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Analysis of experimental designs used in bioassays

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ANALYSIS OF EXPERIMENTAL DESIGNS USED IN BIOASSAYS

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CHAPTER I

BIOASSAYS

1.1 Introdnction

This dissertation is intended to provide a unified method of analysis of data from bioassays. Bioassays are useful in estimating the relative potency of a new drug or material or preparation as compared to a standard one. This comparison is done by modeling the dose-response relationships of the two drugs or preparations. If possible, the dose as well as the response are first suitably transformed to linearize this relationship and then the models are exploited to obtain doses producing the same response. To estimate the parameters of this model, data is collected on several units (animals, patients, etc.) and extraneous sources of variation are eliminated. This constitutes bioassay experimental designs.

There are several types of assays and there are several different statistical experimental designs. Before the advent of computers, due to the difficulties in inverting matrices and obtaining eigenvectors and values, several designs were constructed that produced patterned design matrices and different formulas were developed for different designs. However, now this is no longer necessary. A unified method applicable for any design can be used. This dissertation deals with such a unified method of

analysis, provides an algorithmic program for this purpose and the use of this method is illustrated by several case studies.

In this chapter, we first describe briefly how assaying is done and in the next chapter, a brief account of contrasts of treatments in designs that are needed in bioassays is given. The third chapter will describe the unified method of analysis of such bioassay designs and subsequent chapters will provide the necessary case studies as illustrations.

1.2 Bioassays

Finney (1978) defines a bioassay as an experiment for estimating the potency of a drug, material, preparation or process by means of the reaction that follows its application to living matter. The emphasis in bioassays is on comparing the potencies of treatments rather than estimating the difference between the effects of treatments. For example, Finney points out that an investigation into the effects of different samples of corticotrophin on the ascorbic acid in rat adrenals is not necessary an assay; it becomes one if the interest lies in using the changes in ascorbic acid for estimating the potencies of the samples in standard units of corticotrophin.

The typical bioassay involves a stimulus (for example, a vitamin or a drug) applied to a subject (animal, tissue or some such experimental unit). The level of the stimulus can be varied and the effect of the stimulus on the subject can be measured in terms of a characteristic which we call as response. The relationship between stimulus and response is a statistical relation subject to a random error. The relationship can be used to study the potency of a drug from the response it produces.

The estimate of potency is always relative to a standard preparation of the stimulus. A new test preparation is then assayed to find the mean response to a selected

drug. Next, we find the dose of the standard preparation that produces the same mean response. The ratio ρ of the dose z_s of the standard preparation equally effective as this dose z_t of the test preparation is the potency of the test preparation relative to the standard preparation. Thus, if $\rho = 4$, one unit of the test preparation is as effective as 4 units of the standard preparation.

There are three main types of biological assays: Direct Assays, Indirect Assays and Assays based on quantal (all or nothing) responses. Direct assays are of fundamental importance. Here, the response is measured for a number of doses to establish a statistical dose-response relation and from this the potency of a test preparation is determined.

1.3 Parallel-Line Bioassays

An important type of bioassay is the parallel-line bioassay, where the dose-response relation is linear for the standard as well as the test preparation and the lines are parallel or that they have a common slope. This linearity can often be achieved by a logarithmic or Box-Cox (1964) type transformation of the dose and/or the response. The treatments are then applied to the experimental units by using a suitable experimental design such as Randomized blocks, Latin squares, Cross-over, Split-plot or even an incomplete block design (such as, Balanced incomplete block design, Partially balanced incomplete block design, or a Lattice design). Whether linearity of the dose-response relation is achieved or not and if so, whether the slopes of the lines for the standard and test preparations are the same or not is tested first by what are known as tests of validity which include the test of parallelism also.

Thus if t_s, t_t denote respectively the effects of the standard (S) and the test (T)

preparation, the parallel lines representing the dose-response relation can be taken as

$$t_s = \alpha_s + \beta x_s,$$
$$t_t = \alpha_t + \beta x_t \tag{1.1}$$

where x_s, x_t are respectively $\log z_s$ and $\log z_t$ where z_s, z_t are the doses of S and T in original units. If ρ is the relative potency of T with respect to S, ρz_t and z_s produce the same effect and this leads to

$$\log \rho = (\alpha_t - \alpha_s)/\beta \tag{1.2}$$

and this can be expressed in terms of the treatment contrasts, using

$$\alpha_t - \alpha_s = \bar{t}_t - \bar{t}_s - \beta(\bar{x}_t - \bar{x}_s),$$
$$\beta = \frac{\sum t_m(x_m - \bar{x}_m) + t_s(x_s - \bar{x}_s)}{\sum(x_m - \bar{x}_m)^2 + (x_s - \bar{x}_s)} \tag{1.3}$$

where $\bar{t}_t$, $\bar{t}_s$, and etc. are the average and the summation is over the employed doses of S and T. The contrast $\alpha_t - \alpha_s$ is called the preparation contrast and β is called the regression contrast. These contrasts can be estimated from the observations in the experimental design as will be seen in later chapter.

1.4 Slope Ratio Assays

If the linearizing transformation of dose is not logarithmic but of the type $x = z^\lambda$, the relative potency turns out to be $\rho = (\beta_t/\beta_s)^{\frac{1}{\lambda}}$, provided the dose-response lines for T and S intersect, have slopes β_t, β_s and provided the intercepts α_t, α_s of the two lines are equal. The validity of these assumptions in such slope-ratio assays will have to be tested by testing the departure from linearity of the dose-response regression lines and a test of significance of $\alpha_t - \alpha_s$. It is expected that when $x_s = x_t = 0$, the

response should be the same for S and T and this is tested by introducing blanks (neither S nor T) in the experiment.

1.5 Planning an Assay

Finney (1978) has discussed the principles of planning an assay. He stresses the importance of validity or consistency of an estimate of the relative potency, the economics of the assay design, the necessity of pilot investigations before choosing an optimum design, the simplicity of a systematic design and the cost of statistical analysis. He has given specific detailed recommendations for parallel-line and slope-ratio assays as well as quantal assays.

For some assays, the measured response is time: usually the time that elapse between application of the stimulus and the occurrence of some reaction. In such time-response assays the main difficulty is that the assay may end before a response has been measured for every subject. Finney suggests converting the data to a quantal form or assigning an arbitrary value as the response for subjects that have not reacted when the assay ends or using a mathematical model for reaction time in such cases.

CHAPTER II

TREATMENT CONTRASTS USED IN BIOASSYS

2.1 Introduction

Experimental Designs are used to compare two or more conditions and these are called treatments. Examples of such treatments are different doses of a drug, different hospitals, different ethnic groups, different fertilizers, different aspirin preparations and so on. The linear model underlying a design is such that only contrasts or comparisons of treatment effects are estimable from the observations on response variables. These treatments may be qualitative or quantitative and if they are quantitative like the doses of a drug, they may be equally spaced or uot. Sometimes the treatments used in a bioassay are combinations of the levels of several factors. A meaningful analysis of data in such cases requires formulation of suitable contrasts of treatments. By contrasts, we mean a linear function of the treatment effects $t_1, t_2, \cdots, t_i$ such that the coefficients of the t_i's add up to zero. Bioassays, basically require two main contrasts: one is called a preparation contrast and the other is called the regression contrast. But this is only if the response curve is linear. In geueral, therefore, one also

requires what are known as validity contrasts which test the linearity assumption. In the next section, we describe these aspects in more detail.

2.2 Treatment Contrasts

There are various types of contrasts and different contrasts are meaningful in different practical situations. If there is no structure imposed on the treatments, only elementary contrasts; namely, contrasts of the form $t_i - t_u$ are useful. But otherwise, the structure has to be taken into account.

For example, if the v treatments correspond to v equally spaced doses, it is meaningful to assume a functional relationship between the treatment effect t and the doses x and this function can be approximated by a polynomial of degree at most equal to $v-1$. (of course, equal spacing is not essential for this but it makes the mathematics easier by the use for orthogonal polynomials) First, without loss of generality, by changing the origin and scale x can be assumed to have the values

$$x_1 = -\frac{v-1}{2}, x_2 = -\frac{v-3}{2}, \cdots, 0, \cdots, x_v = \frac{v-1}{2}, \tag{2.1}$$

if v is odd, and

$$x_1 = -(v-1), x_2 = -(v-3), \cdots, -1, 1, \cdots, x_v = (v-1), \tag{2.2}$$

if v is even. The polynomial relation can then be expressed as

$$t(x) = \alpha_0 P_0(x) + \alpha_1 P_1(x) + \cdots + \alpha_{v-1} P_{v-1}(x) \tag{2.3}$$

where $P_r(x)$ is a polynomial of degree r in x and these polynomials are such that

$$P_0(x) \equiv 1 \tag{2.4}$$

$$\sum_x P_r(x) P_s(x) = 0, \quad r \neq s \tag{2.5}$$

$$\sum_x P_r^2(x) = d_{v,r} \quad (r = 0, 1, 2, \cdots, v-1). \tag{2.6}$$

These polynomials are tabulated for various values of v. Then, from (2.3), it follows that

$$\alpha_r = \frac{\sum_x t(x)P_r(x)}{d_{v,r}} \tag{2.7}$$

and α_r is then a treatment contrast as

$$\sum_x P_r(x) = \sum_x P_r(x)P_0(x) = 0 \tag{2.8}$$

whenever $r \neq 0$.

In parallel line bioassays, linearity of dose-effect relationship is necessary and this is sometimes obtained by transforming form the actual dose to the transformed dose-metameter, x and the linearity can be checked by testing whether $\alpha_2, \cdots, \alpha_{v-1}$ are all null.

These treatment contrasts α_r are thus extremely useful and meaningful in bioassys. In particular,

$$P_1(x) = x \tag{2.9}$$

$$P_2(x) = \lambda_2(x^2 - \frac{v^2-1}{12}) \tag{2.10}$$

$$P_3(x) = \lambda_3(x^3 - \frac{3v^2-7}{20}x) \tag{2.11}$$

$$P_4(x) = \lambda_4(x^4 - \frac{3v^2-13}{14}x^2 + \frac{3(v^2-1)(v^2-9)}{560}) \tag{2.12}$$

where $\lambda_2, \lambda_3, \lambda_4$ are suitable multipliers, to have integral values for $P_r(x)$. In bioassays, sometimes the treatments form different groups. For example, a control treatment and several new treatments at different levels will form different groups.

Consider a parallel line bioassay, involving a standard drug S and a new drug T, each at v equally spaced levels (again, equal spacing or equal number of levels are not necessary but only convenient). If the treatment effects are $t_1, \cdots, t_v$ for the standard and $t'_1, \cdots, t'_v$ for the new one, the following contrasts will be meaningful using $x_1, \cdots, x_v$ for the values of x, the dose (after transformation, as

in (2.1)). Consider the following sets of coefficients $l_1, \cdots, l_v, l'_1, \cdots, l'_v$ in the contrast $l_1 t_1 + \cdots + l_v t_v + l'_1 t'_1 + \cdots + l'_v t'_v$ which is summarized in Table 2.1.

Table 2.1: Contrast in parallel line bioassays

Contrast	$l_1, \cdots, l_v$	$l'_1, \cdots, l'_v$
Preparation	$-1, -1, \cdots, -1$	$1, 1, \cdots, 1$
Linear regression	$x_1, \cdots, x_v$	$x_1, \cdots, x_v$
Diff in linear regression	$-x_1, \cdots, -x_v$	$x_1, \cdots, x_v$
$\vdots$	$\vdots$	$\vdots$
r-th degree	$P_r(x_1), \cdots, P_r(x_v)$	$P_r(x_1), \cdots, P_r(x_v)$
Diff in r-th degree	$-P_r(x_1), \cdots, -P_r(x_v)$	$P_r(x_1), \cdots, P_r(x_v)$

Consider the polynomials representing the standard and the new drug. They are

$$t = \alpha P_0(x) + \cdots + \alpha_{v-1} P_{v-1}(x) \tag{2.13}$$

$$t' = \alpha' P_0(x) + \cdots + \alpha'_{v-1} P_{v-1}(x). \tag{2.14}$$

Then the following questions naturally arise. Are $\alpha_2, \cdots, \alpha_{v-1}, \alpha'_2, \cdots, \alpha'_{v-1}$ all null? If so, the regressions will be linear and we can employ the techniques of a parallel line bioassay to estimate the relative potency of the new drug with respect to the standard one. This is the validity test. If the answer is negative, and we want to find out, which coefficients are non-null. If the answer is yes, we can test whether $\alpha_1 = \alpha'_1$ to find out whether the regression lines are parallel. This is tested by the parallelism contrast

$$\alpha'_1 - \alpha_1 = \sum_{i=1}^{v} x_i t'_i - \sum_{i=1}^{v} x_i t_i \tag{2.15}$$

If $\alpha_1 = \alpha'_1$, the common slope is proportional to

$$\alpha'_1 + \alpha_1 = \sum x_i (t_i + t'_i) \tag{2.16}$$

which is the regression contrast in the table. Similarly, $\sum P_2(x_i)(t'_i - t_i)$ measures the difference in the quadratic coefficients and $\sum P_2(x_i)(t'_i + t_i)$ is proportional to the common second degree coefficient and so on.

Sometimes, the coefficients $\alpha_3, \cdots, \alpha_{v-1}, \alpha'_3, \cdots, \alpha'_{v-1}$ are negligible and in that case, the relative potency will be determined by using $\alpha_1 + \alpha'_1$ and the difference in the response of the new and standard drug, namely,

$$-\sum t'_i + \sum t_i \tag{2.17}$$

which is also called the preparation contrast.

If several new drugs and a standard are to be assayed simultaneously and if each preparation is tested only at two doses, a high and a low, then the elementary contrasts $t_{ih} - t_{il}$ and $(t_{ih} + t_{il}) - (t_{0h} + t_{0l})$ where i is the index of the preparation ($i = 0$ for standard) and h, l stand for high and low levels, will represent the regression contrast and the preparation contrast for the i-th drug. The differences of the contrasts $t_{ih} - t_{il}$, will test parallelism of all the regression lines and if there is no evidence of lack of parallelism, their average will be the common slope of the regression lines and can be used in computing the relative potencies. In all this discussion, it is assumed that the original natural doses are transformed by a logarithmic or similar transformation to a dose metameter and then by a change of scale and origin transformed further to have the values -1 and +1 for the high and low doses. The relative potency is then expressed in terms of the original or natural doses by transforming back to them. In a subsequent chapter, the specific details will be spelled out.

In slope ratio assays, the assumption is that the regression lines of response on dose intercept at the same point on the response axis and the validity test for this purpose is based on the contrasts that represents the intercepts on the y-axis of the standard and test regressions.

Returning again to parallel line bioassays, if the number of doses of the standard and test preparation are not the same, but are equally spaced, we proceed as follows for the appropriate contrasts useful in validity tests and in estimating the relative potency.

Let m and m^* denote the number of doses of the standard (S) and the test (T) preparation respectively and let the doses be denoted by

$$x_i = \begin{cases} 2i-(m-1) & \text{if } m \text{ is even} \\ i-\frac{1}{2}(m-1) & \text{if } m \text{ is odd,} \quad (i=1,2,\cdots,m) \end{cases} \tag{2.18}$$

for the standard preparation and by

$$x_i^* = \begin{cases} 2i-(m^*-1) & \text{if } m^* \text{ is even} \\ i-\frac{1}{2}(m^*-1) & \text{if } m^* \text{ is odd,} \quad (i=1,2,\cdots,m^*) \end{cases} \tag{2.19}$$

for the test preparation.

