Calculation of LD_{50} and Other LD Values Using Methods of Finney, Miller-Tainter and Comparison with OECD Modifications

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ABSTRACT

A test example for LD_{50} determination for thymoquinone administered intraperitoneally in rats, demonstrates the implementation of interactive computer programs for computing LD_{50} and other LD values using Finney method (Program 'LD_{50}-mortality-Finney') and Miller-Tainter method (Program 'LD_{50}-mortality-Miller-Tainter'), written in MATLAB. Detailed program algorithms and their execution, as well as the differences between them, resulting from differences between the methods, are presented. The Finney method transforms results obtained for mortality (in %) to probit values, where probit values for 0% and 100% mortality depend on the number of experimental animals in the group, and then continues processing. The Miller-Tainter method also transforms mortality results (in %) to probit values, but previously corrects percentage values, relevant to the number of experimental animals, if mortality for the lowest dose is 0% and for the highest dose 100%, the corrected values are changed to probit values, and then continues processesing. In the case of OECD Modifications 420, 423 and 425, once the doses for the determination of LD_{50} and other LD values are chosen, Litchfield-Wilcoxon statistical program is usually used to estimate these values and their confidence limits. In the test example of thymoquinone given intraperitoneally to rats LD_0, LD_{16}, LD_{50}, LD_{84} and LD_{100} values obtained by applying the programs "LD_{50}-mortality-Finney", "LD_{50}-mortality-Miller-Tainter" and those calculated by Litchfield-Wilcoxon method were very similar and Fischer's coefficient (F) value showed no statistically significant differences between them ($P>0.05$).

Keywords: Acute toxicity; LD_{50} and other LD values; probit analysis; Finney method; Miller-Tainter method; interactive programs; MATLAB; OECD modifications; Litchfield-Wilcoxon method; modified one-way analysis of variance

Introduction

The LD_{50} value was first introduced by Trevan [1]. The method was designed to estimate acute toxicity of compounds from the dose-response curve by using several animals (usually 5/sex) at each of many test doses, thus consuming a huge number of animals and a lot of time. Since then various improvements have been made in the original method to reduce the number of animals and the time required for the experiment, for example "staircase method" [2] and Organization for Economic Cooperation and

Development (OECD) guide lines 420 [3], 423 [4] and 425 [5]. Once appropriate doses have been chosen that can kill from 0% to 100% of animals when administered to a limited number of animals, LD_{50} and other LD values can be calculated by various graphic or mathematical methods to include: Kärber [6], Bliss [7], Miller and Tainter [8], Finney [9], Litchfield and Wilcoxon [10], Thompson and Weil [11], Weil [12], Berkson [13], Bruce [14], Dixon [15] and Gad [16].

In a previous paper results obtained with the Miller-Tainter [8] and Finney [9] methods for the calculation of LD_{50} and other LD values (LD_0, LD_{16}, LD_{84} and LD_{100}) were compared using the data for the estimation of LD_{50} of thymoquinone given intraperitoneally [17] as an example. The obtained results were very similar showing no statistically significant difference, P>> 0.05 [18].

In the present paper, using the data of the same example for the determination of LD_{50} and other LD values for thymoquinone, the detailed algorithms and execution of the computer programs in MATLAB, "LD_{50}-mortality-Miller-Tainter" and "LD_{50}-mortality-Finney" , are presented, which have been written on the basis of methods of Miller-Tainter [8] and Finney [9], respectively. The results of LD_{50} and other LD values obtained with these programs have been compared with those calculated with the method of Litchfield and Wilcoxon [10] (according to the algorythm by Blazka and Hayes [19]). The methods of Miller-Tainter [8] and Finney [9] are 'Probit Methods', while the method of Litchfield-Wilcoxon [10] is a 'Graphic Method' and has been frequently used for the calculation of LD_{50} values once suitable doses have been determined for the quantal dose response curve after the application of OECD guidelines, particularly OECD 425, based on Up-and-Down proceedure, originally proposed by Bruce [14]. Moreover, with all three methods, the LD_{50} values are presented as estimated doses (mg/kg) with confidence limits.

It is hoped that the present work will help investigators to calculate LD_{50} and other LD values of compounds by the 'Probit Methods' of Miller-Tainter and Finney more conveniently.

MATERIALS AND METHODS

Experimental animals

The data for the estimation of LD_{50} of thymoquinone given intraperitoneally to rats [17] was used as an example. In this example, approximate LD_{50} was initially determined by a 'staircase method' using a small number of animals, 2 each dose, starting from usual effective dose reported in the literature and doubling the dose till 100% mortality. Then 5 doses were selected for the determination of LD_{50} starting from zero to 100% deaths and given intraperitoneally to 5 groups of rats, 10 in each group. The animals were observed for first 2 hours and then at 6th and 24th hour for any toxic symptoms. After 24 hours, the number of deceased rats was counted in each group and percentage of mortality calculated.

Computer programs

Programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter" are written in MATLAB [20]. Programs were executed using the 7.13 version (MATLAB® & Simulink® Installation Guide, R2011b, online only, the MathWorks®, Inc). Detailed program algorithms are presented in the original form (the command text has characteristic, automatically generated colors for the default mode of work).

The program "LD_{50}-mortality-Finney" has been originated by modification of the interactive program "LD_{50}-mortality" written in program language BASIC for execution on a Commodore-64 computer [21]. Modification was done to enable the use of the program on PCs. Modification required significant changes to program commands because of the specificity of differences in syntax and commands between BASIC and MATLAB (see execution of the program "LD_{50}-mortality" [21].

