VARIANCE FUNCTIONS AND THE MINIMUM DETECTABLE CONCENTRATION IN ASSAYS

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Abstract

Assay data are often fit by a nonlinear regression model incorporating heterogeneity of variance, as in radioimmunoassay, for example. Typically, the standard deviation of the response is taken to be proportional to a power θ of the mean. There is considerable empirical evidence suggesting that for assays of a reasonable size, how one estimates the parameter θ does not greatly affect how well one estimates the mean regression function. An additional component of assay analysis is the estimation of auxillary constructs such as the minimum detectable concentration, for which many definitions exist; we focus on one such definition. The minimum detectable concentration depends both on θ and the mean regression function. We compare three standard methods of estimating the parameter θ due to Rodbard (1978), Raab (1981a) and Carroll and Ruppert (1982b). When duplicate counts are taken at each concentration, the first method is only 20% efficient asymptotically in comparison to the third, and the resulting estimate of the minimum detectable concentration is asymptotically 3.3 times more variable for first than the third. Less dramatic results obtain for the second estimator compared to the third; this estimator is still not efficient, however. Simulation results and an example are supportive of the asymptotic theory.

1. Introduction

The analysis of assay data has long been an important problem in clinical chemistry and the biological sciences; see, for example, Finney (1964) and Oppenheimer, et al. (1983). The most common method of analysis is to fit a nonlinear regression model to the data. Much recent work suggests that these data can be markedly heteroscedastic; in radioimmunoassay, for example, this characteristic has been observed repeatedly and incorporated into the analysis as discussed by Finney (1976), Rodbard (1978), Tiede and Pagano (1979) and Raab (1981a,b) and Butt (1984). Such analyses are for the most part special cases of the heteroscedastic nonlinear regression model. Specifically, we observe independent counts Y_{ij} at concentrations x_i for $i = 1,\ldots,N$ and $j = 1,\ldots M_i$ with mean and variances given by

$$(1.1) \qquad E\, Y_{ij} = \mu_i = f(x_i,\beta) \quad : \quad var(Y_{ij}) = \{\sigma g(x_i,\beta,\theta)\}^2,$$

where β is the unknown regression parameter vector of length p and θ is the structural variance parameter. A fairly standard model for the mean in a radioimmunoassay is the four parameter logistic model

$$(1.2) \qquad f(x,\beta) = \beta_1 + (\beta_2 - \beta_1)/[1 + \exp\{\beta_4(\log x - \beta_3)\}].$$

Almost without exception, the variances have been modeled as functions of the mean response, usually either as a quadratic or as a power of the mean, e.g.,

$$(1.3) \qquad \sigma_i = \text{Standard deviation of } Y_{ij} = \sigma g(x_i,\beta,\theta) = \sigma f(x_i,\beta)^{\theta}.$$

The fundamental contribution of Rodbard and other workers has been to

incorporate the heterogeneity into the analysis; the result of their contribution has been a great improvement in the quality of statistical analysis.

Methods of estimating β and θ are discussed in Section 2. The most common method of estimating β is generalized least squares. By various devices, one forms estimates $\hat{\sigma}_i$ of the variances σ_i and then estimates β by weighted least squares. As discussed by Jobson and Fuller (1980) and Carroll and Ruppert (1982b), under quite general circumstances for large enough sample sizes how one estimates the variance does not matter, and $\hat{\beta}$ is asymptotically normally distributed with mean β and variance $(\sigma^2/N_S)S_G^{-1}$, where N_S is the total sample size and

$$S_G = N_S^{-1} \Sigma_{i=1}^{N} \Sigma_{j=1}^{M_i} \{f_\beta(x_i,\beta) f_\beta(x_i,\beta)^T\}/g^2(x_i,\beta,\theta), \tag{1.4}$$

and f_β the derivative of f with respect to β. It has been shown that for estimation of β, how one estimates the variance function, in particular the parameter θ, has only a second order effect asymptotically, see, for example, Rothenberg (1984). The general asymptotic result (1.4) can be optimistic, but in our experience for RIA and ELISA assays, the asymptotics are often rather reasonable.

What the previous discussion suggests is that if our only interest is to estimate β, then in many assays the method of estimating the variance function may not be crucial. However, the assay problem does not always stop with estimating β, but rather also addresses issues of calibration. These issues include confidence intervals for a true x_* given a new Y_*, the classic calibration problem. Also of interest is determining the sensitivity of the assay using such concepts as the minimum detectable concentration of Rodbard

(1978) and the critical level, detection level and determination limit of Oppenheimer, et al. (1983). A unique feature of these calibration problems is that the efficiency of estimation is essentially determined by how well one estimates the variance parameter θ; the purpose of this paper is to justify this claim.

To the best of our knowledge, our paper is one of the first which shows explicitly that how one estimates the structural variance parameter θ can be important in determining the behavior of estimates of interesting quantities. Far from being only a nuisance parameter as it is often thought to be, θ is a quantity which has an important role in the analysis of calibration and prediction problems. In addition, θ can be important in itself, as in for example off line quality control, see Box and Meyer (1986). In this latter application, one might want to find the levels of x which give minimum variance subject to a constraint on the mean.

Our general qualitative conclusion is that how well one estimates θ really matters. Instead of pursuing a fully general theory, we focus on the determination of minimum detectable concentration. There is no unique definition of this concept, and for illustration we pick one of the possible candidates.

Definition. Let $\overline{Y}(x,M)$ be the mean response based on M replicates at concentration level x, taken independently of the calibration data set $\{Y_{ij}\}$. Let $f(0,\beta)$ be the expected response at zero concentration based on the calibration data set. The minimum detectable concentration x_c at level $(1-\alpha)$ is the smallest concentration x for which

$$\Pr\{\overline{Y}(x,M) \geq f(0,\beta)\} > 1 - \alpha. \qquad (1.5)$$

□

Qualitatively, the minimum detectable concentration is arrived at as illustrated in Figure 1. One first constructs the estimated regression function $f(x,\hat{\beta})$, and then attaches to it an estimated lower $(1-\alpha)$ confidence line for the new response $\overline{Y}(x,M)$ at concentration x based on M replicates. Starting from the estimated zero concentration mean $f(0,\hat{\beta})$, one does a standard calibration by drawing a horizontal line until it intersects the lower confidence interval, the value of x at which this intersection occurs being the minimum detectable concentration. In Figure 1, we illustrate why getting a good handle on the variance function is important. Assuming as is natural that the variance is smallest where the mean count is smallest, we see that the prediction interval based on an unweighted analysis is much too conservative for low concentrations. This translates immediately into a large bias in the estimated minimum detectable concentration. Even if the heterogeneity of variance is taken into account, Figure 1 makes clear that a poor estimate of θ can have considerable impact on the estimated minimum detectable concentration.