Let $P_{mr}(x)$ denote the orthogonal polynomial of degree r for m equally spaced values above. It should be noted that the original natural doses of S and T are first transformed to a log scale and then again by changing the origin and scale are made to assume the above set of values. Also,

$$\begin{aligned} P_{m0}=1 \quad &\text{and} \quad P_{m^*0}=1 \quad \text{and} \\ P_{m1}(x)=x \quad &\text{and} \quad P_{m^*1}(x^*)=x^*. \end{aligned} \tag{2.20}$$

Then, the preparation contrast is

$$\frac{1}{m^*}\sum_1^{m^*} t_i^* - \frac{1}{m}\sum_1^{m} t_i \tag{2.21}$$

where t_i, t_i^* are the effects of the i-th treatment (corresponding to the i-th dose), and the regression contrast, in case of parallel regression lines is proportional to

$$\sum_1^{m^*} t_i^* x_i^* - \sum_1^{m} t_i x_i \tag{2.22}$$

while the contrasts that tests parallelism is

$$\frac{\sum t_i^* x_i^*}{\sum x_i^{*2}} - \frac{\sum t_i x_i}{\sum x_i^2} \tag{2.23}$$

Other contasts useful in validity tests (such as linearity of regression, etc) are of the form

$$\frac{\sum t_i^* P_{m^*r}(x_i^*)}{\sum P_{m^*r}^2(x_i^*)} - \frac{\sum t_i P_{mr}(x_i)}{\sum P_{mr}^2(x_i)} \quad (r=2,3,\cdots) \tag{2.24}$$

More details of derivation of these contrasts and their appropriateness are given in the appendix, for the sake of reference and completion.

2.3 Eigenvector contrasts

In section 2.2, we described the treatment contrasts that are useful in bioassays. There are, of course, many other types of contrasts such as main effects and interactions among several factors where the treatments are combinations of levels of different factors; but this is not the topic of this dissertation. However, there is one more set of contrasts that are extremely important in experimental designs and this is the set of eigenvector contrasts. A matrix C that occurs in the reduced normal equations for estimating treatment contrasts, provides a basic reference set of contrasts that are estimable and all other estimable contrasts are linear combinations of them. This matrix and their eigenvector contrasts are described in detail in the next chapter and then the relations between the contrasts we need for a bioassay and these contrasts is exploited to provide a unified concise method of analyzing bioassay designs and estimating the relative potency of a new preparation.

CHAPTER III

A UNIFIED TREATMENT OF THE ANALYSIS OF EXPERIMENTAL DESIGNS USING EIGENVECTORS

3.1 Introduction

The main objective in the analysis of any experimental design is to estimate certain treatment contrasts, obtain their variances and estimate them, test their significance, and obtain confidence intervals. These contrasts are of various types depending on whether it is a bioassay experiment for relative potency of a drug or whether it is a response surface experiment intended to estimate the response relation or it is a factorial experiment for comparing different factors and studying their interations.

The estimability and estimates of treatment contrasts in an experimental design model depends on a certain matrix C related to the incidence matrix N of the design. Before the advent of computers, most of the research in this area was focused on constructing suitable designs so that this C matrix has a nice invertible pattern and different sets of formulas were developed for different such patterns. Now that the computers can find the eigenvalues and eigenvectors and a generalized inverse, it is possible to provide a unified method of statistical inference on these contrasts. All

questions, such as, whether a contrast is estimable or partially confounded or confounded or totally unconfounded can now be easily answered through this approach.

3.2 C-matrix of an Experimental Design

Let there be v treatments tested in b blocks. Let there be k_j plots in the j-th block and let the i-th treatment occur n_{ij} (0 or 1 only) times in the j-th block and r_i times ($i = 1, \cdots, v$) in the whole experiment. The vxb matrix of the elements n_{ij} is called the incidence matrix of the design and satifies

$$\begin{aligned} N\mathrm{E}_{b1} &= [r_1, \cdots, r_v]' \\ \mathrm{E}_{1v}N &= [k_1, \cdots, k_b] \\ n &= \sum r_i = \sum r_j \end{aligned} \tag{3.1}$$

where E_{ab}, in general, stands for an axb matrix of unit elements. Let D_k, D_r be, respectively, diagonal matrices of elements k_j and r_i. The model for an experimental design when block effects are fixed is

$$\mathbf{y} = \Delta_1 \mathbf{t} + \Delta_2 \boldsymbol{\beta} + \boldsymbol{\epsilon} \tag{3.2}$$

where $\mathbf{y}$ is the nx1 vector of the observation $\mathbf{t}$ is the vx1 vector of treatment effects, $\boldsymbol{\beta}$ is the bx1 vector of block effects, $\boldsymbol{\epsilon}$ is the nx1 vecor of random errors assumed to be independent normal with zero means and variance σ^2 (that is $\boldsymbol{\epsilon} \sim N(\mathbf{0}, \sigma^2 \mathbf{I_n})$), where $\sim$ means distributed as. Δ_1 is the nxv matrix and its element in the u-the row and i- th column is 1 if the u-th observation is on the i-th treatment and zero otherwise ($u = 1, 2, \cdots, n; i = 1, \cdots, v$). Δ_2 is the nxb matrix such that its element in the u-th row and j-th column is 1 if the u-th observation belongs to the j-th block

$(j = 1, \cdots, b)$ and zero otherwise. It is obvious that

$$\mathrm{E}_{1n}\Delta_1 = [r_1, \cdots, r_v]' \tag{3.3}$$

$$\Delta_1 \mathrm{E}_{v1} = \mathrm{E}_{n1} \tag{3.4}$$

$$\Delta_2 \mathrm{E}_{b1} = \mathrm{E}_{n1} \tag{3.5}$$

$$\mathrm{E}_{1n}\Delta_2 = [k_1, \cdots, k_b] \tag{3.6}$$

$$\Delta_1'\Delta_2 = N \quad . \tag{3.7}$$

Further,

$$\mathbf{T} = \Delta_1'\mathbf{y} \quad \text{is the vector of treatment totals of the observations} \tag{3.8}$$

$$\mathbf{B} = \Delta_2'\mathbf{y} \quad \text{is the vector of block totals of the observations} \tag{3.9}$$

$$\mathbf{Q} = \mathbf{T} - ND_k^{-1}\mathbf{B} \quad \text{is the vector of adjusted treatment totals} \tag{3.10}$$

$$g = \mathrm{E}_{1v}\mathbf{T} = \mathrm{E}_{1b}\mathbf{B} \quad \text{is the grand total of all the } n \text{ observations.} \tag{3.11}$$

The matrix

$$C = D_r - ND_k^{-1}N' \tag{3.12}$$

plays an important role in the estimation of any treatment contrast $\mathbf{l}'\mathbf{t}$, where $\mathrm{E}_{1v}\mathbf{l} = 0$ is necessary for estimation. The model leads to the reduced least squares or normal equations

$$Q = C\hat{\mathbf{t}} \tag{3.13}$$

on eliminating $\hat{\boldsymbol{\beta}}$. Here, $\hat{\mathbf{t}}, \hat{\boldsymbol{\beta}}$ denote solutions of the least squares equations and <u>not estimates</u> of $\mathbf{t}$ and $\boldsymbol{\beta}$.

$$\mathrm{E}(\mathbf{Q}) = C\mathbf{t}; \quad \mathrm{var}(\mathbf{Q}) = \sigma^2 C \tag{3.14}$$

where 'E' stands for expected value and 'var' for variance-covariance matrix.

The error S.S. (sum of squares) for the model comes out as

$$E = \mathbf{y}'\mathbf{y} - \mathbf{B}'D_k^{-1}\mathbf{B} - \mathbf{Q}'\hat{\mathbf{t}} \tag{3.15}$$

and has

$$f = n - b - \text{rank}C \tag{3.16}$$

degrees of freedom (d.f.) and, therefore, $E/f = \hat{\sigma^2}$ provides an unbiased estimator of σ^2. If $r_1 = \cdots = r_v (= r)$, it is an equireplicate design and if $k_1 = \cdots = k_b = (= k)$, it is a proper design. If rank$C = v - 1$, it is connected design (see for example, M.C. Chakrabarti (1965)) and then every treatment contrast is estimable.

A necessary and sufficient condition of estimability $\mathbf{l't}$ is

$$\mathbf{l}'C^{-}C = \mathbf{l}' \tag{3.17}$$

where C^{-} is a generalized inverse of C, that is, it is a vxv matrix such that

$$CC^{-}C = C \quad . \tag{3.18}$$

3.3 Eigenvector Contrasts

It has been shown that

$$C\mathrm{E}_{v1} = 0 \tag{3.19}$$

so that zero is always an eigenvalue of C, with the corresponding unit eigenvector $v^{-\frac{1}{2}}\mathrm{E}_{v1}$. If the rank of C is only $v - s - 1$, there are other s eigenvalues which are zero. Let

$$\Theta_1 \geq \Theta_2 \geq \cdots \geq \Theta_{v-s-1} \tag{3.20}$$

be the non-zero eigenvalues of C, with the corresponding unit orthogonal eigenvectors $\mathbf{f}_i (i = 1, 2, \cdots, v - s - 1)$. Let also $\mathbf{f}_i (i = v - s, \cdots, v - 1)$ and $\frac{1}{\sqrt{v}}\mathrm{E}_{v1}$ be the other orthogonal unit eigenvectors of C corresponding to its zero eigenvalues. Then, from (3.13), it has been shown that the only estimable treatment contrasts are

$\mathbf{f}'\mathbf{t}(i = 1, 2, \cdots, v - s - 1)$ and any treatment contrast must be a linear combination of only these, in order to be estimable.

Any given contrast

$$l_1 t_1 + l_2 t_2 + \cdots + l_v t_v \tag{3.21}$$

is always expressible as

$$m_1 \mathbf{f}_1' \mathbf{t} + \cdots + m_{v-1} \mathbf{f}_{v-1}' \mathbf{t} \tag{3.22}$$

because $\mathbf{f}_i' \mathbf{t}(i = 1, \cdots, v - 1)$ is a complete set of linearly independent contrasts, but it will be estimable only if

$$m_{v-s} = m_{v-s+1} = \cdots = m_{v-1} = 0. \tag{3.23}$$

Also, the best estimate of $\mathbf{f}_i' \mathbf{t}(i = 1, \cdots, s)$ is $\Theta_i^{-1} \mathbf{f}_i' \mathbf{Q}$ and its variance is σ^2 / Θ_i.

Therefore, a unified method of analysis of any experimental design can be based on obtaining Θ_i's, $\mathbf{f}_i$'s and expressing the desired or required set of contrasts in terms of these and then obtain the best estimates and sums of squares of the desired contrasts through their relationship with the $\mathbf{f}_i' \mathbf{t}(i = 1, \cdots, v - s - 1)$.

In the next chapter, we describe this unified method and provide a computer program for implementing these.

CHAPTER IV

A UNIFIED APPROACH OF ANALYSIS OF BIOASSAY DESIGNS

4.1 Introduction

In the earlier chapters, we considered the treatment contrasts that are useful in bioassays, and the general principles of analysis of any experimental design. We observed that the C-matrix of a design, its eigenvalues and vectors provide a unifying tool of inference associated with treatment contrasts. This method expresses the desired contrast in terms of the eigenvector contrasts and exploits this relationship to obtain estimates and sums of squares (S.S.) for testing the significance of desired contrasts.

In the next section, we provide the necessary steps to carry out the objectives. In other words, the next section gives a kind of tree diagram of the intended procedure and then the corresponding computer program based on SAS is provided thereafter.

Extension of this method to two-way designs (that is designs where two types of blocking are introduced) is also discussed in this chapter.

4.2 A Procedural Routine for Analysis of Any One-Way Design

Step 1

Identify the treatments and number them from 1 through v.

Step 2

Identify the blocks and note b, their number. Also, if the blocks are grouped in replicates, note r the number of replications.

Step 3

From the lay-out of the design, form the incidence matrix N of order vxb whose elements are

$$n_{ij} = \begin{cases} 1 & \text{if the } i\text{-th treatment occurs in the } j\text{-th block} \\ 0 & \text{otherwise} \end{cases}$$

Step 4

Evaluate

$$\begin{aligned} N\mathrm{E}_{b1} &= \mathbf{r} = [r_1, \cdots, r_v]' \\ \mathrm{E}_{1v}N &= \mathbf{k} = [k_1, \cdots, k_b]' \\ \text{and form} \quad D_r &= \mathrm{diag}(r_1, \cdots, r_v), \quad D_k = \mathrm{diag}(k_1, \cdots, k_b). \end{aligned}$$

Step 5

Evaluate

$$C = D_r - ND_k^{-1}N'$$

check $C\mathrm{E}_{v1} = 0$. (restrict evaluation to 8 decimal places or so)

Step 6

Find the eigenvalues $\Theta_1 \geq \Theta_2 \geq \cdots \geq \Theta_{v-s-1} > 0$ and $\Theta_{v-s} = \Theta_{v-s+1} = \cdots = \Theta_v = 0$ and note s.

Step 7

Find the eigenvalues and vectors of

$$C + \frac{a}{v}\mathrm{E}_{vv}$$

where $a = \Theta_1 + 10$. Verify that the first eigenvector has all elements equal to $\frac{1}{\sqrt{v}}$. Throw out this vector and the corresponding eigenvalue, a.

Step 8

Note the number of non-zero roots of C. Call it $v - s - 1$.

Step 9

After the eigenvector $v^{-\frac{1}{2}}\mathrm{E}_{v1}$ is thrown out, write down the remaining eigenvectors as a matrix

$$F_1 = [\mathbf{f}_1, \cdots, \mathbf{f}_{v-1}]$$

$$\text{and} \quad F = [\mathbf{f}_1, \cdots, \mathbf{f}_{v-1}, \frac{1}{\sqrt{v}}\mathrm{E}_{v1}].$$

Step 10

From the matrix

$$C^- = F\mathrm{diag}(\frac{1}{\Theta_1}, \cdots, \frac{1}{\Theta_{v-s-1}}, 0, \cdots, 0)F',$$

$$H = C^-C.$$

and $H = C^{-}C$.

Step 11

Write down the desired set of d contrasts that one needs in your bioassay, as $L'\mathbf{t}$ where $L'$is dxv. It is convenient to arrange the rows of L' such that the first contrast is the preparation contrast, the second is the regression one, the third one is parallelism and the rest correspond to the validity tests.

Step 12

Find $L' - L'C^{-}C$ and if this matrix is null, $L't$ is estimable. If a particular row, say u-th row of this matrix is not null, the u-th contrast $\mathbf{l}_u'\mathbf{t}$ in $L't$ is not estimable.

Step 13

From the vector $\mathbf{y}$ of observations, starting from the first block and first plot and going on to the last plot in the last block, form the matrices Δ_1 and Δ_2 as in chapter 3.

Step 14

Obtain

$$\begin{aligned} \mathbf{t} &= \Delta_1'\mathbf{y} \\ \mathbf{B} &= \Delta_2'\mathbf{y} \\ \mathbf{Q} &= \mathbf{T} - ND_k^{-1}\mathbf{B} \\ g &= \mathbf{E}_{1v}\mathbf{t}. \end{aligned}$$

Step 15

Find the Error S.S.

$$E = \mathbf{y}'\mathbf{y} - \mathbf{B}'D_k^{-1}\mathbf{B} - \mathbf{Q}'\hat{\mathbf{t}},$$

f = d.f. of Error S.S. = $\mathbf{r}'\mathrm{E}_{v1} - b - (v - s - 1)$,

$\hat{\sigma}^2 = E/f$ = estimate of σ^2.