The program "LD_{50}-mortality-Miller-Tainter" has been originated by modification of the interactive program "LD_{50}-mortality-Finney". Modifications are caused by differences between the methods reflected in the sequence of processing of obtained experimental results for mortality. As a result of these differences, initial values for pairs *X* (logarithm for concentra tion) and *Y* (mortality [probits]) for regression analysis differ (Table 1).

THYMOQUINONE CONCENTRATION (mg/kg)	LOG. CONCENTRATION (X)	MORTALITY				
		ANIMALS	FINNEY'S METHOD		MILLER-TAINTER'S METHOD	
			%	PROBIT (Y)	%	PROBIT (Y)
25	1.398	0/10	0	2.60 *	2.5 corrected **	3.04
50	1.699	4/10	40	4.75	40	4.75
75	1.875	7/10	70	5.52	70	5.52
100	2.000	9/10	90	6.28	90	6.28
150	2.176	10/10	100	7.40 *	97.5 corrected **	6.96

Table 1. Comparative presentation of the initial data (shaded) for regression analysis according to the methods of Finney and Miller-Tainter. (*) Probits values for mortality of 0% and 100% according to the number of animals (N=10). (**) Equations for corrected % values for mortality 0% and 100% were presented in Randhawa [17].

Mortality trends, as a function of thymoquinone concentrations, were compared using a modified one-way analysis of variance for comparing linear regression [22].

Presentation of the detailed algorhytm and execution of the interactive program "LD_{50}-mortality-Finney"

```
%calculation of LD50 according to the mortality (Finney, 1971)
clear,clc
disp('"CALCULATION OF LD50 ACCORDING TO THE MORTALITY, FINNEY (1971)"')
fprintf('\n');
disp('SAME NUMBER OF ANIMALS IN DOSES ')
fprintf('\n');
N=input(' enter number of doses (concentrations) =');
W=input(' number of animals in dose (concentration) =');
X=input(' enter values of dose (concentration) =');
Y=input (' enter values of mortality in dose (concentration) =');
Y5=(Y/W)*100;
fprintf('\n');
disp('    conc.                mortality          log.conc.              mortality (%)')
fprintf('\n');fprintf('%8g %10g %15g %15g\n',[X;Y;loglO(X);Y5])
fprintf('\n');
Zl=input('enter tabelar values of probit for the relevant value of mortality (in %) = ') ;
fprintf('\n');
fprintf ('pairs of (X (log.conc.),Y (mortality in probits) for regression analisys\n')
fprintf('\n');
disp('          log.conc.              mortality ')
disp('                    (in probits tabel.)')
fprintf('\n');fprintf('%15g %20g\n',[loglO(X);Zl])
% regression analisys
SX=sum(loglO(X));SXX=dot(loglO(X),loglO(X));
SY=sum(Zl);SYY=dot(Zl,Zl);
SXY=dot(loglO(X),Zl);
fprintf('\n');fprintf('\n');
fprintf('number of samples =');disp(N);
fprintf('sum of X =');disp(SX);
fprintf('sum of X*X =');disp(SXX);
fprintf('sum of Y =');disp(SY);
fprintf('sum of Y*Y =');disp(SYY);
```

```
33 fprintf('sum of X*Y =');disp(SXY);
34 SXSY=SX*SY;SXSY1=SXSY/N;SXY1=SXY-SXSY1;
35 SX1=SX^2; SX2=SX1/N; SXX1=SXX-SX2 ;
36 A=SXY1/SXX1;
37 YS=SY/N;XS=SX/N;
38 fprintf('\n');fprintf('\n');
39 B=YS-(A*XS);
40 fprintf ('slope (a) =');disp(A);
41 fprintf('intercept (b)=');disp(B);
42 Zll=A*logl0(X)+B;
43 disp('regression equation ')
44 fprintf('Y (mortality [probit] = %g * log X %g',A,B);
45 fprintf ('\n');fprintf ('\n');
46 % calculation of the square error
47 KG=Z1-Z11;KGl=dot(KG,KG);
48 fprintf('square error =');disp(KG1);
49 % calculation of coefficient of correlation and T-tests
50 SY1=SY^2;NSXY=N*SXY;NSXY1=NSXY-SXSY;
51 NSXX=N*SXX;NSYY=N*SYY;
52 NSXX1=NSXX-SX1;NSYY1=NSYY-SY1;NSXSY=NSXX1*NSYY1;
53 NSXSYl=sqrt(NSXSY);R=NSXY1/NSXSY1;
54 T=abs(R)*sqrt(N-2)/sqrt(1-R*R);
55 fprintf('koefficient of correlation (r)=');disp(R);
56 fprintf('T (r) =');disp(T);
57 fprintf ('D.F. =');disp(N-2);
58 fprintf('\n');
59 disp('          log.conc.                mortality          mortality')
60 disp('                    (in probits tabel.) (in probits calc.)')
61 fprintf('\n');fprintf('%15g %15g %21g\n',[loglO(X);Zl;Zll])
62 fprintf('\n');
63 W10=input('enter tabelar value of weight factor W for relevant values of probit calc. = ') ;
64 fprintf('\n');
65 disp(' subscores ')
66 fprintf('\n');
67 K=sum(W*W10);Kl=sum(W*W10.*logl0(X));K2=sum(W*W10.*(loglO(X).*logl0(X)));
68 fprintf('sum (number        of animals *      weight)=');disp(K);
69 fprintf('sum (number        of animals *      weight * log.cone.)=');disp(Kl);
70 fprintf('sum (number        of animals *      weight * sguare of log.cone.)=');disp(K2);
71 % calculation of values LDO, LD16, LD50, LD84 and LD100
72 P3=(5.000-B)/A;
73 K5=K1/K;
74 K15=K2- ( (Kl)^2/K) ;
75 K25=l/(A^2);
76 K35=l/K;
77 K45=((P3-K5)^2)/K15;
78 K55=K25*(K35+K45);
79 K65=sqrt(K55);
80 K75=(10^P3)*2.30*K65;
81 % calculation of confidence limits
```

```
H5=10^(P3+(1.96*K65));
H15=10^(P3-(1.96*K65));
if Y5(1)==0
if Y5(N)==100
P1=10^((Zl(1)-B)/A) ;
P5=10^((Zl(N)-B)/A) ;
end
end
if Y5(1)==0
if Y5(N)<100
P1=10^((Zl(1)-B)/A) ;
fprintf('\n');
fprintf('number of animals in dose =');disp(W);
ZZl=input('correction values of probit for mortality 100% according to the number of animals =');
P5=10^((ZZ1-B)/A);
end
end
if Y5(1)>0
if Y5(N)==100
fprintf ('\n');
fprintf('number of animals in dose =');disp(W);
ZZ2=input('correction values of probit for mortality 0% according to the number of animals =');
P1=10^((ZZ2-B)/A);
P5=10^((Zl(N)-B)/A) ;
end
end
if Y5(1)>0
if Y5(N)<100
fprintf('\n') ;
fprintf('number of animals in dose =');disp(W);
ZZ2=input('correction values of probit for mortality 0% according to the number of animals =') ;
P1=10^( (ZZ2-B) /A) ;
fprintf('number of animals in dose =');disp(W);
ZZl=input('correction values of probit for mortality 100% according to the number of animals =');
P5=10^( (ZZ1-B) /A) ;
end
end
fprintf ('\n');
fprintf('LD0 =');disp(PI);
P2=10^((4.0055-B)/A);fprintf('LD16 =');disp(P2);
P31=10^(P3);fprintf('LD50 +/- S.E. (LD50) = %g +/- %g',P31,K75);
P4=10^((5.9945-B)/A);
fprintf ('\n');fprintf ('\n');fprintf ('LD84 =');disp(P4);
fprintf('LD100 =');disp(P5);
fprintf ('\n');
% calculation of CHI square value
disp('     mortality')
disp('       (in probit calc.)')
fprintf('\n');fprintf('%15g\n', [Zll])
```

```
131 fprintf ('\n');
132 U=input('enter tabelar value of mortality (in %) for relevant values of mortality (in probit calc.) = ');
133 fprintf('\n');
134 Q=(U*W)/100;
135 Q5=(Y-Q);
136 Q10=Q.*(1.—(U/100));
137 Q11=Q5.*Q5;
138 D=sum(Qll./Q10);
139 fprintf('value of CHI square =');disp(D);
140 fprintf('D.F. =');disp(N-2);
141 % plot of diagram
142 plot(loglO(X),Z1,1s1,loglO(X),Zll,'-r')
143 xlabel('log. of concentration ')
144 ylabel('mortality (in probits)')
145 grid on
```