We now outline the standard method for estimating the minimum detectable concentration. To be more precise and follow the outline given in the preceeding paragraph, one would replace the t-percentage point to follow with an asymptotically negligible correction based on the limit distribution of the estimates of (θ,β,σ). This program has not been followed in practice since the last limit distribution has been unknown, and in any case the effect is asymptotically unimportant. If $t(\alpha,N_s-p)$ is the $(1-\alpha)^{th}$ percentile of the t-distribution with N_s-p degrees of freedom, the usual estimate $\hat{x}_c$ of x_c satisfies

$$(1.6)\quad \{f(\hat{x}_c,\hat{\beta}) - f(0,\hat{\beta})\}^2 = \{t(\alpha,N_s-p)\}^2\{\hat{\sigma}^2 g^2(\hat{x}_c,\hat{\beta},\hat{\theta})/M + \hat{var}[f(0,\hat{\beta})]\},$$

where $\hat{var}[f(0,\hat{\beta})]$ is an estimate of the variance of $f(0,\hat{\beta})$ and $\hat{\sigma}^2$ is the usual mean squared error from the weighted fit:

$$\hat{\sigma}^2 = (N_S-p)^{-1}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}\{Y_{ij} - f(x_i,\hat{\beta})\}^2 f^{-2\theta}(x_i,\hat{\beta}).$$

In Section 2 of this paper, we discuss three standard methods for estimating the variance parameter θ. Under relatively general conditions, two of these can be quite a bit less efficient than the third, and we discuss in Section 3 how this difference translates theoretically to the minimum detectable concentration problem. In Sections 4 and 5, we present a small Monte-Carlo study and an example to illustrate the results. The key conclusion is that how one estimates θ can affect the relative efficiency of estimated quantities useful in the calibration of assays.

2. Methods of estimating mean and variance parameters

The problem of estimating θ in models (1.1) and (1.3) has been discussed in many places in the literature. A nice introduction is given by Judge, et al. (1935, Chapter 11). More specialized and formal treatments include those by Rodbard (1978), Jobson and Fuller (1980), Raab (1981a), Carroll and Ruppert (1982b) and Davidian and Carroll (1986), although these only represent a sampling of the possible selection. We focus our attention on three methods with quite different motivations. For simplicity, we will discuss only the case of equal replication $M_i = M \geq 2$. The first two methods require some replication.

2.1 Log-linearized estimation

Model (1.3) implies upon taking logarithms that the log standard deviation is linear in the log mean with slope θ. Letting $(\overline{Y}_{i\cdot}, S_i^2)$ be the within concentration sample means and variances, this suggests that one estimate θ as the slope from regressing log S_i on log $\overline{Y}_{i\cdot}$. If we denote this estimate as $\hat{\theta}_{LL}$, Rodbard (1978) suggests forming estimated standard deviations as the sample mean to the power $\hat{\theta}_{LL}$, and then applying weighted least squares to estimate β.

2.2 Modified maximum likelihood

Raab (1981a) suggests a method for estimating θ using normal theory maximum likelihood but without making any assumptions about the form of the mean function. Raab assumes independence and proposes estimation of θ by joint maximization of the "modified" normal likelihood

$$(2.1) \qquad \Pi_{i=1}^{N}\{2\pi\sigma^2 g(\mu_i,\theta)\}^{(m-1)/2}\exp[-\Sigma_{j=1}^{m}(Y_{ij}-\mu_i)^2/(2\sigma^2 g(\mu_i,\theta))]$$

in the parameters σ, θ, $\mu_1,\ldots,\mu_N$, where we have written $g(x_i,\beta,\theta)$ as $g(\mu_i,\theta)$ to emphasize the dependence of the variance function on the mean response. The modification serves to make the estimator of σ^2 unbiased. Estimation of β may now proceed via weighted least squares in a fashion analagous to the log-linearized method.

2.3 Pseudo-likelihood

For given θ, the pseudo-likelihood estimator of θ is the normal theory maximum likelihood estimate, maximizing

$$(2.2) \qquad -\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}\log\{g(x_i,\hat{\beta},\theta)\} - (N/2)\log[N^{-1}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}\{Y_{ij}-f(x_i,\hat{\beta})\}^2 g(x_i,\hat{\beta},\theta)^{-2}],$$

see Carroll and Ruppert (1982b). One can devise many ways to estimate θ and β jointly. For example, one can

(i) Set $\hat{\beta}$ = unweighted least squares;

(ii) Estimate θ by pseudo-likelihood;

(iii) Form estimated variances $g^2(x_i,\hat{\beta},\hat{\theta})$;

(iv) Re-estimate β by weighted least squares;

(v) Iterate (ii) - (iv) one or more times.

The number of cycles $\mathcal{C}$ of this algorithm is the number of times one hits step (iv). One can do step (ii) by direct maximization or by weighted least squares as in Davidian and Carroll (1986).

A key point to note is that pseudo-likelihood requires no replication and hence easily copes with unequal replication.

2.4 Other methods

Various other methods have been proposed; see, for example, Jobson and

Fuller (1980) and Box and Hill (1974). Robust variance function estimation methods have also been developed, see Carroll and Ruppert (1982b) and Giltinan, Carroll and Ruppert (1986).

A final method of jointly estimating (β,θ) is normal theory maximum likelihood. There are important issues of robustness which complicate routine use of this method, see McCullagh (1983), Carroll and Ruppert (1982a) and Davidian and Carroll (1986) for further discussion. For assay data, pseudo-likelihood and maximum likelihood estimates of θ have similar asymptotic behavior, and we will use the former largely for its ease of calculation.

3. Asymptotic theory

The asymptotic theory of the log-linearized estimator $\hat{\theta}_{LL}$ is complicated because regressing log S_i on log $\overline{Y}_{i\cdot}$ is not a standard linear regression problem. Likewise, the asymptotic theory for the modified maximum likelihood estimator $\hat{\theta}_{MML}$ is complicated because the dimension of the parameter space increases with N. Both of these problems are nonlinear functional errors-in-variables problems of kind addressed by Wolter and Fuller (1982), Amemiya and Fuller (1985) and Stefanski and Carroll (1985). The error in estimating μ_i by $\overline{Y}_{i\cdot}$ in $\hat{\theta}_{LL}$ or by the joint estimator $\hat{\mu}_i$ in $\hat{\theta}_{MML}$ causes these estimators to be biased asymptotically. This bias is typically negligible, because in most of the assays we have seen the parameter σ in (1.1) is quite small. Thus, empirically it makes sense to define an asymptotic theory where the sample size $N_S = NM$ becomes large and σ simultaneously is small. Because in most assays the number of replicates M is small, we shall let $N \to \infty$ and $\sigma \to 0$ while keeping M fixed; Raab (1981a) suggests that M = 2 is the most common

case.