Step 16

Find the estimates of the desired estimable contrasts from $L'\hat{\mathbf{t}}$.

Step 17

Find the matrix $L'C^{-}L$ and the estimated variance-covariance matrix $L'C^{-}L\hat{\sigma}^2$ of the estimates.

After carrying out these steps, the preliminary requirements are completed. This should be followed by validity tests and estimation of relative potency. This is illustrated by actual case studies for different types of designs in the subsequent chapters.

4.3 Some Additional Remarks

• For two-way designs the following changes are necessary:

For designs in which heterogeneity is eliminated in two directions and plots are arranged in rows and columns, there are slight changes in the model and the C-matrix. The model becomes

$$\mathbf{y} = \Delta_1'\mathbf{t} + \Delta_2'\boldsymbol{\alpha} + \Delta_3'\boldsymbol{\beta} + \boldsymbol{\epsilon} \tag{4.1}$$

where $\mathbf{y}, \boldsymbol{\epsilon}, \text{and}\Delta_1$ are as in (3.2) and Δ_2, Δ_3 are respectively nxp and nxq matrices corresponding to the p rows and q columns of the design, defined in the same way as Δ_2 for blocks in (3.2) and $n = pq$ is the number of observations. Instead of only one incidence matrix N as in (3.7), we have a treatment-row incidence matrix N_1 and a

treatment-column incidence matrix N_2 defined by

$$N_1 = \Delta_1'\Delta_2, \quad N_2 = \Delta_1'\Delta_3 \quad . \tag{4.2}$$

They satify

$$\mathrm{E}_{1n}\Delta_2 = q\mathrm{E}_{1p}, \quad \mathrm{E}_{1n}\Delta_3 = p\mathrm{E}_{1q} \quad . \tag{4.3}$$

The role of C of (3.12) is taken by the matrix

$$C = D_r - \frac{1}{q}N_1N_1' - \frac{1}{p}N_2N_2' + \frac{1}{pq}N_1\mathrm{E}_{pp}N_1' \tag{4.4}$$

and the vector of adjusted treatment totals is now

$$\mathbf{Q} = \mathbf{T} - \frac{1}{q}N_1\mathbf{R} - \frac{1}{p}N_2\mathbf{C} + \frac{N_1\mathrm{E}_{p1}g}{pq} \tag{4.5}$$

where

$$\mathbf{T} = \Delta_1'\mathbf{y}, \quad \mathbf{R} = \Delta_2'\mathbf{y}, \quad \mathbf{C} = \Delta_3'\mathbf{y}, \quad g = \mathrm{E}_{1v}\mathbf{T}.$$

Everything else then is as before for estimation of treatment contrasts $L'\mathbf{t}$. The Error S.S. is

$$E_1 = \mathbf{y}'\mathbf{y} - \frac{1}{q}\mathbf{R}'\mathbf{R} - \frac{1}{p}\mathbf{C}'\mathbf{C} - \mathbf{Q}'C^{-}\mathbf{Q} + \frac{g^2}{pq} \tag{4.6}$$

with d.f.

$$\begin{aligned} f &= (pq-1) - (p-1) - (q-1) - (v-s-1) \\ &= pq - p - q - v + s + 2. \end{aligned}$$

```
*************************************************************;
*******      Analysis of any one-way design          *******  ;
*************************************************************;

%MACRO ONEWAY;

/*****---------  DO NOT CHANGE THE LINES BELOW  ---------*****/

*** Generate a blk(block) vector;
*** Summation of obs by block;
proc sort data=design out=design;
  by block;
proc means noprint data=design;
  var y;
  by block;
output out=out_blk sum=blk;
run;

*** Generate a trt(treatment) vector;
*** Summation of obs by treat;
proc sort data=design out=design;
  by treat;
proc means noprint data=design;
  var y;
  by treat;
output out=out_trt sum=trt;
run;


proc iml;
  use design;   read all var {y};   * y : vector of obs;
  use design;   read all var {block treat} into bt; *bt: totx2 matrix;
  use contrast; read all into L;    * L` : dxv matrix ;
  use out_blk;  read all var {blk};  * blk:vector of block;
  use out_trt;  read all var {trt};  * trt:vector of trt;

  print, "Observations", y;
  print, "Block", blk;
  print, "Treatment", trt;

  b=max(bt[,1]);  * # of columns in matrix N;
  v=max(bt[,2]);  * # of rows    in matrix N;
  tot = nrow(bt); * # of total observations;

  *** Create a vxb incidence matrix N;
  N=j(v,b,0);
  do i = 1 to v;
     do j = 1 to b;
        do k=1 to tot;
           if bt[k,1]=j & bt[k,2]=i then N[i,j]=N[i,j]+1;
        end;
     end;
  end;

  r=N[,+];   *row size;
  k=N[+,];   *col size;
  print N ;
  print, "Row Size", r;
  print, "Column Size", k;

  Dr=diag(r);  *diagonal matrix for r;
  Dk=diag(k);  *diagonal matrix for k;

  C=Dr-N*inv(Dk)*N`;
  Cinv=ginv(C);
  print C Cinv;

  * Find eigenvaluas and eigenvectors of ;
  * Ca = C+(Max(r)+5)(1/v)Evv                ;
  call teigen(theta,e_vec,C+(max(r)+5)*j(v,v,1/v));
  print theta[format=8.2]  e_vec[format=8.2];

  * Find # of non-zero roots of Ca;
  s=0;
  do i=2 to v;
     if int(abs(theta[i,])*10**4)=0 then s=s+1;
  end;
  print, "s+1 = # of zero roots of Ca", s;

  * If s=0, then compute (2/(v-1)) x sum(1/theta_i)[i=2 to v];
  if s=0 then do;
     O=(2/(v-1))*(sum(1/theta)-1/theta[1,]);
     print, "(2/(v-1)) x sum(1/theta_i)(i=2 to v)", O(format=8.2];
  end;

  * Construct F for e_vec(1) thru e_vec(v-1);
  F=e_vec[,2:v];
  Fprime=F`;
  print Fprime[format=8.2];

  * Compute M`=L`F;
  Mprime=L`*F;
  print Mprime[format=8.2];

  * Partition M` to M1` and M2`;
  M1prime=Mprime[,1:v-s-1];
  print M1prime[format=8.2];
  if s > 0 then do;
     M2prime=Mprime[,v-s:v-1];
     print M2prime[format=8.2];
     end;

  * Print Cinv x C  &  L`A - L`;
  A = Cinv*C;
  H = L`*A-L`;
  print, "ginv(C)*C",    A[format=8.2];
  print, "L`*A - L`",    H[format=8.2];

  * Print trace(ginv(C)xC);
  tr=trace(Cinv*C);
  print, "trace(ginv(C)xC)", tr;


  *** The program below is using observations from;
  *** design, out_blk and out_trt data sets;
        * Notes *;
  ***** a1=total SS;
  ***** a2=adj. treat SS;
  ***** a3=unadj. block SS;
  ***** a4=unadj. treat SS;
  ***** a5=adj. block SS;
```

```
   ***** E =error SS;
   ***** fe=d.f. for error;
   ***** ft=d.f. for adj. treat SS;
   ***** fb=d.f. for adj. block SS;


   *** Create a new L' matrix when s>0 ;
   if s > 0 then do;
      d    = nrow(M2prime);
      newd = 0;
      newL = shape(1,nrow(L),1);
      do i=1 to d;
         null=0;
         do j=1 to s;
            if int(abs(M2prime[i,j])*10**4)=0 then null=null+1;
         end;
         if s=null then do;
                   newd=newd+1;
                   newL=newL||L[,i];
                   end;
      end;
   L=newL[,2:1+newd];
   print, "New contrast matrix L', L;
   end;

   /***
   *** The alternative way;
   *** To create a new L' matrix when s>0 ;
   if s > 0 then do;
      d    = nrow(M2prime);
      newd = 0;
      newL = shape(1,nrow(L),1);
      w=vecdiag(diag(M2prime*M2prime`)*ginv(diag(M2prime*M2prime`)));
      do i=1 to d;
         if w[i,]=0 then do;
            newd=newd+1;
            newL=newL||L[,i];
            end;
      end;
      if new > d then do;
         L=newL[,2:1+newd];
         print, "New contrast matrix L', L;
      end;
   end;
  ***/

   g=sum(blk);  *grand total;

   Q=trt-N*inv(Dk)*blk;
   t_hat=Cinv*Q;
   Lt_hat=L`*t_hat;
   SSLt=(L`*t_hat)`*inv(L`*Cinv*L)*(L`*t_hat);
   print, "T - N*inv(Dk)*B", Q[format=8.2];
   print, "ginv(C)*Q",       t_hat[format=8.2];
   print, "L`*t_hat",        Lt_hat[format=8.2];
   print, "(L`*t_hat)`*inv(L`*ginv(C)*L)*(L`*t_hat)", SSLt[format=8.2];

   a1=y`*y-g**2/tot;
   a2=Q`*t_hat;
   a3=blk`*inv(Dk)*blk-g**2/tot;
   a4=trt`*inv(Dr)*trt-g**2/tot;
   print, "total SS : y`y - g**2/n",                  a1[format=8.2];
   print, "adj. treat SS : Q`*t_hat",                 a2[format=8.2];
   print, "unadj. block SS : B`*inv(Dk)*B - g**2/n",  a3[format=8.2];
   print, "unadj. treat SS : T`*inv(Dr)*T - g**2/n",  a4[format=8.2];

   E=a1-a2-a3;
   a5=a1-E-a4;
   print, "error SS : a1 - a2 - a3",       E[format=8.2];
   print, "adj. block SS : a1 - a4 - E",   a5[format=8.2];

   fe=(tot-1)-(b-1)-(v-s-1);
   ft=v-s-1;
   fb=b-s-1;
   print, "d.f. of for error",     fe;
   print, "d.f. of adj. treat SS", ft;
   print, "d.f. of adj. block SS", fb;

   cov=L`*Cinv*L;
   print, "L`*ginv(C)*L", cov[format=8.2];

   *** Dl=diagonal matrix of elements of L`*t_hat;
   *** Dc=diagonal matrix of elements of L`*ginv(C)*L;
   Dl=diag(Lt_hat);
   Dc=diag(cov);
   sscont=vecdiag((Dl**2)*inv(Dc));
   print, "vector of Dl**2*inv(Dc)", sscont[format=8.2];

   if fe > 0 then do;
      EMS=E/fe;
      capf  =inv(EMS)*sscont;
      print "EMS=E/fe",               EMS;
      print, "inv(EMS)*sscont",       capf  [format=8.2];
   end;

close;

%MEND;
```

```
*************************************************************;
*******      Analysis of any two-way design          *******  ;
*************************************************************;

%MACRO TWOWAY;

/*****---------  DO NOT CHANGE THE LINES BELOW  ---------*****/

*** Generate a trt(treatment) vector;
*** Summation of obs by treat;
proc sort data=design out=design;
  by treat;
proc means noprint data=design;
  var y;
  by treat;
output out=out_trt sum=trt;
run;

*** Generate a row(row) vector;
*** Summation of obs by row;
proc sort data=design out=design;
  by row_;
proc means noprint data=design;
  var y;
  by row_;
output out=out_row sum=row;
run;

*** Generate a col(column) vector;
*** Summation of obs by col;
proc sort data=design out=design;
  by col_;
proc means noprint data=dasign;
  var y;
  by col_;
output out=out_col sum=col;
run;


proc iml;
  use design;   read all var {y};    * y : vector of obs;
  use dasign;   read all var {treat row_ col_} into trc; *trc: totx3 matrix;
  use contrast; read all into L;    * L' : dxv matrix ;
  use out_trt;  read all var {trt};  * trt:vector of trt;
  use out_row;  read all var {row};  * row:vector of row;
  use out_col;  read all var {col};  * col:vector of col;

  print, "Observations", y;
  print, "Treatment",    trt;
  print, "Row #",        row;
  print, "Col #",        col;

  v=max(trc[,1]);  * # of rows    in matrix N1 or N2;
  p=max(trc[,2]);  * # of cols    in matrix N1;
  q=max(trc[,3]);  * # of cols    in matrix N2;
  tot = nrow(trc); * # of total observations;

  *** Create a vxp incidence matrix N1;
  N1=j(v,p,0);
  do i = 1 to v;
     do j = 1 to p;
        do k=1 to tot;
           if trc[k,1]=i & trc[k,2]=j then N1[i,j]=N1[i,j]+1;
        end;
     end;
  end;

  *** Create a vxq incidence matrix N2;
  N2=j(v,q,0);
  do i = 1 to v;
     do j = 1 to q;
        do k=1 to tot;
           if trc[k,1]=i & trc[k,3]=j then N2[i,j]=N2[i,j]+1;
        end;
     end;
  end;

  r=N1[,+];   *Treatment Replication;
  print N1 N2 ;
  print, "Treatment Replication", r;

  Dr=diag(r);  *diagonal matrix for r;

  C=Dr-N1*N1`/q-N2*N2`/p+r*r`/(p*q);
  Cinv=ginv(C);
  print C Cinv;

  * Find eigenvalues and eigenvectors of ;
  * Ca = C+(Max(r)+5)(1/v)Evv                ;
  call teigen(theta,e_vec,C+(max(r)+5)*j(v,v,1/v));
  print theta[format=8.2]  e_vec[format=8.2];

  * Find # of non-zero roots of Ca;
  s=0;
  do i=2 to v;
     if int(abs(theta[i,])*10**4)=0 then s=s+1;
  end;
  print, "s+1 = # of zero roots of Ca", s;

  * If s=0, then compute (2/(v-1)) x sum(1/theta_i)(i=2 to v];
  if s=0 then do;
     O=(2/(v-1))*(sum(1/theta)-1/theta[1,]);
     print, "(2/(v-1)) x sum(1/theta_i)[i=2 to v]", O[format=8.2];
  end;

  * Construct F for e_vec(1) thru e_vec(v-1);
  F=e_vec[,2:v];
  Fprime=F`;
  print Fprime[format=8.2];

  * Compute M'=L'F;
  Mprime=L`*F;
  print Mprime[format=8.2];

  * Partition M` to M1' and M2';
  M1prime=Mprime[,1:v-s-1];
  print M1prime[format=8.2];
  if s > 0 then do;
     M2prime=Mprime[,v-s:v-1];
     print M2prime[format=8.2];
     end;
```

```
   * Print Cinv x C  &  L'A - L';
   A = Cinv*C;
   M = L'*A-L';
   print, 'ginv(C)*C',    A[format=8.2];
   print, 'L'*A - L'',    M[format=8.2];

   * Print trace(ginv(C)xC);
   tr=trace(Cinv*C);
   print, "trace(ginv(C)xC)", tr;


   *** The program below is using observations from;
   *** design, out_blk and out_trt data sets;
         * Notes *;
   ***** a1=total SS;
   ***** a2=adj. treat SS;
   ***** a3=unadj. block SS;
   ***** a4=unadj. treat SS;
   ***** a5=adj. block SS;
   ***** E =error SS;
   ***** fe=d.f. for error;
   ***** ft=d.f. for adj. treat SS;


   *** Create a new L' matrix when s>0 ;
   if s > 0 then do;
      d    = nrow(M2prime);
      newd = 0;
      newL = shape(1,nrow(L),1);
      do i=1 to d;
         null=0;
         do j=1 to s;
            if int(abs(M2prime[i,j])*10**4)=0 then null=null+1;
         end;
         if s=null then do;
                  newd=newd+1;
                  newL=newL||L[,i];
                  end;
      end;
      if newd > 0 then do;
         L=newL[,2:1+newd];
         print, "New contrast matrix L', L;
      end;
   end;

   /***
   *** The alternative way;
   *** To create a new L' matrix when s>0 ;
   if s > 0 then do;
      d    = nrow(M2prime);
      newd = 0;
      newL = shape(1,nrow(L),1);
      w=vecdiag(diag(M2prime*M2prime')*ginv(diag(M2prime*M2prime')));
      do i=1 to d;
         if w[i,]=0 then do;
            newd=newd+1;
            newL=newL||L[,i];
            end;
      end;
   L=newL[,2:1+newd];
   print, 'New contrast matrix L', L;
   end;
  ***/

   g=sum(trt);  *grand total;

   *** Rename the variables;
       p_=p; q_=q;

   Q=trt-N1*row/q_-N2*col/p_+N1*j(p,1,1)*g/(p_*q_);
   t_hat=Cinv*Q;
   Lt_hat=L'*t_hat;
   SSLt=(L'*t_hat)'*inv(L'*Cinv*L)*(L'*t_hat);
   print, 'T - N1row/q - N2col/p + N1Ep1g/pq', Q[format=8.2];
   print, 'ginv(C)*Q',                         t_hat[format=8.2];
   print, 'L'*t_hat',                          Lt_hat[format=8.2];
   print, "(L'*t_hat)'*inv(L'*ginv(C)*L)*(L'*t_hat)", SSLt[format=8.2];

   a1=y'*y-g**2/tot;
   a2=Q'*t_hat;
   a3=row'*row/q_-g**2/tot;
   a4=col'*col/p_-g**2/tot;
   print, 'total SS : y'y - g**2/n',                  a1[format=8.2];
   print, 'adj. treat SS : Q'*t_hat',                 a2[format=8.2];
   print, 'unadj. block SS : row'*row/q - g**2/n',    a3[format=8.2];
   print, 'unadj. treat SS : col'*col/p - g**2/n',    a4[format=8.2];

   E=a1-a2-a3-a4;
   a5=a1-E-a4;
   print, 'error SS : a1 - a2 - a3-a4',      E[format=8.2];
   print, 'adj. block SS : a1 - a4 - E',     a5[format=8.2];

   fe=(tot-1)-(p_-1)-(q_-1)-(v-s-1);
   ft=v-s-1;
   print, 'd.f. of for error',     fe;
   print, 'd.f. of adj. treat SS', ft;

   cov=L'*Cinv*L;
   print, "L'*ginv(C)*L', cov[format=8.2];

   *** D1=diagonal matrix of elements of L'*t_hat;
   *** Dc=diagonal matrix of elements of L'*ginv(C)*L;
   D1=diag(Lt_hat);
   Dc=diag(cov);
   sscont=vecdiag((D1**2)*inv(Dc));
   print, "vector of D1**2*inv(Dc)", sscont[format=8.2];

   if fe > 0 then do;
      EMS=E/fe;
      capf  =inv(EMS)*sscont;
      print "EMS=E/fe",                EMS;
      print, 'inv(EMS)*sscont',       capf  [format=8.2];
   end;

close;

%MEND;
```