Figure 1. presents the detailed algorithm of the interactive program "LD_{50}-mortality-Finney".

Program execution begins by data input: number of doses (concentration) and number of animals per dose (concentration). This is followed by inputing experimental values for concentrations and values for deaths per concentration (steps 7-10). Because of the specific MATLAB syntax, values for concentration, and values for deaths per concentration are input separately. Mentioned values, as well as all data having several values are input in medium brackets. Based on input data, the program calculates the logarithms of concentration and mortality (in %) per concentration and presents these values. Tabular values of probits for corresponding mortality values (in %) (steps 11-16), are entered afterwards.

Since the probit values for 0% and 100% are not final, it is necessary to enter, in order to calculate LD_0 and LD_{100}, the table probit values for 0% and 100% mortality, for the specified number of animals. Tables for transformation of percentage values (in this case mortality) to probit values are given in [17] (for mortality from 1-99%), and [18] (for mortality 0% and 100%, depending on the number of experimental animals). Tables for transformation of percent values from 0.1-99.99% to probit values are given in Finney [9, Table I].

The logarithm transformation of experimental data for concentration and transformation of mortality data (in %) to probit values linearizes obtained experimental data. Calculated values for the logarithm of concentration and input values for mortality (in probits) are starting value pairs for regression analysis: *X* (logarithm of concentration) and *Y* (mortality [probits]) (Table 1), while the mathematical model is a linear equation in the form of a logarithm function with a free member:

$$Y = a * \log X + b \qquad \text{Eq. 1}$$

The parameters (*b* is intercept on *Y* axis, *a* is slope of line) are determined based on the entered experimental data and are processed using the least squares method [23]. Based on the obtained values for *a* and *b*, program calculates the regression line which aproximates the experimental results and estimates the values for LD_0, LD_{16}, LD_{50}, LD_{84} and LD_{100}.

In order to calculate the standard error (S.E.) of the calculated LD_{50} value, the program presents the calculated values of concentrations, entered tabelar values of probit and the calculated probitcalc values.

