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Comparison of the Methods of Finney and Miller-Tainter for the Calculation of LD_{50} Values

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Abstract: Comparative results obtained with the Finney and Miller-Tainter methods for the calculation of LD_{50} of a compound are presented, using the example of the assessment of LD_{50} of thymoquinone following its intraperitoneal administration to rats. The obtained results are very similar showing no statistically significant difference ($P > 0.05$) in mortality trends between the methods of Finney and Miller-Tainter. Minor differences in mortality trends, therefore, in the obtained values for LD_0, LD_{16}, LD_{50} ±S.E.(LD_{50}), LD_{84} and LD_{100} are the result of slight methodological differences reflected in the order of processing of the obtained mortality experimental results. The method of Finney is used to transform the obtained mortality results (in %) into probit values, where the probit values for 0% and 100% mortality depend on the number of experimental animals per group, which are then further processed. The Miller-Tainter method also transforms mortality results (in %) into probit values but first, the percentage values are corrected against the number of experimental animals, if mortality of the lowest and/or the highest dose is 0% and/or 100%, respectively; such corrected values are then transformed into probit values that are used for further processing. Therefore, the differences were relatively more around LD_0 and LD_{100} and very negligible around LD_{50} values, usually reported. To conclude the methos of Finney and Miller-Tainter give similar results for the determination of LD_{50} of a compound.

Key words: Acute Toxicity · LD_{50} · Probit Analysis · Finney Method · Miller-Tainter Method · Thymoquinone · Interactive Computer Programs · Matlab

INTRODUCTION

The assessment of LD_{50}, as a measure of toxicity of the tested product, can be conducted by the assessment of a mathematical model that best describes the experimental results (X – different concentrations of the testes product and Y – experimental animal mortality (%) at certain concentration of the tested product (Regression analysis). The size of the square error is used for the selection criterion of the most suitable mathematical model [1]. The values for parameters of the mathematical model that best describes experimental data are used to establish, by interpolation, the value for independent variable X (LD_{50}) at which the dependent variable Y is 50% [2].

As a modification of the regression analysis, the probit analysis is used for linear expression of experimental data by logarhythmic transformation of the independent variable X, this transforming the value for dependent variable Y (%) into probit values [3]. The regression and probit analyses have an important advantage over other LD_{50} calculation methods consisting in that the inter-dose interval of the tested product and the number of experimental animals per experimental dose need not be the same. Similar to those methods are the Litchfield and Wilcoxon [4] and Miller and Tainter methods [5].

The Miller and Tainter [5] method is particularly interesting because it also leads to experimental data linearization by logarhythmic transformation of the

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independent variable *X*, transforming the value for dependent variable *Y* (%) into probit values. Randhawa [6] presents the detailed procedure for Miller-Tainter method implementation using the example of the assessment of LD_{50} of thymoquinone following intraperitoneal administration to rats. Contrary to those, the Kärber [7], Behrens [8] and Behrens and Schlosser [9] methods require, in order to be implemented, that the inter dose interval of the tested product and the number of experimental animals per experimental dose, are identical.

In this paper, the authors provided comparative presentation of the results obtained by the Finney [3] and Miller and Tainter [5] methods implemented in order to assess the LD_{50} of thymoquinone following its intraperitoneal administration to rats.

MATERIAL AND METHODS

The experiments were conducted on rats divided into 10-animal groups. The thymoquinone was administered in intraperitoneal doses of 25, 50, 75, 100 and 150 mg/kg. The animals were observed during the period of 2 hours and at 6 and 24 hours from administration. Mortality (%) was calculated after 24 hours [6]. The results were processed using the interactive LD_{50} calculation programs according to Finney [10] (The current BASIC software was modified for PC performance) and Miller-Tainter. The softwares were written in the MatLab software language. The assessments were made for LD_0, LD_{16}, LD_{50} ±S.E. (LD_{50}), LD_{84} and LD_{100}. Mortality trends, as a function of thymoquinone concentrations, were compared by comparing the linear correlation coefficient [11].

RESULTS AND DISCUSSION

Table 1 shows comparative presentation of the values for LD_0, LD_{16}, LD_{50} ±S.E. (LD_{50}), LD_{84} and LD_{100} of thymoquinone following peritoneal administration to rats, obtained by processing of those experimental results using the methods of Finney and Miller-Tainter. The obtained differences are the consequence of methodological differences between the methods reflected in the order of processing of the obtained experimental mortality results. Both methods are associated with the linearization of the experimental results achieved by logarhythmic transformation of the independent variable *X*, the dependent variable *Y* (%), being, according to Finney, transformed into probit values, where the probit mortality values of 0% and 100% depend on the number of experimental animals per group and are read from the table (Table 2). According to Miller-Tainter, the values for independent variable *Y* (%) are transformed into probit values only after the correction of the percentage value against the number of experimental animals, if mortality result of lowest and highest dose is 0% and/or 100%, respectively. Such correction is made using the formulas:

for 0% mortality: 100* (0.25/N) (1)

for 100% mortality: 100* (N-0.25/N) (2)

Where 'N' is the number of experimental animals per group [6]. The methodological differences result in different starting values for independent variable *Y* for regression analysis (Table 3) resulting, in turn, in different values for regression equation parameters (slope and intercept) (Table 4). According to the Goset (Student) T test values, there is no statistically significant difference as a function of thymoquinone concentration ($P > 0.05$; $T_{exp.} = 0.001 << T_{0.05}$ (d.f. 4) = 2.776) (Figure 1). The logaryhthmic transformation of the independent variable *X* and the calculation of the probits of dependent variable *Y* allow for the application of the methods for a wider reange of values and the presentation of the sample for a population.

