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A MANUAL OF STATISTICAL METHODS FOR DETERMINING DOSE AT A LEVELING OFF POINT IN THE GROWTH RESPONSE TO EXPERIMENTAL CONCENTRATIONS OF ANTIBIOTIC FEED ADDITIVES

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PURPOSE

The manual has been developed to provide a review with worked examples of methodology for the statistical determination of the maximum dose which a producer of antibiotic feed additives should be permitted to recommend. This determination is to be based upon a dose response feeding trial replicated at several locations, where the response variable is either weight gain or feed efficiency, and dose is measured in grams of the antibiotic additive per ton of feed. The test animals might be poultry, swine, or cattle, and an experimental unit consists of a pen of animals fed for a fixed period of time at a constant dose level. Typically, the treatments include a control and 3 to 6 levels of dose which are usually equally spaced on the logarithmic scale.

Data from such a replicated experiment are amenable to analysis of variance, which produces an estimate of experimental error variance, and to linear or non-linear regression analysis as a method of fitting a dose response curve to the treatment means. The latter method produces a residual mean square which may be compared to the independent experimental error mean square to test the goodness of fit of the regression model. Such data are also amenable to non-parametric methods for analyzing a two-way classification of replicates x treatments, where treatments represent ordered levels of single factor.

The biological basis for the growth promoting effects of antibiotic feed additives has been extensively studied but is poorly understood, and provides no basis for developing a theoretical growth response function of dose. Typically, the average daily weight gain increases through the lower doses and then tends to level off at the higher doses, but with disturbing frequency experiments reveal a dip in the response at the next to highest dose. The empirical basis even for assuming complete monotonicity is therefore somewhat suspect.

Growth promotion through the addition of antibiotics to animal feeds is aimed at improving profits in animal production, and a cost/benefit analysis of a dose response curve would determine the economically optimum dose. Concern over the biological hazards of excessive use of antibiotics, however, demands a more conservative approach to dose determination, and the statistical approaches to be reviewed here will serve to conservatively determine the first dose level at which response tends to level off.

PREVIEW OF METHODS

Statistical methods available for adaptation to this problem are the parameteric and nonparametric tests against ordered alternatives, and regression methods for locating a plateau or peak. Conservatism may be invoked by utilizing a lower confidence limit rather than a actual point estimate of the dose level at the plateau or peak. A regression method is also suggested for setting a lower confidence limit on the dose at which the slope of an increasing but concave regression function attains some specified small value ϵ, and a suggestion is offered for objectively determining ϵ. Published nonparametric procedures for testing against monotone alternatives have also been supplemented here by providing tests against monotone-concave alternatives.

Plateau regression models. In its simplest form this method consists of fitting the intersecting two-phase linear regression model shown in Figure 1 with a specified slope of zero in the second phase, then calculating a lower confidence limit on the dose level at the join point. In the field of animal nutrition, for example, such a model is sometimes applied to the weight gain response to increasing levels of a nutrient factor as a method of estimating the required level of that factor. The relatively small number of dose levels available in an antibiotic feed additive trial demands that parsimony be exercised in choosing a regression model with few parameters. This linear-linear model contains 3 parameters: the slope of the first segment, the dose level at the join point, and the height of the final horizontal segment. An alternative 3-parameter segmented polynomial is the quadratic-linear with the two constraints that the function has a continuous first derivative and that the linear segment has zero slope. In the notation of Gallant and Fuller (JASA, 1973, p. 144) this model is

$$f(x;\underset{\sim}{\theta}) = \theta_1 + \theta_2(\alpha-x)^2 I_+(\alpha-x)$$

where

$$I_+(\alpha-x) = \begin{cases} 1 & \text{for } x \leq \alpha \\ 0 & \text{for } x > \alpha \end{cases} .$$

Walker-Carmer method. An alternative to the device of imposing a plateau on the regression model was proposed by Walker and Carmer (Agron. Jour., 1967, p. 161) who suggested determining the highest dose level at which the estimated slope differs significantly from zero. Applied to a 3-parameter quadratic model on some scale T(x),

$$f(x;\underset{\sim}{\theta}) = \theta_1 + \theta_2 T(x) - \theta_3 T(x)^2 ,$$

which attains a maximum within the range of test doses ($\theta_2 > 0, \theta_3 > 0$), this procedure generates a lower confidence limit x_L on the maximizing dose $x = T^{-1}(\theta_2/2\theta_3)$ in the form

$$T(x_L) = \frac{\hat{\theta}_2\hat{\theta}_3 + t^2 s_{23} - t s_2 s_3 \sqrt{(1-r_{23}^2)(F_{23}-t^2)}}{2\hat{\theta}_3^2(1 - \frac{t^2}{T_3^2})}$$

x_L = lower confidence limit on $T^{-1}(\frac{\theta_2}{2\theta_3})$

t = critical value of Student's t with experimental error d.f.

s_2^2, s_3^2, s_{23} = estimated variances and covariance of $\hat{\theta}_2, \hat{\theta}_3$

$r_{23} = s_{23}/\sqrt{s_2^2 s_3^2}$

$T_3 = \hat{\theta}_3/s_3$, a noncentral t-statistic

$$F_{23} = \frac{1}{1-r_{23}^2}\left[\frac{\hat{\theta}_2^2}{s_2^2} + \frac{\hat{\theta}_3^2}{s_3^2} - 2r_{23}\frac{\hat{\theta}_2\hat{\theta}_3}{s_2 s_3}\right] , \quad \text{a noncentral F .}$$

The Walker-Carmer approach is not restricted to quadratic curves but applies to any regular (e.g., analytic) response function of dose which achieves its maximum values within the range of experimental doses. In practice, however, the demands of parsimony in parameter estimation are likely to restrict applications to quadratic functions on some scale of dose.

Specification of a minimum rate of increase in response. Dose response relationships in this field of application are more typically monotonic in appearance within the usual range of test doses, and forcing a maximum to occur in the fitted curve below the highest test dose would constitute an unjustified, penalizing restriction. If the curve to be fitted is monotonically strictly increasing within the range of experimental doses, but with strictly decreasing slope, then the Walker-Carmer approach may be modified to provide a lower confidence limit on that dose level where the slope achieves some specified small value $\epsilon > 0$. A cost/benefit analysis would utilize the slopes of both the weight gain and feed efficiency dose response curves, and the economic optimum dose level would occur when these slopes become sufficiently small in relation to other factors. There is consumer-economic justification therefore in adopting such a criterion for determining maximum recommendable dose level.

The specification of ϵ is a critical step in this procedure; since the slope presumably converges toward zero with increasing dose, then the smaller is ϵ the greater will be the dose level at which a slope of size ϵ is attained. If this assumption of strict monotoncity with a decreasing slope is valid then as the precision of the experiment increases to provide increasingly supportive evidence of monotonicity, the specified value of ϵ should be allowed to decrease and thereby increase the recommendable range of doses over which response is claimed to increase with dose. The specification of ϵ should thus be required to depend upon such precision factors as the amount of replication, the number and range of test dose levels, and the goodness of fit to the postulated monotonic dose response function.

Such requirements would seem to be satisfied by specifying ϵ to be the smallest slope which could, with reasonable likelihood, be detected with the available experimental resources if these resources were optimally allocated for measuring slope. Under ideal circumstances the optimal design for estimating slope is to assign half of the resources to the lowest dose level and half to the highest. In an experiment where k test doses $x_1 < x_2 < \cdots < x_k$ and the control $x_0 = 0$ are each replicated r times, the hypothetical and idealistic optimum allocation of these resources would assign $r(k+1)/2$ to $x_0 = 0$ and $r(k+1)/2$ to x_k. The standard error of the resulting slope estimator would then be

$$\sigma_b = \frac{2\sigma_{y\cdot x}}{x_k\sqrt{r(k+1)}} .$$

The slope ϵ which could be detected with probability β by a one-tailed, size-α t-test on $r(k+1)-2$ degrees of freedom could then be read from the power curves of the t-test, and is given approximately by $\epsilon \doteq \sigma_b(t_{1-\alpha} - t_{1-\beta})$. The curve that is actually fitted to the treatment means provides an estimate $s^2_{y\cdot x}$ of $\sigma^2_{y\cdot x}$ which is independent of the replicate x treatment experimental error mean square, and which provides a measure of goodness of fit. This measure may be incorporated into the specification of ϵ simply by substituting $s_{y\cdot x}$ for $\sigma_{y\cdot x}$, giving

$$\epsilon = \frac{2s_{y\cdot x}}{x_k\sqrt{r(k+1)}} (t_{1-\alpha} - t_{1-\beta})$$

where the t's are percentiles of the t-distribution on $r(k+1)-2$ degrees of freedom. The choice of β is somewhat arbitrary; a generous interpretation of "reasonable likelihood" would be $\beta = .25$.

Confidence limits on the dose with a specified slope. Implementation of such a procedure consists of fitting a selected monotonic response function $y = f(x;\underset{\sim}{\theta})$ and calculating a lower confidence limit on that dose level $x(\epsilon;\underset{\sim}{\theta})$ at which $dy/dx = \epsilon$. The dose response function $f(x;\underset{\sim}{\theta})$ will in general be nonlinear in $\underset{\sim}{\theta}$ but usually simple in form, and its derivative $f'(x;\underset{\sim}{\theta}) = dy/dx$ will be montonic with $f'(\infty;\underset{\sim}{\theta}) = 0$ so that $f'(x;\underset{\sim}{\theta}) = \epsilon$ has a unique solution $x(\epsilon;\underset{\sim}{\theta})$. Examples of 3-parameter curves which are reasonable candidates in this context are $(y-\theta_1)/\theta_2 = g(x;\theta_3)$ or $(\theta_1-y)/\theta_2 = h(x;\theta_3)$ where h is decreasing in x and g is increasing; specifically, for example,

$$\frac{y-\theta_1}{\theta_2} = \log(x+\theta_3) \quad \text{or} \quad \frac{\theta_1-y}{\theta_2} = e^{-\theta_3 x} .$$

In both of these examples g and h are symmetric in x and θ_3, where θ_3 is a location parameter in the first, log-linear case and is a scale parameter in the second, Mitcherlich case. In either case the nonlinear estimation reduces to the simple

problem of iteratively maximizing the (2 d.f.) sum of squares $R(\theta_3)$ due to regression,

$$R(\theta_3) = \frac{[\Sigma(Y_j-\bar{Y})Z_j]^2}{\Sigma(Z_j-\bar{Z})^2}$$

where Z_j is either $g(x_j;\theta_3)$ or $h(x_j;\theta_3)$.

Methodology for constructing a lower confidence limit on $x(\epsilon;\underset{\sim}{\theta})$ is open to several alternative choices, all of which produce only asymptotically valid confidence limits in this case of nonlinear estimation. One obvious possibility is use of the t-approximation to the sampling distribution of $[x(\epsilon;\hat{\underset{\sim}{\theta}}) - x(\epsilon;\underset{\sim}{\theta})]/\sqrt{\hat{V}(x(\epsilon;\hat{\underset{\sim}{\theta}}))}$, where the approximate variance formula $V(x(\epsilon;\hat{\underset{\sim}{\theta}}))$ is derived in the usual manner from a first order Taylor series expansion of $x(\epsilon;\hat{\underset{\sim}{\theta}})$ about $x(\epsilon;\underset{\sim}{\theta})$. Other possibilities utilize Fieller's Theorem to obtain the lower limit as a solution to the equation

$$\frac{f'(x;\hat{\underset{\sim}{\theta}}) - \epsilon}{\sqrt{\hat{V}(f'(x;\hat{\underset{\sim}{\theta}}))}} = t_{1-\alpha} \qquad (a)$$

or, for example,

$$\frac{\log[f'(x;\hat{\underset{\sim}{\theta}})/\epsilon]}{\sqrt{\hat{V}(\log[f'(x;\hat{\underset{\sim}{\theta}})/\epsilon)}} = t_{1-\alpha} \qquad (b)$$

Upper and lower Fieller limits are not symmetric about $x(\epsilon;\hat{\underset{\sim}{\theta}})$, and in this context the lower Fieller limit will generally be closer $x(\epsilon;\hat{\underset{\sim}{\theta}})$ than the lower limit of the symmetric interval mentioned above. Some care must be exercised in applying Fieller's Theorem, however; if the equation under consideration has only finite roots when $\epsilon = 0$ then no root is a valid confidence limit on $x(\epsilon;\underset{\sim}{\theta})$. Applied to the Mitcherlich model, for example, equation (a) has the two roots

$$\frac{\widehat{Cov}(\hat{\theta}_2\hat{\theta}_3,\hat{\theta}_3)}{\hat{\theta}_2\hat{\theta}_3\hat{V}(\hat{\theta}_3)} \pm \frac{t_{1-\alpha}}{\hat{\theta}_2\hat{\theta}_3\hat{V}(\hat{\theta}_3)}\sqrt{[\widehat{Cov}(\hat{\theta}_2\hat{\theta}_3,\hat{\theta}_3)]^2 - \hat{V}(\hat{\theta}_3)\hat{V}(\hat{\theta}_2\hat{\theta}_3) + \frac{(\hat{\theta}_2\hat{\theta}_3)^2\hat{V}(\hat{\theta}_3)}{t^2_{1-\alpha}}}$$

when $\epsilon = 0$, where the smaller root is negative if $\hat{\theta}_2\hat{\theta}_3$ differs significantly from zero and the larger root is positive. Since $x(\epsilon;\hat{\underset{\sim}{\theta}})$ increases without bound as ϵ approaches zero, a lower confidence limit must do likewise and hence the positive root of (a) in this case is not a lower confidence limit. Equation (b), which has no finite roots at $\epsilon = 0$, does produce a valid lower (and upper) confidence limit in the Mitcherlich case, and is closer to the point estimate $x(\epsilon;\hat{\underset{\sim}{\theta}}) = (1/\hat{\theta}_3)\log(\hat{\theta}_2\hat{\theta}_3/\epsilon)$ than the lower limit of the symmetric interval estimator. The same holds true in the log-linear case, $y = \theta_1 + \theta_2 \log(x + \theta_3)$, where $x(\epsilon;\underset{\sim}{\theta}) = (\theta_2/\epsilon) - \theta_3$; when θ_3 is specified, say $\theta_3 = 0$, then equation (b) produces the lower confidence limit $(\hat{\theta}_2/\epsilon)\exp(-t/T_2)$ while the symmetric interval gives $(\hat{\theta}_2/\epsilon)[1 - (t/T_2)]$, where $T_2 = \hat{\theta}_2/\sqrt{\hat{V}(\hat{\theta}_2)}$. This example, however, illustrates the nature of the approximation errors arising in nonlinear estimation theory; these two confidence limits are asymptotically equal since $V(\hat{\theta}_2)$ converges to zero, but the symmetric interval estimator may be expected to have greater validity in small samples. Statistical discretion is thus required in selecting an appropriate interval estimator.

