NUMERICAL COMPARISONS OF BIOASSAY METHODS IN ESTIMATING LC50

by

TIANHONG ZHOU

B.S., China Agricultural University, 2003
M.S., China Agricultural University, 2006

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Statistics
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2010

Approved by:

Major Professor
Weixing Song

Abstract

The potency of a pesticide or some materials is widely studied in agricultural and biological fields. The level of a stimulus that results in a response by 50% of individuals in a population under study is an important characterizing parameter and it is denoted by the median lethal concentration (LC50) or the median lethal dose (LD50) or median. Estimation of LC50 is a type of quantal response assays that belong to qualitative indirect bioassays. In this report, seven methods of estimating LC50 are reviewed with reference to two normal distributions of tolerance in four different cases. Some modified methods are also discussed. Simulation shows that the maximum likelihood method generally outperforms all other traditional methods, if the true tolerance distribution is available. The comparison results indicate that the modified Dragstedt-Behrens method and modified Reed-Muench method are good substitutes for the original ones in most scenarios.

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Acknowledgements

I would like to take this opportunity to thank all the people who have supported my study, inspired me on my research, and given me their selfless friendship in my Master study in the Department of Statistics at Kansas State University.

First of all, I would like to express my deepest gratitude to my major professor Dr. Weixing Song for his great support, warm encouragement, invaluable comments and patient guidance of my study. He was always there to help me to design and discuss my report, revise the manuscripts repeatedly. Without his guidance and persistent help this dissertation would not have been possible.

I would express my special gratitude to Dr. John Boyer and Dr. Weixin Yao for their continuous support and guidance throughout my Master study and their valuable time serving as my graduate committee. My appreciation also goes to Dr. Leigh Murray, Dr. Paul Nelson, Dr. James Neill, Dr. Dallas Johnson, Dr. Abigail Jager and Dr. Gary Gadbury for their great help with my courses when I was pregnant.

I would like to thank Dr. Guihua Bai for his great support and help during my graduate study. I thank Dr. Amy Bernardo for her patient guidance in the KSU DNA Sequencing and Genotyping Facility.

I also want to acknowledge all my former classmates Lei Dong, Lianqing Zheng, Xiuqin Bai, Hyounojin Jun, Lisi Xue and Dalin Zhu for their help, cooperation, and friendship. I will always remember the friendship and support from staff and secretaries in the Department of Statistics.

Finally, I thank my parents, Xiaonian Guo and Jianjun Zhou, for their infinite love bestowed upon me. Furthermore, this work is dedicated to my beloved husband Xin Deng and my adorable son Renfu Deng, for their love, support, and understanding. They have been always the motivational force in my life.

CHAPTER 1 - Introduction

1.1 Bioassay

Finney (1978) defined a biological assay as "an experiment for estimating the nature, constitution, or potency of a material (or of a process), by means of the reaction that follows its application to living matter." A variety of procedures are developed in bioassay to estimate the potency. In those procedures, the amount or strength of an agent (usually is a drug) or stimulus is determined by a response (particular characteristic changes such as death) of a subject (usually is an animal or animal tissue). According to Webster's International Dictionary, bioassay is defined as "the estimation of the strength of a drug, etc., by comparing its effect on biological material, as animals or animal tissue, with those of a standard product." For example, ρX_t units of drug perform as X_s units of the standard. When $\rho = 2$, it means one unit of the test drug is equivalent to 2 units of the standard with respect to biological activity.

Bioassay includes the stochastic assay and the non-stochastic assay. For the stochastic assay, ρ is affected by other factors besides the preparations. It's very difficult to control those factors. Non-stochastic assays assume that ρ is a constant that is independent of the subject. For the majority of cases, the preparations are influenced by multiple factors such as species of the animal and environmental differences. So biometricians always talk about the stochastic assay.

The stochastic assay can be further classified into two categories: direct bioassay and indirect bioassay. In direct assays, the amount of stimulus is measured by holding the response fixed. In this situation, the dose is not the response but the variable of interest. However, the most common assay is indirect assay in which people observe a variable related to an event.

Indirect assay includes qualitative assay and quantitative assay. In qualitative assay, response is always some desired symptoms shown by the subjects, such as death, certain heart rate, and so on. The number of responses is the variable of interest. In quantitative indirect assays, both the response and the level of dosage are variables of interest.

1.2 LC50

Quantal assays belong to the qualitative indirect bioassays. In quantal response assays, a stimulus (e.g., dose of a drug) is applied to n experimental units, then there will be r units that are responding (e.g., death) and $n - r$ that are not. Through these assays, we can obtain information about the amount of stimulus that produces a response. The quantal response assays are used to estimate the tolerance of individual, which refers as the individual effective dose (IED). The result will be the level of a stimulus corresponding to a particular response. A very important parameter, median lethal concentration (LC50), is the level of a stimulus that causes a response of 50% of individuals. There are some similar parameters such as median lethal dose (LD50), median effective dose (ED50) and median effective concentration (EC50). We'll concentrate on LC50 in the sequel.

Why is LC50 so important? Why not use the minimal lethal dose under which all individuals response or the maximum lethal dose under which no one responses? Theoretically, these two definitions seem to be reasonable, but practically extreme members always exist in most samples no matter how many experimental units they have. Hence, the 100% or zero of lethal dose won't be accurate. In some cases, LC20, LC90, etc. are also used, but LC50 is usually estimated more precisely.

1.3 Methods of estimating LC 50

In the following chapters, we will review and compare seven classical methods of estimating LC50:

(1). Dragster-Behrens method

(2). Spearman-Kärber method

(3). Reed-Muench method

(4). Thompson moving average method

(5). Shuster-Dietrich method

(6). Shuster-Yang method

(7). Maximum Likelihood method

In all the simulations conducted in this report, the dose level is chosen to equally spaced log dose levels, which are $10^{0.1}$, $10^{0.2}$, $10^{0.3}$, $10^{0.4}$, $10^{0.5}$, $10^{0.6}$, $10^{0.7}$, and unequally spaced dose levels, which are 1, 2, 3, 3.5, 5, 7 and 9. The measurement units of these dose levels are deliberately omitted for the sake of generality. When it comes to the number of subjects at each dose level, we select two cases: equal numbers of subjects at each dose level (n = 10, 20, 30, 40, 50), and unequal numbers of subjects at each dose level (n is generated randomly based on $n = m \pm j$, where m =10, 20, 30, 40, 50, and j is a random integer uniformly generated from 1, 2 and 3). Therefore, four different experimental scenarios will be applied to each LC50 estimation method mentioned above. The point estimates, together with their mean square errors (MSE), and confidence intervals, if any, in some cases from each method, so one can have a better idea about the goodness of these classical estimation methods. In the simulation studies, we use normal, Cauchy and logistic as the tolerance distribution. In each simulation, we repeat estimation 500 times, then the mean and MSE are calculated by 500 LC50s. Furthermore, in some simulation studies, we calculate the coverage rate (number of true LC50s in the 95% CI/500) and length of CI.

Note that the first six methods are nonparametric ones, we will not compare the goodness of them with the tolerance distribution, but the simulation results will be still present in this report.

1.4 Other Methods of estimating LC 50

There are still some methods that we won't discuss in this report such as the Dixon-Mood method (1948) and Litchfield-Wilcoxon method (1949). The Dixon-Mood method is also known as the "up-and-down method" because of its unique procedure. In the procedure, if the subject survives, the dose for the next one will be increased; if it dies, the dose will be decreased. This method needs much few experiment subjects than others, so it's very popular in many expensive and time-consuming experiments.

The Litchfield-Wilcoxon method uses a line drawn by eye to fit points for each dose and response data. This is a rapid graphic method for estimating LD50 and confidence limits. For

the Litchfield-Wilcoxon method, accuracy is a conspicuous merit. Finney (1971) states, "The results are often very close to those from maximum likelihood estimation".

In addition, many researchers including Ramsey (1972), Chmiel (1976), Freeman (1980), Davis (1972), R. Bhattacharya and M. Kong (2007), Hans-Georg Müller (1998), and Joan G. Staniswalis (1988) studied other methods that require more statistical background.

1.5 **Data Structure**

In all the simulation studies conducted below, we shall assume that the bioassay experiment produces the following data structure:

Dose Level (X)	Size (n)	Response (r)
x_1	n_1	r_1
x_2	n_2	r_2
…	…	…
x_k	n_k	r_k

where k is the number of dose levels used in the experiment, and X is the log-transformed dose level. Usually, x_i's are arranged in the ascending order. n_i is the number of subjects at each dose level. r_i is the number of response subject at each dose level.

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CHAPTER 2 - Methods of Estimating LC50

2.1 The Dragstedt-Behrens Method

Dragstedt-Behrens method was independently introduced by Dragstedt and Lang (1928) and Behrens (1929). The algorithm of Dradstedt-Behrens method is very simple and is very easy to use. However its numerical simplicity is considered to be the "only merit" and it has many limitations. For example, it "may behave reasonably well if the data are equally spaced in X have equal n at each X, and are moderately symmetric" (Finney 1971). Besides, Dragstedt-Behrens method has no sound theoretical basis.

2.1.1 Algorithm

The Dradstedt-Behrens method uses the following procedure to find out the estimate of LC50.

(1). At each dose level, calculate

$T_1(x) =$ Total of all values of r's for doses equal to or less than x,

$T_2(x) =$ Total of all values of $(n-r)$'s for doses equal to or greater than x,

and $p^*(x) = \dfrac{T_1(x)}{T_1(x) + T_2(x)}$.

(2). If there is a dose level, say x_i, such that $p^*(x_i) = 0.5$, then let $m = x_i$.

(3). If there is no value of p^* being equal to 0.5, then find the biggest dose level, say x_i, which p^* is less than 0.5. Then let

$$m = x_i + \frac{(x_{i+1} - x_i)(0.5 - p^*(x_i))}{p^*(x_{i+1}) - p^*(x_i)}.$$

(4). The LC50 is estimated by $LC50 = 10^m$.

2.1.2 Confidence Interval

If the X values are equally spaced and the numbers of subject in each dose level are the same, then a crude estimate of the standard error of m can be estimated by

$$SE(m) = \sqrt{\frac{0.79h \times IR}{n}},$$

where h is the difference between two adjacent dose levels, n is the number of subjectss in each dose level and IR is an estimate of the inter-quartile range of log(LC75) – log(LC25), which can be obtained by using similar algorithm as finding the LC50. Then a confidence interval of LC50 with confidence level 1- α can be constructed as

$$10^{m \pm z_{\alpha/2} SE(m)},$$

where $z_{\alpha/2}$ is the upper 100α/2-th percentile of the standard normal distribution.