This is needed to enter the relevant value of the specified weight factor (*W*) , for each probitcalc value. Table for transformation of probit values to weight factor (*W*) are given in Finney [9, Table II]. Based on these values, the program calculates and presents the following subscores values: a) Sum of (number of animals * weight) (Abb. *Snw*), b) Sum of (number of animals * weight * log. conc.) (Abb. *SnwlogX*) and c) Sum of (number of animals * weight * log. conc.^{2}) (Abb. *SnwlogX*^{2}).

Based on these values, program calculates the standard error (S.E.) of the calculated LD_{50} value using the following equations as the mathematical model [9]:

$$V(\log LD_i) = \frac{1}{a^2} * \left\{\frac{1}{Snw} + \frac{(\log LD_i - \bar{X})^2}{Snw\,(X-\bar{X})^2}\right\}$$ Eq. 2

$$S.E.(\log LD_i) = \sqrt{V(\log LD_i)}$$ Eq. 3

$$S.E.(LD_i) = LD_i * 2.30 * S.E.(\log LD_i)$$ Eq. 4

$$\bar{X} = \frac{SnwlogX}{Snw}$$ Eq.5

$$Snw\,(X - \bar{X})^2 = SnwlogX^2 - \frac{(SnwlogX)^2}{Snw}$$ Eq. 6

LD_i - different LD values (in this case LD_{50})

a – slope of regression line

In order to make the assessment of whether the calculated regression line represents the experimental results in a satisfactory way, CHI square value need to be calculated. In order to do that, the program present probitcalc values of mortality. The relevant table values of the expected mortality (in %) should be entered, for each probitcalc value (Table I, [9]). Based on these data, the program calculates and presents the CHI square value for the specific degree of freedom. At the end of executio of the program, program present the plot of diagram (Figure 2).

"CALCULATION OF LD50 ACCORDING TO THE MORTALITY, FINNEY (1971)"
SAME NUMBER OF ANIMALS IN DOSES
enter number of doses (concentrations) = 5
number of animals in dose (concentration) = 10
enter values of dose (concentration) = [25 50 75 100 150];
enter values of mortality in dose (concentration) = [0 4 7 9 10];

conc.	mortality	log.conc.	mortality (%)
25	0	1.39794	0
50	4	1.69897	40
75	7	1.87506	70
100	9	2	90
150	10	2.17609	100

enter tabelar values of probit for the relevant value of mortality (in %) = [2.60 4.75 5.52 6.28 7.40];
pairs of (X (log.conc.),Y (mortality in probits) for regression analisys

log.conc.	mortality (in probits tabel.)
1.39794	2.6
1.69897	4.75
1.87506	5.52
2	6.28
2.17609	7.4

number of samples = 5
sum of X = 9.1481
sum of X*X = 17.0920
sum of Y = 26.5500
sum of Y*Y = 153.9913

sum of X*Y = 50.7182

slope (a)= 6.0413
intercept (b) = -5.7432

regression equation
Y (mortality [probit] = 6.04127 * log X -5.74318
square error = 0.0707

koefficient of correlation (r) = 0.9973
T (r) = 23.4341
D.F. = 3

log.conc.	mortality (in probits tabel.)	mortality (in probits calc.)
1.39794	2.6	2.70215
1.69897	4.75	4.52075
1.87506	5.52	5.58457
2	6.28	6.33936
2.17609	7.4	7.40317

enter tabelar value of weight factor W for relevant values of probit calc. = [0.076 0.545 0.508 0.321 0.062];

subscores
sum (number of animals * weight) = 15.1200
sum (number of animals * weight * log.conc.) = 27.6163
sum (number of animals * weight * square of log.conc.) = 50.8531

LD0 = 24.0453
LD16 = 41.0848
LD50 +/- S.E. (LD50) = 60.0204 +/- 6.12137
LD84 = 87.6832
LD100 = 149.8188

mortality (in probit calc.)
2.70215
4.52075
5.58457
6.33936
7.40317

enter tabelar value of mortality (in %) for relevant values of mortality (in probit calc.) =[1.1 31.5 72.1 91.0 99.19];