These regression methods rely upon empirical curve fitting techniques and may be criticized on this basis, though the bulk of all applications of statistics would have to share such criticism. A restraining force does exist to inhibit rampant curve fitting since the target confidence limit estimator is a function of all shape parameters of the fitted curve, and generally speaking the more parameters included in the model the less precisely is each estimated and the longer is the target confidence interval. A desirable property of these methods is their exploitation of the mathematical regularity of the dose response relationship; this relationship is a smooth curve, and regression methods permit interpolation of response between the sparsely scattered set of points representing the test dose levels.

Tests for ordered alternatives. Other approaches besides regression methods are available which require less regularity in the dose response function but which are then correspondingly less efficient if the dose response curve is in fact a smooth, slowly changing function. This class of procedures may be collectively called "tests for ordered alternatives", which are based only upon the assumption that the expected values of response are simply ordered with respect to dose

levels tested. Behavior of the response function between test doses is thus formally irrelevant with these procedures which restrict inference to the doses tested, but informally the requirement that responses at test doses constitute a monotone sequence is logically equivalent to the requirement that over the dose continuum covered by experimental doses, the dose response function must be monotonic. Monotonicity is clearly a much weaker requirement than is necessary for the regression approach, but common sense and impirical evidence support the conjecture that if monotonicity does obtain then the dose response curve is very likely to be highly regular and well approximated by some simple, continuous regression function. From a practical point of view, then, the assumptions underlying tests for ordered alternatives are virtually the same as those underlying the regression approach in this context where the ordering is based upon quantitative levels of a treatment factor.

Procedures available for testing whether response increases monotonically over the highest m test doses include the tests developed by D. J. Bartholomew (JRSS (Ser. B), 1961, p. 239) and by D. A. Williams (Biometrics, 1971, p. 103). Both of these tests are based upon isotonic regression of the m normally distributed treatment means; i.e., the observed treatment means $\bar{Y}_{k-m+1},\cdots,\bar{Y}_k$ are replaced by the maximum likelihood estimates $\hat{M}_{k-m+1},\cdots,\hat{M}_k$ estimated under the parameter restrictions $M_{k-m+1} \leq \cdots \leq M_k$. Thus, if $\bar{Y}_i > \bar{Y}_{i+1}$, then $\bar{Y}_i$ and $\bar{Y}_{i+1}$ are both replaced by $(\bar{Y}_i + \bar{Y}_{i+1})/2$ and, further, if $\bar{Y}_{i-1} > (\bar{Y}_i + \bar{Y}_{i+1})/2$ then all three of these observed treatment means are replaced by their average $(\bar{Y}_{i-1} + \bar{Y}_i + \bar{Y}_{i+1})/3$. Completion of this amalgamation process throughout the sequence $\bar{Y}_{k-m+1},\cdots,\bar{Y}_k$ produces the monotone sequence $\hat{M}_{k-m+1},\cdots,\hat{M}_k$ of length m. Bartholomew's test statistic is given by

$$\bar{E}^2_m = \frac{r \sum_{k-m+1}^{k} (\bar{Y}_i - \bar{Y})^2 - r \sum_{k-m+1}^{k} (\bar{Y}_i - \hat{M}_i)^2}{r \sum_{k-m+1}^{k} (\bar{Y}_i - \bar{Y})^2 + \text{Expt'l Error SS}}$$

and critical values are tabulated by Shorack (Annals Math. Stat., 1967, p. 1744) who also presents a nonparametric version of this test which is the isotonic analogue of Friedman's chi-square test based upon the treatment means of the

within-block ranks. Transformation from ranks to normal scores might be expected to improve the power of this nonparametric test. Such a procedure is to be applied first to all m = k+1 treatment means including the control, then the control is deleted and the test is applied to the remaining m = k treatment means, then to the highest m = k-1 doses, and so on until the first time that $\tilde{E}_m^2$ is not statistically significant. That dose level x_{k-m+1} then becomes the highest recommendable dose level.

Williams proposed a test statistic which in form is identical to Student's t-statistic,

$$\bar{t}_m = \frac{(\hat{M}_k - \bar{Y}_{k-m+1})}{\sqrt{2s^2/r}}$$

where s^2 is experimental error mean square in the randomized block experiment with k + 1 treatments in r complete blocks. His table of critical values of this statistic includes the case of infinite error degrees of freedom, and thus the nonparametric analogue of this test if available, based either upon sums of within-block ranks or sums of the corresponding normal scores.

Simpler procedures with similar but not identical power characteristics may be generated by application of the Abelson and Tukey (Ann. Math. Stat., 1963, p. 1347) linear contrasts for testing ordered alternatives. In this case the linear contrast $\sum_{k-m+1}^{k} c_i \bar{Y}_i = \sum_{1}^{m} c_{k-m+i} \bar{Y}_{k-m+i}$ given by

$$c_i = \sqrt{i-1}\sqrt{\frac{m-i+1}{m}} - \sqrt{i}\sqrt{\frac{m-i}{m}}$$

is tested in the conventional manner by a one-tailed t-test,

$$t = \frac{\sum_{1}^{m} c_{k-m+1} \bar{Y}_{k-m+i}}{\sqrt{\frac{s^2}{r}\sum_{1}^{m} c_i^2}}$$

Again, the nonparametric analogue of this test may be applied to rank sums or sums of tramsformed ranks.

The above procedures do lean heavily upon monotonicity and suffer power losses when monotonicity is violated, as in the empirically suggested case when the weight gain response appears to show a dip at the second highest dose level in a number of antibiotic feed additive trials. Protection against this type of violation of monotonicity may be achieved by turning to Dunnett's procedure for multiple comparisons with a control as an alternative to William's procedure. Thus, the "control" mean $\bar{Y}_{m-k+1}$ is compared with the maximum of the remaining m-1 treatment means $\bar{Y}_{m-k+2},\cdots,\bar{Y}_{k}$ by a Dunnett t-test, using only the upper tail of this test statistic. Again, nonparametric analogues of this test are available.

A substantial disparity still exists between the specificity of assumptions underlying regression procedures and those underlying tests for ordered alternatives. Regression procedures exploit more (assumed) prior information about the dose response relationship and are thereby potentially more efficient when the assumptions are (approximately) correct. The particular assumption that dy/dx is positive and monotonic has wide empirical support and is the basis for selecting certain explicit regression functions with this property; monotonicity of dy/dx is not exploited at all, however, by existing techniques for testing against ordered alternatives. A need was therefore seen to exist for tests against ordered alternatives with the additional restriction that $(M_{i+1} - M_i)/(x_{i+1} - x_i)$ is also monotonic. Such procedures for testing against monotone-concave or convex alternatives have recently been developed by J. L. Rosenberger (unpublished Ph.D. Thesis, Cornell University, 1977).

ILLUSTRATIONS OF REGRESSION METHODOLOGY

Least squares methods will be used to fit three-parameter models of the form

$$Y = a + bg(X;c).$$

Such models might be regarded as linear on a transformed dose scale $g(X;c)$; but as functions of the parameters, these models are nonlinear in b and c. In certain special cases such as $g(X;c) = X + cX^2$, the model may be reparameterized into a form which is linear in the new parameters; thus

$$Y = a + b(X + cX^2) = a^* + b^*X + c^*X^2$$

is linear in the new parameters a^*, b^*, and c^*, where

$$a^* = a \quad b^* = b \quad c^* = bc.$$

When such a linear reparameterization is not possible, then nonlinear least squares methods must be employed in estimating the unknowns a, b, and c.

The particular examples of g-functions to be illustrated here are

Unrestricted quadratic in X : $g(X;c) = X + cX^2$

Unrestricted quadratic in $\sqrt{X}$: $g(X;c) = \sqrt{X} + cX$

Quadratic-horizontal in X : $g(X;c) = (c-X)^2 I_+(c-X)$

$$I_+(c-X) = \begin{cases} 1 & \text{if } X \le c \\ 0 & \text{if } X > c \end{cases}$$

Quadratic-horizontal in $\sqrt{X}$: $g(X;c) = (c - \sqrt{X})^2 I_+(c - \sqrt{X})$

$$I_+(c-\sqrt{X}) = \begin{cases} 1 & \text{if } \sqrt{X} \le c \\ 0 & \text{if } \sqrt{X} > c \end{cases}$$

Mitcherlich model : $g(X;c) = e^{cX}$

Log-linear model : $g(X;c) = \log_e(X + c)$

Nonlinear least squares. A formula $Y = f(X;a,b,c)$ for predicting a "dependent variable" Y from the "independent variable(s)" X will involve some "unknown parameters", say a, b, and c. The formula is said to be nonlinear in the parameters if any of the partial derivatives $\partial Y/\partial a$, $\partial Y/\partial b$, $\partial Y/\partial c$ depend upon any of the unknown parameters. When this occurs, then the least squares

solution for the unknowns a, b, and c can ordinarily be calculated only by iterative methods.

Each stage of iteration produces a revised set of estimates of a, b, and c. The next stage then consists of (i) calculating the prediction errors $e = Y - f(X;a,b,c)$ and the partial derivatives $\partial Y/\partial a$, $\partial Y/\partial b$, $\partial Y/\partial c$ at each dose level X, and (ii) calculating the multiple linear regression of the "dependent variable" e on the three "independent variables" $\partial Y/\partial a$, $\partial Y/\partial b$, $\partial Y/\partial c$. The three corresponding multiple linear regression coefficients then constitute the corrections which (iii) are algebraically added to a, b, and c, respectively, to form the revised estimates for the next stage. The process continues until such corrections become negligible.

Models of the form $Y = a + bg(X;c)$ are nonlinear because

$$\frac{\partial Y}{\partial a} = 1 \qquad \frac{\partial Y}{\partial b} = g(X;c) \qquad \frac{\partial Y}{\partial c} = b\,\frac{\partial g(X;c)}{\partial c}$$

and except for $\partial Y/\partial a = 1$, these partial derivatives do depend upon unknown parameters. In the log-linear case, for example, where $Y = a + b\log(X + c)$ we have

$$\frac{\partial Y}{\partial a} = 1 \qquad \frac{\partial Y}{\partial b} = \log(X + c) \qquad \frac{\partial Y}{\partial c} = \frac{b}{X + c}\,,$$

or when fitting $Y = a + b(c - \sqrt{X})^2 I_+(c - \sqrt{X})$ then

$$\frac{\partial Y}{\partial a} = 1 \qquad \frac{\partial Y}{\partial b} = (c - \sqrt{X})^2 I_+(c - \sqrt{X}) \qquad \frac{\partial Y}{\partial c} = 2b(c - \sqrt{X}) I_+(c - \sqrt{X}).$$

Estimation in these nonlinear cases of $Y = a + bg(X;c)$ begins with any reasonable guesses for the values of a, b, and c. The guessed values are then successively adjusted by a three-variable linear regression algorithm to ultimately produce the least squares estimates. The dependent variable in this regression algorithm is the calculated residual

$$e = Y - a - bg(X;c),$$

which is to be linearly regressed upon the two independent variables

$$X_b = \frac{\partial Y}{\partial b} = g(X;c)$$

and

$$X_c = \frac{\partial Y}{\partial c} = b\,\frac{\partial g(X;c)}{\partial c}\,.$$

The coefficients (including the intercept as a coefficient of $X_a = \partial Y/\partial a = 1$) obtained in the regression equation

$$e = A + BX_b + CX_c$$

are the adjustments which will give the revised estimates a', b', and c':

$$a' = a + A \qquad b' = b + B \qquad c' = c + C.$$

The coefficients A, B, and C all converge to zero in successive applications of this algorithm, and the iteration process then terminates.

