ESTIMATION OF LD50 BY MOVING AVERAGES

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1. *Introduction and summary.* Methods of graduation of a series of observations by means of moving averages were discussed by Sheppard (1914), and subsequently by Sherriff (1920) and a number of other writers. These methods based on least squares or weighted least squares solutions differ from actuarial or summation methods. Thompson (1947) has proposed that the method of moving averages be considered a 'basic' one in the estimation of the median effective dose (LD 50) of bioassay data. On the basis of an empirical study of the data of Topley and Wilson he recommended in particular the use of a three-term moving average. In a recent paper, Finney (1950) has discussed the efficiency of Thompson's moving average method generally.

In this paper, I investigate further the possible uses of moving average estimates in situations where unequal numbers of observations are taken at successive dose levels. The efficiency of such estimates is discussed with reference to several logit or probit distributions of tolerance or threshold in the symmetric case. The use of the arc-sine transformation in combination with moving average methods is also discussed.

2. *Method of moving averages.* We consider a series of $(2m+1)$ observations

$$u_{-m}, \ldots, u_{-1}, u_0, u_1, \ldots, u_m$$

taken at equally spaced points (e.g. in time)

$$t = -m, \ldots, -1, 0, 1, \ldots, m$$

respectively. A series of unequal weights

$$n_t \quad (t = 0, \pm 1, \ldots, \pm m)$$

is associated with the observations. According to the method of moving averages curves are fitted by weighted least squares and then the central value of the series represented by the corresponding computed value of the curve. Thus we determine a polynomial of degree p,

$$u(t) = b_0 + b_1 t + \ldots + b_p t^p, \tag{2·1}$$

which minimizes

$$\sum_{t=-m}^{m} n_t(u_t - b_0 - b_1 t - \ldots - b_p t^p)^2. \tag{2·2}$$

The central value $u(0) = b_0$ will then be the 'smoothed' value corresponding to u_0. From (2·2) the normal equations are:

$$(\Sigma t^i n_t) b_0 + (\Sigma t^{i+1} n_t) b_1 + \ldots + (\Sigma t^{i+p} n_t) b_p = \Sigma t^i n_t u_t, \tag{2·3}$$

for $i = 0, 1, \ldots, p$. Solving for b_0, we have

$$b_0 = \sum_{t=-m}^{m} c_t u_t,$$

https://doi.org/10.1017/S0022172400019513 Published online by Cambridge University Press

where c_t is the ratio of the determinants

$$c_t = \frac{\begin{vmatrix} n_t & (\Sigma t n_t) & \dots & (\Sigma t^p n_t) \\ t n_t & (\Sigma t^2 n_t) & \dots & (\Sigma t^{p+1} n_t) \\ \dots & \dots & \dots & \dots \\ t^p n_t & (\Sigma t^{p+1} n_t) & \dots & (\Sigma t^{2p} n_t) \end{vmatrix}}{\begin{vmatrix} (\Sigma n_t) & (\Sigma t n_t) & \dots & (\Sigma t^p n_t) \\ (\Sigma t n_t) & (\Sigma t^2 n_t) & \dots & (\Sigma t^{p+1} n_t) \\ \dots & \dots & \dots & \dots \\ (\Sigma t^p n_t) & (\Sigma t^{p+1} n_t) & \dots & (\Sigma t^{2p} n_t) \end{vmatrix}} \tag{2·4}$$

which may be evaluated then for values of m, n_t; here $\Sigma = \sum_{t=-m}^{m}$.

In practice, then, the moving averages may be obtained by successively applying the values $c_{-m}, \dots, c_{-1}, c_0, c_1, \dots, c_m$ to the original series of observations. The determinants in (2·4) may be further simplified, but since I shall restrict the polynomials (2·1) to linear ones ($p = 1$) this will not be done at this time.