value of CHI square = 0.5619
D.F. = 3

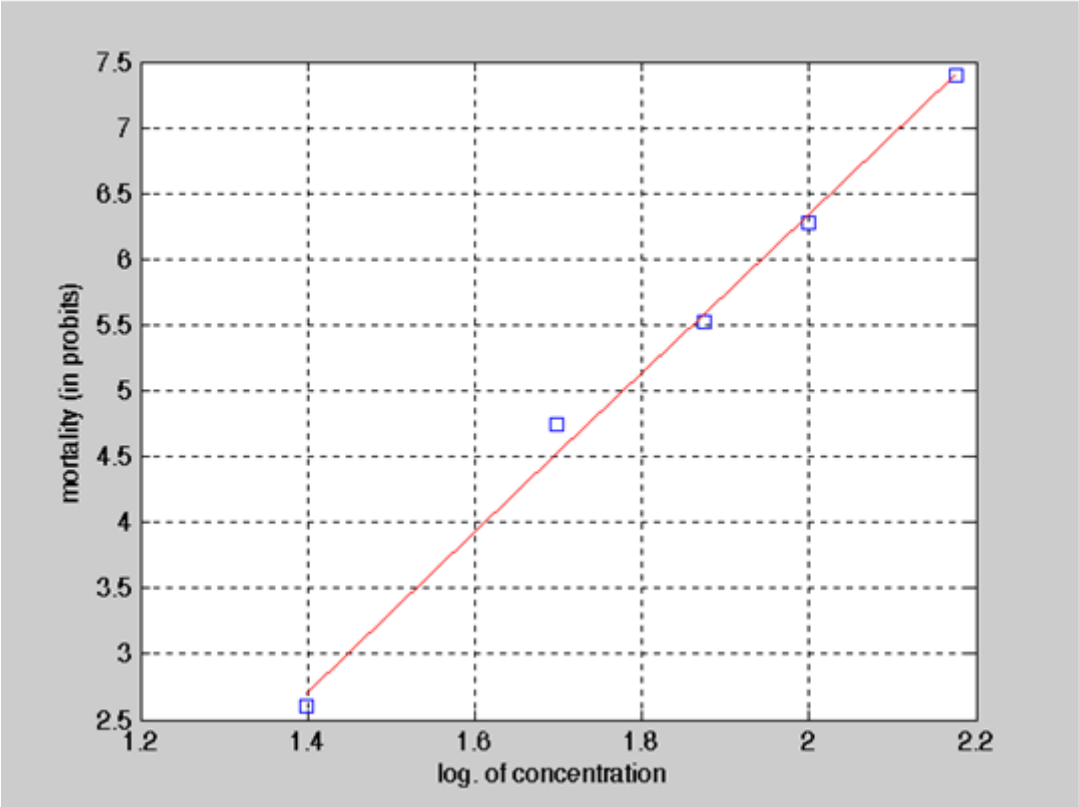

Figure 2. Execution of "LD_{50}-mortality-Finney" program on the test-example

Presentation of the detailed algorhytm and execution of the interactive program LD_{50}-mortality-Miller-Tainter"

```
%calculation of LD50 according to the mortality (Miller-Tainter, 1944)
clear,clc
disp('"CALCULATION OF LD50 ACCORDING TO THE MORTALITY, MILER-TAINTER (1944)"')
fprintf(' \n ' ) ;
disp('SAME NUMBER OF ANIMALS IN DOSES ')
fprintf('\n');
N=input(' enter number of doses (concentrations) =');
W=input(' number of animals in dose (concentration) =');
X=input(' enter values of dose (concentration) =');
Y=input(' enter vales of mortality in dose (concentration) =');
Y5=(Y/W)*100;
fprintf('\n');
disp('  conc.  mortality  log.conc.  mortality  (%)')
fprintf('\n');fprintf('%8g %10g %15g %15g\n',[X;Y;loglO(X);Y5])
fprintf('\n');
if Y5(1)>0
Y5(1)==Y5 (1);
if Y5(N)<100
Y5(N)==Y5(N);
end
```

```
end
if Y5(1)==0
Y5(1)=(0.25/W)*100;
end
if Y5(N)==100
Y5(N)=((W-0.25)/W)*100;
end
fprintf('\n');
disp('       corrected values of percent for mortality 0% and 100% or 0% only, or 100%  only according to the number of animals')
disp('       conc.    mortality        log.conc.          mortality          (%)')
fprintf('\n');fprintf('%8g %10g %15g %15g\n',[X;Y;loglO(X);Y5])
fprintf('\n');
Zl=input('enter tabelar values of probit for the relevant value of mortality (in %)= ');
fprintf('\n');
fprintf ('pairs of (X (log.conc.),Y (mortality in probits) for regression analisys \n')
fprintf('\n');
disp('       log.conc.          mortality ')
disp('       (in       probits  tabel.)')
fprintf('\n');fprintf('%15g %20g\n',[loglO(X);Zl])
% regression analisys
SX=sum(loglO(X));SXX=dot(loglO(X),loglO(X));
SY=sum(Zl);SYY=dot(Zl,Zl);
SXY=dot(loglO(X),Zl);
fprintf ('\n');fprintf ('\n');
fprintf('number of samples =');disp(N);
fprintf('sum of X =');disp(SX);
fprintf('sum of X*X =');disp(SXX);
fprintf('sum of Y =');disp(SY);
fprintf('sum of Y*Y =');disp(SYY);
fprintf('sum of X*Y =');disp(SXY);
SXSY=SX*SY; SXSY1=SXSY/N; SXY1=SXY-SXSY1 ;
SX1=SX^2; SX2=SX1/N; SXX1=SXX-SX2 ;
A=SXY1/SXX1;
YS=SY/N;XS=SX/N;
fprintf('\n');fprintf('\n');
B=YS-(A*XS);
fprintf('slope (a)=');disp(A);
fprintf ('intercept (b) = ');disp(B);
Zll=A*loglO(X)+B;
disp('regression eguation ')
fprintf('Y (mortality [probit] = %g * log X %g',A,B);
fprintf('\n');fprintf('\n');
```

```
% calculation of the square error
KG=Z1-Z11;KGl=dot(KG,KG);
fprintf('square error =');disp(KG1);
% calculation of coefficient of correlation and T-tests
SY1=SY^2;NSXY=N*SXY;NSXY1=NSXY-SXSY;
NSXX=N*SXX; NSYY=N*SYY;
NSXX1=NSXX-SX1;NSYY1=NSYY-SY1;NSXSY=NSXX1*NSYY1;
NSXSYl=sqrt(NSXSY);R=NSXY1/NSXSY1;
T=abs(R)*sqrt(N-2)/sqrt(1-R*R);
fprintf('koefficient of correlation (r)=');disp(R);
fprintf('T (r) =');disp(T);
fprintf('D.F. =');disp(N-2);
fprintf('\n');
disp('          log.conc.          mortality                mortality')
disp('                          (in probits tabel.) (in probits calc.)')
fprintf('\n');fprintf('%15g %15g %21g\n',[loglO(X);Zl;Zll])
fprintf('\n');
% calculation of values for LDO, LD16, LD50, LD84 and LD100
P0=10^ ((Zl(1)-B)/A) ;
P16=10^((4.0055-B)/A);
P50=(5.000-B)/A;P501=10^(P50);
P84=10^((5.9945-B)/A);
P100=10^((Zl(N)-B)/A) ;
SE1=P84-P16;SE2=sqrt(6*N);
SE4=SE1/SE2;
fprintf('\n');
fprintf('LDO =');disp(P0);
fprintf ('LD16 =');disp(PI6);
fprintf('LD50 +/- S.E. (LD50) = %g +/- %g',P501,SE4);
fprintf('\n');fprintf('\n');fprintf('LD84 =');disp(P84);
fprintf ('LD100 =');disp(P100);
fprintf('\n');
% calculation of CHI square value
disp('          mortality')
disp('       (in probit calc.)')
fprintf('\n');fprintf('%15g\n',[Zll])
fprintf('\n');
U=input ('enter tabelar value of mortality (in %) for relevant values of mortality (in probit calc.) =');
fprintf ('\n');
Q=(U*W)/100;
Q5=(Y-Q);
Q10=Q.*(1.-(U/100));
Q11=Q5.*Q5;
```

```
106 D=sum(Qll./Q10);
107 fprintf('value of CHI square =');disp(D);
108 fprintf('D.F. =');disp(N-2);
109 % plot of diagram
110 plot(loglO(X),Z1,'s',logl0(X),Zll,'-r')
111 xlabel('log. of concentration ')
112 ylabel('mortality (in probits)')
113  grid on
```