At termination, the second degree statistics of the final multiple linear regression analysis come into use for calculating standard errors and covariances of the least squares estimates a, b, and c, as well as the residual mean square. Since 3 parameters are estimated in the regression equation, degrees of freedom in the residual sum of squares are 3 less than the number levels including control; thus, if the treatment design consisted of k levels plus the control then df for residuals are k + 1 - 3 = k - 2. Expressed on a "per experimental unit" scale, the residual mean square $s^2_{y\cdot x}$ is then given by the formula

$$s^2_{y\cdot x} = \frac{r\Sigma e^2}{k - 2}$$

if the Y-variable in the regression calculations was the mean for r replicates (locations) or

$$s^2_{y\cdot x} = \frac{\Sigma e^2}{r(k - 2)}$$

if the Y-variable was the total for r replicates. Expressed on the "per experimental unit" scale, the residual mean square $s^2_{y\cdot x}$ is directly comparable to the experimental error mean square (EMS = levels X locations MS) calculated from an analysis of variance (anov) of the complete (k + 1) x r data table.

The other second degree statistics from the final regression of e on X_b and X_c are:

$$\Sigma x_b^2 = \Sigma X_b^2 - \frac{(\Sigma X_b)^2}{k + 1} \qquad \Sigma x_c^2 = \Sigma X_c^2 - \frac{(\Sigma X_c)^2}{k + 1}$$

$$\Sigma x_b e = \Sigma X_b e - \frac{(\Sigma X_b)(\Sigma e)}{k + 1} \qquad \Sigma x_c e = \Sigma X_c e - \frac{(\Sigma X_c)(\Sigma e)}{k + 1}$$

$$\Sigma x_b x_c = \Sigma X_b X_c - \frac{(\Sigma X_b)(\Sigma X_c)}{k + 1}.$$

Correction terms involving the factor Σe should, in fact, be zero since Σe should have converged to zero. The regression coefficients B and C as well as the intercept A should likewise have converged to zero:

$$A = (\Sigma e - B\Sigma X_b - C\Sigma X_c)/(k + 1) \approx 0$$

$$B = v_{bb}\Sigma x_b e + v_{bc}\Sigma x_c e \approx 0$$

$$C = v_{bc}\Sigma x_b e + v_{cc}\Sigma x_c e \approx 0 .$$

Coefficients denoted here by v's are the elements of the inverse of the 2 x 2 matrix of corrected sums of squares and cross products of X_b and X_c:

$$\begin{bmatrix} v_{bb} & v_{bc} \\ v_{bc} & v_{cc} \end{bmatrix} = \begin{bmatrix} \Sigma x_b^2 & \Sigma x_b x_c \\ \Sigma x_b x_c & \Sigma x_c^2 \end{bmatrix}^{-1}$$

or

$$v_{bb} = \frac{\Sigma x_c^2}{\Sigma x_b^2 \Sigma x_c^2 - (\Sigma x_b x_c)^2}$$

$$v_{cc} = \frac{\Sigma x_b^2}{\Sigma x_b^2 \Sigma x_c^2 - (\Sigma x_b x_c)^2}$$

$$v_{bc} = \frac{-\Sigma x_b x_c}{\Sigma x_b^2 \Sigma x_c^2 - (\Sigma x_b x_c)^2} .$$

These v-coefficients also appear in the formulas for the standard errors of the least squares estimates; in particular,

$$s_b^2 = \frac{EMS}{r} v_{bb} \qquad s_c^2 = \frac{EMS}{r} v_{cc}$$

$$s_{bc} = \frac{EMS}{r} v_{bc}$$

where EMS is the error mean square (levels x locations MS) from the analysis of variance.

A data set. The methods are illustrated on two different data sets, A and B, the first of which exhibits a relatively small error variance and produces satisfactory results under most methods. Experimental error variance is relatively large in data set B, and all methods then indicate a low (or even negative) maximum recommendable dose. In both cases the data are synthetic, having been derived from real data but modified for these purposes of illustration.

The data are presented in the format of a two-way table with columns representing replicates (locations or blocks) and are analyzed by analysis of variance (anov) as a randomized complete block design (Snedecor, G. W. and Cochran, W. G., 1967, p. 300). A summary statistic required from the analysis of variance table is the standard error of a treatment mean,

$$s_{\bar{y}} = \sqrt{\frac{\text{Error mean square}}{\text{number of replicates}}}$$

$$= \sqrt{\frac{EMS}{r}}$$

which is listed below the anov table. Also listed here is the coefficient K in the formula for calculating the smallest detectable slope $\epsilon = Ks_{y \cdot x}$,

$$K = \frac{2(t_{.95} - t_{.75})}{X_k\sqrt{r(k+1)}} \qquad df = r(k+1) - 2.$$

The residual mean square $s^2_{y \cdot x}$ required to complete the calculation of ϵ is obtained from regression analysis as illustrated below.

Data Set A

Level X	Location 1	2	3	Mean Y
0	1.47	1.33	1.52	1.44
10	1.59	1.56	1.62	1.59
20	1.65	1.57	1.58	1.60
40	1.66	1.65	1.70	1.67
80	1.61	1.67	1.76	1.68

anov

	df	SS	MS
Level	4	.11076	.02769
Location	2	.016	.008
Error	8	.0218	.002725

$$s_{\bar{y}} = \sqrt{\frac{.002725}{3}} = .030139$$

$$K = \frac{2(1.7709 - .6938)}{80\sqrt{3(5)}} = .006953$$

Analysis of an unrestricted quadratic in X. Conventional, non-iterative, multiple linear regression methods may be used to fit the unrestricted quadratic model

$$Y = a^* + b^*X + c^*X^2$$

to the mean responses Y at dose levels X. If the estimate b^* is positive and c^* is negative then this quadratic curve peaks at the level $-b^*/(2c^*)$. If this estimated level falls within the range of test levels (0 to 80 in case A), then the maximum recommendable level is given by the lower, one-sided 95% confidence limit

$$X_L = \frac{-b^*c^* + t^2 s_{b^*c^*} - t\sqrt{(c^* s_{b^*})^2 + (b^* s_{c^*})^2 - 2b^*c^* s_{b^*c^*} - t^2(s^2_{b^*}s^2_{c^*} - s^2_{b^*c^*})}}{2\left((c^*)^2 - t^2 s^2_{c^*}\right)}$$

where t denotes the 95th percentile of Student's t on k(r - 1) df.

If the estimate $-b^*/(2c^*)$ exceeds the maximum tested level then the method for determining maximum recommendable dose level utilizes the above formula for X_L but with b^* replaced by $b^* - \epsilon$, $\epsilon = Ks_{y\cdot x}$. The estimates $s^2_{b^*}$ and $s_{b^*c^*}$ as well as $s^2_{c^*}$ remain unchanged, and $s^2_{y\cdot x}$ is the residual mean square (on a per experimental unit basis) obtained from fitting the quadratic regression function.

Fitting this "unrestricted quadratic in X" model to the data set A by three-variable linear regression methods, our dependent variable Y is the mean response at dose level X, and our two independent variables may be notationally defined as

$X_{b^*} = X$ and $X_{c^*} = X^2$, giving:

	X_{b^*}	X_{c^*}	Y
	0	0	1.44
	10	100	1.59
	20	400	1.60
	40	1600	1.67
	80	6400	1.68
Mean	30	1700	1.596

$$\Sigma x_{b^*}^2 = 4000 \qquad \Sigma x_{c^*}^2 = 2924 \times 10^4$$

$$\Sigma x_{b^*} y = 9.7 \qquad \Sigma x_{c^*} y = 657$$

$$\Sigma x_{b^*} x_{c^*} = 33 \times 10^4$$

$$\Sigma x_{b^*}^2 \Sigma x_{c^*}^2 - (\Sigma x_{b^*} x_{c^*})^2 = 806 \times 10^7$$

$$v_{b^*b^*} = 3.628 \times 10^{-3} \qquad v_{c^*c^*} = 4.963 \times 10^{-7}$$

$$v_{b^*c^*} = -4.0943 \times 10^{-5}$$

$$b^* = v_{b^*b^*}\Sigma x_{b^*} y + v_{b^*c^*}\Sigma x_{c^*} y = 8.290 \times 10^{-3}$$

$$c^* = v_{b^*c^*}\Sigma x_{b^*} y + v_{c^*c^*}\Sigma x_{c^*} y = -7.109 \times 10^{-5}$$

$$a^* = \bar{Y} - b^*\bar{X}_{b^*} - c^*\bar{X}_{c^*} = 1.46815$$

$$s_{b^*} = s_y\sqrt{v_{b^*b^*}} = .030139\sqrt{.008290} = 2.744 \times 10^{-3}$$

$$s_{c^*} = s_y\sqrt{v_{c^*c^*}} = 2.123 \times 10^{-5}$$

$$s_{b^*c^*} = s_y^2 v_{b^*c^*} = -3.719 \times 10^{-8} .$$

The estimated quadratic regression function

$$Y = 1.46815 + .008290X - .0000/109X^2$$

achieves its maximum value at

$$\frac{-b^*}{2c^*} = 58.3$$

as illustrated in Figure 2. The sum of squares due to regression,

$$b^{*}\Sigma x_b{}^{*}y + c^{*}\Sigma x_c{}^{*}y = .033707$$

when expressed on a "per experimental unit" basis by applying the factor r = 3,

$$.033707r = .101121,$$

is seen to account for a major fraction of the sum of squares "among levels" from the anov:

	df	SS	MS
Regression	2	.10112	
Residual	2	.00964	.004820
Levels	4	.11076	
Error	8	.0218	.002725

Regression thus accounts for a fraction

$$R^2 = \frac{.10112}{.11076} = .913$$

of sum of squares among levels, and the residual mean square

$$s^2_{y\cdot x} = .004820$$

does not differ significantly from the experimental error mean square. The F-statistic

$$F = \frac{.004820}{.002725} = 1.77$$

on 2 and 8 df is not statistically significant.

Since the maximum of the estimated quadratic curve does occur within the range of test dose levels, $0 < 58.3 < 80$, then the formula for the lower confidence limit X_L is directly applicable. The required 95^{th} percentile from the tables of Student's t distribution on 8 df is (see, for example, Documenta Geigy Scientific Tables, Seventh Edition)

$$t = 1.8595.$$

Calculating other component parts of X_L, we get

$$(c^* s_{b^*})^2 + (b^* s_{c^*})^2 - 2b^* c^* s_{b^* c^*}$$

$$= 380.5268 \times 10^{-16} + 309.7484 \times 10^{-16} - 438.3482 \times 10^{-16}$$

$$= 251.9270 \times 10^{-16}$$

$$t^2(s^2_{b^*} s^2_{c^*} - s^2_{b^* c^*}) = (1.8595)^2(33.9366 \times 10^{-16} - 13.831 \times 10^{-16})$$

$$= 69.5201 \times 10^{-16}$$

and then

$$X_L = \frac{58.9336 \times 10^{-8} - 12.8593 \times 10^{-8} - 1.8595\sqrt{182.4069 \times 10^{-16}}}{2(50.5379 \times 10^{-10} - 15.5845 \times 10^{-10})} = 30.$$

Except insofar as the unrestricted quadratic curve does come reasonably close to all five data points in Figure 2, the data otherwise give no hint of a maximum response between $40 \leq X \leq 80$. The quadratic peak is therefore relatively flat, and consequently difficult to estimate with any precision, resulting in a lower confidence limit, 30, at about one-half the value of the point estimate, 58.3.

Analysis of the quadratic-horizontal in X. The occurrence of a peak between $40 \leq X \leq 80$ may indeed be simply an artifact of quadratic curvature, and may be altered from a peak to a plateau by imposing such a restriction on the fitted quadratic function. An unrestricted quadratic curve is symmetric about its peak, first increasing to its maximum value and then decreasing in exactly the same manner. The second, decreasing half of the curve will now be replaced by a horizontal line extending to the right of the peak and forming a plateau, as shown in Figure 3. The two segments of such a curve may be expressed by the formula

$$Y = \begin{cases} a + b(c - X)^2 & \text{for } X \leq c \\ a & \text{for } X > c \end{cases}.$$

In the present context, a and c will be positive numbers and b will be negative.

The parameter c in this model corresponds to $-b^*/(2c^*)$ in the previous model, being the location at which the plateau begins. As in the unrestricted case, the

quadratic-horizontal curve which best fits the data (in the least squares sense) may involve a c-value which exceeds the maximum tested dose level. The segment of the curve which does fall within the range of test levels is the pure quadratic segment, and is in fact then identical to the first half of the least squares unrestricted quadratic curve. Thus, whenever a least squares, unrestricted quadratic curve produces a peak beyond the test level range, then a quadratic-linear curve will produce exactly the same curve. In such circumstances the procedure for determining a maximum recommendable dose level reverts to the method described earlier, where b^* is replaced by $b^* - \epsilon$ in the formula for calculating X_L.