It is important for the reader to understand that letting $N \to \infty$ and $\sigma \to 0$ simultaneously is dictated by the problems of studying the log-linearized estimator $\hat{\theta}_{LL}$ and the modified maximum likelihood estimator $\hat{\theta}_{MML}$: the pseudo-likelihood estimator $\hat{\theta}_{PL}$ has a routine asymptotic theory even for fixed σ.

The asymptotic distribution of these estimates of θ can be obtained from the general theory of Davidian and Carroll (1986). Define

$$\epsilon_{ij} = \{Y_{ij} - f(x_i,\beta)\}/\{\sigma f(x_i,\beta)^{\theta}\}, \qquad v_i = \log f(x_i,\beta),$$

$$q_i^2 = \{(M-1)^{-1}\Sigma_{i=1}^{M}(\epsilon_{ij} - \bar{\epsilon}_{i\cdot})^2\}, \quad \sigma_v^2 = \lim_{N\to\infty} (N-1)^{-1}\Sigma_{i=1}^{N}(v_i - \bar{v})^2.$$

Theorem 1. As $N \to \infty$ and $\sigma \to 0$ simultaneously and $N^{1/2}\sigma = O(1)$, if the random variables $\{\epsilon_{ij}\}$ are symmetric and independent and identically distributed, then

$$N^{1/2}(\hat{\theta}_{LL} - \theta) \xrightarrow{\mathcal{L}} N(0,\ \mathrm{var}\{\log q_i\}/(4\sigma_v^2)),$$

$$N^{1/2}(\hat{\theta}_{MML} - \theta) \xrightarrow{\mathcal{L}} N(0,\ \mathrm{var}(q_i^2)/(4\sigma_v^2)),$$

$$N^{1/2}(\hat{\theta}_{PL} - \theta) \xrightarrow{\mathcal{L}} N(0,\ \mathrm{var}(\epsilon_{ij}^2)/(4M\sigma_v^2)). \quad \square$$

Under these asymptotics, the symmetry condition is necessary to ensure that the asymptotic distributions of $\hat{\theta}_{LL}$ and $\hat{\theta}_{MML}$ have zero mean; symmetry is unnecessary for the result for $\hat{\theta}_{PL}$. Sadler and Smith (1985) note that the estimator obtained by replacing μ_i by $\bar{Y}_{i\cdot}$ in (2.1) and maximizing in σ^2 and θ is virtually indistinguishable in practice from the full modified maximum

likelihood estimator of θ. It can be shown that these two estimators are asymptotically equivalent under the above asymptotics and that this second estimator is equivalent to the pseudo-likelihood estimator with $f(x_i,\hat{\beta})$ replaced everywhere by $\bar{Y}_{i\cdot}$ in (2.2); thus, as an approximation to $\hat{\theta}_{MML}$ one could compute the pseudo-likelihood estimator using $\bar{Y}_{i\cdot}$.

From Theorem 1, the asymptotic relative efficiencies of the log-linearized method and the modified maximum likelihood method relative to pseudo-likelihood can be computed. Note that

$$\text{(3.1)} \qquad \operatorname{var}(q_i^2) = \operatorname{var}(\epsilon_{ij})/M + 2/\{M(M-1)\}$$

so that $\hat{\theta}_{MML}$ has uniformly larger asymptotic variance than $\hat{\theta}_{PL}$ for all $M \geq 2$ regardless of the distribution of the $\{\epsilon_{ij}\}$.

We have tabulated the relative efficiencies of $\hat{\theta}_{LL}$ and $\hat{\theta}_{MML}$ to $\hat{\theta}_{PL}$ for various numbers of replications M, assuming normally distributed data.

Asymptotic relative efficiencies of estimators of θ for small σ

M	$\hat{\theta}_{LL}$ to $\hat{\theta}_{PL}$	$\hat{\theta}_{MLL}$ to $\hat{\theta}_{PL}$	$\hat{\theta}_{LL}$ to $\hat{\theta}_{MLL}$
2	0.203	0.500	0.405
3	0.405	0.667	0.608
4	0.535	0.750	0.713
9	0.783	0.889	0.881
10	0.804	0.900	0.893

In our experience, these numbers slightly exaggerate the inefficiency of $\hat{\theta}_{LL}$ relative to pseudo-likelihood, especially when the assay is rather small. For duplicates M = 2, in two assay simulations, we have found that the variance efficiency of the log-linearized method was 0.35 and 0.31; the former number is reported in the next section. The asymptotic relative efficiencies of $\hat{\theta}_{LL}$ relative to $\hat{\theta}_{MML}$ agree quite well with the efficiencies of 39%, 62% and 77% and 39%, 64% and 74% for M = 2, 3 and 4 reported by Raab (1981a) and Sadler and Smith (1985), respectively, in two Monte-Carlo studies. While modified maximum likelihood represents an improvement over the log-linearized method, the theory clearly points to the inefficiency of both the log-linearized and modified maximum likelihood methods when the number of replicates is small and the data are nearly normally distributed.

For the minimum detectable concentration, note that in (1.6) the term $\hat{\text{var}}\{f(0,\hat{\beta})\}$ is of the order $(NM)^{-1}$ and is hence rather small relative to all the other terms. Of course, for normally distributed data, the solution to (1.6) is the quantity x_c^*, where

$$(3.2) \qquad 0 = \{z(\alpha)\}^2\sigma^2 f^{2\theta}(x_c^*,\beta)/M - \{f(x_c^*,\beta) - f(0,\beta)\}^2,$$

ans $z(\alpha)$ is the $(1 - \alpha)^{th}$ percentile point of the standard normal distribution.