CHAPTER V

RELATIVE POTENCY FROM AN INCOMPLETE BLOCK DESIGN

5.1 Introduction

In this chapter, we present a case study for illustrating our program of determining the relative potency, when the experimental design is an incomplete block design. The data is from Gridegeman (1944) for a vitamin A assay.

The responses were weight increases in rats in a period of three weeks. The investigator used blocks only, because they have a higher growth rate and in consequence many litters could not accommodate more than two subjects. He, therefore, arranged that the blocks should be pairs of litter-mates. He assigned randomly chosen 10 of his 30 litters to comparisons of the lowest doses of the two preparations, 10 to comparisons of the highest doses and 10 to those of the middle doses. The doses of the test preparation were chosen in the belief that its potency was about 2.0 units/mg; successive doses of either preparation were in the ratio 5:3. The data is shown in Table 5.1.

The other parameters of the design are $v = 6, b = 30, r = 10, k = 2$. The incidence matrix N, the matrix C, its eigenvlaues, vectors are all shown in the attached computer program. The eigenvalues of C are all non-zero (except one, the always occurring zero value for any C) and all the five contrasts in $L_1't$ are estimable.

From the computer program, we reproduce the ANOVA and the individual S.S. for each contrast in Table 5.2.

The subdivision of the treatment S.S. from the matrix

$$(L_1'\hat{t})'(L_1'C^-L_1)^{-1}(L_1'\hat{t})$$

is in Table 5.3.

It is obvious that the validity test of linearity is satisfactory and that the regression lines for the standard and the test preparations are parallel.

For the standard preparation, the actual doses are 0.9, 1.5 and 2.5 units while for the test preparation, they are 0.45, 0.75, 1.25 mg. After taking logs and subtracting means and dividing by spacing they become -1, 0, 1 for each preparation. Specifically, the transformation from z_s to x_s and z_t to x_t is

$$x_s = (\log z_s - \bar{\log z_s})/\log d$$

$$x_t = (\log z_t - \bar{\log z_t})/\log d$$

where d is the difference between any two successive values of $\log z_s$ or $\log z_t$. If we denote the treatments by $t_1, t_2, t_3, t_4, t_5, t_6$ (the first three for the standard preparation and the last three for the test preparation) and use orthogonal polynomials, it is clear that the preparation contrast is

$$\mathbf{l}_1'\mathbf{t} = -t_1 - t_2 - t_3 + t_4 + t_5 + t_6.$$

The regression contrast is

$$\mathbf{l}_2'\mathbf{t} = -t_1 + 0 \cdot t_2 + t_3 - t_4 + 0 \cdot t_5 + t_6.$$

The parallelism contrast is

$$\mathbf{l}_3'\mathbf{t} = -(-t_1 + 0 \cdot t_2 + t_3) + (-t_4 + 0 \cdot t_5 + t_6)$$

Table 5.1: Data of incomplete block design

Observation	Treatment	Block	Observation	Treatment	Block
20	1	1	43	2	16
33	2	1	35	5	16
18	1	2	43	2	17
36	2	2	50	6	17
16	1	3	37	2	18
44	3	3	33	6	18
22	1	4	44	3	19
33	3	4	26	4	19
29	1	5	48	3	20
35	4	5	28	4	20
26	1	6	35	3	21
14	4	6	43	5	21
47	1	7	43	3	22
48	5	7	33	5	22
30	1	8	46	3	23
30	5	8	23	6	23
16	1	9	51	3	24
38	6	9	51	6	24
30	1	10	20	4	25
41	6	10	37	5	25
40	2	11	12	4	26
47	3	11	30	5	26
40	2	12	21	4	27
59	3	12	33	6	27
35	2	13	25	4	28
4	4	13	40	6	28
47	2	14	39	5	29
16	4	14	43	6	29
27	2	15	18	5	30
35	5	15	27	6	30

Table 5.2: ANOVA Table

Source	d.f.	S.S.	M.S.
Blocks	29	4248	146.48
Treatments	5	2564	512.80
Error	25	1039	41.56

Table 5.3: Breakdown of the treatment S.S.

Source	d.f.	S.S.
Preparation	1	245
regression	1	2081
Parallelsim	1	0
Validity	2	238

and the remaining 2 contrasts are the validity test contrasts. Specially, they are, the quadratic contrast

$$\mathbf{l}_4'\mathbf{t} = (t_1 - 2t_2 + t_3) + (t_4 - 2t_5 + t_6)$$

and the difference in quadratics is

$$\mathbf{l}_5'\mathbf{t} = -(t_1 - 2t_2 + t_3) + (t_4 - 2t_5 + t_6).$$

Collecting the coefficients of $t_1, \cdots, t_6$, the matrix L_1' of order 5x6 of these five contrast

is

$$L_1' = \begin{bmatrix} l_1' \\ l_2' \\ l_3' \\ l_4' \\ l_5' \end{bmatrix} = \begin{bmatrix} -1 & -1 & -1 & 1 & 1 & 1 \\ -1 & 0 & 1 & -1 & 0 & 1 \\ 1 & 0 & -1 & -1 & 0 & 1 \\ 1 & -2 & 1 & 1 & -2 & 1 \\ -1 & 2 & -1 & 1 & -2 & 1 \end{bmatrix}$$

We, therefore, proceed to calculate the relative potency. For equispaced log-doses and symmetric bioassays, the general formula for the relative potency reduced to (for odd number of doses) (Finney, 1978)

$$\log c\hat{\rho} = \frac{(\log d)(k^2-1)L_{\text{prep}}}{6L_{reg}}$$

where d is the spacing of the log doses and c is ratio of any z_t to the corresponding z_s. Here,

$$\begin{aligned} c &= \frac{z_{t_1}}{z_{s_1}} = \frac{.45}{.9} = \frac{1}{2} \\ d &= \log 1.5 - \log .9 = \log \frac{5}{3} \\ k &= \text{number of doses of } S \text{ or } T = 3 \\ L_{\text{reg}} &= \text{regression contrast} = l_2'\hat{t} = 48(9.3125) \\ L_{\text{prep}} &= \text{preparation contrast} = l_1'\hat{t} = 36(-5.2222) \end{aligned}$$

substituting these values, we find $\hat{\rho} = 1.502$. Also, $\hat{\sigma}^2 = \text{MSE} = 41.56$ based on 25 d.f.

$$\begin{aligned} \text{var}(l_1'\hat{t}) &= l_1'C^{-}l_1\hat{\sigma}^2 = 36^2 \cdot \frac{\hat{\sigma}^2}{9} = 144\hat{\sigma}^2 \\ \text{var}(l_2'\hat{t}) &= l_2'C^{-}l_2\hat{\sigma}^2 = 96\hat{\sigma}^2 \end{aligned}$$

By Feiller's theorem (1954), the 98 % confidence interval for ρ is

$$\frac{1}{c}\exp[\frac{(\log d)(k^2-1)}{6l_2't(1-g)}\{l_1't \pm t_o[N\{(1-g)+\frac{(k^2-1)(l_1'\hat{t})^2}{12(l_2'\hat{t})^2}\}]^{3'}\}]$$

where

$$\begin{aligned} g &= \frac{Nt_o^2\hat{\sigma}^2(k^2-1)}{12(l_2'\hat{t})^2} = 0.0847 \\ N &= \text{total no. of observations} \\ t_o &= \text{the 5 \% pt. of a } t \text{ with 25 d.f.} \end{aligned}$$

The upper and lower limits of ρ are 1.915 and 1.117, respectively.

Example 2:

The previous design was a well chosen design as all the eigenvalues of C were equal and none was zero, showing a good balance in estimating contrasts.

We will now consider the same data, when we have a different design, which is not a well chosen one. The data and allocation of treatments to units is shown on the next page. Again, we have $b = 30, v = 6, k = 2, r = 10$ but the incidence matrix N and hence the C matrix is different as shown on the computer program. Two of the eigenvalues of C are non zero (in addition to the usual zero value corresponding to the vector E_{v1}), showing some two contrasts are not estimable, cautioning us to be careful in analyzing the data. The contrast matrix L_1' is the same as before as the doses are the same. From the estimability condition, we find that l_1' is estimable but $l_2't$ and $l_3't$ are not. It is thus not possible to estimate the relative potency.

There is a way-out of this difficulty and that will be available only if we could assume that the 30 litters are chosen at random out of an infinite collection of litters, having a normal distribution. But, we will not go into this complication here. Our aim is to show how the difficulty of non-estimability is detected by our computer program.

Chapter V appendix 1.

Data p184 of from Finney

A balanced incomplete block design
Standard and Test preparations each have 2 doses
A design with 30 blocks.
Well Chosen design
Contrast matrix on p. 29.

Observations

Y
20
18
16
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46
51
35
14
4
16
26
28
20
12
21
25
48
30
35
35
43
33
37
30

39
18
38
41
50
33
23
51
33
40
43
27

Block

BLK
53
54
60
55
64
40
95
60
54
71
87
99
39
63
62
78
93
70
70
76
78
76
69
102
57
42
54
65
82
45

Treatment

TRT
254
381
450
201
348
379

N

	1	1	1	1	1	1	1
:	1	1	1	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0					
	1	1	0	0	0	0	0
:	0	0	0	1	1	1	1
:	1	1	1	1	0	0	0
:	0	0	0	0	0	0	0
:	0	0					
	0	0	1	1	0	0	0
:	0	0	0	1	1	0	0
:	0	0	0	0	1	1	1
:	1	1	1	0	0	0	0
:	0	0					
	0	0	0	0	1	1	0
:	0	0	0	0	0	1	1
:	0	0	0	0	1	1	0
:	0	0	0	1	1	1	1
:	0	0					
	0	0	0	0	0	0	1
:	1	0	0	0	0	0	0
:	1	1	0	0	0	0	1
:	1	0	0	1	1	0	0
:	1	1					
	0	0	0	0	0	0	0
:	0	1	1	0	0	0	0
:	0	0	1	1	0	0	0
:	0	1	1	0	0	1	1
:	1	1					

Row Size

R

10
10
10
10
10
10

Column Size

K						
2	2	2	2	2	2	2
2	2	2	2	2	2	2
2	2	2	2	2	2	2
2	2	2	2	2	2	2
2	2					

C					
5	-1	-1	-1	-1	-1
-1	5	-1	-1	-1	-1
-1	-1	5	-1	-1	-1
-1	-1	-1	5	-1	-1
-1	-1	-1	-1	5	-1
-1	-1	-1	-1	-1	5

CINV					
0.1388889	-0.027778	-0.027778	-0.027778	-0.027778	-0.027778
-0.027778	0.1388889	-0.027778	-0.027778	-0.027778	-0.027778
-0.027778	-0.027778	0.1388889	-0.027778	-0.027778	-0.027778
-0.027778	-0.027778	-0.027778	0.1388889	-0.027778	-0.027778
-0.027778	-0.027778	-0.027778	-0.027778	0.1388889	-0.027778
-0.027778	-0.027778	-0.027778	-0.027778	-0.027778	0.1388889

THETA	E_VEC					
15.00	0.41	-0.12	0.82	-0.26	-0.24	-0.13
6.00	0.41	-0.24	-0.53	-0.26	-0.64	-0.13
6.00	0.41	-0.47	-0.17	-0.26	0.70	-0.13
6.00	0.41	0.84	-0.13	-0.26	0.18	-0.13
6.00	0.41	0.00	0.00	0.21	0.00	0.89
6.00	0.41	0.00	0.00	0.83	0.00	-0.38

s+1 = # of zero roots of Ca

S
0

(2/(v-1)) x sum(1/theta_i)[i=2 to v]

0
0.33

FPRIME

-0.12	-0.24	-0.47	0.84	0.00	0.00
0.82	-0.53	-0.17	-0.13	0.00	0.00
-0.26	-0.26	-0.26	-0.26	0.21	0.83
-0.24	-0.64	0.70	0.18	0.00	0.00
-0.13	-0.13	-0.13	-0.13	0.89	-0.38

MPRIME

1.68	-0.25	1.56	0.36	0.76
-1.19	-0.87	1.09	0.77	-0.25
-0.49	1.12	1.09	-1.12	-0.25
0.72	1.59	0.16	1.93	-2.29
0.96	-1.84	0.16	-1.57	-2.29