2.1.3 Modification

(1). Non-equally spaced X or different number of subjectss at each dose level.
If the values of X are roughly equally spaced or the numbers of subjects at the chosen dose levels are roughly same, the above procedure still works. The algorithm to find LC50 stays unchanged. To construct the confidence interval using the above mentioned SE(m), one can use the following formulas to calculate h and n.

$$h = \frac{X_k - X_1}{k-1}, \text{ and } n = \frac{\sum_{i=1}^{k} n_i}{k}.$$

(2). Cubic-Fitting method of finding LC50.

The Dragstedt-Behrens method uses linear interpolation to find the estimation of LC50. It only uses the information from two points (x_i, $p*(x_i)$), and (x_{i+1}, $p*(x_{i+1})$), where x_i is the biggest dose level for which $p*(x_i) \le 0.5$. The following is a typical plot of $p*$ versus x.

The general pattern of the plot shows a sigmoid trend. Therefore, a cubic polynomial,

estimated from all data, can be used to find a better estimation of LC50. One can easily show that $p^*(x)$ is an increasing function of the dose level x.

Figure 2.1 plot of p* versus x, Dragstedt-Behrens method and Modified Dragstedt-Behrens method.
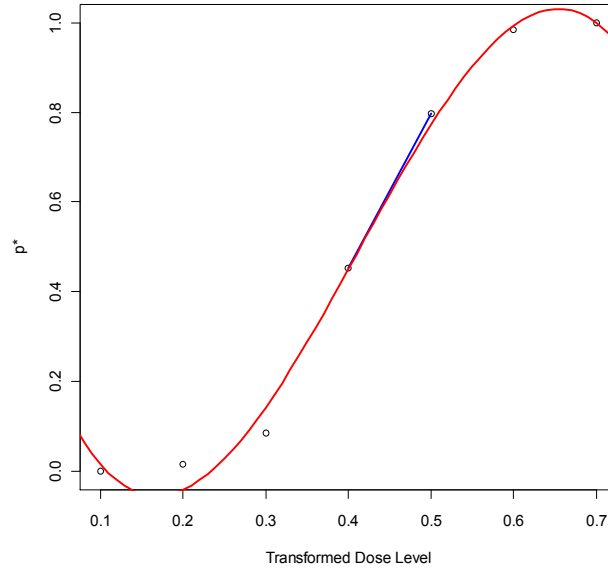
2.1.4 Simulation Study

Case 1. Equally spaced dose levels, same number of subjects at each dose level

Table 2.1 Dragstedt-Behrens Method, Case 1, normal distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10	2.8179	0.0379	0.968	1.3836	2.7727	0.0322
n=20	2.7920	0.0161	0.972	1.2512	2.7662	0.0158
n=30	2.7890	0.0104	0.986	1.1988	2.7618	0.0106
n=40	2.7892	0.0067	0.984	1.1680	2.7617	0.0073
n=50	2.7877	0.0059	0.986	1.1492	2.7597	0.0065

Table 2.2 Dragstedt-Behrens Method, Case 1, Cauchy distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10	2.7766	0.0563	0.966	1.4581	2.7263	0.0539
n=20	2.7506	0.0275	0.960	1.3033	2.7231	0.0282
n=30	2.7470	0.0195	0.948	1.2377	2.7160	0.0208
n=40	2.7458	0.0143	0.954	1.2033	2.7155	0.0161
n=50	2.7388	0.0125	0.956	1.1804	2.7137	0.0147

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.3 Dragstedt-Behrens Method, Case 2, normal distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10+j	2.7914	0.0392	0.968	1.3840	2.7522	0.0341
n=20+j	2.7996	0.0145	0.978	1.2458	2.7719	0.0142
n=30+j	2.7902	0.0095	0.986	1.1973	2.7613	0.0098
n=40+j	2.7906	0.0063	0.990	1.1691	2.7617	0.0070
n=50+j	2.7861	0.0063	0.984	1.1503	2.7581	0.0069

Table 2.4 Dragstedt-Behrens Method, Case 2, Cauchy distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10+j	2.7014	0.0668	0.948	1.4651	2.6528	0.0647
n=20+j	2.7659	0.0230	0.974	1.2960	2.7413	0.0256
n=30+j	2.7491	0.0171	0.966	1.2353	2.7222	0.0190
n=40+j	2.7423	0.0127	0.964	1.2038	2.7118	0.0157
n=50+j	2.7292	0.0137	0.946	1.1826	2.7056	0.0161

Case 3. Unequally spaced dose levels, same number of subjects at each dose level

Table 2.5 Dragstedt-Behrens Method, Case 3, normal distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10	4.1028	0.4093	0.914	1.7975	4.2918	0.2894
n=20	4.1246	0.2331	0.918	1.5073	4.2754	0.1478
n=30	4.1150	0.1805	0.884	1.3967	4.2596	0.1012
n=40	4.1177	0.1391	0.890	1.3362	4.2566	0.0744
n=50	4.1197	0.1257	0.864	1.2937	4.2546	0.0651

Table 2.6 Dragstedt-Behrens Method, Case 3, Cauchy distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10	3.9584	0.4703	0.930	1.8622	4.1578	0.3164
n=20	3.9696	0.3201	0.876	1.5494	4.1449	0.1857
n=30	3.9642	0.2728	0.808	1.4293	4.1311	0.1437
n=40	3.9651	0.2351	0.786	1.3626	4.1258	0.1186
n=50	3.9550	0.2363	0.730	1.3166	4.1212	0.1110

Case 4. Unequally spaced dose levels, different number of subjects at each dose level

Table 2.7 Dragstedt-Behrens Method, Case 4, normal distribution.

Sample Size	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=10+j	3.8968	0.4800	0.870	1.7948	4.0754	0.3173
n=20+j	4.1847	0.1934	0.944	1.5037	4.3324	0.1371
n=30+j	4.1207	0.1627	0.898	1.3934	4.2596	0.0949
n=40+j	4.1114	0.1351	0.902	1.3351	4.2406	0.0737

Sample Size	Mean	MSE	Coverage Rate	Length of CI	Mean	MSE
n=50+j	4.0807	0.1462	0.826	1.2989	4.2185	0.0727

Table 2.8 Dragstedt-Behrens Method, Case 4, Cauchy distribution.

	Dragstedt-Behrens Method				Cubic Polynomial Fitting	
Sample Size	**Mean**	**MSE**	**Coverage Rate**	**Length of CI**	**Mean**	**MSE**
n=10+j	3.7138	0.6480	0.884	1.8735	3.8938	0.4568
n=20+j	4.0620	0.2609	0.914	1.5452	4.2217	0.1608
n=30+j	3.9849	0.2528	0.832	1.4218	4.1430	0.1314
n=40+j	3.9607	0.2306	0.790	1.3618	4.1102	0.1220
n=50+j	3.9181	0.2627	0.660	1.3209	4.0866	0.1276

Conclusion: From above simulation study, when the tolerance distribution is normal, one can see that in the case of equally spaced dose levels, Dragstedt-Beherens method and the cubic polynomial fitting method provide similar results; in the case of unequally spaced dose levels, the cubic polynomial fitting method outperforms Dragstedt-Behrens method.

If the case of equally spaced dose levels, Dragstedt-Beherens method provides coverage rate which is slightly larger than the nominal level 95%, but it performs poorly in the case of unequally spaced dose levels, the coverage rate are all significantly smaller than the nominal level 95%, and more seriously, the empirical confidence levels are decreasing when the numbers of subject at each dose level increases.

When it comes to Cauchy distribution, the result is not as good as the normal distribution, which is not out of expectation. The Cauchy distribution is more "variable" than the normal distribution.

2.2 The Spearman-Kärber Method

The Spearman-Kärber method was first proposed by Spearman (1908) and independently

reintroduced by Kärber (1931). This method is very precise and particularly easy to use, so it is still very popular in many fields, such as analysis of psychometric functions. In some experimental conditions, it can provide more power to detect differences in parameters across. There are some methods that are quite easy to calculate, such as Reed-Muench method and Dragstedt-Behrens method, but Spearman-Kärber is easier and often markedly superior.

The Spearman-Kärber method obtains the estimate of m (m=log(LC50)). Although the method does not need equal spacing of doses level and equal replications, it has two requirements:1) the doses extend over the range from 0% to 100% response; 2) the response increasing rate between successive doses should concentrate on the center of the interval.

2.2.1 Algorithm

(1). Calculate

$$X_i = \log(r_i/n_i), \ p_i = r_i / n_i.$$

Let $p_1 = 0\%$ and $p_k = 100\%$.

(2). The estimation of $m = \log_{10}(\text{LD50})$ is

$$m = \sum_{i=1}^{k-1} \frac{(p_{i+1} - p_i)(x_i + x_{i+1})}{2}.$$

(3). In the case of equally spaced doses with $X_{i+i} - X_i = d$, the formula can be reduced to the simple expression:

$$m = x_k - d\left(\frac{1}{2} - \sum_{i=1}^{k} p_i\right).$$

(4). If the number of replications is also constant, then the m is

$$m = x_k - d\left(\frac{1}{2} - \frac{1}{n}\sum_{i=1}^{k} r_i\right).$$

2.2.2 Confidence Interval

The variance of m is obtained by replacing the variance of p_i with $p_i(1-p_i)$:

$$Var(m) = \sum_{i=2}^{k-1} \frac{p_i(1-p_i)(x_{i+1}-x_{i-1})^2}{4n_i},$$

$$SE(m) = \sqrt{Var(m)},$$

the 95% confidence interval of m is $m \pm z_{\alpha/2} SE(m)$, where $z_{\alpha/2}$ is the upper 100 $z_{\alpha/2}$ percentile of the standard normal distribution. Then, the 95% confidence interval of LC50 is $10^{m \pm z_{\alpha/2} SE(m)}$.