Here is the major result, the technical details for which are given in the appendix. Define

$$(3.3) \qquad d_0 = \log f(0,\beta) - \lim_{N\to\infty} N^{-1}\Sigma_{i=1}^{N} \log f(x_i,\beta).$$

Theorem 2. Let $\hat{x}_c(LL)$, $\hat{x}_c(MML)$ and $\hat{x}_c(PL)$ denote the estimated minimum detectable concentrations using the log-linearized estimate $\hat{\theta}_{LL}$, the modified

maximum likelihood estimate $\hat{\theta}_{MML}$ and the pseudo-likelihood estimate $\hat{\theta}_{PL}$ respectively. Then under regularity conditions, there is a constant A_0 and a sequence b_N for which

$$(3.4)\quad b_N A_0 N^{1/2}(\hat{x}_c(LL) - x_c^*)/\sigma \xrightarrow{\mathcal{L}} N(0,\ var(\epsilon_{ij}^2) + var(\log q_i^2)d_0^2M/\sigma_v^2),$$

$$(3.5)\quad b_N A_0 N^{1/2}(\hat{x}_c(MML) - x_c^*)/\sigma \xrightarrow{\mathcal{L}} N(0,\ var(\epsilon_{ij}^2) + var(q_i^2)d_0^2M/\sigma_v^2),$$

$$(3.6)\quad b_N A_0 N^{1/2}(\hat{x}_c(PL) - x_c^*)/\sigma \xrightarrow{\mathcal{L}} N(0,\ var(\epsilon_{ij}^2)\{1 + d_0^2/\sigma_v^2\}). \quad \square$$

From (3.1), the asymptotic relative efficiency of the modified maximum likelihood estimate to minimum detectable concentration relative to the pseudo-likelihood estimate is

$$(3.7)\quad var(\epsilon_{ij}^2)(\sigma_v^2+d_0)/\{var(\epsilon_{ij}^2)(\sigma_v^2+d_0) + 2d_0^2/(M-1)\}$$

which is less than 1 for all M regardless of the value of $var(\epsilon_{ij}^2)$. Similarly, the asymptotic relative efficiency of the log-linearized estimate of minimum detectable concentration to the pseudo-likelihood estimate is

$$(3.8)\quad var(\epsilon_{ij}^2)(\sigma_v^2+d_0^2)/\{\sigma_v^2 var(\epsilon_{ij}^2) + Md_0^2 var(\log q_i^2)\}.$$

It follows from Theorem 1 and (3.7) and (3.8) that the ordering in efficiency of estimated minimum detectable concentration is the same as the ordering for estimating θ and thus will favor pseudo-likelihood for normally distributed data in the case of the log-linearized estimator and for all distributions in the case of the modified maximum likelihood method. For distributions other

than normal, calculations with other symmetric distributions such as double exponential and various contaminated normal distributions show very few cases where $\hat{\theta}_{LL}$ is more efficient than $\hat{\theta}_{PL}$, see Davidian (1986). The numerical efficiencies depend on the logarithm of the true means through d_0^2 and σ_v^2. For example, in the simulation discussed in the next section, the asymptotic relative efficiency of the log-linearized estimate is 27% for M = 2 and 63% for M = 4.

The asymptotic theory thus suggests that inefficiencies in estimating the variance parameter θ translate into inefficiencies for estimating the minimum detectable concentration.

4. A simulation

To check the qualitative nature of the asymptotic theory, we ran a small simulation. We restrict our focus here to the log-linearized method and pseudo-likelihood.

The responses Y_{ij} were normally distributed with mean and variance satisfying (1.2), (1.3), where $\beta_1 = 29.5274$, $\beta_2 = 1.8864$, $\beta_3 = 1.5793$, $\beta_4 = 1.0022$, $\theta = 0.7$ and $\sigma = .0872$. The 23 concentrations chosen are given in Table 4. We studied the case M = 2 or duplicates and M = 4 or quadruplicates. For each situation, there were 500 simulated data sets. A limited second simulation was run with the larger value $\sigma = 0.17$, but there did not appear to be significant qualitative differences from the case reported here.

The estimators chosen were unweighted least squares for β, the Rodbard log-linearized method and the pseudo-likelihood/generalized least squares combination which we report only for $\ell = 1$ and 2 cycles of the algorithm. The

methods of estimating the minimum detectable concentration are as discussed in Section 1. The estimates of θ were constrained to lie in the interval $0 \leq \theta \leq 1.50$.

In Table 1, we compare the estimators of θ on the basis of bias and variance. The biases are large relative to the standard error, so that mean-squared error comparisons are artificial and dramatic. The bias in the pseudo-likelihood estimate of θ when doing only $\ell = 1$ cycles of the algorithm has been observed by us in other problems. One sees here that the effect of doubling the replicates from two to four for a given set of concentrations improves the Monte-Carlo efficiency of the log-linearized estimate of θ from 35% to 58%, compared to the theoretical asymptotic increase from 20% to 54%. This example indicates that pseudo-likelihood estimation of θ can in some circumstances be a considerable improvement over the log-linearized method.

For the minimum detectable concentration we chose $\alpha = 0.05$. For all of the methods used in the study, the probability requirement (1.5) was easily satisfied; rather than 95% exceedance probability, every case was more than 97%. The mean values of the minimum detectable concentrations are reported in Table 2, with variances given in Table 3. Note that in both of these tables, we give results for the case that θ is known as well as estimated. The relatively poor behavior of unweighted least squares is evident. To quote from Oppenheimer, et al. (1983): "Rather dramatic differences have been observed depending on whether a valid weighted or inappropriate unweighted analysis is used." When the variance parameter θ is known, there is little difference between any of the weighted methods.

When θ is unknown, there are rather large proportional differences. The figures in Table 4 show that the mean minimum detectable concentration for the log-linearized method is 10% larger than for the pseudo-likelihood method based

on ℓ = 2 cycles; whether the raw numerical difference is of any practical consequence will depend on the context.

For M = 2 replicates, the pseudo-likelihood estimate of minimum detectable conentration with unknown θ has mean 3.934×10^{-2} and standard deviation 0.05×10^{-4}; the corresponding figures for M = 4 are 2.722×10^{-2} and 0.028×10^{-4}. Proportionately, when θ is unknown, the method of estimating it seems to have important consequences for the estimate of minimum detectable concentration, particularly in the variability of the estimate. For the case of duplicates, the Monte-Carlo variance of pseudo-likelihood is only 37% as large as that based on the log-linearized estimate, while the asymptotics suggest 27%, increasing to 71% and 63% respectively for quadruplicates.

The point here is that the relative efficiency of the estimated minimum detectable concentration can be affected by the algorithm used to estimate the variance parameter θ.