Figure 3. presents the detailed algorithm of the interactive program "LD_{50}-mortality-Miller-Tainter".

The program algorithm and its execution are similar to the algorithm and execution of the program "LD_{50}-mortality-Finney" and differences resulting from the differences between the methods will be presented. Based on input data (number of concentrations (doses), number of animals per dose and animal mortality by doses), the program calculates logarithms of concentration and mortality (in %) by concentration, and presents these values. If mortality for the lowest dose is 0% and/or for the highest dose 100%, the program previously corrects percentage values for mortality 0% and/or 100%, in relation to the number of experimental animals. Correction is done using following formulas:

for mortality 0% = 100* (0.25/N) Eq. 7
for mortality 100% = 100* (N-0.25/N) Eq.8

(N – number of experimental animals in the group [17]. After the correction of percentage values, the program presents values for the logarithm of concentration and corrected values for mortality (in %). Calculation of values for the logarithm of concentration and input of tabular values of probits for corresponding values for mortality (in %) linearize obtained experimental data representing starting value pairs for analysis: *X* (logarithm of concentration) and *Y* (mortality [probits]) (Table 1), while the mathematical model is a linear equation in the form of a logarithm function with a free member.

$$Y = a * \log X + b \qquad \text{Eq. 1}$$

Further execution of the program "LD_{50}-mortality-Miller-Tainter", using mentioned equations (eqs. 2-6), as mathematical models, is identical to the execution of the program "LD_{50}-mortality-Finney". At the end of executio of the program, program present the plot of diagram (Figure 4).

```
"CALCULATION OF LD50 ACCORDING TO THE MORTALITY, MILER-TAINTER (1944)"
SAME NUMBER OF ANIMALS IN DOSES

 enter number of doses (concentrations) = 5
 number of animals in dose (concentration) = 10
 enter values of dose (concentration) = [25  50  75  100  150];
 enter values of mortality  in dose (concentration) = [0  4  7  9  10];

 conc.    mortality    log.conc.      mortality (%)

   25          0        1.39794          0
   50          4        1.69897         40
   75          7        1.87506         70
  100          9        2               90
```

150	10	2.17609	100

corrected values of percent for mortality 0% and 100% or 0% only, or
100% only according to the number of animals

conc.	mortality	log.conc.	mortality (%)
25	0	1.39794	2.5
50	4	1.69897	40
75	7	1.87506	70
100	9	2	90
150	10	2.17609	97.5

enter tabelar values of probit for the relevant value of mortality (in %) = [3.04 4.75 5.52 6.28 6.96];

pairs of (X (log.conc.),Y (mortality in probits) for regression analisys

log.conc.	mortality (in probits tabel.)
1.39794	3.04
1.69897	4.75
1.87506	5.52
2	6.28
2.17609	6.96

number of samples = 5
sum of X = 9.1481
sum of X*X = 17.0920
sum of Y = 26.5500
sum of Y*Y = 150.1545
sum of X*Y = 50.3758

slope (a)= 5.0756
intercept (b)= -3.9764

regression equation
Y (mortality [probit] = 5.07558 * log X -3.97635
square error = 0.0402

koefficient of correlation (r) = 0.9978
T (r) = 26.1240
D.F. = 3

log.conc.	mortality (in probits tabel.)	mortality (in probits calc.)
1.39794	3.04	3.11901
1.69897	4.75	4.64691
1.87506	5.52	5.54068

2 6.28 6.17482
2.17609 6.96 7.06858

LD0 = 24.1198
LD16 = 37.3764
LD50 +/- S.E. (LD50) = 58.6862 +/- 9.99941
LD84 = 92.1454
LD100 = 142.7902

mortality
(in probit calc.)