M1PRIME

1.68	-0.25	1.56	0.36	0.76
-1.19	-0.87	1.09	0.77	-0.25
-0.49	1.12	1.09	-1.12	-0.25
0.72	1.59	0.16	1.93	-2.29
0.96	-1.84	0.16	-1.57	-2.29

ginv(C)*C

A

0.83	-0.17	-0.17	-0.17	-0.17	-0.17
-0.17	0.83	-0.17	-0.17	-0.17	-0.17
-0.17	-0.17	0.83	-0.17	-0.17	-0.17
-0.17	-0.17	-0.17	0.83	-0.17	-0.17
-0.17	-0.17	-0.17	-0.17	0.83	-0.17
-0.17	-0.17	-0.17	-0.17	-0.17	0.83

L`*A - L`

H

-0.00	-0.00	0.00	0.00	0.00	0.00
-0.00	0.00	-0.00	-0.00	0.00	0.00
0.00	-0.00	0.00	-0.00	0.00	0.00
0.00	0.00	0.00	0.00	0.00	-0.00
-0.00	0.00	0.00	0.00	0.00	-0.00

trace(ginv(C)xC)

TR
5

T - N*inv(Dk)*B

Q
-49.00
32.00
64.00
-84.00
10.50
26.50

ginv(C)*Q

T_HAT
-8.17
5.33
10.67
-14.00
1.75
4.42

L`*t_hat

LT_HAT
-15.67
37.25
-0.42
-21.25
-4.92

(L`*t_hat)`*inv(L`*ginv(C)*L)*(L`*t_hat)

SSLT
2564.92

total SS : y`y - g**2/n

A1
7850.85

adj. treat SS : Q`*t_hat

A2
2564.92

unadj. block SS : B`*inv(Dk)*B - g**2/n

A3
4248.35

unadj. treat SS : T`*inv(Dr)*T - g**2/n

A4
4196.15

error SS : a1 - a2 - a3

E
1037.58

adj. block SS : a1 - a4 - E

A5
2617.12

d.f. of for error

FE
25

d.f. of adj. treat SS

FT
5

d.f. of adj. block SS

FB
29

L`*ginv(C)*L

COV				
1.00	0.00	-0.00	0.00	-0.00
0.00	0.67	0.00	-0.00	-0.00

-0.00	0.00	0.67	-0.00	-0.00
-0.00	-0.00	-0.00	2.00	-0.00
-0.00	0.00	-0.00	-0.00	2.00

vector of Dl**2*inv(Dc)

SSCONT
245.44
2081.34
0.26
225.78
12.09

EMS=E/fe

EMS
41.503333

inv(EMS)*sscont

CAPF
5.91
50.15
0.01
5.44
0.29

The SAS System

Chapter 5
Appendix 2.

Observations

Y
20
22
18
26
30
29
47
16
16
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43
37
38
40
47
27
41
43
35
30
51
45
46
48
55
35
49
51
43
27
15
8
18
14
15
35
34
20
19
23
35
30
37
37
31
35
48
47
18

X-9 Data from p. 179 of Finney

An ill chosen Incomplete block design where needed contrasts are not estimable.

3 doses of S
3 doses of T

Regression contrast Not estimable.

30
37
34
23
44
39
46
41
51
36
28

Block

BLK
35
30
36
40
45
64
81
36
35
53
78
67
75
77
78
62
89
90
53
60
86
79
69
92
94
81
90
102
79
55

Treatment

TRT
254

381
450
201
348
379

	N						
	1	1	1	1	1	1	1
:	1	1	1	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0					
	0	0	0	0	0	0	0
:	0	0	0	1	1	1	1
:	1	1	1	1	1	1	0
:	0	0	0	0	0	0	0
:	0	0					
	0	0	0	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0	0	0	0	0	1
:	1	1	1	1	1	1	1
:	1	1					
	1	1	1	1	1	1	1
:	1	1	1	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0					
	0	0	0	0	0	0	0
:	0	0	0	1	1	1	1
:	1	1	1	1	1	1	0
:	0	0	0	0	0	0	0
:	0	0					
	0	0	0	0	0	0	0
:	0	0	0	0	0	0	0
:	0	0	0	0	0	0	1
:	1	1	1	1	1	1	1
:	1	1					

Row Size

R
10
10
10

10
10
10

Column Size

K

2	2	2	2	2	2	2
2	2	2	2	2	2	2
2	2	2	2	2	2	2
2	2	2	2	2	2	2
2	2					

C

5	0	0	-5	0	0
0	5	0	0	-5	0
0	0	5	0	0	-5
-5	0	0	5	0	0
0	-5	0	0	5	0
0	0	-5	0	0	5

CINV

0.05	0	0	-0.05	0	0
0	0	0	0	-0.1	0
0	0	0.05	0	0	-0.05
-0.05	0	0	0.05	0	0
0	0	0	0	0.1	0
0	0	-0.05	0	0	0.05

THETA	E_VEC					
15.00	0.41	-0.10	0.54	-0.44	-0.45	-0.37
10.00	0.41	-0.07	0.44	0.55	0.54	-0.20
10.00	0.41	0.70	0.12	-0.01	-0.09	0.57
10.00	0.41	0.10	-0.54	0.44	-0.45	-0.37
0.00	0.41	0.07	-0.44	-0.55	0.54	-0.20
-0.00	0.41	-0.70	-0.12	0.01	-0.09	0.57

s+1 = # of zero roots of Ca

S
2

FPRIME

-0.10	-0.07	0.70	0.10	0.07	-0.70
0.54	0.44	0.12	-0.54	-0.44	-0.12

-0.44	0.55	-0.01	0.44	-0.55	0.01
-0.45	0.54	-0.09	-0.45	0.54	-0.09
-0.37	-0.20	0.57	-0.37	-0.20	0.57

MPRIME

-1.05	-2.20	-0.20	-0.00	0.00
-0.00	-0.00	0.00	0.70	1.87
-1.59	0.84	-0.87	0.00	0.00
-0.00	-0.00	-0.00	-3.24	1.22
-1.47	0.43	3.11	0.00	-0.00

M1PRIME

-1.05	-2.20	-0.20
-0.00	-0.00	0.00
-1.59	0.84	-0.87
-0.00	-0.00	-0.00
-1.47	0.43	3.11

M2PRIME

-0.00	0.00
0.70	1.87
0.00	0.00
-3.24	1.22
0.00	-0.00

ginv(C)*C

A

0.50	0.00	0.00	-0.50	0.00	0.00
0.00	0.50	0.00	0.00	-0.50	0.00
0.00	0.00	0.50	0.00	0.00	-0.50
-0.50	0.00	0.00	0.50	0.00	0.00
0.00	-0.50	0.00	0.00	0.50	0.00
0.00	0.00	-0.50	0.00	0.00	0.50

L`*A - L`

H

0.00	0.00	0.00	0.00	0.00	0.00
1.00	0.00	-1.00	1.00	0.00	-1.00
0.00	0.00	0.00	0.00	0.00	0.00
-1.00	2.00	-1.00	-1.00	2.00	-1.00
0.00	0.00	0.00	0.00	0.00	0.00

→ Regression Contrast not estimable

trace(ginv(C)xC)

TR
3

New contrast matrix L

L		
-1	1	-1
-1	0	2
-1	-1	-1
1	-1	1
1	0	-2
1	1	1

T - N*inv(Dk)*B

Q
26.50
16.50
35.50
-26.50
-16.50
-35.50

ginv(C)*Q

T_HAT
2.65
1.65
3.55
-2.65
-1.65
-3.55

L`*t_hat

LT_HAT
-15.70
-1.80
-5.80

(L`*t_hat)`*inv(L`*ginv(C)*L)*(L`*t_hat)

SSLT
446.95

total SS : y`y - g**2/n

A1
7920.85

adj. treat SS : Q`*t_hat

A2
446.95

unadj. block SS : B`*inv(Dk)*B - g**2/n

A3
6411.35

unadj. treat SS : T`*inv(Dr)*T - g**2/n

A4
4196.15

error SS : a1 - a2 - a3

E
1062.55

adj. block SS : a1 - a4 - E

A5
2662.15

d.f. of for error

FE
27

d.f. of adj. treat SS

FT
3

d.f. of adj. block SS

FB
27

L`*ginv(C)*L

COV		
0.60	0.00	0.00
0.00	0.40	0.00
0.00	0.00	1.20

vector of Dl**2*inv(Dc)

SSCONT
410.82
8.10
28.03

EMS=E/fe

EMS
39.353704

inv(EMS)*sscont

CAPF
10.44
0.21
0.71

CHAPTER VI

BIOASSAY IN A TWO-WAY DESIGN

6.1 Introduction

In this chapter, we provide an illustration of the program we created, in the case of a two-way design, and that too for a multiple bioassay. In a multiple bioassay, a standard preparation is assayed against several test preparations simultaneously, instead of carrying out several single assays. Obviously, it is more economical to do so, though the analysis becomes more complex. However, our program can take care of this complexity, provided we form appropriate contrasts that may be necessary for estimation of the relative potencies. The internal homogeneity for linearity and parallelism of regression lines for the preparations and the combinations of the several estimates of the common slope is in general the requirement in such cases.

In addition, this illustration in the next section deals with a 2-way design that eliminates two rather than one source of extraneous variation. Since bioassay experiments are performed on human beings or animals, these are bound to be more than one sources of variations such as age, sex, initial weight etc. and better comparisons can be achieved between any test treatments, if these sources are eliminated.

But this causes other problems, because two- way designs such as Latin Squares and Yonden Squares impose other severe restrictions such as the requirement of the same number of rows, columns and treatments or completeness (i.e. accommodation of all treatments) of either rows or columns. However, these restrictions were necessary for the ease of analysis, and solutions of the least squares solutions. With the advent of computers, this is no longer any problem and our program can handle the complexity of the analysis easily. The actual bioassay design and the test preparations and the sources of variation eliminated are described in the next section.

6.2 A Multiple Bioassay in a Two-way Design

The data for this bioassay is borrowed from Lees and Tootill (1955). A standard and seven test preparations of vitamin B_{12} are assayed simultaneously. Each preparation was tested only at two doses and the resulting 16 treatments were tested in a two-way design, where the rows eliminate the variation due to drift, that is decrease in zone size from one end of the experimental plate to the other. The other source of variation was the difference in time trend, due to the inability of carrying out all tests at the same time in a large experiment.

With 16 treatments, each replicated 4 times only, it was impossible to be able to estimate all the treatment contrasts of interest and confounding or lack of estimability was bound to occur. The analysis presented Lee and Tootil or Finney (1978) is more complex, because they had to spend a considerable amount of time in identifying which contrasts are estimable and which are not. But our unified method is capable of doing this in the regular course of analysis and it is just a matter of looking at the

zero eigenvlaues and their effect on the contrasts of our interest.

6.3 Treatment Contrasts

The preparation contrast, that compare each test preparation $A, B, \cdots, G$ with the standard S are to

$$\begin{aligned} (A_1 + A_2) &- (S_1 + S_2) \\ (B_1 + B_2) &- (S_1 + S_2) \\ &\vdots \\ (G_1 + G_2) &- (S_1 + S_2). \end{aligned}$$

As regards the regression contrasts or the slopes of the regression lines that depict the relationship between the treatment effect and the dose, it is clear that the slope is $(S_2 - S_1)/(1 - (-1))$ where -1, 1 are the two coded doses (metameters) for each preparation with a similar result for the other preparations. The regression contrasts are, thus, proportional to $S_2 - S_1, A_2 - A_1, B_2 - B_1, \cdots$ etc.

The validity tests, in this experiment, consists only of parallelism as a departure from linearity cannot be tested, there being only two doses for each treatment. A comprehensive test of parallelism for all these eight preparations consists in testing the hypothesis that

$$S_2 - S_1 = A_2 - A_1 = \cdots = G_2 - G_1$$

As a result, the following seven contrasts provide the validity tests of parallelism.

$$\begin{aligned} 1.\quad (A_2 - A_1) &- (S_2 - S_1) \\ 2.\quad (B_2 - B_1) &- (S_2 - S_1) \\ &\vdots \end{aligned}$$

$$7. \quad (G_2 - G_1) \quad - \quad (S_2 - S_1)$$

It is possible that all these 7 contrasts may not be estimable and this may not be a testable hypothesis. Only a smaller number of combinations of true contrasts may be estimable and the validity tests may have to be based on fewer degrees of freedom than 7.

6.4 Analysis of the Bioassay Design

In this example, the number of treatment is $v = 16$, they being $S_1, S_2, A_1, A_2, B_1, B_2, \cdots, G_1, G_2$. We will number them from 1 to 16 in the above order.

$$n = \text{the total number of observations} = 64 \quad .$$

The number of rows $p = 8$ and the number of columns $q = 8$; they representing the two extraneous blocking factors to be eliminated. Each treatment is replicated $r = 4$ times.

The data treatment-row and treatment-column incidence matrices N_1 and $N - 2$ are in table 1, 2, and 3 respectively. The treatment, row and column totals are also shown and labeled in the computer program attached. The C matrix and C^- are also shown. The contrast matrix $L_i't$ of interest is

$$
L't = \begin{bmatrix} l_1't \\ l_2't \\ \vdots \\ l_7't \\ \cdots \\ l_8't \\ l_9't \\ \vdots \\ l_{14}'t \\ \cdots \\ l_{15}'t \end{bmatrix} = \begin{bmatrix} -1 & -1 & 1 & 1 & & & & & \\ -1 & -1 & & & 1 & 1 & & & \\ \vdots & & & & & & \ddots & & \\ -1 & -1 & & & & & & 1 & 1 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ 1 & -1 & -1 & 1 & & & & & \\ 1 & -1 & & & -1 & 1 & & & \\ \vdots & & & & & & \ddots & & \\ 1 & -1 & & & & & & -1 & 1 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ -1 & 1 & -1 & 1 & -1 & 1 & \cdots & -1 & 1 \end{bmatrix}
$$

$$
= \begin{bmatrix} \mathrm{E}_{71} & \otimes & [-1,1] & \vdots & \mathrm{I}_7 & \otimes & \mathrm{E}_{12} \\ \cdots & \cdots & \cdots\cdots & \cdots & \cdots & \cdots & \cdots\cdots \\ \mathrm{E}_{71} & \otimes & [1,-1] & \vdots & \mathrm{I}_7 & \otimes & [-1,1] \\ \cdots & \cdots & \cdots\cdots & \cdots & \cdots & \cdots & \cdots\cdots \\ -1 & & 1 & \vdots & \mathrm{E}_{17} & \otimes & [-1,1] \end{bmatrix}
$$

where $l_1't, \cdots, l_7't$ are the preparation contrasts $(A_2 + A_1) - (S_2 + S_1)$ etc. and $l_8't, \cdots, l_{14}'t$ are the parallelsim contrasts $(A_2 - A_1) - (S_2 - S_1)$ etc. and $l_{15}'t = (A_2 - A_1) + \cdots + (G_2 - G_1) + (S_2 - S_1)$ is the common slope if the regression lines are parallel.

The test of estimability, namely, $L'C^-C = L'$ shows that all the preparation contrasts $l_1', \cdots, l_7't$ and the regression contrast $l_{15}'t$ are estimable but all the parallelism contrasts are not. This is supported by the fact that C has two eigenvalues equal to zero (besides the usual value 0 corresponding to E_{v1}). Hence, it is obvious that two linear combinations of $l_8't, \cdots, l_{14}'t$ are confounded and the remaining 5 are not. Thus, the parallelism test will have only 5 d.f. and not 7. The sum of squares corresponding

to these 5 d.f. can be calculated indirectly, after all other S.S. are found out. The treatment S.S has thus not 16-1 = 15 but 15-2 = 13 d.f. We get the following ANOVA tables which are in Table 6.1, 6.2 and 6.3., where c = ratio of any dose of the test drug to the corresponding dose of the standard, and d is the spacing of the log-doses.