5. An example

Differences among the three estimators of θ and the subsequent estimators of minimum detectable concentration which are reminiscent of the qualitative implications of the asymptotic theory and simulation can be seen in the following example. The data are from a radioimmunoassay and are presented in Table 4. The analysis presented here is for illustrative purposes only; we do not claim to be analyzing these data fully. Our aim is to exhibit the fact that the three methods of analysis considered in this paper can lead to nontrivially different results.

We assumed in all cases the model (1.2) and (1.3). For the full data set

and reduced data sets considering all possible permutations of duplicates (except one set for which an $S_i^2 = 0$, complicating the application of the log-linearized method), we computed the estimates of θ, σ^2 and x_c using the pseudo-likelihood and log-linearized methods and the estimate of Sadler and Smith (1985) as described in Section 3 in place of the more computationally difficult modified maximum likelihood estimate. We also computed the estimate of minimum detectable concentration based on ordinary least squares. The results are given in Table 5.

An investigation of both the full and reduced data sets suggests that there are no massive outliers and that design points 1, 22 and 23 are possible high leverage points. For our purposes of illustration we do not pursue this point; see Davidian and Carroll (1986) for discussion on accounting for leverage in estimation of θ.

The results of Table 5 show that the three estimates can vary greatly. As a crude measure of this, consider the means and standard deviations of $\hat{\theta}$ and $\hat{x}_c$ for the five data sets obtained by considering duplicates (ignoring the fact that these data sets are not strictly independent). Below we list "relative efficiencies" for the estimators based on these crude measures:

"Relative efficiencies" for estimators of θ and x_c

for data in example when M =2

	LL to PL	MML to PL	LL to MML
$\hat{\theta}$	.222	.351	.632
$\hat{x}_c$	.529	.659	.802

Qualitatively, the estimates exhibit the type of behavior predicted by the

asymptotic theory; quantitatively, the values compare favorably with what the theory would predict given the crudity of the comparison.

This example shows that there can be wide differences among the various estimation methods for θ and minimum detectable concentration in application and that the qualitative way in which the differences manifest themselves is predicted by the asymptotic theory of Section 3.

6. Incorporating unknowns and standards

In many assays, along with the known standards $\{Y_{ij}, x_i\}$ there is an additional set $\{Y^*_{ij}\}$ of proportional size at unknown concentrations $\{x^*_i\}$, $i = 1,\ldots,N^*$, $j = 1,\ldots,M_i$. It is common to assume that these unknowns satisfy

$$\text{Standard Deviations } (Y^*_{ij}) = \sigma_*(E\ Y^*_{ij})^{\theta}. \tag{6.1}$$

The power of the log-linearized or modified likelihood methods is that they can incorporate the responses at unknown concentrations to obtain a better estimate of θ; pseudo-likelihood and other similar techniques cannot easily incorporate this information because they rely on knowing the concentrations $\{x^*_i\}$. A simple way to improve pseudo-likelihood to take into account the unknowns is to incorporate the additional information about the variances in the unknowns by exploiting an estimator that does not depend on the form of the mean response. For the exposition here, consider the log-linearized estimator; we could equally well employ the same idea using the modified maximum likelihood estimator. Let $\hat{\theta}_u$ and $\hat{\theta}_{s,u}$ denote the log-linearized estimates of θ based on the unknowns alone or the full data respectively, and let $\hat{\theta}_{PL}$ denote the

pseudo-likelihood estimate based on the standards alone. Let S_u^2 denote the variance estimate of $\hat{\theta}_u$ produced by the linear least squares program and let S_{PL}^2 be the estimated variance of $\hat{\theta}_{PL}$, where following Theorem 1

$$S_{PL}^2 = (4N_S)^{-1} \frac{\{\text{sample variance of standardized squared residuals } r_{ij}\}}{\{\text{sample variance of log predicted values log } f(x_i,\beta)\}},$$

$$r_{ij} = \{Y_{ij} - f(x_i,\hat{\beta})\}/\{\hat{\sigma} f(x_i,\hat{\beta})^{\hat{\theta}}\}.$$

Then a weighted estimate of θ is simply

$$\hat{\theta} = w\hat{\theta}_{PL} + (1-w)\hat{\theta}_u, \quad w = S_u^2/(S_u^2 + S_{PL}^2); \tag{6.2}$$

we prefer to replace $\hat{\theta}_u$ by $\hat{\theta}_{s,u}$ in (6.2). The weighted estimate (6.2) will improve upon the log-linearized estimate $\hat{\theta}_{s,u}$ based on all the data, although the degree of improvement will be smaller than that found in Sections 3 or 4. For example, if there are exactly as many unknowns as standards and duplicates are used, then $\hat{\theta}_{s,u}$ has asymptotic relative efficiency of 34% versus 20% when only standards are available.

7. Discussion

We have addressed the general issue of estimating calibration quantities in assays which exhibit large amounts of heterogeneity. We have shown that not weighting at all leads to large decreases in the efficiency of analysis. Even

when weighting is used, we have shown that changes in relative efficiency occur depending on the method of estimating the variances, especially the parameter θ in (1.3). The key point is that while for estimation of β the effect of how one estimates the variance function is only second order, for estimation of other quantities such as minimum detectable concentration, the effect is first order.

We have had success using the idea of pseudo-likelihood in Carroll and Ruppert (1982b); this method applies in general heteroscedastic models and is easy to compute as shown in the appendix, although the reader should be aware that it is not robust against outliers.

One can also consider data transformation rather than weighting. The transform-both-sides idea in Carroll and Ruppert (1984) applies to the assay problem.

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Appendix

The analysis of the minimum detectable concentration is complicated by the behavior of the derivative of $f(x,\beta)$ with respect to β at x=0, especially for the standard model (1.2). We will write $f(x,\beta) = h(\eta,\beta)$, where $\eta = \ell(x,\beta)$, $\eta_c^* = \ell(x_c^*,\beta)$ and $\hat{\eta}_c = \ell(\hat{x}_c,\beta)$. In the model (1.2), $\eta = \ell(x,\beta) = \exp(\beta_4 \log x)$. We assume throughout that $f(0,\beta) > 0$, and that all functions are sufficiently smooth. Assume further that

(A.1) $$\ell(0,\beta) = 0 ;$$

(A.2) $$\partial/\partial\eta\ h(0,\beta) = h_{\eta}(0,\beta) \neq 0\ ;$$

(A.3) $$\ell_{\beta}(0,\beta) = 0\ ;$$

(A.4) If $w \to 0$ and v is a random variable such that

$$\ell(v,\beta)/\ell(w,\beta) \xrightarrow{p} 1, \text{ then}$$

$$\sup\{\ |\ \ell_{\eta}(\alpha v+(1-\alpha)w,\beta)/\ell_{\eta}(w,\beta) - 1\ |\ \text{for } 0\leq\alpha\leq 1\ \} \xrightarrow{p} 0\ .$$

These assumptions are satisfied for the model (1.2) if $\beta_4 > 0$.