2.2.3 Simulation study

Case 1. Equally spaced dose levels, same number of subjects at each dose level

Table 2.9 Spearman-Kärber method, Case 1, normal distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	2.8114	0.0385	0.920	1.276
n=20	2.8054	0.0189	0.926	1.1937
n=30	2.8046	0.0126	0.944	1.1576
n=40	2.8043	0.0087	0.946	1.1357
n=50	2.8004	0.0074	0.944	1.1203

Table 2.10 Spearman-Kärber method, Case 1, Cauchy distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	2.8331	0.076	0.428	1.1127
n=20	2.8273	0.0355	0.368	1.0566
n=30	2.8242	0.0245	0.26	1.0379
n=40	2.8227	0.018	0.252	1.0284
n=50	2.8175	0.0148	0.172	1.0226

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.11 Spearman-Kärber method, Case 2, normal distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	2.8140	0.0413	0.914	1.2818
n=20+j	2.8044	0.0179	0.924	1.1906
n=30+j	2.8046	0.0120	0.934	1.1544
n=40+j	2.8059	0.0088	0.952	1.1357
n=50+j	2.8004	0.0076	0.946	1.1215

Table 2.12 Spearman-Kärber method, Case 2, Cauchy distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	2.8329	0.0774	0.452	1.1121
n=20+j	2.8268	0.0355	0.346	1.0567
n=30+j	2.8268	0.0243	0.264	1.0379
n=40+j	2.8234	0.0185	0.246	1.0284
n=50+j	2.8176	0.0151	0.188	1.0226

Case 3. Unequally spaced dose levels, same number of subjects at each dose level

Table 2.13 Spearman-Kärber method, Case 3, normal distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	3.9410	0.4000	0.848	1.5753
n=20	3.9368	0.2952	0.758	1.3927
n=30	3.9280	0.2629	0.666	1.3132
n=40	3.9192	0.2493	0.570	1.2684
n=50	3.9290	0.2332	0.522	1.2364

Table 2.14 Spearman-Kärber method, Case 3, Cauchy distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	3.7014	0.6491	0.188	1.1667
n=20	3.6955	0.5572	0.080	1.0826
n=30	3.6865	0.5279	0.016	1.0549
n=40	3.6752	0.5235	0.010	1.0412

n=50	3.6835	0.5042	0.004	1.0328

Case 4. Unequally spaced dose levels, different number of subjects at each dose level

Table 2.15 Spearman-Kärber method, Case 4, normal distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	3.9417	0.3743	0.842	1.5533
n=20+j	3.9384	0.2917	0.754	1.3886
n=30+j	3.9302	0.2584	0.678	1.3085
n=40+j	3.9203	0.2484	0.586	1.2692
n=50+j	3.9250	0.2354	0.516	1.2363

Table 2.16 Spearman-Kärber method, Case 4, Cauchy distribution.

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	3.7073	0.6345	0.196	1.1677
n=20+j	3.6984	0.5535	0.080	1.0827
n=30+j	3.6857	0.5261	0.014	1.0549
n=40+j	3.6748	0.5241	0.012	1.0412
n=50+j	3.6840	0.5023	0.006	1.0328

Conclusion: The Spearman-Kärber method provides smaller MSE when the dose levels are equal. In addition, in the case of equally spaced dose levels, the confidence intervals with empirical levels are almost the nominal level 95%, but they are significantly smaller than the nominal 95% when the space of log dose levels are not equal.

Note that when the tolerance distribution is Cauchy distribution, the coverage rate is much smaller than 95%. It is because the 95% confidence interval is related to limiting distribution, which needs that the second moment exists. However, Cauchy does not qualify this condition, so CI formula is not applicable to Cauchy distribution.

2.3 The Reed-Muench Method

Reed and Muench (1938) proposed "a simple method of estimating fifty per cent endpoints". It does not need large numbers tests at dilutions near the value for LD50, but use a wide range of possible variations. This method was very popular because of its numerical simplicity and no sound theoretical basis. Pittman and Lieberman (1948) showed evidence of the inferiority of this method relative to maximum likelihood. However, this method (as well as the extreme effective dose and Dragstedt-Behrens methods) is not recommended. They "ought never to be used" because their tests were not valid and less efficient then other simple methods. They should be forgotten, "except as part of statistical history" (Finney 1978). The Reed-Muench method assumes that any subject responding to a given dose of an agent would respond to all higher doses; and that any subject not responding to a given dose would not respond to a lower dose.

2.3.1 Algorithm

(1). Calculate

$T_1(x)$ = Total of all values of r's for doses equal to or less than x,

$T_2(x)$ = Total of all values of $(n-r)$'s for doses equal to or greater than x,

(2). If one of the doses used in the experiment has $T_1(x_i) = T_2(x_i)$, then let $m = x_i$.

(3). If there is no x_i, satisfying $T_1(x_i) = T_2(x_i)$,, then find x_i and x_{i+1} such that

$T_1(x_i) < T_2(x_i)$, $T_1(x_{i+1}) > T_2(x_{i+1})$.

(4). Using bi-linear interpolation to find out the x-coordinate of the intersection. m will be estimated by

$$m = x_i + \frac{(x_{i+1} - x_i)[T_2(x_i) - T_1(x_i)]}{T_i(x_{i+1}) - T_2(x_{i+1}) - T_1(x_i) + T_2(x_i)}.$$

(5). The LC50 is estimated by $LC50 = 10^m$.

2.3.2 Modification and Improvement

The Reed-Muench method only uses the information around the intersection. We expect a better estimator that will be obtained if it will involve all the information in the data set. For example, we can fit quadratic curves for $\{x_i, T_1(x_i)\}$, $i=1, 2, \ldots, k$, and $\{x_i, T_2(x_i)\}$, $i=1, 2, \ldots, k$, respectively. The x-coordinate of the intersection of these two quadratic curves can be used to estimate LC50. If the estimated curve from (x, T_1) is $a_1x^2 + b_1x + c_1$, and the estimated curve from (x, T_2) is $a_2x^2 + b_2x + c_2$, then

$$m = \begin{cases} m' = \dfrac{b_2 - b_1 - \sqrt{(b_1 - b_2) - 4(a_1 - a_2)(c_1 - c_2)}}{2(a_1 - a_2)} & \text{If } x_i < m' < x_{i+1} \\[4mm] m'' = \dfrac{b_2 - b_1 + \sqrt{(b_1 - b_2) - 4(a_1 - a_2)(c_1 - c_2)}}{2(a_1 - a_2)} & \text{otherwise} \end{cases}$$

Figure 2.2 Plot of T versus x for Reed-Muench method and Modified Reed-Muench method.

Trasformed Dose Level
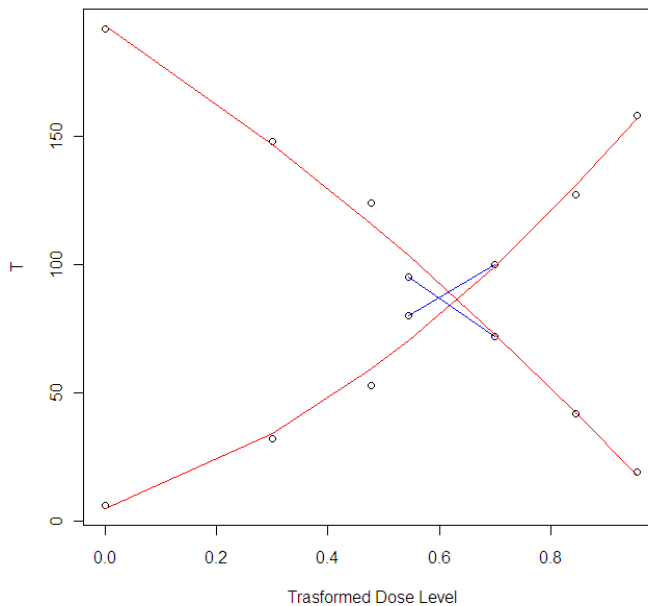
2.3.3 Simulation study

Case 1. Equally spaced dose levels, same number of subjects at each dose level

Table 2.17 Reed- Muench method, Case 1, normal distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10	2.7901	0.0368	2.8069	0.0377
n=20	2.7898	0.0176	2.8053	0.0183
n=30	2.7860	0.0118	2.8004	0.0126
n=40	2.7875	0.0078	2.8021	0.0084
n=50	2.7858	0.0070	2.8003	0.0073

Table 2.18 Reed- Muench method, Case 1, Cauchy distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10	2.7400	0.0531	2.7489	0.0573
n=20	2.7408	0.0273	2.7472	0.0291
n=30	2.7355	0.0192	2.7408	0.0203
n=40	2.7366	0.0140	2.7435	0.0144
n=50	2.7346	0.0128	2.7411	0.0129

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.19 Reed- Muench method, Case 2, normal distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10+j	2.7721	0.0379	2.7828	0.0358
n=20+j	2.7944	0.0159	2.8106	0.0183
n=30+j	2.7836	0.0108	2.8100	0.0127
n=40+j	2.7888	0.0074	2.8054	0.0089
n=50+j	2.7831	0.0074	2.8000	0.0076

Table 2.20 Reed- Muench method, Case 2, Cauchy distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10+j	2.6712	0.0628	2.6714	0.0626
n=20+j	2.7618	0.0239	2.7686	0.0276
n=30+j	2.7416	0.0175	2.7567	0.019
n=40+j	2.7369	0.0128	2.7415	0.0146
n=50+j	2.7258	0.0142	2.7337	0.0138

Case 3. Unequally spaced dose levels, same number of subjects at each dose level

Table 2.21 Reed- Muench method, Case 3, normal distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10	4.1725	0.3609	4.4655	0.3094
n=20	4.1472	0.2160	4.4592	0.1592
n=30	4.1286	0.1701	4.4514	0.1088
n=40	4.1275	0.1357	4.4510	0.0785
n=50	4.1251	0.1260	4.4492	0.0683

Table 2.22 Reed- Muench method, Case 3, Cauchy distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10	4.0279	0.4144	4.321	0.318
n=20	3.9946	0.3018	4.3156	0.1629
n=30	3.9774	0.2616	4.3084	0.111
n=40	3.9702	0.2334	4.3062	0.0791
n=50	3.9628	0.2292	4.3006	0.0689

Case 4. Unequally spaced dose levels, different number of subjects at each dose level

Table 2.23 Reed- Muench method, Case 4, normal distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10+j	3.9529	0.4272	4.2815	0.2797
n=20+j	4.2132	0.1811	4.5148	0.1678
n=30+j	4.1335	0.1582	4.4771	0.1107
n=40+j	4.1187	0.1323	4.4530	0.0787
n=50+j	4.0849	0.1467	4.4220	0.0638

Table 2.24 Reed- Muench method, Case 4, Cauchy distribution.

	Reed- Muench method		Modified method	
Sample size	Mean	MSE	Mean	MSE
n=10+j	3.7643	0.5892	4.0705	0.385
n=20+j	4.086	0.2411	4.3926	0.1613
n=30+j	3.9969	0.2392	4.3445	0.1049
n=40+j	3.966	0.2289	4.3041	0.0788
n=50+j	3.9217	0.2626	4.2724	0.0724

Conclusion: From the above simulation study, one can see that in the case of equally spaced dose levels, Reed-Muench method and the modified method provide similar results; In

the case of unequally spaced dose levels, the modified method outperforms Reed-Muench method.

Based on the comparisons, it appears that the modified method is a good substitute for the original one in most conditions.