The segmented, or two-phase, quadratic-horizontal model is a nonlinear function in terms of the unknown parameters b and c, and requires nonlinear least squares estimation procedures. As outlined earlier, this procedure is iterative and consists of successive adjustments to the estimates a, b, and c until the adjustments converge to zero. The process begins with "reasonable guessed values" for a, b, and c, and in the quadratic-horizontal case these initial values for a, b, and c may be taken from the coefficients a^*, b^*, and c^* of the easily calculated least squares unrestricted quadratic curve. As noted above, the parameter c of the quadratic-horizontal curve corresponds to $-b^*/(2c^*)$, and hence the initial value for c becomes

$$c = -b^*/(2c^*).$$

Initial values for a and b are then given by

$$b = c^* \qquad a = a^* - bc^2.$$

Applying these formulas to data set A gives the initial estimates:

$$a = 1.71 \qquad b = -.00007 \qquad c = 58.3.$$

Adjustments to these initial estimates are obtained by a three-stage process which consists first of calculating the two independent variables

$$X_b = \frac{\partial Y}{\partial b} = g(X;c) = \begin{cases} (c - X)^2 & \text{if } X \le c \\ 0 & \text{if } X > c \end{cases}$$

$$X_c = \frac{\partial Y}{\partial c} = b\,\frac{\partial g(X;c)}{\partial c} = \begin{cases} 2b(c - X) & \text{if } X \le c \\ 0 & \text{if } X > c \end{cases}$$

and the dependent variable

$$e = Y - a - bg(X;c) = \begin{cases} Y - a - b(c - X)^2 & \text{if } X \leq c \\ Y - a & \text{if } X > c \end{cases}.$$

Using data set A and the above initial values for a, b, and c we obtain

X	Y	X_b	X_c	e
0	1.44	3398.89	-.008162	-.0320777
10	1.59	2332.89	-.006762	.0433023
20	1.60	1466.89	-.005362	-.0073177
40	1.67	334.89	-.002562	-.0165577
80	1.68	0	0	-.03

At X = 10, for example,

$$X_b = (58.3 - 10)^2 = 2332.89 \qquad X_c = -.00014(58.3 - 10) = -.006762$$

$$e = 1.59 - 1.71 + .00007(2332.89) = .0433023.$$

The next and major step consists of calculating the multiple linear regression of the dependent variable e on the two independent variables X_b and X_c. Denoting the intercept of this regression by A, the slope with respect to X_b by B, and the slope with respect to X_c by C, this calculation gives

$$A = -.0365664 \qquad B = -.0000341 \qquad C = -17.3745586$$

where extended decimals are being carried to allow the reader to check the arithmetic operations. These represent algebraic adjustments to be applied to the initial estimates, and the third step in the iteration then produces the revised estimates with which to begin the next iteration,

$$a = 1.71 - .0365664 = 1.6734336 \qquad b = -.00007 - .0000341 = -.0001041 \qquad c = 58.3 - 17.3745586 = 40.9254414 .$$

All three steps must now be repeated, recalculating X_b, X_c, and e using data set A and these revised estimates of a, b, and c:

X_b	X_c	e
1674.89175	-.00851957	-.05909997
956.38292	-.00643784	.01611294
437.87410	-.00435611	-.02785684
85.64417	-.00019265	-.00334449
0	0	.00656637

A = -.00076095	B = -.0000513?	C = -4.81260096
1.6734336	-.0001041	40.9254414
a = 1.67267265	b = -.00015547	c = 36.11284044

The iteration process thus continues in this manner. If the above calculations are referred to as the first and second iterations, then the following calculations constitute the tenth iteration:

X_b	X_c	e
1335.364551	-.01203629966	-.01228341091
704.5117479	-.008742531176	.03382243489
273.6589442	-.005448762690	-.02713403444
0	0	-.0022024 94768
0	0	.007797505232

$A = 4 \times 10^{-10}$	$B = 4 \times 10^{-12}$	$C = 4.258 \times 10^{-7}$
1.6722024 94	-.0001646 8842	36.54264018
a = 1.6722025	b = -.0001647	c = 36.54264

This tenth set of adjustments are negligible, and the above values for a, b, and c are taken to be the least squares estimates. Second degree statistics obtained from this tenth multiple linear regression analysis are:

$$\Sigma x_b^2 = 1283935.441 \qquad \Sigma x_c^2 = 1.134160433 \times 10^{-4}$$

$$\Sigma x_b x_c = -11.58747406 \qquad \Sigma e^2 = .0020967472$$

$$v_{bb} = .09932 \times 10^{-6} \qquad v_{cc} = 113128.8196$$

$$v_{bc} = 1.0209838$$

The plateau is estimated to start at the level c = 36.5426, and the standard error of c is estimated by

$$s_y\sqrt{v_{cc}} = .030139(336.3463) = 10.1371.$$

The maximum recommendable dose level is now given by the lower confidence limit

$$36.5426 - 1.8595(10.1371) = 17.7.$$

Analysis of an unrestricted quadratic in $\sqrt{X}$. The reason for spacing dose levels at ever wider intervals is the anticipated decrease in slope of the response function at higher dose levels. A nonlinear transformation $g(X;c)$ of the dose scale which induces relatively greater contractions at higher dose levels would tend to counteract this tendency for slope to decrease. Slope with respect to this transformed scale would thus tend to be more nearly constant. The square root transformation does have greater effect at the higher levels; the difference between 1 and 4, for example, being the same as the difference between 64 and 81 when plotted on the square root scale. Failure to exactly stabilize slope by this transformation may be roughly accommodated by adding a quadratic term to the model. A quadratic term on the $\sqrt{X}$ scale is linear in X, thus producing:

$$Y = a^* + b^*\sqrt{X} + c^*X.$$

When b^* is positive and c^* is negative, this curve peaks at

$$\sqrt{X} = -\frac{b^*}{2c^*} \quad \text{or} \quad X = \left(\frac{b^*}{2c^*}\right)^2.$$

If $(b^*)^2/(2c^*)^2$ lies within the range of test levels, then a lower confidence limit $\sqrt{X_L}$ on $(-b^*)/(2c^*)$ may be computed as before and then squared to obtain the recommendable dose level X_L. If $(b^*)^2/(2c^*)^2$ exceeds the largest test level, then we may revert to calculating the smallest detectable slope ϵ and a lower confidence limit on the dose level at which the slope ϵ is achieved. This slope ϵ is calculated with respect to the original scale X, which is proportional to both the price and the mass of the antibiotic,

$$\frac{dY}{dX} = \frac{b^*}{2\sqrt{X}} + c^* .$$

The least squares fit of data set A to this model is obtained by applying three-variable linear regression analysis to

$$Y = a^* + b^* X_{b^*} + c^* X_{c^*}$$

where

Y	X_{b^*}	X_{c^*}
1.44	0	0
1.59	$\sqrt{10}$	10
1.60	$\sqrt{20}$	20
1.67	$\sqrt{40}$	40
1.68	$\sqrt{80}$	80

$$\Sigma y^2 = .03692 \qquad \Sigma x_{b^*}^2 = 45.08831175 \qquad \Sigma x_{c^*}^2 = 4000$$

$$\Sigma x_{b^*} y = 1.218250812 \qquad \Sigma x_{c^*} y = 9.7 \qquad \Sigma x_{b^*} x_{c^*} = 402.4922359$$

$$\Sigma x_{b^*}^2 \Sigma x_{c^*}^2 - (\Sigma x_{b^*} x_{c^*})^2 = 18353.24704$$

$$v_{b^*b^*} = .2179450858 \qquad v_{c^*c^*} = .002456694$$

$$s_{b^*} = s_y \sqrt{v_{b^*b^*}} = .0140702683 \qquad s_{c^*} = .001493841$$

$$v_{b^*c^*} = -.0219303012$$

$$s_{b^*c^*} = s_y^2 v_{b^*c^*} = -.00001992$$

$$a^* = 1.440797670 \qquad b^* = .05278785592 \qquad c^* = -.0028866755$$

$$\Sigma y^2 - b^* \Sigma x_{b^*} y - c^* \Sigma x_{c^*} y = \Sigma e^2 = .00061190$$

$$s_{y \cdot x}^2 = \frac{\Sigma e^2}{5 - 3} = .00030595$$

$$s_{y \cdot x}^2 = r s_{y \cdot x}^2 = .0009179$$

$$s_{y \cdot x} = .030296 .$$

A peak in the response curve is estimated to occur at

$$\left(\frac{-b^*}{2c^*}\right)^2 = 83.6,$$

which is beyond the largest test level of 80. Rather than construct a lower confidence limit on this extrapolated peak location we may calculate

$$\epsilon = .006953 s_{y \cdot x} = .00021065$$

and then

$$t\sqrt{(c^* - \epsilon)^2 s_{b^*}^2 + (b^* s_{c^*})^2 - 2b^*(c^* - \epsilon) s_{b^* c^*} - t^2(s_{b^*}^2 s_{c^*}^2 - s_{b^* c^*}^2)}$$

$$= .0000707634$$

$$-b^*(c^* - \epsilon) + t^2 s_{b^* c^*} = .000094623$$

$$2[(c^* - \epsilon)^2 - t^2 s_{c^*}^2] = .0000037545 .$$

The lower confidence limit X_L then becomes

$$\sqrt{X_L} = \frac{.000094623 - .0000707634}{.0000037545} = 6.3547$$

or

$$X_L = 40.38 .$$

Exactly this same solution would be obtained if we were to fit a restricted, quadratic-horizontal in $\sqrt{X}$. The quadratic segment of this two-phase model would be

$$Y = 1.440797670 + .0527875592\sqrt{X} - .0028866755X$$

for $X \leq 83.6$, exactly as in the above unrestricted model, and the horizontal segment would then begin at this level. The quadratic segment is seen to fit the means of data set A quite closely in Figure 4, the multiple correlation coefficient being

$$R^2 = 1 - \frac{\Sigma e^2}{\Sigma y^2} = .9834 .$$

Analysis of the Mitcherlich model. A concave quadratic on any scale T(X) incorporates the feature of a maximum response at a finite dose level. The Mitcherlich model

$$Y = a + be^{cX} \qquad b < 0,\ c < 0$$

retains the feature of increasing to a definite maximum, but this curve achieves its maximum (a) only at an infinite dose level. The earlier question of whether to construct a lower confidence limit on the maximizing dose level, where the slope is zero, or on the dose level at which the slope is ϵ, cannot be an issue when the Mitcherlich model is used.

The dose level x_ϵ at which the derivative of the Mitcherlich function equals ϵ is given by

$$x_\epsilon = -\frac{1}{c} \ln \frac{bc}{\epsilon} .$$

This solution depends upon the two unknown parameters b and c, but depends upon b only through the function

$$d = \ln bc.$$

In terms of the estimates c and d, a lower confidence limit X_L for x_ϵ is given by

$$X_L = \frac{x_\epsilon - (\frac{t}{c})^2 s_{cd} + \frac{t}{c}\sqrt{x_\epsilon^2 s_c^2 + s_d^2 - 2x_\epsilon s_{cd} - (\frac{t}{c})^2(s_c^2 s_d^2 - s_{cd}^2)}}{1 - (\frac{t}{c})^2 s_c^2} .$$

The parameter a is the asymptotic maximum of the Mitcherlich function with negative b and c parameters, and a reasonable initial value for the asymptotic maximum is readily obtained by inspection of the data. Initial values for b and c, needed to begin the nonlinear least squares iterations, may then be obtained by calculating a simple linear regression of $\ln(a - Y)$ on dose level X, since

$$Y = a + be^{cX}$$

$$a - Y = -be^{cX}$$

$$\ln(a - Y) = \ln(-b) + cX .$$

The intercept of this simple linear regression thus provides an initial value for $\ell n(-b)$, which may be transformed into an initial value for b, and the slope provides an initial value for c. Estimation then proceeds iteratively by calculating the two independent variables

$$X_b = \frac{\partial Y}{\partial b} = e^{cX} \qquad X_c = \frac{\partial Y}{\partial c} = bXe^{cX}$$

and the dependent variable

$$e = Y - a - bX_b \ .$$

The adjustments A, B, and C to be applied to the estimates a, b, and c are then obtained from the linear regression of e on X_b and X_c,

$$e = A + BX_b + CX_c \ .$$

Inspection of data set A shows that a = 1.7 is a reasonable, rough guess of the maximum response. An equally rough calculation of $\ell n(a - Y)$:

X	Y	1.7 - Y	$\ell n(1.7 - Y)$
0	1.44	.26	-1.35
10	1.59	.11	-2.21
20	1.60	.10	-2.30
40	1.67	.03	-3.51
80	1.68	.02	-3.91

regressed on X gives:

slope = - .030725 = c

intercept = -1.73425 = $\ell n(-b)$ b = -.17653 .