Case (i). As the simplest possible special case consider first a moving average based on three successive points. In this case for $p = 1, m = 1$,

$$b_0 = \frac{u_{-1} + \tfrac{1}{2} n_0 \left(\dfrac{1}{n_{-1}} + \dfrac{1}{n_1}\right) u_0 + u_1}{2 + \tfrac{1}{2} n_0 \left(\dfrac{1}{n_{-1}} + \dfrac{1}{n_1}\right)}$$

$$= \frac{u_{-1} + \dfrac{n_0}{\bar{n}_0} u_0 + u_1}{\left(2 + \dfrac{n_0}{\bar{n}_0}\right)}, \tag{2·5}$$

where $\dfrac{1}{\bar{n}_0} = \dfrac{1}{2}\left(\dfrac{1}{n_{-1}} + \dfrac{1}{n_1}\right)$ is the harmonic mean of the two extreme weights n_{-1}, n_1. We note that if the weights $n_t = n$, b_0 reduces to the arithmetic mean of the three successive terms.

Case (ii). If $p = 1, m = 2$, a moving average based on five successive points, since

$$c_t = n_t[\Sigma t^2 n_t - t(\Sigma t n_t)]/D,$$

or

$$\begin{aligned} b_0 = \{ & n_{-2}(-n_{-1} + 3n_1 + 8n_2)\, u_{-2} \\ & + n_{-1}(2n_{-2} + 2n_1 + 6n_2)\, u_{-1} \\ & + n_0(4n_{-2} + n_{-1} + n_1 + 4n_2)\, u_0 \\ & + n_1(6n_{-2} + 2n_{-1} + 2n_2)\, u_1 \\ & + n_2(8n_{-2} + 3n_{-1} - n_1)\, u_2\}/D, \end{aligned} \tag{2·6}$$

where D = sum of coefficients of the u's. If the weights are all equal, (2·6) reduces to the arithmetic mean as before.

In view of the fact that the differences between the successive coefficients (in parentheses) of the u's are constant $= (n_1 - n_{-1}) + 2(n_2 - n_{-2})$ in (2·6), b_0 reduces to

$$b_0 = \frac{n_{-2} u_{-2} + n_{-1}(1 - \rho_0) u_{-1} + n_0(1 - 2\rho_0) u_0 + n_1(1 - 3\rho_0) u_1 + n_2(1 - 4\rho_0) u_2}{n_{-2} + n_{-1} + n_0 + n_1 + n_2 - \rho_0(n_{-1} + 2n_0 + 3n_1 + 4n_2)}, \tag{2·7}$$

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where $\rho_0 = \frac{(n_1 - n_{-1}) + 2(n_2 - n_{-2})}{(-n_{-1} + 3n_1 + 8n_2)}$. In this form the five-term moving average may be most easily computed.

The use of these moving averages will be restricted to odd numbers of terms ($m = 1, 2$), i.e. three- and five-term weighted moving averages. If the observations are equally weighted, then the appropriate coefficients c_t ($t = 0, \pm 1, \ldots, \pm m$) are available for various values of p (cf. Kendall, 1946; Sherriff, 1920).

When the t's are unequally spaced, e.g. in the case of three terms u_{-1}, u_0, $u_{1+\delta}$ over the points $t = -1, 0, 1+\delta$ ($\delta > 0$), the corresponding weighted least squares solution is

$$b_0 = \frac{n_{-1}n_{1+\delta}(1+\delta)(2+\delta)\,u_{-1} + n_0\{n_{-1} + n_{1+\delta}(1+\delta)^2\}\,u_0 + n_{-1}n_{1+\delta}(2+\delta)\,u_{1+\delta}}{n_{-1}n_0 + n_{-1}n_{1+\delta}(2+\delta)^2 + n_0 n_{1+\delta}(1+\delta)^2}$$

$$= \frac{(1+\delta)(2+\delta)\,u_{-1} + n_0\left\{\frac{1}{n_{1+\delta}} + \frac{(1+\delta)^2}{n_{-1}}\right\}u_0 + (2+\delta)\,u_{1+\delta}}{(2+\delta)^2 + n_0\left\{\frac{1}{n_{1+\delta}} + \frac{(1+\delta)^2}{n_{-1}}\right\}}, \qquad (2\cdot8)$$

which reduces to (2·5) in case $\delta = 0$. If the interval consists of the points

$$t = -(1+\delta), 0, 1$$

instead, then the weights in (2·8) become interchanged.

3. *Applications to biological assay.* One of the statistical problems of biological assay may be typically described as follows. A certain substance (e.g. a drug) is administered to different series of animals at a number of different levels or concentrations d_i ($i = 1, \ldots, k$). At each level is observed the number, or rather the proportion p_i, of the animals in which a characteristic effect (e.g. death) was noted. On the basis of the observed proportions p_i it is required to estimate the median dose (i.e. LD 50), or that dose estimated to produce the characteristic effect in 50 % of the animals.