We need the following results. The proofs of Lemmas A.2 and A.3 will be at the end of the appendix. Let $c = \{z(\alpha)\}^2$.

Lemma A.1 As $\sigma \to 0$ for $f(0,\beta) > 0$,

$$\eta_c^* = \sigma a_c + \mathcal{O}(\sigma^2), \qquad a_c = (c/M)^{1/2} f^{\theta}(0,\beta)\{\partial/\partial\eta\ h(0,\beta)\}^{-1}.$$

Proof: A Taylor series expansion of (3.2) in η_c^* and around zero. □

Lemma A.2 Assume that as $N \to \infty$, $\sigma \to 0$, $(\hat{\eta}_c - \eta_c^*) = \mathcal{O}_p(\sigma N^{1/2})$. Define

$$A_1 = 2Ma_c\{\partial/\partial\eta\ h(0,\beta)\}^2/\{cf^{2\theta}(0,\beta)\}.$$

Then as $N \to \infty$, $\sigma \to 0$, if $N^{1/2}(\hat{\theta} - \theta) = \mathcal{O}_p(1)$, we have the asymptotic expansion

(A.5) $$A_1 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma$$
$$= N^{1/2}(\hat{\sigma}^2 - \sigma^2)/\sigma^2 + 2\{\log f(0,\beta)\}\ N^{1/2}(\hat{\theta} - \theta) + o_p(1). \quad \square$$

Lemma A.3 Consider Lemma A.2. Then

$$(NM)^{1/2}(\hat{\sigma}^2 - \sigma^2)/\sigma^2$$
$$= (NM)^{-1/2}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}(\epsilon_{ij}^2 - 1) - 2\bar{v}(NM)^{1/2}(\hat{\theta} - \theta) + o_p(1)$$

so that if $A_0 = M^{1/2}A_1$,

$$A_0 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma$$
$$= (NM)^{-1/2}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}(\epsilon_{ij}^2 - 1) + 2d_0(NM)^{1/2}(\hat{\theta}-\theta) + o_p(1),$$

where d_0 is defined in (3.3). □

Proposition 1 : The limit results (3.4) - (3.6) hold for $A_0\ N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma$, where $\hat{\eta}_c = \hat{\eta}_c(LL)$, $\hat{\eta}_c(MML)$ or $\hat{\eta}_c(PL)$.

Proof of Proposition 1 : From Davidian and Carroll (1986), using Theorem 1 we have that

$$N^{1/2}(\hat{\theta}_{LL} - \theta) = (1/2)\ N^{-1/2}\ \Sigma_{i=1}^{N}\ \{\log q_i^2 - E(\log q_i^2)\}\ (v_i - \bar{v}) + o_p(1)\ ;$$
$$N^{1/2}(\hat{\theta}_{MML} - \theta) = (1/2)\ N^{-1/2}\ \Sigma_{i=1}^{N}\ \{q_i^2 - E(q_i^2)\}\ (v_i - \bar{v}) + o_p(1)\ ;$$
$$N^{1/2}(\hat{\theta}_{PL} - \theta) = (1/2)\ N^{-1/2}\ M^{-1}\ \Sigma_{i=1}^{N}\Sigma_{j=1}^{M}\ (\epsilon_{ij}^2 - 1)\ (v_i - \bar{v}) + o_p(1)\ ,$$

so that by Lemmas A.1 - A.3 and equation (A.5), we have

$$A_0 N^{1/2}(\hat{\eta}_c(PL) - \eta_c^*)/\sigma$$
$$= (NM)^{-1/2}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}(\epsilon_{ij}^2 - 1)\{1 + d_0(v_i - \bar{v})/\sigma_v^2\} + o_p(1),$$

which with the central limit theorem gives the same limit distribution as in (3.6). We also have that

$$A_0 N^{1/2}(\hat{\eta}_c(LL) - \eta_c^*)/\sigma = (NM)^{-1/2}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}(\epsilon_{ij}^2 - 1)$$
$$+ d_0 M^{1/2} N^{-1/2}\Sigma_{i=1}^{N}(v_i - \bar{v})\log q_i^2/\sigma_v^2 + o_p(1)$$

$$A_0 N^{1/2}(\hat{\eta}_c(MML) - \eta_c^*)/\sigma = (NM)^{-1/2}\Sigma_{i=1}^{N}\Sigma_{j=1}^{M}(\epsilon_{ij}^2 - 1)$$
$$+ d_0 M^{1/2} N^{-1/2}\Sigma_{i=1}^{N}(v_i - \bar{v})q_i^2/\sigma_v^2 + o_p(1).$$

Simple central limit theorem calculations yield the same limit distribution as in (3.4) and (3.5). □

Remark: Result (3.5) is based on $\hat{\sigma}$ obtained from the residuals of the final fit of the mean response function as for the log-linearized method and pseudo-likelihood, so that Lemma A.3 holds. The modified maximum likelihood method also provides a joint estimate of σ along with the estimate of θ. If one considers this estimator in place of $\hat{\sigma}$ in Lemma A.3, it can be shown that the resulting estimator of minimal detectable concentration has even larger asymptotic variance than that in (3.5). For reasons of space we do not prove this, but it certainly has interesting implications for practice.