Using these values for b and c together with a = 1.7 we then calculate

X	X_b	X_c	e
0	1	0	-.08347
10	.73547	-1.29832	.01983
20	.54091	-1.90974	-.00451
40	.29259	-2.06600	.02165
80	.08561	-1.20896	-.00489

and

A = -.05933	B = -.01204	C = -.04276
1.7	-.17653	-.030725
a = 1.64067	b = -.18857	c = -.073485

These revised values for a, b, and c then give the recalculated:

X_b	X_c	e
1	0	-.01210
.47958	-.90434	.03976
.22999	-.86740	.00270
.05290	-.39899	.03930
.00280	-.04221	.03986

and

A = .03614	B = -.04317	C = -.002278
1.64067	-.18857	-.073485
a = 1.67681	b = -.23170	c = -.075763

After two more iterations the results are

A = -.0000003	B = -.0000008	C = .0000027
1.676577	-.231667	-.075774
a = 1.676577	b = -.231668	c = -.075771

$\Sigma x_b^2 = .66546$ $\Sigma x_c^2 = 1.0712$ $\Sigma x_b x_c = .14800$

$$\Sigma x_b^2 \Sigma x_c^2 - (\Sigma x_b x_c)^2 = .69094$$

$v_{bb} = 1.55035$ $v_{cc} = .96312$ $v_{bc} = -.21420$

$\Sigma e^2 = .001205$ $s_{y \cdot x} = \sqrt{\frac{r\Sigma e^2}{5 - 3}} = .04251$

From the anov of data set A we then compute

$$\epsilon = .006953 s_{y \cdot x} = .0002956$$

$$s_b = .030139\sqrt{v_{bb}} \qquad s_c = .030139\sqrt{v_{cc}}$$

$$= .037527 \qquad = .029578$$

$$s_{bc} = (.030139)^2 v_{bc}$$

$$= -.0001946 .$$

The estimated dose level x_ϵ at which $dY/dX = \epsilon$ is now

$$x_\epsilon = -\frac{1}{c} \ln \frac{bc}{\epsilon} = 53.9.$$

In order to calculate a lower confidence limit from the formula for X_L we also need:

$$s_d^2 = \frac{s_b^2}{b^2} + \frac{s_c^2}{c^2} + \frac{2s_{bc}}{bc} = .156449$$

$$s_{cd} = -\frac{s_{bc}}{c} - \frac{s_c^2}{c} = .010707 .$$

Substitution into the formula for X_L with $t = 1.8595$ then gives

$$X_L = 36.1 .$$

Analysis of the log-linear model. The common use of logarithmically equal spacing of test dose levels to accommodate that anticipated reduction in slope at higher dose levels, suggests that the anticipated form of the response curve is approximately log-linear:

$$Y = a + b \log(X + c).$$

Like the Mitcherlich, this function increases forever but at a decreasing rate,

$$\frac{dY}{dX} = \frac{b}{X + c}$$

which converges to zero. The dose level

$$x_\epsilon = \frac{b}{\epsilon} - c$$

at which dY/dX has diminished to the value ϵ is estimated to exceed the limit

$$X_L = x_\epsilon - t\sqrt{\frac{s_b^2}{\epsilon^2} + s_c^2 - 2\frac{s_{bc}}{\epsilon}} \quad .$$

The value of c seemingly anticipated in choosing a geometric series of dose levels is c = 0; this would provide a reasonable and convenient initial value to start the nonlinear iterations while also providing a statistical basis for testing whether c does differ significantly from zero. Regressing Y on log X at the test levels $X > 0$,

$$Y = a + b \log X$$

also provides initial values of a and b to start the nonlinear least squares iterations. These iterations consist of calculating

$$X_b = \frac{\partial Y}{\partial b} = \log(X + c) \qquad X_c = \frac{\partial Y}{\partial c} = \frac{b}{X + c}$$

$$e = Y - a - bX_b$$

and the multiple linear regression of e on X_b and X_c,

$$e = A + BX_b + CX_c$$

to obtain the adjusted parameter estimates a + A, b + B, and c + C.

Regression of Y on ℓn X in data set A produces the equation

$$Y = 1.471 + .049 \ell n\ X$$

with a residual sum of squares of .00072 on 2 df and a correlation coefficient of r = .943 between Y and ℓn X with one df. This correlation is statistically significant when tested by anov:

	df	SS	MS	F
Control vs. Test levels	1	.09126		
Among Test levels	3	.0195		
Regression on ℓn X	1	.01734	.01734	6.36
Residual	2	.00216	.00108	0.40
Error	8	.0218	.002725	

The residual mean square, expressed on a "per experimental unit" basis as $3(.00072)/2 = .00108$, is of the same order of magnitude as the experimental error mean square. The evidence thus provided by the two indicated F-tests is sufficient to conclude that c = 0 provides an adequate fit.

The value for x_ϵ in this special case is calculated as follows:

$$\Sigma(\ell n\ X - \overline{\ell n\ X})^2 = 2.402265 \qquad v_{bb} = \frac{1}{2.402265} = .41627$$

$$s_{y\cdot x} = \sqrt{.00108} = .03286 \qquad s_b = .030139\sqrt{v_{bb}} = .01945$$

$$\epsilon = .006953 s_{y\cdot x} = .0002285 \qquad x_\epsilon = \frac{b}{\epsilon} = 214.44$$

$$X_L = x_\epsilon - t\frac{s_b}{\epsilon} = 56.2$$

Data Set B

Level X	I	Location II	III	IV	Mean Y
0	8.07	7.58	8.29	8.19	8.0325
1	8.28	8.23	8.36	8.61	8.37
2	8.96	8.54	8.49	8.63	8.655
4	8.00	9.44	9.01	8.75	8.8
8	9.18	7.65	9.75	8.04	8.655
16	9.13	8.86	9.73	9.02	9.185

anov B

	df	SS	MS	F
Levels	5	3.0466	.609	2.32
Locations	3	.9838	.328	
Error	15	3.9250	.262	

$$s_{\bar{y}} = \sqrt{\frac{.262}{4}} = .256$$

$$K = \frac{2(1.717 - .686)}{16\sqrt{4(6)}} = .0263$$

$$t_{.95} = 1.753 \quad \text{on 15 df.}$$

Regression analyses of a second data set. A substantially more erratic data set will now be analyzed by some of the preceding methods to illustrate the ways in which the methods break down. Both the number of levels tested and the number of replicates are increased by one in data set B, but the experimental error is so large relative to treatment differences that an F-test of treatment effects is not significant at the 5 percent level.

A quadratic-horizontal model fitted to these treatment means produces the segmented curve

$$Y = \begin{cases} 8.8952 - .0389(4.6758 - X)^2 & \text{for } X \leq 4.6758 \\ 8.8952 & \text{for } X > 4.6758 \end{cases}$$

but the estimated standard error attaching to 4.6758 is 2.9878, resulting in a negative lower confidence limit on the dose level at the start of the plateau.

The Mitcherlich model fitted to data set B accounts for 82.44 percent of the treatment sum of squares, leaving a residual mean square $s^2_{y \cdot x} = .178344$. The dose level x_ϵ at which the slope of the fitted curve

$$Y = 8.9665 - .9067e^{-.4175X}$$

achieves a slope of $\epsilon = .00896$ is $x_\epsilon = 28.26$. The estimated standard error attaching to 28.26 is 18.16, resulting in a negative lower confidence limit.

The most revealing case is the quadratic regression on $\sqrt{X}$:

	df	SS	MS	F
Quad. regression on $\sqrt{X}$	2	2.6843	1.342	5.12
Linear on $\sqrt{X}$	1	2.6402	2.640	10.08
Quad. elim. linear	1	.0441	.044	0.17
Residual	3	.3623	.121	0.46
Error	15	3.9250	.262	

The "linear on $\sqrt{X}$ " model

$$Y = 8.133 + .258\sqrt{X}$$

clearly provides an adequate fit to the treatment means. The level x_ϵ at which the slope

$$\frac{dY}{dX} = \frac{b}{2\sqrt{X}} = \frac{.258}{2\sqrt{X}}$$

is equal to

$$\epsilon = .0263\sqrt{.102} = .0084$$

is

$$x_\epsilon = \left(\frac{.258}{.0168}\right)^2 = 235.8 .$$

A lower confidence limit is given by

$$\sqrt{X_L} = \frac{b - ts_b}{2\epsilon}$$

where

$$s_b = \frac{.256}{\sqrt{\Sigma X - \frac{1}{6}(\Sigma\sqrt{X})^2}} = .081$$

giving

$$\sqrt{X_L} = 6.80 \qquad X_L = 46.24 .$$

ILLUSTRATIONS OF METHODOLOGY FOR ORDERED ALTERNATIVES

Methods of testing against ordered alternatives are extensions of methods for testing the null hypothesis $M_0 = M_1$ against the one-sided alternative hypothesis that $M_0 < M_1$. Such extensions are required for testing differences among several treatment means $M_0, M_1, M_2, \cdots, M_k$ in circumstances where it may be assumed that $M_0 \leq M_1 \leq M_2 \leq \cdots \leq M_k$. In the present circumstance this latter assumption concerning mean responses to successively higher dose levels is much less specific than the assumption that, for example, a quadratic-horizontal regression model is an adequate approximation to the dose-response relationship. Analyses based upon this less specific assumption of simply an increasing dose-response relationship have a correspondingly greater range of applicability and validity, but at the cost of reduced efficiency in extracting information from the data.

The methods referred to are the sequential $\bar{t}$-tests proposed by D. A. Williams (Biometrics, 1971, p. 116), and corresponding multiple-comparison extensions of the Abelson and Tukey maximin contrast for monotone alternatives. A test procedure proposed by D. J. Bartholomew (Biometrika, 1961, pp. 325-332) is also applicable in this context but does require some further development.

Williams' method in this context consists of successively comparing the mean response at the highest test level with the control mean, then with the mean at the lowest test level, then with the mean at the next lowest level, etc., until a level is reached where the mean response does not differ significantly from that at the highest dose level. If the maximum observed mean response occurs at the second highest rather than at the highest dose level then the comparison in each case is made with the average response at the two highest test levels. Likewise, if the largest observed mean occurred at the third highest test level then the comparison in each case would be with the average response at the three highest test levels, and so on.

Each test in the sequence of tests proposed by Williams has the simple form of a one-sided t-test, but the Student t-tables are not applicable when means at the higher dose levels may be amalgamated in the manner described above. Williams tabulated critical values at the .05 and .01 significance levels (Biometrics, 1971, pp. 107-108) taking into account such amalgamations. When the comparisons are, in effect, performed in a prescribed sequence so that a particular comparison is made only if all preceding tests were statistically significant, then each of the one-

sided comparisons may be tested at the same significance level – say, at the .05 level. The nominal size of each test is then .05, and the actual size is at most .05, so the test procedure is conservative.

The same reasoning would apply to a predetermined sequence of single degree of freedom contrasts among the k + 1 treatment means. One reasonable procedure of this form, for example, might consist of testing for positive slopes in linear trend lines; first linear through all the data, then log-linear through all but the control, then all but the control and the lowest test dose, and so on until a log-linear slope is found to be not significantly greater than zero. Alternatively, the sequence of contrasts might be the maximin contrasts developed by R. P. Abelson and J. W. Tukey, applied first to all k + 1 treatment means, then to all but the control, then to all but the control and the lowest dose level, and so on until a contrast is found to be not significantly greater than zero when tested by an ordinary t-test.

Contrast coefficients for the latter procedure are given in Table 1. These coefficients were derived to produce the largest minimum power for monotone alternatives; i.e., for any other contrast such as the log-linear contrast mentioned above, there exists a monotone configuration of "true" means where the power of the log-linear test is smaller than the minimum power of the Abelson-Tukey test. For this reason the Abelson-Tukey coefficients are referred to in Table 1 as the monotone (M) maximin coefficients.

Table 1. Contrast coefficients of the monotone (M) maximin contrasts (from R. P. Abelson and J. W. Tukey, Ann. Math. Stat., 1963, 1358*).

number of levels								
2	3	4	5	6	7	8	9	10
-.707	-.707	-.699	-.690	-.682	-.674	-.668	-.662	-.656
.707	0	-.108	-.155	-.180	-.196	-.206	-.214	219
	-.707	.108	0	-.052	-.083	-.103	-.117	.127
		.699	.155	.052	0	-.032	-.053	-.069
			.690	.180	.083	.032	0	-.022
				.682	.196	.103	.053	.022
					.674	.206	.117	.069
						.668	.214	.127
							.662	.219
								.656

* The coefficients tabulated by these authors have here been normalized to have a sum of squares equal to unity.

The maximin criterion may be applied to the still more restricted region of the parameter space defined by monotone concave alternatives. Such an approach would exploit the prior information that a dose-response function increases at a decreasing rate; i.e., if the dose levels are $0 < X_1 < X_2 < \cdots < X_k$, then

$$\frac{M_1 - M_0}{X_1 - 0} \geq \frac{M_2 - M_1}{X_2 - X_1} \geq \cdots \geq \frac{M_k - M_{k-1}}{X_k - X_{k-1}} \geq 0.$$

Table 2 presents the coefficients of the monotone concave (MC) maximin contrasts for the two most common spacings of levels – equal spacing and logarithmically equal spacing. Of all single df contrasts, a test using these coefficients has the largest minimum power over the region of monotone concave alternatives.