Although the essential mathematical solutions to the problems of estimation of the LD 50 from quantal response data have been available for some time under the assumption of a fundamental distribution of tolerance or threshold, nevertheless there still appears to be some need for further study of simple interpolation methods of estimation (cf. Armitage & Allen, 1950; Finney, 1950).

In the present paper I propose to investigate some specific applications of the methods of moving averages (as discussed in §2) to the problem of estimating the LD 50. Suppose then that p_i ($i = 1, \ldots, k$) represents the proportion of the animals in which the specified effect was noted at the ith dose level d_i. In particular the 'weights' n_t of §2 will coincide with the number of animals tested at the successive dose levels. Also we shall assume that the doses are equally spaced (e.g. on a log scale), i.e. $d_{r+1} = d_1 + rd$ ($r = 1, \ldots, k-1$), d being the constant dose interval.

According to (2·5), then, when there are four or more dose levels ($k \geqslant 4$) we shall use as the smoothed value of p_i ($i = 2, \ldots, k-1$) the three-term moving average

$$p'_i = \frac{p_{i-1} + \frac{n_i}{\bar{n}_i}p_i + p_{i+1}}{\left(2 + \frac{n_i}{\bar{n}_i}\right)} \qquad (3\cdot1)$$

for $i = 2, \ldots, k-1$, with corresponding doses

$$d'_i = \frac{d_{i-1} + \frac{n_i}{\bar{n}_i} d_i + d_{i+1}}{\left(2 + \frac{n_i}{\bar{n}_i}\right)}$$

$$= d_1 + (i-1)\,d \quad (i = 2, \ldots, k-1). \tag{3·2}$$

If now two successive proportions p'_i, p'_{i+1} are such that $p'_i < 0{\cdot}5 < p'_{i+1}$ then the estimated value of log LD 50 will be

$$m' = d_1 + (i-1)\,d + \frac{(0{\cdot}5 - p'_i)}{(p'_{i+1} - p'_i)}\,d$$

$$= d_1 + \left\{(i-1) + \frac{(0{\cdot}5 - p'_i)}{(p'_{i+1} - p'_i)}\right\} d \tag{3·3}$$

as determined by linear interpolation. The approximate variance of (3·3) may be written as

$$\sigma^2_{m'} \cong d^2 .\, \text{variance}\, \frac{(0{\cdot}5 - p'_i)}{(p'_{i+1} - p'_i)} = d^2 \sigma^2_f, \tag{3·4}$$

where f represents the sample fraction $\dfrac{(0{\cdot}5 - p'_i)}{(p'_{i+1} - p'_i)}$.

If π'_i $(i = 2, \ldots, k-1)$ are the corresponding moving averages of the true probabilities π_i $(i = 1, \ldots, k)$ of the number of animals killed, i.e.

$$\pi'_i = \frac{\left(\pi_{i-1} + \frac{n_i}{\bar{n}_i} \pi_i + \pi_{i+1}\right)}{\left(2 + \frac{n_i}{\bar{n}_i}\right)} \quad (i = 2, \ldots, k-1), \tag{3·5}$$

then

$$\sigma^2_f \cong \frac{(0{\cdot}5 - \pi'_i)^2}{(\pi'_{i+1} - \pi'_i)^2} \left[\frac{\sigma^2_{p'i}}{(0{\cdot}5 - \pi'_i)^2} - 2\frac{(\sigma^2_{p'i} - \sigma_{p'ip'i+1})}{(0{\cdot}5 - \pi'_i)(\pi'_{i+1} - \pi'_i)} + \frac{(\sigma^2_{p'i} - 2\sigma_{p'ip'i+1} + \sigma^2_{p'i+1})}{(\pi'_{i+1} - \pi'_i)^2}\right]$$

and noting that

$$\sigma^2_{p'i} = \frac{1}{\left(2 + \frac{n_i}{\bar{n}_i}\right)^2} \left[\frac{\pi_{i-1}(1 - \pi_{i-1})}{n_{i-1}} + \frac{\frac{n_i}{\bar{n}_i} \pi_i (1 - \pi_i)}{\bar{n}_i} + \frac{\pi_{i+1}(1 - \pi_{i+1})}{n_{i+1}}\right]$$