2.4 The Thompson Moving Average Method

Moving average method was first discussed by Sheppard (1914). Thompson (1947) proposed a method utilizing moving average to estimate the LC50 of bioassay data. It has some relationship to the Spearman–Kärber method. The dose interval for the Spearman–Kärber method should be from almost 0 to 100%. It's wasteful if there is an existing approximation to the LC50. The Thompson method is sufficiently different because it would average all sets of successive values of p and plot average versus middle dose. Finally an estimate of the LC50 can be obtained by linear interpolation. Unlike other methods of estimating LC50, the Thompson method cannot estimate any other percentage points. The Thompson moving average method was considered a 'basic' one in finding LC50. For example, $\log_{10}$(LC50) can be obtained by plotting $(p_i + p_{i+1})/2$ versus $(x_i + x_{i+1})/2$ and simple linear interpolation. A three-term moving average was recommended by Topley and Wilson.

2.4.1 Algorithm

(1). Calculate

$$p*_i = \frac{p_{i+1} - \dfrac{n_i}{n_i} p_i + p_{i+1}}{(2 + \dfrac{n_i}{n_i})},$$

$$X^*_i = \frac{x_{i-1} - \frac{n_i}{\overline{n}_i} x_i + x_{i+1}}{(2 + \frac{n_i}{\overline{n}_i})}, \text{ where } i=2, \ldots, k\text{-}1 \text{ and } \frac{1}{\overline{n}_i} = \frac{1}{2}(\frac{1}{n_{i-1}} + \frac{1}{n_{i+1}}).$$

(3). If there is a successive proportions $p_i^* = 0.5$, then $m = x_i$.

(2). If there are p_i^* and p_{i+1}^* following $p_i^* < 0.5 < p_{i+1}^*$, then the estimated value of $\log_{10}(\text{LC50})$ is

$$m = X^*_i + \frac{(0.5 - P^*_i)}{(P^*_{i+1} - P^*_i)}(X^*_{i+1} - X^*_i).$$

(4). The LC50 is estimated by $LC50 = 10^m$.

2.4.2 Modification

If the value of p^* is not monotonic increasing near 0.5, there will be more than one m, which is unreasonable. If significant information will not be lost, we drop some p_i^* to make the rest of them to be monotonic increasing, thus the Thompson moving average method can be used. However, we do not recommend this method.

The Thompson moving average method uses linear interpolation to find the estimation of LC50, which only uses the information from two points(x_i, p_i^*)and (x_{i+1}, p_{i+1}^*), where $p_i^* < 0.5 < p_{i+1}^*$. The following is a typical plot of p^* versus x.

Figure 2.3 Plot of p*versus x for moving average method
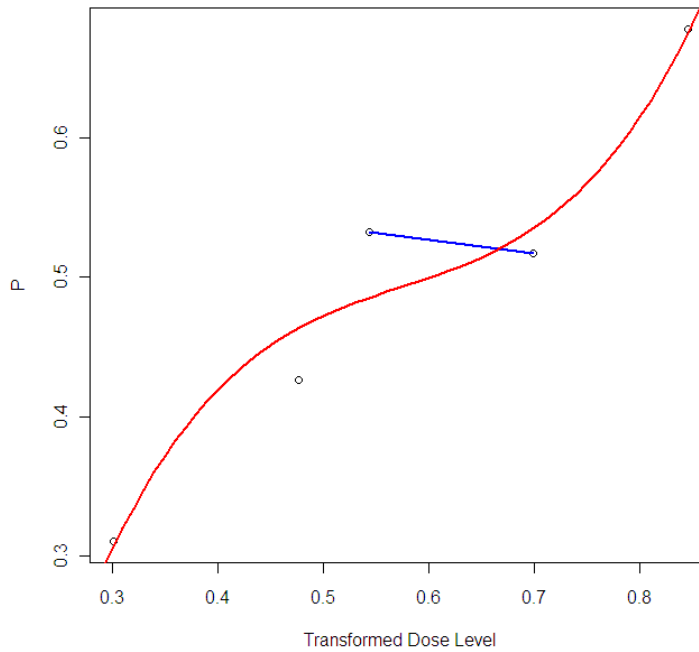
The general pattern of the plot shows a sigmoid trend. Therefore, a cubic polynomial, estimated from all data, can be used to find a better estimation of LC50.

2.4.3 Simulation study

Case 1. Equally spaced dose levels, same number of subjectss at each dose level

Table 2.25 Thompson Moving Average Method, Case 1, normal distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	**Mean**	**MSE**	**Mean**	**MSE**
n=10	2.7936	0.0460	2.7957	0.0476
n=20	2.7934	0.0203	2.7939	0.0216
n=30	2.7862	0.0141	2.7850	0.0150
n=40	2.7894	0.0091	2.7883	0.0098
n=50	2.7883	0.0085	2.7873	0.0091

Table 2.26 Thompson Moving Average Method, Case 1, Cauchy distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	Mean	MSE	Mean	MSE
n=10	2.8070	0.0871	2.8214	0.0822
n=20	2.8765	0.0778	2.8798	0.072
n=30	2.7761	0.0286	2.7907	0.0278
n=40	2.8496	0.0235	2.8470	0.0242
n=50	2.7234	0.0221	2.7244	0.0209

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.27 Thompson Moving Average Method, Case 2, normal distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	Mean	MSE	Mean	MSE
n=10+j	2.7952	0.0466	2.7979	0.0480
n=20+j	2.7920	0.0190	2.7913	0.0206
n=30+j	2.7853	0.0133	2.7833	0.0143
n=40+j	2.7906	0.0090	2.7890	0.0098
n=50+j	2.7876	0.0087	2.7867	0.0093

Table 2.28 Thompson Moving Average Method, Case 2, Cauchy distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	Mean	MSE	Mean	MSE
n=10+j	2.7978	0.0419	2.8072	0.0398
n=20+j	2.8441	0.0388	2.8455	0.0365
n=30+j	2.7757	0.014	2.7818	0.0132
n=40+j	2.8316	0.0104	2.8291	0.0112
n=50+j	2.7406	0.011	2.7376	0.0111

Case 3. Unequally spaced dose levels, same number of subjects at each dose level

Table 2.29 Thompson Moving Average Method, Case 3, normal distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	Mean	MSE	Mean	MSE
n=10	4.4014	0.7447	4.4273	0.7973
n=20	4.5839	0.7979	4.6754	0.9481
n=30	4.3549	0.3565	4.3667	0.3167
n=40	4.5744	0.2913	4.5073	0.2797
n=50	4.1539	0.2196	4.1215	0.1977

Table 2.30 Thompson Moving Average Method, Case 3, Cauchy distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	Mean	MSE	Mean	MSE
n=10	4.3754	0.5083	4.3999	0.6410
n=20	4.8471	1.0924	4.9262	1.2033
n=30	4.2201	0.3060	4.1802	0.3012
n=40	4.6529	0.8472	4.7736	1.2175
n=50	4.5439	0.6286	4.5791	0.6578

Case 4. Unequally spaced dose levels, different number of subjects at each dose level

Table 2.31 Thompson Moving Average Method, Case 4, normal distribution

	Thompson Moving Average Method		Cubic Polynomial Fitting	
Sample Size	Mean	MSE	Mean	MSE
n=10+j	4.4312	0.8003	4.4223	0.8800
n=20+j	4.6886	0.8087	4.7146	1.0268
n=30+j	4.3464	0.3731	4.3713	0.3420
n=40+j	4.5828	0.3029	4.5164	0.2944
n=50+j	4.1673	0.2197	4.1356	0.1985

Table 2.32 Thompson Moving Average Method, Case 4, Cauchy distribution

Sample Size	Thompson Moving Average Method		Cubic Polynomial Fitting	
	Mean	MSE	Mean	MSE
n=10+j	14.3184	0.7420	4.1472	0.9897
n=20+j	4.4294	0.5234	4.4134	0.5278
n=30+j	4.4922	0.7897	4.4992	1.0387
n=40+j	4.4758	0.4392	4.4085	0.4208
n=50+j	4.8537	0.6107	4.8739	0.6225

Conclusion: The modified method doesn't have significant improvement comparing to the original one.

2.5 The Shuster-Dietrich Method

The method was recommended by Shuster and Dietrich (1976) for estimating the dose response curves in quantal response assays. It is a general inverse regression procedure.

2.5.1 Algorithm

(1). Calculate

$X_i = \log_{10} d_i$, where $i = 1, 2, \ldots, k$, d_i is the dose level.

$$Y_i = \left(\frac{n_i}{n}\right)^{1/2} X_i$$

$$n = \sum_{i=1}^{k} n_i$$

$$\widetilde{Y} = \sum \left(\frac{n_i}{n}\right)^{1/2} Y_i = \frac{1}{n}\sum_{i=1}^{k} n_i \log_{10} d_i$$

$$Z_i = \left(\frac{n_i}{n}\right)^{1/2} \sin^{-1} \hat{p}_i^{1/2} 180/\pi$$

$$\tilde{Z} = \sum_{i=1}^{k} z_i \sqrt{\frac{n_i}{n}}$$

$$\hat{b} = S_{YZ}/S_{ZZ}$$

$$S_{YZ} = \sum_{i=1}^{k} Y_i Z_i - \tilde{Y}\tilde{Z}$$

$$S_{ZZ} = \sum_{i=1}^{k} Z_i^2 - \tilde{Z}^2$$

$$S_n^2 = 820.7\left[(\tilde{Z}-45)^2 \frac{S_{YY}}{S_{ZZ}^2} + \tilde{b}^2\right]$$

(2). The estimated of $\log_{10}$LC50, m, is

$$m = \tilde{Y} - \hat{b}(\tilde{Z}-45) .$$

(3). The LC50 is estimated by $LC50=10^m$.

(4) The 95% confidence interval for m is $m \pm z_{\alpha/2} \times S_n/\sqrt{n}$. Hence the approximate 95%

confidence interval for LC50 is $10^{m \pm z_{\alpha/2} \times S_n/\sqrt{n}}$.