3.11901
4.64691
5.54068
6.17482
7.06858

enter tabelar value of mortality (in %) for relevant values of mortality (in probit calc.) = [3.0 36.3 70.6 88.0 98.07];

value of CHI square = 0.6049
D.F. = 3

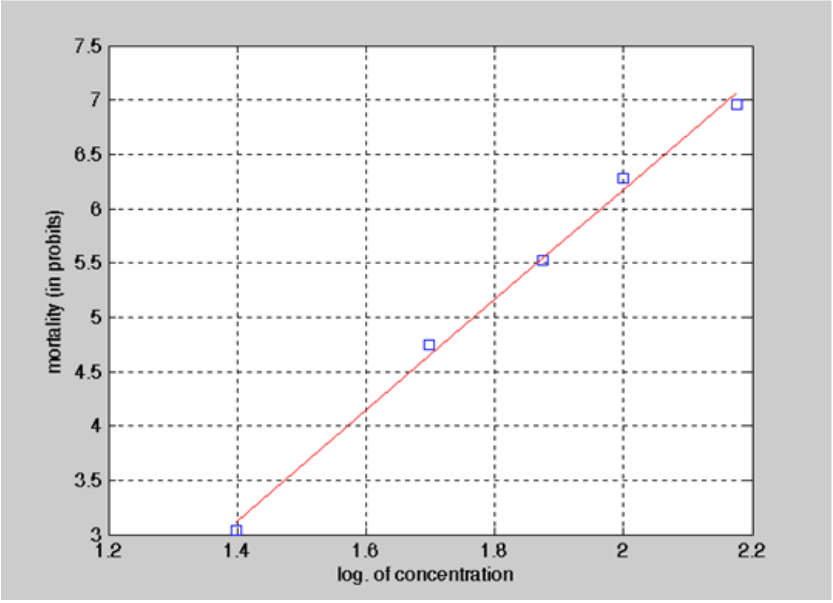

Figure 4. Execution of "LD_{50}-mortality-Miller-Tainter" program on the test-example

RESULTS

Validation of execution of the programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter" using the example of processing of the test-example of thymoquinone given intraperitoneally in rats are presented in Figure 2 and Figure 4, respectively.

T H Y M O Q U I N O N E (mg/kg)				
	TEST EXAMPLE (Randhawa, 2009) (Graphycal method, Miller-Tainter, 1944)	"LD_{50}-mortality-Finney" program	"LD_{50}-mortality-Miller-Tainter" program	Litchfield-Wilcoxon (1949) (algorytm by Blazka and Hayes, 2008)
LD_0	15.750	24.045	24.120	19.085 (extrapolation)
LD_{16}	37.970	41.085	37.376	36.208
$LD_{50} \pm$ S.E. (LD_{50})	57.54 ± 11.9	60.020 ± 6.121	58.686 ± 9.999	57.201 ± 8.107
LD_{84}	87.040	87.683	92.145	90.365
LD_{100}	152.580	149.819	142.790	171.356 (extrapolation)

Table 2 shows a comparative review of LD_0, LD_{16}, $LD_{50} \pm$S.E. (LD_{50}), LD_{84} and LD_{100} values obtained by pro cessing of the same data by applying the programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter", and the method of Litchfield-Wilcoxon.

LD_{50} and other LD values, obtained by processing the test-example of thymoquinone with the programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter", and the method of Litchfield-Wilcoxon were very similar, as is indicated by the comparative presentation of trends for mortality (Figure 5). Based on Fischer's coefficient (F) value, there is no statistically significant difference between trends of mortality as a function of thymoquinone doses ($P > 0.05$; $F_{exp} = 5.060 < F_{0.05}$ (d.f. 3;10) = 8.79).