and also
$$\sigma_{p'ip'i+1} = \frac{1}{\left(2 + \frac{n_i}{\bar{n}_i}\right)\left(2 + \frac{n_{i+1}}{\bar{n}_{i+1}}\right)} \left[\frac{\pi_i(1 - \pi_i)}{\bar{n}_i} + \frac{\pi_{i+1}(1 - \pi_{i+1})}{\bar{n}_{i+1}}\right]$$

the approximate variance is

$$\sigma^2_{m'} \cong \frac{d^2}{(\pi'_{i+1} - \pi'_i)^2} \left[(1 - \tau')^2 \sigma^2_{p'i} + 2\tau'(1 - \tau')\sigma_{p'ip'i+1} + \tau'^2 \sigma^2_{p'i+1}\right] \tag{3·6}$$

if we denote the fraction $\tau' = \dfrac{(0{\cdot}5 - \pi'_i)}{(\pi'_{i+1} - \pi'_i)}$.

For the corresponding sample estimate $s^2_{m'}$ of (3·6) we substitute in (3·6) the values p'_i, p'_{i+1}, f and the (unbiased) estimates of $\sigma^2_{p'_i} = s^2_{p'_i}$ in terms of $s^2_{p'_i} = \dfrac{p_i(1-p_i)}{n_i - 1}$, i.e.

$$s^2_{m'} = \frac{d^2}{(p'_{i+1} - p'_i)^2}[(1-f)^2 s^2_{p'_i} + 2f(1-f)\, s_{p'_i p'_{i+1}} + f^2 s^2_{p'_{i+1}}]. \tag{3·7}$$

The expressions (3·6) or (3·7) may be further simplified to give the coefficients of the respective terms in $\pi_i(1-\pi_i)$ or $p_i(1-p_i)$.

When the n_i's are all equal ($= n$) then (3·6) reduces to

$$\sigma^2_{m'} = \frac{d^2}{(\pi_{i+2} - \pi_{i-1})^2} \frac{(1-\tau)^2 \pi_{i-1}(1-\pi_{i-1}) + \pi_i(1-\pi_i) + \pi_{i+1}(1-\pi_{i+1}) + \tau^2 \pi_{i+2}(1-\pi_{i+2})}{n}$$

which coincides with the expression given by Thompson (1947).

4. *Use of five-term weighted moving average.* The five-term weighted moving average suggested by (2·7) is defined as the sequence $\{p''_i\}$ ($i = 3, \ldots, k-2$) for $k \geqslant 6$, where

$$p''_i = \frac{\sum_{t=-2}^{2} n_{i+t}\{1-(t+2)\rho_i\} p_{i+t}}{\sum_{t=-2}^{2} n_{i+t}\{1-(t+2)\rho_i\}} \tag{4·1}$$

and $\rho_i = \dfrac{(n_{i+1} - n_{i-1}) + 2(n_{i+2} - n_{i-2})}{(-n_{i-1} + 3n_{i+1} + 8n_{i+2})}$. The estimate of log LD 50 may then be obtained by linear interpolation to be

$$m'' = d_1 + \left\{(i-1) + \frac{(0·5 - p''_i)}{(p''_{i+1} - p''_i)}\right\} d, \tag{4·2}$$

whenever $p''_i < 0·5 < p''_{i+1}$. The approximate variance of m'' is given by

$$\sigma^2_{m''} \cong \frac{d^2}{(\pi''_{i+1} - \pi''_i)^2} [(1-\tau'')^2 \sigma^2_{p''_i} + 2\tau''(1-\tau'')\sigma_{p''_i p''_{i+1}} + \tau''^2 \sigma^2_{p''_{i+1}}], \tag{4·3}$$

where $\tau'' = \dfrac{(0·5 - \pi''_i)}{(\pi''_{i+1} - \pi''_i)}$, π''_i being the expression (4·1) evaluated for π_{i+t} instead of p_{i+t} and

$$\sigma^2_{p''_i} = \frac{\sum_{t=-2}^{2} n_{i+t}\{1-(t+2)\rho_i\}^2 \pi_{i+t}(1-\pi_{i+t})}{\left[\sum_{t=-2}^{2} n_{i+t}\{1-(t+2)\rho_i\}\right]^2},$$

$$\sigma_{p''_i p''_{i+1}} = \frac{\sum_{t=-1}^{2} n^2_{i+t}\{1-(t+2)\rho_i\}\{1-(t+1)\rho_{i+1}\}\pi_{i+t}(1-\pi_{i+t})}{\left[\sum_{t=-2}^{2} n_{i+t}\{1-(t+2)\rho_i\}\right]\left[\sum_{t=-1}^{3} n_{i+t}\{1-(t+1)\rho_{i+1}\}\right]}. \tag{4·4}$$