Proof of Theorem 2 : By (A.5), for any of the estimators $\hat{x}_c$, since $\eta = \ell(x,\beta)$, we have the limit result that for some Δ,

(A.6) $$N^{1/2}\{\ \ell(\hat{x}_c,\beta) - \ell(x_c^*,\beta)\ \}/\sigma \xrightarrow{\mathcal{L}} N(0,\Delta).$$

Thus, for γ_c between $\hat{x}_c$ and x_c^*, defining

$$W_N = N^{1/2}\ \ell_x(x_c^*,\beta)\ (\hat{x}_c - x_c^*)\ /\ \sigma, \text{ we have}$$

$$W_N \, \ell_x(\gamma_c,\beta) \; / \; \ell_x(x_c^*,\beta) \quad \xrightarrow{\mathcal{L}} N(0,\Delta)\,.,$$

where $\ell_x(v,\beta)$ is the derivative of the first component of $\ell(v,\beta)$. It thus suffices through (A.4) to prove that

$$\ell(\gamma_c,\beta) \; / \; \ell(x_c^*,\beta) \xrightarrow{p} 1.$$

But this follows from (A.6) since $\eta_c^*/\sigma = \ell(x_c^*,\beta)/\sigma \to a_c$, see Lemma A.1. The result now follows from Propostion 1. □

Proof of Lemma A.2: By a series of Taylor expansions and using Lemma A.1,

(A.7)
$$\begin{aligned} & N^{1/2}\{h(\hat{\eta}_c,\hat{\beta}) - h(0,\hat{\beta})\}^2/\sigma^2 \\ &= N^{1/2}\{h(\hat{\eta}_c,\beta) - h(0,\beta)\}^2/\sigma^2 + o_p(N^{-1/2}) \\ &= N^{1/2}\{h(\eta_c^*,\beta) - h(0,\beta)\}^2/\sigma^2 \\ &\quad + 2\{h(\eta_c^*,\beta) - h(0,\beta)\}\{\partial/\partial\eta \; h(\eta_c^*,\beta)\}N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma^2 + o_p(N^{-1/2}) \\ &= N^{1/2}\{h(\eta_c^*,\beta) - h(0,\beta)\}^2/\sigma^2 \\ &+ 2\eta_c^*\{\partial/\partial\eta \; h(0,\beta)\}^2 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma^2 + o_p(1) \\ &= N^{1/2}\{h(\eta_c^*,\beta) - h(0,\beta)\}^2/\sigma^2 \\ &+ 2a_c\{\partial/\partial\eta \; h(0,\beta)\}^2 N^{1/2}(\hat{\eta}_c - \eta_c^*)/\sigma + o_p(1). \end{aligned}$$

Similar calculations taking into account that $N^{1/2}(\hat{\beta} - \beta) = o_p(\sigma)$ and $\eta_c^* \to 0$ yield

$$
\begin{aligned}
(A.8)\qquad & N^{1/2}h^{2\hat{\theta}}(\hat{\eta}_c,\hat{\beta})\hat{\sigma}^2/(M\sigma^2) \\
&= N^{1/2}h^{2\theta}(\eta_c^*,\beta)/M + h^{2\theta}(\eta_c^*,\beta)(N^{1/2}/M)(\hat{\sigma}^2-\sigma^2)/\sigma^2 \\
&\quad + (2/M)h^{2\theta}(\eta_c^*,\beta)\{\log h(\eta_c^*,\beta)\}N^{1/2}(\hat{\theta}-\theta) \\
&\quad + (2\theta/M)h^{2\theta-1}(\eta_c^*,\beta)h_\beta(\eta_c^*,\beta)N^{1/2}(\hat{\beta}-\beta) + o_p(1) \\
&= \{h^{2\theta}(\eta_c^*,\beta)/M\}N^{1/2} + (c/M)h^{2\theta}(0,\beta)N^{1/2}(\hat{\sigma}-\sigma)/\sigma^2 \\
&\quad + (2c/M)h^{2\theta}(0,\beta)\{\log h(0,\beta)\}N^{1/2}(\hat{\theta}-\theta) + o_p(1).
\end{aligned}
$$

Combining (1.6), (3.2), (A.7) and (A.8) yields (A.5). □

Proof of Lemma A.3: Define

$$\hat{\sigma}_0^2 = (NM)^{-1}\Sigma_{i=1}^N\Sigma_{j=1}^M[\{Y_{ij}-f(x_i,\beta)\}/f^\theta(x_i,\beta)]^2 = (NM)^{-1}\sigma^2\Sigma_{i=1}^N\Sigma_{j=1}^M\epsilon_{ij}^2.$$

Then, since $(NM)^{1/2}(\hat{\beta}-\beta) = o_p(1)$,

$$
\begin{aligned}
(NM)^{1/2}(\hat{\sigma}^2-\hat{\sigma}_0^2)/\sigma^2 &= (NM)^{-1/2}\Sigma_{i=1}^N\Sigma_{j=1}^M[\{Y_{ij}-f(x_i,\hat{\beta})\}^2/f^{2\hat{\theta}}(x_i,\hat{\beta}) \\
&\qquad -\{Y_{ij}-f(x_i,\beta)\}^2/f^{2\theta}(x_i,\beta)]\sigma^{-2}
\end{aligned}
$$

$$
\begin{aligned}
(A.7)\qquad &= (NM)^{-1/2}\Sigma_{i=1}^N\Sigma_{j=1}^M[\{Y_{ij}-f(x_i,\beta)\}^2/f^{2\hat{\theta}}(x_i,\beta) \\
&\qquad -\{Y_{ij}-f(x_i,\beta)\}^2/f^{2\theta}(x_i,\beta)]\sigma^{-2} + o_p(1) \\
&= -2\bar{v}(NM)^{1/2}(\hat{\theta}-\theta) + o_p(1),
\end{aligned}
$$