2.5.2 Simulation study

Case 1. Equally spaced dose levels, same number of subjects at each dose level

Table 2.33 Shuster-Dietrich Method, Case 1, normal distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	2.7315	0.0264	0.918	1.2311
n=20	2.7379	0.0157	0.910	1.1674
n=30	2.7476	0.0110	0.902	1.1366
n=40	2.7462	0.0084	0.912	1.1238
n=50	2.7431	0.0079	0.802	1.0910

Table 2.34 Shuster-Dietrich Method, Case 1, Cauchy distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	2.7116	0.0582	0.866	1.3088
n=20	2.7290	0.0317	0.980	1.3284
n=30	2.7283	0.0229	0.926	1.2247
n=40	2.7296	0.0173	0.932	1.1898
n=50	2.7280	0.0155	0.844	1.1434

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.35 Shuster-Dietrich Method, Case 2, normal distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	2.7161	0.0296	0.916	1.2444
n=20+j	2.7419	0.0148	0.912	1.1642
n=30+j	2.7526	0.0104	0.908	1.1354
n=40+j	2.7478	0.0084	0.880	1.1128
n=50+j	2.7440	0.0080	0.812	1.0911

Table 2.36 Shuster-Dietrich Method, Case 2, Cauchy distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	2.6697	0.0666	0.936	1.4465
n=20+j	2.7365	0.0312	0.976	1.3249
n=30+j	2.7372	0.0218	0.930	1.2167
n=40+j	2.7296	0.0174	0.940	1.1946
n=50+j	2.7280	0.0156	0.842	1.1443

Case 3. Unequally spaced dose levels, same number of subjects at each dose level

Table 2.37 Shuster-Dietrich Method, Case 3, normal distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	4.2048	0.4309	0.916	1.7450
n=20	4.2723	0.2583	0.972	1.6843
n=30	4.2850	0.1771	0.954	1.4784
n=40	4.2927	0.1310	0.972	1.4521
n=50	4.2923	0.1105	0.878	1.2772

Table 2.38 Shuster-Dietrich Method, Case 3, normal distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10	4.0114	0.5539	0.894	1.7939
n=20	4.1583	0.4455	0.970	1.9772
n=30	4.1898	0.3123	0.944	1.6734
n=40	4.2029	0.2424	0.966	1.6036
n=50	4.1987	0.2130	0.852	1.3903

Case 4. Unequally spaced dose levels, different number of subjectss at each dose level

Table 2.39 Shuster-Dietrich Method, Case 4, normal distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	4.1218	0.4972	0.910	1.7299
n=20+j	4.2904	0.2420	0.968	1.6163
n=30+j	4.2979	0.1683	0.950	1.4534
n=40+j	4.2936	0.1309	0.972	1.4484
n=50+j	4.2866	0.1113	0.880	1.2864

Table 2.40 Shuster-Dietrich Method, Case 4, Cauchy distribution

Sample Size	Mean	MSE	Coverage rate	Length of CI
n=10+j	3.9089	0.6973	0.858	1.8010
n=20+j	4.1885	0.4373	0.982	2.0477
n=30+j	4.2035	0.2993	0.960	1.7262
n=40+j	4.2026	0.2422	0.964	1.6056
n=50+j	4.2024	0.2146	0.850	1.3856

Conclusion: When the space of log dose level is equal, the Shuster-Dietrich method estimates LC50 with very small MSEs, but it obtains much larger MSEs in the case of unequally spaced dose level. There is no significant difference between different or same number of subjectss at each dose level. The Shuster-Dietrich method seems more suitable for the case of equally spaced dose level.

2.6 The Shuster-Yang Method

Shuster and Yang (1975) have developed a nonparametric method to estimate the minimum dose level to induce a given response rate. They made a comparison between this method and other well-known nonparametric methods to show that the Shuster-Dietrich method is better in certain conditions.

They also introduced some practical applications of this method in particular conditions. It can estimate LC50 when the dose level is discrete. Even based on a preliminary scan of the dosage levels, this method can get exact inferences about the location of LC50. Since interpolation is applied to this procedure, there is no need to make a distribution-free estimator for LC50, but it needs to construct a distribution-free statement for the LC50 interval.

This method provides an easy algorithm for locating the interval where the estimate of LC50 can be found by liner interpolation. For this method, the only assumption is that the response rate is non-decreasing as a function with respect to dose level.

2.6.1 Algorithm

(1). Calculate

$A_i = r_i - 0.5n_i$, where $i = 1, \ldots, k$

$S_i = \sum_{u=0}^{i} A_u$, where $i = 1, \ldots, k$, $j = \min\{i : p_i > 0.5\}$.

(2). Find the first integer s such that $S_s = \min\{S_1, S_2, \ldots, S_k\}$, where $\hat{j} = s + 1$.

(3). LC50 is in the interval (x_s, x_{s+1}).

(4). By linear interpolation on x and p, the estimated of LC50 is

$$LC50 = x_s + (x_{s+1} - x_s)\frac{0.5 - p_s}{p_{s+1} - p_s}.$$

2.6.2 Simulation study

Case 1. Equally spaced dose levels, same number of subjects at each dose level

Table 2.41 Shuster-Yang Method, Case 1, normal distribution

Sample Size	Mean	MSE
n=10	2.7961	0.0773
n=20	2.8052	0.0434
n=30	2.8018	0.0283
n=40	2.8027	0.0192
n=50	2.8053	0.0170

Table 2.42 Shuster-Yang Method, Case 1, Cauchy distribution

Sample Size	Mean	MSE
n=10	2.7800	0.1366
n=20	2.8029	0.0761
n=30	2.8003	0.0471
n=40	2.8073	0.0345
n=50	2.8045	0.0302

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.43 Shuster-Yang Method, Case 2, normal distribution

Sample Size	Mean	MSE
n=10+j	2.8034	0.0812
n=20+j	2.8068	0.0406
n=30+j	2.7977	0.0253
n=40+j	2.8047	0.0183
n=50+j	2.8049	0.0177

Table 2.44 Shuster-Yang Method, Case 2, Cauchy distribution

Sample Size	Mean	MSE
n=10+j	2.8262	0.1431
n=20+j	2.8110	0.0701
n=30+j	2.7990	0.0450
n=40+j	2.8050	0.0323
n=50+j	2.8067	0.0324

Case 3. Unequally spaced dose levels, same number of subjectss at each dose level

Table 2.45 Shuster-Yang Method, Case 3, normal distribution

Sample Size	Mean	MSE
n=10	4.4294	1.6622
n=20	4.5022	1.0458
n=30	4.4032	0.6570
n=40	4.4610	0.5157
n=50	4.4382	0.4872

Table 2.46 Shuster-Yang Method, Case 3, Cauchy distribution

Sample Size	Mean	MSE
n=10	4.3503	2.1878
n=20	4.5429	1.6543
n=30	4.4130	1.0115
n=40	4.4810	0.8383
n=50	4.4549	0.7388

Case 4. Unequally spaced dose levels, different number of subjects at each dose level

Table 2.47 Shuster-Yang Method, Case 4, normal distribution

Sample Size	Mean	MSE
n=10+j	4.5263	1.7224
n=20+j	4.5546	1.0631
n=30+j	4.4123	0.6190
n=40+j	4.4656	0.5010
n=50+j	4.4496	0.4850

Table 2.48 Shuster-Yang Method, Case 4, Cauchy distribution

Sample Size	Mean	MSE
n=10+j	4.5458	2.4126
n=20+j	4.6041	1.6767
n=30+j	4.4266	0.9560
n=40+j	4.4742	0.8066
n=50+j	4.4619	0.7484

Conclusion: Comparing the case of equally spaced dose levels, the unequal space case get significant greater MSEs. It indicates that the Shuster-Yang Method works much better in equal space case than in unequal case.

2.7 Maximum Likelihood Method

Fisher recommended and analyzed Maximum-likelihood estimation in 1912. This method became very popular since then. Researchers always prefer it for estimating LC50 today because of its high accuracy, but its disadvantage lies in the fact that one must know the exact tolerance distribution in advance.

2.7.1 Algorithm
(1) For the normal model

$$\pi(x) = F(\alpha + \beta x) = \int_{-\infty}^{\alpha+\beta x} \frac{1}{\sqrt{2\pi}} \exp(-t^2/2)\,dt = \Phi(\alpha + \beta x),$$

For Cauchy and other tolerance distributions, $\pi(x)$ will change accordingly.

(2) The likelihood function is proportional to $P_i^{ri} Q_i^{ni-ri}$, i= 1, 2,… , k , where $P_i = F(\alpha + \beta x_i), Q_i = 1 - P_i$.

(3) The log-Likelihood function is $L = \sum_{i=1}^{k} [r_i \log P_i + (n_i - r_i) \log Q_i]$.

(4) Let $f_i = dF(y)/dy|_{y=\alpha+\beta x_i}$. The parameters α and β are the solution of

$$\frac{\partial L}{\partial \alpha} = \sum n_i w_i (p_i - P_i)/f_i = 0$$
$$\frac{\partial L}{\partial \beta} = \sum n_i w_i X_i (p_i - P_i)/f_i = 0$$

where $w_i = f_i^2 / P_i Q_i$, $p_i = r_i/n_i$.

(5) $m = -\dfrac{\alpha}{\beta}$, the estimation of LC50=10^m.

2.7.2 Simulation Study

In the following simulation studies, we will treat all the tolerance distribution as normal in the calculation. Therefore, we can investigate the robustness of the maximum likelihood method. Because we assume the tolerance distribution is normal distribution, the simulation that

uses Cauchy as tolerance distribution cannot perform well for sure.

Case 1. Equally spaced dose levels, same number of subjects at each dose level

Table 2.49 Maximum Likelihood Method, Case 1, normal distribution

Sample Size	Mean	MSE
n=10	2.7979	0.0346
n=20	2.7920	0.0168
n=30	2.7901	0.0110
n=40	2.7894	0.0074
n=50	2.7868	0.0064

Table 2.50 Maximum Likelihood Method, Case 1, Cauchy distribution

Sample Size	Mean	MSE
n=10	2.7824	0.08442
n=20	2.7703	0.03811
n=30	2.7625	0.02511
n=40	2.7617	0.01772
n=50	2.7574	0.01514

Case 2. Equally spaced dose levels, different number of subjects at each dose level

Table 2.51 Maximum Likelihood Method, Case 2, normal distribution

Sample Size	Mean	MSE
n=10+j	2.7985	0.0356
n=20+j	2.7912	0.0162
n=30+j	2.7901	0.0106
n=40+j	2.7909	0.0075
n=50+j	2.7870	0.0066

Table 2.52 Maximum Likelihood Method, Case 2, Cauchy distribution

Sample Size	Mean	MSE
n=10+j	2.7858	0.0891
n=20+j	2.7688	0.0384
n=30+j	2.7646	0.0246
n=40+j	2.7622	0.0179
n=50+j	2.7578	0.0154

Case 3. Unequally spaced dose levels, same number of subjects at each dose level

Table 2.53 Maximum Likelihood Method, Case 3, normal distribution

Sample Size	Mean	MSE
n=10	4.5441	1.1543
n=20	4.4308	0.3693
n=30	4.3997	0.2149
n=40	4.3949	0.1517
n=50	4.3801	0.1199

Table 2.54 Maximum Likelihood Method, Case 3, Cauchy distribution

Sample Size	Mean	MSE
n=10	320.2301	3.069878e+07
n=20	4.4544	1.0112
n=30	4.3817	0.4673
n=40	4.3681	0.3320
n=50	4.3271	0.2499

Case 4. Unequally spaced dose levels, different number of subjects at each dose level

Table 2.55 Maximum Likelihood Method, Case 4, normal distribution

Sample Size	Mean	MSE
n=10+j	4.5754	1.4268
n=20+j	4.4271	0.3420
n=30+j	4.4040	0.2080
n=40+j	4.3981	0.1535
n=50+j	4.3752	0.1205

Table 2.56 Maximum Likelihood Method, Case 4, Cauchy distribution

Sample Size	Mean	MSE
n=10+j	9.4356	5642.3981
n=20+j	4.4477	1.0011
n=30+j	4.3795	0.4593
n=40+j	4.3699	0.3381
n=50+j	4.3314	0.2549

Conclusion: Comparing the case of equally spaced dose levels, the equal space case get similar MSEs. In addition, they give the smallest MSEs compared to other methods, indicating that the Maximum Likelihood method works much better than other methods. This is not unexpected, since ML uses the information from the tolerance distribution. Note that the simulation results show the performance of normal is much better than the Cauchy.