Table 2. Contrast coefficients of the monotone-concave (MC) maximin contrasts for equal spacing and for logarithmically equal spacing (from J. L. Rosenberger, Ph.D. thesis, Cornell University, 1977).

Equal spacing of levels

number of levels

2	3	4	5	6	7	8	9	10
-.707	-.789	-.816	-.826	-.830	-.831	-.831	-.829	-.828
.707	.212	.035	-.050	-.097	-.125	-.142	-.154	-.160
	.577	.272	.121	.035	-.019	-.055	-.080	-.097
		.509	.292	.166	.086	.032	-.006	-.034
			.463	.297	.191	.119	.067	.029
				.429	.296	.206	.140	.092
					.402	.292	.214	.155
						.379	.287	.218
							.361	.281
								.344

Equal spacing of logarithmic levels

-.707	-.765	-.773	-.769	-.761	-.754	-.746	-.738	-.731
.707	.135	-.021	-.070	-.098	-.119	-.135	-.146	-.153
	.630	.188	.025	-.037	-.075	-.098	-.114	-.126
		.606	.216	.066	.013	-.026	-.052	-.071
			.598	.241	.097	.044	.007	-.019
				.589	.258	.119	.064	.031
					.580	.270	.136	.079
						.572	.279	.148
							.564	.284
								.558

Parametric methods. The sequential procedure proposed by Williams and the sequence of t-tests of maximin contrasts are parametric methods based upon the normality and additivity assumptions underlying anov. Critical values of Williams' $\bar{t}$-statistic under these assumptions are listed here for analyzing data sets A and B:

		number of means				
df		2	3	4	5	6
8	1%	2.90	2.99	3.01	3.03	
	5%	1.86	1.96	2.00	2.01	
15	1%	2.60	2.66	2.68	2.69	2.70
	5%	1.75	1.84	1.87	1.88	1.89

The form of his $\bar{t}$ statistic is

$$\bar{t} = \frac{\hat{M}_k - \bar{Y}_i}{s_{\bar{y}}\sqrt{2}}$$

where

$\hat{M}_k$ = amalgamated mean at the highest dose level

$\bar{Y}_i$ = mean at the i^{th} dose level

$s_{\bar{y}} = \sqrt{\frac{EMS}{r}}$.

In the case of data set A where the treatment means are monotonic to begin with:

$$X = \quad 0 \qquad 10 \qquad 20 \qquad 40 \qquad 80$$

$$\bar{Y} = 1.44 < 1.59 < 1.60 < 1.67 < 1.68$$

there is no amalgamation needed. Applying Williams' sequential procedure with

$$s_{\bar{y}}\sqrt{2} = .030139\sqrt{2} = .04262$$

we get first:

$$\bar{t} = \frac{1.68 - 1.44}{.04262} = 5.63.$$

This is compared to the critical value 3.03 or 2.01 on 8 df and seen to be significant at the 1 percent level, so the sequential procedure continues with:

$$\bar{t} = \frac{1.68 - 1.59}{.04262} = 2.11.$$

Compared with the table entries 3.01 and 2.00, this is seen to be significant at the 5 percent level, so we continue to:

$$\bar{t} = \frac{1.68 - 1.60}{.04262} = 1.88.$$

Compared with the table entries 2.99 and 1.96, this test results in termination of testing with the conclusion that no significant increase in response occurs beyond the dose level X = 20.

Maximin contrasts are tested by an ordinary t-test,

$$t = \frac{\Sigma c\bar{Y}}{s_{\bar{y}}}$$

where the c's are the tabulated contrast coefficients and the critical values of t are read from Student's t-table. The spacings in data set A are logarithmically equal so the second half of Table 2 is applicable. The first test in the sequence includes the control level X = 0, however, so Table 2 is not applicable at this first step. Using Table 1, instead, we get

X =	0	10	20	40	80
$\bar{Y}$ =	1.44	1.59	1.60	1.67	1.68
c =	-.690	-.155	0	.155	.690

$$t = \frac{\Sigma c\bar{Y}}{s_{\bar{y}}} = \frac{.178}{.030139} = 5.906$$

and from the t-table on 8 df, P < .001. Now applying Table 2 to the logarithmically equally spaced:

X =	10	20	40	80
$\bar{Y}$ =	1.59	1.60	1.67	1.68
c =	-.773	-.021	.188	.606

$$t = \frac{.06937}{.030139} = 2.302 \qquad P < .025$$

and then proceeding with:

$$X = \quad 20 \qquad 40 \qquad 8$$

$$\bar{Y} = 1.60 \qquad 1.67 \qquad 1.68$$

$$c = -.765 \qquad .135 \qquad .630$$

$$t = \frac{.05985}{.030139} = 1.986 \qquad P < .05 .$$

The process then terminates with

$$X = \quad 40 \qquad 80$$

$$\bar{Y} = 1.67 \qquad 1.68$$

$$c = -.707 \qquad .707$$

$$t = \frac{.00701}{.030139} = .233$$

and the conclusion that no significant increase occurs beyond the dose level $X = 40$.

Applying the same methods to data set B

X =	0	1	2	4	8	16
$\bar{Y}$ =	8.0325	8.37	8.655	8.8	8.655	9.185
$\hat{M}_i$ =	8.0325	8.37	8.655	8.7275	8.7275	9.185

and calculating the amalgamated monotone sequence of estimated means $\hat{M}_i$ by averaging the responses at $X = 4$ and $X = 8$, we see that amalgamation again has no effect upon Williams' sequential test. The first step,

$$\bar{t} = \frac{9.185 - 8.0325}{s_{\bar{y}}\sqrt{2}} = \frac{1.1525}{.362} = 3.18, \qquad P < .01$$

exceeds the 1 percent point 2.70, and the second step

$$\bar{t} = \frac{9.185 - 8.37}{.362} = 2.25, \qquad .05 > P > .01$$

also gives statistical significance. The next step, however, leads to termination

$$\bar{t} = \frac{9.185 - 8.655}{.362} = 1.46$$

and the conclusion that no significant increase in response occurs beyond the dose level X = 2.

The sequence of maximin contrasts leads to the same conclusion:

X =	0	1	2	4	8	16
$\bar{Y}$ =	8.0325	8.37	8.655	8.8	8.655	9.185
c =	-.682	-.180	-.052	.052	.180	.682

$$t = \frac{.8448}{.256} = 3.300, \qquad P < .0025$$

and then using Table 2:

X =	1	2	4	8	16
$\bar{Y}$ =	8.37	8.655	8.8	8.655	9.185
c =	-.769	-.070	.025	.216	.598

$$t = \frac{.5397}{.256} = 2.108, \qquad .05 > P > .025$$

X =	2	4	8	16
$\bar{Y}$ =	8.655	8.8	8.655	9.185
c =	-.773	-.021	.188	.606

$$t = \frac{.3181}{.256} = 1.220, \qquad .15 > P > .10 .$$

Nonparametric methods. Ordered alternative methodology may also be applied to rank order statistics. Assigning ranks to the dose levels at a location according to the rank order of the observed responses at that location, and then basing the statistical analysis simply upon the ranks, clearly results in a loss of information. Monotonicity is preserved in ranks, however, and with respect to ordered

alternative methodology the loss in efficiency may be expected to be relatively small. Concavity of a monotone sequence is a parametric property which is not preserved in the rank-order transformation, however, and Table 2 is therefore not applicable to nonparametric methods.

Loss in efficiency resulting from the rank-order transformation may be reduced by the further transformation from ranks to the normal scores listed in Table 3. As for any other nonparametric method, error variance is known *a priori* and is listed for the normal scores in Table 3. Error mean square, which must be estimated, with $k(r - 1)$ df in the parametric case is perfectly known or, in effect, estimated with infinite df in the nonparametric case. The tables of Williams or the Student t-tables are therefore entered with infinite df when analyzing means of normal scores at several dose levels, as given at the bottom of Table 3.

Table 3. Normal score transformation of rank order (from CRC Handbook for Probability and Statistics).

Rank order	number of treatments 2	3	4	5	6
1	.56419	.84628	1.02938	1.16296	1.26721
2	-.56419	0	.29701	.49502	.64176
3		-.84628	-.29701	0	.20155
4			02938	-.49502	-.20155
5				-1.16296	-.64176
6					-1.26721
S^2	.63662	.71619	.76523	.79876	.82332
	Williams' table for infinite df				
1%	2.326	2.366	2.377	2.382	2.385
5%	1.645	1.716	1.739	1.750	1.756

Table 4. Rank-order transformation of data set A.

Level X	Location 1	2	3	Sum of normal scores	Abelson-Tukey coefficients
0	5	5	5	-3.48888	-.690
10	4	4	3	-.99004	-.155
20	2	3	4	0	0
40	1	2	2	2.15300	.155
80	3	1	1	2.32592	.690

Data set A is transformed to normal scores in Table 4, and the treatment sums of normal scores are calculated by referring to Table 3; at the level X = 10, for example,

$$(-.49502) + (-.49502) + (0) = -.99004.$$

Since the largest sum of normal scores occurs at the highest dose level, no amalgamation is needed in calculating Williams' $\bar{t}$-test:

$$\bar{t} = \frac{2.32592 - (-3.48388)}{\sqrt{2rS^2}} = \frac{5.81480}{\sqrt{2(3)(.79876)}} = 2.656$$

where $S^2 = .79876$ is read from Table 3. Compared to the critical values 2.382 and 1.750 listed in Table 3, this calculated value $\bar{t} = 2.656$ is statistically significant. The test is therefore repeated on the remaining levels, excluding the control and using normal scores for 4 treatments in Table 3:

10	4	4	3	-2.35577	-.699
20	2	3	4	-1.02938	-.108
40	1	2	2	1.62340	.108
80	3	1	1	1.76175	.699

$$\bar{t} = \frac{1.76175 - (-2.355[illegible]}{\sqrt{2(3)(.765225)}} \qquad 1.922 \qquad .05 > P > .01 .$$

This value of $\bar{t}$ is again statistically significant, indicating that response does increase between the level X = 10 and the level X = 80. The test procedure therefore continues with:

20	2	3	3	-1.69256	-.707	
40	1	2	2	.84628	0	$\bar{t} = 1.225$.
80	3	1	1	.84628	.707	

The critical values of $\bar{t}$ shown in Table 3, 2.366 and 1.716, are not usable in this case of only 3 treatments in 3 blocks; with such small numbers, the only statistically significant outcome would be the configuration

20	3	3	3
40	2	2	2
80	1	1	1

at P = 1/216 or $\bar{t}$ = 1.637 at P = 11/216. The observed rank-order configuration is thus not statistically significant and the test terminates with the conclusion that no further increase in response occurs between X = 20 and X = 80.

Exactly the same conclusions would be reached applying the Abelson-Tukey contrasts to the normal scores. From Table 4

$$t = \frac{(-.690)(-3.48888) + (-.155)(-.99004) + 0(0) + (.155)(2.15300) + (.690)(2.32592)}{\sqrt{rs^2}}$$

$$= \frac{4.9938}{\sqrt{3(.79876)}} = 2.907 > 2.326 \qquad (P < .01)$$

and compared to the critical values of Student's t with infinite df, 2.326 and 1.645, this value t = 2.907 is significant at the 1 percent level. Continuing the test procedure:

$$t = \frac{(-.699)(-2.35577) + (-.108)(-1.02935) + (\quad 8)(1.62340) + (.699)(1.76175)}{\sqrt{3(.765225}}$$

$$= 2.089 \qquad .05 > P > .01$$

and then the process terminates with the non-significant 3 x 3 configuration of ranks, as before.

Table 5. Rank-order transformation of data set B.

Level X	Location 1	2	3	4	Sum of normal scores	Abelson-Tukey coefficients
0	5	6	6	5	-3.81794	-.682
1	4	4	5	4	-1.24641	-.180
2	3	3	4	3	.40310	-.052
4	6	1	3	2	.84331	.052
8	1	5	1	6	.62543	.180
16	2	2	2	1	3.19249	.682

Applying Williams' procedure to the normal score sums of data set B, first to compare the control with the highest dose level X = 16 (which also gives the highest normal score sum), we have:

$$\bar{t} = \frac{3.19249 - (-3.81794)}{\sqrt{2(4)(.82332)}} = 2.732 \qquad P < .01$$

which is significant when compared to the entries 2.385 and 1.756 in Table 3. The Abelson-Tukey test in this case:

$$t = \frac{5.14094}{\sqrt{4(.82332)}} = 2.833$$

when compared to the critical values 2.326 and 1.645 would lead to the same conclusion.