completing the proof. □

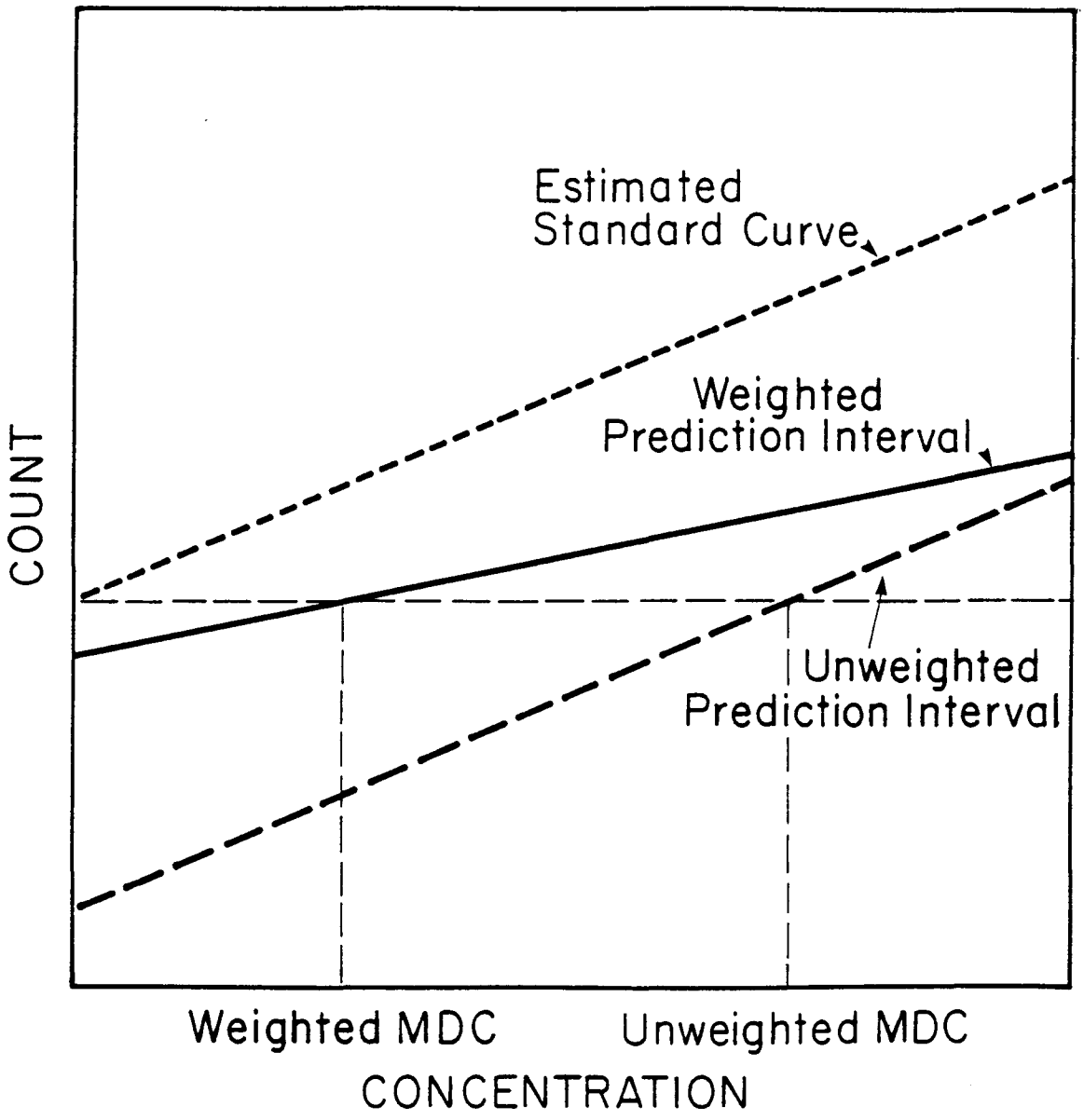

FIGURE 1

Schematic Representation of Estimated MDC for Small Concentrations

Table #1

Three Estimates of the Variance Parameter ϑ

Monte-Carlo Bias

	M = 2	M = 4
Log-linearized	0.15	0.001
Pseudo-likelihood		
ℓ = 1	0.045	0.022
ℓ = 2	0.000	0.001
ℓ = 3	0.004	0.000

Variance Relative to Pseudo-likelihood with ℓ = 2

	M = 2		M = 4	
	Monte-Carlo	Asymptotic	Monte-Carlo	Asymptotic
Log-linearized	2.85	4.93	1.71	1.87
Pseudo-likelihood				
ℓ = 1	0.99		0.99	
ℓ = 3	1.00		1.00	

Table #2

100 × Mean Minimum Detectable Concentrations

	M = 2 Replicates		M = 4 Replicates	
	θ Known	θ Estimated	θ Known	θ Estimated
Unweighted Least Squares	13.106	13.106	9.173	9.173
Log-Linearized	3.937	4.346	2.718	2.785
Pseudo-likelihood				
$\ell = 1$	-	4.216	-	2.809
$\ell = 2$	3.927	3.934	2.715	2.722

Table #3

Ratio of Monte-Carlo Variance of the Estimate of

Minimum Detectable Concentration Relative to Pseudo-likelihood

with ℓ = 2 Cycles

	M = 2 Replicates		M = 4 Replicates	
	θ Known	θ Estimated	θ Known	θ Estimated
Unweighted Least Squares	14.90	8.46	18.83	13.72
Log-linearized	1.02	2.72	1.00	1.41
Pseudo-likelihood				
ℓ = 1	-	1.19	-	1.10

Table #4

Data for Example of Section 5

Concentration (x)	Response (Y)
0.000	1.700, 1.660, 1.950, 2.070
0.075	1.910, 2.270, 2.110, 2.390
0.1025	2.220, 2.250, 3.260, 2.920
0.135	2.800, 2.940, 2.380, 2.700
0.185	2.780, 2.640, 2.710, 2.850
0.250	3.540, 2.860, 3.150, 3.320
0.400	3.910, 3.830, 4.880, 4.210
0.550	4.540, 4.470, 4.790, 5.680
0.750	6.060, 5.070, 5.000, 5.980
1.000	5.840, 5.790, 6.100, 7.810
1.375	7.310, 7.080, 7.060, 6.870
1.850	9.880, 10.120, 9.220, 9.960
2.500	11.040, 10.460, 10.880, 11.650
3.250	13.510, 15.470, 14.210, 13.920
4.500	16.070, 14.670, 14.780, 15.210
6.000	17.340, 16.850, 16.740, 16.870
8.250	18.980, 19.850, 18.750, 18.510
11.250	21.666, 21.218, 19.790, 22.669
15.000	23.206, 22.239, 22.436, 22.597
20.250	23.922, 24.871, 23.815, 24.871
27.500	25.748, 25.874, 24.907, 24.871
37.000	24.441, 25.874, 25.748, 27.270
50.000	29.580, 26.698, 26.536, 27.181

Table #5

Estimates of θ, σ and x_c based on example of Section 5

	Least Squares	Pseudo-likelihood $\ell=2$		Log-Linearized		Modified Max. Likelihood	
	$\hat{x}_c$	$\hat{x}_c$	$\hat{\theta}$	$\hat{x}_c$	$\hat{\theta}$	$\hat{x}_c$	$\hat{\theta}$
Full Duplicates	.1554	.0790	.4750	.0793	.4757	.0822	.4500
1 & 2	.2230	.0728	.7000	.0476	.9404	.0659	.7500
2 & 3	.2385	.1555	.3500	.1870	.1950	.1739	.2500
3 & 4	.2513	.1324	.5750	.1112	.6940	.1104	.7000
1 & 4	.1593	.0612	.5500	.0601	.5931	.0695	.5000
1 & 3	.1859	.0938	.4500	.0981	.4233	.0909	.4750
Mean	.2116	.1031	.5250	.1008	.5692	.1021	.5350
SD	.0763	.0357	.1183	.0491	.2511	.0439	.1997

Note: Means and SDs are based only on the five reduced permutations of the data with duplicates.