Table 6.1: ANOVA Table

Source	d.f.	S.S.	M.S.
Rows(unadj.)	7	5.6400	0.8057
Columns(unadj.)	7	0.3760	0.0537
Treatments(unadj.)	13	$Q'C - Q = 85.2683$	6.3283
Error	36	2.3045	$\hat{\sigma}^2$=0.0640

Table 6.2: Treatment S.S.

Source	d.f.	S.S.
Treatments	13	85.2683

The S.S. in Table 6.2 and 6.3 are found as follows. From the values of $\hat{\mathbf{t}}$ in the computer program, we obtain

$$(L_2'\hat{t})'(L_2'C^{-}\hat{t})^{-1}(L_2'\hat{t})$$

where L_2' is the matrix of the row vectors, $l_1', \cdots, l_7'$, as the S.S. due to the preparation contrasts. The S.S. due to the regression contrast is

$$(l_{15}'\hat{t})^2/(l_{15}'C^{-}l_{15}).$$

Table 6.3: Breakdown of the treatment S.S.

Source	d.f.	S.S.	M.S.
Preparation	7	9.3336	1.3334
Regression	1	75.4727	75.4727
Parallelism	5	0.4620	0.0924

These two are additive because $l'_{15}C^{-}l_2 = 0$. The estimates of the preparation contrasts are: $l'_1\hat{t} = -1.50, l'_2\hat{t} = -2.20, l'_3\hat{t} = -1.18, l'_4\hat{t} = -2.42, l'_5\hat{t} = -1.00, l'_6\hat{t} = -0.45$. The estimate of the regression contrast is $l'_{15}\hat{t} = 17.37$.

6.5 Relative Potency

The general formula for a relative potency in a symmetric parallel line bioassay with k equidistant log-doses is

$$\begin{aligned} \log(c\hat{\rho}) &= d\frac{L_{\text{prep}(k^2-1)}}{3L_{\text{reg}}} \\ &= \frac{l'_1\hat{t}(2^2-1)}{3l'_{15}\hat{t}} \end{aligned}$$

where

$$\begin{aligned} d &= \log(\text{high dose}) - \log(\text{low dose}) \\ c &= \frac{\text{low dose of the test preparation}}{\text{low dose of the standard}}. \end{aligned}$$

The 95% confidence interval for ρ, using Feiller's theorem is

$$\frac{1}{c}\exp[\frac{(\log d)(k^2-1)}{3\hat{L_{\text{reg}}}(1-g)}\{\hat{L_{\text{prep}}} \pm t_o\hat{\sigma}[N\{(1-g)+\frac{(k^2-1)\hat{L^2_{\text{prep}}}}{3\hat{L^2_{\text{reg}}}}\}]^{\frac{1}{2}}]$$

where

$$g = Nt^2\hat{\sigma}^2(k^2-1)/3\hat{L^2_{\text{reg}}}$$

t_0 = upper (0.025) 100 % pt. of a t-dist with d.f. of the error S.S.

N = total no. of observations on the test and standard = 16.

Observations

Chapter 6
Appendix 1.

Y
4
3.8
4.3
1
1.6
1.9
0.9
3.6
3.6
4.4
3
1
1.5
1.9
1
3.8
1.9
0.5
2.6
4
3.3
3.4
3.4
0.3
3.6
4.2
2.8
1.3
1.9
1
1
3.5
2.3
1.5
1.6
3.3
3
2.8
3.8
1
2.5
1.5
1.7
3
3.6
3.4
3.4
0.3
2.3

Lees and Tootill data
Multiple parallel lines bioassay
one standard and seven test
Preparations, each at high + low doses
Latin square of side 8
Incomplete 2-way design
Confounding expected.

16 Treatments & their numbers

S_1	1
S_2	2
A_1	3
A_2	4
B_1	5
B_2	6
C_1	7
C_2	8
D_1	9
D_2	10
E_1	11
E_2	12
F_1	13
F_2	14
G_1	15
G_2	16

S_1 low of standard
S_2 high " "
+ so on.

$V = 16$ # treats
$p = 8$ # rows
$q = 8$ # columns
$r = 4$ # repl. of each tre

Contrasts of interest
Preparation contrasts $(L_1't, \ldots, L_7't)$
$(A_1 + A_2) - (S_1 + S_2)$
and similarly for $B, \ldots, G$
Parallelism contrasts $(L_8't, \ldots, L_{14}t)$
$(A_2 - A_1) - (S_2 - S_1)$
+ similarly for $B, \ldots, G$.
Regression contrast $(L_{15}'t)$
$(S_2 - S_1) + (A_2 - A_1) + (B_2 - B_1) + \cdots + (G_2 - G_1)$

$$L't_{15\times 16}\ {}_{16\times 1} = \begin{bmatrix} L_1't \\ \vdots \\ L_{15}'t \end{bmatrix} = \left[\begin{array}{c|c} E_{7,1} \otimes [-1,-1] & I_7 \otimes E_{12} \\ \hline E_{7,1} \otimes [1,-1] & I_7 \otimes [-1,1] \\ \hline [-1,1] & E_{1,7} \otimes [-1,1] \end{array}\right]$$

$d = 15$
$v = 16$

where $\otimes$ is Kronecker Product.

$$= \left[\begin{array}{cccccc} -1 & -1 & 1 & 1 & & \\ & & & & 11 \cdots & 11 \\ \hline 1 & -1 & -1 & 1 & & \\ \vdots & & & & \ddots & \\ 1 & -1 & & & & -1\ 1 \\ \hline -1 & 1 & \cdots & & -1 & 1 \end{array}\right] \begin{array}{l} \rightarrow \text{Prep. Contrasts} \\ \\ \rightarrow \text{Parallelism Contrast} \\ \\ \rightarrow \text{Regression Contrast} \end{array}$$

1.2
1.6
3.8
3.3
2.9
3.4
1
4.3
4.3
3.6
0.9
2.2
1
0.6
3.4

Treatment totals

TRT
9.2
15.6
4.5
14.3
3.4
12.5
4.5
15.6
2.6
12.5
6.2
14.6
7.7
15.3
6.4
13.6

Row #

ROW totals
24.5
21.4
21.2
18.3
20.4
18.3
17.5
16.9

Col #

COL totals
21.1
20.2
19.4
19.3
19.3
19.4
19.5
20.3

N1

1 0 1 0 1 1 0
: 0

0 1 0 1 0 0 1
: 1

0 1 0 1 0 0 1
: 1

1 0 1 0 1 1 0
: 0

0 1 0 1 0 0 1
: 1

1 0 1 0 1 1 0
: 0

0 1 0 1 0 0 1
: 1

1 0 1 0 1 1 0
: 0

0 1 0 1 0 0 1
: 1

1 0 1 0 1 1 0
: 0

1 0 1 0 1 1 0
: 0

0 1 0 1 0 0 1
: 1

1 0 1 0 1 1 0
: 0

0 1 0 1 0 0 1

```
:       1

        1       0       1       0       1       1       0
:       0

        0       1       0       1       0       0       1
:       1



        N2
        1       0       1       0       0       1       0
:       1

        0       1       0       1       1       0       1
:       0

        0       1       0       1       1       0       1
:       0

        1       0       1       0       0       1       0
:       1

        1       0       1       0       0       1       0
:       1

        0       1       0       1       1       0       1
:       0

        0       1       0       1       1       0       1
:       0

        1       0       1       0       0       1       0
:       1

        1       0       1       0       0       1       0
:       1

        0       1       0       1       1       0       1
:       0

        1       0       1       0       0       1       0
:       1

        0       1       0       1       1       0       1
:       0

        0       1       0       1       1       0       1
:       0

        1       0       1       0       0       1       0
:       1
```

	0	1	0	1	1	0	1
:	0						
	1	0	1	0	0	1	0
:	1						

Treatment Replication

R
4
4
4
4
4
4
4
4
4
4
4
4
4
4
4
4
4
4

C						
3.25	0.25	0.25	-0.75	-0.25	-0.25	0.25
: -0.75	-0.25	-0.25	-0.75	0.25	-0.25	-0.25
: -0.25	-0.25					
0.25	3.25	-0.75	0.25	-0.25	-0.25	-0.75
: 0.25	-0.25	-0.25	0.25	-0.75	-0.25	-0.25
: -0.25	-0.25					
0.25	-0.75	3.25	0.25	-0.25	-0.25	-0.75
: 0.25	-0.25	-0.25	0.25	-0.75	-0.25	-0.25
: -0.25	-0.25					
-0.75	0.25	0.25	3.25	-0.25	-0.25	0.25
: -0.75	-0.25	-0.25	-0.75	0.25	-0.25	-0.25
: -0.25	-0.25					
-0.25	-0.25	-0.25	-0.25	3.25	0.25	-0.25
: -0.25	-0.75	0.25	-0.25	-0.25	0.25	-0.75
: 0.25	-0.75					
-0.25	-0.25	-0.25	-0.25	0.25	3.25	-0.25
: -0.25	0.25	-0.75	-0.25	-0.25	-0.75	0.25

```
:      -0.75       0.25

        0.25      -0.75      -0.75       0.25      -0.25      -0.25       3.25
:       0.25      -0.25      -0.25       0.25      -0.75      -0.25      -0.25
:      -0.25      -0.25

       -0.75       0.25       0.25      -0.75      -0.25      -0.25       0.25
:       3.25      -0.25      -0.25      -0.75       0.25      -0.25      -0.25
:      -0.25      -0.25

       -0.25      -0.25      -0.25      -0.25      -0.75       0.25      -0.25
:      -0.25       3.25       0.25      -0.25      -0.25       0.25      -0.75
:       0.25      -0.75

       -0.25      -0.25      -0.25      -0.25       0.25      -0.75      -0.25
:      -0.25       0.25       3.25      -0.25      -0.25      -0.75       0.25
:      -0.75       0.25

       -0.75       0.25       0.25      -0.75      -0.25      -0.25       0.25
:      -0.75      -0.25      -0.25       3.25       0.25      -0.25      -0.25
:      -0.25      -0.25

        0.25      -0.75      -0.75       0.25      -0.25      -0.25      -0.75
:       0.25      -0.25      -0.25       0.25       3.25      -0.25      -0.25
:      -0.25      -0.25

       -0.25      -0.25      -0.25      -0.25       0.25      -0.75      -0.25
:      -0.25       0.25      -0.75      -0.25      -0.25       3.25       0.25
:      -0.75       0.25

       -0.25      -0.25      -0.25      -0.25      -0.75       0.25      -0.25
:      -0.25      -0.75       0.25      -0.25      -0.25       0.25       3.25
:       0.25      -0.75

       -0.25      -0.25      -0.25      -0.25       0.25      -0.75      -0.25
:      -0.25       0.25      -0.75      -0.25      -0.25      -0.75       0.25
:       3.25       0.25

       -0.25      -0.25      -0.25      -0.25      -0.75       0.25      -0.25
:      -0.25      -0.75       0.25      -0.25      -0.25       0.25      -0.75
:       0.25       3.25




        CINV
    0.203125   0.015625   0.015625  -0.046875  -0.015625  -0.015625   0.015625
:  -0.046875  -0.015625  -0.015625  -0.046875   0.015625  -0.015625  -0.015625
:  -0.015625  -0.015625

    0.015625   0.203125  -0.046875   0.015625  -0.015625  -0.015625  -0.046875
:   0.015625  -0.015625  -0.015625   0.015625  -0.046875  -0.015625  -0.015625
:  -0.015625  -0.015625
```

```
   0.015625 -0.046875  0.203125  0.015625 -0.015625 -0.015625 -0.046875
:  0.015625 -0.015625 -0.015625  0.015625 -0.046875 -0.015625 -0.015625
: -0.015625 -0.015625

  -0.046875  0.015625  0.015625  0.203125 -0.015625 -0.015625  0.015625
: -0.046875 -0.015625 -0.015625 -0.046875  0.015625 -0.015625 -0.015625
: -0.015625 -0.015625

  -0.015625 -0.015625 -0.015625 -0.015625  0.203125  0.015625 -0.015625
: -0.015625 -0.046875  0.015625 -0.015625 -0.015625  0.015625 -0.046875
:  0.015625 -0.046875

  -0.015625 -0.015625 -0.015625 -0.015625  0.015625  0.203125 -0.015625
: -0.015625  0.015625 -0.046875 -0.015625 -0.015625 -0.046875  0.015625
: -0.046875  0.015625

   0.015625 -0.046875 -0.046875  0.015625 -0.015625 -0.015625  0.203125
:  0.015625 -0.015625 -0.015625  0.015625 -0.046875 -0.015625 -0.015625
: -0.015625 -0.015625

  -0.046875  0.015625  0.015625 -0.046875 -0.015625 -0.015625  0.015625
:  0.203125 -0.015625 -0.015625 -0.046875  0.015625 -0.015625 -0.015625
: -0.015825 -0.015625

  -0.015625 -0.015625 -0.015625 -0.015625 -0.046875  0.015625 -0.015625
: -0.015625  0.203125  0.015625 -0.015625 -0.015625  0.015625 -0.046875
:  0.015625 -0.046875

  -0.015625 -0.015625 -0.015625 -0.015625  0.015625 -0.046875 -0.015625
: -0.015625  0.015625  0.203125 -0.015625 -0.015625 -0.046875  0.015625
: -0.046875  0.015625

  -0.046875  0.015625  0.015625 -0.046875 -0.015625 -0.015625  0.015625
: -0.046875 -0.015625 -0.015625  0.203125  0.015625 -0.015625 -0.015625
: -0.015625 -0.015625

   0.015625 -0.046875 -0.046875  0.015625 -0.015625 -0.015625 -0.046875
:  0.015625 -0.015625 -0.015625  0.015625  0.203125 -0.015625 -0.015625
: -0.015625 -0.015625

  -0.015625 -0.015625 -0.015625 -0.015625  0.015625 -0.046875 -0.015625
: -0.015625  0.015625 -0.046875 -0.015625 -0.015625  0.203125  0.015625
: -0.046875  0.015625

  -0.015625 -0.015625 -0.015625 -0.015625 -0.046875  0.015625 -0.015625
: -0.015625 -0.046875  0.015625 -0.015625 -0.015625  0.015625  0.203125
:  0.015625 -0.046875

  -0.015625 -0.015625 -0.015625 -0.015625  0.015625 -0.046875 -0.015625
: -0.015625  0.015625 -0.046875 -0.015625 -0.015625 -0.046875  0.015625
:  0.203125  0.015625
```

```
  -0.015625 -0.015625 -0.015625 -0.015625 -0.046875  0.015625 -0.015625
: -0.015625 -0.046875  0.015625 -0.015625 -0.015625  0.015625 -0.046875
:  0.015625  0.203125
```

THETA
9.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
4.00
0.00
0.00

Two eigenvalues = 0, showing 2 contrasts are non-estimable.
d.f. of treat. S.S = (6−1) − 2 = 13
= (v−1) − s