CONCLUSIONS

The differences between methos of Finney and Miller-Tainter were rlatively more around LD_0 and LD_{100} and very negligible around LD_{50} values, usually reported. Both methods give similar results for the determination of LD_{50} of a compound.

Table 1: Comparative presentation of LD_0, LD_{16}, LD_{50}±S.E. (LD_{50}), LD_{84} and LD_{100} values obtained by the methods of Finney and Miller-Tainter.

THYMOQUINONE (mg/kg)	FINNEY'S METHOD	MILLER-TAINTER'S METHOD
LD_0	24.045	24.120
LD_{16}	41.085	37.376
LD_{50} ± S.E (LD_{50})	60.020 ± 6.121	58.686 ± 9.999
LD_{84}	87.683	92.145
LD_{100}	149.819	142.790

Table 2: Values of probits for 0% and 100% according to the number of biological objects

	Values of Probits for 0% and 100%				
	Probits			Probits	
Number of Biological Objects (N)	0%	100%	Number of Biological Objects (N)	0%	100%
1	3.36	6.64	18	2.41	7.59
2	3.13	6.87	20	2.38	7.62
3	2.99	7.01	24	2.32	7.68
4	2.90	7.10	25	2.31	7.69
5	2.82	7.18	30	2.26	7.74
6	2.76	7.24	40	2.17	7.83
7	2.71	7.29	50	2.10	7.90
8	2.67	7.33	60	2.05	7.95
9	2.63	7.37	70	2.01	7.99
10	2.60	7.40	80	1.97	8.03
12	2.54	7.46	90	1.93	8.07
15	2.47	7.53	100	1.90	8.10

Table 3: 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 (10). (**) Equations for corrected % values for mortality 0% and 100% were presented in Randhawa [6].

		Mortality				
			Finney's Method		Miller-Tainter's Method	
Thymoquinone concentration (mg/kg)	**Log. Concentration (X)**	Animals	%	**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 4: Comparative presentation of the parameters of regression equations described mortality according to the methods of Finney and Miller-Tainter.

	Regressions of Mortality Y (mortality) [probit] = a * log X + b						
	Slope (a)	Intercept (b)	Square error	Coefficient correlation (r)	Student T test of (r)	D.F. of (r) (N-2)	P for (r)
FINNEY	6.04127	-5.74318	0.0707	0.9973	23.4341	3	<< 0.001
MILLER-TAINTER	5.07558	-3.97635	0.0402	0.9978	26.1240	3	<< 0.001

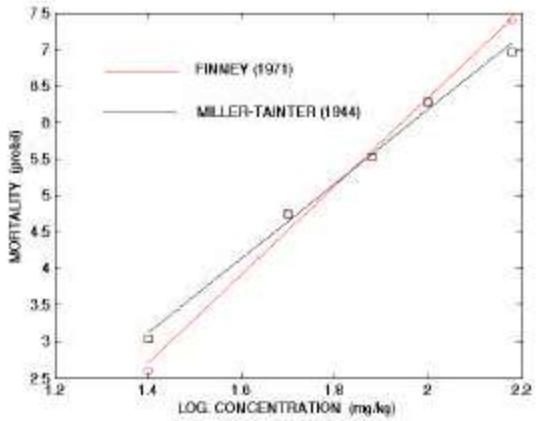

Fig. 1: Comparative presentation of the regressions of mortality as a function of thymoquinone concentrations according to the methods of Finney and Miller-Tainter.

REFERENCES

1. Arambašić, M., D. Ristanović and A. Kalauzi, 1988. The comparison of some empirical functions of growth using pond snail Lymnaea stagnalis L. as an example. Biom. J., 30(8): 975 -983.
2. Arambašić, M.B., S. Kondić, L.J. Pitić and M. Stojanović, 1991. Review of some mathematical statistical methods for processing toxicological-pharmacological experimental results. Acta Pharm. Jugosl., 41(3): 177-190.
3. Finney, D.J., 1971. Probit Analysis, 3rd ed., University Press, Cambridge.
4. Litchfield, J.T. and F.J. Wilcoxon, 1949. Simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 96: 99-113.
5. Miller, C.L. and M.L. Tainter, 1944. Estimation of LD_{50} and its error by means of log-probit graph paper. Proc. Soc. Exp. Biol. Med., 57: 261-264.
6. Randhawa, M.A., 2009. Calculation of LD50 values from the method of Miller and Tainter, 1944. J. Ayub. Med. Coll. Abbottabad, 21(3): 184-185.
7. Kärber, G., 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmakol., 162: 480-483.
8. Behrens, B., 1929. Zur Auswertung der Digitalisblätter im Froschversuch. Arch. Exp. Path. Pharmakol., 140: 237-256.
9. Behrens, B. and L. Schlosser, 1957. Beitrag zur Bestimmung der LD_{50} und der Berechnung ihrer Fehlerbreite. Arch. Exp. Path. Pharmakol., 230: 59-72.
10. Arambašić, M.B., L.J. Pitić, D. Jeremić and D. Adnađević, 2002. Applicational possibilities of the application of regression analysis and analysis of variance.II. Assessment and comparison of acute toxicity: Presentation and practical application of interactive computer programm "LD_{50}-mortality". Boll. Chim. Farmaceutico, 141(4): 290-298.
11. Petz, B., 1981. Razlika između dva koeficijenta korelacije r. In: Osnovne statističke metode za nematematičare. SNL, Zagreb, pp: 218-219.