5. *Relative efficiency of three- and five-term weighted moving averages.* Finney (1950) has investigated in some detail the question of the bias and relative efficiency of Thompson's methods in the estimation of the LD 50 with moving averages of varying spans. His results provide approximate bounds for the corresponding weighted moving averages with the same spans. The following brief numerical investigation, undertaken before Finney's results became available to the author,

was designed to compare the (approximate) variances of estimates (3·3), (4·2) for several theoretical bioassay experiments. A series of four different tolerance or threshold distributions each based on the logistic (cf., for example, Armitage & Allen, 1950) were employed, a total of eight symmetrically placed doses being used. Table 1 indicates the true proportions ($=\pi_i$) at each of the successive dose levels. As indicative of a typical bioassay experiment the column ($=n_i$), or the numbers of animals tested at each of the dose levels ($=d_i$), was obtained from a table of random numbers in the interval 10–20.

Table 1. *True proportions* ($=\pi_i$)

		Series			
Dose ($=d_i$)	n_i	1	2	3	4
−3·5	12	0·100	0·200	0·300	0·400
−2·5	11	0·172	0·270	0·353	0·430
−1·5	18	0·280	0·355	0·410	0·455
−0·5	14	0·422	0·450	0·470	0·485
0·5	15	0·578	0·550	0·530	0·515
1·5	10	0·720	0·645	0·590	0·545
2·5	12	0·838	0·730	0·647	0·570
3·5	16	0·900	0·800	0·700	0·600
Slope	—	0·3140	0·1980	0·1211	0·0580

Table 2. *Comparison of variances*

Series	Logit	3-term moving average (Thompson)	Rel. eff.	3-term weighted average	Rel. eff.	5-term weighted average	Rel. eff.
1	0·139	0·215	65	0·207	67	0·155	89
2	0·286	0·516	55	0·502	57	0·364	79
3	0·691	1·34	52	1·29	53	0·933	74
4	2·81	5·72	49	5·59	50	4·04	70

In Table 2 are given the approximate variance for the log LD 50 as estimated from the three- and five-term weighted moving averages. In the column 'Relative efficiency' (%) these are compared with the corresponding 'exact' logit variance. Since each of these estimates is essentially a ratio estimate, their respective variances correspond to the same order of approximation.

It is to be noted that the relative efficiency of the estimate from a weighted three-term moving average is slightly greater than the corresponding unweighted average of Thompson, i.e. from the sequence $\{p_i^*\}$ $(i = 2, \ldots, k-1)$ where

$$p_i^* = \tfrac{1}{3}(p_{i-1}+p_i+p_{i+1}) \quad (i = 2, \ldots, k-1). \tag{5·1}$$

The approximate variance of the estimate of LD 50 obtained by linear interpolation from the sequence p_i^* is given by Thompson (1947).

From the Table 2 above, it appears that for this typical series of experiments the range of efficiency for the three-term weighted moving average is 50–67 %, and for the five-term average 70–89 %.

In order to investigate further the effect of the sample size n_i upon the relative efficiency of the three-term moving average in particular, a further series of bioassay

https://doi.org/10.1017/S0022172400019513 Published online by Cambridge University Press

experiments was used, the fundamental distribution of threshold or tolerance being the integrated normal or probit ($0{\cdot}10 \leqslant \pi_i \leqslant 0{\cdot}90$). Table 3 indicates the results of these experiments.