CHAPTER 3 - Conclusion and Discussion

To draw conclusions, we put some the simulation result in following table, where case 1: d_i is a constant, n_i=50; case 2: d_i is a constant, n_i is not a constant; case 3: d_i is not a constant, n_i=50; and case 4: d_i is not a constant, n_i is not a constant.

The Maximum likelihood generally performs best among all seven traditional methods. This is not out of expectation, because we know the exact distribution of data in advance. However, in practice, we never know the exact real model. The best data for estimation should be sufficiently checked before fitting the parametric model. Different data demand different methods.

By comparison, one can see the modified Dragstedt-Behrens method and modified Reed-Muench method also perform well. They are good substitutions for the original ones. Since all those methods except maximum likelihood method are nonparametric model, the modified Dragstedt-Behrens method and modified Reed-Muench method can be used in practice.

Normal distribution as tolerance distribution (n=50 or n=50+j)

		Dragstedt-Behrens	Modified Dragstedt-Behrens	Spearman-Karber	Reed-Muench	Modified Reed-Muench	Moving Average	Modified Moving Average	Shuster-Dietrich	Shuster-Yang	Maximum likelihood
Case 1	Mean	2.7877	2.7597	2.8004	2.7858	2.8003	2.7883	2.7873	2.7431	2.8053	2.7868
	|Mean-2.7866|	0.0011	0.0269	0.0138	0.0008	0.0137	0.0017	0.0007	0.0435	0.0187	0.0002
	MSE	0.0059	0.0065	0.0074	0.0070	0.0073	0.0085	0.0091	0.0079	0.0170	0.0064
	Empirical 95% CI	0.986		0.944					0.802		
	Mean of Empirical Length	1.1492		1.1203					1.0910		
Case 2	Mean	2.7861	2.7581	2.8004	2.7831	2.8000	2.7876	2.7867	2.7440	2.8049	2.7870
	|Mean-2.7866|	0.0005	0.0285	0.0138	0.0035	0.0134	0.001	0.0001	0.0426	0.0183	0.0004
	MSE	0.0063	0.0069	0.0076	0.0074	0.0076	0.0087	0.0093	0.0080	0.0177	0.0066
	Empirical 95% CI	0.984		0.946					0.812		
	Mean of Empirical Length	1.1503		1.1215					1.0911		
Case 3	Mean	4.1197	4.2546	3.9290	4.1251	4.4492	4.1539	4.1215	4.2923	4.4382	4.3801
	|Mean-4.3571|	0.2374	0.1025	0.4281	0.232	0.0921	0.2032	0.2356	0.0648	0.0811	0.0230
	MSE	0.1257	0.0651	0.2332	0.1260	0.0683	0.2196	0.1977	0.1105	0.4872	0.1199
	Empirical 95% CI	0.864		0.522					0.878		
	Mean of Empirical Length	1.2937		1.2364					1.2772		
Case 4	Mean	4.0807	4.2185	3.9250	4.0849	4.4220	4.1673	4.1356	4.2866	4.4496	4.3752
	|Mean-4.3571|	0.2764	0.1386	0.4321	0.2722	0.0649	0.1898	0.2215	0.0705	0.0925	0.0181
	MSE	0.1462	0.0727	0.2354	0.1467	0.0638	0.2197	0.1985	0.1113	0.4850	0.1205
	Empirical 95% CI	0.826		0.516					0.880		
	Mean of Empirical Length	1.2989		1.2363					1.2864		

Cauchy distribution as tolerance distribution (n=50 or n=50+j)

Case		Dragstedt-Behrens	Modified Dragstedt-Behrens	Spearman-Karber	Reed-Muench	Modified Reed-Muench	Moving Average	Modified Moving Average	Shuster-Dietrich	Shuster-Yang	Maximum likelihood
Case 1	Mean	2.7388	2.7137	2.8175	2.7346	2.7411	2.7234	2.7244	2.7280	2.8045	2.7574
	MSE	0.0125	0.0147	0.0148	0.0128	0.0129	0.0221	0.0209	0.0155	0.0302	0.0151
	Empirical 95% CI	0.956		0.172					0.844		
	Mean of Empirical Length	1.1804		1.0226					1.1434		
Case 2	Mean	2.7292	2.7056	2.8176	2.7258	2.7337	2.7406	2.7376	2.728	2.8067	2.7578
	MSE	0.0137	0.0161	0.0151	0.0142	0.0138	0.011	0.0111	0.0156	0.0324	0.0154
	Empirical 95% CI	0.946		0.188					0.842		
	Mean of Empirical Length	1.1826		1.0226					1.1443		
Case 3	Mean	3.9550	4.1212	3.6835	3.9628	4.3006	4.5439	4.5791	4.1987	4.4549	4.3271
	MSE	0.2363	0.1110	0.5042	0.2292	0.0689	0.6286	0.6578	0.213	0.7388	0.2499
	Empirical 95% CI	0.730		0.004					0.852		
	Mean of Empirical Length	1.3166		1.0328					1.3903		
Case 4	Mean	3.9181	4.0866	3.6840	3.9217	4.2724	4.8537	4.8739	4.2024	4.4619	4.3314
	MSE	0.2627	0.1276	0.5023	0.2626	0.0724	0.6107	0.6225	0.2146	0.7484	0.2549
	Empirical 95% CI	0.660		0.006					0.850		
	Mean of Empirical Length	1.3209		1.0328					1.3856		

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Appendix   R-code

The simulation study is conducted by using the following R program.

A.1 Dragstedt-Behrens Method

```
rm(list=ls())
set.seed(987654)


Eqx.or.Not=1      # 0=No, 1=Yes
Eqn.or.Not=0      # 0=No, 1=Yes

# Define Dose Levels

  # Equally Spaced Transformed Dose Levels

  Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+
              (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);

  Num.Dose.Level=length(Dose.Level);

# Dose Level Transformations

  Tran.Dose.Level=log10(Dose.Level);

# Define Tolerance Distribution

  Log.True.Mean=log10(mean(Dose.Level));
  Log.True.Sd=log10(sd(Dose.Level));
  Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};         # normal distribution
  #Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)}; # Cauchy distribution
# Generate True Response Rate

  True.Res.Rate=Tol.Dist(Tran.Dose.Level);

# Generate Sample
  Mean.LC50=Mean.LC50.new=Ep.Level=Mean.Ep.Length=MSE.LC50=MSE.LC50.new=rep(0,5);
```

```r
j=1;
for(size in c(10,20,30,40,50))
 {
 n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1-
     Eqn.or.Not)*(size+round(3*runif (Num.Dose.Level,-1,1)))


 Rep.in.Sim=500;


 m50=m50.new=m25=m75=SEs=rep(0,Rep.in.Sim);    # Initial values of Log(LC50,LC25,LC75)


 for(run in seq(Rep.in.Sim))
  {
    Sim.Res=rep(0,Num.Dose.Level)
    for(i in seq(Num.Dose.Level))
     {
       Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
     }

    # Dragstedt-Behrens Method

      T1=cumsum(Sim.Res);
      T2=rev(cumsum(rev(n-Sim.Res)));
      pstar=T1/(T1+T2);

      plow50=max(pstar[pstar<=0.5])
      pupper50=min(pstar[pstar>=0.5])
      xlow50=Tran.Dose.Level[pstar==plow50]
      xupper50=Tran.Dose.Level[pstar==pupper50]

      m50[run]=ifelse(
                      pupper50==plow50, plow50,
                          {
                           w=(pupper50-0.5)/(pupper50-plow50);
                           w*xlow50+(1-w)*xupper50
                          }
                      )

      z1=Tran.Dose.Level;
      z2=z1*z1;
      z3=z2*z1;
      my3reg=lm(pstar~z1+z2+z3);
```

```r
    a0=my3reg$coefficient[1]-0.5;
    a1=my3reg$coefficient[2];
    a2=my3reg$coefficient[3];
    a3=my3reg$coefficient[4];
    myfun=function(x){a0+a1*x+a2*x^2+a3*x^3};
    root=uniroot(myfun, lower=min(z1),upper=max(z1),tol = 0.0001)
    m50.new[run]=root$root;

    plow25=max(pstar[pstar<=0.25])
    pupper25=min(pstar[pstar>=0.25])
    xlow25=Tran.Dose.Level[pstar==plow25]
    xupper25=Tran.Dose.Level[pstar==pupper25]
    m25[run]=ifelse(
                    pupper25==plow25, plow25,
                      {
                        w=(pupper25-0.25)/(pupper25-plow25);
                        w*xlow25+(1-w)*xupper25
                        }
                    )

    plow75=max(pstar[pstar<=0.75])
    pupper75=min(pstar[pstar>=0.75])
    xlow75=Tran.Dose.Level[pstar==plow75]
    xupper75=Tran.Dose.Level[pstar==pupper75]
    m75[run]=ifelse(
                    pupper75==plow75, plow75,
                      {
                        w=(pupper75-0.75)/(pupper75-plow75);
                        w*xlow75+(1-w)*xupper75
                      }
                    )
   # h=(max(Tran.Dose.Level)-min(Tran.Dose.Level))/(Num.Dose.Level-1);
   # SE[run]=(h/size)*sqrt(sum(Sim.Res*(size-Sim.Res))/(size))
 }

h=(max(Tran.Dose.Level)-min(Tran.Dose.Level))/(Num.Dose.Level-1);
IR=m75-m25;
SE=sqrt(0.79*h*IR/round(mean(n)))

L.end=10^(m50-1.96*SE)
R.end=10^(m50+1.96*SE)
```

```
freq=sum((10^Log.True.Mean>=L.end)*(10^Log.True.Mean<=R.end))
Mean.Ep.Length[j]=mean(10^(2*1.96*SE))
Ep.Level[j]=freq/Rep.in.Sim;
Mean.LC50[j]=mean(10^m50)
Mean.LC50.new[j]=mean(10^m50.new)

MSE.LC50[j]=mean((10^m50-10^Log.True.Mean)^2);
MSE.LC50.new[j]=mean((10^m50.new-10^Log.True.Mean)^2);

j=j+1;
}
Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50,Ep.Level,Mean.Ep.Length,Mean.LC50.new,
        MSE.LC50.new))
dimnames(Result)=list(c("","","","",""), c("Sample.Size","Mean","MSE","Empirical.CL",
            "Mean.Ep.Length", "Mean.Cubic","MSE.Cubic"))
{
cat("                    Simulation Result","\n");
cat("        Equally Spaced X:", Eqx.or.Not,","," ", "Same Number of subjectss:",
    Eqn.or.Not,"\n\n")
Result
}

pp=as.matrix(cbind(z1,pstar))
plot(z1,pstar,xlab="Transformed Dose Level",ylab="p*")
xx=c(pp[4,1],pp[5,1])
yy=c(pp[4,2],pp[5,2])
lines(xx,yy,col="blue",lwd=2)
xx=seq(0,1,by=0.01);
lines(xx,a0+0.5+a1*xx+a2*xx^2+a3*xx^3,col="red",lwd="2");
```