At the next step in the analysis of Table 5 we thus eliminate the control group and recalculate ranks and sums of new normal scores:

X	1	2	3	4		
1	4	4	5	4	-2.64802	-.690
2	3	3	4	3	-.49502	-.155
4	5	1	3	2	.49502	0
8	1	5	1	5	0	.155
16	2	2	2	1	2.64802	.690

giving for Williams' $\bar{t}$

$$\bar{t} = \frac{2.64802 - (-2.64802)}{\sqrt{2(4)(.79876)}} = 2.095 \qquad .05 > P > .01$$

and for the Abelson-Tukey contrast

$$t = \frac{3.73100}{\sqrt{4(.79876)}} = 2.087 \qquad .05 > P > .01 \ .$$

By either procedure the process continues to:

X	1	2	3	4		
2	3	3	4	3	-1.92041	-.699
4	4	1	3	2	0	-.108
8	1	4	1	4	0	.108
16	2	2	2	1	1.92041	.699

Neither procedure would allow continuation beyond this level, since

$$\bar{t} = \frac{1.9[illegible] - (-1.92041)}{\sqrt{(4)(.76523)}} = 1.552 \qquad P > .05$$

and for the Abelson-Tukey contrast

$$t = \frac{2.68473}{\sqrt{4(.76523)}} = 1.535 \qquad P > .05 \ .$$

Both procedures would thus lead to the conclusion that no increase in response occurs beyond the recommendable level X = 2.

COMMENTS

Adjustment of ϵ. Perhaps the most striking feature in the preceding examples is that the largest recommendable dose levels X_L were achieved in cases where a simple linear regression model on a transformed scale $g(X;0)$ provided an adequate fit to the treatment means. In the case of data set A, the regression of Y on $\ell n(X + 0)$ provided an adequate fit when tested by anov, and resulted in $X_L = 56.2$; in case B, simple linear regression of Y on the square root of dose level X provided an adequate fit and produced a lower confidence limit $X_L = 46.2$ exceeding the largest test level $X_k = 16$.

The latter extrapolation is undefendable, and in practice X_L would presumably be forced back to $X_K = 16$, but these two results do serve to demonstrate, first, that parsimony in modeling does offer advantages and, second, that the choice $\beta = .25$ may be too small. Increasing β to .50 and thus requiring probability of at least .50 for detecting a slope of size ϵ would, for example, alter the K-value for data set B from $K = .0263$ to $K = .0438$ and thereby reduce the limit from $X_L = 46.2$ to $X_L = 16.7$. In the log-linear case where $X_L = 56.2$ for data set A, the effect of increasing β to .50 would be to reduce X_L from 56.2 to 34.2.

Validation of the ϵ-procedure. The validity of the proposed procedure for constructing ϵ and the lower confidence limit X_L rests primarily upon large sample theory, and no small sample validation studies have been attempted. In the course of demonstrating the nonvalidity of the Walker-Carmer method applied without modification to the Mitcherlich regression function, however, some indication of validity of the ϵ-procedure was obtained.

The latter investigation was restricted to the unrealistic case of a single unknown parameter in the Mitcherlich function,

$$Y = 1 - e^{-cX}$$

with the remaining parameters set equal to unity. The estimate of c was, moreover, assumed to be exactly normally distributed with a known standard error σ_c. A lower confidence limit on the dose level

$$x_\epsilon = \frac{1}{c} \log \frac{c}{\epsilon}$$

at which $dY/dX = \epsilon$ is then given by

$$X_L = \frac{\log \frac{\hat{c}}{\epsilon} + t \frac{\sigma_c}{\hat{c}}}{\hat{c} + t\sigma_c}$$

where $\hat{c}$ is a normally distributed random variable with mean c and standard error σ_c.

Exact confidence levels were calculated for some combinations of ϵ, c and σ_c when t = 1.645 and the nominal confidence level is 95 percent:

	Achieved confidence level (percent)					
	c = 1			c = 10		
ϵ	$\sigma_c = .1$	$\sigma_c = .05$	$\sigma_c = .01$	$\sigma_c = 1$	$\sigma_c = .5$	$\sigma_c = .1$
.01	94.49	94.78	94.96	94.70	94.87	94.98
.0001	94.78	94.91	94.98	94.83	94.93	94.99
.000001	94.86	94.94	94.99	94.88	94.95	94.99

Dose-additive effects of locations. Curvilinear regression methodology used in the above examples followed the customary practice of fitting a curve to the treatment means (or, equivalently, to treatment totals) across replicates. The rationale for this convention would follow from the rationale for applying an additive linear model to the treatment x location cross classification. In the context of antibiotic feed additive trials, however, the treatment is recognized to serve as an alternative or substitute for other management practices and is virtually ineffective, for example, in a germ-free environment where response is already at a high level (The Use of Drugs in Animal Feeds, Proc. of Symp., Nat. Acad. Sci. Publ. 1679, 1969, p. 17).

Since management practices and microflora populations do differ among the locations (replicates) in a trial there may be more reason to suppose that such

differences are manifested by translation effects along a single dose response curve rather than by vertical translations of the same curve. In view of the multi-factor differences among locations, however, a still more reasonable approach might be to incorporate both kinds of additivity into the regression model.

Applied to the Mitcherlich function, for example, the conventional model used earlier expresses the mean response M_{ij} to dose level X_j at location i in the form

$$M_{ij} = \alpha_i + a + be^{cX_j} \qquad \sum_{1}^{r} \alpha_i = 0 \ .$$

The term α_i represents the response-additive effect of the i^{th} location, and conveys the assumption that the dose response curve at this particular location differs from the average response curve

$$M_j = a + be^{cX_j}$$

by the same amount α_i at every dose level.

A strictly dose-additive model of location effects would, on the other hand, take the form

$$M_{ij} = a + be^{c(X_j+\delta_i)} \qquad \sum_{1}^{r} \delta_i = 0 \ ,$$

implying that management practices and other factors at the i^{th} location have the combined effect of substituting for a "background dose" or size δ_i, when compared to an average background dose defined to be zero. Operationally, fitting such a model to the observed responses Y_{ij} at k + 1 levels ($j=0,1,2,\cdots,k$) in r replicates ($i=1,2,\cdots,r$) would require iterative multiple linear regression of residuals

$$e_{ij} = Y_{ij} - a - b_i e^{cX_j}$$

on r + 2 independent variables defined by the partial derivations of M_{ij} with respect to $b_1, b_2, \cdots, b_r$, a and c. At termination, location effects δ_i are

estimated by

$$\delta_i = \frac{\ell nb_i - \ell nb}{c}$$

where

$$\ell nb = \frac{1}{r} \sum_{1}^{r} \ell nb_i \quad .$$

With the exception of the 'unrestricted quadratic in X' model, the other regression models illustrated earlier with a response-additive model for locations would also require iterative computations with r + 2 independent variables if a dose-additive model were used for location effects.

The latter method is not illustrated here because of its unconventionality, but is mentioned as a possibility worthy of future investigation with real data. Dose-additive replicate effects which are small do not seriously bias the regression methods illustrated here, but act only to increase the magnitude of experimental error variance and thereby increase the length of confidence intervals.

Extensions of ordered alternative methodology. Monotonicity and concavity are invariant with respect to dose-additivity of replicate effects; in fact, a much more general kind of invariance holds. If the true dose response curve at each location is monotone and concave then these same properties automatically hold for the sum or average across locations. Exploitation of these properties, insofar as they are applicable to dose response curves at several locations, does require further development and more extensive comparisons of methods for restricted alternatives.

Some guidance in this matter is offered by the power comparisons given by Williams (loc. cit.) and by Bartholomew (Barlow, R. E., Bartholomew, D. J., Bremner, J. M. and Brunk, H. D., Statistical Inference Under Order Restrictions, J. Wiley and Sons, Ltd., London, 1972). Rosenberger (loc. cit.) has also made some power comparisons between maximin contrasts and likelihood ratio tests (extensions of Bartholomew's test) against monotone concave alternatives. Power differences are small in all such comparisons, and more extensive investigations including the comparison of sequences of tests, should be carried out before recommendations are made. If sufficient power advantage accrues from the monotone concave restriction as compared to simple monotonicity then efficient

computer algorithms will be needed to extend both William's test and the likelihood ratio test with estimated error variance to accomodate arbitrary but specified spacings of levels.

Validity of nonparametric tests against monotone alternatives. The nonadditivity which certainly exists to some extent, and which reduces sensitivity of parametric procedures through heterogeneity and inflation of error variance estimates, may be expected to have much less effect upon nonparametric procedures. The error variance used in construction of such procedures is predetermined by the null hypothesis and is uneffected by nonadditivity; and the power depends upon monotonicity of the dose response relationship at each location, and upon the true and undistorted error variance at each location. Under conditions which are less than ideal for normal theory parametric tests, the nonparametric procedure may indeed out-perform the parametric procedure.

Small sample validity of the normal approximation used in determining critical values of the nonparametric test statistics proposed here has been verified. Exact, discrete probability distributions of these statistics have been tabulated for comparison with the normal approximation. The smallest sample size configuration for which the normal approximation was here intended is the case of 4 treatment levels in 3 blocks. Using the normal score transformation of ranks results in the following degree of agreement between nominal and exact probability levels of the nonparametric Abelson-Tukey contrast among 4 treatments:

t	Nominal P	3 blocks Exact P	4 blocks Exact P
1.6449	.05	.0515047	.0496479
1.7507	.04	.0402199	.0403766
1.8808	.03	.0298033	.0296766
2.0537	.02	.0169994	.0187837
2.3262	.01	.0055700	.0078909

The exact frequency distribution of the nonparametric Williams test under the same circumstances is:

r = 3		r = 4	
$\bar{t}$	Exact P	$\bar{t}$	Exact P
1.45	.1381652	1.425	.0957057
1.55	.0956306	1.510	.0875677
1.65	.0643807	1.595	.0635276
1.75	.0591724	1.680	.0574030
1.85	.0574363	1.765	.0432490
1.95	.0234375	1.850	.0305899
2.05	.0211227	1.935	.0255021
2.15	.0176505	2.020	.0226086
2.25	.0081019	2.105	.0187114
2.35	.0046296	2.190	.0090965
		2.275	.0079391
		2.360	.0050456

where the nominal critical values are

$\bar{t}$	Nominal P
1.739	.05
2.377	.01

The number of distinct rank-order configurations that are possible with 4 treatments is $4! = 24$, and in r blocks the number is 24^r. There are $.0055700 \times (24)^3 = 77$ configurations in 3 blocks producing an Abelson-Tukey contrast t-statistic as large as $t = 2.3263$, and in 4 blocks there are $.0050456 \times (24)^4 = 1674$ configurations of normal scores producing a Williams-$\bar{t}$ statistic as large as $\bar{t} = 2.360$.

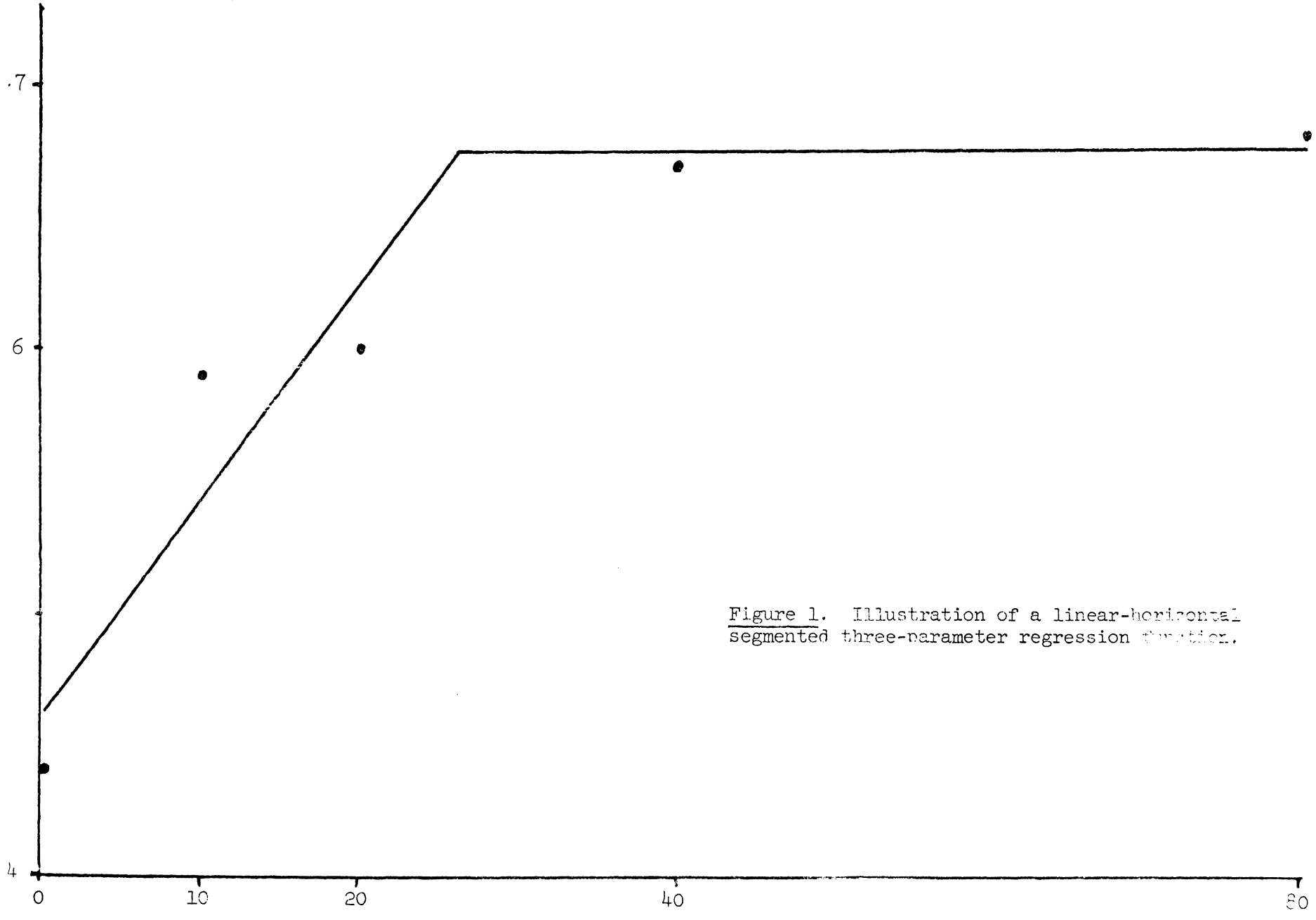

Figure 1. Illustration of a linear-horizontal segmented three-parameter regression function.