E_VEC						
0.25	-0.21	0.04	-0.11	-0.42	-0.20	-0.03
0.65	0.11	-0.06	-0.03	-0.15	0.28	0.02
0.00	0.35					
0.25	0.19	-0.15	0.63	-0.15	-0.22	-0.03
0.08	0.11	-0.47	-0.19	0.11	-0.03	-0.04
0.00	-0.35					
0.25	0.19	-0.15	-0.02	-0.15	-0.22	-0.03
0.08	0.11	0.79	-0.19	0.11	-0.03	-0.04
0.00	-0.35					
0.25	-0.21	0.04	0.08	-0.42	-0.20	-0.03
-0.53	0.11	0.04	-0.03	-0.15	-0.48	0.02
0.00	0.35					
0.25	-0.01	0.09	0.06	0.25	0.01	-0.37
0.36	-0.37	0.03	0.22	-0.04	-0.53	-0.05
0.35	0.00					
0.25	-0.05	0.57	0.23	0.03	0.47	0.04
0.05	-0.08	0.12	-0.32	-0.12	0.00	-0.27
-0.35	0.00					
0.25	0.19	0.55	-0.40	-0.15	-0.22	-0.03
-0.14	0.11	-0.21	0.39	0.11	0.09	-0.04

```
:      0.00    -0.35

       0.25    -0.21     0.13     0.23     0.37    -0.20    -0.03
:     -0.10     0.11     0.12     0.11     0.64     0.23     0.02
:      0.00     0.35

       0.25    -0.01    -0.07    -0.04    -0.01     0.01    -0.37
:     -0.36    -0.37    -0.02    -0.22    -0.22     0.55    -0.05
:      0.35     0.00

       0.25     0.03    -0.28     0.27     0.01     0.14     0.04
:     -0.06    -0.08     0.14     0.70    -0.29     0.18    -0.03
:     -0.35     0.00

       0.25     0.70    -0.03    -0.08     0.01     0.27    -0.03
:      0.00     0.11    -0.04    -0.08     0.06    -0.03     0.46
:      0.00     0.35

       0.25    -0.51    -0.08    -0.09    -0.01     0.34    -0.03
:      0.00     0.11    -0.05    -0.04     0.08    -0.03     0.63
:      0.00    -0.35

       0.25    -0.03    -0.05    -0.21     0.56    -0.45     0.08
:      0.00     0.02    -0.11    -0.25    -0.40    -0.06     0.12
:     -0.35     0.00

       0.25    -0.04    -0.20    -0.14     0.22     0.29     0.04
:     -0.02     0.64    -0.07     0.02    -0.14    -0.02    -0.42
:      0.35     0.00

       0.25    -0.01    -0.41    -0.41    -0.13     0.15    -0.04
:     -0.01    -0.29    -0.21    -0.11     0.41    -0.12    -0.33
:     -0.35     0.00

       0.25     0.00     0.00     0.00     0.00     0.00     0.84
:      0.00    -0.34     0.00     0.00     0.00     0.00     0.00
:      0.35     0.00
```

s+1 = # of zero roots of Ca

S
2

FPRIME

```
       -0.21     0.19     0.19    -0.21    -0.01    -0.05     0.19
:     -0.21    -0.01     0.03     0.70    -0.51    -0.03    -0.04
:     -0.01     0.00

       0.04    -0.15    -0.15     0.04     0.09     0.57     0.55
```

```
:      0.13     -0.07     -0.28     -0.03     -0.08     -0.05     -0.20
:     -0.41      0.00

      -0.11      0.63     -0.02      0.08      0.06      0.23     -0.40
:      0.23     -0.04      0.27     -0.08     -0.09     -0.21     -0.14
:     -0.41      0.00

      -0.42     -0.15     -0.15     -0.42      0.25      0.03     -0.15
:      0.37     -0.01      0.01      0.01     -0.01      0.56      0.22
:     -0.13      0.00

      -0.20     -0.22     -0.22     -0.20      0.01      0.47     -0.22
:     -0.20      0.01      0.14      0.27      0.34     -0.45      0.29
:      0.15      0.00

      -0.03     -0.03     -0.03     -0.03     -0.37      0.04     -0.03
:     -0.03     -0.37      0.04     -0.03     -0.03      0.08      0.04
:     -0.04      0.84

       0.65      0.08      0.08     -0.53      0.36      0.05     -0.14
:     -0.10     -0.36     -0.06      0.00      0.00      0.00     -0.02
:     -0.01      0.00

       0.11      0.11      0.11      0.11     -0.37     -0.08      0.11
:      0.11     -0.37     -0.08      0.11      0.11      0.02      0.64
:     -0.29     -0.34

      -0.06     -0.47      0.79      0.04      0.03      0.12     -0.21
:      0.12     -0.02      0.14     -0.04     -0.05     -0.11     -0.07
:     -0.21      0.00

      -0.03     -0.19     -0.19     -0.03      0.22     -0.32      0.39
:      0.11     -0.22      0.70     -0.08     -0.04     -0.25      0.02
:     -0.11      0.00

      -0.15      0.11      0.11     -0.15     -0.04     -0.12      0.11
:      0.64     -0.22     -0.29      0.06      0.08     -0.40     -0.14
:      0.41      0.00

       0.28     -0.03     -0.03     -0.48     -0.53      0.00      0.09
:      0.23      0.55      0.18     -0.03     -0.03     -0.06     -0.02
:     -0.12      0.00

       0.02     -0.04     -0.04      0.02     -0.05     -0.27     -0.04
:      0.02     -0.05     -0.03      0.46      0.63      0.12     -0.42
:     -0.33      0.00

       0.00      0.00      0.00      0.00      0.35     -0.35      0.00
:      0.00      0.35     -0.35      0.00      0.00     -0.35      0.35
:     -0.35      0.35

       0.35     -0.35     -0.35      0.35      0.00      0.00     -0.35
```

:	0.35	0.00	0.00	0.35	-0.35	0.00	0.00
:	0.00	0.00					

MPRIME

	0.00	-0.00	-0.46	0.00	0.00	0.00	-1.17
:	0.00	1.35	-0.00	0.00	-0.76	0.00	0.00
:	0.00						
	-0.04	0.77	-0.23	0.85	0.90	-0.27	-0.32
:	-0.67	0.68	0.12	-0.12	-0.78	-0.29	-0.00
:	-0.00						
	0.00	0.79	-0.69	0.79	0.00	0.00	-0.96
:	0.00	0.44	0.72	0.79	0.06	0.00	0.00
:	0.00						
	0.04	-0.24	-0.29	0.57	0.57	-0.27	-1.15
:	-0.67	0.65	0.70	-0.48	0.47	-0.05	-0.00
:	-0.00						
	0.22	0.01	-0.69	0.56	1.03	0.00	-0.72
:	0.00	0.44	0.09	0.18	-0.32	1.12	0.00
:	-0.00						
	-0.04	-0.13	-0.86	1.35	0.26	0.18	-0.74
:	0.45	0.35	-0.01	-0.50	-0.34	-0.28	0.00
:	-0.00						
	0.02	-0.30	-0.93	0.44	0.57	0.85	-0.73
:	-0.85	0.32	0.10	0.45	-0.37	-0.31	0.00
:	-0.00						
	-0.81	0.37	-0.64	-0.54	0.05	0.00	-0.04
:	0.00	-0.33	0.31	-0.51	-0.14	0.12	0.00
:	1.41						
	-0.44	0.66	-0.57	-0.50	0.48	0.42	0.26
:	0.29	0.50	-0.38	-0.34	0.84	-0.17	-0.71
:	0.71						
	-0.81	-0.24	-0.11	0.25	0.05	0.00	0.60
:	0.00	0.74	-0.12	0.28	0.45	0.12	0.00
:	1.41						
	-0.36	-0.03	-0.43	-0.25	0.15	0.42	0.87
:	0.29	0.57	1.07	-0.33	-0.05	0.08	-0.71
:	0.71						
	-1.61	0.13	-0.75	-0.29	0.09	0.00	0.56
:	0.00	0.41	0.19	-0.23	0.31	0.23	0.00

```
:      0.00

      -0.41      0.03     -0.67     -0.60      0.76     -0.05      0.55
:      0.62      0.45      0.42     -0.00      0.36     -0.48      0.71
:      0.71

      -0.39      0.60     -0.33     -0.14     -0.13      0.88      0.57
:     -0.04      0.63      0.27     -0.66      0.43      0.39      0.71
:      0.71

      -1.62      0.06      2.40      0.09      1.26      1.66     -1.17
:      1.15     -0.35      0.52      0.26     -0.29     -0.18      0.00
:      0.00



   M1PRIME
       0.00     -0.00     -0.46      0.00      0.00      0.00     -1.17
:      0.00      1.35     -0.00      0.00     -0.76      0.00

      -0.04      0.77     -0.23      0.85      0.90     -0.27     -0.32
:     -0.67      0.68      0.12     -0.12     -0.78     -0.29

       0.00      0.79     -0.69      0.79      0.00      0.00     -0.96
:      0.00      0.44      0.72      0.79      0.06      0.00

       0.04     -0.24     -0.29      0.57      0.57     -0.27     -1.15
:     -0.67      0.65      0.70     -0.48      0.47     -0.05

       0.22      0.01     -0.69      0.56      1.03      0.00     -0.72
:      0.00      0.44      0.09      0.18     -0.32      1.12

      -0.04     -0.13     -0.86      1.35      0.26      0.18     -0.74
:      0.45      0.35     -0.01     -0.50     -0.34     -0.28

       0.02     -0.30     -0.93      0.44      0.57      0.85     -0.73
:     -0.85      0.32      0.10      0.45     -0.37     -0.31

      -0.81      0.37     -0.64     -0.54      0.05      0.00     -0.04
:      0.00     -0.33      0.31     -0.51     -0.14      0.12

      -0.44      0.66     -0.57     -0.50      0.48      0.42      0.26
:      0.29      0.50     -0.38     -0.34      0.84     -0.17

      -0.81     -0.24     -0.11      0.25      0.05      0.00      0.60
:      0.00      0.74     -0.12      0.28      0.45      0.12

      -0.36     -0.03     -0.43     -0.25      0.15      0.42      0.87
:      0.29      0.57      1.07     -0.33     -0.05      0.08

      -1.61      0.13     -0.75     -0.29      0.09      0.00      0.56
:      0.00      0.41      0.19     -0.23      0.31      0.23
```

	-0.41	0.03	-0.67	-0.60	0.76	-0.05	0.55
:	0.62	0.45	0.42	-0.00	0.36	-0.48	
	-0.39	0.60	-0.33	-0.14	-0.13	0.88	0.57
:	-0.04	0.63	0.27	-0.68	0.43	0.39	
	-1.62	0.06	2.40	0.09	1.26	1.66	-1.17
:	1.15	-0.35	0.52	0.26	-0.29	-0.18	

M2PRIME

0.00	0.00
-0.00	-0.00
0.00	0.00
-0.00	-0.00
0.00	-0.00
0.00	-0.00
0.00	-0.00
0.00	1.41
-0.71	0.71
0.00	1.41
-0.71	0.71
0.00	0.00
0.71	0.71
0.71	0.71
0.00	0.00

ginv(C)*C

	A						
	0.81	0.06	0.06	-0.19	-0.06	-0.06	0.06
:	-0.19	-0.06	-0.06	-0.19	0.06	-0.06	-0.06
:	-0.06	-0.06					
	0.06	0.81	-0.19	0.06	-0.06	-0.06	-0.19
:	0.06	-0.06	-0.06	0.06	-0.19	-0.06	-0.06
:	-0.06	-0.06					
	0.06	-0.19	0.81	0.06	-0.06	-0.06	-0.19
:	0.06	-0.06	-0.06	0.06	-0.19	-0.06	-0.06
:	-0.06	-0.06					
	-0.19	0.06	0.06	0.81	-0.06	-0.06	0.06
:	-0.19	-0.06	-0.06	-0.19	0.06	-0.06	-0.06
:	-0.06	-0.06					
	-0.06	-0.06	-0.06	-0.06	0.81	0.06	-0.06
:	-0.06	-0.19	0.06	-0.06	-0.06	0.06	-0.19
:	0.06	-0.19					

	-0.06	-0.06	-0.06	-0.06	0.06	0.81	-0.06
:	-0.06	0.06	-0.19	-0.06	-0.06	-0.19	0.06
:	-0.19	0.06					
	0.06	-0.19	-0.19	0.06	-0.06	-0.06	0.81
:	0.06	-0.06	-0.06	0.06	-0.19	-0.06	-0.06
:	-0.06	-0.06					
	-0.19	0.06	0.06	-0.19	-0.06	-0.06	0.06
:	0.81	-0.06	-0.06	-0.19	0.06	-0.06	-0.06
:	-0.06	-0.06					
	-0.06	-0.06	-0.06	-0.06	-0.19	0.06	-0.06
:	-0.06	0.81	0.06	-0.06	-0.06	0.06	-0.19
:	0.06	-0.19					
	-0.06	-0.06	-0.06	-0.06	0.06	-0.19	-0.06
:	-0.06	0.06	0.81	-0.06	-0.06	-0.19	0.06
:	-0.19	0.06					
	-0.19	0.06	0.06	-0.19	-0.06	-0.06	0.06
:	-0.19	-0.06	-0.06	0.81	0.06	-0.06	-0.06
:	-0.06	-0.06					
	0.06	-0.19	-0.19	0.06	-0.06	-0.06	-0.19
:	0.06	-0.06	-0.06	0.06	0.81	-0.06	-0.06
:	-0.06	-0.06					
	-0.06	-0.06	-0.06	-0.06	0.06	-0.19	-0.06
:	-0.06	0.06	-0.19	-0.06	-0.06	0.81	0.06
:	-0.19	0.06					
	-0.06	-0.06	-0.06	-0.06	-0.19	0.06	-0.06
:	-0.06	-0.19	0.06	-0.06	-0.06	0.06	0.81
:	0.06	-0.19					
	-0.06	-0.06	-0.06	-0.06	0.06	-0.19	-0.06
:	-0.06	0.06	-0.19	-0.06	-0.06	-0.19	0.06
:	0.81	0.06					
	-0.06	-0.06	-0.06	-0.06	-0.19	0.06	-0.06
:	-0.06	-0.19	0.06	-0.06	-0.06	0.06	-0.19
:	0.06	0.81					

L`*A - L`

	H						
	0.00	-0.00	0.00	0.00	0.00	0.00	-0.00
:	-0.00	-0.00	-0.00	-0.00	-0.00	0.00	-0.00
:	0.00	-0.00					

$\underline{l}'\underline{t}$ not confounded estimables

	0.00	0.00	0.00	0.00	0.00	0.00	-0.00	$l_2't$ estimable
:	0.00	-0.00	-0.00	0.00	-0.00	-0.00	-0.00	
:	-0.00	-0.00						
	0.00	-0.00	0.00	-0.00	0.00	0.00	0.00	$l_3't$ estimable
:	-0.00	-0.00	-0.00	-0.00	-0.00	0.00	-0.00	
:	-0.00	0.00						
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	$l_4't$ estimable
:	-0.00	0.00	-0.00	0.00	-0.00	-0.00	-0.00	
:	0.00	-0.00						
	0.00	0.00	0.00	-0.00	0.00	0.00	-0.00	$l_5't$ estimable
:	-0.00	0.00	-0.00	0.00	0.00	-0.00	-0.00	
:	0.00	-0.00						
	0.00	0.00	0.00	0.00	0.00	-0.00	0.00	$l_6't$ estimable
:	0.00	-0.00	-0.00	0.00	-0.00	0.00	0.00	
:	-0.00	-0.00						
	0.00	0.00	0.00	0.00	0.00	-0.00	-0.00	$l_7't$ estimable
:	0.00	-0.00	0.00	-0.00	0.00	-0.00	-0.00	
:	0.00	0.00						All Preparation Contrasts estimable
	-0.50	0.50	0.50	-0.50	0.00	0.00	0.50	$l_8't$ Not estimable
:	-0.50	-0.00	-0.00	-0.50	0.50	0.00	0.00	
:	0.00	-0.00						
	-0.25	0.25	0.25	-0.25	0.25	-0.25	0.25	$l_9't$ estimable
:	-0.25	0.25	-0.25	-0.25	0.25	-0.25	0.25	
:	-0.25	0.25						
	-0.50	0.50	0.50	-0.50	0.00	0.00	0.50	$l_{10}'t$ not estimable
:	-0.50	0.00	0.00	-0.50	0.50	-0.00	-0.00	
:	0.00	0.00						
	-0.25	0.25	0.25	-0.25	0.25	-0.25	0.25	$l_{11}'t$ Not estimable
:	-0.25	0.25	-0.25	-0.25	0.25	-0.25	0.25	
:	-0.25	0.25						
	-0.00	-0.00	0.00	-0.00	0.00	0.00	-0.00	$l_{12}'t$ estimable
:	0.00	-0.00	-0.00	0.00	0.00	-0.00	-0.00	
:	0.00	-0.00						
	-0.25	0.25	0.25	-0.25	-0.25	0.25	0.25	$l_{13}'t$ not estimable
:	-0.25	-0.25	0.25	-0.25	0.25	0.25	-0.25	
:	0.25	-0.25						
	-0.25	0.25	0.25	-0.25	-0.25	0.25	0.25	$l_{14}'t$ not estimable
:	-0.25	-0.25	0.25	-0.25	0.25	0.25	-0.25	
:	0.25	-0.25						

	0.00	0.00	-0.00	-0.00	0.00	0.00	-0.00
:	0.00	-0.00	-0.00	-0.00	0.00	-0.00	-0.00
:	0.00	0.00					

list Regression Contrast estimable

trace(ginv(C)xC)

TR
13

= d.f. of adj. treat s.s.