A.2 Spearman-Karber Method

```
rm(list=ls())
set.seed(987654)

Eqx.or.Not=0      # 0=No, 1=Yes
Eqn.or.Not=0      # 0=No, 1=Yes
```

```
# Define Dose Levels

 Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+ (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);

  Num.Dose.Level=length(Dose.Level);

# Dose Level Transformations

  Tran.Dose.Level=log10(Dose.Level);

# Define Tolerance Distribution

  Log.True.Mean=log10(mean(Dose.Level));
  Log.True.Sd=log10(sd(Dose.Level));
  Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};                        # normal distribution
  #Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)};    # Cauchy distribution

# Generate True Response Rate

  True.Res.Rate=Tol.Dist(Tran.Dose.Level);

# Generate Sample

 m=Mean.LC50=Ep.Level=Mean.Ep.length=MSE.LC50=Var=rep(0,5);
 j=1;
 for(size in c(10,20,30,40,50))
 {
  n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1-Eqn.or.Not) * ( size+round( 3*runif(Num.Dose.Level,-1,1)))

  #n=rep(size,Num.Dose.Level); # Population Size at Each Dose Level
  Rep.in.Sim=500;
  m=rep(0,Rep.in.Sim); # Initial values of Log(LC50)
  for(run in seq(Rep.in.Sim))
    {
     Sim.Res=rep(0,Num.Dose.Level)
     for(i in seq(Num.Dose.Level))
        {
          Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
        }
```

```
# Spearman-Karber Method
p=Sim.Res/n;
P=c(0,p,1);
K=length(P);
X=c(0,Tran.Dose.Level,1);
N=rep(size,K)
m[run]=sum((P[2:K]-P[1:(K-1)])*(X[1:(K-1)]+X[2:K]))/2
Var[run]=sum(P[2:(K-1)]*(1-P[2:(K-1)])*(X[3:(K)]-X[1:(K-2)])^2/(4*N[2:(K-1)]))
                                                  # Var of each m

}


SE=sqrt(Var/N)


L.end=10^(m-1.96*SE)
R.end=10^(m+1.96*SE)


freq=sum((10^Log.True.Mean>=L.end)*(10^Log.True.Mean<=R.end))
Mean.Ep.length[j]=mean(10^(2*1.96*SE))
Ep.Level[j]=freq/Rep.in.Sim;


Mean.LC50[j]=mean(10^m)
MSE.LC50[j]=mean((10^m-10^Log.True.Mean)^2);


j=j+1;
}


Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50,Ep.Level, Mean.Ep.length))
dimnames(Result)=list(c("","","","",""), c("Sample.Size","Mean","MSE","Empirical.CL",  "Mean.Ep.length"))
{
cat("                  Simulation Result","\n");
cat("      Equally Spaced X:", Eqx.or.Not,","," ", "Same Number of subjectss:",
    Eqn.or.Not,"\n\n")
Result
}
```

A.3 Reed-Muench Method

```
rm(list=ls())
set.seed(987654)
```

```
Eqx.or.Not=1      # 0=No, 1=Yes
Eqn.or.Not=1      # 0=No, 1=Yes

# Define Dose Levels

Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+
          (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);

Num.Dose.Level=length(Dose.Level);

# Dose Level Transformations

Tran.Dose.Level=log10(Dose.Level);

# Define Tolerance Distribution

Log.True.Mean=log10(mean(Dose.Level));
Log.True.Sd=log10(sd(Dose.Level));
Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};        # normal distribution
#Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)};  # Cauchy distribution

# Generate True Response Rate

True.Res.Rate=Tol.Dist(Tran.Dose.Level);

# Generate Sample

Mean.LC50=Mean.LC50.new=Ep.Level=MSE.LC50=MSE.LC50.new=rep(0,5);
j=1;
for(size in c(10,20,30,40,50))
{
n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1-Eqn.or.Not) * ( size+round(3*runif(Num.Dose.Level,-1,1)))

  #n=rep(size,Num.Dose.Level); # Population Size at Each Dose Level
  Rep.in.Sim=500;
  m=m.new=rep(0,Rep.in.Sim); # Initial values of Log(LC50,LC25,LC75)
  for(run in seq(Rep.in.Sim))
    {
    Sim.Res=rep(0,Num.Dose.Level)
    for(i in seq(Num.Dose.Level))
```

```
  {
    Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
  }

# Dragstedt-Behrens Method
X=Tran.Dose.Level;
T1=cumsum(Sim.Res);
T2=rev(cumsum(rev(n-Sim.Res)));

T1.low=max(T1[T1<=T2])
T2.low=min(T2[T1<=T2])
Xlow=Tran.Dose.Level[T1==T1.low]

T1.upper=min(T1[T1>T2])
T2.upper=max(T2[T1>T2])
Xupper=Tran.Dose.Level[T1==T1.upper]

m[run]=(Xlow+(Xupper-Xlow)*(T2.low-T1.low)/(T1.upper-T2.upper-T1.low+T2.low))

# Modified Reed-muench Method
X=Tran.Dose.Level;
T1=cumsum(Sim.Res);
T2=rev(cumsum(rev(n-Sim.Res)));
X2=X^2;

myreg1=lm(T1~X+X2);
myreg2=lm(T2~X+X2);
summary(myreg1)
c1=myreg1$coeff[1]  #coefficients for reg1
b1=myreg1$coeff[2]
a1=myreg1$coeff[3]
summary(myreg2)
c2=myreg2$coeff[1]  #coefficients for reg2
b2=myreg2$coeff[2]
a2=myreg2$coeff[3]
delta=sqrt((b1-b2)^2-4*(a1-a2)*(c1-c2))

 root1=(b2-b1-delta)/(2*(a1-a2))
 root2=(b2-b1+delta)/(2*(a1-a2))

 if(root1>=Xlow & root1<=Xupper) #get the real root by regression methord
```

```
            {
              m.new[run]=root1
            }
        else
          {
              m.new[run]=root2
          }
      }
    Mean.LC50[j]=mean(10^m)
    Mean.LC50.new[j]=mean(10^m.new)
    MSE.LC50[j]=mean((10^m-10^Log.True.Mean)^2);
    MSE.LC50.new[j]=mean((10^m.new-10^Log.True.Mean)^2);

    j=j+1;
}


    plot(X, T1, xlim=range(X), ylim=range(T1, T2), xlab=' Trasformed Dose Level', ylab='T')
    points(X, T2, xlab='Trasformed Dose Level', ylab='T')
    lines(X,myreg1$fitted,col=c("red"))
    lines(X,myreg2$fitted,col=c("red"))
    lines(c(Xlow, Xupper),c(T1.low,T1.upper),col=c("blue"));
    lines(c(Xlow, Xupper),c(T2.low,T2.upper),col=c("blue"))

Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50,Mean.LC50.new,MSE.LC50.new))
    dimnames(Result)=list(c("","","",""),("Sample.Size","Mean","MSE","Mean.LC50.new","MSE.LC50
                    .new"))
Result
```

A.4 Thompson Moving Average Method

```
    rm(list=ls())
    set.seed(987654)

    Eqx.or.Not=1      # 0=No, 1=Yes
    Eqn.or.Not=0      # 0=No, 1=Yes

  #  Define Dose Levels

    # Equally Spaced Transformed Dose Levels
```

```
   Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+
                (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);

   Num.Dose.Level=length(Dose.Level);

#  Dose Level Transformations

   Tran.Dose.Level=log10(Dose.Level);

#  Define Tolerance Distribution

   Log.True.Mean=log10(mean(Dose.Level));
   Log.True.Sd=log10(sd(Dose.Level));
   Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};              # normal distribution
  #Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)};   # Cauchy distribution

#  Generate True Response Rate

   True.Res.Rate=Tol.Dist(Tran.Dose.Level);

#  Generate Sample
  Mean.LC50=Mean.LC50.new=Ep.Level=MSE.LC50=MSE.LC50.new=rep(0,5);
  j=1;
  for(size in c(10,20,30,40,50))
   {
    n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1-
         Eqn.or.Not)*(size+round(3*runif(Num.Dose.Level,-1,1)))

    Rep.in.Sim=25;

    m=m.new=SE=rep(0,Rep.in.Sim);      # Initial values of Log(LC50)

    for(run in seq(Rep.in.Sim))
      {
       Sim.Res=rep(0,Num.Dose.Level)
       for(i in seq(Num.Dose.Level))
         {
          Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
         }
```

```
# Thompson Moving Average Method
  X=Tran.Dose.Level;
  p=Sim.Res/n
  n.mean=mean(n)
  k=Num.Dose.Level

  P=rep(0,k-2)
  for(i in seq(k-2))
   {
    P[i+1]=(p[i]+n[i+1]*p[i+1]/n.mean+p[i+2])/(2+n[i+1]/n.mean)
   }

  D=rep(0,k-2)
  for(i in seq(k-2))
   {
    D[i+1]=(X[i]+n[i+1]*X[i+1]/n.mean+X[i+2])/(2+n[i+1]/n.mean)
   }

  Plow=max(P[P<=0.5])
  Pupper=min(P[P>=0.5])
  I=length(Plow)
  Dlow=D[P==Plow]
  Dupper=D[P==Pupper]
  Xlow=X[P==Plow]
  Xupper=X[P==Pupper]
  plow=p[P==Plow]
  pupper=p[P==Pupper]

  if (Plow==0.5)
   {
    m[run]=Xlow
   }
  else if (I==1)                    #when p's are increasing
   {
    m[run]=Dlow+(Dupper-Dlow)*(0.5-Plow)/(Pupper-Plow)
   }
  else                    #when p's are not increasing
   {
    p=p(-c(pupper))
    X=X(-c(Xupper))
   }
```