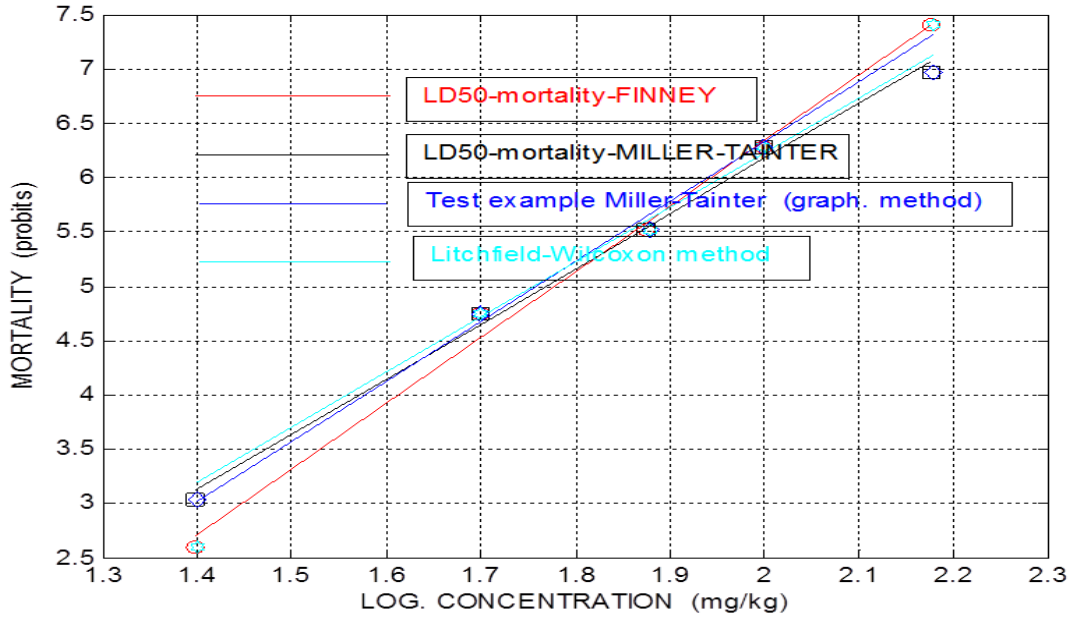

Figure 5. Comparative presentation of the trends of mortality as a function of concentrations of thymoquinone according to the Miller-Tainter graphycal method (test-example, Randhawa [17]) and programs "LD_{50}-mortality-Finney" and "LD_{50}- mortality-Miller-Tainter" and by the method of Litchfield- Wilcoxon

DISCUSSION

Methods commonly used for the calculation of LD_{50} value are graphic or mathematecal and are based on the assumption that the effect is a quantal one (all or none), is dose related and the cumulative effect is distributed normally. The LD_{50} values are presented as estimated doses (mg/kg) with confidence limits. As a measure of toxicity, LD_{50} is considered as somewhat unreliable and results may vary greatly between testing facilities due to factors such as the genetic characteristics of the sample population, animal species tested, environmental factors and mode of administration. However, when properly conducted in standard environmental conditions; a) yields not only the LD_{50} but also provide information on other acute effects such as cause of death, time of death, symptomatology, nonlethal acute effects, organs affected, and reversibility of nonlethal effects;

b) the results can form the basis for the design of subsequent subchronic studies; c) is useful as a first approximation of hazards to workers and d) is rapidly completed [24].

Recently, OECD has suggested guidlines for the determination of acute toxicity , to include: a fixed dose pro- cedure [3], an acute toxic class method [4] and an up-and-down procedure [5]. These guide lines are primarily aimed at reducing the number of animals and the duration of experiment for the estimation of acute toxicity. The up-and-down procedure [5] generates a point estimate of lethality and confidence intervals of the LD_{50} and therefore may be useful in a wider set of applications. The test employs sequential dosing, using only a single animal at each step (usually female rats), the dose is increased or decreased depending on whether the previously dosed animal lives or dies and can be used to evaluate lethality up to 5000 mg/kg. The main test incorporates elements of range finding and uses a flexible stopping point. A sequential limit test uses up to five animals at each dose level selected. Default dose spacing is 3.2 times the previous dose. The starting dose should be slightly below the estimated LD_{50}, judged from previously conducted *in vivo* or *in vitro* experiments. If no information is available to estimate the LD_{50}, the starting dose is 175 mg/kg [3]. Once the data of the number (or %) of animals died at each dose level of the final limit test is available a graphical or mathematical method can be employed for the calculation of LD_{50} with confidence intervals. Methods usually employed are those of Dixon's and Finney's; while other methods, like Miller-Tainter and Litchfield-Wilcoxon, can also be used [5].

In the example of thymoquinone given intraperitoneally in rats [17] 'staircase method' described by Ghosh [2] was used for the estimation of dose range for the determination of LD_{50}, which helped to reduce the number of animals. When the data of % animals died at each dose level selected for the final experiment was available the method of Miller and Tainter [8] was employed for the calculation of LD_{50} with confidence Intervals.

In the present manuscript, LD_{50} and other LD values, obtained by processing the test-example of thymoquinone with the programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter", and the method of Litchfield-Wilcoxon were compared with each other and found to be very similar (Figure 5) and had statistically non-significant differences between trends of mortality as a function of thymoquinone doses, as estimated by Fischer's coefficient (F) value ($P > 0.05$; $F_{exp} = 5.060 < F_{0.05}$ (d.f. 3;10) = 8.79). Therefore, it is suggested that comparable results can be achieved for the estimation of LD_{50} and other LD values with the method of Miller-Tainter [8], Finney [9]; or Litchfield-Wilcoxon [10], once appropriate doses have been chosen by the adaptation of OECD guidelines or staircase method. Moreover, it is hoped that the programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter" will help researchers for prompt calculation of LD_{50} and other LD values.

CONCLUSIONS

In the present article interactive computer programs for computing LD_{50} and other LD values using Finney method (Program 'LD_{50}-mortality-Finney') and Miller-Tainter method (Program 'LD_{50}-mortality-Miller-Tainter'), written in MATLAB are presented. LD_{50} and other LD values, obtained by processing the test example of thymoquinone given intraperitoneally to rats with programs "LD_{50}-mortality-Finney" and "LD_{50}-mortality-Miller-Tainter" and the method of Litchfield-Wilcoxon were found to be very similar and had statistically non-significant differences between trends of mortality as a function of thymoquinone doses. Therefore, comparable results can be achieved for the estimation of LD_{50} and other LD values with the method of Miller-Tainter, Finney, or Litchfield-Wilcoxon; once appropriate doses have been known by the application of OECD guidelines or other suitable procedures.

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