New contrast matrix L

	L						
	-1	-1	-1	-1	-1	-1	-1
:	1	-1					
	-1	-1	-1	-1	-1	-1	-1
:	-1	1					
	1	0	0	0	0	0	0
:	0	-1					
	1	0	0	0	0	0	0
:	0	1					
	0	1	0	0	0	0	0
:	0	-1					
	0	1	0	0	0	0	0
:	0	1					
	0	0	1	0	0	0	0
:	0	-1					
	0	0	1	0	0	0	0
:	0	1					
	0	0	0	1	0	0	0
:	0	-1					
	0	0	0	1	0	0	0
:	0	1					
	0	0	0	0	1	0	0
:	-1	-1					
	0	0	0	0	1	0	0
:	1	1					
	0	0	0	0	0	1	0

```
:     0     -1

      0      0      0      0      0      1      0
:     0      1

      0      0      0      0      0      0      1
:     0     -1

      0      0      0      0      0      0      1
:     0      1
```

T - N1row/q - N2col/p + N1Ep1g/pq

Q
-1.47
6.46
-4.64
3.63
-5.98
2.07
-4.64
4.93
-6.78
2.07
-4.47
5.46
-2.73
5.92
-4.03
4.22

$\hat{\underline{t}} =$ ginv(C)*Q Least-squares solutions

T_HAT
-0.37
1.61
-1.16
0.91
-1.50
0.52
-1.16
1.23
-1.70
0.52
-1.12
1.36
-0.68
1.48
-1.01

1.05

L`*t_hat Estimates of estimable Contrasts.

LT_HAT
-1.50
-2.23
-1.18
-2.43
-1.00
-0.45
-1.20
0.50
17.38

(L`*t_hat)`*inv(L`*ginv(C)*L)*(L`*t_hat) S.S. due to all estimable Contrasts

SSLT
85.06

total SS : y`y - g**2/n

A1
93.59

adj. treat SS : Q`*t_hat

A2
85.27

unadj. ~~block~~ Row SS : row`*row/q - g**2/n

A3
5.65

unadj. ~~treat~~ Column SS : col`*col/p - g**2/n

A4
0.38

error SS : a1 - a2 - a3-a4

E
2.30

~~adj. block SS : a1 - a4 - E~~

~~A5~~
~~90.784~~

d.f. of for error

f_e = FE
36

d.f. of adj. treat SS

FT
13

L`*ginv(C)*L

	COV						
	1.00	0.50	0.50	0.50	0.50	0.50	0.50
:	0.00	-0.00					
	0.50	1.00	0.50	0.50	0.50	0.50	0.50
:	-0.00	-0.00					
	0.50	0.50	1.00	0.50	0.50	0.50	0.50
:	0.00	-0.00					
	0.50	0.50	0.50	1.00	0.50	0.50	0.50
:	-0.00	-0.00					
	0.50	0.50	0.50	0.50	1.00	0.50	0.50
:	0.00	-0.00					
	0.50	0.50	0.50	0.50	0.50	1.00	0.50
:	0.00	-0.00					
	0.50	0.50	0.50	0.50	0.50	0.50	1.00
:	0.00	-0.00					
	0.00	0.00	0.00	0.00	0.00	0.00	0.00
:	1.00	-0.00					
	-0.00	0.00	-0.00	-0.00	-0.00	-0.00	-0.00
:	0.00	4.00					

vector of Dl**2*inv(Dc)

SSCONT
2.25
4.95
1.38
5.88
1.00
0.20
1.44
0.25
75.47

EMS=E/fe

EMS
0.0640104

inv(EMS)*sscont

CAPF
35.15
77.34
21.57
91.87
15.62
3.16
22.50
3.91
1179.07

CHAPTER VII

CONCLUDING REMARKS

7.1 Summary

In the first chapter, we described what a bioassay is and defined the concept of relative potency of a drug as compared with a standard one. In the second chapter, we described the concepts of contrasts in experimental designs and the contrasts that are needed for a bioassay. In the third chapter, we described the use of the C-matrix of a design in analyzing it and in the fourth chapter, we gave an algorithm for a unified method of analysis of any bioassay design. In the fifth and sixth chapters, we considered two incomplete block designs and a 2-way design with a multiple bioassay experiment.

7.2 Remarks

With the help of three case studies, one dealing with a well-planned balanced incomplete block design, the other dealing with an ill-chosen design and the third dealing with a 2-way design and multiple assay, we have shown how a single unified

program based on the eigenvalues and eigenvectors of the C-matrix of a design. We can find out which contrasts are estimable, which are confounded, and which are unconfounded and have simple estimates. The C-matrix provides estimates of estimable contrasts, their variances, covariances and the sums of squares. Once these essential tasks are carried out, computation of the relative potency with the help of standard formulas (which we have derived in the appendices) is routine. The last case study was an example that dealt with several complications : two-way design, incomplete rows and columns, anon-testable hypothesis of validity tests that needed to be replaced by and effective testable hypothesis.

We did not included in this dissertation slope-ratio assays. We considered only the parallel-line bioassays, because there is no conceptual difficulty in using our method for the slope-ratio assay; only the contrasts are different.

Also, we did not include the changes in the analysis of a design when the blocks are chosen at random and the block effects are random variables. If so, even the confounded contrasts can be estimated but with a different variance. This extension of analysis is not difficult and this addition to our program is being worked out.

APPENDIX I

DERIVATION OF SUITABLE CONTRASTS IN PARALLEL-LINE BIOASSAYS

Let $z_{s1}, z_{s2}, \cdots, z_{sm}$ be the original doses of the standard preparation (S) and $z^*_{t1}, z^*_{t2}, \cdots, z^*_{tm}$ be the original dose of the test preparation (T). These doses are then transformed by a logarithmic transformation to achieve linearity of regression. We also further assume that on a log scale they are equally spaced. The dose metameters are then

$$x_{si} = \begin{cases} \log_{\sqrt{d}} z_{si} - \frac{1}{m}\sum_{i=1}^{m} \log_{\sqrt{d}} z_{si} & \text{if } m \text{ is even} \\ \log_d z_{si} - \frac{1}{m}\sum_{i=1}^{m} \log_d z_{si} & \text{if } m \text{ is odd} \end{cases}$$

where d is the spacing of the doses on the log-scale i.e.

$$d = z_{s(i+1)}/z_{si} \quad (i = 1, 2, \cdots, m)$$

Then, it follows that

$$x_{si} = \begin{cases} 2i - (m-1) & \text{if } m \text{ is even} \\ i - \frac{1}{2}(m-1) & \text{if } m \text{ is odd} \end{cases}$$

A similar transformation is made from the $z^*_{ti} (i = 1, 2, \cdots, m^*)$ to x^*_{ti}.

Now, consider the regression of the treatment effects t_{si} on the dose metameters x_{si} for S. If the regression is linear, we can write

$$t_{si} = \alpha_s + \beta_s x_{si} \quad (i = 1, 2, \cdots, m)$$

for S, and similarly

$$t_{ti} = \alpha_t + \beta_t x^*_{ti} \quad (i = 1, 2, \cdots, m^*)$$

Observe that

$$\sum_i x_{si} = 0, \quad \sum_i x^*_{ti} = 0.$$

Hence,

$$\beta_s = \frac{\sum t_{si} x_{si}}{\sum x^2_{si}},$$
$$\beta_t = \frac{\sum t_{ti} x^*_{ti}}{\sum x^{*2}_{ti}}.$$

Also,

$$\alpha_s = \sum_i t_{si}/m$$
$$\alpha_t = \sum_i t_{ti}/m.$$

Parallelism of regression lines is tested by the difference in slopes $\beta_t - \beta_s$ which is the parallelism contrast

$$\frac{\sum t_{ti} x^*_{ti}}{\sum x^{*2}_{ti}} - \frac{\sum t_{si} x^*_{si}}{\sum x^{*2}_{si}}.$$

If, however, $\beta_s = \beta_t (= \beta)$ the contrast that represents the common slope β is the weighted average

$$\beta = \frac{\sum t_{ti} x^*_{ti} + \sum t_{si} x_{si}}{\sum x^{*2}_{ti} + \sum x^2_{si}}$$

which is called the regression contrast.

APPENDIX II

RELATIVE POTENCY

Let z_s, z_t denote the original doses of the standard (S) and the Test (T) preparations and let

$$x_s = \log z_s, \quad x_t = \log z_t$$

be the transformed dose metameters. If the treatment effects of S and T are denoted by t_s and t_t and if the parallel regression lines of the preparations S and T are

$$t_s = \alpha_s + \beta_s$$

$$t_t = \alpha_t + \beta_t.$$

We find, from the usual regression theory

$$\alpha_s = \bar{t}_s - \beta \bar{x}_s$$

$$\alpha_t = \bar{t}_t - \beta \bar{x}_t$$

where $\bar{t}_s, \bar{t}_t, \bar{x}_s, \bar{x}_t$ are averages and

$$\beta = \frac{\sum t_s(x_s - \bar{x}_s) + \sum t_t(x_t - \bar{x}_t)}{\sum (x_s - \bar{x}_s)^2 + \sum (x_t - \bar{x}_t)^2}$$

the summations being over all the values of t_s and t_t.

If ρ is the relative potency of T with respect to S, a dose $z_s = \rho z_t$ of S and z_t of T produce the same effect and hence

$$\alpha_s + \beta \log(\rho z_t) = \alpha_t + \beta \log z_t.$$

This leads to

$$\begin{aligned}\log\rho &= \frac{\alpha_t - \alpha_s}{\beta} \\ &= \frac{\bar{t}_t - \bar{t}_s}{\beta} - (\bar{x}_t - \bar{x}_s) \\ &= \frac{L_{\text{prep}}}{L_{\text{reg}}} \cdot \frac{S_{xxt} + S_{xxs}}{k} - (\bar{x}_t - \bar{x}_s)\end{aligned}$$

where

$$\begin{aligned}L_{\text{prep}} &= \text{Preparation Contrast} = \sum t_t - \sum t_s \\ L_{\text{reg}} &= \text{Regression Contrast} = \sum t_t(x_t - \bar{x}_t) + \sum t_s(x_s - \bar{x}_s) \\ k &= \text{Number of doses of } S \text{ and } T \text{ in the experiment.}\end{aligned}$$

If in a parallel line bioassay, the doses used are $z_s = h, hd, hd^2, \cdots, hd^{k-1}$ and $z_t = ch, chd, chd^2, \cdots, chd^{k-1}$, so that $\log d$ is the spacing between equally spaced log doses, the transformation

$$\begin{aligned}x_t &= \frac{\epsilon(\log z_t - \bar{\log z_t})}{\log d} \\ x_s &= \frac{\epsilon(\log z_s - \bar{\log z_s})}{\log d} \\ \epsilon &= \begin{cases} 2 & \text{if } k \text{ is even} \\ 1 & \text{if } k \text{ is odd} \end{cases}\end{aligned}$$

makes the values of x_s, x_t as

$$\begin{aligned}x_s &= x_t = \epsilon(i - \frac{k-1}{2}), \quad i = 0, 1, 2, \cdots, k-1 \\ \bar{x}_s &= \bar{x}_t = 0\end{aligned}$$

As a result, the formula for $\log\rho$ simplifies to

$$\frac{\log c\rho}{\log d} = \frac{\epsilon(k^2-1)}{6} \cdot \frac{L_{\text{prep}}}{L_{\text{reg}}}$$

where

$$\begin{aligned}L_{\text{reg}} &= \sum_{i=0}^{k-1} \epsilon(i - \frac{k-1}{2})(t_{si} + t_{ti}) \\ L_{\text{prep}} &= \sum_i t_{ti} - \sum_i t_{si}.\end{aligned}$$

If now $\hat{L_{\text{prep}}}$ and $\hat{L_{\text{reg}}}$ are estimatable, the data in our experimental design, the estimate of ρ is

$$\frac{1}{c}\text{Antilog}\{(\log d)\cdot\frac{\epsilon(k^2-1)}{6}\cdot\frac{\hat{L_{\text{prep}}}}{\hat{L_{\text{reg}}}}\}$$

where $c = z_{ti}/z_{si}$ for any i. By using Feiller's result for the confidence interval of a ratio, we find the 95 % confidence interval for ρ as

$$\frac{1}{c}\text{Antilog}\{(\log d)\frac{\epsilon(k^2-1)}{6}\cdot(R_u, R_l)\}$$

where R_u, R_l are given by

$$\frac{1}{1-g}\{\frac{\hat{L_{\text{prep}}}}{\hat{L_{\text{reg}}}} - g\frac{C_{12}}{C_{22}} \pm \frac{t_o\hat{\sigma}}{\hat{L_{\text{reg}}}}[C_{11}(1-g) - 2C_{12}\frac{\hat{L_{\text{prep}}}}{\hat{L_{\text{reg}}}} + C_{22}\frac{\hat{L_{\text{prep}}}^2}{\hat{L_{\text{reg}}}^2} + g\frac{C_{12}^2}{C_{22}}]^{\frac{1}{2}}\}$$

and

$$\begin{aligned} C_{11}\sigma^2 &= \text{var}(\hat{L_{\text{prep}}}) \\ C_{22}\sigma^2 &= \text{var}(\hat{L_{\text{reg}}}) \\ C_{12}\sigma^2 &= \text{cov}(\hat{L_{\text{prep}}}, \hat{L_{\text{reg}}}) \\ g &= \frac{t_o^2\hat{\sigma}^2 C_{22}}{\hat{L_{\text{reg}}}^2} \end{aligned}$$

t_o is given by P($|t| < t_o$)=.95 and t has a t distribution with d.f., same as the ones on which $\hat{\sigma}^2$ is based.

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