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```
# Quadratic-Fitting Method
   z1=Tran.Dose.Level[2:(k-1)];
   z2=z1*z1;
   z3=z2*z1;
   Pstar=P[2:(k-1)]
   my3reg=lm(Pstar~z1+z2+z3);
   a0=my3reg$coefficient[1]-0.5;
   a1=my3reg$coefficient[2];
   a2=my3reg$coefficient[3];
   a3=my3reg$coefficient[4];
   myfun=function(x){a0+a1*x+a2*x^2+a3*x^3};
   root=uniroot(myfun, lower=min(z1),upper=max(z1),tol = 0.0001)
   m.new[run]=root$root;
   }


   Mean.LC50[j]=mean(10^m)
   Mean.LC50.new[j]=mean(10^m.new)

   MSE.LC50[j]=mean((10^m-10^Log.True.Mean)^2);
   MSE.LC50.new[j]=mean((10^m.new-10^Log.True.Mean)^2);

   j=j+1;
}


Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50,Mean.LC50.new,MSE.LC50.new))
dimnames(Result)=list(c("","","","",""),c("Sample.Size","Mean","MSE","Mean.Cubic","MSE.Cubic"))
   {
   cat("                    Simulation Result","\n");
   cat("       Equally Spaced X:", Eqx.or.Not,","," ", "Same Number of subjectss:", Eqn.or.Not,"\n\n")
   Result
   }

   pp=as.matrix(cbind(z1,Pstar))
   plot(z1,Pstar,xlab="Transformed Dose Level",ylab="p")
   xx=c(pp[2,1],pp[3,1],pp[4,1])
   yy=c(pp[2,2],pp[3,2],pp[4,2])
   lines(xx,yy,col="blue",lwd=2)
   xx=seq(0,1,by=0.01);
   lines(xx,a0+0.5+a1*xx+a2*xx^2+a3*xx^3,col="red",lwd="2");
```

A.5 Shuster-Dietrich Method

```
rm(list=ls())
set.seed(987654)

Eqx.or.Not=1      # 0=No, 1=Yes
Eqn.or.Not=0      # 0=No, 1=Yes

#  Define Dose Levels

  # Equally Spaced Transformed Dose Levels

  Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+
              (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);

  Num.Dose.Level=length(Dose.Level);

#  Dose Level Transformations
  Tran.Dose.Level=log10(Dose.Level);

#  Define Tolerance Distribution
  Log.True.Mean=log10(mean(Dose.Level));
  Log.True.Sd=log10(sd(Dose.Level));
 #Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};        # normal distribution
  Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)};    # Cauchy distribution

#  Generate True Response Rate

  True.Res.Rate=Tol.Dist(Tran.Dose.Level);

#  Generate Sample
 Mean.LC50=Ep.Level= Mean.Ep.Length =MSE.LC50=rep(0,5);
 j=1;
 for(size in c(10,20,30,40,50))
 {
  n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1- Eqn.or.Not)*(size+round(3*runif(Num.Dose.Level,-1,1)))

  Rep.in.Sim=500;
```

```
m=SE=rep(0,Rep.in.Sim);      # Initial values of Log(LC50)

for(run in seq(Rep.in.Sim))
  {
   Sim.Res=rep(0,Num.Dose.Level)
   for(i in seq(Num.Dose.Level))
     {
       Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
     }
   #  Shuster-Dietrich Method
   N=sum(n);
   Y=(sqrt(n/N))*Tran.Dose.Level;
   Yhat=sum((sqrt(n/N))*Y);
   p=Sim.Res/n;
   Z=(sqrt(n/N))*asin(sqrt(p))*180/pi;
   Zhat=sum(Z*sqrt(n/N));
   Syz=sum(Y*Z)-Yhat*Zhat;
   Szz=sum(Z*Z)-Zhat*Zhat;
   bhat=Syz/Szz;

   m[run]=Yhat-bhat*(Zhat-45);
  }

Syy= sum(Y^2)-Yhat^2
Sn=sqrt(820.7*((Zhat-45)^2*Syy/Szz^2+bhat^2))
SE=Sn/sqrt(N)
L.end=10^(m-1.96*SE)
R.end=10^(m+1.96*SE)

freq=sum((10^Log.True.Mean>=L.end)*(10^Log.True.Mean<=R.end))

Ep.Level[j]=freq/Rep.in.Sim;
Mean.Ep.Length[j]=mean(10^(2*1.96*SE));

Mean.LC50[j]=mean(10^m);
MSE.LC50[j]=mean((10^m-10^Log.True.Mean)^2);

j=j+1;
}
```

```
Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50,Ep.Level, Mean.Ep.Length))
dimnames(Result)=list(c("","","","",""), c("Sample.Size","Mean","MSE","Empirical.CL "," Mean.Ep.Length "))
    {
    cat("                    Simulation Result","\n");
    cat("       Equally Spaced X:", Eqx.or.Not,","," ", "Same Number of subjectss:",
        Eqn.or.Not,"\n\n")
    Result
     }
```

A.6 Shuster-Yang Method

```
    rm(list=ls())
    set.seed(987654)

    Eqx.or.Not=0     # 0=No, 1=Yes
    Eqn.or.Not=1     # 0=No, 1=Yes

 #  Define Dose Levels

   # Equally Spaced Transformed Dose Levels

   Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+
              (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);

   Num.Dose.Level=length(Dose.Level);

 #  Dose Level Transformations

   Tran.Dose.Level=log10(Dose.Level);

 #  Define Tolerance Distribution

   Log.True.Mean=log10(mean(Dose.Level));
   Log.True.Sd=log10(sd(Dose.Level));
   Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};                         # normal distribution
   #Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)};   # Cauchy distribution

 #  Generate True Response Rate
```

```
    True.Res.Rate=Tol.Dist(Tran.Dose.Level);

#  Generate Sample
    Mean.LC50=Mean.LC50.new=Ep.Level=Mean.Ep.Length=MSE.LC50=MSE.LC50.new
              =rep(0,5);
 j=1;
 for(size in c(10,20,30,40,50))
  {
 n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1-Eqn.or.Not)*(size+round(3*runif(Num.Dose.Level,-1,1)))

  Rep.in.Sim=500;
  LC50=rep(0,Rep.in.Sim);
  for(run in seq(Rep.in.Sim))
   {
     Sim.Res=rep(0,Num.Dose.Level)
     for(i in seq(Num.Dose.Level))
      {
        Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
      }

    # Shuster-Yang Method
     d=Dose.Level
     p=Sim.Res/n;
     A=Sim.Res-0.5*n
     S=cumsum(A)
     Ss=min(S)
     s=which(S==Ss)

     J=function(s)
       (
         if (length(s)>=2)
           (return(min(s)+1))
         else
           (return(s+1))
        )
     h=J(s)

     LC50[run]=d[h-1]+(d[h]-d[h-1])*(0.5-p[h-1])/(p[h]-p[h-1])
   }

  Mean.LC50[j]=mean(LC50,na.rm=TRUE)
```

```
                      #remove the unavailable value of LC50 because h>length of dose levers


      MSE.LC50[j]=mean((LC50-10^Log.True.Mean)^2, na.rm=TRUE);
                           #remove the unavailable value of LC50 because h>length of dose levers


      j=j+1;
}


 Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50))
 dimnames(Result)=list(c("","","","",""), c("Sample.Size","Mean","MSE"))
     {
     cat("                      Simulation Result","\n");
     cat("       Equally Spaced X:", Eqx.or.Not,","," ", "Same Number of subjectss:",
         Eqn.or.Not,"\n\n")
     Result
      }
```

A.7 Maximum Likelihood

```
 rm(list=ls())
 set.seed(987654)


 Eqx.or.Not=0      # 0=No, 1=Yes
 Eqn.or.Not=0     # 0=No, 1=Yes
# Define Dose Levels
 Dose.Level=Eqx.or.Not*c(10^0.1, 10^0.2, 10^0.3, 10^0.4, 10^0.5, 10^0.6, 10^0.7)+
          (1-Eqx.or.Not)*c(1,2,3,3.5,5,7,9);


 Num.Dose.Level=length(Dose.Level);


# Dose Level Transformations


 Tran.Dose.Level=log10(Dose.Level);


# Define Tolerance Distribution


 Log.True.Mean=log10(mean(Dose.Level));
 Log.True.Sd=log10(sd(Dose.Level));
```

```
#Tol.Dist=function(u){pnorm(u,Log.True.Mean,Log.True.Sd)};
 # normal distribution
 Tol.Dist=function(u){pcauchy(u, location=Log.True.Mean, scale, scale=Log.True.Sd)};                    # Cauchy
distribution


 # Generate True Response Rate

  True.Res.Rate=Tol.Dist(Tran.Dose.Level);


 # Generate Sample

  Mean.LC50=Mean.LC50.new=Ep.Level=MSE.LC50=MSE.LC50.new=rep(0,5);
  j=1;
  for(size in c(10,20,30,40,50))
   {
    n=(Eqn.or.Not)*rep(size,Num.Dose.Level)+(1-Eqn.or.Not) * ( size+round(
        3*runif(Num.Dose.Level,-1,1)))

    #n=rep(size,Num.Dose.Level); # Population Size at Each Dose Level
    Rep.in.Sim=500;
    m=m.new=rep(0,Rep.in.Sim);
     for(run in seq(Rep.in.Sim))
      {
       Sim.Res=rep(0,Num.Dose.Level)
       for(i in seq(Num.Dose.Level))
          {
            Sim.Res[i]=rbinom(1, n[i], True.Res.Rate[i])
          }

       # Maximum Likelihood Method
        X=Tran.Dose.Level;
        RRes=Sim.Res/n
        myglm=glm(RRes~X, family=binomial(link=probit))
        m[run]=-myglm$coefficients[1]/myglm$coefficients[2]
       }
     Mean.LC50[j]=mean(10^m)
     MSE.LC50[j]=mean((10^m-10^Log.True.Mean)^2);

     j=j+1;
}
```

```
Result=as.matrix(cbind(seq(5)*10,Mean.LC50,MSE.LC50))
dimnames(Result)=list(c("","","","",""), c("Sample.Size","Mean","MSE"))
    {
     cat("                    Simulation Result","\n");
     cat("        Equally Spaced X:", Eqx.or.Not,","," ", "Same Number of subjectss:",
       Eqn.or.Not,"\n\n")
     Result
     }
```