Table 3. *Relative efficiency* (%)

Number ($=n_i$)	Weighted 3-term moving average	3-term moving average
5–10	64	58
20–30	61	60

6. *Use of the angular transformation.* The arc-sine transformation has been suggested by Knudsen & Curtis (1947) as an alternative to the probit or logit method in order to utilize the property that the variances resulting from this transformation are approximately constant. Thus if p_i ($i = 1, \ldots, k$) are the individual sample proportions and n_i the numbers on which they are based, the transformation: $y_i = \sin^{-1}\sqrt{p_i}$ is such that $\sigma^2_{y_i} = c^2/n_i$, where

$$c^2 \begin{cases} = 0{\cdot}25, & \text{if } y \text{ in radians,} \\ = 821, & \text{if } y \text{ in degrees.} \end{cases} \tag{6·1}$$

It is of interest to combine the arc-sine transformation with the method of moving averages in order to estimate the LD50. Thus if, for example, we denote by y'_i the three-term weighted moving average

$$y'_i = \frac{y_{i-1} + \dfrac{n_i}{\bar{n}_i} y_i + y_{i+1},}{\left(2 + \dfrac{n_i}{\bar{n}_i}\right)} \tag{6·2}$$

it may be verified that

$$\sigma^2_{y'_i} = \frac{c^2}{\bar{n}_i\left(2 + \dfrac{n_i}{\bar{n}_i}\right)}. \tag{6·3}$$

If two successive values y'_i, y'_{i+1} of the series $y'_2, \ldots, y'_{k-1}$, are such that $y'_i < \frac{1}{4}\pi < y'_{i+1}$ then the estimated value of log LD50 will be

$$m' = d_1 + \left\{(i-1) + \frac{(\frac{1}{4}\pi - y'_i)}{(y'_{i+1} - y'_i)}\right\} d. \tag{6·4}$$

The approximate variance of the estimate (6·4) may be written as

$$\sigma^2_{m'} \cong \frac{d^2}{(\vartheta'_{i+1} - \vartheta'_i)^2}\left[(1-\nu)^2 \sigma^2_{y'_i} + 2\nu(1-\nu)\,\sigma_{y'_i y'_{i+1}} + \nu^2 \sigma^2_{y'_{i+1}}\right], \tag{6·5}$$

where

$$\theta_i = \sin^{-1}\sqrt{\pi_i},$$

$$\sigma^2_{y'_i} = \frac{c^2}{\bar{n}_i\left(2 + \dfrac{n_i}{\bar{n}_i}\right)},$$

$$\sigma_{y'_i y'_{i+1}} = \frac{\left(\dfrac{1}{\bar{n}_i} + \dfrac{1}{\bar{n}_{i+1}}\right) c^2}{\left(2 + \dfrac{n_i}{\bar{n}_i}\right)\left(2 + \dfrac{n_{i+1}}{\bar{n}_{i+1}}\right)},$$

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ϑ'_i is the weighted average of the ϑ_i's corresponding to (6·2), and ν is the fraction

$$\frac{(\frac{1}{4}\pi - \vartheta'_i)}{(\vartheta'_{i+1} - \vartheta'_i)}.$$

7. *Discussion.* In this article, I have presented in detail some of the possibilities of improving the estimates of the LD 50 by moving averages as originally proposed by Thompson, for situations where unequal numbers of animals are tested at a number of equally spaced doses (or log doses). In particular, only three- and five-term weighted moving averages are considered. The sequence $\{p'_i\}$ of three-term averages, as defined by (3·1) in case four or more dose levels are involved, results in an interpolation estimate of LD 50 which appears from several numerical studies to be slightly more efficient generally than the unweighted three-term moving average (5·1) of Thompson. An increase in the efficiency of the weighted moving average may be expected when the numbers of animals vary considerably at the different levels. Similar results are obtained on the efficiency of the estimation of the LD 50 by means of a five-term weighted moving average, though such averages are rather more difficult to compute in view of the nature of the coefficients or weights involved (equation 4·1).

It is of interest to consider the method of moving averages in combination with the arc-sine transformation in order to utilize the variance stabilizing property of this transformation. Equation (6·2) defines a three-term moving average sequence $\{y'_i\}$ in terms of the transformed percentages. The corresponding estimate of the LD 50 is then obtained by linear interpolation.

This study has been restricted to a consideration of weighted moving averages with odd spans in only the simplest nontrivial cases (spans of 3 and 5). Further work might certainly be done on the efficiency of such estimates in the case of dose-effect relationships other than the integrated normal or logit distributions.

I wish to acknowledge the assistance of Mr Henry Peterson in the computation of Tables 2 and 3.

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(*MS. received for publication* 30. VII. 51.)