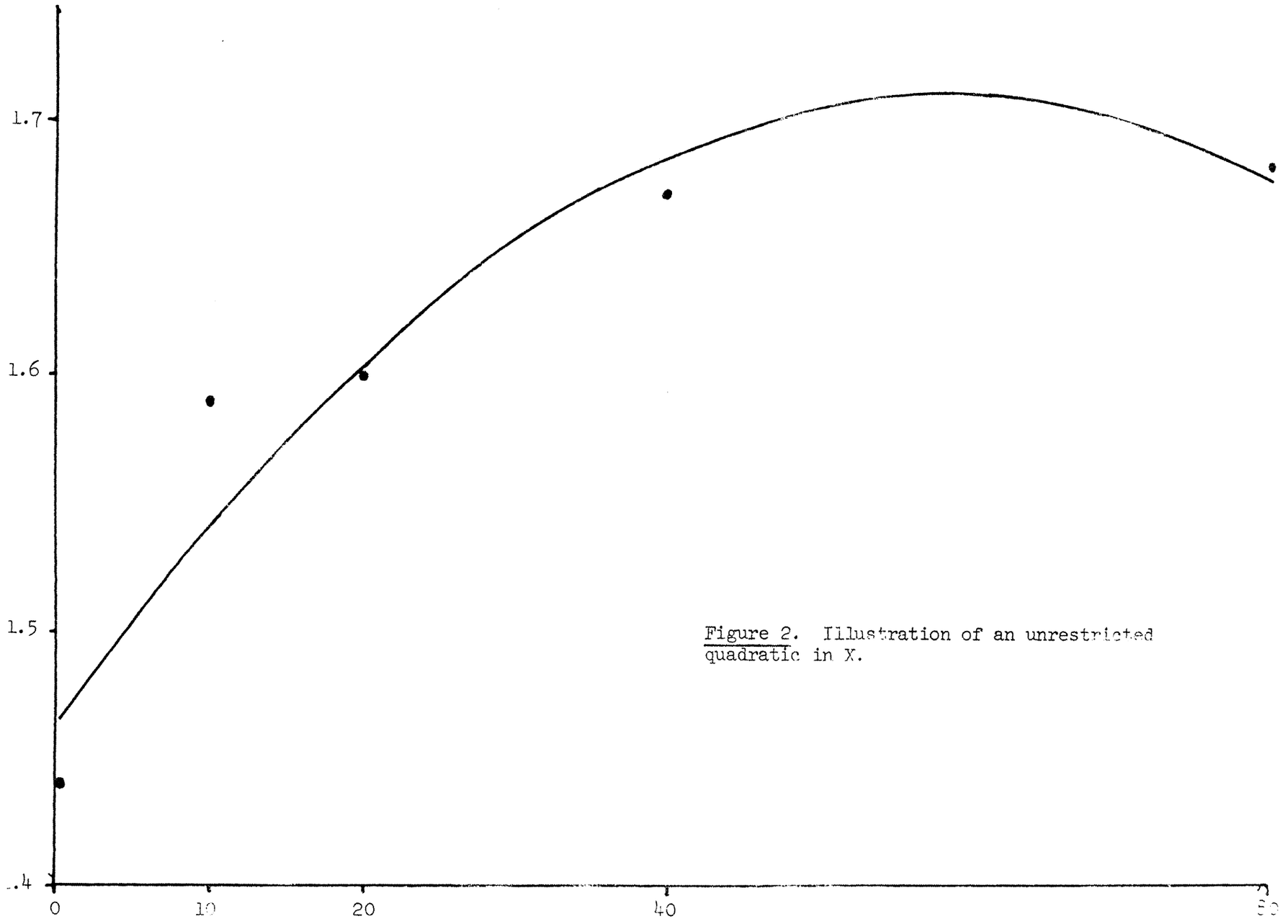

Figure 2. Illustration of an unrestricted quadratic in X.

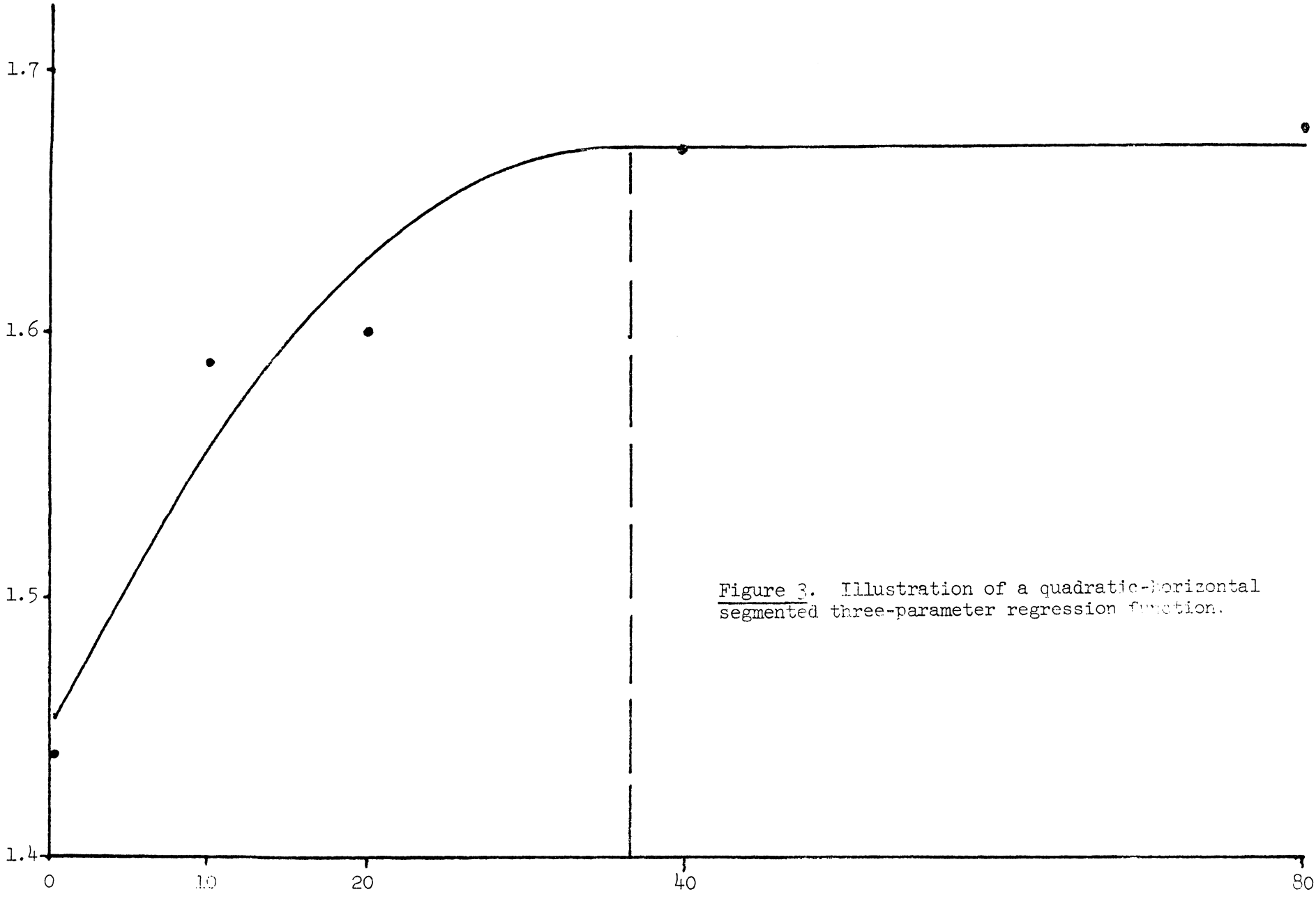

Figure 3. Illustration of a quadratic-horizontal segmented three-parameter regression function.

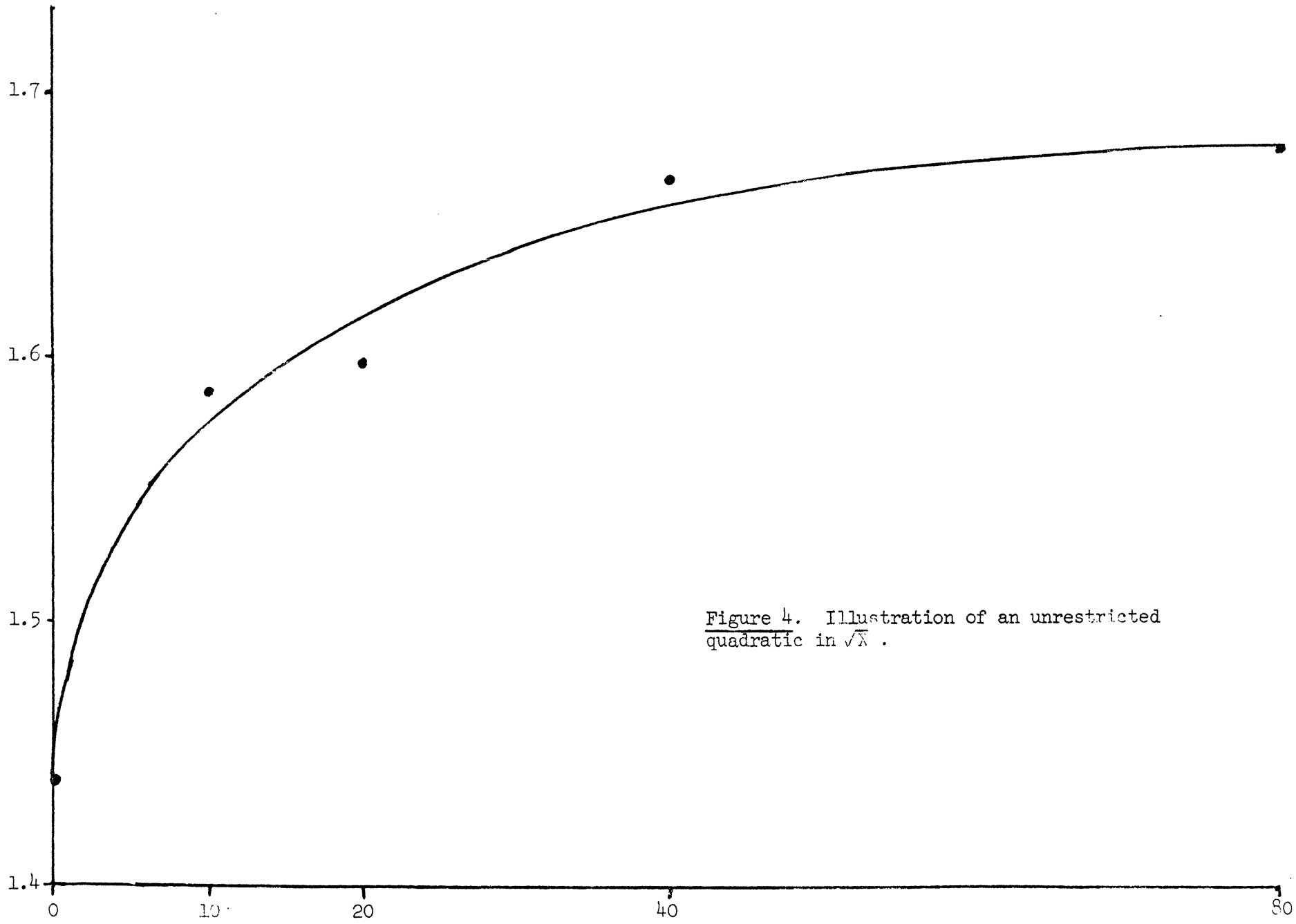

Figure 4. Illustration of an unrestricted quadratic in $\sqrt{X}$.

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